Optimising agronomic management of the pseudocereals buckwheat (*Fagopyrum esculentum* Moench.) and quinoa (*Chenopodium quinoa* Willd.) for improved yield and nutritional quality

Thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

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Abstract

Buckwheat (*Fagopyrum esculentum* Moench.) and quinoa (*Chenopodium quinoa* Willd.) are gluten-free pseudocereals which have shown increasing consumer demand in recent years. Both crops can provide agronomic benefits to farmers but remain underutilised, particularly in the UK, because of agroecological limitations and/or limited knowledge/research. The aim of this study was therefore to identify genetic variation and optimise the agronomic management of both crops for improved yield and nutritional quality. Field experiments were carried out over 3 seasons (2016-18) at Nafferton Farm in North-east England to evaluate the effects of sowing date (mid-April vs early-May) and fertilisation (zinc and nitrogen source and rate) on the performance of four buckwheat and three quinoa genotypes.

There is clear potential to grow buckwheat and quinoa in the UK where early-May sowing combined with high nitrogen fertilisation rate (150 kg N/ha) are used. The average yields were about 1 t/ha which was similar to the average global production. Both crops were characterised by poor germination in all seasons irrespective of sowing date with an average of 58% across both species in the field trials. The low temperatures in this cool temperate climate leads to an extended life-cycle of 150 – 190 days, resulting in relatively late harvest with high seed losses. Genotype × environment interactions indicated that Cebelica (buckwheat) and Atlas (quinoa) may be suited to the UK agroecological conditions as they produced the highest yields with relatively high grain quality in terms of protein and minerals. In general, buckwheat and quinoa showed concentrations of Fe, Zn, total polyphenols and flavonoids approximately 2-3 times higher than those published for the major cereals wheat, rice and maize. Therefore, despite relatively low yields, there is the potential to develop a UK supply chain of buckwheat and quinoa with relatively high grain quality. Both crops show clear potential especially for low input and organic growers through wider genetic screening programmes, improvements in plant breeding (with the development of more cold tolerant genotypes) and optimisation of agronomic management and harvesting techniques.

Keywords: *Fagopyrum esculentum* Moench., *Chenopodium quinoa* Willd., G × E, nutritional quality, nitrogen fertilisation, zinc biofortification.

Dedication

To my sons and wife (Rubem, Venâncio and Ilda Nongando),

To my parents (Lúcia and Domingos Chipindula),

To my siblings (Rosa, Helder and Arlete Nongando),

Our journeys may have taken us to different places but never away from one another.

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Chapter 1 – General Introduction

Increasing global population, high malnutrition (Lee *et al.*, 2013; Olsen and Palmgren, 2014; Chattha *et al.*, 2017), narrow genetic base, decreasing mineral content of staple food crops (Murphy *et al.*, 2008; Velu *et al*., 2014; Joshi *et al*., 2019), over-reliance on agrochemical inputs and climate change are currently considered major challenges to global food systems. It is estimated that the global population will increase up to or more than 9.2 billion by 2050 (Kuijten, 2013; Borrill *et al.*, 2014; Cakmak and Kutman, 2018), thus putting an unprecedented pressure on agriculture resources to provide food security. Estimates indicate that approximately 25% of the world's population suffers from anaemia, 17.3% is at risk of inadequate Zn intake (Borrill *et al.*, 2014; Cakmak and Kutman, 2018) while one-third of the global population suffers from Zn deficiency and malnutrition (Ghasemi *et al.*, 2013; Olsen and Palmgren, 2014; Chattha *et al.*, 2017). Estimates also indicate that Fe deficiency is the most common cause of anaemia which affects nearly 1.6 billion people globally whereby pre-school children and pregnant women are the group of people at high risk. Moreover, cereal food crops have low concentration and bioavailability of Fe (Murphy *et al.*, 2008; Aciksoz *et al*., 2011). It is also estimated that three billion people have a nutrient deficient diet (Murphy *et al.*, 2008; de Valencia *et al.*, 2017) which often results in impaired physical growth, immune system function, brain development (Boonchuay *et al.*, 2013; Cakmak and Kutman, 2018) as well as chronic diseases such as hypertension (Li and Zhang, 2001; Lee *et al.*, 2013). Therefore, nutritional security is becoming an increasing threat in addition to food security. Most importantly, with increasing awareness of healthier diets, nutritional quality of crops is becoming a key driver to maintaining human health (Janssen *et al*., 2017).

To address the occurrence of malnutrition in humans, scientists are devising strategies including genetic and agronomic biofortification which have become mainstream research endeavours especially in Africa and Asia. These initiatives have been supported by various programmes from various institutions such as the Consultative Group for International Agricultural Research (CGIAR) through the International Maize and Wheat Improvement Centre (CIMMYT) or the International Food Policy Research Institute (IFPRI) where the aim is to develop improved mineral-dense varieties to contribute to global food security and introduce efficient and sustainable agricultural practices. However, much of these efforts have been limited to a number of staple crops (e.g. wheat, rice and maize) despite evidence pointing to a number of crops with relatively high nutritional value being currently underutilised. Hence, there is the concern that staple cereal crops have lower nutritional value than many underutilised crops which have been used as staple foods in the past. Among these underutilised crops are the

pseudocereals buckwheat (*Fagopyrum* spp.) and quinoa (*Chenopodium* spp.), which although cultivated in specific regions (often because they are essential for traditional food products or because they are linked to nutritional benefits), are often not supported by international research and extension programmes to increase their productivity, processing and marketing potential (Jarvis *et al*., 2017) probably due to various factors including limited knowledge and relatively difficult management.

In recent years, there has been an increased focus on improving and diversifying human diets globally by identifying and selecting alternatives to the staple cereal crops. There is evidence indicating that the pseudocereals buckwheat and quinoa can show clear agronomic properties and provide important health benefits to consumers. Indeed, several studies (Holasova *et al*., 2002; Bonafaccia *et al*., 2003; Zhang *et al*., 2012b; Joshi *et al*., 2019) have shown that buckwheat and quinoa are suitable for the production of gluten-free products, nutraceuticals and functional foods with health benefits to consumers beyond basic nutrition. For example, 30g daily intake of buckwheat has the potential to reduce the risk of cardiovascular diseases and mitigate the effects of coeliac disease and gluten sensitivity symptoms (Bonafaccia *et al*., 2003). However, there is little information on the potential for agroecological adaptation outside the specific regions of origin especially in cooler environments such as the UK. Most importantly, much of the global production is still confined to a few countries e.g. Russia and China for buckwheat, Bolivia and Peru for quinoa which may not be able to meet the increasing global demand.

It is well established that optimisation of agronomic management (e.g. sowing date and fertilisation practices) is key to cultivation of any crop. While sowing date plays a key role in the growth and development of crops (Bhargava and Srivastava, 2013; Joshi *et al*., 2019) and hence the management of crop rotation systems, fertilisation is arguably the most important management factor to optimise crop yield and quality (Fang *et al*., 2018). Data available from various studies show that sowing date and fertilisation practices vary in different regions of the world due to different soil types, weather conditions, laws and regulations, or fertiliser availability. Therefore, the central hypothesis of the present study was to test whether buckwheat and quinoa can successfully grow in the UK, particularly NE-England, and produce satisfactory yields with high quality in terms of protein, Zn and bioactive compounds. The specific aims of this study were:

- To evaluate the effectiveness of current agronomic biofortification strategies for increasing grain Zn concentrations of the major cereals via a meta-analysis approach.

- To evaluate the effects of sowing date on the growth, yield and quality of buckwheat (*Fagopyrum esculentum* Moench.) and quinoa (*Chenopodium quinoa* Willd.) genotypes grown in NE-England.
- To evaluate the effects of source and rate of N fertiliser and, foliar Zn fertilisation on the growth, yield and quality of buckwheat and quinoa.
- To provide an overview of the key factors limiting production of buckwheat (*Fagopyrum esculentum* Moench.) and quinoa (*Chenopodium quinoa* Willd.) in NE-England.

CHAPTER 2 – Literature Review

2. The Pseudocereals Buckwheat and Quinoa

2.1. Buckwheat

2.1.1. Botanical and morphological characteristics

Buckwheat (*Fagopyrum* spp.) is a dicotyledonous broad-leaved plant species member of the *Polygonaceae* family whose seeds resemble in function and composition (especially with respect to carbohydrates) those of the staple cereals (wheat, rice and barley) and therefore is referred to as pseudocereal (Alvarez-Jubete *et al*., 2010; Katar *et al*., 2016). The genus *Fagopyrum* is often classified according to the photosensitivity of flowering and is highly heterogeneous, composed of 26 diploid/tetraploid species of which *Fagopyrum esculentum* Moench. (common buckwheat) and *Fagopyrum tataricum* Gaertn. (tartary buckwheat) are the most cultivated species (**Fig. 2.1**). *F. esculentum* and *F. tataricum* species belong to the large achenes group (Cawoy *et al.*, 2009; Pan and Chen, 2010; Barcaccia *et al*., 2016; Joshi *et al*., 2019). The difference between the two species is that *F. esculentum* is a self-incompatible species with sweet taste whereas *F. tataricum* is self-pollinating species with bitter taste (Iwata *et al*., 2005; Pan and Chen, 2010; Bonafaccia *et al*., 2003; Cawoy *et al.*, 2009; Sytar *et al*., 2016; Janssen *et al*., 2017; Ahmad *et al*., 2018). Although common buckwheat has been widely cultivated, the evolution of the genus *Fagopyrum* remains not fully understood (Sytar *et al*., 2016).

Common buckwheat is a diploid heteromorphic self-infertile species with an indeterminate growth habit (**Fig. 2.2 and 2.3**). The dimorphic self-incompatibility is due to its allogamous characteristic controlled by a group of genes (i.e. S supergene) that discriminates the inability to form a zygote or infertile zygotes. This is the cause of loss of reproductive function and determines the flower morphology. The inflorescence is composed of numerous clusters of flowers (panicles in long axillary spikes) containing 5-6 flowers attached to the nodes of the stem (Halbrecq *et al*., 2005; Woo *et al*., 2010). While thrum floral type species have long pollen tubes, pin floral type species have short pollen tubes (Adhikari and Campbell, 1998; Miljuš-Dukić *et al*., 2004; Matsui *et al*., 2004; Cawoy *et al*., 2009; Woo *et al*., 2010). The thrum floral type species produce 1-2 times fewer and larger pollen grains than pin floral type species. Whilst the thrum floral species is a heterozygote (*Ss*) type, the pin floral species is a recessive homozygote (*ss*) type; thus, both species match morphologically and genetically for seed production which would not occur otherwise (Ahmad *et al*., 2018; Joshi *et al*., 2019). Buckwheat has a branched hollow stem, 20 to 70 cm high with internodes. The root system is

shallow, which together with weak stem make the plant prone to lodging (Halbrecq *et al*., 2005; Cawoy *et al*., 2009; Pan and Chen, 2010; Woo *et al*., 2010; Joshi *et al*., 2019).

2.1.2. Origin and domestication of buckwheat

Buckwheat is one of the oldest domesticated food crops from Asia (Joshi *et al*., 2019). It originates from central Asia i.e. Nepal, India and south-west China, in the Himalayan foothills, probably in the first millennium BC, and then established in the rest of the world around the 1500's both as a summer crop in various crop rotations or as an experimental crop on new farmlands (Jacquemart *et al*., 2012; Popović *et al*., 2013; 2014; Mariotti *et al*., 2016). However, with the development of higher yielding staple food crops such as wheat, rice and maize in the 19 and 20th centuries, buckwheat was mostly cultivated only by resource-poor farmers (Mariotti *et al*., 2016).

Data available show that production of buckwheat in the traditional areas such as China has declined substantially over the last 30 years (Jacquemart *et al*., 2012). Currently, buckwheat cultivation is diffused from central Asia to Europe (e.g. Russia, Ukraine), Africa (e.g. South Africa) and America (e.g. Canada, USA) at least partly due to the increasing demand for gluten-free products.

2.1.3. Global production of buckwheat

Buckwheat production remains relatively low in terms of quantity and quality probably due to several factors including the development of higher yielding staple cereal crops, little genetic progress via conventional plant breeding (Joshi *et al*., 2019) and limited agronomic research for crop improvement. Moreover, difficult management and crop specific constraints (e.g. frost tolerance) remain key challenges for cultivation particularly outside the traditional areas of origin.

Global production of buckwheat is around 3 million tonnes with relative stability over the last 50 years. Europe (59%) and Asia (34%) together account for 93% of global buckwheat production (**Table 2.1**), with Russia (37%) and China (28%) the major producers. The average yield of buckwheat is currently around 0.96 t/ha. At a country level, the highest buckwheat yields 3.66 t/ha were obtained in France (Guglielmini *et al*., 2019) where the yields have been three times higher than the world average whereas in China (one of the largest producers) yields remain lower than global average (FAOSTAT, 2019). This difference in yields is at least partly due to the yield potential of the varieties cultivated in each country or region (Campbell, 1997).

Fig. 2.1 Morphological differences between *F. esculentum* (left) and *F. tataricum* (right) buckwheat as described by Woo *et al*., (2010).

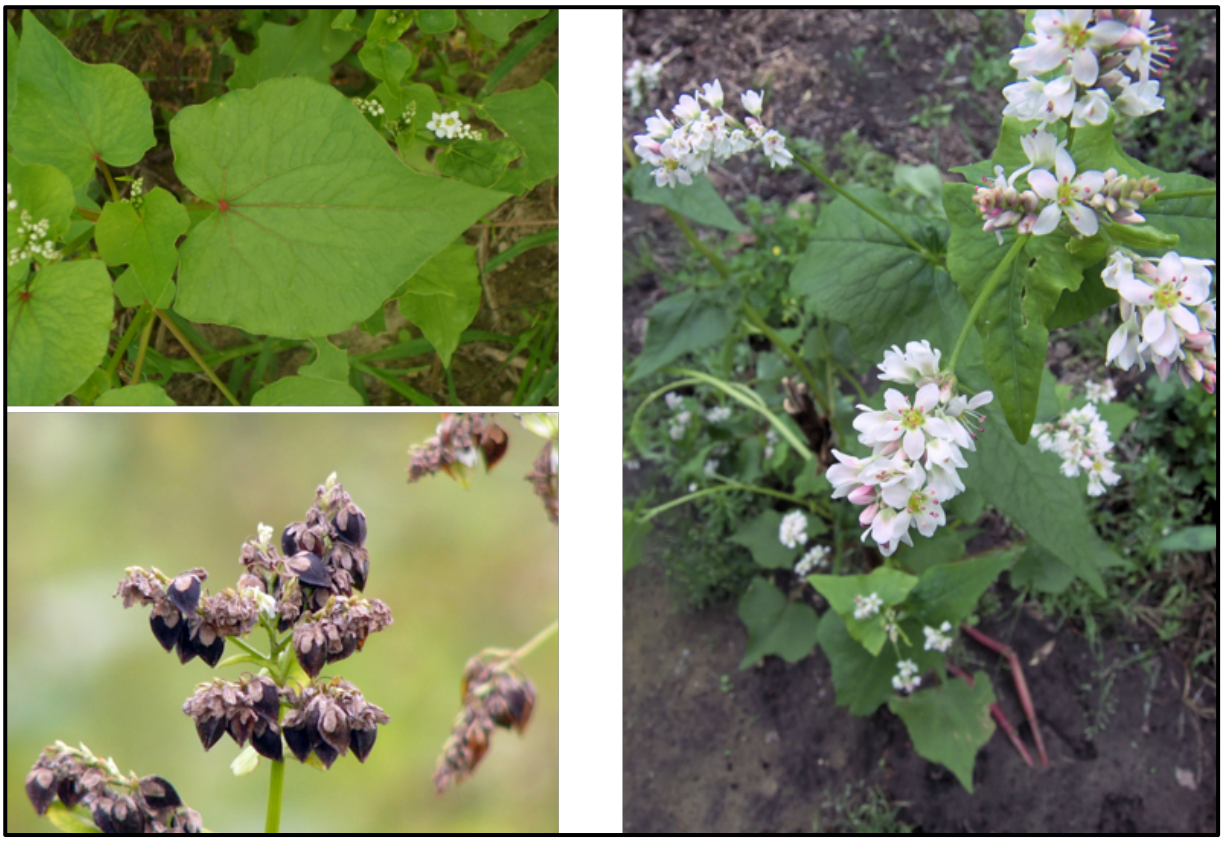

Fig. 2.2 *Fagopyrum esculentum* Moench. images. Photograph by Dalgial and Rasbak. Image credit to Australian National Botanic Gardens.

Fig. 2.3 Mature buckwheat (*Fagopyrum esculentum* Moench.) plant at flowering stage. Photograph by Dalgal. Image credit to Australian National Botanic Gardens.

For example, the French variety La Harpe is probably the highest-yielding buckwheat variety which produces larger and heavier seeds than varieties cultivated elsewhere (Brunori *et al*., 2005). That may be one of the reasons why yields in France are three times higher than in Russia and China even though the total production in France is only about one fourth of that in Russia and China. Therefore, exploiting genetic variability in buckwheat yield and breeding buckwheat varieties for higher seed yield seems to be key to optimising or maximising current yields.

Table 2.1 Global production of buckwheat 1961 - 2017. (FAOSTAT 2019)

	1961	1965	1970	1975	1980	1985	1990	1995	2000	2005	2010	2011	2012	2013	2014	2015	2016	2017
Yield (t/ha)																		
Africa	0.73	0.71	0.77	1.08	0.95	0.52	0.33	0.47	0.60	0.82	0.82	0.95	0.98	0.98	0.99	0.99	1.00	1.02
Asia	0.60	0.68	0.75	1.33	1.27	1.24	1.26	1.59	1.62	0.86	0.69	0.98	0.92	0.90	0.79	0.76	0.84	0.85
Europe	0.43	0.48	0.51	0.30	0.54	0.60	0.78	0.59	0.78	0.80	0.78	1.03	0.88	1.05	1.08	1.04	1.15	1.08
America	1.12	0.97	1.05	1.09	0.83	1.03	1.19	1.05	1.02	1.05	1.09	1.11	1.13	1.14	1.16	1.15	1.15	1.16
World	0.53	0.60	0.66	0.90	0.89	0.92	1.00	1.04	1.09	0.84	0.76	1.01	0.91	1.00	0.96	0.88	1.01	0.97
Total production (million tonnes)																		
Africa	0.01	0.01	<0.01	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	0.02	0.03	0.03	0.02	0.02
Asia	1.55	1.74	1.93	2.73	2.18	2.13	1.93	2.29	2.01	0.84	0.57	0.87	0.79	0.77	0.66	1.52	1.15	1.62
Europe	0.87	0.90	1.01	0.46	0.98	1.05	1.48	1.02	1.64	1.12	0.74	1.35	1.32	1.33	1.09	1.24	1.70	2.05
America	0.05	0.04	0.09	0.09	0.10	0.13	0.21	0.15	0.13	0.12	0.14	0.14	0.14	0.14	0.15	0.14	0.14	0.14
World	2.47	2.70	3.03	3.29	3.26	3.31	3.62	3.47	3.78	2.08	1.46	2.37	2.26	2.26	1.93	2.94	3.00	3.83

There is little or no commercial production of buckwheat in the UK. A few UK farmers have tried buckwheat as a spring break crop over the past 10 years or so. A group of farmers at Coastal Grains Ltd, Belford, Northumberland have grown buckwheat over the past 2 years (i.e. 2018-19) with some of the buckwheat being marketed in 2019. However, in general, much buckwheat production in the UK (except where grown as a cover crop) has not been a success as farmers found it unattractive for commercial production for human consumption because of the indeterminate growth habit, low yield, and lack of recommended agrochemicals, despite recognising that there is a potential market in the UK especially if produced organically. Therefore, a better understanding of the effects of local agroecological conditions on growth and development of buckwheat and quinoa remains an important task which would aid UK farmers with robust evidence-based information.

2.1.4. Agronomic management of buckwheat

Buckwheat is a crop species which requires 17 - 18°C and 50 – 70mm of average daily temperature and monthly rainfall, respectively, for successful cultivation (Cheng *et al*., 2018). Under these climatic conditions, buckwheat can complete its life-cycle in 70 – 90 days (Joshi *et al*., 2019; Guglielmini *et al*., 2019), with the potential to be cultivated twice a year, depending on the crop rotation pattern and weather conditions. In the case of climatic conditions such as those in the UK, the life-cycle of buckwheat is likely to be longer than 90 days, thus there is a need to identify and develop short life-cycle frost resistant genotypes (Woo *et al*., 2010; Ahmad *et al*., 2018; Joshi *et al*., 2019) which could successfully grow with lower than optimal temperatures.

Tartary (*F. tataricum*) and common (*F. esculentum*) buckwheat have shown different responses to cooler (i.e. lower than 17 - 18°C) climatic conditions, whereby tartary buckwheat has been reported to have a better crop growth and development than common buckwheat (Bulan *et al*., 2015). Therefore, agronomic management of common buckwheat in particular is a challenging task especially in cool/temperate environments; hence, determining the appropriate sowing time becomes the most important factor for optimal crop performance. This is probably one of the reasons why common buckwheat is currently cultivated in the UK not as much for food production but as a cover crop, usually in a mixture with other species such as vetch, black oat, brassicas and phacelia.

The appropriate sowing date for buckwheat depends on a number of factors including temperature, moisture, latitude and altitude. For example, while in some regions of China the appropriate sowing date of buckwheat is likely to be late May, in western Europe it is likely to

be between late-March and mid-July. Since susceptibility and severity of frost or chilling conditions vary from place to place, the key is to determine a sowing date in which climatic conditions such as temperature and moisture are suitable for optimal plant germination and survival (Farooq *et al*., 2016). On the other hand, it is equally important to sow at appropriate rate and depth (Juszczak and Wesolowski, 2011; Farooq *et al*., 2016). It has been suggested that while seed rate of buckwheat generally depends on the crop use (30 – 40 kg/ha when used as a grain crop or about 50 kg/ha when used as a cover/fodder crop), sowing depth depends on the climatic conditions (4 – 6 cm or deeper during optimal or dry conditions, respectively) (Farooq *et al*., 2016). However, it is also important to note that while deep sowing has the potential to delay seedling emergence and provide uneven crop establishment (Farooq *et al*., 2016), low seed rate has the potential to increase weed competition due to low plant population and hence result in low yield production.

Buckwheat is reported in the literature to have low fertiliser requirements. Although previous studies have shown that application of 50 kg N/ha resulted in satisfactory buckwheat yields (i.e. about 1 t/ha) (Mariotti *et al*., 2016; Siracusa *et al*., 2017; Fesenko and Mazalov, 2017), it is also proven that higher rates of nitrogen application (e.g. 100 kg N/ha) can result in similar or even higher buckwheat yields (Vazhov *et al*., 2013; Sobhani *et al*., 2014). However, as is the case with most arable crops, high rate of nitrogen application has the potential to delay crop development and increase lodging thus resulting in significant yield losses. This is particularly important because buckwheat is morphologically prone to lodging due to the weak stem that can be strengthened with application of phosphorus i.e. K_2O at moderate rates (Farooq *et al*., 2016). Therefore, determining the appropriate fertilisation practice (i.e. rate and source) is particularly important for optimising yield performance of buckwheat.

Buckwheat can reach maturity 10 – 12 or 20 – 25 weeks after sowing in temperate or cold regions, respectively (Farooq *et al*., 2016). However, regardless of the region, crop harvesting of buckwheat is challenging because of its indeterminate growth habit whereby ripened seeds coexist with green seeds and few flowers in the same plant (Joshi *et al*., 2019). This also highlights another challenge related to the critical period for yield determination which also depends on the climatic conditions and genotype (Guglielmini *et al*., 2019). So that, harvest of buckwheat is usually done when 70 – 75% of the seeds are ripened.

Pollination is another key challenge for the agronomic management of buckwheat because buckwheat requires cross-pollination to produce seeds; therefore, insect pollinators (such as *Hymenoptera*, *Diptera*, *Syrphidae* and *Calliphoridae*) are required due to the dimorphic self-

incompatibility characteristic of buckwheat plants (Halbrecq *et al*., 2005; Cawoy *et al*., 2009; Woo *et al*., 2010; Joshi *et al*., 2019). In fact, a previous study carried out in Japan (Kasijima *et al*., 2017) found significant differences in yield and harvest index between buckwheat pollinated with and without flies for which the genotypes pollinated without flies tended to produce lower yields and harvest index. Another previous study (Vazhov *et al*., 2013) carried out in Russia also found that pollination resulted in significantly higher yield than crops without pollination (i.e. 1.65 vs 0.42 t/ha). The efficiency of pollination depends especially on insect abundance, flower morphology and temperature (Farooq *et al*., 2016). While honey bees (*Apis mellifera* L.) are the main type of *Hymenoptera* insect pollinators because of the amount/type of pollen they can collect at once, thrum flowers produce more nectar than pin flowers hence attracting more insect pollinators with 20°C as the optimum temperature for effective pollination (Cawoy *et al*., 2009; Woo *et al*., 2010; Farooq *et al*., 2016).

An important agronomic management benefit that buckwheat can provide to farmers is generally associated with its weed suppressive ability which results from the allelopathic activity in the crop residues, thus allowing farmers the potential to grow and use buckwheat for weed control in succeeding crops (Bulan *et al*., 2015; Cheng *et al*., 2018). However, although buckwheat residues have been shown to have allelopathic activity in both field and laboratory studies with several allelochemical compounds identified (Falquet *et al*., 2015), it remains unresolved whether the weed suppressive ability can be observed in-season as well as during the following season and often allelopathy has been identified against specific weeds rather than a range of weeds (Bulan *et al*., 2015). Other agronomic management benefits that buckwheat can provide to farmers include an adequate, uniformly distributed mulch particularly if minimum surface soil disturbance is sought (Bulan *et al*., 2015; Cheng, 2018), beneficial insect habitats, soil improvement in terms of nutrient availability, fodder for livestock and green manure (Halbrecq *et al*., 2005; Bulan *et al*., 2015).

2.1.5. Genetic progress

Improving seed yield remains the most important objective in breeding programmes of buckwheat genotypes as this crop is a low-yielding species at least partly due to small seed size (Joshi *et al*., 2019). The most significant genetic progress achieved in common buckwheat breeding programmes is the increase of seed size and shattering resistance. One example is the Canadian Koban variety, considered a breakthrough in the development of high yielding varieties in 1996 (Woo *et al*., 2010; Izydorczyk *et al*., 2014). Other large seed varieties include Manor (Canada), Winsor Royal (USA), Hashikamiwase (Japan), Yangjeal Meamil (Korea) and

La Harpe (France), which could be used as prototypes for increasing the yield ceiling of buckwheat (Woo *et al*., 2010). Despite this progress, various characteristics such as the dimorphic sporophytic self-incompatibility and seed abortion remain unresolved. For example, to address the dimorphic sporophytic self-incompatibility, breeders tried to develop self-pollinating homomorphic *Fagopyrum esculentum* (common buckwheat) lines which often resulted in inbreeding depression possibly due to deleterious recessive genes which do not occur in a homozygous state (Halbrecq *et al*., 2005; Ahmad *et al*., 2018; Joshi *et al*., 2019). Hence, the genetic homogeneity of common buckwheat tends to decrease over time.

Another significant genetic progress achieved is related with seed shattering. In fact, low-shattering and tetraploid buckwheat genotypes were developed in Russia, Canada and Japan (Campbell, 1997; Woo *et al*., 2010) which was a significant improvement towards higher yields. However, there is another challenge to be crossed which is associated with frost resistance especially if the crop is going to be an option in frost sensitive countries and regions. The frost tolerance trait is only found in *Fagopyrum tataricum* genotypes whereas the *Fagopyrum esculentum* genotypes have little frost tolerance (Campbell, 1997). This is particularly important for UK farmers because of high risk of frost occurrence early in the season for spring sown crops. Therefore, identifying a progenitor buckwheat species with a self-compatible pollination and frost tolerance mechanisms would be a priority to develop a high-yielding *Fagopyrum esculentum* genotype through backcrossing or out-crossing breeding, possibly involving interspecific hybridisation with *Fagopyrum tataricum* or other crop species (Woo et al., 2010; Ahmad *et al*., 2018).

2.1.6. Food products and nutritional value of buckwheat

Buckwheat is grown for human consumption as flour, whole seeds, sprouts, shoots and honey. Buckwheat is consumed in a wide range of food products including noodles, spaghetti, porridge, soup, biscuits, breads, cakes, pastry, drinks and beverages (Bai *et al*., 2015). However, consumption of these food products varies from region to region. For example, while buckwheat noodle is popular in Japan, buckwheat porridge is popular in Central Europe and buckwheat crêpes called “galettes” are popular in France (Jacquemart *et al*., 2012). Buckwheat is also sold as pancakes, crispbreads, biscuits and salads in the UK market.

There is increasing evidence suggesting that buckwheat can have a higher nutritional value than the major cereals wheat, rice and maize. This assertion is based on the type of protein, concentration of minerals, vitamins, essential amino acids and bioactive compounds (Čepková *et al*., 2009; Angioloni and Collar, 2011).

Protein

The composition of proteins is often used as an indicator to distinguish the nutritional quality of different crops. The main types of proteins are albumins, globulins and prolamins (Jansen *et al*., 2017). The relative concentration of proline and glutamine in these types of protein is generally used to determine their nutritional value especially in the context of human health. It is well established that prolamin is the type of protein which contains relatively high concentrations of proline and glutamine, generically described as gluten. It is also established that buckwheat proteins are composed mainly of globulins and albumins with little or no prolamins (Ahmed *et al*., 2014). This is particularly important because excess of proline and gluten increase indigestibility and induces gluten sensitivity symptoms, coeliac disease or irritable bowel syndrome (IBS) in consumers who can only mitigate by consumption of a gluten-free diet (Brouns *et al*., 2019). In fact, while approximately 80% of buckwheat protein is composed of soluble albumin and globulin fractions (Radovic *et al*., 1999) hence easily digestible by the human body, approximately 50% of cereal crops (e.g. wheat, rice and maize) protein is the insoluble prolamin fraction (Jansen *et al*., 2017). Therefore, consumers are at high risk of developing gluten sensitivity symptoms, coeliac disease or irritable bowel syndrome from cereal-based diets which they can prevent by a gluten-free buckwheat diet.

Another important aspect associated with the nutritional value of buckwheat proteins is localization in the grain. On average, concentration of proteins in buckwheat grains is similar to that of wheat (i.e. 12 – 13%). But the difference is that a large proportion of buckwheat protein is located in the endosperm fraction of the grain whereas protein in cereal crops is generally located in the bran fraction of the grain (Ahmed *et al*., 2014). While a substantial amount of protein in wheat and rice is lost when the bran is separated in the process of refining grain (Sytar *et al*., 2016; Brouns *et al*., 2019), approximately 90 – 95% is retained in buckwheat due to differences in allocation of protein in the kernels as described above (Alvarez-Jubete *et al*., 2010). Therefore, this suggests that buckwheat could supply more protein than wheat and rice.

Minerals

Buckwheat grains are important sources of Fe, Zn and Se within the range of 60 – 100, 20 – 30 and 0.02 – 0.05 mg/kg, respectively (Steadman *et al*., 2001a; Alvarez-Jubete *et al*., 2010; Alonso-Miravalles and O'Mahony, 2018; Joshi *et al*., 2019). These minerals are essential micronutrients for human health whose concentrations in modern cereal crops are declining over time, particularly Fe and Zn (Murphy *et al*., 2008). Not only is there a decline of essential

micronutrients in modern cereal crops, especially modern wheat varieties, gluten-free cereal diets in general are often deficient in these essential micronutrients in addition to Ca and Mg. This is particularly important in the context of the health of the global population because deficiency in Ca, Fe and Mg, can induce prevalence of osteopenia and osteoporosis as well as neurodegenerative diseases among genetically predisposed and coeliac disease patients (Alvarez-Jubete *et al*., 2010). Therefore, buckwheat could be used as a promising treatment for micronutrient malnutrition and other chronic diseases in humans if more people consume buckwheat (Bai *et al*., 2015).

Bioactive compounds

The most important bioactive compound in buckwheat is rutin (Steadman *et al*., 2001; Kreft *et al*., 2006; Joshi *et al*., 2019). Rutin is a citrus flavonoid glycoside polyphenolic compound with various physiological and pharmacological functions in human health including anti-inflammatory, antihypertensive, antitumor cytoprotective and antibacterial. Other sources of rutin include vegetables, citrus fruits and berries such as *Ruta graveolens* and *Morus elba*. Rutin content varies significantly between buckwheat species whereby *F. esculentum* has lower rutin content than *F. tataricum* (Joshi *et al*., 2019). Nonetheless, common buckwheat (*F. esculentum* Moench.) also contains essential amino acids, fatty acids, phenolic acids, antioxidants and flavonoids (Krkošková and Mrázová, 2005; Sun and Ho, 2005; Kreft *et al*., 2006; Čepková *et al*., 2009; Bai *et al*., 2015; Kiprovski *et al*., 2015). The level of these bioactive compounds is key to the development of functional foods with a potential role in the prevention of degenerative diseases such as cancer and cardiovascular diseases (Janovská *et al*., 2010; Alvarez-Jubete *et al*., 2010; Sytar *et al*., 2016). Most importantly, the balanced composition of these bioactive compounds (especially amino acids and flavonoids) increases the nutritional value of buckwheat over major cereals (Joshi *et al*., 2019).

There is evidence showing that buckwheat bran and hulls can have 2–7 times higher antioxidant activity than barley, triticale and oats (Inglett *et al*., 2011) and the bran is a rich source of dietary fibre (Kreft *et al*., 2006). Indeed, there has been an increasing interest in buckwheat for production of nutraceuticals because buckwheat has the potential for functional food products rich in dietary fibre, polyunsaturated fatty acids and antioxidants which play significant roles in modifying and/or maintaining physiological functions of the human body associated with health problems such as obesity, osteoporosis and diabetes (Čepková *et al*., 2009; Janovská *et al*., 2010; Angioloni and Collar, 2011; Ahmed *et al*., 2014; Joshi *et al*., 2019).

2.2. Quinoa

2.2.1. Botanical and morphological characteristics

Quinoa (*Chenopodium* spp.) is a self-pollinating small grain pseudocereal with a hard coat, member of the *Amaranthaceae* family. The *Amaranthaceae* family is a complex allotetraploid taxa composed of 160 genera and 2400 species including wild, weedy and other forms of seed crops. The genus *Chenopodium* is composed of approximately 150 species but there are about 6000 genotypes cultivated by farmers which can grow under a wide range of agroecological conditions (Bazile *et al*., 2016; Zhang *et al*., 2017) of which *Chenopodium quinoa* Willd. is currently the most cultivated species. However, it is not clear if *Chenopodium quinoa* Willd. descended from the hybrid diploids or tetraploids *Chenopodium hircinum, Chenopodium carnosolum, Chenopodium incisum* or *Chenopodium petiolare* (Sosa-Zuniga *et al*., 2017).

Based on loci and morphological traits, quinoa (*Chenopodium quinoa* Willd.) species are generally divided into sea level and highland types (Ruiz *et al*., 2014; Bazile *et al*., 2016). The plant architecture is highly variable between and within species (**Fig. 1.4**). Some species do not develop branches depending on the genotype and growing conditions, thus resulting in the following growth habits: simple, branched to bottom third, branched to second third and branched with undefined main panicle (Sosa-Zuniga *et al*., 2017). The leaves are polymorphic, covered by cells rich in calcium oxalate, which improves water retention and lowers evapotranspiration; the stem is profusely branched up to 3 metres tall depending on the genotype and plant population. The inflorescence architecture is determined by the length of the pedicels and is composed of numerous hermaphrodite and unisexual female flowers arranged in a glomerular and/or elongated shape (Tapia, 2015; Bazile and Baudron, 2015; Sosa-Zuniga *et al*., 2017; Zhang *et al*., 2017). The structure of the grain (i.e. starch-rich perisperm surrounded by the embryo), particularly the percentage of bran fraction (i.e. seed coat and embryo), explains why quinoa grain can have higher protein and fat concentration than wheat, rice and maize (Alvarez-Jubete *et al*., 2010).

2.2.2. Origin and domestication

Quinoa originates from the Andean highlands of South America where it has been cultivated for thousands of years (Bois et al., 2006; Ruiz *et al*., 2014; Bazile *et al*., 2016), yet it is not known exactly where and when quinoa was domesticated first. Nonetheless, it is well established that once domesticated, quinoa became a subsistence and staple food crop in several countries including Argentina, Bolivia, Chile, Ecuador and Peru (Ramzani *et al*., 2017). Quinoa also became a relatively common food crop in Europe, Canada, the USA, Australia, Africa and

Southeast Asia particularly because of its wide genetic diversity and range of uses of the food products. So, Bolivia and Peru remain the main areas of quinoa cultivation (Nurse *et al*., 2016). In various regions of the world particularly in Denmark, the Netherlands, Greece, Italy, Spain , UK, France, Namibia and Brazil agroecological adaptability tests are being carried out with promising results but currently there is little commercial cultivation due to several reasons including limited knowledge of farmers, consumption habits and institutional support (Ruiz *et al*., 2014; Noulas *et al*., 2017). In particular, quinoa was introduced in the UK for cultivation in the late 1970s (Jacobsen, 2015) where it has been used as cover crop (Ruiz *et al*., 2014).

2.2.3. Global production of quinoa

Historical data of quinoa production is available only for three countries Bolivia, Ecuador and Peru (**Table 2.2**). In fact, production of quinoa is largely confined to these three South American countries, where it is a staple food crop either due to agroecological or sociocultural reasons (Jacobsen, 2003; Zhang *et a*l., 2017), which account for more than 90% of global production whereby Bolivia remains the largest exporter (Kuijten, 2013). The rest of global production is shared among small producers such as Australia, Canada, and the United States of America. It is also suggested that quinoa production in these countries (i.e. Australia, Canada, the United States of America and European countries) will have little or no impact on the income of South American farmers due to global scarcity (Kuijten, 2013).

While the average yield of quinoa is currently around 0.85 t/ha, with the highest yield of 1.68 t/ha obtained in Peru, global production of quinoa is around 160 thousand tonnes (**Table 2.2**) (Alvarez-Jubete *et al*., 2010; Janssen *et al*., 2017). Global production of quinoa increased by 65.8% from 2013 to 2014 (**Table 2.2**) probably due to a global priority given by the United Nations in 2013 as the 'International year of quinoa' to promote consumption of quinoa (FAO & CIRAD, 2015). However, the data also show that global production decreased by 19.2% from 2014 to 2017 possibly due to decrease in cultivation area (because low-yielding crops are being replaced by high-yielding food crops). It is also conceivable that the decrease in the global production could be due to unsustainable models of crop production in some countries (Quiroga *et al*., 2015) or because little genetic progress was achieved.

2.2.4. Agronomic management of quinoa

One of the most critical management tasks in crop production is to determine the appropriate sowing date, as it impacts crop establishment, growth and development.

Table 2.2 Global production of quinoa 1961-2017 (FAOSTAT 2019)

	1961	1965	1970	1975	1980	1985	1990	1995	2000	2005	2010	2011	2012	2013	2014	2015	2016	2017
Yield (t/ha)																		
Bolivia	0.42	0.40	0.80	0.79	0.57	0.44	0.42	0.51	0.65	0.64	0.63	0.65	0.39	0.43	0.60	0.62	0.55	0.60
Ecuador	0.38	0.74	0.74	0.72	0.39	0.45	0.76	0.34	0.50	0.70	0.85	0.87	0.91	0.97	0.90	1.78	1.76	1.46
Peru	0.79	0.95	0.45	0.62	0.88	0.68	0.78	0.74	0.98	1.14	1.16	1.16	1.15	1.16	1.68	1.53	1.23	1.27
World	0.62	0.70	0.60	0.72	0.72	0.49	0.48	0.58	0.79	0.85	0.83	0.83	0.57	0.60	1.00	0.98	0.80	0.85
Total production (thousand tonnes)																		
Bolivia	9.2	6.8	9.7	15.2	8.9	21.1	16.1	18.8	23.8	25.2	36.7	40.9	50.9	63.1	67.7	75.5	65.6	66.8
Ecuador	0.7	0.9	0.7	1.3	0.5	0.03	0.7	0.4	0.7	0.7	1.8	2.1	2.3	2.5	3.7	12.7	3.9	1.3
Peru	22.5	18.5	7.3	8.1	16.3	8.0	6.3	13.8	28.2	32.6	41.1	41.2	44.2	52.1	100.0	100.0	79.3	78.7
World	32.4	26.2	17.8	24.6	25.8	29.2	23.0	33.0	52.6	58.4	79.6	84.2	97.4	117.7	186.2	193.8	148.7	146.7

While it is important to plan the sowing date with target to optimal weather conditions (i.e. higher than 10°C) (Nurse *et al*., 2016), it is also important to note that in general quinoa is sensitive to air temperatures higher than 30°C or lower than 15°C especially during the flowering stage with varietal differences (Bhargava and Srivastava, 2013; Jacobsen, 2015; Bazile *et al*., 2016; Nurse *et al*., 2016; Hinojosa *et al*., 2018). Hence, determining the appropriate time of sowing is key to obtain satisfactory plant germination, growth and development.

As is the case for most arable crops, initial plant establishment is critical in the cultivation of quinoa, which is determined by sowing date and conditions (Risi and Galgwey, 1991; Bhargava and Srivastava, 2013; Jacobsen, 2015). The optimum sowing date of quinoa varies from place to place depending on weather conditions but it is likely to be during spring or early summer (Jacobsen, 2015; Isobe *et al*., 2016; Nurse *et al*., 2016). There is evidence indicating that some quinoa genotypes can resist temperatures below zero e.g. down to -16°C during the vegetative growth stage, probably due to the presence of epidermal vesicles in the young leaves and buds which are absent in mature leaves (Bois *et al*., 2006; Kuijten, 2013; Hinojosa *et al*., 2018). Optimal sowing conditions generally include sowing at 1-2 cm depth in a uniform humid seedbed with soil temperature above 0°C (Jacobsen, 2015), ideally 10 – 15°C to provide consistent seed germination and seedling emergence (Nurse *et al*., 2016). Therefore, planning the sowing date which avoids extreme temperatures particularly at germination and flowering stages, remains the key to quinoa cultivation (Hirich *et al*., 2014; Jacobsen, 2015; Bazile *et al*., 2016).

Quinoa is generally cultivated without fertiliser application especially in the altiplanos of Bolivia, Chile, Ecuador and Peru because quinoa is a crop species which can grow successfully on marginal lands (Bhargava and Srivastava, 2013; Vilcacundo and Hernadez-Ledesma, 2017; Hinojosa *et al*., 2018). However, recent studies (Jacobsen, 2015; De Santis et al., 2016; Garrido *et al*., 2016) have shown that quinoa was highly responsive to moderate or high rates of nitrogen application (i.e. 40 to 200 kg N/ha) in terms of yield production. Therefore, although quinoa is considered a low-yielding crop species, there is the potential for increasing its yield ceiling if nitrogen fertilisation is used (Jacobsen, 2015). Nonetheless, while data available show that quinoa yield can be increased by nitrogen fertilisation practices, there is no clear indication to the optimum fertilisation rate as it depends on genotype, soil type and environmental conditions which vary from place to place.

Quinoa can provide important benefits to farmers as it is a short-season crop species which can complete its life-cycle in 70 – 90 days (Curti *et al*., 2016; Nurse *et al*., 2016), thus showing the potential to be cultivated twice a year (Jung *et al*., 2015) depending on the crop rotation pattern and weather conditions. Nonetheless, there is also evidence for life-cycles of 150 days or more depending on variety, climate, fertilisation and geographical location (Kuijten, 2013; Bazile *et al*., 2015; Sosa-Zuniga *et al*., 2017). Therefore, considering the climatic conditions in the UK, it would be important to understand the effects of local agroecological conditions on growth and development to identify and select suitable quinoa cold tolerant genotypes which could successfully grow in this environment.

Another important agronomic management benefit of quinoa is associated with its forage properties e.g. digestibility and high protein (Callisaya, 2015; Vilcacundo and Hernadez-Ledesma, 2017). Considering the paucity of forage in many regions, quinoa is a multipurpose crop and viable option for animal feed (e.g. ruminants) whose consumption is not affected by the presence of saponins in the grain (Callisaya, 2015).

Despite various agronomic management benefits, there is also a number of challenges associated with agronomic management of quinoa. The major challenge relates to harvest due to adverse impacts of environmental factors (Jacobsen, 2015; Bazile *et al*., 2016) and lack of technologies in the farming areas such as mechanised crop harvesting, thus pointing to the need for optimisation of the commonly used harvest techniques to reduce grain impurities, damage and losses (Quiroga *et al*., 2015; Jacobsen, 2015). Another challenge relates to seed rate and the impact of low temperatures on seed germination (Bois *et al*., 2006; Bhargava and Srivastava, 2013; Jacobsen, 2015; Nurse *et al*., 2016). This is particularly important in the UK where the intensity and duration of low temperatures especially for spring sown crops can ultimately result in cold-induced photoinhibition.

2.2.5. Genetic progress

Although breeding programmes have focused on improving yields by developing genotypes with relatively uniform maturity and bigger seed size, a significant genetic progress is the development of genotypes with high plasticity of photoperiodism through cross breeding between neutral and photosensitive genotypes. The most significant progress achieved so far is the development of the sweet variety from the bitter variety by reducing the content of saponins in the seeds (Kuijten, 2013; Sosa-Zuniga *et al*., 2017; Jarvis *et al*., 2017). Saponins are strong bitter tasting and/or toxic active compounds known as triterpene glycosides which accumulate in the seed pericarp of quinoa 20 – 24 days after anthesis (Jarvis *et al*., 2017). Despite being

beneficial to plant growth by deterring herbivory (Kuijten, 2013; Jarvis *et al*., 2017), saponins must be removed to be suitable for human consumption generally by wet or dry methods such as hulling, washing and drying (Planella *et al*., 2015; Tapia, 2015; Jarvis *et al*., 2017). However, this process of saponin removal is costly, often water-intensive and likely to reduce the nutritional value. Therefore, the development of saponin-free lines became a major breeding objective (Kuijten, 2013; Jarvis *et al*., 2017).

Fig. 2.4 Mature quinoa plants at grain filling.

With the development of saponin-free lines (i.e. sweet quinoa varieties) loss of mineral nutrients and other nutritional compounds which occurred during the removal of the outer layers of the bitter quinoa seeds is now prevented. Additionally, new quinoa sweet varieties were developed for large seed number (Kuijten, 2013; Planella *et al*., 2015; Tapia, 2015; Sosa-Zuniga *et al*., 2017). Nevertheless, the underlying genes regulating the absence of saponins in saponin-free lines remain unknown (Jarvis *et al*., 2017).

Genotype x environment interactions showed that genotypes differ in the ability to cope with contrasting environmental conditions (Bhargava and Srivastava, 2013; Sosa-Zuniga *et al*., 2017; Zhang *et al*., 2017). This allows the development of short and early-maturing varieties based on growth (life-cycle and plant architecture) and yield (seed number, size and weight) responses to specific environmental conditions. For example, it is suggested that short early-maturing and tall late-maturing quinoa genotypes have a contrasting performance (Jarvis *et al*., 2017) in response to cold environments due to differences in the duration of the phenological stages necessary to complete the growth cycle. Therefore, it is important to evaluate genetic variation to aid selection of genotypes bred for specific environments.

2.2.6. Food products and nutritional value of quinoa

Quinoa can be processed into various food products including pearled quinoa, granules, flakes, flour, cakes, pasta, bread, crackers, soups and salads. Production of these food products depends on specific quality parameters. For example, relatively stable amylase and amylopectin are desirable for production of custard desserts, jellies and instant quinoa noodles. However, it remains unknown which quinoa variety is best suited for the noodle and pasta industry (Quiroga *et al*., 2015). Furthermore, there is the key question of understanding the health benefits of quinoa nutritional compounds to consumers in addition to genetic variation in nutritional quality between genotypes (Kuijten, 2013). Nonetheless, there has been an increasing global interest in quinoa because of the nutritional value of its seeds which have shown properties such as gluten-free, low glycaemic index, balance of essential amino acids, fibre, lipids, carbohydrates, vitamins and minerals. Therefore, quinoa is regarded as having the potential to provide nutritious food products and contribute to global food security (Jarvis *et al*., 2017).

Protein

Quinoa protein content is generally higher than in the common staple cereals. It is widely established that proteins are the most important reservoirs of amino acids and this is particularly important because it distinguishes quinoa proteins from those of the staple cereals. Quinoa proteins are more similar to those of legumes and animals than cereals in terms of composition (Janssen *et al*., 2017). Contrary to staple cereals, approximately 80% of quinoa protein is mainly composed of the globulin and albumin fractions whereas the prolamin fraction is lower than 10%. Moreover, quinoa proteins (as is the case of buckwheat and amaranth) have a well-balanced and unique amino acids composition (Alvarez-Jubete *et al*., 2010; Janssen *et al*., 2017). In general, the concentration of the most limiting amino acid lysine is highest in the albumin and lowest in the prolamin proteins in food crops whereas the least essential amino

acid threonine is highest in the prolamin and lowest in the globulin protein (Janssen *et al*., 2017). Furthermore, the globulin and albumin fractions contain less glutamic acid and proline than the prolamin fractions (Alvarez-Jubete *et al*., 2010). Hence, considering the relative proportions of globulins, albumins and prolamins between quinoa and staple cereal proteins, quinoa proteins have a higher nutritional value than those of wheat, rice and maize (Janssen *et al*., 2017).

Another important aspect of the biological value of quinoa proteins is related to the structure of the grain. Quinoa grain has a starch-rich perisperm surrounded by the embryo where a large proportion of proteins is stored, whereas in the staple cereals such as wheat is located in the outer layers of the grain. This may help to explain why quinoa grain can have higher protein and fat concentration than wheat, rice and maize (Alvarez-Jubete *et al*., 2010; Janssen *et al*., 2017; Alonso-Miravalles and O'Mahony, 2018). Most importantly, in general quality of proteins of crops can also be affected by processing under conditions that decrease the availability of essential nutritional compounds (Alvarez-Jubete *et al*., 2010; Quiroga *et al*., 2015) whereby the outer layers of the grain (i.e. bran fraction) are more likely to be affected by processing (e.g. refining, cooking) than the endosperm. Therefore, processing has little negative effect on protein quality of quinoa because protein losses by refining is minimised while solubility and digestibility by cooking is increased (Brady *et al*., 2007; Alvarez-Jubete *et al*., 2010; Alonso-Miravalles and O'Mahony, 2018).

Minerals

Minerals play important roles in most of the physiological and metabolic functions of the human body. Thus, an understanding of the mineral concentration in various crops is also a good indicator of the potential to diversify and improve human diets dominated by a small number of staple crops. In fact, staple cereal grains are currently the primary dietary sources of food energy including minerals, especially in those countries where access to diverse food crops is rather limited (Alonso-Miravalles and O'Mahony, 2018). Nonetheless, quinoa grains contain generally higher concentrations of Ca, Fe, K, Mg and P than common cereals and hence has relatively higher nutritional function of supplying nutrients in the human diets (Hirose *et al*., 2010). For example, Nascimento *et al*., (2014) investigated the nutrient profile of quinoa grown in Argentina and found that quinoa grains had higher concentrations of Fe, Mg and Zn than white rice. There is also evidence showing that a 40g daily intake of quinoa can supply more essential micronutrients such as Fe, Mn and Zn than a 40g daily intake of staple cereals (Vilcacundo and Hernandez-Ledesma, 2017). This is particularly important because Fe and Zn

deficiencies affect approximately 2 billion people in the world thus it has become a key challenge for public health and clinical medicine (Ghasemi *et al.*, 2013; Olsen and Palmgren, 2014; Borrill *et al.*, 2014; Cakmak and Kutman, 2018).

Bioactive compounds

The content of phenolic compounds in quinoa grains is generally associated with high antioxidant activity. Despite the increasing evidence for the presence of these bioactive compounds in quinoa grains linked to important health benefits (e.g. prevention of diet-induced obesity) there is still limited evidence demonstrating these health benefits in humans or animals (Kuijten, 2013; Vilcacundo and Hernandez-Ledesma, 2017). Moreover, while there is substantial information about the presence of bioactive compounds in quinoa food products, there is also a limited understanding about genetic diversity within and between species with respect to grain concentrations of the bioactive compounds (Repo-Carrasco-Valencia *et al*., 2010; Sytar *et al*., 2016). Nonetheless, it is well established that these bioactive compounds are key to the development of functional foods for their role in the prevention of degenerative diseases such as cancer and cardiovascular diseases (Alvarez-Jubete *et al*., 2010; Sytar *et al*., 2016).

2.3. Coeliac disease, gluten sensitivity and irritable bowel syndrome

Coeliac disease is a chronic health condition of the small intestine, which results from long-term exposure to dietary gluten particularly in genetically predisposed individuals (Brouns *et al*., 2019). The gastrointestinal symptoms of coeliac disease (i.e. abdominal pain, bloating and chronic diarrhoea) are similar to those of IBS. Therefore, there is a possible overlap between coeliac disease and IBS so that there is a high risk of coeliac disease in IBS patients and vice versa.

Gluten is one of the various components of storage proteins, whose concentration in food products depends on the crop species and type of protein. Gluten is present in many food products but it can be found in relatively high concentrations in grains such as wheat, rye and barley whereas the gluten-free grains include buckwheat, quinoa, amaranth, sorghum and millet. The maximum amount of gluten allowed in gluten-free products is 2 mg/100g (Jassen *et al*., 2017; Brouns *et al*., 2019).

Some people may be exposed to dietary gluten and not develop coeliac disease, but they will develop some reactivity of the immune system to food products containing gluten which can result in gastrointestinal and/or extra-intestinal symptoms described as gluten sensitivity (Brouns *et al*., 2019). Both coeliac disease, gluten sensitivity (and to some extent IBS) can only

be treated with a gluten-free diet (Zevallos *et al*., 2015; Brouns *et al*., 2019). Therefore, since gluten-free cereal diets are often deficient in micronutrients such as Fe and Zn, which could result in other chronic health problems, buckwheat and quinoa are likely to be attractive options for a gluten-free diet.

The maximal concentration of gluten allowed in food products is 2 mg/100g. Buckwheat and quinoa contain up to 1 and 0.3 mg/100g of gluten, respectively (Zevallos *et al*., 2015). Hence, buckwheat and quinoa are suitable for the production of "gluten-free" food products (Alvarez-Jubete *et al*., 2010; Janssen et al., 2017). Inclusion of buckwheat and quinoa can help mitigation of coeliac disease, gluten sensitivity symptoms and/or irritable bowel syndrome by supplementing the nutritional value of gluten-free diets (Zevallos *et al*., 2015). The potential for mitigation of coeliac disease has been a key driver for the increasing demand for buckwheat and quinoa in the global market especially by gluten-intolerant and coeliac disease patients (Alvarez-Jubete *et al*., 2010; Janssen *et al*., 2017).

There is evidence suggesting that ancient cereal grains e.g. spelt, emmer, einkorn could also be attractive alternatives to help in the combat of coeliac disease and gluten-intolerance symptoms. However, it remains unproven that ancient grains such as spelt would be preferable over bread-wheat products for gluten sensitive individuals because these are closely related forms of the same hexaploid species (*Triticum aestivum*) which contain gluten as well as fermentable carbohydrates (Brouns *et al*., 2019). In particular, Brouns *et al*. (2019) also highlighted that in addition to gluten, other components present in wheat such as fermentable carbohydrates can potentially contribute to non-coeliac wheat sensitivity symptoms.

2.4. Market demand for the pseudocereals buckwheat and quinoa

The market for buckwheat and quinoa is driven particularly by the system of production and the application in the food industry. An organic system of production of any crop can achieve a price premium (Čepková *et al*., 2009), while potential for development of "functional/dietary foods" drives the price in the nutraceutical market (Zhang *et al*., 2017). Thus, buckwheat and quinoa are perceived dietary food crops with health benefits (Joshi *et al*., 2019; Guglielmini *et al*., 2019) in the global market and therefore the demand increases proportionally to the increasing awareness of healthy diets among consumers. However, there is still a small but increasing market for buckwheat and quinoa probably due to limited knowledge of these crops outside the traditional regions. This is challenging because it limits the market of buckwheat and quinoa to the specific countries merely because of cultural reasons while millions of people who could have benefited from it are not aware of these crops nor of their health benefits.

CHAPTER 3 – Effectiveness of Current Agronomic Biofortification Strategies for Increasing Grain Zn Concentrations of the Major Cereals via a Meta-analysis Approach[1]

3.1 Introduction

Food biofortification is an integral approach to provide foods that meet dietary daily requirements of human energy needs via several strategies including agronomic and genetic biofortification, dietary supplementation and diversification (Cakmak and Kutman, 2018). A key driver for food biofortification strategies over the last 20 years has been the increasing risk of Zn malnutrition in humans associated with chronic and neurodegenerative diseases, particularly in children and women in the developing countries (Chattha *et al*., 2017; Cakmak and Kutman, 2018). Zinc malnutrition is due to Zn deficiencies in human diets as a result of lower than optimal Zn concentration in crops grown on Zn marginal or deficient soils (Alloway, 2009; Cakmak *et al.*, 2010; Zou *et al.*, 2012; Velu *et al.*, 2014). In fact, on the one hand, two thirds of the world's agriculture lands are marginal or severely Zn deficient (Zou *et* al., 2012; Cakmak and Kutman, 2018); on the other hand, besides the current loss of genetic diversity in crops being used for human nutrition, Zn concentrations of the major cereals, particularly that of wheat, has declined over time (Murphy *et al*., 2008). Therefore, to address the occurrence of Zn malnutrition, scientists are devising various strategies including genetic and agronomic biofortification. An agronomic biofortification strategy prescribes judicious use of Zn fertiliser (i.e. appropriate method, rate and timing) as the primary agricultural practice not only for correcting deficiencies in soils but also for increasing Zn concentration in crops (Alloway, 2009; Cakmak *et al.*, 2010; Velu *et al.*, 2014; Cakmak and Kutman, 2018). An agronomic biofortification strategy can also involve selecting for species and varieties with high Zn concentration linked to breeding for further improvement (Velu *et al*., 2014; Borrill *et al*., 2014). As a result, on the one hand, different methods of Zn fertilisation (e.g. soil, foliar and/or soil+foliar) may differ significantly in efficiency and efficacy for improving the Zn nutritional status of crops. On the other hand, different crop species and varieties may differ in their response to different methods of Zn fertilisation (Cakmak and Kutman, 2018).

Various previous studies have shown that Zn fertilisation (soil, foliar and/or soil+foliar) results in statistically significant increase of grain Zn concentrations of wheat (Gogos *et al*., 2013), rice (Ram et al., 2016) and maize (Manzeke *et al*., 2012). For example, Zn fertilisation was shown

[1] A paper was published from this chapter in the Campbell Collaboration platform in 2017 with title: Agronomic biofortification strategies to increase grain zinc concentrations for improved nutritional quality of wheat, maize and rice.

to result in grain Zn concentrations of wheat up to 50 mg/kg (Khoshgoftarmanesh *et al*., 2004; Cakmak *et al*., 2010; Zou *et al*., 2012). Also, Zn fertilisation was also shown to have a significant but more limited effect on increasing grain yield than grain Zn concentration of cereal crops (Zou *et al*., 2012; Ram *et al*., 2016). While the majority of studies have focused on the two common Zn fertilisation methods (i.e. soil and foliar), in recent years a large number of studies have also compared the efficiency and efficacy of soil+foliar Zn fertilisation application for improving Zn concentration and grain yield and have shown substantial improvement with this method (Cakmak and Kutman, 2018). Most importantly, previous studies and reviews have highlighted different responses of wheat, rice and maize to Zn fertilisation methods (Palmgren *et al*., 2008; Olsen and Palmgren, 2014) while suggesting that soil Zn fertilisation has little effect on grain Zn concentration under field conditions (Zou *et al*., 2012; Ram *et al*., 2016; Cakmak and Kutman, 2018).

Biofortification remains a mainstream development activity (Pfeiffer and McClafferty, 2007; Chattha *et al*., 2017). Therefore, identifying efficiencies in biofortification remains an important development and food nutritional security objective. Although there are a large number of studies on Zn biofortification through soil and foliar application of Zn fertilisers, to my knowledge, no systematic review has been published using systematic data collection, critical appraisal and statistical synthesis using network meta-analysis which is a synthesis of data from various heterogenous sources to determine an overall trend. The only existing evidence synthesis undertook a cost effectiveness analysis of the potential of Zn-enriched fertilisers to alleviate human dietary Zn deficiency in sub-Saharan countries (Joy *et al*., 2015). Whilst useful, this synthesis did not report/adopt the standard methodologies of a systematic review to minimise bias (Popay *et al*., 2010). Another existing systematic review focused on quantifying the effect of Zn deficiency on human health in relation to the incidence and related mortality risk of diseases such as diarrhoea, pneumonia and malaria, particularly among children in developing countries (Caulfield and Black, 2004), yet did not assess the evidence regarding biofortification strategies to mitigate the prevalence of inadequate Zn intake.

A network meta-analysis is highly informative for decision-making purposes as it provides information about the relative efficiency of multiple interventions for pairwise comparisons and ranks the interventions with confidence (Salanti *et al*., 2014). However, assessing efficiency of Zn fertilisation practices in improving Zn nutritional status of crops through a research synthesis (i.e. network meta-analysis) is challenging because the majority of eligible studies (1) use different experimental designs and (2) are carried out in contrasting environments. This heterogeneity is likely to result in substantial differences in the direction and magnitude of the

effect of the interventions and hence not holding, at least partly, the assumption of transitivity of the effect in the context of network meta-analyses (Salanti *et al*., 2014). Moreover, many eligible studies for meta-analyses do not adequately report measures of variation, thus reducing the within- and between-study power of the analyses.

Therefore, the main objective of the present review and meta-analysis was to evaluate current agronomic biofortification strategies to ascertain the effectiveness of soil, foliar, and soil+foliar Zn fertilisation for increasing grain Zn concentrations and yield of the major cereals wheat, rice and maize. Nutritional quality or biological value of buckwheat and quinoa is often compared to that of the major cereals wheat, rice and maize in order to address the prevalence of micronutrient (especially Fe and Zn) malnutrition in humans usually via agronomic or genetic biofortification strategies. Therefore, this chapter provides evidence for comparison between major cereals (wheat, rice and maize as the main sources of dietary Zn) and the pseudocereals buckwheat and quinoa used in the present investigation.

3.2 Materials and Methods

3.2.1 Study selection and data extraction

The inclusion criteria (type of study, interventions, comparator and outcomes) are summarized in **Table 3.1**. Type of study included in the review consisted only of field-based experiments in which the method of Zn fertilisation was clearly described and the crops of interest were wheat, rice and maize. The search strategy included electronic databases limited to the period 1990 – 2018. Information extracted from the eligible studies included means (across sites and genotypes), sample size (replications) and standard deviation or standard error. Study location (country), initial soil pH and Zn status were also extracted and used as potential effect modifiers to investigate both between-study heterogeneity and inconsistency.

3.2.2 Critical appraisal, measure of treatment effect and data synthesis

Eligible studies were critically assessed in terms of clarity of aims, clarity and appropriateness of methodology, objectivity of outcome measurements, use of controls, and clarity of findings. The critical appraisal informed the overall strength of the evidence. No study was excluded based on the results of the critical appraisal tool.

Table 3.1 Summary of the inclusion criteria

Study design	Quantitative empirical data from field-based experiments assessing the efficacy of agronomic strategies for Zn biofortification in wheat, rice and maize.
Intervention	Zn fertilisation: soil, foliar and soil + foliar application of Zn as $ZnSO_4 \cdot 7H_2O$. Other forms of Zn fertiliser were also eligible for inclusion where the rate and method of application was clearly defined. Basal fertilisers (NPK) were not eligible for inclusion except where it was used as co-intervention.
Comparator	Absolute control (no fertiliser of any kind) or control with basal fertilisers.
Outcome	Zn concentration in grain (where concentration in whole grain was reported, this was prioritized) and grain yield.

Considering that the eligible studies measured grain Zn concentration and grain yield on the same scale, the efficiency of the Zn fertilisation methods was compared by computing mean differences (MDs) of the interventions with their 95% confidence intervals (CIs). As for efficiency, an MD greater than 1.0 favours the intervention (relative to the comparator). A negative MD favours the comparator.

A pairwise meta-analysis was carried out using a random-effects model (for wheat and rice) and a fixed-effects model (for maize due to the low number of studies). Effect sizes were pooled across studies followed by meta-regression using a hierarchical model framework to determine 'a summary effect size' which was calculated using weighted random effects network meta-analysis to explore heterogeneity between studies attributable to effect modifiers and estimate the treatment effects obtained from several potentially heterogeneous sources of evidence (Jansen *et al.*, 2008). Pairwise meta-regressions using AIC/DIC were performed to avoid overfitting. Pairwise meta-regressions were also conducted to further explore the sources of heterogeneity where appropriate. Between-study heterogeneity was quantified using the I^2 statistic. The effect size was then interpreted in relation to the minimum increase of 8 mg/kg from the baseline of 16, 25 and 25 for rice, wheat and maize, respectively, (Pfeiffer and McClafferty, 2007; Cakmak and Kutman, 2018).

Data were processed using RStudio, version 3.3.1 (R Foundation) using the packages netmeta version 6.6-6 (Rücker, 2019) and metafor version 2.0-0 (Wolfgang, 2017), for network and pairwise meta-analysis, respectively. For data synthesis purposes, crop species and the effects

of Zn fertilisation method on grain Zn concentration and grain yield were treated separately. For the network meta-analysis, a common heterogeneity variance was assumed, that is, a single heterogeneity variance for the entire network was considered. The main findings of the review were set out in a summary of findings table to explain the significance of the findings.

3.3 Results

3.3.1 Studies included

The literature search returned 628 records (**Fig. 3.1**). After full-text review, only 24 records were eligible, corresponding to 44 independent studies (i.e. field experiments) that fulfilled the inclusion criteria and were used in the qualitative and quantitative analyses.

The 44 studies were conducted across 55 site locations on 8 soil types with pH 4.8-8.8 and Zn status 0.1-6.5 mg/kg soil. Usually, Zn status lower than 1 mg/kg soil is considered deficient. The majority of studies were carried out in China (13), Turkey (7), Pakistan (6) and India (4) between 1993 and 2013. There were 27 studies on wheat, 13 on rice and four on maize. There were 92 wheat varieties (of which only four where used in at least three different studies), 13 rice varieties (of which only three were used at least in two different studies) and two maize varieties.

Soil Zn fertilisation consisted of 10, 23, 25, 40, 50, 100 and/or 150 kg/ha of $ZnSO_4 \cdot 7H_2O$ as a single application on the soil surface then incorporated (15-20 cm depth) into the soil by disc-ploughing before sowing/planting. Foliar Zn fertilisation consisted of 0.2%, 0.3%, 0.4% and 0.5% w/v (usually at 4 kg/ha) solution of ZnSO4.7H2O at 500-1000 L/ha application rate with 1-3 applications at different growth stages. Co-interventions were also administered in four studies, which included insecticides and fungicides, urea and superphosphate, phosphorus blend, ammonium nitrate, cattle manure, woodland litter and urea. Basal fertilisation treatments included application of ammonium nitrate, ammonium sulphate, urea, superphosphate and triple superphosphate applied before sowing/planting and/or before flowering based on each study where local common crop management practices were carried out.

3.3.2 Risk of bias in the studies

Overall, the included studies were judged to have a low risk of bias. Nonetheless, the risk of selection and reporting biases were unclear as some studies did not report the methods and findings adequately. The included studies were heterogeneous in terms of crop species, varieties, soil acidity and Zn availability. This heterogeneity was taken into consideration when performing meta-analyses and interpreting the findings.

3.3.3 Increase of grain Zn concentrations

Analyses were based on data summarised in **Table 3.2 – 3.4**. The average grain Zn concentration was 41, 25 and 23 mg/kg for wheat, rice and maize, respectively, across all countries, whereby the highest concentrations in wheat were observed in Kazakhstan and Portugal (49 and 46 mg/kg, respectively) whereas the highest concentrations in rice were observed in Lao and China (26 mg/kg). Overall, grain Zn concentrations of wheat, rice and maize increased by at least 5.4, 1.6 and 2.1 mg/kg, respectively, in response to Zn fertilisation method. Nonetheless, the relative effectiveness of Zn fertilisation method was significantly different in magnitude depending on crop species.

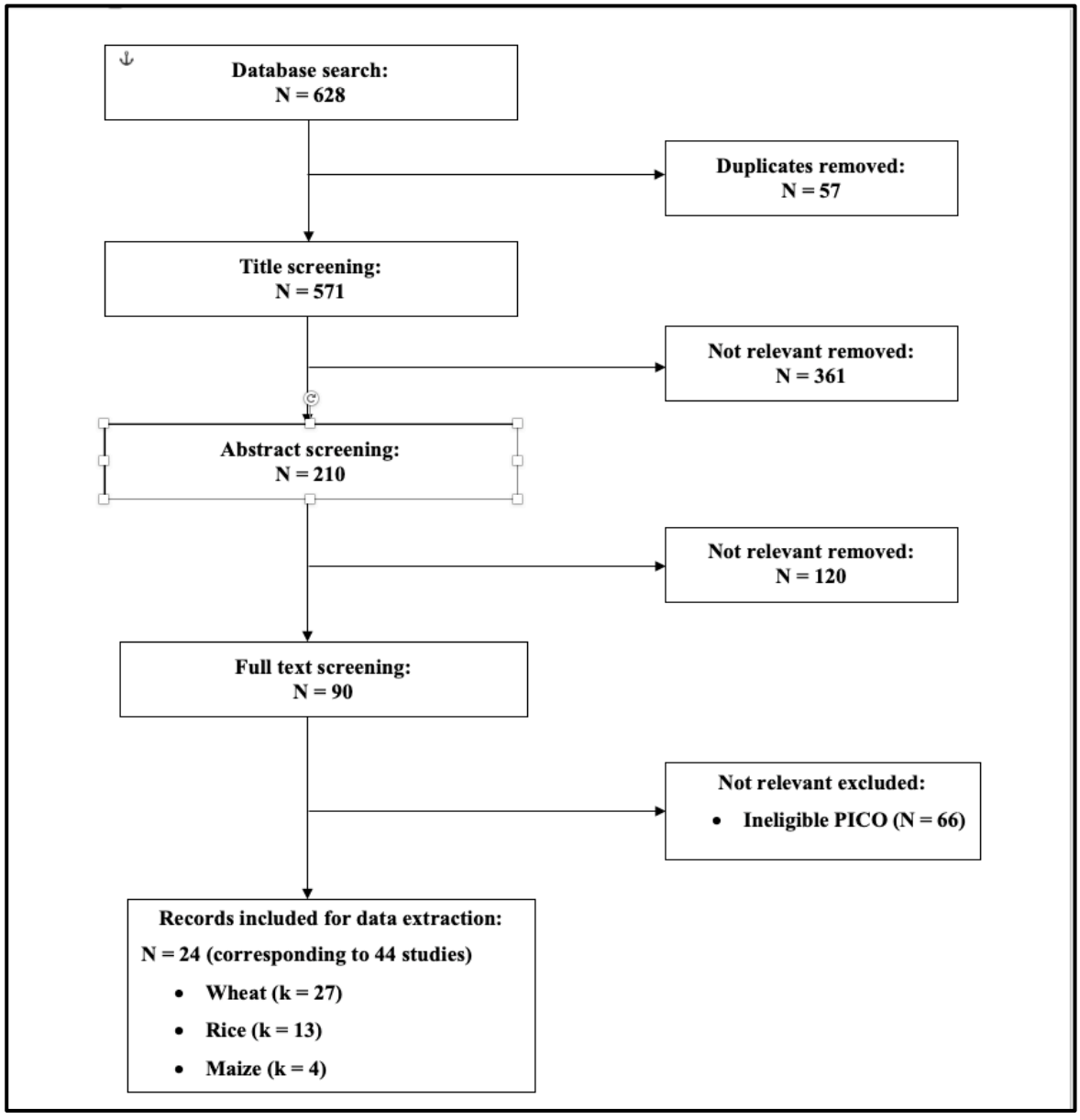

Fig. 3.1 Flow chart of literature search and study selection

Table 3.2 Grain Zn concentrations of wheat as affected by Zn fertilisation method together with an average concentration for each country.

Reference (country)	Method			
	Control	Soil	Foliar	Soil + Foliar
Wang *et al*., 2012 (China)	21.0±0.94	24.3±0.95	30.0±0.97	33.8±1.03
Zou *et al*., 2012 (China)	23.9±0.96	29.1±1.01	37.4±1.11	40.6±1.06
Zou *et al*., 2012 (India)	31.9±1.03	35.5±0.94	53.9±0.98	55.8±1.02
Zou *et al*., 2012 (Kazakhstan)	21.5±0.87	29.5±0.87	68.0±0.93	76.5±1.26
Zou *et al*., 2012 (Mexico)	21.0±0.95	25.0±0.84	43.0±1.07	45.0±1.09
Zou *et al*., 2012 (Pakistan)	32.9±0.91	34.2±1.05	52.2±0.94	50.7±1.01
Zou *et al*., 2012 (Turkey)	19.3±1.08	19.5±0.98	34.6±1.06	35.4±1.01
Zou *et al*., 2012 (Zambia)	23.0±1.13	24.0±0.99		43.0±1.18
Li *et al*., 2015 (China)	22.3±1.12		40.7±0.90	
Zhang *et al*., 2012a (China)	27.9±0.67		39.1±1.10	
Cakmak, 2008 (Turkey)	10.0±0.95	18.0±1.16	27.0±0.97	35.0±0.65
Kalayci *et al*., 1999 (Turkey)	8.0±1.15	12.0±0.97		
Coronado *et al*., 2015 (Portugal)	36.0±1.14	35.0±0.86	56.0±1.03	57.0±0.84
Cakmak *et al*., 2010 (Turkey)	19.0±1.01	33.0±0.94	47.0±1.18	69.0±1.10
Khoshgoftarmanesh *et al*., 2004 (Iran)	14.1±1.21	22.8±0.66		
Ram *et al*., 2016 (India)	29.2±0.81		40.0±1.09	
Ram *et al*., 2016 (Pakistan)	25.3±0.89		32.7±0.88	
Ram *et al*., 2016 (Brazil)	28.3±1.18		49.0±1.13	
Ram *et al*., 2016 (China)	26.1±1.18		40.2±0.93	
Ram *et al*., 2016 (Turkey)	29.4±1.05		38.2±0.86	
Ram *et al*., 2016 (Zambia)	32.8±1.06		49.7±1.10	
Ajiboye *et al*., 2015 (Turkey)	27.5±0.71		52.5±1.10	
Cakmak, 2010a (Turkey)	23.9±0.95	21.6±0.99	43.9±1.06	
Li *et al*., 2016 (China)	34.8±0.81			53.1±0.96
Mabesa *et al*., 2013 (Philippines)	18.6±0.83		22.6±0.73	
Brazil	28.3		49.0	
China	26.0	26.7	37.5	42.5
India	30.5	35.5	46.9	55.8
Iran	14.1	22.8		
Kazakhstan	21.5	29.5	68.0	76.5
Mexico	21.0	25.0	43.0	45.0
Pakistan	30.5	40.1	48.4	56.7
Philippines	18.6		22.6	
Portugal	36.0	35.0	56.0	57.0
Turkey	19.7	20.9	39.9	41.6
Zambia	27.9	24.0	49.7	43.0

Table 3.3 Grain Zn concentrations of rice as affected by Zn fertilisation method together with an average concentration for each country

Reference (country)	Method			
	Control	Soil	Foliar	Soil + Foliar
Ram *et al*., 2016 (India)	18.9±0.78		23.6±1.11	
Ram *et al*., 2016 (China)	20.0±0.72		24.5±1.11	
Ram *et al*., 2016 (Thailand)	18.4±1.01		23.5±1.08	
Phattarakul *et al*., 2012 (China)	20.5±0.87	22.1±1.04	25.5±0.86	27.1±0.75
Phattarakul *et al*., 2012 (India)	20.0±1.04	23.9±0.94	27.4±0.98	27.6±0.90
Phattarakul *et al*., 2012 (Lao)	23.1±1.06	24.2±1.17	28.3±0.82	29.1±0.89
Phattarakul *et al*., 2012 (Thailand)	16.2±1.06	15.8±0.80	20.6±0.95	22.1±1.06
Phattarakul *et al*., 2012 (Turkey)	14.5±0.97	14.6±0.74	16.7±1.23	17.3±0.94
Wei *et al*., 2012 (China)	29.5±0.97		31.3±1.12	
Yin *et al*., 2016 (China)	20.4±1.10	24.7±0.97	27.6±1.06	28.8±0.82
Boonchuay *et al*., 2013 (Thailand)	16.7±0.88		43.2±1.31	
Guo *et al*., 2016 (China)	22.9±0.98	24.4±0.95	31.4±0.66	32.7±0.81
Imran *et al*., 2015 (Pakistan)	22.0±1.16	23.0±0.85	26.0±0.87	29.0±1.34
China	22.6	23.7	28.0	29.5
India	19.4	23.9	25.5	27.6
Lao	23.1	24.2	28.3	29.1
Pakistan	22.0	23	26.0	29.0
Thailand	17.1	15.8	29.1	22.1
Turkey	14.5	14.6	16.7	17.3

Table 3.4 Grain Zn concentrations of maize as affected by Zn fertilisation method.

Reference (country)	Method			
	Control	Soil	Foliar	Soil + Foliar
Wang et al., 2012 (China)	15.4±0.90	17.4±1.16	23.0±1.07	23.3±0.93
Manzeke et al., 2014 (Zimbabwe)	15.8±1.06	22.8±1.05		
Liu et al., 2017 (China)	13.7±0.77	18.8±0.99		
Kanwal et al., 2010 (Pakistan)	24.0±1.01	29.5±1.00		

Pairwise meta-analyses

All included studies used a control (no Zn application + basal fertilisers) for comparison with proposed interventions. However, only three studies (Li *et al*., 2015; Manzeke *et al*., 2014; Zhang *et al*., 2012a) used absolute controls (no fertiliser application of any kind) for comparison with the proposed interventions. All pairwise meta-analyses detected a statistically significant increase in grain Zn concentration. The results showed that soil Zn fertilisation increased grain Zn concentration by 4.7, 1.6 and 4.9 mg/kg in wheat, rice and maize, respectively. Foliar Zn fertilisation showed a greater response and increased grain Zn concentration by 18, 6.7 and 9 mg/kg in wheat, rice and maize, respectively.

The results also indicated that soil+foliar Zn fertilisation increased grain Zn concentrations by 25.4, 6.8 and 7.9 mg/kg in wheat, rice and maize respectively. In particular, the effect sizes of foliar and soil+foliar Zn fertilisation were at least 2 mg/kg smaller on wheat and rice when outliers were removed from the analysis. The certainty of the evidence was moderate due to high inconsistency ($I^2 > 80\%$). Meta-regression tests indicated that neither study location, soil pH or Zn availability modified the effect size (magnitude and direction) of the intervention significantly.

All eligible studies indicated that soil+foliar Zn fertilisation increased grain Zn concentrations more than soil Zn fertilisation with a mean difference of 21 and 5 mg/kg in wheat and rice, respectively. The fixed-effects model showed that soil+foliar Zn fertilisation increased grain Zn concentrations in maize more than soil Zn fertilisation with a mean difference of 5.9 mg/kg. Data from 21 studies indicated that foliar Zn fertilisation increased grain Zn concentrations more than soil Zn fertilisation with a mean difference of 16.9 and 3.9 mg/kg in wheat and rice, respectively. The fixed-effects model showed that foliar Zn fertilisation increased grain Zn concentrations in maize more than soil Zn fertilisation with a mean difference of 5.6 mg/kg. This review also found that soil+foliar Zn fertilisation increased grain Zn concentrations more than foliar Zn fertilisation with a mean difference of 4.8 and 1.2 mg/kg in wheat and rice respectively. The fixed-effects model showed that soil+foliar Zn fertilisation increased grain Zn concentrations in maize more than foliar Zn fertilisation with a mean difference of 0.2 mg/kg.

Network meta-analyses

The network meta-analysis showed that grain Zn concentration of wheat was significantly increased by soil+foliar and foliar Zn fertilisation but not by soil Zn fertilisation, based on the upper and lower confidence intervals of the pooled effects (**Table 3.5** – **3.7**). In terms of

effectiveness, the network meta-analysis showed that Zn fertilisation methods differed substantially with respect to increasing grain Zn concentrations in wheat, and thus the methods were ranked as follows: soil+foliar > foliar > soil. On the other hand, fertilisation methods did not differ substantially with respect to increasing grain Zn concentrations in rice and maize. Nonetheless, this result should be interpreted with caution due to high inconsistency and low number of studies on maize.

3.3.4 Increase of grain yield

The network meta-analysis showed that none of the Zn fertilisation methods was likely to increase grain yield significantly regardless of the crop species (**Table 3.8**). The mean difference was not statistically significant in any of the possible comparisons. The between-study heterogeneity or the inconsistency of point estimates was negligible and therefore the certainty of evidence was very high.

3.4 Discussion

The results show that Zn fertilisation methods for increasing grain Zn concentration are more effective in wheat than rice and maize. This is probably due to crop genetics but the biochemical, molecular or physiological reasons for the different responses of these three crops to Zn fertilisation methods, especially foliar application, remain unresolved (Cakmak and Kutman, 2018).

A previous review (Cakmak and Kutman, 2018) indicated that foliar Zn applications are particularly effective in increasing grain Zn concentration of wheat and rice substantially whereby timing of foliar application is the key factor for the success at increasing grain Zn concentrations. Additionally, a previous meta-analysis study (Joy *et al*., 2015) also found that foliar Zn application increased grain Zn concentration of wheat, rice and maize significantly by up to 30%. However, based on the upper and lower confidence intervals, the present meta-analysis study showed that foliar Zn application only increased grain Zn concentration of wheat substantially but not that of rice and maize. Whilst the findings of the present meta-analysis allow conclusions on wheat and rice, the data for maize was more variable most probably due to the fact that only four studies were eligible for analysis.

Table 3.5 Summary of findings 1 – Network meta -analysis for the effects of Zn fertilisation methods on grain Zn concentration in wheat.

Setting: field-based trials

Crop species: wheat

Intervention: soil, foliar and/or soil+foliar application of $ZnSO_4 \cdot 7H_2O$

Comparison	Mean difference [95%-CI] in mg/kg	I^2	Tau^2	No. of studies	Certainty of evidence
Soil versus control	3.1 [-1.15; 7.38]	98.1%	22.2	15	Low
Foliar versus control	18.0 [14.31; 21.60]	99.5%	80.8	22	Low
Soil+foliar versus control	23.9 [19.41; 28.42]	99.6%	164.3	13	Low
Foliar versus soil	14.8 [10.32; 19.36]	99.4%	72.3	12	Low
Soil+foliar versus soil	20.8 [15.97; 25.63]	99.6%	109.5	12	Low
Soil+foliar versus foliar	6.0 [1.24; 10.67]	98.9%	40.8	11	Low

Number of studies: k = 26

Number of treatments: n = 4

Number of pairwise comparisons: m = 85

Number of designs: d = 6

Heterogeneity / inconsistency: $Tau^2 = 78.8$; $I^2 = 99.5\%$

Test of heterogeneity (within designs): Q = 7376.8 (p = 0.0000)

Test of inconsistency (between designs): Q = 1260.5 ($p < 0.0001$)

95%-CI: 95% confidence intervals

Table 3.6 Summary of findings 2 - Network meta-analysis for the effects of Zn fertilisation method on grain Zn concentration in maize

Setting: field-based trials

Crop species: maize

Intervention: soil, foliar and/or soil+foliar application of $ZnSO_4 \cdot 7H_2O$

Comparison	Mean difference [95%-CI] in mg/kg	I^2	QM	No. of studies	Certainty of evidence
Soil versus control	4.4 [3.75; 5.12]	88.6%	27.9	4	Low
Foliar versus control	8.5 [7.47; 9.59]			1	Very low
Soil+foliar versus control	8.8 [7.80; 9.74]			1	Very low
Foliar versus soil	4.1 [2.98; 5.21]			1	Very low
Soil+foliar versus soil	4.3 [3.30; 5.37]			1	Very low
Soil+foliar versus foliar	0.2 [-0.90; 1.38]			1	Very low

Number of studies: k = 4

Number of treatments: n = 4

Number of pairwise comparisons: m = 9

Number of designs: d = 2

Heterogeneity / inconsistency: $Tau^2 = 4.2$; $I^2 = 89.2\%$

Test of heterogeneity (within designs): Q = 3.3 (p = 0.1971)

Test of inconsistency (between designs): Q = 24.6 (p < 0.0001)

95%-CI: 95% confidence intervals

Table 3.7 Summary of findings 3 - Network meta -analysis for the effects of Zn fertilisation methods on grain Zn concentrations in rice

Setting: field-based trials

Crop species: rice

Intervention: soil, foliar and/or soil+foliar application of $ZnSO_4 \cdot 7H_2O$

Comparison	Mean difference [95%-CI] in mg/kg	I^2	QM	No. of studies	Certainty of evidence
Soil versus control	2.2 [-0.04; 4.47]	83.9%	7.78	8	Low
Foliar versus control	6.6 [4.75; 8.48]	98.7%	14.8	13	Low
Soil+foliar versus control	7.3 [5.08; 9.59]	89.1%	84.6	8	Low
Foliar versus soil	4.4 [2.15; 6.65]	82.8%	51.4	8	Low
Soil+foliar versus soil	5.1 [2.75; 7.48]	87.2%	67.2	8	Low
Soil+foliar versus foliar	0.7 [-1.54; 2.97]	14%	25.1	8	Moderate

Number of studies: k = 13

Number of treatments: n = 4

Number of pairwise comparisons: m = 53

Number of designs: d = 2

Heterogeneity / inconsistency: $Tau^2 = 11.3$; $I^2 = 96.3\%$

Test of heterogeneity (within designs): $Q = 684.7$ ($p < 0.0001$)

Test of inconsistency (between designs): $Q = 10.7$ ($p = 0.0011$)

95%-CI: 95% confidence intervals

Table 3.8 Summary of findings 4 - Network meta -analysis for the effects of Zn fertilisation method on grain yield of wheat, rice and maize

Comparison	Mean difference [95%-CI] in t/ha			Certainty of evidence
	Wheat	Rice	Maize	
Soil vs control	0.12 [-0.22; 0.45]	0.20 [-0.25; 0.65]	0.02 [-0.80; 0.84]	High
Foliar vs control	0.16 [-0.13; 0.45]	0.01 [-0.38; 0.40]	0.10 [-0.93; 1.14]	High
Soil+foliar vs control	0.13 [-0.24; 0.50]	0.20 [-0.27; 0.67]	0.16 [-1.04; 1.36]	High
Foliar vs soil	0.04 [-0.32; 0.40]	-0.19 [-0.65; 0.27]	0.08 [-0.90; 1.06]	High
Soil+foliar vs soil	0.01 [-0.38; 0.40]	-0.002 [-0.49; 0.48]	0.14 [-1.02; 1.30]	High
Soil+foliar vs foliar	-0.03 [-0.42; 0.36]	0.19 [-0.28; 0.66]	0.06 [-1.15; 1.27]	High
Number of studies:	22	11	3	
Number of treatments:	4	4	4	
Number of pairwise comparisons:	71	46	8	
Number of designs:	5	2	2	
Heterogeneity / inconsistency:	$Tau^2 = 0$; $I^2 = 0\%$	$Tau^2 = 0$; $I^2 = 0\%$	$Tau^2 = 0$; $I^2 = 0\%$	
Test of heterogeneity (within designs):	Q = 8.90 (p = 1.0000)	Q = 10.3 (p = 0.9744)	Q = 0.01 (p = 0.9186)	
Test of inconsistency (between designs):	Q = 10.3 (p = 0.1131)	Q = 0.27 (p = 0.6012)	Q = 1.08 (p = 0.2981)	

95%-CI: 95% confidence intervals

Nonetheless, in accordance with Joy *et al*. (2015), the present findings suggest that all three methods of Zn fertilisation (soil+foliar, foliar and soil) significantly increased grain Zn concentrations of maize but without significant differences between soil+foliar and foliar applications. Hence, the results of the present meta-analysis suggest that although there was a small increase in grain Zn concentrations of rice, it has a lower baseline concentration than both wheat and maize.

The network meta-analyses showed that soil + foliar Zn applications increased grain Zn concentrations of wheat by approximately 2-fold relative to the soil and foliar only treatments. The increase in grain Zn concentrations of wheat in response to soil + foliar Zn applications was 24 mg/kg which indicated a potential to increase current Zn concentrations from 25 to approximately 50 mg/kg. Hence, the soil + foliar Zn fertilisation is the most effective method for increasing grain Zn concentrations of wheat and to a lesser extent of rice.

Data available from the studies included in the present meta-analysis show that there is substantial genetic variation in grain Zn concentration within each species (especially wheat and rice) which highlights the potential for selection and improvement via breeding (Welch and Graham, 2002; Gomez-Coronado *et al*., 2016; Cakmak and Kutman, 2018). Hence, it is important to complement the Zn fertilisation strategy for increasing grain Zn concentration of cereals with strategies via wider screening programmes for genetic variation in Zn concentrations. Moreover, considering that foliar Zn fertilisation strategy may be rather difficult to deploy (Joy *et al*., 2015; Ram *et al*., 2016) particularly for resource poor farmers, thus genetic strategies may be more cost effective. This is especially important because grain Zn concentrations of modern cereal varieties are declining over time at least partly due to genetic erosion and development of high-yielding varieties (Murphy *et al*., 2008). Therefore, alternative crop species such as buckwheat and quinoa with a high Zn concentration and with large variation within a species could be potential target crops for improving Zn supply in the human diet.

The network meta-analyses also detected high inconsistency in effect sizes as reflected in I^2, which is at least partly due to pooling treatment effects from diverse and potentially heterogenous sources. The high inconsistency in effect sizes indicates that there is high variation in point estimates and direction of effect attributable to effect modifiers. However, none of the effect modifiers (i.e. soil pH, soil Zn availability and study location) explained the observed variance in point estimates and direction of effect. Hence, it was assumed that there was a true variance in point estimates and direction of effect which could be due to factors such

as crop genetics and time of Zn application. It is well established that crop genetics determine the mechanisms and pathways for translocation of nutrients (Tsonev and Lidon, 2012; Borrill *et al*., 2014) and the time of Zn application can affect the amount taken up, remobilised and stored in the grain (Cakmak and Kutman, 2018). So, the high inconsistency in effect sizes can be addressed in the future by synthesis and evidence of genotype performance and the effect of time of Zn application including rate of application. Whilst it is important to detect genetic variation within and between crop species in Zn concentration from a breeding perspective, the challenge from a statistical point of view is that across the eligible studies of the 105 genotypes used, only seven were used in more than one study and in not more than two growing seasons; thus it is rather difficult to draw a definite conclusions with confidence as the majority of the genotypes have not been tested in contrasting environments over multiple years to ascertain their efficiency and stability with respect to Zn fertilisation.

Unfortunately, paucity of data did not enable estimation of the effects of Zn fertilisation method on grain Zn concentration post-processing (bioavailability). Hence, this study also highlights a gap in direct evidence of the effect of Zn fertilisation on Zn bioavailability which is needed to assess the efficiency of Zn fertilisation strategies in combating Zn deficiency and related-malnutrition in humans (Hussain *et al*., 2012). This is particularly important because large amounts of grain Zn in cereal crops (wheat and rice particularly) can be lost when the outer layers (where large proportion of Zn is stored) are removed during processing. Despite the relatively high number of studies dealing with agronomic Zn biofortification of wheat, rice and maize, only two of the eligible studies estimated grain Zn bioavailability. Many of the studies that met the eligibility criteria drew conclusions based only on non-processed whole grain Zn concentrations, thus indicating an overreliance on whole grain concentrations rather than grain fractions or flour post-processing. Therefore, future data syntheses should consider Zn concentrations in grain fractions post-processing because grain fractions reflect the proportion that is available to consumers, as Zn is likely to be lost during milling (Borrill *et al*., 2014) in some species more than others.

The risk of selection bias was predominantly unclear within and across studies, thus reducing the strength of the evidence. Most studies, especially those dealing with rice and maize, reported only the effect of single interventions on grain Zn concentration suggesting a selection or reporting bias. Such bias may be likely due to a generalisation of the assumption that the effect of foliar or soil + foliar Zn fertilisation on grain Zn concentrations is always greater than other interventions. Hence, the assumption that Zn fertilisation can address the occurrence of Zn malnutrition is dependent on evidence from single methods of fertilisation and indirect

comparisons rather than multiple direct comparisons from multiple potentially heterogenous sources. Moreover, lack of detailed and complete information limits the ability to detect biases with confidence, thus resulting in uncertainty. The certainty of evidence could be improved with further direct evidence.

The network meta-analysis showed that there was a small or no significant effect of Zn application on grain yield. However, interestingly, the small effect of Zn applications on grain yield was not modified by soil Zn status even when grown on Zn-deficient soils (<1.0 mg Zn/kg soil). Zinc fertilisation is an attractive short-term agronomic strategy to combat Zn deficiencies in soils and crops. Evidence of the effects of Zn fertilisation on the bioavailability of Zn needs further study. Most importantly, it would be important to test the potential of other crop species such as buckwheat and quinoa as these crops have been shown to have inherently higher Zn concentrations than wheat, rice and maize.

CHAPTER 4 – Materials and Methods

4.1 Site Description

The study was conducted at Nafferton Farm, Northumberland, UK (54º 59' 09'' N; 1º 43' 56'' W) (**Fig. 4.1**) over three seasons (2016-18). Nafferton Farm is an agricultural research facility within the School of Natural and Environmental Sciences at Newcastle University, focusing on organic and conventional approaches to crop and livestock management. From 2004 half of the farm has been used for conventional farming and the other half certified for organic use. More information about trials and the site can be obtained from https://www.ncl.ac.uk/nu-smart-farms/long-term-field-trials/.

Soil characteristics (top 15 cm layer) of the trial plots are listed in **Table 4.1**. All weather data were obtained from an automated weather station located on site (about 500 m from the trials). The weather conditions were typical of the UK which is defined as a temperate oceanic climate, characterised by mild winters and warm summers.

Fig. 4.1 Site location of the field trials in the 3 years. Source: www.google.co.uk/intl/en_uk/earth

Table 4.1 Summary of soil chemical properties for each trial

	Trial 1 - 2016	Trial 2 - 2017	Trial 3 - 2018
pH	6.5	6.4	5.9
Available N (kg/ha)	7.9	7.7	7.5
Organic matter (%)	4.7	4.7	4.7
Extractable Zn (mg/L)	2.4	1.3	1.9
Extractable Fe (mg/L)	82.8	124.1	105.3
Extractable P (mg/L)	8.2	17.1	17.0
Extractable K (mg/L)	74.3	102.5	119.0

4.2 Experimental design

4.2.1 Trial 1 – 2016

The experiment was laid in randomised split block design with four replicates using sowing date as the main plot, foliar zinc fertilisation as sub-plot and genotype as sub-sub-plot to examine the effects of, and interaction between, sowing date, zinc fertilisation and genotype on growth, yield and quality of buckwheat and quinoa.

The experiment consisted of two sowing dates (mid-April vs early-May), two zinc fertilisation regimes (foliar fertilisation vs no-Zn fertilisation), two buckwheat (Bamby vs Cebelica) and quinoa (Atlas vs Jessie) genotypes. The design generated a layout with four treatment combinations: (1) mid-April sowing with foliar Zn fertilisation; (2) mid-April sowing without foliar Zn fertilisation; (3) early-May sowing with foliar Zn fertilisation; (4) early-May sowing and without foliar Zn fertilisation. There were 32 plots for each crop species (64 plots in total) with each plot being 12.0 x 2.6 m (**Fig. 4.2**).

Soil samples were taken on 25th March after ploughing prior to sowing. Samples were taken in a W-shape from the top-15 cm soil layer by bulking 15 soil cores and producing a composite sample for each experimental block. The samples were then sent to a commercial laboratory NRM, Bracknell, Berkshire for analysis.

Sowing was carried out with a 2-week interval (i.e. 19th April and 3rd May) at the seed rate of 90 kg/ha (i.e. 450 and 380 seeds/m^2 for Bamby and Cebelica, respectively) and 10 kg/ha (320 seeds/m^2 for Atlas and Jessie) using a semiautomatic 5-row seed planter with 10 cm between rows in a 2.6m drill. Buckwheat seeds (Bamby and Cebelica) were obtained from the Agricultural Institute of Slovenia with Bamby being bred in Austria and Cebelica in Slovenia. Quinoa seeds (Atlas and Jessie) were obtained from the company British Quinoa Ltd, UK, with

both genotypes being bred in Holland. Both buckwheat and quinoa seeds were of the sweet varieties.

Zinc treatment consisted of foliar application of 4 kg/ha of zinc sulphate ($ZnSO_4 \cdot 7H_2O$), a solution containing 14% of zinc, applied on 11th July. The solution was sprayed at flowering. All plots were also fertilised with 150 kg N/ha of biogas digestate as baseline treatment on 15th June. The biogas digestate consisted of 0.2 % w/v total N, 1156 and 5.3 mg/kg NH_4^+ and Zn, respectively, and <10 mg/kg of NO_3-. The digestate was obtained from a commercial Anaerobic Digestion plant of DJ and SJ Enderby, Codlaw Farm Codlaw Hill, Hexham, Newcastle which uses energy crops (maize, rye, whole-crop silage) as feedstock. No crop protection treatments were applied. The preceding crops were spelt and rye grown as part of a research trial. Bee hives were installed by a commercial bee keeper in the field to aid the pollination of buckwheat.

4.2.2 Trial 2 – 2017

The experiment was arranged in a split-split plot design with four replications. Sowing date was the main-plot, nitrogen fertilisation as sub-plot and genotype as sub-sub-plot to examine the effects of, and interaction between, sowing date, nitrogen fertilisation (rate and source) and genotype on growth, yield and quality of buckwheat and quinoa. The experiment consisted of four buckwheat (Bamby, Cebelica, Zamira and Zita) and three quinoa (Atlas, Duches and Jessie) genotypes, three nitrogen fertilisation rates (0, 75 and 150 kg/ha), two sources of nitrogen fertiliser (mineral N and biogas digestate), and two sowing dates (mid-April and early-May). The design generated a layout with eight treatment combinations: (1) mid-April sowing and zero-N treatment; (2) mid-April sowing and low rate mineral nitrogen (75 kg N/ha); (3) mid-April sowing with high rate mineral nitrogen (150 kg N/ha;) (4) mid-April sowing with high rate biogas digestate (150 kg N/ha); (5) early-May sowing and zero-N treatment; (6) early-May sowing and low rate mineral nitrogen (75 kg N/ha); (7) early-May sowing and high rate mineral nitrogen (150 kg N/ha); and (8) early-May sowing and high rate biogas digestate (150 kg N/ha). Therefore, there were 8 treatments x 4 genotypes x 4 replicates = 128 plots for buckwheat whereas for quinoa there were 8 treatments x 3 genotypes x 4 replicates = 96 plots, which resulted in 224 plots in total with each plot 6.0 x 2.1m (**Fig. 4.3**).

Soil samples were taken on 4th April after ploughing and prior to sowing. Samples were composites from each experimental block taken in a W-shape from the top-15 cm soil layer. Additionally, samples from three depths (i.e. 0-30, 30-60 and 60-60 cm) were taken for analysis of available nitrogen. The samples were then sent to NRM Ltd for analysis.

Sowing was carried out with a 2-week interval (i.e. 13th April and 2nd May) at the seed rate of 90 kg/ha (i.e. 450, 380, 300 and 300 seeds/m^2 for Bamby, Cebelica, Zita and Zamira, respectively) and 10 kg/ha (320, 320 and 300 seeds/m^2 for Atlas, Jessie and Duches, respectively) using a commercial seed drill. Buckwheat seeds Zamira and Zita were obtained from the Crop Research Institute, Prague, Czech Republic. Quinoa seeds (Duches) were obtained from British Quinoa Ltd, UK, all of which were sweet varieties (low saponin content).

Nitrogen treatments (i.e. biogas digestate and mineral N) were applied at the leaf development stage (GS15-20). The biogas digestate consisted of 0.4 % w/v total N, 1959 and 10 mg/kg NH_4^+ and Zn, respectively, and <10 mg/kg of NO_3^-. No crop protection treatments were applied. The preceding crop was winter wheat. Bee hives were installed in the field to aid the pollination of buckwheat.

4.2.3 Trial 3 – 2018

The experiment was arranged in a split-split plot design with four replications, with the same experimental design as in 2017, with the exception that only two buckwheat genotypes (Bamby and Cebelica) were used. Therefore, there were 8 treatments x 2 genotypes x 4 replicates = 64 plots for buckwheat whereas for quinoa there were 8 treatments x 3 genotypes x 4 replicates = 96 plots, which resulted in 160 plots in total (**Fig. 3.4**). Sowing was carried out with a 2-weeks interval (i.e. 20th April and 9th May). The preceding crop was spring barley.

4.3 Crop Growth Assessments

4.3.1 Phenology

Phenology was assessed by observing key features of the life-cycle using a two-digit growth scale adapted from the growth keys for quinoa (**Table 4.2**) described by Sosa-Zuniga *et al*. (2017) and for buckwheat (**Table 4.3**) described by Arduini *et al*. (2016). A simplified/modified two-digit scale (**Table 4.4**) was used to record growth stages of buckwheat and quinoa. Growth cycle was recorded every week based on visible changes in the morphology of the plant from emergence to ripening. The cycle was measured in days after sowing (DAS) and principal growth stages were recorded. The list of assessments at each growth stage is described (**Table 4.5)**.

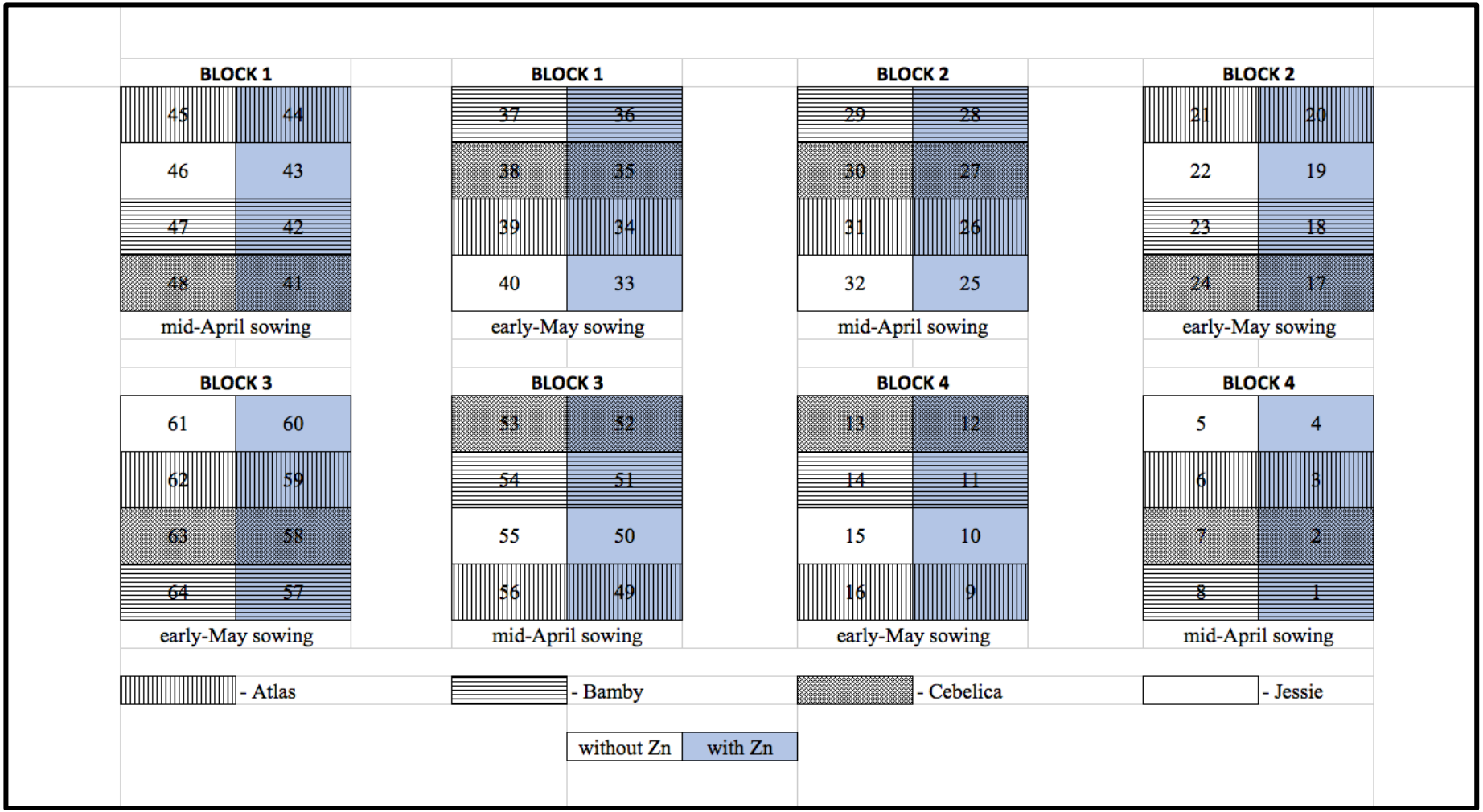

Fig. 4.2 Field layout and experimental design of the buckwheat (Bamby, Cebelica) and quinoa (Atlas, Jessie) trial in 2016.

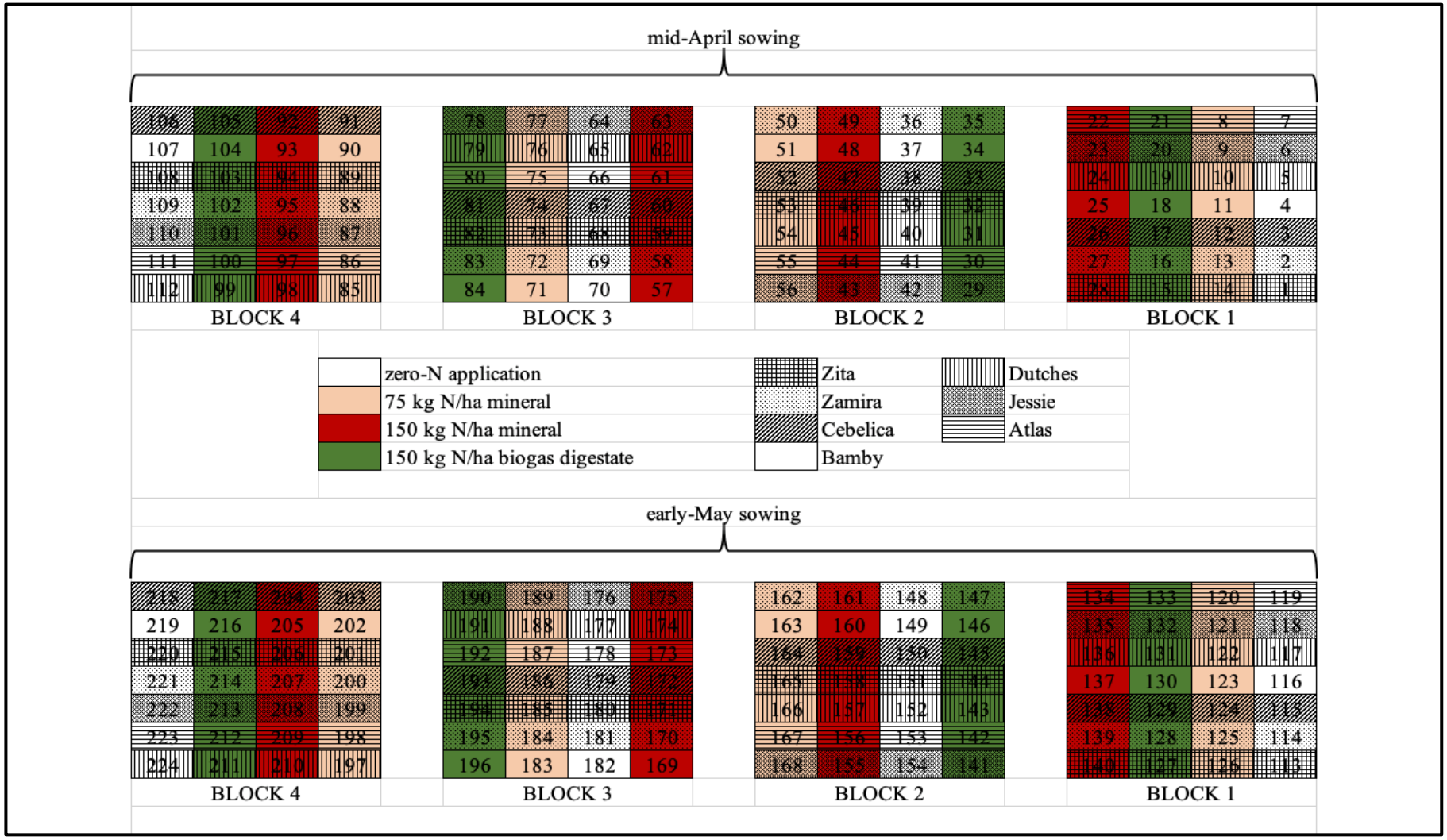

Fig. 4.3 Field layout and experimental design of the buckwheat (Bamby, Cebelica, Zita, Zamira) and quinoa (Atlas, Jessie, Dutches) trial in 2017.

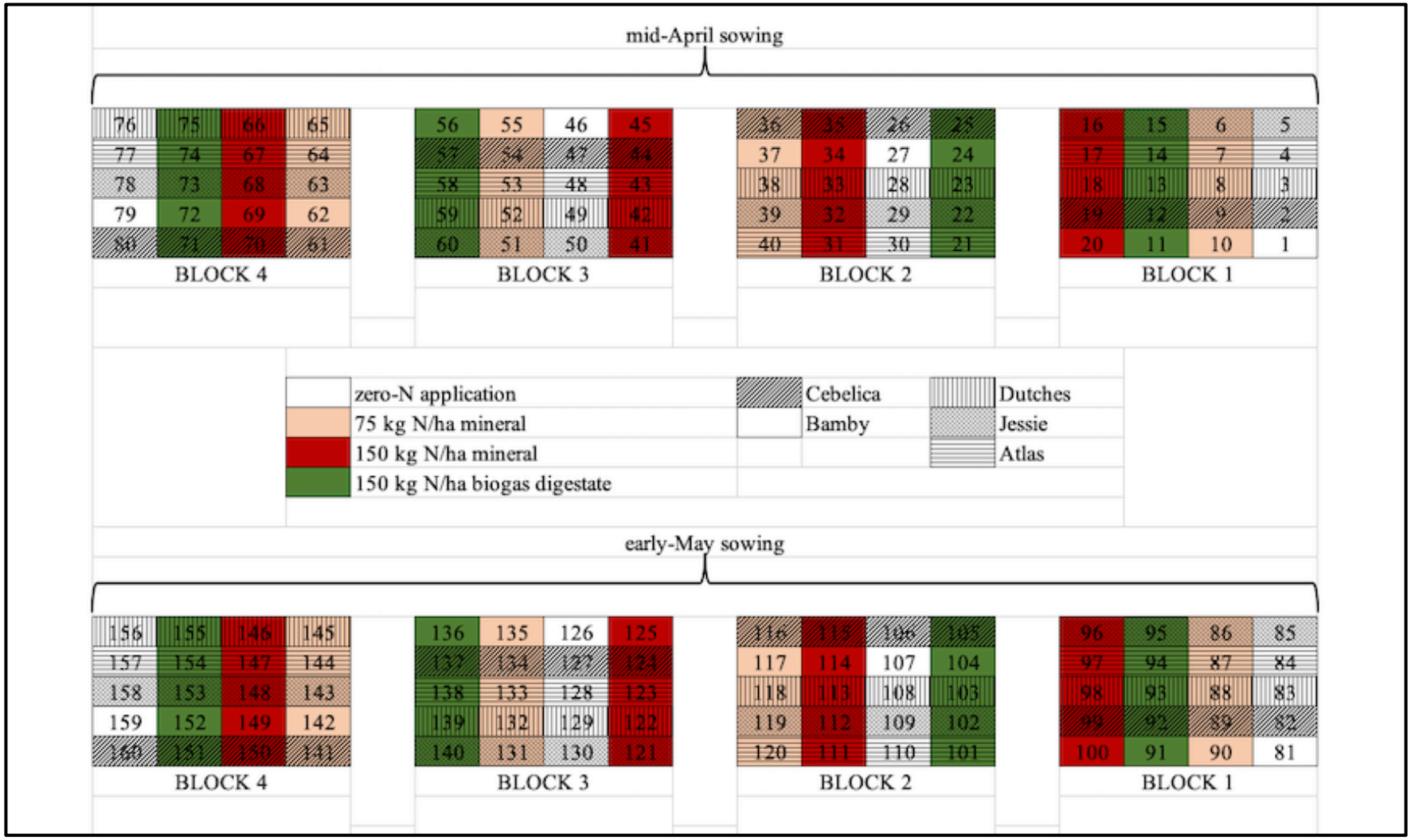

Fig. 4.4 Field layout and experimental design of the buckwheat (Bamby, Cebelica) and quinoa (Atlas, Jessie, Dutches) trial in 2018.

Table 4.2 BBCH growth scale for quinoa (Sosa-Zumiga *et al*. 2017)

Code	Description
Principal growth stage 0: germination	
00	Dry seed
01	Initiation of seed imbibition
03	Seed imbibition completed
05	Radicle emergence from seed
07	Emergence of hypocotyl
08	Hypocotyl with cotyledons growing towards soil surface
09	Emergence of cotyledons through soil
Principal growth stage 1: leaf development	
10	Cotyledons fully emerged
11	First pair of leaves visible
12	Second pair of leaves visible
1.	Coding continues with the same scheme
19	Nine pair of leaves visible. If required, coding can continue following the same scheme
Principal growth stage 2: formation of side shoots	
20	Visible lateral buds or expanded leaves without lateral stems
21	One side shoot visible
22	Two side shoots visible
2.	Coding continues with the same scheme
29	Nine side shoots visible. If required, coding can continue following the same scheme
Principal growth stage 3: stem elongation (omitted)	
Principal growth stage 4: development of harvestable vegetative parts (omitted)	
Principal growth stage 5: inflorescence emergence	
50	Inflorescence present but still enclosed by leaves
51	Leaves surrounding inflorescence separated, inflorescence visible from above
59	Inflorescence visible, but all the flowers are still closed
Principal growth stage 6: flowering	
60	Beginning of anthesis: main inflorescence flowers with first extruded anthers
67	Early end of anthesis: main inflorescence flowers with first senesced anthers
69	Complete anthesis: main inflorescence flowers with senesced anthers

Table 4.2 *continued...*

Principal growth stage 7: fruit development	
70	Fruit set: ovary thickening and first visible grains in the main stem
Principal growth stage 8: ripening	
81	Milky grain, easily crushed with fingernails, liquid content and green pericarp
85	Thick grain, easily crushed with fingernails, white pasty content, green, beige, red or black pericarp
89	Ripe grain, difficult to crush with fingernails, dry content, the grain has a beige, red or black colour on its outside. Ready to harvest
Principal growth stage 9: senescence	
91	Only basal leaves are dry
93	Leaves of the first half portion of the plant, starting from the base, are dead
95	All leaves are dead; stem colour turns from yellow to brown
97	Plant dead and dry
99	Harvested product

4.3.2 Seed germination %

Seed germination % for both sowing date treatments was assessed on 17th May in 2016, 5th and 30th May in 2017, and 22nd May and 5th June in 2018 at GS10-15 (beginning of leaf development) using a 0.5 m^2 quadrat.

4.3.3 Plant height

Plant height was recorded at GS60 and GS80 by randomly selecting three plants from each plot and measuring height from the base of the stem to the tip of the terminal raceme and the average value was recorded. Only data recorded at GS80 was used for analysis of variance.

4.3.4 Chlorophyll content

Leaf chlorophyll content was measured using a Soil Plant Analysis Diagnostic (SPAD-502) meter. Chlorophyll content was recorded as the average of readings taken from young fully expanded leaves of ten plants in each plot. SPAD readings were taken at least every two weeks before foliar Zn application in 2016 and weekly until the grain filling (GS50-60) in 2017 and 2018.

Table 4.3 A scale for buckwheat growth stages (Arduini *et al*. 2016)

Code		Principal growth stage	Description
00		Dry see	Sowing date
09		Emergence	Cotyledons break through soil surface
10		Cotyledon	Cotyledons completely unfolded
11		First leaf	First true leaf at node 1 (N1) unfolded
12		Second leaf	Two true leaves unfolded at N1 and N2
13		Third leaf	Three true leaves unfolded at N1, N2 and N3
14		Fourth leaf	Four true leaves unfolded at N1, N2, N3 and N4
1.		… leaf	Stages continuous till …
21		Branching	First side shoot visible
50		Blossoming	First inflorescence bud visible through an unfolding leaf at the main stem apex (+INF)
60		First flowers open	1-2 flowers open at the base of the first formed inflorescence (+INF)
62		Beginning of flowering	1-2 flowers open in the terminal inflorescence (TINF)
65		Full flowering	Open flowers in most inflorescences
	70	First green fruits	1-2 well-developed green achenes visible at the base of +INF
	71	Fruits begin to develop	1-2 well-developed green achenes in TINF
	80	Beginning of fruit ripening	Angles of oldest achenes in TINF turn brownish-red
66		Advanced flowering	No more flowers open in +INF
67		Late main stem flowering	No more than 1-2 flowers open at the same time in TINF. Flowers open in branches
	85	First brown fruits	1-2 brown achenes visible at the base of +INF
68		End of main stem flowering	No more flowers open in TINF. Still a few flowers open in branches
69		End of branch flowering	No more flowers open in the whole plant
	86	Advanced fruit ripening	All achenes brown in +INF
	87	Late fruit ripening	All achenes in TINF and in other main stem inflorescence are dark brown
	88	End of fruit ripening	All achenes dark brown or aborted
	90	Beginning of plant senescence	Leaf margins turn yellow and start wither
	97	Plant dead	The stem turns brown, dries up and become fragile

Table 4.4 Simplified scale for growth assessment for buckwheat and quinoa, adapted from Meier (2001), Arduini *et al*. (2016) and Sosa-Zumiga *et al*. (2017).

Scale code	Description
00 – 10	Seedling emergence
10 – 20	Leaf development
20 – 30	Inflorescence
30 – 40	Flowering
40 – 50	Seed setting
50 – 60	Grain filling
60 – 70	Ripening
70 – 80	Maturity

4.3.5 Crop biomass

Crop biomass was recorded as the average of ten readings per plot generated using a Green Seeker hand held (NDVI) sensor. The NDVI readings were taken weekly until grain filling (GS50-60) in 2017 and 2018.

4.3.6 Weed proliferation and disease

Weed cover was assessed at GS50-80 (grain filling – maturity) by estimation of the percent of total cover and dominant species present per unit area using a 0.5 m^2 quadrat. Plant heath was assessed visually and the severity of disease infection (*Erysipphe polygoni* and *Peronospora ducumeti* in buckwheat, *Peronospora variabilis* Gaum, formerly called *Peronospora farinosa* Fr., in quinoa) estimated at the leaf and whole plant level on a weekly basis as described by Danielsen and Munk (2004).

4.4 Grain Yield Assessments

At GS70-80 (maturity), plant population (plants/m^2), seed number (seeds/plant, seeds/m^2), panicle number (panicles/plant, panicles/m^2), total above-ground biomass, thousand-grain weight (TGW) and harvest index (HI) were assessed based on a 0.5 m^{-2} quadrat. Plants were oven-dried at 70ºC for 72 hours to determine individual yield component dry weights. Harvest index (HI) was calculated as the ratio between total seed weight and total above-ground plant biomass. Thousand-grain weight (TGW) was recorded using an Elmor C3 seed counter.

Seeds from the combine (CLASS Dominator 38) were weighed, and oven-dried at 35-40ºC for 7 days. Due to the very small seed size of both crops and the large amount of crop debris arising from the combine, samples were cleaned in two phases prior to yield calculations: (1) samples were cleaned using a Lainchbury semi-automatic seed cleaner with two sieves (4 and 3 mm); this phase was repeated at least three times until a clean sample was obtained. (2) samples were then sieved manually through two sieves (2 and 1 mm); this phase was repeated until 90-95% of debris was removed. Seeds were milled and sieved (Retsch Ultra Centrifugal Mill ZM 200) through a 0.50-mm sieve to approximately 20 g for sample and stored at room temperature in sterilin vials prior to further quality analyses.

4.5 Grain Quality Assessments

4.5.1 Protein and ash content

Total nitrogen content in grain was determined by the Dumas combustion method using a Vario Macro Cube Automated C/N analyser. Approximately 0.5g of flour (milled sample) was weighed into a sample cup on an analytical balance and placed into the auto-sampler of the Elementar Vario Macro Cube analyser. Then N and C were quantified by gas chromatography using a thermal conductivity analyser detector and expressed as the percent nitrogen of each sample. Protein content was determined indirectly from total nitrogen content multiplied by 6.25 as recommended by ISO/TS 16634-2:2009.

Ash content was determined by incineration using a muffle furnace. Approximately 1.5 g of flour was incinerated in the muffle furnace at 550°C overnight and then cooled in a desiccator. The weight was recorded and ash content was calculated:

$$\%\, Ash = \frac{Weight\ of\ the\ sample\ after\ incineration}{Weight\ of\ the\ sample\ before\ incineration} \times \frac{100}{1}$$

4.5.2 Minerals

Mineral content was determined by microwave digestion using a Microwave digester CEM Mars 6. Acid digestion was carried out using concentrated nitric acid (purity 69.0% for trace analysis) purchased from WVR International.

Table 4.5 List of assessments carried out at each growth stage

	2016	2017	2018
GS10	seedling emergence	seedling emergence	seedling emergence
GS20	leaf development	leaf development	leaf development
GS30	leaf chlorophyll	leaf chlorophyll	leaf chlorophyll
		crop biomass	crop biomass
GS40	plant disease	plant disease	plant disease
	leaf chlorophyll	leaf chlorophyll	leaf chlorophyll
		crop biomass	crop biomass
GS50	plant disease	plant disease	plant disease
	leaf chlorophyll	leaf chlorophyll	leaf chlorophyll
		crop biomass	crop biomass
	weed proliferation	weed proliferation	weed proliferation
GS60	plant disease	plant disease	plant disease
	leaf chlorophyll	leaf chlorophyll	leaf chlorophyll
		crop biomass	crop biomass
	plant height	plant height	plant height
	weed proliferation	weed proliferation	weed proliferation
GS70	plant disease	plant disease	plant disease
	leaf chlorophyll	leaf chlorophyll	leaf chlorophyll
		crop biomass	crop biomass
	weed proliferation	weed proliferation	weed proliferation
	plant disease	plant disease	plant disease
	leaf chlorophyll	leaf chlorophyll	leaf chlorophyll
GS80	plant disease	plant disease	plant disease
		crop biomass	crop biomass
	plant height	plant height	plant height
	weed proliferation	weed proliferation	weed proliferation
	branches and nodes	branches and nodes	
	plant population	plant population	plant population
		panicle number	panicle number
	seed number	seed number	seed number
	thousand grain weight	thousand grain weight	thousand grain weight
	above-ground biomass	above-ground biomass	above-ground biomass
	seed yield	seed yield	seed yield
	harvest index	harvest index	harvest index

Approximately 0.25g of flour (milled sample) was digested with 5 mL of concentrated HNO_3 for 30 minutes at 200°C in the Microwave digester CEM Mars 6. The Microwave digester CEM Mars 6 ran with the following operational settings:

- Temperature: 200ºC
- Time: 30 minutes = ramp:15minutes; hold: 15 minutes

- Pressure: 800 (psi)
- Power: 900 – 1050 (W)

Then extracts were filtered and diluted with distilled water to a volume of 25-mL and sample extracts were stored in sterilin acid-resistant vials at 4°C.

Sample extracts were analysed by Inductively Coupled Plasma Optical Emission Spectrophotometry (ICP-OES) using a Vista-MPX CCD simultaneous ICP-OES machine to determine total concentration of Al, Ca, Cd, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, S and Zn with the following limits of detection [in parts per million (ppm)]: 0.0005, 0.00001, 0.0002, 0.0009, 0.0003, 0.0003, 0.00005, 0.0001, 0.0005, 0.0002, 0.0007, 0.004, 0.004 and 0.0002, respectively. The measure of absorbance in sample extracts occurred by correction with background. The absorbance average from three repeats was converted into element concentration (milligrams per kilogram) according to the corresponding calibration curves and expressed on a dry weight (DW) basis. The efficiency of digestion and machine operation was checked using standards in each batch of samples

4.5.3 Secondary metabolites

Total polyphenols, total antioxidants and total flavonoids were determined by colorimetric absorbance. Method and procedures for extract preparation and total assays were adapted from Li *et al*. (2008) and Dziadek *et al.* (2016). Hydrochloric acid (purity 69.0% for trace analysis), methanol (technical), and acetone (99.6% purity) were used as internal standards purchased from Fisher Scientific company and WVR International. The method was validated for repeatability and stability. Repeatability was tested by preparing three random samples from each crop species and main plots (12 samples). Stability was tested by measuring the content on at least two different days.

Phenolic acids (polyphenols, antioxidants and flavonoids) were extracted from the flour fractions (soluble free and bound). Approximately 0.05 g of flour (milled sample) was extracted with 1 mL of 0.08M hydrochloric acid in 80% methanol. The solution was homogenised by vortex for 10 minutes and sonic bath for further 10 minutes then centrifuged at 1320 rpm for five minutes. Supernatant was transferred into micro-centrifuge Eppendorf acid resistant tubes. The residues were re-extracted with 1 mL of 70% acetone, then supernatants were combined and stored at -20°C.

Working solutions were: Galic acid, Folin-Ciocialteu and 25% $NaCO_3$ using distilled water as a solvent for estimation of total polyphenols in the sample extracts; Trolox, 2.45mM $K_2S_2O_8$,

7mM ABTS and a 5mM phosphate buffer solution (NaCl + $NaH_2PO_4.H_2O$ + NaH_2PO_4 .$12H_2O$) using 50% MeOH and distilled water as solvents for estimation of total antioxidants; Rutin, Catechin, 5% $NaNO_2$, 10% $AlCl_3$. $6H_2O$ and 1M NaOH using distilled water as a solvent for estimation of total flavonoids.

Total polyphenol concentration in the extracts was estimated by the Folin-Ciocialteu reagent. After preparation of Galic acid standard solution, 130 uL of 10% Folin-Ciocialteu solution was reacted with 20 uL of sample extracts and 100 uL of 25% sodium carbonate solution. The absorbance was measured at 760 nm at 37ºC using a spectrophotometer SPECTRAmax ^{384}Plus. A linear relationship between absorbance readings and the amount of Galic acid was obtained. The linearity range of standard Galic acid was 1.5625 – 100 µg and the equation of linear regression was y = 0.0037x - 0.0132 with a correlation coefficient (R^2) 0.9978. Then final concentration of total polyphenols was calculated from x = (absorbance reading + 0.0132)/0.0037 for each sample.

Total antioxidant activity was determined by quantifying the amount of the ABTS (2, 2'-azinobis-(3-ethylbenzothiazoline-6 sulfonic acid) solution reduced by the antioxidants present in the extracts. After preparation of reagents, 10 mL of 2.45 mM potassium peroxydisulphate solution was mixed with 90 mL of 7 mM ABTS solution and the absorbance (734 nm) reading was adjusted to 0.7 using a 5-mM phosphate buffer solution at pH 7.4. After preparation of standards (0.5 mM Trolox standard solution), 290 uL of the adjusted ABTS working solution was added to 20 uL of sample extracts and incubated at 37ºC for 6 minutes. Then the absorbance was measured at 734 nm at 37ºC using a spectrophotometer SPECTRAmax ^{384}Plus. The linearity range of standard Trolox was 1.5625 – 100 µg and the equation of linear regression was y = 0.0042x + 0.0155 with a correlation coefficient (R^2) 0.9972. Then final concentration of total antioxidants was calculated from x = (absorbance reading – 0.0155)/0.0042 for each sample.

Total flavonoid content was determined after preparation of Rutin (for buckwheat) and Catechin (for quinoa) standard solutions. Then, 25 uL of sample extract was reacted first with 7.5 uL of a 5% sodium nitrate solution, secondly with 15 uL of a 10% aluminium chloride hexahydrate solution and 50 uL of a 1M sodium hydroxide solution, then further diluted with 150 uL of double distilled water. The absorbance was measured at 510 nm at 25ºC using a spectrophotometer SPECTRAmax ^{384}Plus. The linearity range of standard Rutin and Catechin was 0.9375 – 3.0 µg and the equation of linear regression was y = 0.01x + 0.0016 and y = 0.0017x + 0.0065 with correlation coefficients (R^2) 0.9867 and 0.9996, respectively. Then final

concentration of total flavonoids was calculated from x = (absorbance reading – 0.0016)/0.01 for each sample.

4.6 Statistical Analysis

Data were processed using R, version 3.3.1 (R Foundation) and analysed for normality. Data were checked for normal distribution using Shapiro–Wilk test. Seed germination and some minerals data (e.g. Al, Fe and Ni) were not normally distributed therefore were transformed using the standardize method before parametric test to meet the criteria of normal data distribution statistics. Unexpected outliers were excluded from the analysis.

Significance was established by *p*-values in addition to measure of dispersions (i.e. confidence intervals measured by standard errors) where all *p*-values less than 0.05 were considered statistically significant and all *p*-values >0.05 and < 0.10 were considered as trends.

For data synthesis purposes, buckwheat and quinoa were analysed and presented separately. All data were analysed using the linear mixed-effect model ANOVA fit by Residual Effect Maximum Likelihood (REML) to determine the effects of, and interaction between, the experimental variables. The general linear model (GLM) was used whenever the REML reached singularity due to excessive missing values or unbalanced design.

Tukey's (HSD) test was used to determine differences between treatments in a multiple-way ANOVA model with multiple mean comparisons. Correlation analyses were carried out by calculating the Pearson correlation coefficients.

CHAPTER 5 – Effects of, Sowing Date, Zinc Fertilisation and Genotype on Growth, Yield and Quality of Buckwheat (*Fagopyrum esculentum* Moench.) in 2016

5.1 Introduction

Buckwheat is a pseudocereal largely cultivated for human consumption as a summer crop in diverse rotations because of its rapid growth, ability to suppress weeds (allelopathic activity) and relative ease of management (Falquet *et al*., 2015; Bulan *et al*., 2015; Mariotti *et al*., 2016). Buckwheat is sensitive to photoperiod and temperature especially at the germination and flowering growth stages. The optimal growth conditions for buckwheat are environments with temperature ranging between 17 and 21ºC for flowering and fruit maturation. The time from germination to physiological maturity can vary between 9 and 24 weeks, depending on temperature (Jung *et al*., 2015; Arduini *et al*., 2016; Mariotti *et al*., 2016; Siracusa *et al*., 2017), indicating that buckwheat genotypes may have differential sensitivity to specific agroecological conditions (Hara and Ohsawa, 2013). Therefore, sowing date plays a key role in determining its agroecological suitability.

Buckwheat is a relatively new crop in the UK agricultural landscape. Besides the weather conditions, the key limitations include the indeterminate crop growth and limited knowledge, research and development associated with this crop. However, there has been an increasing interest in growing buckwheat, especially in European countries, not only due to its nutritional value and the fact that it is gluten free (Siracusa *et al*., 2017) but also because it could fit into various cropping rotations (Arduini *et al*., 2016). Most importantly, with the increasing consumption of buckwheat products in the UK in recent years, assessment of yield and grain quality is key in determining the potential and suitability for production in the UK, providing potential as a spring sown break-crop for UK growers who currently operate very intensive cereal-based cropping rotations. Moreover, buckwheat is considered a low-input crop. Therefore, the potential for a new spring grown low-input crop with clear market potential offers an opportunity for farmers in the fight against the increasing threat of black-grass and ever-increasing inputs of pesticides and fertilisers into most UK grown crops.

Various studies have reported that buckwheat shows genetic variation in grain yield and grain quality, indicating that variability in phenology and duration of growth cycle can also exist. Hence, it is expected that buckwheat genotypes will show contrasting crop performance in

response to the local weather conditions. To our knowledge, no data are available about buckwheat cultivation in the UK. Therefore, the aim of this experiment was to:

- Identify buckwheat genotypes suited to NE-England, and
- Evaluate how the productivity and quality of buckwheat can be affected by sowing date, and Zn fertilisation.

5.2 Results

5.2.1 Weather data

The average monthly temperature was 12°C and there was a relatively homogeneous distribution of rainfall with a total average of 57.2 mm over the entire growth cycle (**Table 5.1**). Assuming 15 days of germination time from sowing, minimum temperature was near or below zero degree Celsius for the mid-April sowing date whereas for the early-May sowing date minimum temperature was 2.7 and the maximum 19.1°C. Whilst rainfall increased significantly towards the end of the germination period for the mid-April sowing date, the germination period for early-May sowing was characterised by limited water availability (**Table 5.2**).

5.2.2 Crop growth

The growth cycle of buckwheat was 12-14 days shorter when sown early-May than mid-April and lasted up to 170-180 days. No significant difference between genotypes was observed with respect to the duration of the growth cycle. Both Bamby and Cebelica were harvested on the same day i.e. 27th of October.

Table 5.1 Summary of weather conditions (average temperature, total rainfall and total solar radiation) from sowing to harvest of buckwheat in 2016.

	Temperature (°C)	Rainfall (mm)	Solar radiation (W/m^2)
April	6.2	50.8	132.5
May	10.6	21.4	162.1
June	12.7	88.0	175.2
July	15.1	63.6	188.6
August	15.2	66.4	162.3
September	14.7	52.8	104.7
October	9.8	57.4	51.3

Table 5.2 Weather conditions (minimum and maximum temperature and total rainfall) over germination period of buckwheat for early and late sowing date (white and shaded area, respectively) in 2016.

Date	Min (°C)	Max (°C)	Rain (mm)
19/04	2.0	10.7	0.0
20/04	-0.1	14.7	0.0
21/04	1.6	15.3	0.2
22/04	1.1	9.1	0.4
23/04	0.1	8.8	0.0
24/04	1.3	8.7	0.4
25/04	1.5	7.6	1.0
26/04	1.0	7.0	9.4
27/04	0.4	7.7	0.8
28/04	-0.6	6.9	8.8
29/04	0.3	7.6	1.4
30/04	0.9	10.9	1.8
1/05	2.1	12.6	0.6
2/05	6.4	13.9	1.0
3/05	4.4	13.1	0.0
4/05	6.2	14.7	0.0
5/05	8.1	16.5	0.0
6/05	6.0	13.8	0.0
7/05	7.3	14.2	0.0
8/05	7.3	18.2	0.0
9/05	5.5	14.8	0.2
10/05	8.1	14.8	0.0
11/05	9.4	15.2	0.0
12/05	8.6	15.0	0.2
13/05	6.9	19.1	0.0
14/05	2.7	11.9	0.0
15/05	2.7	12.2	0.0
16/05	3.3	12.9	0.0
17/05	4.6	17.4	0.6
18/05	9.1	16.9	0.0

All three main factors sowing date, fertility and genotype significantly affected seed germination and plant height but not plant number at harvest. No significant interactions were detected. The average germination % of buckwheat across sowing dates and genotypes was 55%. Seed germination was 7% higher when sown mid-April than early-May with significant differences between genotypes also detected wherein seed germination of Bamby was 11% higher than Cebelica (**Table 5.3**).

The average plant height was 77 cm across all treatments and genotypes wherein plants sown early-May were about 8 cm taller than mid-April while Cebelica was 13 cm taller than Bamby.

Surprisingly, there was a significant effect of foliar Zn application on plant height. Plants were approximately 8 cm taller with foliar Zn application than without foliar Zn application (**Table 5.3**).

Chlorophyll content of buckwheat over the vegetative period (GS30-50) was generally higher when sown in early-May compared with mid-April but differences between genotypes were not statistically significant (**Table 5.3**). A significant sowing date × genotype interaction on SPAD at GS40 and GS50, showed that the highest chlorophyll content was detected in Bamby when sown in early-May. There was approximately 50% of flowers that did not produce grains, of which a larger proportion was detected in Bamby than Cebelica (data not shown because it was based on visual observation).

Only sowing date had a significant effect on total above-ground biomass (**Table 5.3**) whereby late sowing increased total above-ground biomass by up to 47% compared with early sowing. No significant interaction effects were detected.

Overall, both genotypes were relatively clean with little or no foliar disease identified. Intensity and severity of leaf and/or plant infection by powdery (*Erysiphe polygoni*) and downy mildew (*Peronospora ducumeti*) was lower than 10% of the whole plant and approximately 5% of leaf area in all plots and so data is not presented.

The weed population was dominated by black-bindweed, chickweed, thistle and wild oat. There were no significant differences between genotypes in the ability to suppress these weed species but weed colonisation was significantly different with respect to sowing date whereby weed colonisation decreased by 20% with delayed sowing date (**Table 5.3**).

5.2.1 Yield and yield components

On average across all treatments, grain yield from the combine ranged between 0.23 and 0.39 t/ha. All three main factors sowing date, fertility and genotype did not affect grain yield significantly (**Table 5.4**). However, there was a small but not significant increase in grain yield with late sowing. The interaction effects were not significant on grain yield. Nonetheless, the results showed a trend ($p \leq 0.10$) for the sowing date × genotype interaction, which indicated that late sowing resulted in the highest grain yield which was produced by the variety Cebelica.

Grain yield from the biomass sample was much higher (up to 5-fold) than that from the combine, and it was up to 60% higher in the early-May than mid-April sowing date plots.

Table 5.3 Effects of sowing date, zinc fertilisation and genotype on % germination, plant height, total above-ground biomass, chlorophyll content (GS30-50) of buckwheat and weed % of ground cover in 2016.

	Germination (%)	Height (cm)	Biomass (g/m^2)	Weed (% of ground cover)	Chlorophyll content (SPAD-units)		
					GS30	GS40	GS50
Sowing date (S)							
Mid-April	59.0±2.74	73.6±2.03	234.4±17.22	61.9±3.16	32.8±0.30	29.4±0.44	28.3±0.23
Early-May	52.0±1.71	81.2±2.30	344.0±19.84	41.6±4.36	32.1±0.18	30.1±0.47	30.3±0.35
Zinc (Zn)							
+ Zn		81.6±2.01	306.2±20.07	55.0±4.19			
- Zn		73.2±2.26	272.2±21.64	48.4±4.19			
Genotype (G)							
Bamby	61.3±2.23	70.4±2.10	263.6±22.54	51.4±3.96	32.1±0.30	31.2±0.46	29.7±0.39
Cebelica	49.7±2.00	83.7±1.74	314.8±18.41	52.0±4.49	32.9±0.16	28.3±0.27	29.0±0.28
ANOVA							
Sowing date (S)	*0.056*	**0.033**	**0.007**	**0.005**	ns	ns	**0.001**
Zinc (Zn)		**0.017**	ns	ns			
Genotype (G)	**0.049**	**0.033**	ns	ns	ns	**0.027**	ns
S * Zn		ns	ns	ns			
S * G	ns	ns	ns	ns	ns	ns	**0.023**
G * Zn		ns	ns	ns			
S * Zn * G		ns	ns	ns			

Cebelica had a significantly higher thousand-grain weight (TGW) than Bamby (**Table 5.4**). Sowing date and Zn fertilisation did not significantly affect TGW but the interaction effect was statistically significant which indicated that the highest TGW was obtained from plots with foliar Zn application sown early i.e. mid-April. Although the ANOVA *p*-value for the interaction effect between sowing date and zinc application was statistically significant, Tukey's test for multiple comparisons of the means was not statistically significant at $p \leq 0.05$, showing that the differences were not substantive.

Sowing date and genotype did not affect harvest index (HI) but a significantly higher HI was obtained from the plots without than with foliar Zn application (**Table 5.4**). There was a statistically significant sowing date × fertility interaction which indicated that early sowing resulted in the highest HI of 0.31 obtained in the plots without foliar Zn application (**Table 5.5**).

Late sowing increased the seed number per plant by up to 50% compared with early sowing (**Table 5.4**). The effect of Zn fertilisation was not statistically significant. There was a trend indicating that Cebelica had a significantly higher seed number per plant than Bamby and the interaction effect between sowing date and genotype was also statistically significant, showing that late sowing resulted in the highest seed number per plant produced by the variety Cebelica (**Table 5.6**).

Plant number at harvest was not significantly affected by any of the three main factors. On average, Bamby had more plants per unit area than Cebelica, which reflected to some extent the differences in germination % (**Table 5.4**).

5.2.2 Grain quality

Protein, ash and mineral content

There was only a significant effect of sowing date on ash content and Zn fertilisation on grain concentrations of Mo (**Table 5.7, Table 5.8**). Late sowing decreased the ash content compared with early sowing and grain concentrations of Mo decreased with foliar Zn application. Grain Zn concentrations were not affected by the experimental factors. The interactions were not significant.

Total polyphenols, antioxidants and flavonoids

Only the effect of sowing date was statistically significant whereby late sowing increased grain concentrations of total polyphenols by up to 26.4% but decreased the concentrations of total antioxidants and flavonoids by up to 7- and 34-fold, respectively. The interactions were not statistically significant (**Table 5.9**).

Table 5.4 Effects of sowing date, zinc fertilisation and genotype on yield and yield components of buckwheat in 2016.

	Plant number (plants/m^2)	Seed number /plant	Seed number /m^2	TGW (g)	Seed yield (t/ha)		HI
					Biomass sample	Combine	
Sowing date (S)							
Mid-April	133.3±5.50	33.4±2.12	4579.7±394.68	21.2±0.28	0.96±0.08	0.30±0.03	0.23±0.02
Early-May	144.3±5.73	50.1±4.95	7415.8±914.07	21.2±0.26	1.60±0.21	0.31±0.02	0.19±0.02
Zinc (Zn)							
+ Zn	141.6±5.89	37.7±4.32	5446.0±639.59	21.3±0.27	1.25±0.14	0.30±0.02	0.16±0.01
- Zn	135.9±5.46	45.8±3.74	7081.0±952.53	21.0±0.27	1.41±0.20	0.30±0.03	0.26±0.03
Genotype (G)							
Bamby	146.4±6.00	32.9±1.64	4914.5±373.90	20.0±0.17	0.99±0.08	0.28±0.02	0.18±0.01
Cebelica	131.1±5.02	50.6±5.08	7081.0±952.53	22.3±0.17	1.57±0.22	0.33±0.03	0.24±0.03
ANOVA							
Sowing date (S)	ns	**0.024**	*0.058*	ns	0.058	ns	ns
Zinc (Zn)	ns	ns	ns	ns	ns	ns	**0.011**
Genotype (G)	ns	*0.081*	ns	**0.006**	ns	ns	ns
S * Zn	ns	ns	ns	**0.047**	ns	ns	*0.066*
S * G	ns	*0.066*	ns	ns	ns	*0.075*	ns
G * Zn	ns	ns	ns	ns	ns	ns	ns
S * Zn * G	ns	ns	ns	ns	ns	ns	ns

Table 5.5 Interaction between sowing date and zinc fertilisation on harvest index (HI) of buckwheat in 2016. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey test.

	+ Zn	- Zn
mid-April	0.15±0.01aB	0.31±0.02aA
early-May	0.17±0.02aA	0.20±0.02aA

Table 5.6 Interaction between sowing date and genotype on seed/plant of buckwheat in 2016. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey test.

	Bamby	Cebelica
mid-April	31.2±0.29aA	35.4±0.20bA
early-May	34.6±0.28aB	65.7±0.38aA

Table 5.7 Effects of, sowing date, zinc fertilisation and genotype on protein and ash content of buckwheat in 2016.

	Protein (%)	Ash (%)
Sowing date (S)		
Mid-April	11.4±0.19	3.2±0.23
Early-May	11.7±0.22	2.5±0.05
Zinc (Zn)		
+ Zn	11.5±0.18	2.8±0.21
- Zn	11.7±0.22	2.8±0.13
Genotype (G)		
Bamby	11.5±0.24	2.9±0.19
Cebelica	11.7±0.16	2.7±0.15
ANOVA		
Sowing date (S)	ns	*0.051*
Zinc (Zn)	ns	ns
Genotype (G)	ns	ns
S * Zn	ns	ns
S * G	ns	ns
G * Zn	ns	ns
S * Zn * G	ns	ns

Table 5.8 Effects of, sowing date, zinc fertilisation and genotype on the concentration of minerals in buckwheat in 2016.

	Ca	K	Mg	P	S	Al	Cd	Cu	Fe	Mn	Mo	Na	Ni	Zn
			(%)							(mg/kg)				
Sowing date (S)														
Mid-April	0.11	0.68	0.23	0.46	0.15	35.8	0.07	7.1	61.0	25.6	0.46	108.5	1.8	29.2
Early-May	0.11	0.73	0.24	0.47	0.16	60.0	0.07	7.2	53.5	27.5	0.48	71.6	1.2	27.0
Zinc (Zn)														
+ Zn	0.11	0.78	0.24	0.49	0.15	36.1	0.07	7.5	57.4	27.0	0.56	65.4	1.7	26.8
- Zn	0.11	0.63	0.23	0.43	0.15	59.7	0.07	6.8	57.5	26.0	0.34	114.7	1.3	29.5
Genotype (G)														
Bamby	0.11	0.67	0.23	0.45	0.15	23.8	0.06	6.3	51.5	23.0	0.41	100.2	1.4	27.0
Cebelica	0.11	0.74	0.24	0.47	0.16	69.7	0.07	8.1	63.8	29.8	0.52	79.9	1.7	29.2
ANOVA														
Sowing date (S)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Zinc (Zn)	ns	*0.086*	ns	ns	ns	ns	ns	ns	ns	ns	**0.014**	ns	ns	ns
Genotype (G)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
S * Zn	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
S * G	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
G * Zn	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
S * Zn * G	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table 5.9 Effects of, sowing date, zinc fertilisation and genotype on the concentrations of total polyphenols, antioxidants and flavonoids of buckwheat in 2016.

	Polyphenols (µg GA/g DW)	Antioxidants (µg TE/g DW)	Flavonoids (µg Rutin/g DW)
Sowing date (S)			
Mid-April	5012.4±333.29	4908.4±148.56	2829.4±218.06
Early-May	5805.5±245.70	683.4±34.38	84.0±2.57
Zinc (Zn)			
+ Zn	5972.5±285.93	2883.2±401.66	1549.0±280.23
- Zn	5845.4±378.17	2708.5±398.67	1364.4±306.72
Genotype (G)			
Bamby	6413.3±343.83	2942.5±413.96	1504.0±300.09
Cebelica	5404.6±299.77	2649.2±384.66	1409.3±288.05
ANOVA			
Sowing date (S)	**0.003**	**<0.001**	**<0.001**
Zinc (Zn)	ns	ns	ns
Genotype (G)	ns	ns	ns
S * Zn	ns	ns	ns
S * G	ns	ns	ns
G * Zn	ns	ns	ns
S * Zn * G	ns	ns	ns

5.2.3 Correlation coefficients

Correlation tests indicated that total above-ground biomass showed a strong positive correlation with plant height and grain yield. The tests also indicated that total antioxidants showed a strong positive correlation with total flavonoids but negative correlation with total polyphenols (**Table 5.10 – 5.12**).

Table 5.10 Correlation coefficients for growth traits of buckwheat in 2016. Stars in the shaded area represent significance at p<0.05. Significance codes: 0.001 '***', 0.01 '**', 0.05 '*', ns 'not significant'.

	Height	SPAD-GS50	Biomass	Yield	HI
Height	1.00	ns	**	ns	ns
SPAD-GS50	-0.04	1.00	ns	ns	ns
Biomass	**0.55**	0.11	1.00	*	ns
Yield	0.25	-0.18	**0.39**	1.00	*
HI	-0.09	-0.10	-0.07	**-0.38**	1.00

Table 5.11 Correlation coefficients for yield traits of buckwheat in 2016. Stars in the shaded area represent significance at p<0.05. Significance codes:', 0.001 '***', 0.01 '**', 0.05 '*', ns 'not significant'.

	Plants/m^2	Seeds/m^2	Biomass	TGW	Yield	HI
Plants/m^2	1.00	***	***	ns	ns	ns
Seeds/m^2	**0.57**	1.00	***	ns	ns	**
Biomass	**0.61**	**0.68**	1.00	ns	*	ns
TGW	-0.10	**0.19**	**0.28**	1.00	ns	ns
Yield	0.03	0.09	**0.39**	**0.22**	1.00	*
HI	0.10	**0.53**	-0.07	0.02	**-0.38**	1.00

Table 5.12 Correlation coefficients for quality traits of buckwheat in 2016. Stars in the shaded area represent significance at p<0.05. Significance codes: 0.001 '***', 0.01 '**', 0.05 '*', ns 'not significant'.

	Yield	Protein	Fe	Zn	Phenols	Antioxidants	Flavonoids
Yield	1.00	ns	ns	ns	ns	ns	ns
Protein	0.12	1.00	ns	ns	ns	ns	ns
Fe	-0.22	-0.11	1.00	ns	ns	ns	ns
Zn	-0.07	0.21	0.06	1.00	ns	ns	ns
Phenols	-0.24	-0.14	0.14	-0.05	1.00	*	ns
Antioxidants	0.03	-0.12	0.04	0.12	**-0.38**	1.00	***
Flavonoids	-0.16	-0.11	0.12	0.10	-0.11	**0.87**	1.00

5.3 Discussion

5.3.1 Crop growth

Weather conditions in the present study were sufficient to create a suitable growing environment for buckwheat in terms of temperature and water supply. These weather conditions were below the optimal conditions for buckwheat cultivation at least in terms of temperature (17 – 19ºC) as reported by Jung *et al*. (2015) and Mariotti *et al*. (2016). The short drought period that occurred from May 26th to June 11th might have created a condition of limited water availability during early seedling growth as there was zero rainfall during that period. Therefore, differences in seedling emergence and growth rates between sowing dates could be attributed to low soil moisture content due to limited water availability, at least for the early growth stages, because limited water availability can negatively affect buckwheat (Horbowicz and Obendorf, 2005; Mariotti *et al*., 2016).

Seed germination in the field (50 – 60%) was variable and low. Various reasons could explain the relatively low germination rates observed in this experiment particularly low temperatures during germination. There were two days (April 23rd and 28th) with minimum temperatures below zero degree Celsius i.e. -0.2 and -1.2ºC, respectively, which might have affected seed germination of the mid-April sowing date. Temperature during the germination period (April 19th – May 17th) for both sowing dates varied from -0.2 to 5.5ºC and from 4.7 to 11.8ºC for night and day temperatures, respectively. This is likely to be a major reason for the low seed germination rates in this study especially the night temperatures because buckwheat needs a base temperature of 5 – 10ºC for optimal germination rates (Arduini *et al*., 2016; Mariotti *et al*., 2016). Nonetheless, the base temperature for both sowing dates was on average (7 – 9ºC) which is within the optimal temperature for germination.

Although the germination rates were low, these results are in agreement with the results from other studies on buckwheat (Sakata and Ohsawa, 2006). Moreover, despite the limiting germination conditions, the 50% germination rate occurred 2 – 4 weeks after sowing which was relatively faster than the germination rates observed by Sakata and Ohsawa, (2006). Sakata and Ohsawa (2006) studied 17 Japanese common buckwheat (*Fagopyrum esculentum* Moench) genotypes and found that seed germination rates three weeks after sowing ranged between 20 and 57% with significant differences between crops sown on June 25th (29.9%) and August 6th (40 – 42%) sowing dates.

There was high variability in the data with respect to germination not only because of variation due to treatment effect but also because of variation within blocks. Some of this variation ascribed to blocks was due to areas within each block (particularly in the early-May sowing date plots) where there was either poor germination or no germination at all. It appeared that the patches were associated with the depth of drilling (possibly due to uneven tilling which is likely to be more critical in smaller seeded crops like buckwheat), thus reducing seed germination rates. Nonetheless, there were no clear patterns of this variability within blocks that could be also associated with soil fertility problems linked to management practices of the preceding cropping seasons to explain the effects on current crops.

Sowing date

The buckwheat growth cycle was 150 – 190 days long and varied by up to 9% in response to sowing date. The delay in sowing time from mid-April to early-May resulted in a shorter growth cycle. Despite the difference in response to sowing date, the length of the growth cycle was longer compared with other studies (Jung *et al*., 2015; Arduini *et al*., 2016; Siracusa *et al*., 2017) which reported growth cycles of 90 – 140 days. The reason for such a long growth cycle was attributed to longer daylength and especially the lower UK temperatures which contributed to slower crop development and the need for more thermal time. Mean temperatures of the entire growth cycle (12.8ºC and 13.3ºC for early and late sowing respectively) were below the optimal range of 18 – 23ºC for cultivation of buckwheat (Mariotti *et al*., 2016). This confirms that buckwheat requires a longer growth period and more thermal time when grown in environments with low temperatures and longer daylength (Arduini *et al*., 2016; Mariotti *et al*., 2016), thus imposing serious limitations to cultivation of buckwheat in the UK where fruit maturation and harvest date would be delayed. Nonetheless, the response of buckwheat in terms of length of the growth cycle did not vary greatly between sowing dates and it was similar to those published by Mariotti *et al*. (2016) under similar or different growing conditions. Therefore, considering

the weather conditions in the UK particularly in NE-England, it is crucial to identify and select genotypes with faster phenological development and shorter growth cycle for successful commercial production of buckwheat

In the current field trial, April 19th and May 3rd sowing dates (described here as mid-April and early-May, or early and late sowing, respectively) were used, assuming that sowing date is a major factor affecting variation of germination and different traits of buckwheat across a wide range of production systems. The results showed that germination rates decreased significantly with the delay in sowing time from April to May. The highest germination rate was obtained by sowing in April. The most likely reason for this difference was the lower temperature and limited water availability that occurred towards the end of the germination period particularly for the early-May sowing date. Thus, water availability (i.e., soil moisture content) was probably the most limiting factor for seed germination in response to early-May sowing in 2016 rather than temperature. Usually, when there is sufficient water availability and optimal temperatures, late sowing (July – August in Japan) was shown to result in higher germination rates (Sakata and Ohsawa, 2006). Differences in germination rates between the two sowing dates did not result in significant differences in crop biomass at later growth stages, particularly at harvest. Plant number at harvest was not significantly different between sowing dates suggesting that plant survival rate was higher with the delay in sowing from April to May.

The effect of sowing date was also significant on plant height, chlorophyll content and total above-ground biomass. Early-May sowing resulted in increments of up to 8 – 10 cm in plant height, 2 – 3 SPAD units in chlorophyll and 47% greater above-ground biomass. These results indicate that despite the water stress in May influenced germination %, the higher temperature with delayed drilling played a key role to sustain growth and biomass production in the early and late growth stages, respectively. Therefore, although germination rates were low, delaying sowing from April to May supports the optimal growth of buckwheat under the conditions of the present study. Nonetheless, it is well established that buckwheat is particularly susceptible to low temperature frost damage (Jacquemart *et al*., 2012; Farooq *et al*., 2016), hence, based on the weather data, it is conceivable that frost damage/kill was one of the key reasons for the poorer crop performance of the mid-April sowing relatively to early-May sowing.

Genotype

Genotype did not significantly affect many of the growth traits except germination and plant height. Although seed germination of Cebelica was 11% lower than that of Bamby, such a difference did not result in significant differences in plant number at later growth stages,

particularly at harvest, suggesting that Cebelica had a higher survival rate than Bamby. These results are in agreement with those published by Sakata and Ohsawa (2006) who found up to 43% differences in germination rate between genotypes. There was only a significant interaction effect between sowing date × genotype on chlorophyll content which did not necessarily correlate with improved grain yield or crop growth.

5.3.2 Yield and yield components

Buckwheat is a low-yielding crop with an average yield ranging between 0.8 and 1.2 t/ha worldwide (Popović *et al*., 2014). In this experiment, grain yield from the combine was very low (i.e. 0.23 – 0.39 t/ha) compared with the average global yield. However, similar results were observed by Brunori *et al.* (205) and Siracusa *et al.* (2017) who observed grain yields of 0.12 – 0.98 t/ha and 0.32 – 0.70 t/ha, respectively. Thus, despite being relatively low, the results of the present study were within the range of average grain yield found by other studies.

In this experiment, grain yield was very low at least primarily due to high seed loss and flower abortion. Seed loss was associated with delayed harvest due to difficulties with ensuring even ripening (because of the indeterminate growth habit of buckwheat and the deteriorating weather conditions). The seed loss was estimated based on the difference between yields obtained from the biomass samples and the combine harvested samples. Whilst the latter ranged from 0.23 to 0.39 t/ha, the former was 1.82 t/ha, which was more consistent with other findings in the literature (Erley *et al.*, 2005; Popović *et al.*, 2013; Ghiselli *et al.*, 2016). Erley *et al.* (2005) found an average grain yield of 1.43 t/ha, Popović *et al.* (2013) found 1.32 – 1.40 t/ha, and Guiselli *et al.* (2016) found 1.76 – 1.99 t/ha. Therefore, seed loss was considered the primary reason for the relatively low grain yield of 0.23 – 0.39 t/ha obtained from the combine harvested samples in the present study.

Another factor to consider was flower abortion, that is, the proportion of flowers that did not produce seeds and mature grains. Despite installation of bee hives in the field to encourage and help pollination, approximately 50% of flowers did not produce seeds. Taking into consideration the weather data, it was assumed that the reason for low seed set was the relatively low temperatures and wet weather conditions in June-August (flowering-seed development), contrary to previous studies (Kalinova and Vrchotova, 2011; Siracusa *et al*., 2017) which attributed the high proportion of flower abortion in buckwheat to heat stress due to relatively high summer temperatures during flower development. Similar results of high flower abortion were observed by Halbrecq *et al.* (2005) who found that only 28 – 40% of buckwheat flowers produced seeds and mature grains. In addition to heat stress (Kalinova and Vrchotova, 2011),

flower abortion has been associated with age (late flowers in anthesis) and position (lower part of the cymes) of aborted flowers in the inflorescence as suggested elsewhere (Halbrecq *et al.*, 2005; Jacquemart *et al.*, 2012; Jacquemart *et al.,* 2012; Mariotti *et al.*, 2016).

Sowing date

The effect of sowing date on grain yield was not statistically significant in the present study despite the early-May sowing having significantly higher seed number per plant than the mid-April sowing date. In contrast, previous studies (Jung *et al*., 2015; Mariotti *et al*., 2016, Siracusa *et al*., 2017) carried out in Korea and Italy investigated the effects of spring (March, April, and May) and summer (July, August and September) sowing times on buckwheat yield and observed significant differences. Jung *et al*. (2015) obtained the highest yield (235 kg/ha) by sowing on August 25th in central Korea; Mariotti *et al*. (2016) obtained the highest yield (2 t/ha) by sowing mid-April in Italy; Siracusa *et al*. (2017) obtained the highest yield (517.2 kg/ha) by sowing in May in southern Italy. Both studies attributed the differences observed at least partly to temperature and water availability.

Foliar Zn application

The effect of foliar Zn application on yield was not statistically significant. This result is in agreement with previous studies showing that Zn application did not cause significant effects on yield even under Zn-deficient soil conditions except in few instances (Zou *et al*., 2012; Zhang *et al*., 2012a). This conclusion is also supported by the results of meta-analysis about the effect of Zn application on grain Zn and yield of wheat, rice and maize presented in **Chapter 3**, which showed that the effect of Zn application on yield was not statistically significant regardless of the method of application. Another reason to consider was that the level of Zn (higher than 1.5 mg/L) in the field trial was not critical for Zn deficiency such that levels of Zn in the soil did not appear to be limiting to yield in the present study. However, there are studies reporting small but significant increases of grain yield of cereal crops up to 3% - 13% caused by foliar Zn application despite high levels of soil Zn availability (Zou *et al*., 2012).

There was a significant effect of Zn fertilisation on HI. The results showed that HI decreased with foliar Zn application and was highest in mid-April sowing without foliar Zn application. The difference in HI in response to Zn fertilisation was attributed to a random variation probably due to sampling error because neither of the yield components, which could have ultimately affected the HI value, were significantly affected by Zn fertilisation. Nonetheless, the average HI across all treatment and genotypes was 0.21 in the present study which was

lower than 0.35 but similar to 0.23 (average across three genotypes) for field and pot-grown buckwheat, respectively, in Japan in 2013 (Kasajima *et al*. 2017).

Genotype

Genotype is one of the factors that has a major effect on yield (Jung et al., 2015) as is generally the case for crops. However, in the present study genotype choice did not cause a significant effect on yield of buckwheat despite the significant difference in TGW. Previous studies (Erley *et al*., 2005; Siracusa *et al*., 2017; Kasajima *et al*., 2017) had similar observations where yield was not affected by genotypic variation. The TGW values in the present study were similar to the average 21 g across four buckwheat genotypes grown in Italy published by Siracusa *et al*. (2017). The present study also showed that Bamby (20 g) and Cebelica (22 g) had higher TGW values than those (i.e. 17 and 18 g respectively) for the same genotypes grown in Serbia published by Filipčev *et al*. (2013).

A trend of interaction effect between sowing date and genotype was detect by ANOVA on grain yield (p=0.07) total seed number (p=0.06) showing that Cebelica produced up to 35% more yield and up to 47% more seeds per plant than Bamby in the later sowing i.e. early-May. The ANOVA results also indicated significant interactions between sowing date and Zn application on TGW and HI showing that there was a small but non-significant 3.3 - 4.2% increase of TGW and a significant 15 – 52% decrease of HI with foliar Zn application.

5.3.3 Grain quality

The present study found concentrations of total polyphenols, antioxidants and flavonoids within the following ranges 5012.4 – 6805.5 (polyphenols), 683.4 – 4908.4 (antioxidants) and 84.0 – 2829.4 µg/g (flavonoids). These concentrations were similar to those published for several studies on buckwheat (Quettier-Deleu *et al*., 2000; Holasova *et al*., 2002; Gorinstein *et al*., 2007; Guo *et al*., 2011; Inglett *et al*., 2011; Sobhani *et al*., 2014; Zhu, 2016; Siracusa *et al*., 2017). A simple correlation test showed a significant (p=0.03) negative non-linear correlation between the concentration of total polyphenols and total antioxidants while total antioxidants and total flavonoids showed a significant (p< .001) strong positive non-linear correlation. Therefore, the negative correlation contradicts, at least partly, previous studies which suggested that increasing concentration of total polyphenols in buckwheat may be positively and necessarily correlated with high concentration of antioxidants and vice-versa (Holasova *et al*., 2002; Vollmannová *et al*., 2013).

The present study found an average protein content of 11.6% across all treatments. This protein content was approximately 1% below the range of 12 – 13% published by several studies (Angiolonni and Collar, 2011; Inglett *et al*., 2011; Siracusa *et al*., 2017) as the expected range for common buckwheat *Fagopyrum esculentum* Moench. However, other studies have also reported protein content above 16% and below 11% (Guo *et al*., 2007; Filipčev *et al*., 2013; Zhu, 2016). All treatments did not affect mineral concentrations. Nonetheless, mineral concentrations were similar or higher (particularly K and Mg) than those published in other studies (Bonafaccia *et al*., 2003; Huang *et al*., 2014).

Sowing date

Sowing date had a significant and variable effect on total polyphenols, antioxidants and flavonoids. The highest concentration of total polyphenols (0.68 mg/g) was detected in the late i.e. early-May sown plots whereas the highest concentrations of total antioxidants (0.49 mg/g) and flavonoids (0.28 mg/g) were detected in the early i.e. mid-April sown plots.

These results were consistent with other studies which reported significant differences between sowing dates in the concentrations of bioactive compounds (Sobhani *et al*., 2014). However, there are other studies showing that sowing date did not have a significant effect on the concentration of bioactive compounds such as phenols, antioxidants or flavonoids (Siracusa *et al*., 2017) as well as studies showing that high concentration of polyphenols is related to high concentration of antioxidants ((Holasova *et al*., 2002; Vollmannová *et al*., 2013). Therefore, it was assumed that the differences detected in the present study were most likely due to an increased metabolic activity for crop defence against heavy rain and cool temperature stress at the time of seed formation and development as the weather data indicated and has been suggested in the literature (Inglett *et al*., 2011; Siracusa *et al*., 2017).

Sowing date did not significantly affect protein nor mineral concentrations but did have a significant effect on the ash content. It is well established that nitrogen supply (i.e. rate and timing) is the major factor affecting protein content; however, some studies (Siracusa *et al*., 2017) have found significant differences between sowing dates with respect to protein content likely attributable to dilution effects. Although the difference was not statistically significant in the present study, protein content in both sowing date plots was within the normal range of 11 – 15% for buckwheat as reported in previous studies (Campbell, 1997; Siracusa *et al*., 2017).

With regard to mineral concentrations, the most likely reason was that there was little or no dilution effect of any of the minerals is the fact that the yield was very low with no significant

differences between sowing dates. There is clear evidence for arable crops showing that mineral concentrations are generally related to yield so that high yield dilutes the concentrations of protein and minerals.

Foliar Zn application

The effect of foliar Zn application was not significant on the concentration of Zn. Foliar Zn application was not significant on the concentration of total polyphenols, antioxidants and flavonoids. Abundance or deficiency of these bioactive compounds is not generally linked to Zn supply, therefore, the effect of late foliar Zn application on the concentration of polyphenols, antioxidants or flavonoids was unlikely at least under the conditions of this experiment.

Genotype

No significant differences between the two genotypes with respect to total polyphenols, antioxidants and flavonoids were detected. Although the differences were not significant, the concentration of these bioactive compounds was generally higher in Bamby than Cebelica. However, other studies found significant differences between genotypes (Guo *et al*., 2011; Siracusa *et al*., 2017). For example, Guo *et al*. (2011) examined phenolic and flavonoid content of two buckwheat genotypes grown in different locations and found significant effect of genotype choice with concentrations ranging between 5150 – 9660, 2077 – 3149 and $1.2x10^5$ – $1.4x10^5$ µmol/100g for total phenolics, total antioxidants and total flavonoids, respectively. Nonetheless, these concentrations are similar to or even lower than the ones obtained in the present study.

Protein and mineral content did not vary significantly between genotypes. However, significant differences in protein content were found between genotypes, particularly between Bamby (17.6%) and Cebelica (18.3%) grown in Serbia in 2010 (Filipčev *et al*., 2013).

No significant interaction effects between sowing date, fertility and genotype on total polyphenols, antioxidants and flavonoids were detected. Most importantly, since in the present study there was only a 2-weeks gap between sowing dates and crops were exposed to similar environmental conditions, no significant differences in response to sowing date were expected considering that these bioactive compounds are highly responsive to environmental stresses especially temperature and water stress (Guo *et al*., 2011).

CHAPTER 6 – Effects of, Sowing Date, Nitrogen Fertilisation and Genotype on Growth, Yield and Quality of Buckwheat (*Fagopyrum esculentum* Moench.)

6.1.Introduction

Buckwheat originates from China with much higher temperatures during the growing season compared with the UK particularly NE-England. Whilst the optimal temperature for seed germination of buckwheat is 10ºC, optimal temperature for growth and development is 17 – 21ºC (Arduini *et al*., 2016; Mariotti *et al*., 2016). In contrast, temperatures in NE-England are generally lower than the optimal temperature for both seed germination and crop growth. Hence, low temperature may be the key limiting factor to growing buckwheat successfully in NE-England. In addition, there is evidence showing that genetic variation in the ability to adapt to a wide range of climatic conditions may exist among buckwheat genotypes (Sytar *et al*., 2016). Most importantly, if buckwheat can grow successfully in NE-England, then a key requirement for commercial production is to determine the appropriate sowing time that optimises crop growth and yield.

Buckwheat is considered a low-yielding crop species compared to the major cereals. The average seed yield of buckwheat is 0.8 – 1.2 t/ha (Popović *et al*., 2014) compared to 3 – 5 t/ha for wheat and rice (FAOSTAT, 2019). However, there is the potential for increasing buckwheat seed yield by 50% from the current average seed yield, compared with 20% for that of wheat and rice (Li and Siddique, 2018; Li *et al*., 2019). For centuries, nitrogen fertilisers have been a major route to increasing crop yield, but is often associated with increased lodging and risk of foliar disease. Foliar diseases and lodging limit crop yields, reduce photosynthetic and harvesting efficiency (Tang *et al*., 2015). Therefore, there is a need to determine the optimal nitrogen fertiliser rate for optimising seed yield.

Buckwheat is a rich source of macro and micronutrients such as Ca, K, Mg, Mn, Se and Zn as well as protein (Huang *et al*., 2014; Mir *et al*., 2018). However, grain concentration of these nutrients may vary between genotypes and can be influenced by agronomic management practices such as sowing date and nitrogen fertilisation. To our knowledge, there is no published study about the effects of sowing date and nitrogen fertilisation on crop growth, yield and quality of buckwheat grown in the UK. Therefore, the aim of this chapter was to:

- Identify buckwheat genotypes suited to NE-England, and

- Evaluate how the productivity and quality of buckwheat can be affected by sowing date and nitrogen fertilisation.

6.2.Results

6.2.1. Weather data

The average temperature over the entire growth cycle (April – October) was 12.8º and 13.6ºC in 2017 and 2018, respectively; the average temperature over the germination period (i.e. assuming a germination period of two weeks after sowing) in 2017 was 7 and 10ºC with 12 and 11 mm total rainfall for the mid-April and early-May sowing dates, respectively. Whereas in 2018 it was warmer and wetter i.e. 9 and 11ºC with 8.6- and 25.2-mm total rainfall for the mid-April and early-May sowing dates, respectively (**Table 6.1** and **6.2**). Temperatures below zero were observed on 2 days in 2017 with temperatures above 20°C also observed twice in 2018.

Total rainfall for the entire growth cycle was similar with 397.6 and 368.6 mm in 2017 and 2018, respectively. During the germination period in 2017, the total rainfall was 12 mm and zero whereas in 2018 it was 8.6 and 25.2 mm for the mid-April and early-May sowing dates, respectively. The 2018 growing season was characterised by a period of low rainfall for the months May – July (i.e. 75.6 mm) which covered the period GS10-40 (from seedling emergence to the beginning of flowering) whereas in 2017 throughout August, covering the period GS50-60 (from flowering to seed setting), the weather was characterised by warm and relatively dry conditions (**Table 6.1**).

Table 6.1 Summary of weather conditions (average monthly temperature, solar radiation and monthly cumulative rainfall) from sowing to harvest of buckwheat in 2017 and 2018.

	2017			2018		
	Temp (°C)	Rain (mm)	Radiation (W/m^2)	Temp (°C)	Rain (mm)	Radiation (W/m^2)
April	8.0	14.8	147.3	8.1	67.6	123.5
May	11.9	19.8	189.7	12.0	31.0	216.4
June	14.4	127.2	179.5	10.9	38.6	225.0
July	14.4	68.4	160.2	16.9	25.2	208.2
August	14.7	31.6	152.3	15.3	108.6	136.5
September	12.3	84.4	88.5	12.4	53.0	113.3
October	11.5	51.4	45.1	9.5	44.6	56.5

Table 6.2 Weather conditions (minimum and maximum temperature and total rainfall) over the germination period of buckwheat for the early and late sowing dates (white and shaded area, respectively) in 2017and 2018.

Date	2017			2018		
	Min (°C)	Max (°C)	Rain (mm)	Min (°C)	Max (°C)	Rain (mm)
13/04	4.5	11.3	0.0			
14/04	5.9	12.1	0.6			
15/04	3.7	10.9	0.0			
16/04	3.6	7.5	5.4			
17/04	1.3	9.2	0.6			
18/04	-1.4	9.7	0.0			
19/04	4.4	11.7	0.0			
20/04	9.6	15.5	0.0	8.3	16.8	0.0
21/04	6.9	13.5	1.8	4.3	17.9	0.0
22/04	4.5	10.1	0.0	9.1	15.6	0.2
23/04	2.6	13.1	0.0	8.1	12.8	1.0
24/04	0.5	10.4	2.0	6.3	12.1	5.4
25/04	-0.9	7.6	0.8	6.2	12.4	0.0
26/04	0.5	8.5	0.4	5.7	11.8	0.4
27/04	3.7	12.0	0.4	3.5	11.0	0.0
28/04				1.8	8.7	1.0
29/04				1.1	9.1	0.2
30/04				1.5	9.7	0.2
1/05				0.7	12.6	0.0
2/05	7.0	12.0	0.0	5.7	12.7	0.2
3/05	4.2	14.1	0.0	4.7	14.0	0.0
4/05	6.0	13.3	0.0	9.3	18.4	0.0
5/05	3.0	13.1	0.0			
6/05	3.6	12.0	0.2			
7/05	3.0	10.6	0.2			
8/05	6.1	10.3	0.0			
9/05	5.3	15.0	0.0	4.1	14.3	3.0
10/05	3.3	16.4	0.0	4.8	13.5	3.0
11/05	3.7	16.0	0.0	2.8	15.1	0.0
12/05	6.7	13.7	0.0	6.6	14.9	0.4
13/05	9.2	14.7	3.8	7.3	16.2	10.6
14/05	8.9	17.0	0.2	3.4	18.4	0.0
15/05	6.6	17.3	6.2	4.5	19.0	1.4
16/05	11.1	18.3	0.6	3.6	11.2	6.4
17/05				1.5	12.8	0.2
18/05				1.5	18.2	0.0
19/05				4.6	20.5	0.2
20/05				7.6	21.5	0.0
21/05				6.5	22.0	0.0
22/05				8.0	11.9	0.0
23/05				8.4	11.9	0.0

6.2.2. Crop growth

Overall, the growth cycle was approximately 170 days but it was two weeks shorter in 2018 than 2017. Delayed sowing from mid-April to early-May reduced the growth cycle by on average 20 days across both seasons and plants required 30-35 days from flowering to seed set and from seed set to maturity.

Differences in duration of phenological phases with respect to nitrogen application rate were clear from GS50 to GS80, particularly between the 150 kg N/ha and zero-N treatments. Phenological phases were longer with application of 150 kg N/ha relative to zero-N, with differences in duration up to 7 days from one growth stage to another. Nitrogen source (mineral N vs biogas digestate) had no effect on crop development.

Average seed germination was low at about 53% with approximately 75% of germinated seed surviving through to harvest across all treatments in the two years (2017 and 2018). Seed germination % was significantly affected by year and sowing date but was not significantly different between Cebelica and Bamby (**Table 6.3**). Seed germination was 10% higher in 2017 than 2018 and 18% higher in the mid-April than early-May sowing. Significant year × genotype and sowing date × genotype interactions showed that the genotype Bamby had the highest seed germination (66%) in the mid-April sowing in 2017 (**Table 6.4** and **6.5**).

The average plant height was approximately 90 cm across all treatments. Plant height was significantly affected by year of cultivation, sowing date, nitrogen rate and genotype but not by nitrogen source (**Table 6.3**). Plants were 23 cm taller in 2017 than 2018, 20 cm taller in the early-May than mid-April sown plots, approximately 10 cm taller at 150 kg N/ha than at lower nitrogen application rates and the genotype Cebelica was 10 cm taller than Bamby. There was a significant year × sowing date interaction showing that plants were tallest (up to 110 cm) in the early-May sown plots in 2017 (**Table 6.6**).

Total above-ground plant biomass was significantly affected by year of cultivation, sowing date and nitrogen rate but it was not significantly different between nitrogen source and between Cebelica and Bamby (**Table 6.3**). Total above-ground was two times higher in 2017 than 2018, 67% higher in the early-May than mid-April sown plots and at higher nitrogen application rates. Significant year × sowing date, year × nitrogen rate, year × genotype, sowing date × nitrogen rate and sowing date × genotype interactions showed that the application rate of 150 kg N/ha resulted in the highest total above-ground biomass production in the early-May sown plots in 2017. The interactions also showed that whilst Bamby produced the highest total above-ground

biomass (644 g/m^2) in 2017, Cebelica produced the highest above-ground biomass (614 g/m^2) in the early-May sowing date (**Table 6.7–6.11**).

Overall, there was 51% of ground covered by weeds with a significant difference only between years of cultivation. There was a higher weed cover (more than 90%) in 2018 than 2017 (**Table 6.3**). The weed population was dominated by chickweed in 2017 and oilseed rape in 2018. A significant year × sowing date interaction showed that early-May sowing resulted in the lowest weed cover in 2018 (**Table 6.12**).

Chlorophyll content (SPAD) was generally higher at flowering (GS50) than during leaf development and stem elongation (GS30 and GS40). Chlorophyll content at GS50 was significantly affected by sowing date and nitrogen fertilisation rate but not by year of cultivation, nitrogen source and genotype whereby it was significantly higher in the early-May than mid-April sowing date and increased with increasing nitrogen rate particularly at 150 kg N/ha (**Table 6.13**). Significant year × sowing date, year × nitrogen rate, year × genotype and sowing date × genotype interactions at GS50 showed that early-May sowing combined with application of 150 kg N/ha resulted in the highest chlorophyll content in 2018. The interactions also showed that whilst the genotype Cebelica had the highest chlorophyll content in 2018, Bamby had the highest chlorophyll content in the early-May sown plots (**Table 6.14 – 6.17**).

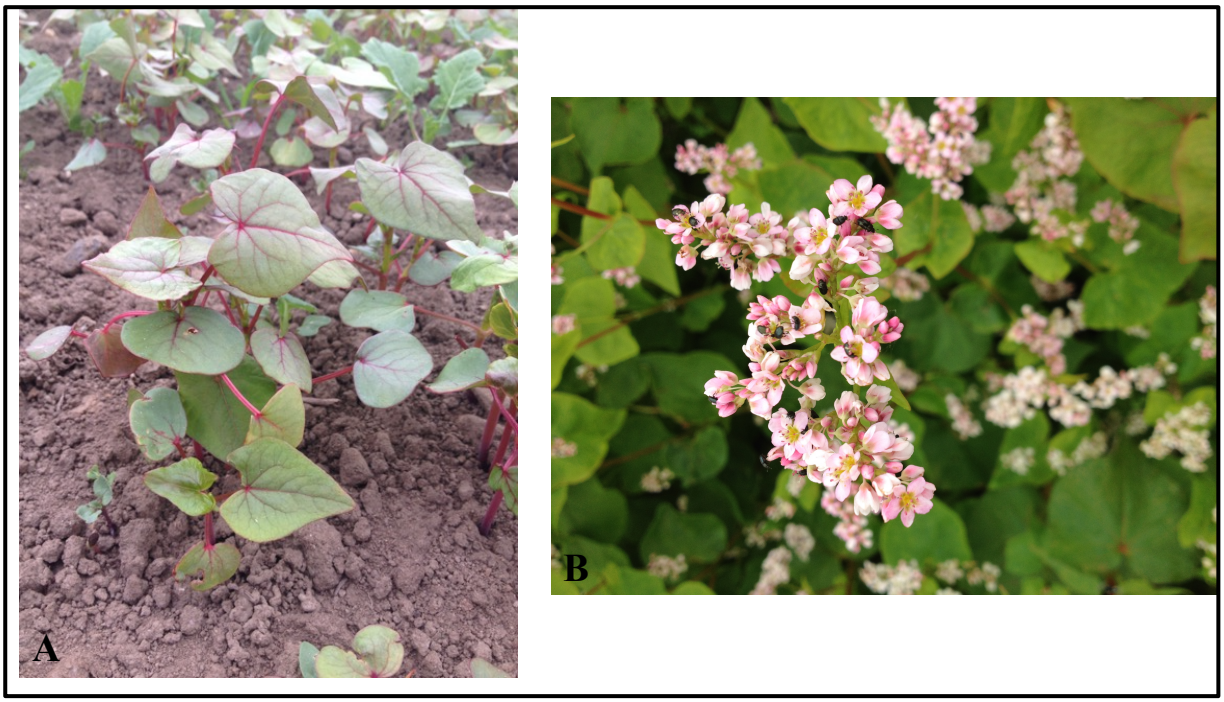

Fig. 6.1 Second leaf (A) and full flowering (B) stages of *Fagopyrum esculentum* Moench.

Fig. 6.2 Inflorescence and flowering stage of *Fagopyrum esculentum* Moench.

Crop biomass (NDVI) was significantly higher during leaf development (GS30) than stem elongation and flowering (GS40 and GS50). Crop biomass at GS50 was significantly affected by year of cultivation, sowing date and nitrogen rate whereby it was significantly higher in 2017 than 2018, in the early-May than mid-April sowing date and at higher nitrogen fertilisation rates (**Table 6.18**). A significant year × nitrogen rate interaction at GS50 showed that application of 150 kg N/ha resulted in the highest crop biomass in 2017 (**Table 6.19**).

Overall, both genotypes were relatively clean with little or no foliar disease identified. Intensity and severity of leaf and/or plant infection by powdery (*Erysiphe polygoni*) and downy mildew (*Peronospora ducumeti*) at GS60 was lower than 10% of the whole plant and approximately 5% of leaf area of young fully expanded leaves in all plots and so data is not presented. Nonetheless, it was higher in 2017 than 2018.

Table 6.3 Effects of year, sowing date, nitrogen rate, nitrogen source and genotype on % germination, plant height, total above-ground biomass of buckwheat and weed % of ground cover. Means followed by the same letter within each column within each trait are not significantly different at $p \leq 0.05$.

	Germination (%)	Seedling survival (%)	Height (cm)	Biomass (g/m^2)	Weed (% of ground cover)
Year (Y)					
2017	53.1±1.56	71.7±3.52	97.5±1.17	639.3±18.80	8.63±1.19
2018	43.5±1.15	86.9±2.59	74.0±1.02	292.5±9.21	100.0±0.00
Sowing date (S)					
Mid-April	58.8±1.35	76.3±2.10	79.4±0.98	419.6±15.65	40.6±3.25
Early-May	41.4±1.32	82.3±3.93	99.9±1.32	627.8±21.24	57.4±3.51
Nitrogen rate (R)					
Zero		83.5±3.94a	82.2±1.47b	419.0±17.76b	50.2±3.37a
75 kg/ha		73.9±2.04a	91.1±1.28ab	476.0±17.13ab	43.4±3.40a
150 kg/ha		78.7±3.54a	91.8±1.31a	623.2±22.25a	45.5±3.49a
Nitrogen source (T)					
Mineral N		78.7±3.54	91.8±1.31	623.2±22.25	45.5±3.49
Biogas digestate		81.1±2.84	93.5±1.34	576.5±19.88	48.5±3.40
Genotype (G)					
Cebelica	43.6±1.38	78.2±2.50	90.5±1.34	495.4±18.48	62.3±3.36
Bamby	53.0±1.44	80.4±3.71	80.6±1.36	440.0±21.11	63.0±3.33

Table 6.3 *continued ...*

	Germination (%)	Seedling survival (%)	Height (cm)	Biomass (g/m2)	Weed (% of ground cover)
ANOVA					
Year (Y)	**<0.001**	**0.012**	**<0.001**	**<0.001**	**<0.001**
Sowing date (S)	**<0.001**	ns	**<0.001**	**<0.001**	ns
Nitrogen rate (R)		ns	**<0.001**	**0.002**	*0.073*
Nitrogen source (T)		ns	ns	ns	ns
Genotype (G)	ns	ns	**0.036**	ns	ns
Y*S	ns	ns	**<0.001**	**<0.001**	**0.039**
Y*R		ns	ns	**0.005**	ns
Y*T		ns	ns	ns	ns
Y*G	**0.012**	**0.016**	ns	**0.046**	ns
S*R		ns	ns	**0.034**	ns
S*T		ns	ns	ns	ns
S*G	**0.011**	ns	ns	**0.034**	ns
R*T		ns	ns	ns	ns
R*G		ns	ns	ns	ns
T*G		ns	ns	ns	ns
Y*S*R	ns	ns	ns	ns	ns
Y*S*T	ns	ns	ns	ns	ns
S*R*T	ns	ns	ns	ns	ns
Y*S*G	ns	ns	ns	ns	ns
S*R*G	ns	ns	ns	ns	ns
R*T*G	ns	ns	ns	ns	ns
Y*S*R*T	ns	ns	ns	ns	ns
Y*S*R*G	ns	ns	ns	ns	ns
Y*S*R*T*G	ns	ns	ns	ns	ns

Table 6.4 Interaction between year and genotype on germination % of buckwheat. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Cebelica	Bamby
2017	52.1±1.68aA	54.4±1.60aA
2018	35.3±0.55bB	51.7±1.30aA

Table 6.5 Interaction between sowing date and genotype on germination % of buckwheat. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Cebelica	Bamby
Mid-April	48.6±1.68aB	65.7±1.10aA
Early-May	38.6±1.59bA	41.1±1.63bA

Table 6.6 Interaction between year and sowing date on plant height (cm) of buckwheat. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	mid-April	early-May
2017	84.0±0.87aB	110.2±0.70aA
2018	68.6±0.53bB	79.3±1.14bA

Table 6.7 Interaction between year and sowing date on total above-ground biomass (g/m^2) of buckwheat. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	mid-April	early-May
2017	496.4±21.23aB	789.4±19.45aA
2018	263.9±8.79bA	321.1±12.96bA

Table 6.8 Interaction between year and nitrogen rate on total above-ground biomass (g/m^2) of buckwheat. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Zero	75 kg/ha	150 kg/ha
2017	534.0±23.90aB	564.9±24.65aB	783.7±22.75aA
2018	243.7±10.44bA	318.5±13.36bA	288.6±7.03bA

Table 6.9 Interaction between sowing date and nitrogen rate on total above-ground biomass (g/m^2) of buckwheat. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Zero	75 kg/ha	150 kg/ha
Mid-April	255.8±12.50bC	315.9±10.74bBC	497.9±26.56aA
Early-May	521.9±24.08aA	567.5±25.69aA	574.4±29.30aA

Table 6.10 Interaction between year and genotype on total above-ground biomass (g/m^2) of buckwheat. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Cebelica	Bamby
2017	641.7±23.80aA	644.2±24.67aA
2018	349.1±11.38bA	235.9±8.79bB

Table 6.11 Interaction between sowing date and genotype on total above-ground biomass (g/m^2) of buckwheat. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Cebelica	Bamby
Mid-April	377.0±13.39bA	383.4±23.80aA
Early-May	613.9±25.21aA	496.7±27.20aA

Table 6.12 Interaction between year and sowing date on weed % of ground cover. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	mid-April	early-May
2017	10.9±1.64bA	3.00±0.13bB
2018	100.0±0.00aA	100.0±0.00aA

Table 6.13 Effects of year, sowing date, nitrogen rate, nitrogen source and genotype on chlorophyll content (SPAD) of buckwheat at GS30-50. Means followed by the same lowercase letter within each column within each factor and uppercase letter within each row within each trait are not significantly different at $p \leq 0.05$.

	SPAD (GS30)	SPAD (GS40)	SPAD (GS50)
Year (Y)			
2017	32.8±0.32B	29.6±0.26C	35.6±0.48A
2018	26.5±0.15C	39.4±0.44A	35.6±0.67B
Sowing date (S)			
Mid-April	32.4±0.41A	31.1±0.49A	31.6±0.57A
Early-May	29.0±0.21C	34.7±0.42B	39.5±0.34A
Nitrogen rate (R)			
Zero	30.6±0.32aA	29.0±0.36cA	31.9±0.54bA
75 kg/ha	30.5±0.35aB	32.5±0.48bA	33.2±0.51bA
150 kg/ha	30.9±0.38aC	36.1±0.45aB	39.4±0.46aA
Nitrogen source (T)			
Mineral N	30.9±0.38C	36.1±0.45B	39.4±0.46A
Biogas digestate	31.0±0.35C	34.0±0.44B	37.9±0.49A
Genotype (G)			
Cebelica	29.7±0.34C	34.4±0.47B	36.9±0.47A
Bamby	29.7±0.36B	35.0±0.52A	34.9±0.66A
ANOVA			
Year (Y)	**<0.001**	**<0.001**	ns
Sowing date (S)	**<0.001**	**<0.001**	**<0.001**
Nitrogen rate (R)	ns	**<0.001**	**<0.001**
Nitrogen source (T)	ns	**0.007**	ns
Genotype (G)	ns	ns	ns
Y*S	**<0.001**	ns	**0.002**
Y*R	**0.042**	**<0.001**	**0.004**
Y*T	ns	ns	ns
Y*G	ns	ns	**0.016**
S*R	ns	**<0.001**	ns
S*T	ns	ns	ns
S*G	ns	ns	**0.014**
R*T	ns	ns	ns

Table 6.13 ANOVA *continued...*

	SPAD (GS30)	SPAD (GS40)	SPAD (GS50)
R*G	ns	ns	ns
T*G	ns	ns	ns
Y*S*R	ns	*0.076*	**0.046**
Y*S*T	ns	ns	ns
Y*S*G	ns	ns	**0.038**
S*R*T	ns	ns	ns
S*R*G	ns	ns	ns
R*T*G	ns	ns	ns
Y*S*R*T	ns	ns	ns
Y*S*R*G	ns	ns	ns
Y*S*R*T*G	ns	ns	ns

Table 6.14 Interaction between year and sowing date on chlorophyll content of buckwheat at GS50. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	mid-April	early-May
2017	33.4±0.61aB	39.1±0.41bA
2018	29.8±0.82bB	41.5±0.37aA

Table 6.15 Interaction between year and nitrogen rate on chlorophyll content of buckwheat at GS50. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	zero	75 kg/ha	150 kg/ha
2017	32.1±0.60aB	31.9±0.50aB	41.9±0.29aA
2018	34.3±0.76aA	35.7±0.86aA	36.5±0.81bA

Table 6.16 Interaction between year and genotype on chlorophyll content of buckwheat at GS50. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Cebelica	Bamby
2017	36.0±0.58aA	36.4±0.58aA
2018	37.7±0.57aA	33.5±0.98aB

Table 6.17 Interaction between sowing date and genotype on chlorophyll content of buckwheat at GS50. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Cebelica	Bamby
Mid-April	33.7±0.59bA	29.4±0.82bB
Early-May	40.1±0.40aA	40.5±0.41aA

Table 6.18 Effects of year, sowing date, nitrogen rate, nitrogen source and genotype on crop biomass (NDVI) of buckwheat at GS30-50. Means followed by the same lowercase letter within each column within each factor and uppercase letter within each row within each trait are not significantly different at $p \leq 0.05$.

	NDVI (GS30)	NDVI (GS40)	NDVI (GS50)
Year (Y)			
2017	0.63±0.01A	0.55±0.01B	0.52±0.01C
2018	0.51±0.01A	0.46±0.01B	0.46±0.01B
Sowing date (S)			
Mid-April	0.53±0.01A	0.49±0.01B	0.48±0.01B
Early-May	0.61±0.0A1	0.53±0.01B	0.50±0.01C
Nitrogen rate (R)			
Zero	0.56±0.01aA	0.44±0.01bB	0.43±0.01cB
75 kg/ha	0.58±0.01aA	0.51±0.01aB	0.48±0.01bC
150 kg/ha	0.59±0.01aA	0.53±0.01aB	0.51±0.01aB
Nitrogen source (T)			
Mineral N	0.59±0.01A	0.53±0.01B	0.51±0.01B
Biogas digestate	0.56±0.01A	0.54±0.01AB	0.52±0.01B
Genotype (G)			
Cebelica	0.58±0.01A	0.51±0.01B	0.48±0.01C
Bamby	0.56±0.01A	0.51±0.01B	0.49±0.01B
ANOVA			
Year (Y)	**<0.001**	**<0.001**	**<0.001**
Sowing date (S)	**<0.001**	**<0.001**	**0.021**
Nitrogen rate (R)	ns	**<0.001**	**<0.001**
Nitrogen source (T)	ns	**0.007**	ns
Genotype (G)	ns	ns	ns
Y*S	**<0.001**	ns	ns
Y*R	ns	**0.028**	**0.027**

Table 6.18 ANOVA *continued...*

	NDVI (GS30)	NDVI (GS40)	NDVI (GS50)
Y*T	ns	ns	ns
Y*G	*0.063*	ns	ns
S*R	ns	ns	ns
S*T	ns	ns	ns
S*G	ns	ns	ns
R*T	ns	ns	ns
R*G	ns	ns	ns
T*G	ns	ns	ns
Y*S*R	ns	ns	ns
Y*S*T	ns	ns	ns
Y*S*G	ns	ns	ns
S*R*T	ns	ns	ns
S*R*G	ns	ns	ns
R*T*G	ns	ns	ns
Y*S*R*T	ns	ns	ns
Y*S*R*G	ns	ns	ns
Y*S*R*T*G	ns	ns	ns

Table 6.19 Interaction between year and nitrogen rate on crop biomass (NDVI) of buckwheat at GS50. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	zero	75 kg/ha	150 kg/ha
2017	0.46±0.01aC	0.49±0.00aB	0.56±0.00aA
2018	0.40±0.01bB	0.47±0.01aA	0.47±0.01bA

6.2.3. Yield and yield components

Biomass seed yield was significantly affected by year of cultivation, sowing date and nitrogen fertilisation rate. The average biomass seed yield was 1.02 t/ha across all treatments and was up to 48% higher in 2017 than 2018, 67% higher in the early-May than mid-April treatment and up to 15% higher at 150 kg N/ha compared with the zero-N application.

Combine seed yield was up to 38% higher in the early-May than mid-April sowing date and up to 30% higher at 150 kg N/ha relative to lower nitrogen rates (**Table 6.20**). A significant sowing × genotype interaction showed that the highest combine seed yield was obtained from Cebelica sown early-May (**Table 6.21**). Additionally, significant year × sowing date, year × nitrogen rate, year × genotype and sowing date × nitrogen rate interactions showed that the highest biomass seed yield was obtained from Bamby sown early-May with application of 150 kg N/ha in 2017 (data not shown).

The average HI was 0.23 across all treatments. There was a trend showing that HI was higher in the mid-April than early-May sown plots, with application of biogas digestate than mineral nitrogen and from Bamby than Cebelica (**Table 6.20**). Interestingly, HI was generally higher in the mid-April than early-May sown plots despite significantly lower seed yield. A significant sowing date × nitrogen rate interaction showed that the highest HI of 0.28 was obtained from the mid-April sown plots at zero-N application (**Table 6.22**).

All yield components, except thousand-grain weight (TGW), were significantly affected by year of cultivation, whereby plant number per m^2, cyme number and seed number were up to two times higher in 2017 than 2018. While plant number (plants/m^2) was not significantly different between sowing dates, all other yield components were significantly higher in the early-May than mid-April treatments. Early-May sowing resulted in 8% and 15-fold higher number of cymes/plant and cymes/m^2, respectively, two times higher number of seeds/plant, seeds/m^2 and 7% higher TGW compared with the mid-April sowing (**Table 6.23**).

Whilst the effect of nitrogen application rate was statistically significant only on cyme and seed number per plant, the effect of nitrogen source was significant only on cyme and seed number per m^2. Cyme and seed number per plant increased with increasing nitrogen rate at least by 19% whereas cyme and seed number per m^2 was up to 25% higher when biogas digestate was applied compared with mineral nitrogen. There was no difference between genotypes in yield components (**Table 6.23**).

Significant interaction effects on yield components were observed, especially the year × sowing date (plants/m^2, cymes/plant, seeds/m^2 and TGW), year × genotype (cymes/m^2, seeds/plant and seeds/m^2) and sowing date × nitrogen source (plants/m^2 and cymes/m^2) interactions (**Table 6.24 – 6.32**). The year × sowing date interactions indicated that early-May sowing date resulted in the highest number of plants/m^2, cymes/plant, seeds/m^2 and TGW in 2017 (**Table 6.24 – 6.27**) The year × genotype interactions indicated that the highest number of cymes/m^2, seeds/plant and seeds/m^2 was obtained from the genotype Bamby in 2017 (**Table 6.28 – 6.30**); The sowing

date × nitrogen source interactions indicated that while the mid-April sowing combined with application of biogas digestate at the rate of 150 kg N/ha resulted in the highest number of plants/m^2, early-May sowing combined with application of biogas digestate resulted in the highest number of cymes/m^2 (**Table 6.31** and **6.32**).

Table 6.20 Effects of year, sowing date, nitrogen rate, nitrogen source and genotype on seed yield and harvest index (HI) of buckwheat. Means followed by the same letter within each column within each trait are not significantly different at p≤0.05. Combine yield for 2018 is not presented (n.d.) because of complete seed loss due to desiccation with glyphosate.

	Biomass seed yield (t/ha)	Combine seed yield (t/ha)	HI
Year (Y)			
2017	1.41±0.07	1.09±0.04	0.23±0.01
2018	0.66±0.03	**n.d.**	0.24±0.01
Sowing date (S)			
Mid-April	0.83±0.04	0.90±0.03	0.24±0.01
Early-May	1.23±0.07	1.28±0.05	0.23±0.01
Nitrogen rate (R)			
Zero	0.87±0.04a	0.99±0.05b	0.24±0.01a
75 kg/ha	0.99±0.06a	0.93±0.05b	0.23±0.01a
150 kg/ha	0.98±0.05a	1.23±0.03a	0.20±0.01a
Nitrogen source (T)			
Mineral N	0.98±0.05	1.23±0.03	0.20±0.01
Biogas digestate	1.29±0.08	1.20±0.04	0.26±0.01
Genotype (G)			
Cebelica	0.97±0.05	1.17±0.04	0.21±0.01
Bamby	1.10±0.07	1.01±0.04	0.26±0.01
ANOVA			
Year (Y)	**<0.001**		ns
Sowing date (S)	**<0.001**	**<0.001**	*0.077*
Nitrogen rate (R)	ns	**0.007**	ns
Nitrogen source (T)	**0.003**	ns	*0.063*
Genotype (G)	ns	ns	*0.062*

Table 6.20 ANOVA *continued...*

	Biomass seed yield (t/ha)	Combine seed yield (t/ha)	HI
Y*S	**0.008**		ns
Y*R	**0.031**		ns
Y*T	ns		ns
Y*G	**0.002**		ns
S*R	**0.030**	ns	**0.020**
S*T	ns	ns	ns
S*G	ns	**0.004**	ns
R*T	ns	ns	ns
R*G	ns	ns	ns
T*G	ns	ns	ns
Y*S*R	ns		ns
Y*S*T	ns		ns
Y*S*G	**0.019**		ns
S*R*T	ns	ns	ns
S*R*G	ns	ns	ns
R*T*G	ns	ns	ns
Y*S*R*T	ns		ns
Y*S*R*G	ns		ns
Y*S*R*T*G	ns		ns

Table 6.21 Interaction between sowing date and variety on combine seed yield (t/ha) of buckwheat. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Cebelica	Bamby
mid-April	0.89±0.03aA	0.91±0.03aA
early-May	1.45±0.05aA	1.11±0.05aB

Table 6.22 Interaction between sowing date and nitrogen rate on harvest index (HI) of buckwheat. Means followed by the same letter are not significantly different at $p \leq 0.05$ by Tukey's test.

	Zero	75 kg/ha	150 kg/ha
mid-April	0.28±0.01aA	0.22±0.01aA	0.17±0.01aB
early-May	0.21±0.01bA	0.23±0.01aA	0.23±0.01aA

Table 6.23 Effects of year, sowing date, nitrogen rate, nitrogen source and genotype on plant, cyme and seed number and thousand-grain weight (TGW) of buckwheat. Means followed by the same lowercase letter within each column within each trait are not significantly different at $p \leq 0.05$.

	Plants/m^2	Cymes/plant	Cymes/m^2	Seeds/plant	Seeds/m^2	TGW (g)
Year (Y)						
2017	157.0±3.25	36.5±0.73	6560.9±202.33	360.2±14.41	64166.2±2955.04	21.5±0.16
2018	146.2±3.25	28.9±0.85	426.6±143.04	217.3±9.14	30808.8±1336.17	21.4±0.05
Sowing date (S)						
Mid-April	155.5±3.30	30.9±0.82	4797.9±205.83	238.2±11.03	39793.7±2040.16	20.7±0.09
Early-May	151.3±3.24	37.0±0.74	5789.6±201.53	339.3±14.49	55181.4±3135.49	22.2±0.10
Nitrogen rate (R)						
Zero – N	158.3±3.56a	28.6±0.65b	4810.1±221.18a	225.5±9.63b	39341.9±2066.53a	21.4±0.12a
75 kg N/ha	151.8±2.77a	35.1±0.85a	5363.6±233.52a	291.4±15.43a	46275.9±2626.39a	21.3±0.13a
150 kg N/ha	147.1±2.82a	35.5±0.81a	5035.0±213.50a	290.1±10.70a	44864.4±2260.63a	21.5±0.11a
Nitrogen source (T)						
Mineral N	147.1±2.82	35.5±0.81	5035.0±342.58	290.1±10.70	44864.4±2260.63	21.5±0.11
Biogas digestate	156.4±3.83	36.6±0.82	5966.3±261.48	347.9±15.83	59468.0±3508.44	21.6±0.10
Genotype (G)						
Cebelica	154.6±2.87	32.0±0.75	4921.6±181.67	287.6±11.21	43843.7±1950.19	21.8±0.12
Bamby	171.6±3.91	33.5±0.90	5665.8±227.34	289.8±15.69	51131.4±3304.41	21.2±0.11

Table 6.23 *continued...*

	Plants/m^2	Cymes/plant	Cymes/m^2	Seeds/plant	Seeds/m^2	TGW (g)
ANOVA						
Year (Y)	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	ns
Sowing date (S)	ns	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Nitrogen rate (R)	ns	**0.024**	ns	**0.012**	ns	ns
Nitrogen source (T)	*0.092*	ns	**0.033**	*0.082*	**0.019**	ns
Genotype (G)	ns	ns	ns	ns	Ns	ns
Y*S	**0.002**	**<0.001**	*0.089*	ns	**0.027**	**0.002**
Y*R	ns	**0.023**	ns	ns	ns	ns
Y*T	ns	ns	ns	ns	ns	ns
Y*G	ns	ns	**0.028**	**0.013**	**<0.001**	ns
S*R	ns	ns	ns	*0.083*	ns	ns
S*T	**0.000**	ns	**0.002**	ns	ns	ns
S*G	ns	ns	ns	ns	ns	ns
R*T	ns	ns	ns	ns	ns	ns
R*G	ns	ns	ns	ns	ns	ns
T*G	ns	ns	ns	ns	ns	ns
Y*S*R	ns	**0.018**	ns	**0.056**	ns	ns
Y*S*T	ns	ns	**0.032**	ns	ns	ns
Y*S*G	**0.002**	ns	ns	ns	**0.017**	ns
Y*R*G	ns	ns	ns	ns	**0.017**	ns
S*R*T	ns	ns	ns	ns	ns	ns
S*R*G	ns	ns	ns	ns	ns	ns
S*T*G	ns	ns	ns	ns	ns	ns
R*T*G	ns	ns	ns	ns	ns	ns
Y*S*R*T	ns	ns	ns	ns	ns	ns
Y*S*R*G	ns	ns	ns	ns	ns	ns
Y*S*T*G	ns	ns	ns	ns	ns	ns
Y*S*R*T*G	ns	ns	ns	ns	ns	ns

Table 6.24 Interaction between year and sowing date on plants/m^2 of buckwheat. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	mid-April	early-May
2017	174.8±3.91aA	185.1±4.10aA
2018	163.0±3.96aA	129.4±3.46bB

Table 6.25 Interaction between year and sowing date on cymes/plant of buckwheat. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	mid-April	early-May
2017	36.3±0.85aA	36.8±0.88aA
2018	20.3±0.39bB	37.5±0.94aA

Table 6.26 Interaction between year and sowing date on seeds/m^2 of buckwheat. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	mid-April	early-May
2017	52425.6±2234.78aB	75906.8±3244.80aA
2018	27161.7±943.84bA	34455.9±158836bA

Table 6.27 Interaction between year and sowing date on TGW (g) of buckwheat. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	mid-April	early-May
2017	20.4±0.12bB	22.5±0.14aA
2018	20.9±0.02aB	22.0±0.02bA

Table 6.28 Interaction between year and sowing date on cymes/m^2 of buckwheat. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Cebelica	Bamby
2017	5776.3±151.38aB	7345.6±161.01aA
2018	4067.0±118.08bA	3986.1±117.30bA

Table 6.29 Interaction between year and sowing date on seeds/plant of buckwheat. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Cebelica	Bamby
2017	332.8±11.95aA	387.7±16.34aA
2018	242.5±8.95bA	192.0±8.90bA

Table 6.30 Interaction between year and sowing date on seeds/m^2 of buckwheat. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Cebelica	Bamby
2017	53966.5±2092.56aB	74366.0±3418.98aA
2018	33720.8±1302.92bA	27896.8±1339.37bA

Table 6.31 Interaction between sowing date and nitrogen source on plants/m^2 of buckwheat. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Mineral N	Biogas digestate
mid-April	140.7±3.056aB	172.7±5.63aA
early-May	153.5±4.72bA	140.2±4.75bB

Table 6.32 Interaction between sowing date and nitrogen source on panicles/m^2 of buckwheat. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Mineral N	Biogas digestate
mid-April	4631.4±205.48bB	5967.8±264.45aA
early-May	5698.9±201.37aA	5311.2±239.67bA

6.2.4. Data from 2017 only

The following results came from a separate analysis based on the four genotypes (Bamby, Cebelica, Zamira and Zita) which were grown in 2017 only with the aim to evaluate the potential of Zamira and Zita which were obtained from the Czech Republic and compare with Bamby and Cebelica. Since the results showed similar magnitude and direction of year, sowing date and nitrogen fertilisation effects, this section focuses on the variation between genotypes.

There were no significant differences in seed germination %, above-ground biomass and ability to suppress weeds with the exception that Cebelica and Zamira were the tallest genotypes (**Table 6.33**). Chlorophyll content was not significantly different at GS30-40 but at GS50 Bamby and Cebelica had higher chlorophyll content than Zamira and Zita (**Table 6.34**). Crop biomass was not significantly different over the vegetative period (GS30-50) (**Table 6.35**) nor was there a significant difference with respect to combine seed yield and HI (**Table 6.36**).

There was a trend showing that Cebelica and Zamira produced higher yields than Bamby and Zita in combine seed yield. Biomass seed yield was higher than combine seed yield (1.31 vs 1.13 t/ha) (**Table 6.36**). A significant sowing date × genotype interaction showed that the highest combine seed yield of 1.78 t/ha was obtained from the genotype Zamira sown in early-May. Yield components were not significantly different among genotypes except plant number for which Bamby had the highest number of plants/m^2 at harvest. Nonetheless, the results indicated that yield components (except plant number and TGW) significantly increased with increasing nitrogen rate but they were not significantly different in response to nitrogen type (**Table 6.37**).

Table 6.33 Effects of sowing date, nitrogen rate, nitrogen source and genotype on % germination, plant height, total above-ground biomass of buckwheat and weed % of ground cover in 2017. Means followed by the same letter within each column within each trait are not significantly different at $p \leq 0.05$.

	Germination (%)	Seedling survival (%)	Height (cm)	Biomass (g/m^2)	Weed (% of ground cover)
Sowing date (S)					
Mid-April	62.7±1.71	77.0±2.18	84.8±1.06	497.4±19.28	10.9±1.68
Early-May	44.4±1.78	70.0±3.40	110.3±0.65	781.2±19.43	2.84±0.12
Nitrogen rate (R)					
Zero		79.1±2.94a	88.9±1.70c	506.7±21.88b	16.9±2.53a
75 kg/ha		69.2±1.67a	97.7±1.38a	554.8±20.76b	5.58±0.61b
150 kg/ha		73.4±3.25a	101.6±1.13a	790.5±20.61a	3.90±0.26b
Nitrogen source (T)					
Mineral N		73.4±3.25	101.6±1.13	790.5±20.61	3.90±0.26
Biogas digestate		72.3±2.08	101.9±1.20	705.2±20.46	7.30±0.75
Genotype (G)					
Cebelica	52.1±2.06a	63.4±1.42a	101.2±1.54a	641.7±23.80a	8.36±1.44a
Bamby	54.4±1.96a	80.1±4.70a	92.9±1.35c	644.2±24.67a	9.18±1.45a
Zamira	55.6±2.01a	66.6±1.52a	99.1±1.47a	623.2±23.10a	7.91±1.71a
Zita	50.3±1.67a	83.9±2.20a	96.8±1.33bc	648.1±21.45a	9.09±1.30a

Table 5.33 *continued...*

	Germination (%)	Seedling survival (%)	Height (cm)	Biomass (g/m^2)	Weed (% of ground cover)
ANOVA					
Sowing date (S)	**<0.001**	ns	**<0.001**	**<0.001**	**0.002**
Nitrogen rate (R)		ns	**<0.001**	**<0.001**	**0.017**
Nitrogen source (T)		ns	ns	*0.072*	ns
Genotype (G)	ns	ns	**0.015**	ns	ns
S*R		ns	**0.032**	ns	**0.056**
S*T		ns	ns	ns	ns
S*G	ns	ns	ns	**0.034**	ns
R*T		ns	ns	ns	ns
R*G		ns	ns	ns	ns
T*G		ns	ns	ns	ns
S*R*T		ns	ns	ns	ns
S*R*G		ns	ns	ns	ns
R*T*G		ns	ns	ns	ns

Table 5.34 Effects of sowing date, nitrogen rate, nitrogen source and genotype on chlorophyll content (SPAD) of buckwheat at GS30-50 in 2017. Means followed by the same lowercase letter within each column within each trait and same uppercase letter within each row within each factor are not significantly different at $p \leq 0.05$.

	SPAD (GS30)	SPAD (GS40)	SPAD (GS50)
Sowing date (S)			
Mid-April	35.5±0.38A	28.0±0.32C	32.6±0.57B
Early-May	30.1±0.21	31.2±0.27B	38.6±0.34A
Nitrogen rate (R)			
Zero	32.2±0.38aA	27.4±0.30cA	30.6±0.60bA
75 kg/ha	32.7±0.37aB	28.5±0.29bA	32.0±0.45bA
150 kg/ha	33.5±0.39aC	32.3±0.29aB	40.9±0.34aA
Nitrogen source (T)			
Mineral N	33.5±0.39B	32.3±0.29B	40.9±0.34A
Biogas digestate	33.0±0.41B	30.2±0.26B	38.9±0.35A
Genotype (G)			
Cebelica	32.7±0.43aB	29.8±0.28aC	36.0±0.58aA
Bamby	33.1±0.40aB	30.3±0.37aC	36.4±0.58aA
Zamira	33.2±0.32aB	28.8±0.30aC	34.5±0.60bA
Zita	32.4±0.40aB	29.5±0.34aC	35.4±0.60abA
ANOVA			
Sowing date (S)	**<0.001**	**<0.001**	**<0.001**
Nitrogen rate (R)	ns	**<0.001**	**<0.001**
Nitrogen source (T)	ns	**0.003**	**0.016**
Genotype (G)	ns	ns	ns
S*R	ns	ns	**<0.001**
S*T	ns	ns	ns
S*G	ns	*0.065*	ns
R*T	ns	ns	ns
R*G	ns	ns	ns
T*G	ns	ns	ns
S*R*T	ns	ns	ns
S*R*G	ns	ns	ns
R*T*G	ns	ns	ns

Table 6.35 Effects of sowing date, nitrogen rate, nitrogen source and genotype on crop biomass (NDVI) of buckwheat at GS30-50 in 2017. Means followed by the same lowercase letter within each column within each factor and same uppercase letter within each row within each trait are not significantly different at p≤0.05.

	NDVI (GS30)	NDVI (GS40)	NDVI (GS50)
Sowing date (S)			
Mid-April	0.54±0.01A	0.53±0.01A	0.50±0.01B
Early-May	0.70±0.00A	0.56±0.00B	0.54±0.00C
Nitrogen rate (R)			
Zero	0.62±0.01aA	0.47±0.01cB	0.47±0.00cB
75 kg/ha	0.62±0.01aA	0.54±0.01bB	0.50±0.00bC
150 kg/ha	0.63±0.01aA	0.59±0.00aB	0.56±0.00aC
Nitrogen source (T)			
Mineral N	0.63±0.01A	0.59±0.00B	0.56±0.00C
Biogas digestate	0.62±0.01A	0.58±0.00B	0.55±0.00C
Genotype (G)			
Cebelica	0.65±0.01aA	0.56±0.01aB	0.52±0.00aC
Bamby	0.61±0.01aA	0.54±0.01aB	0.51±0.01aC
Zamira	0.62±0.01aA	0.55±0.00aB	0.52±0.00aC
Zita	0.60±0.01aA	0.53±0.01aB	0.53±0.01aB
ANOVA			
Sowing date (S)	**<0.001**	**<0.001**	**0.021**
Nitrogen rate (R)	ns	**<.001**	**<0.001**
Nitrogen source (T)	ns	ns	**0.004**
Genotype (G)	ns	ns	ns
S*R	ns	*0.068*	ns
S*T	ns	ns	ns
S*G	ns	ns	ns
R*T	ns	ns	ns
R*G	ns	ns	ns
T*G	ns	ns	ns
S*R*T	ns	ns	ns
S*R*G	ns	ns	ns
R*T*G	ns	ns	ns

Table 6.36 Effects of sowing date, nitrogen rate, nitrogen source and genotype on seed yield and harvest index (HI) of buckwheat in 2017. Means followed by the same letter within each column within each trait are not significantly different at p≤0.05.

	Biomass seed yield (t/ha)	Combine seed yield (t/ha)	HI
Sowing date (S)			
Mid-April	1.09±0.05	0.88±0.03	0.24±0.01
Early-May	1.57±0.06	1.36±0.06	0.21±0.01
Nitrogen rate (R)			
Zero	1.04±0.05c	0.94±0.05b	0.23±0.01a
75 kg/ha	1.28±0.06b	0.99±0.05b	0.24±0.01a
150 kg/ha	1.41±0.06a	1.28±0.04a	0.19±0.01a
Nitrogen source (T)			
Mineral N	1.41±0.06	1.28±0.04	0.19±0.01a
Biogas digestate	1.59±0.07	1.26±0.06	0.24±0.01a
Genotype (G)			
Cebelica	1.20±0.05a	1.17±0.04a	0.21±0.00a
Bamby	1.63±0.08a	1.01±0.04a	0.26±0.01a
Zamira	1.19±0.05a	1.31±0.07a	0.21±0.00a
Zita	1.32±0.06a	0.99±0.04a	0.21±0.00a
ANOVA			
Sowing date (S)	**<0.001**	**<0.001**	**0.007**
Nitrogen rate (R)	**<0.000**	**0.005**	ns
Nitrogen source (T)	**0.002**	ns	ns
Genotype (G)	ns	*0.082*	ns
S*R	ns	ns	*0.069*
S*T	ns	ns	*0.069*
S*G	ns	**0.004**	ns
R*T	ns	ns	ns
R*G	ns	ns	ns
T*G	ns	ns	ns
S*R*T	ns	ns	ns
S*R*G	ns	ns	ns
R*T*G	ns	ns	ns

Table 6.37 Effects of sowing date, nitrogen rate, nitrogen source and genotype on plant, cyme number, seed number and thousand-grain weight (TGW) of buckwheat in 2017. Means followed by the same lowercase letter within each column within each trait are not significantly different at $p \leq 0.05$.

	Plants/m^2	Cymes/plant	Cymes/m^2	Seeds/plant	Seeds/m^2	TGW (g)
Sowing date (S)						
Mid-April	151.7±4.07	36.2±0.89	5489.1±203.02	323.9±13.02	48827.6±2346.75	22.1±0.18
Early-May	162.3±3.87	36.8±0.90	5934.3±191.20	404.3±113.33	65153.6±2717.77	24.4±0.23
Nitrogen rate (R)						
Zero	164.1±4.23a	29.4±0.63b	4849.8±175.36b	258.7±9.66b	43710.5±2095.10c	23.2±0.23a
75 kg N/ha	152.9±3.37a	39.3±0.86a	5983.8±176.51a	272.6±14.64b	56107.3±2477.13a	22.9±0.25a
150 kg N/ha	150.4±3.81a	38.5±0.94a	5751.3±186.48a	402.7±12.40a	59840.7±2302.50b	23.5±0.23a
Nitrogen source (T)						
Mineral N	150.4±3.81	38.5±0.94	5751.3±186.48	402.7±12.40	59840.7±2302.50	23.5±0.23
Biogas digestate	160.6±4.51	38.6±0.84	6262.3±232.22	422.5±12.92	68304.0±3162.13	23.3±0.22
Genotype (G)						
Cebelica	167.0±3.52b	34.7±0.79a	5776.3±185.40a	332.8±11.95a	53966.5±2092.56a	21.9±0.16a
Bamby	192.9±4.17a	38.4±0.90a	7345.6±197.19a	387.7±16.34a	74366.0±3418.98a	21.1±0.16a
Zamira	131.1±2.81c	34.4±0.94a	4431.0±133.90a	357.8±13.46a	46259.4±1787.48a	25.4±0.17a
Zita	137.0±2.76c	38.3±0.91a	5294.3±179.64a	378.3±12.34a	53370.7±2355.06a	24.5±0.18a

Table 6.37 *continued...*

	Plants/m^2	Cymes/plant	Cymes/m^2	Seeds/plant	Seeds/m^2	TGW (g)
ANOVA						
Sowing date (S)	ns	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Nitrogen rate (R)	ns	**<0.001**	**0.034**	**<0.001**	**0.008**	ns
Nitrogen source (T)	ns	ns	ns	ns	ns	ns
Genotype (G)	ns	ns	ns	ns	ns	ns
S*R	**0.022**	ns	ns	**0.048**	ns	ns
S*T	**0.014**	ns	**0.038**	ns	ns	ns
S*G	ns	ns	ns	ns	ns	ns
R*T	ns	ns	ns	ns	ns	ns
R*G	ns	ns	ns	ns	ns	ns
T*G	ns	ns	ns	ns	ns	ns
S*R*T	ns	ns	ns	ns	ns	ns
S*R*G	ns	ns	ns	ns	ns	ns
S*T*G	**0.016**	ns	*0.070*	ns	ns	ns
R*T*G	ns	ns	ns	ns	ns	ns

6.2.5. Grain Quality (data from 2017 only)

On average across all treatments, buckwheat had grain concentrations of: 10.7% protein, 29, 51 and 103 mg/kg of Zn, Mn and Ca, 7.4 and 3.2 mg/g of K and Mg, 4460, 2925 and 1337 µg/g of total polyphenols, antioxidants and flavonoids, (**Table 6.38, 6.39, 6.44**).

Grain quality of buckwheat, with the exception of protein, Fe and Ni, was significantly affected by sowing date. With respect to minerals, early-May sowing resulted in two times higher concentration of macronutrients (Al, Ca, K, Mg, Na, S and P) and micronutrients (Cd, Cu, Mn, Mo and Zn) (**Table 6.38, 6.39**). With respect to the bioactive compounds, early-May sowing significantly increased grain concentration of total polyphenols by 15% but decreased concentration of total antioxidants and flavonoids by at least 7-fold (**Table 6.44**).

The effect of nitrogen fertilisation rate was significant on grain protein, Mn, P, S and total polyphenols. Concentration of protein, Mn and S significantly increased whereas concentration of P and total polyphenols decreased with increasing nitrogen rate (**Table 6.38, 6.39, 6.44**). Only the concentration of S was significantly higher when mineral nitrogen was applied compared to biogas digestate. The concentration of Ca was significantly different between genotypes whereby Bamby had the highest grain Ca concentration (**Table 6.39**).

A significant sowing date × nitrogen source interaction showed that early-May sowing combined with application of mineral nitrogen at the rate of 150 kg/ha resulted in the highest grain concentration of K, Mg and P. A significant nitrogen source × genotype interaction showed that the highest grain concentration of Zn of 38.9 mg/kg was obtained from the genotype Zita with application of biogas digestate at 150 kg/ha. A significant sowing date × genotype interaction showed that the highest grain concentration of total antioxidants was obtained from the genotype Cebelica sown mid-April (**Table 6.40 – 6.43, 6.45**)

Table 6.38 Effects of sowing date, nitrogen rate, nitrogen source and genotype on protein and ash content of buckwheat in 2017. Means followed by the same letter within each column within each trait are not significantly different at $p \leq 0.05$.

	Protein (%)	Ash (%)
Sowing date (S)		
Mid-April	10.8±0.54	2.1±0.01
Early-May	10.8±0.09	2.4±0.02
Nitrogen rate (R)		
Zero	9.9±0.12b	2.3±0.02a
75 kg/ha	10.3±0.12b	2.3±0.02a

Table 6.38 *continued...*

	Protein (%)	Ash (%)
150 kg/ha	11.0±0.09a	2.2±0.03a
Nitrogen source (T)		
Mineral N	11.0±0.09	2.2±0.03
Biogas digestate	12.1±1.51	2.3±0.03
Genotype (G)		
Cebelica	12.1±0.77a	2.3±0.02a
Bamby	10.5±0.10a	2.3±0.02a
Zamira	10.2±0.08a	2.2±0.02a
Zita	10.6±0.09a	2.3±0.02a
ANOVA		
Sowing date (S)	ns	**<0.001**
Nitrogen rate (R)	**<0.001**	ns
Nitrogen source (T)	ns	ns
Genotype (G)	ns	ns
S*R	ns	*0.088*
S*T	ns	ns
S*G	ns	ns
R*T	ns	ns
R*G	ns	ns
T*G	ns	ns
S*R*T	ns	ns
S*R*G	ns	ns
R*T*G	ns	ns
S*R*T*G	ns	ns

Table 6.39 Effects of sowing date, nitrogen rate, nitrogen source and genotype on concentrations of minerals of buckwheat in 2017. Means followed by the same lowercase letter within each column within each trait are not significantly different at $p \leq 0.05$.

	Al	Ca	Cd	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	P	S	Zn
	(mg/kg)					(mg/g)		(mg/kg)				(mg/g)		(mg/kg)
Sowing date (S)														
Mid-April	13.7	67.9	0.08	6.89	201.2	4.48	2.00	25.7	0.38	26.6	1.66	3.15	1.33	19.1
Early-May	139.3	142.2	0.20	13.1	206.9	10.4	4.32	77.1	0.77	51.1	1.95	7.80	2.83	37.9
Nitrogen rate (R)														
Zero	73.0a	106.9a	0.15a	9.73a	122.2a	7.38a	3.12a	46.2b	0.53a	41.3a	1.76a	5.64a	1.99b	30.6a
75 kg N/ha	80.6a	111.4a	0.13a	10.6a	169.2a	7.70a	3.24a	49.3a	0.61a	34.2a	2.28a	5.76a	2.07b	33.0a
150 kg N/ha	70.7a	94.9a	0.12a	9.23a	107.3a	6.93a	3.12a	52.5a	0.59a	45.2a	1.68a	5.02b	2.18a	25.2a
Nitrogen source (T)														
Mineral N	70.7	94.9	0.12	9.23	107.3	6.93	3.12	52.5	0.59	45.2	1.68	5.02	2.18	25.2
Biogas digestate	81.5	104.8	0.15	10.3	406.8	7.49	3.12	55.8	0.55	35.2	1.57	5.34	2.08	25.1
Genotype (G)														
Cebelica	69.1a	98.2b	0.13a	10.0a	281.4a	7.26a	3.14a	51.0a	0.53a	37.0a	1.84a	5.54a	2.08a	30.3a
Bamby	84.4a	110.9a	0.15a	9.92a	124.0a	7.49a	3.15a	51.0a	0.62a	40.7a	1.78a	5.33a	2.08a	26.3a
Zamira	71.3a	96.0bc	0.12a	10.2a	167.2a	7.64a	3.09a	49.0a	0.51a	35.7a	1.91a	5.45a	2.05a	29.3a
Zita	82.3a	94.5c	0.12a	10.3a	224.0a	7.63a	3.18a	53.0a	0.53a	33.7a	2.18a	5.64a	2.09a	33.5a

Table 6.39 *continued...*

	Al	Ca	Cd	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	P	S	Zn
ANOVA														
Sowing date (S)	**<0.001**	**<0.001**	**<0.001**	**<0.001**	ns	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	ns	**<0.001**	**<0.001**	**<0.001**
Nitrogen rate (R)	ns	*0.069*	ns	ns	ns	*0.070*	ns	**0.037**	ns	ns	ns	**0.032**	**<0.010**	ns
Nitrogen source (T)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**0.015**	ns
Genotype (G)	ns	**0.052**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
S*R	ns	ns	ns	ns	ns	*0.066*	ns	*0.080*	ns	ns	ns	ns	ns	ns
S*T	ns	ns	ns	ns	ns	**0.048**	**0.053**	ns	ns	ns	ns	**0.021**	ns	ns
S*G	ns	*0.076*	ns	ns	ns	ns	*0.060*	ns	ns	ns	ns	**0.044**	ns	ns
R*T	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
R*G	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
T*G	ns	ns	ns	*0.077*	ns	ns	ns	ns	ns	ns	ns	ns	ns	**0.042**
S*R*T	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
S*R*G	ns	*0.080*	ns	*0.057*	ns	**0.054**	ns	ns	ns	ns	ns	ns	ns	ns
S*T*G	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**0.015**
R*T*G	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
S*R*T*G	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table 6.40 Interaction between sowing date and nitrogen source on K concentration (mg/g) of buckwheat in 2017. Means followed by the same letter are not significantly different at $p \leq 0.05$ by Tukey's test.

	Mineral N	Biogas digestate
mid-April	4.17±0.18bA	4.98±0.08bA
early-May	10.3±0.09aA	10.1±0.08aA

Table 6.41 Interaction between sowing date and nitrogen source on Mg concentration (mg/g) of buckwheat in 2017. Means followed by the same letter are not significantly different at $p \leq 0.05$ by Tukey's test.

	Mineral N	Biogas digestate
mid-April	1.92±0.06bB	2.04±0.01bA
early-May	4.31±0.04aA	4.12±0.04aA

Table 6.42 Interaction between sowing date and nitrogen source on P concentration (mg/g) of buckwheat in 2017. Means followed by the same letter are not significantly different at $p \leq 0.05$ by Tukey's test.

	Mineral N	Biogas digestate
mid-April	2.87±0.12bB	3.39±0.06bA
early-May	7.72±0.09aA	7.32±0.10aA

Table 6.43 Interaction between nitrogen source and genotype on Zn concentration (mg/kg) of buckwheat in 2017. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Cebelica	Bamby	Zamira	Zita
Mineral N	29.1±2.46aB	20.6±2.01aC	22.2±1.31bC	38.9±2.07aA
Biogas digestate	28.2±2.06aB	22.0±1.52aC	38.6±2.18aA	29.9±1.91bB

Table 6.44 Effects of sowing date, nitrogen rate, nitrogen source and genotype on grain concentrations of total polyphenols, antioxidants and flavonoids of buckwheat in 2017. Means followed by the same letter within each column within each trait are not significantly different at $p \leq 0.05$.

	Polyphenols (µg/g)	Antioxidants (µg/g)	Flavonoids (µg/g)
Sowing date (S)			
Mid-April	4076.5±46.54	5201.5±38.32	2657.7±84.19
Early-May	4844.3±103.45	648.0±12.69	17.0±1.59
Nitrogen rate (R)			
Zero – N	4745.3±73.65a	2910.8±201.83a	1352.1±129.72a
75 kg/ha	4499.4±96.43a	2986.2±211.75a	1405.0±140.87a
150 kg/ha	4146.5±93.86a	2869.9±204.86a	1200.0±116.04a
Nitrogen source (T)			
Mineral N	4146.5±93.86	2869.9±204.86	1200.0±116.04
Biogas digestate	4450.4±76.86	2932.0±207.17	1392.3±142.45
Genotype (G)			
Cebelica	4593.3±108.65a	3057.9±217.48a	1396.8±138.99a
Bamby	4497.7±102.01a	2849.3±200.86a	1157.2±112.37a
Zamira	4434.1±72.16a	2849.9±199.04a	1503.1±144.19a
Zita	4316.7±56.23a	2941.7±207.43a	1292.3±131.84a

Table 6.44 *continued...*

	Polyphenols (µg/g)	Antioxidants (µg/g)	Flavonoids (µg/g)
ANOVA			
Sowing date (S)	**0.013**	**<0.001**	**<0.001**
Nitrogen rate (R)	**0.048**	Ns	ns
Nitrogen source (T)	ns	Ns	ns
Genotype (G)	ns	*0.077*	ns
S*R	ns	ns	ns
S*T	ns	ns	ns
S*G	ns	**0.023**	ns
R*T	ns	ns	ns
R*G	ns	ns	ns
T*G	ns	ns	ns
S*R*T	ns	ns	ns
S*R*G	*0.066*	ns	ns
R*T*G	ns	ns	ns
S*R*T*G	ns	ns	ns

Table 6.45 Interaction between sowing date and genotype on grain concentration of total antioxidants (μg/g) of buckwheat in 2017. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Cebelica	Bamby	Zamira	Zita
mid-April	5469.8±22.58aA	5046.7±51.11aB	5052.0±31.72aB	5237.4±32.30aB
early-May	646.1±16.96bA	652.0±17.29bA	647.7±6.59bA	646.1±6.82bA

6.2.6. Correlation coefficients

Correlation tests indicated that plant height, chlorophyll content, crop and especially total above-ground biomass, showed a strong positive correlation with grain yield. The tests also indicated that grain yield and grain Zn concentration showed a strong negative correlation with total antioxidants and flavonoids (**Table 6.46 – 6.48**).

Table 6.46 Correlation coefficients for growth traits of buckwheat in 2017. Stars in the shaded area represent significance at p<0.05. Significance codes: 0.001 '***', 0.01 '**', 0.05 '*', ns 'not significant'.

	Height	SPAD-GS50	NDVI-GS50	Biomass	Yield	HI
Height	1.00	***	***	***	***	ns
SPAD-GS50	**0.37**	1.00	***	***	**	ns
NDVI-GS50	**0.37**	**0.33**	1.00	***	***	ns
Biomass	**0.79**	**0.31**	**0.46**	1.00	***	***
Yield	**0.47**	**0.42**	**0.45**	**0.43**	1.00	ns
HI	-0.17	0.37	0.37	**-0.26**	-0.14	1.00

Table 6.47 Correlation coefficients for yield traits of buckwheat in 2017. Stars in the shaded area represent significance at p<0.05. Significance codes:', 0.001 '***', 0.01 '**', 0.05 '*', ns 'not significant'.

	Plants/m^2	Cymes/m^2	Seeds/m^2	Biomass	TGW	Yield	HI
Plants/m^2	1.00	***	***	***	ns	ns	ns
Cymes/m^2	**0.60**	1.00	***	***	ns	ns	***
Seeds/m^2	**0.47**	**0.84**	1.00	***	**	ns	***
Biomass	**0.35**	**0.53**	**0.68**	1.00	***	***	***
TGW	0.03	0.16	**0.20**	**0.30**	1.00	***	ns
Yield	0.20	0.22	0.19	**0.43**	**0.46**	1.00	ns
HI	0.16	**0.39**	**0.42**	**-0.26**	-0.01	-0.14	1.00

Table 6.48 Correlation coefficients for quality traits of buckwheat in 2017. Stars in the shaded area represent significance at p<0.05. Significance codes: 0.001 '***', 0.01 '**', 0.05 '*', ns 'not significant'.

	Yield	Protein	Fe	Zn	Phenolics	Antioxidants	Flavonoids
Yield	1.00	ns	ns	ns	ns	***	***
Protein	0.01	1.00	ns	ns	ns	ns	ns
Fe	0.03	-0.02	1.00	ns	ns	ns	ns
Zn	0.22	-0.01	0.03	1.00	***	***	***
Phenolics	-0.02	-0.07	-0.17	**0.55**	1.00	*	ns
Antioxidants	**-0.39**	0.08	-0.86	**-0.53**	**-0.26**	1.00	***
Flavonoids	**-0.40**	-0.04	-0.05	**-0.47**	-0.22	**0.89**	1.00

6.3.Discussion

6.3.1. Crop growth

Crop growth was influenced particularly by year of cultivation, sowing date and nitrogen rate whereas differences between genotypes were only significant with respect to plant height. Overall, there was better crop growth in terms of total biomass and seed yield with delayed sowing from mid-April to early-May and increasing nitrogen rate.

The average seed germination of 53% across all treatments was very low in comparison with many arable crops and is likely due to the very small seed. Year of cultivation and sowing date were the major factors affecting germination % over the two years of the trial. Seed germination % in 2018 declined compared with that of 2017 (53.1 vs 43.5%) and in the early-May compared with the mid-April sowing date (58.8 vs. 41.4%). These results are consistent with the study by Sakata and Ohsawa (2006) which found an average seed germination of buckwheat ranging from 20.4 to 57.4% sown in June, July and August in Japan. The present results are also consistent with the results of the previous experiment in 2016 (**Chapter 5**) where the average seed germination was 50 – 60% with at least 10% lower seed germination in the early-May than mid-April sown plots. Seed germination % declined in response to a later sowing date most likely due to environmental factors such as temperature and water availability. Firstly, although temperatures over the germination period were higher in 2018 than 2017, seed germination % was higher in 2017 compared with 2018. Secondly, temperatures below or close to zero degrees Celsius were observed only over the 15-day germination period of the early sowing date (mid-April) and yet seed germination % was always higher from the early than the late sowing. On average across the two years, 10 and 18 mm of total rain occurred during the germination

periods in the early (April 12th – 27th) and late (May 2nd – 23rd) sowings, respectively. Although temperature was higher at the late compared with early sowing, seed germination % was always higher at the early sowing. However, higher seed germination % in the mid-April sowing did not correlate with better overall crop growth nor crop yield but in fact the reverse occurred. Therefore, limited water availability towards the end of the germination period and especially during the late sowing (i.e. early-May) combined with relatively high temperature, leading to faster drying soil are likely to have been key factors limiting the seed germination of buckwheat under the local agroecological conditions.

The growth cycle of buckwheat was approximately 170 days long in the present study with about 100 days from pre-flowering to physiological maturity. This growth cycle is longer than shown in previous studies (Vazhov *et al*., 2013; Ghiselli *et al*., 2016; Siracusa *et al*., 2017) which observed growth cycles shorter than 110 days long, most likely because those studies were carried out in countries with much higher temperatures throughout the vegetative and reproductive growth stages than in NE-England.

In the present study, the entire growth cycle was 15 days shorter in 2018 than 2017 and in the early-May than mid-April treatments. Whilst a shorter growth cycle resulted in higher yields from the late than early sown plots, a shorter growth cycle in 2018 resulted in much lower yields than 2017. This raises the question of whether duration of growth cycle is a critical factor for yield of buckwheat because the present results suggest that whilst a shorter growth cycle was positively associated with some crop growth traits (chlorophyll content and crop biomass), it did not always result in higher yields. While the difference in the duration of the growth cycle between sowing dates was likely due to higher photosynthetic activity at the late than early sowing (based on SPAD data), the difference between years was probably due to the effect of the long dry spell in the summer of 2018 which had a significant effect on the yields of many UK arable crops (DEFRA, 2019). The drought period accompanied by spells of high temperature in the summer of 2018 could have impaired growth and development of buckwheat due to shallow rooting and hence high drought susceptibility (Woo *et al*., 2010). Therefore, shallow rooting combined with sub-optimal hydrological and thermal conditions played key roles in determining growth and development of buckwheat grown in NE-England in 2017 and 2018.

The average plant height was 90 cm and it was significantly influenced by year of cultivation, sowing date, nitrogen rate and genotype. The increase in plant height with increasing nitrogen rate is consistent with the typical response of crops to N fertiliser (Wang *et al*., 2015b). This is

supported by the SPAD data which showed that chlorophyll content (SPAD) was higher with increasing nitrogen rate. Adequate nitrogen availability resulted in high photosynthetic activity which is likely to contribute to increased cell division and expansion during the vegetative period (stem elongation). There were significant differences between genotypes in plant height in that Cebelica was the tallest genotype but these differences were not linked to SPAD data which showed no differences in chlorophyll content (SPAD) between genotypes. This was most likely due to crop genetics.

With respect to above-ground biomass production, the average was approximately 550 g/m^2 across all treatments, with the highest 644 g/m^2 produced in 2017. There was a significantly higher above-ground biomass production in 2017 and in the early-May sowing date combined with application of mineral N at 150 kg/ha reflected in increased leaf area, stem diameter (visual observation hence data not shown) and plant height which is supported by the SPAD and NDVI data. Most importantly, total above-ground biomass correlated strongly and positively with seed yield. These results suggest that low temperature and mid-April sowing are limiting factors for buckwheat biomass production in NE-England. However, the results did not fully support this suggestion because seedling survival (from low temperature or frost kill) was not significantly different (76 vs 82%, respectively) between early and late sowing except between 2017 and 2018 (72 vs 87%, respectively).

Therefore, although germination % was relatively low, delaying sowing from April to May combined with higher nitrogen rate (150 kg N/ha) provides the optimal conditions for growth of buckwheat in the present study. Nonetheless, the long growth-cycle may increase seed shattering due to indeterminate growth habit and restrict seed maturation later in the season due to low temperatures and relatively high rainfall.

6.3.2. Yield and yield components

The average biomass seed yield was 1.04 t/ha obtained from two genotypes (Cebelica and Bamby) across all treatments over two years (2017 and 2018) whereas the combine seed yield was 1.09 t/ha over one year (2017) because of complete seed loss after desiccation in 2018. These results were similar to the average global yield and that of buckwheat grown in Canada and the USA (0.91, 1.04 t/ha, respectively) but higher than the average seed yield of buckwheat in Asia, Africa and Europe (0.89, 0.85 and 0.90 t/ha, respectively) grown in 2010-2011 (Kalinova and Vrchtova, 2011; Popović *et al*., 2014; Ghiselli *et al*., 2016; 2017; Siracusa *et al*., 2017). The present results are, nonetheless, similar to those published recently (FAOSTAT,

2019) for the average global buckwheat yield and that of Europe, the USA and China (0.96, 1.08, 1.05 and 0.84 t/ha respectively) over the period 2013 – 2017.

In the present study, delaying sowing from April to May and increasing nitrogen rate improved combine seed yield by 30% which was attributed to improved yield components (cyme number, seed number and TGW). The relatively dry and warm period at the end of July and throughout August in 2017, coinciding with the flowering and grain setting/filling growth stage (GS50 – GS60), enhanced a rapid seed development in the early-May sowing treatment likely due to higher photosynthetic activity and distribution of photoassimilates rather than simply due to longer thermal time, whereas the reverse occurred for the mid-April sowing date treatment, resulting in lower grain yield.

Combine seed yield was higher in the early-May than mid-April sowing date (1.36 vs 0.88), which represented an increase of 38% in seed yield. Differences in seed yield with respect to sowing date were also published by previous studies (Jung *et al*., 2015; Mariotti *et al*., 2016) carried out under different experimental conditions and bigger time difference between sowing date treatments. Jung *et al*. (2015) compared the response of two buckwheat genotypes sown in spring (25th March – 10th April) and summer (15th August – 1st September) in the Republic of Korea in 2013 and observed a 33% yield increase by sowing in summer compared to spring but could not determine the optimal sowing date because of contrasting performance of the genotypes. Mariotti *et al*. (2016) assessed the response of buckwheat sown in spring (8th April – 27th May) and summer (3rd – 4th September) under rainfed and irrigated conditions in Italy in 2012-2013 and observed approximately 40% yield reduction by sowing in summer relatively to early spring but when compared with late spring sowing they observed up to 50% yield increase, suggesting that yield reduction at the late summer sowing date treatment was due to an interaction between adverse photothermal conditions and water stress.

The increase in seed yield by delaying sowing from April to May observed in the present study was associated with an increase of TGW, seed number (seeds/plant and seeds/m^2) and cyme number (cymes/plant and cymes/m^2). As indicated before, improvement of TGW, seed number and cyme number are generally related to better growth and development due to higher production and distribution of photoassimilates resulting from a higher photosynthetic activity, often linked to photothermal quotient. Indeed, the results showed that chlorophyll content (SPAD) was higher in the early-May than mid-April sowing, indicating that there was a higher photosynthetic activity with delaying sowing from April to May. Thus, the results indicate that the improvement of TGW, seed number (seeds/plant and seeds/m^2) and cyme number

(cymes/plant and cymes/m^2) was a contributory factor to the higher seed yield obtained in the early-May compared to mid-April sowing date treatment. The results also indicated that TGW (21.2 vs 19.4 g) and cyme number per plant (33.5 vs 28.1) were higher than or similar to those obtained from the same genotype Bamby grown in Italy published by previous studies (Ghiselli *et al*., 2016; 2017). Nonetheless, mid-April sowing could have resulted in lower yields than early-May sowing also partly because of higher seed losses due to late harvest dates.

Seed yield increased in response to higher N rate but was not affected by nitrogen source. The effect of nitrogen rate on biomass seed yield depended on year of cultivation and sowing date. Combine seed yield increased by up to 38% at the rate of 150 kg N/ha relative to zero-N. Nonetheless, it was inconclusive whether 150 kg N/ha would be the optimal nitrogen rate for buckwheat because previous studies (Erley *et al*., 2005; Sobhani *et al*., 2014) obtained similar or much higher seed yields with lower nitrogen rates. Erley *et al*. (2005) obtained seed yields of 1.3 – 1.5 t/ha for buckwheat grown in Germany in 1994-1995 but did not find significant differences among nitrogen rates (0, 30 and 60 kg N/ha); Sobhani *et al*. (2014) obtained seed yields of 2.0 – 2.3 t/ha for buckwheat genotypes grown in Iran in 2011, with significant differences among nitrogen rates (0, 50, 100 and 150 kg N/ha) and obtained the highest seed yield at the rate of 50 kg N/ha. Therefore, it would be important to test whether application of higher rates than 150 kg N/ha would result in higher yields of buckwheat grown in NE-England.

Seed yield was not significantly different among genotypes although the biomass and combine seed yield obtained from Bamby and Zamira in the present study was up to 1.63 and 1.31 t/ha, respectively. Contrary to a previous study carried in Serbia in 2010 and 2011 (Popović *et al*., 2014) showing that seed yield obtained from Bamby and Cebelica was significantly different, the present study did not find significant differences between these 2 varieties in seed yield.

6.3.3. Grain quality

Whilst there is some information on growth and yield traits of buckwheat in response to sowing date, information on grain quality traits is rather limited. Overall, the protein content in the present study was 1-2% lower than for values published elsewhere (Siracusa *et al*., 2017); nonetheless, it was within the cited range of 11 – 19% for buckwheat protein content (Guo *et al*., 2007; Zhang *et al*., 2012b). Differences in protein content of buckwheat in response to sowing time were reported in some previous studies: Sobhani *et al*. (2014) showed that protein content of buckwheat grown in Iran was up to 1% higher in the late (July-August) than early (June-July) sowing date. Siracusa *et al*. (2017) found that the protein content of buckwheat grown in Italy was 0.2% higher in the late (May) than early (September) sown plots and

observed that the protein content in the late sowing treatment was enhanced by high water availability supplied by irrigation. However, in the present study sowing date did not affect protein content of buckwheat significantly.

The results indicated that delaying sowing from April to May resulted in higher grain concentration of minerals, with a close relationship between grain concentration of minerals and seed yield. This was probably due to higher uptake and translocation from the uptake sites into the developing seeds or more likely due to a dilution effect.

In particular, grain concentration of Zn in the present study was similar to that of published by Zhu (2016). However, the present results also indicated a relatively high concentration of heavy metals such as Cd, Cu and Ni. For example, grain concentration of Cd in the present study (0.13 mg/kg) was just a little higher than the maximum amount allowed by the European law No. 220/2004 of 0.10 mg/kg and higher than that obtained from the same genotype Bamby grown in Slovakia (0.15 vs 0.05 mg/kg) published by Vollmannová *et al*. (2013). On the other hand, overall grain concentration of minerals was generally higher than those of historical and modern wheat varieties (Murphy *et al*. 2008). For example, the present study found higher grain concentrations of Fe than the historical and modern wheat varieties (193 vs 35.7 and 32.3 mg/kg, respectively); the present study also found higher grain concentration of Mg and P (3 and 5 mg/g, respectively) than those of the historical and modern wheat varieties (1 and 4 mg/g, respectively).

Previous studies have shown that grain mineral content of buckwheat varies substantially between common buckwheat (*Fagopyrum esculentum* Moench.) and tartary buckwheat (*Fagopyrum tataricum* Gaertn.) species. Bonafaccia *et al*. (2003) found that mineral accumulation is generally higher in tartary than common buckwheat. The present study used common buckwheat species and detected concentrations of micronutrients such as Zn and Fe higher than the concentrations reported by Bonafaccia *et al*. (2003) yet lower than those of tartary buckwheat. However, the present study also showed that the concentration of minerals (except heavy metals) detected in common buckwheat species was higher than the mean values for tartary buckwheat published by Huang *et al*. (2014), thus suggesting that these genotypes could be mineral-dense with potential health benefits.

The average concentration of total polyphenols was 4460 μg GA/g flour DW across all treatments, which was consistent with the range of values published in some previous studies (Vollmannová *et al*., 2013; Mir *et al*., 2018). Although Siracusa *et al*. (2017) found that concentration of most phenolic compounds in buckwheat was generally higher when sown in

September than in May in Italy, the overall effect of sowing time was not statistically significant. In contrast, the present results showed a significant variation in the concentration of total polyphenols in relation to sowing time, for which there was 15% increase by delaying the sowing from April to May. This result is consistent with a previous study (Mariotti *et al*., 2017) which found that delaying sowing time generally increased the concentration of phenolic compounds. Mariotti *et al*. (2017) suggested that the concentration of phenolic compounds such as rutin and quercetin decreased with increasing age of buckwheat plants, which could be linked to a dilution effect due to increased yields, suggesting that plant age could be contributory factor to the higher concentration of total phenolic compounds. However, the present results can only support the suggestion that dilution effect (due to higher yield) was higher at the late sowing compared with early sowing date treatment as crops reached the same stage of maturity regardless of sowing date.

Total antioxidant capacity plays key roles in the reduction of protein and lipid peroxidation parameters and therefore it is an important indicator of nutritional quality (Giménez-Bastida and Zielinski, 2015). Several studies observed a strong positive correlation between total polyphenol content and total antioxidant capacity in buckwheat (Vollmannová *et al*., 2013; Giménez-Bastida and Zielinski, 2015). However, the present study showed that increasing concentration of total polyphenols correlated negatively with the concentration of total antioxidants and total flavonoids in response to late sowing, which was probably indicative of defence responses to oxidative stresses or increased susceptibility to oxidative cell damage (Giménez-Bastida and Zielinski, 2015) associated with abiotic stress conditions such as drought, high temperature or hyperaccumulation of heavy metals. Indeed, the present study showed that grain concentration of all minerals was generally higher in the early-May sowing date, which could have induced a stress tolerance response by increasing the production of phenolic compounds. Alternatively, it could be simply indicative of significantly higher antiperoxidative activity in the phenolic rather than the flavanol fractions of the grains in the late sowing date, whereas the reverse was true in the early sowing date. Nonetheless, it would be important to measure individual antioxidants and markers of oxidative cell damage for a better understanding and definite conclusions.

CHAPTER 7 – Effects of Sowing Date, Zinc Fertilisation and Genotype on Growth, Yield and Quality of Quinoa (*Chenopodium quinoa* Willd.) in 2016

7.1. Introduction

Quinoa is classified into various ecotypes depending on its origin, sensitivity to photoperiod and temperature. However, its agroecological suitability and productivity is mainly determined by the time (and factors that control the time) to flowering and physiological maturity. Previous studies have shown that quinoa can adapt to a diverse range of environments, thus showing a high plasticity in phenology and duration of growth cycle according to latitudinal and longitudinal gradients which ultimately play key roles in determining yield potential and phenotype (Curti *et al*., 2016).

In England, quinoa is a relatively new crop. It is only the British Quinoa group (https://www.britishquinoa.co.uk), a group of farmers around the Midlands, that have started the production and supply of quinoa during the last 5-10 years. Farmers generally do not grow quinoa because of the cool and wet weather, as the crop originates in South America with much higher summer temperatures. Cultivars with short growth cycle (approximately 150 days to harvest) have the potential for cultivation in the UK as shown by the British Quinoa group. Therefore, determining the environmental and management factors that control the phenological development and productivity is key to predicting the suitability of this crop to the NE-England agroecological conditions.

The consumption of quinoa in the UK has increased significantly in recent years and is mainly being supplied by imports from Bolivia and Peru. The grain yield and grain quality are important in determining the potential and suitability to be able to grow this crop in the UK providing potential for UK growers who currently operate very intensive cereal-based cropping rotations. Considering the high plasticity in phenology, it is expected that quinoa genotypes will show large variation and high sensitivity to the local weather conditions.

Although quinoa is considered an important source of mineral nutrients, genetic variation in Zn concentrations may exist. To our knowledge, there is limited information about quinoa cultivation in the UK. Therefore, the aim of this experiment was to:

- Identify quinoa genotypes suited to NE-England, and
- Evaluate how productivity and quality of quinoa can be affected by sowing date, and Zn fertilisation.

7.2. Results

7.2.1. Weather data

The average monthly temperature was 12°C and there was a relatively homogeneous distribution of rainfall with a total average of 57.2 mm over the entire growth cycle. Assuming 15 days of germination time from sowing, minimum temperature was near or below zero degree Celsius for mid-April sowing date whereas for early-May sowing date minimum temperature was 2.7 and the maximum 19.1°C. Whilst rainfall increased significantly towards the end of the germination period for the mid-April sowing date, the germination period for early-May sowing was characterised by limited water availability.

7.2.2. Crop growth

The length of the growth cycle varied significantly between genotypes and sowing date. Atlas and Jessie differed by up to 21 days in the duration from GS50 (flowering) through to GS80 (maturity) (data not shown). The growth cycle of Jessie varied between 150 and 170 days whereas the growth cycle of Atlas varied between 175 and 190 days (early-May and mid-April sowings respectively). Differences in growth duration between both genotypes were more evident from GS40 to GS80 with both genotypes requiring approximately 45 – 60 days to go from GS40 to GS80.

Table 7.1 Summary of weather conditions (average monthly temperature, solar radiation and cumulative monthly rainfall) from sowing to harvest of quinoa in 2016.

	Temperature (°C)	Rainfall (mm)	Solar radiation (W/m^2)
April	6.2	50.8	132.5
May	10.6	21.4	162.1
June	12.7	88.0	175.2
July	15.1	63.6	188.6
August	15.2	66.4	162.3
September	14.7	52.8	104.7
October	9.8	57.4	51.3

Table 7.2 Weather conditions (minimum and maximum temperature and total rainfall) over the germination period of quinoa for the early and late sowing date (white and shaded area, respectively) in 2016.

Date	Min (°C)	Max (°C)	Rain (mm)
19/04	2.0	10.7	0.0
20/04	-0.1	14.7	0.0
21/04	1.6	15.3	0.2
22/04	1.1	9.1	0.4
23/04	0.1	8.8	0.0
24/04	1.3	8.7	0.4
25/04	1.5	7.6	1.0
26/04	1.0	7.0	9.4
27/04	0.4	7.7	0.8
28/04	-0.6	6.9	8.8
29/04	0.3	7.6	1.4
30/04	0.9	10.9	1.8
1/05	2.1	12.6	0.6
2/05	6.4	13.9	1.0
3/05	4.4	13.1	0.0
4/05	6.2	14.7	0.0
5/05	8.1	16.5	0.0
6/05	6.0	13.8	0.0
7/05	7.3	14.2	0.0
8/05	7.3	18.2	0.0
9/05	5.5	14.8	0.2
10/05	8.1	14.8	0.0
11/05	9.4	15.2	0.0
12/05	8.6	15.0	0.2
13/05	6.9	19.1	0.0
14/05	2.7	11.9	0.0
15/05	2.7	12.2	0.0
16/05	3.3	12.9	0.0
17/05	4.6	17.4	0.6
18/05	9.1	16.9	0.0

Seed germination was on average 76% of seed sown across both sowing dates and genotypes (**Table 7.3**). Sowing date had a significant effect (p<0.001) on seed germination with the early-May sowing resulting in 35% lower seed germination than the mid-April sowing. The difference between genotypes was not statistically significant with both genotypes having germination rates > 70%. A significant sowing date ×genotype interaction (p<0.001) on seed germination was detected, which indicated that Jessie had the highest seed germination in the mid-April sown plots (**Table 7.4**). There was block-to-block variation with respect to seed germination wherein seed germination in Blocks 2 and 4 was higher (86.4% and 79.8%, respectively) than in Blocks 1 and 3 (64.6% and 74.6%, respectively). The effect of foliar Zn application was not significant on plant height, total above-ground biomass and wee % of

ground cover. The average plant height was 71 cm across all treatments and genotypes. Plant number at harvest was only significantly different between genotypes, whereby Jessie had a higher plant number than Atlas (154 vs 115 plants/m^2) (**Table 7.3**).

Chlorophyll content in the leaves over the vegetative period (GS20-50) ranged between 33 and 42 SPAD units. The chlorophyll content was significantly different between genotypes at GS40 (p=0.03) and GS50 (p=0.04) with Jessie showing higher mean values than Atlas, but the difference between sowing dates was not statistically significant (**Table 7.3**). There was a significant sowing date × genotype interaction (p=0.01) on chlorophyll content, with the highest mean chlorophyll content detected in Jessie sown in mid-April.

Overall, both genotypes were relatively clean regarding the presence of diseases. There was some evidence of downy mildew (*Peronospora variabilis* Gaum, formerly called *Peronospora farinos*a Fr.) at GS60 but the intensity and severity of infection, whether at leaf or whole plant level (lower than 10%), was negligible. Weed severity was high and covered at least 50% of the area in each plot (**Table 7.3**) with predominance of fat-hen, black-bindweed, chickweed, orache and wild oat (data not shown). There were no significant differences between genotypes in the ability to suppress weeds nor was there a statistically significant difference between sowing dates. The high weed levels are likely due to the non-use of herbicide in these trials with limited products registered for use on quinoa in the UK**.**

7.2.3. Yield and yield components

The overall combine grain yield of quinoa across all treatments was 0.71 t/ha with a harvest index (HI) of 0.31 and thousand-grain weight (TGW) of 2.5 g. Sowing date and Zn application did not significantly affect grain yield and yield components but there were significant differences between genotypes with respect to grain yield (p=0.014) and seed number (p=0.025). Grain yield and seed number of Atlas was 3-4 times higher than that of Jessie (**Table 7.5**). No significant interactions were detected.

Table 7.3 Effects of, sowing date, zinc fertilisation and genotype on % germination, plant height, total above-ground biomass, chlorophyll content (GS20-50) of quinoa and weed % of ground cover in 2016.

	Germination (%)	Height (cm)	Biomass (g/m^2)	Weed (% of ground cover)	Chlorophyll content (SPAD-units)		
					GS30	GS40	GS50
Sowing date (S)							
Mid-April	93.9±2.44	69.2±4.32	436.4±42.96	52.4±4.03	41.4±0.64	35.2±0.66	35.7±0.61
Early-May	58.7±2.35	73.9±4.93	552.2±54.25	42.1±4.57	40.9±0.13	35.2±0.32	34.8±0.37
Zinc (Zn)							
+ Zn		76.6±4.56	561.2±43.78	40.6±4.21			
- Zn		66.4±4.56	427.5±52.90	53.9±4.26			
Genotype (G)							
Atlas	70.2±4.58	86.7±4.96	599.3±59.12	49.2±5.20	40.6±0.61	33.4±0.39	34.0±0.45
Jessie	82.5±2.93	56.3±1.79	389.3±27.96	45.3±3.40	41.7±0.19	36.9±0.42	36.5±0.47
ANOVA							
Sowing date (S)	**<0.001**	ns	ns	ns	ns	ns	ns
Zinc (Zn)		ns	ns	ns			
Genotype (G)	ns	**0.028**	ns	ns	ns	**0.038**	*0.061*
S * Zn		ns	ns	ns			
S * G	**<0.001**	ns	ns	ns	ns	**<0.001**	**0.019**
G * Zn		ns	ns	ns			
S * Zn * G		ns	ns	ns			

Table 7.4 Interaction between sowing date and genotype on germination % of quinoa grown in 2016. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey test.

Sowing date	Atlas	Jessie
Mid-April	92.4±3.01aA	95.6±1.86aA
Early-May	48.0±0.91bA	69.5±1.67bA

7.2.4. Grain Quality

Protein, ash and mineral content

The effect of foliar Zn application was not significant on grain quality. Foliar Zn application did not increase grain Zn concentrations. There was no significant effect of treatments on protein and mineral content, except on grain concentrations of Mo (**Table 7.6, Table 7.7**). There was a trend indicating that grain concentrations of Zn decreased with delayed sowing. Grain concentrations of Mo increased with foliar Zn application and was higher in Jessie than Bamby but with no significant difference between sowing dates. The results also indicated a significant sowing date × genotype interaction (p=0.02) on grain Zn concentration showing that the highest value of 40 mg/kg was obtained in Atlas when sown mid-April (**Table 7.9**, and **7.10**).

Total polyphenols, antioxidants and flavonoids

Only the effect of sowing date was statistically significant whereby late sowing increased grain concentrations of total polyphenols by up to 12.1% but decreased the concentrations of total antioxidants and flavonoids by up to 98% and 74-fold, respectively (**Table 7.8**). The average grain concentrations across all treatments and genotypes was 2361.8, 1245.9 and 412.3 µg/g for total polyphenols, antioxidants and flavonoids, respectively.

Results showed a significant sowing date × genotype (p=0.044) interaction on grain total polyphenols and total flavonoid concentrations, (**Table 7.11**). The interactions showed that the highest concentration of total polyphenols of 2818.7 µg GA/g DW was obtained from late sowing i.e. early-May without foliar Zn application whereas the highest concentration of total flavonoids of 1184.8 µg Catechin/g was obtained from Atlas sown mid-April.

Table 7.5 Effects of, sowing date, zinc fertilisation and genotype on seed yield, thousand-grain weight (TGW), harvest index (HI), seed number and plant number at harvest of quinoa in 2016.

	Plant number/m^2	Seed number/plant	Seed number/m^2	TGW (g)	Seed yield (t/ha)		HI
					Biomass sample	Combine	
Sowing date (S)							
Mid-April	133.6±6.94	583.8±91.56	70701.2±10495.40	2.49±0.05	1.82±0.29	0.62±0.09	0.29±0.04
Early-May	135.4±7.03	827.6±117.89	110284.0±14897.39	2.52±0.06	2.90±0.40	0.79±0.11	0.33±0.03
Zinc (Zn)							
+ Zn	135.6±7.02	825.0±99.14	101125.8±11910.63	2.53±0.05	2.61±0.32	0.75±0.10	0.33±0.04
- Zn	135.4±6.95	586.4±111.77	79859.4±14449.86	2.47±0.06	2.11±0.39	0.66±0.11	0.29±0.04
Genotype (G)							
Atlas	115.3±5.72	1068.9±108.93	127348.8±14010.01	2.52±0.05	3.34±0.38	1.10±0.10	0.31±0.03
Jessie	153.8±6.34	342.4±51.07	53636.4±8447.48	2.59±0.06	1.38±0.22	0.31±0.03	0.30±0.04
ANOVA							
Sowing date (S)	ns	ns	*0.096*	ns	*0.095*	ns	ns
Zinc (Zn)	ns	ns	ns	ns	ns	ns	ns
Genotype (G)	*0.055*	**0.025**	*0.056*	ns	*0.054*	**0.014**	ns
S * Zn	ns	ns	ns	ns	ns	ns	ns
S * G	ns	ns	ns	ns	ns	ns	ns
G * Zn	ns	ns	ns	ns	ns	ns	ns
S * Zn * G	ns	ns	ns	ns	ns	ns	ns

Table 7.6 Effects of, sowing date, zinc fertilisation and genotype on protein and ash content of quinoa in 2016.

	Protein (%)	Ash (%)
Sowing date (S)		
Mid-April	12.5±0.13	3.6±0.10
Early-May	12.9±0.18	3.9±0.11
Zinc (Zn)		
+ Zn	12.2±0.12	3.8±0.11
- Zn	12.2±0.12	3.8±0.12
Genotype (G)		
Atlas	13.2±0.15	3.8±0.09
Jessie	12.2±0.12	3.8±0.13
ANOVA		
Sowing date (S)	ns	ns
Zinc (Zn)	ns	ns
Genotype (G)	**0.032**	ns
S * Zn	ns	ns
S * G	ns	ns
G * Zn	ns	ns
S * Zn * G	ns	ns

Table 7.7 Effects of, sowing date, zinc fertilisation and genotype on mineral concentrations of quinoa grown in 2016.

	Ca	K	Mg	P	S	Al	Cd	Cu	Fe	Mn	Mo	Na	Ni	Zn
	(%)					(mg/kg)								
Sowing date (S)														
Mid-April	0.12	0.98	0.24	0.55	0.16	91.6	0.09	9.0	136.7	26.5	0.49	116.6	2.3	36.8
Early-May	0.10	0.99	0.25	0.57	0.16	73.9	0.08	9.8	104.3	28.2	0.50	64.3	3.7	31.4
Zinc (Zn)														
+ Zn	0.12	0.99	0.24	0.54	0.16	126.2	0.09	8.3	157.6	28.2	0.59	123.4	3.2	34.2
- Zn	0.11	0.98	0.25	0.57	0.16	44.0	0.08	10.3	89.1	26.4	0.44	69.6	2.6	34.5
Genotype (G)														
Atlas	0.11	1.07	0.25	0.58	0.17	32.7	0.07	10.1	107.5	26.1	0.39	84.2	2.0	34.6
Jessie	0.11	0.89	0.24	0.54	0.16	138.3	0.09	8.7	137.8	28.6	0.61	104.7	3.9	34.1
ANOVA														
Sowing date (S)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*0.070*
Zinc (Zn)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*0.050*	ns	ns	ns
Genotype (G)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**0.030**	ns	ns	ns
S * Zn	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
S * G	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**0.020**
G * Zn	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
S * Zn * G	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table 7.8 Effects of, sowing date, zinc fertilisation and genotype on the concentration of total polyphenols, total antioxidants and total flavonoids of quinoa in 2016.

	Polyphenols (µg GA/g)	Antioxidants (µg TE/g)	Flavonoids (µg Catch/g)
Sowing date (S)	2134.8±128.47	2261.1±156.40	813.6±130.82
Mid-April	2588.8±117.11	230.7±19.23	11.0±1.97
Early-May			
Zinc (Zn)	2179.1±76.68	1131.8±166.69	226.0±42.09
+ Zn	2179.1±76.68	1131.8±166.69	226.0±42.09
- Zn			
Genotype (G)			
Atlas	2544.5±159.84	1360.0±254.74	598.6±154.17
Jessie	2179.1±76.68	1131.8±166.69	226.0±42.09
ANOVA			
Sowing date (S)	**0.025**	**<0.001**	**<0.001**
Zinc (Zn)	ns	ns	ns
Genotype (G)	ns	ns	ns
S * Zn	**0.036**	ns	ns
S * G	ns	ns	**0.044**
G * Zn	ns	ns	ns
S * Zn * G	ns	ns	ns

Table 7.9 Interaction between sowing date and genotype on Zn concentration (mg/kg) of quinoa in 2016. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey test.

Sowing date	**Atlas**	**Jessie**
Mid-April	40.4±1.53aA	33.1±1.69aA
Early-May	28.0±1.09bA	35.5±1.05aA

Table 7.10 Interaction between sowing date and zinc fertilisation on the concentration of total polyphenols (µg/g) of quinoa in 2016. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey test.

Sowing date	**+ Zn**	**- Zn**
Mid-April	2324.1±177.86aA	1945.6±34.29bA
Early-May	2358.8±142.94aB	2818.7±71.97aA

Table 7.11 Interaction between sowing date and genotype on the concentration of total flavonoids (µg/g) of quinoa in 2016. Means followed by the same lowercase letter within the column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey test.

Sowing date	**Atlas**	**Jessie**
Mid-April	1184.8±162.41aA	442.3±21.20aB
Early-May	12.4±2.25bA	9.6±1.76bA

7.2.5. Correlation coefficients

Correlation tests indicated that grain yield showed a strong positive and negative correlation with total above-ground biomass and chlorophyll content, respectively. The tests also indicated that grain Zn concentration showed a strong negative correlation with protein and total polyphenols (**Table 7.12 – 7.14**).

Table 7.12 Correlation coefficients for growth traits of quinoa in 2016. Stars in the shaded area represent significance at p<0.05. Significance codes: 0.001 '***', 0.01 '**', 0.05 '*', ns 'not significant'.

	Height	SPAD-GS50	Biomass	Yield	HI
Height	1.00	**	***	***	ns
SPAD-GS50	**-0.49**	1.00	*	**	ns
Biomass	**0.89**	**-0.41**	1.00	***	ns
Yield	**0.88**	**-0.48**	**0.80**	1.00	ns
HI	0.17	-0.28	0.15	0.24	1.00

Table 7.13 Correlation coefficients for yield traits of quinoa in 2016. Stars in the shaded area represent significance at p<0.05. Significance codes: 0.001 '***', 0.01 '**', 0.05 '*', ns 'not significant'.

	Plants/m^2	Seeds/m^2	Biomass	TGW	Yield	HI
Plants/m^2	1.00	ns	ns	Ns	ns	ns
Seeds/m^2	0.09	1.00	***	*	***	***
Biomass	0.15	**0.80**	1.00	*	***	ns
TGW	0.18	**0.45**	**0.44**	1.00	**	*
Yield	-0.19	**0.78**	**0.80**	**0.46**	1.00	ns
HI	0.16	**0.58**	0.15	**0.40**	0.24	1.00

Table 7.14 Correlation coefficients for quality traits of quinoa in 2016. Stars in the shaded area represent significance at p<0.05. Significance codes: 0.001 '***', 0.01 '**', 0.05 '*', ns 'not significant'.

	Yield	Protein	Fe	Zn	Phenols	Antioxidants	Flavonoids
Yield	1.00	ns	ns	ns	ns	ns	ns
Protein	0.30	1.00	ns	*	*	ns	ns
Fe	-0.05	0.00	1.00	ns	ns	ns	ns
Zn	-0.26	**-0.40**	-0.17	1.00	**	ns	ns
Phenols	0.13	**0.40**	0.24	**-0.49**	1.00	ns	ns
Antioxidants	-0.02	-0.20	0.22	0.05	0.10	1.00	***
Flavonoids	0.00	-0.09	0.23	0.02	0.26	**0.86**	1.00

7.3. Discussion

7.3.1. Crop growth

Sowing date

The effect of sowing date was only statistically significant on seed germination. On average, mid-April sowing resulted in germination rates higher than those from the early-May sowing, with averages of 95 and 60%, respectively. Differences in seed germination between sowing dates could be attributed probably to decreasing soil moisture content during the germination period for late i.e. early-May sowing date while differences in seedling growth are likely due to a short period without rain that occurred from May 26th to June 11th. This period without rainfall might have impaired seedling growth for crops from the late sowing i.e. early-May such that there were fewer plants per square meter compared with the mid-April sowing. In fact, as is generally the case for all arable crops, temperature and soil moisture are the key factors limiting seed germination and seedling growth. Nonetheless, since plant count was carried out on May 17th (same date for both sowing dates), this could be the reason early-May resulted in lower % seed germination assuming it may have not reached the full germination %. However, providing optimal conditions (i.e. moisture and temperature), it has been shown that quinoa seeds can germinate two days after sowing. Therefore, the most likely reason could be that seeds indeed germinated with cotyledons emerging from the soil surface but died at the seedling stage before germination assessment or radicles and hypocotyl emerged from the seeds but did not emerge through the soil surface (Sosa-Zuniga *et al*., 2017). Early-May sowing date not only resulted in lower % seed germination, it also resulted in lower plant number at harvest. However, the survival rate increased with early-May sowing date compared with mid-April sowing date.

The effects of temperature, water and salinity on quinoa seed germination have been investigated by several studies (Bois *et al.,* 2006; Adolf *et al.,* 2013; Hirich *et al.,* 2014; Ceccato *et al.,* 2015). In particular, some studies (Boero *et al.,* 2000; Bois *et al.,* 2006) concluded that the optimal temperature for maximum quinoa seed germination is 18 – 23ºC depending on the genotype of study, whereas other studies (Jacobsen and Bach, 1998) concluded that highest germination rates occur at temperatures around 30 – 35ºC. However, the present study showed that quinoa seeds were exposed to much lower germination temperature and yet resulted in a relatively high % seed germination in contrast to Ceccato *et al*. (2015) who found that quinoa seeds did not germinate at 5 – 10ºC.

Delay in sowing time from mid-April to early-May affected the length of the growth cycle by up to 15%. The entire growth cycle was 190 and 150 days long when sown in mid-April and early-May respectively. The length of the growth cycle was similar to (or shorter than) other studies which also investigated the effects of sowing date on the growth cycle of quinoa, such as 77 – 169 days in a multiple environment trial including Peru, Brazil, Bolivia, Vietnam and Kenya (Bertero *et al*., 2004), 109 – 163 days in India (Bhargava *et al.*, 2007) and 180 – 200 days in Argentina (Curti *et al.*, 2016). These results are supported by the assertion that quinoa requires a maximum growth cycle duration of 150 days to secure seed harvest (Gęsiński, 2008; De Santis *et al.*, 2016). However, whilst these studies observed mean monthly temperatures of 22 – 30ºC, especially at flowering and seed maturation growth stages, in the present study the temperature was 9 – 15ºC, thus explaining why the growth cycle was relatively long (requiring more than 150 days) in the present study because crops needed more thermal time due to the effect of low temperatures (Bertero and Ruiz, 2008; Curti *et al.*, 2016; De Santis *et al.*, 2016). This result raises the question of whether quinoa would be agroecologically suitable to the NE-England weather conditions.

Foliar Zn application

There were no significant effects of Zn application on growth because it was a late foliar application (GS50) with the aim to try and increase the grain Zn concentration. Zinc was applied at late growth stages (full flowering) when plant demand for nutrient, especially to support growth, is reduced.

Genotype

Both genotypes showed a significant decrease of 38% in germination rates and 8% in chlorophyll content when sowing time was delayed from mid-April to early-May. However, this decrease did not correlate directly with growth rates as it was associated with an overall better crop performance of both genotypes in response to delayed sowing.

There were significant differences between both genotypes with respect to plant height and chlorophyll content whereby Jessie had 2 – 3 SPAD units higher than Atlas in chlorophyll content whereas Atlas grew on average up to 30 cm taller than Jessie. In particular, the plant height of both genotypes was similar to those of other genotypes published elsewhere (Gonzalez *et al*., 2011; de Vasconcelos *et al*., 2012; Curti *et al*., 2014; Alandia *et al*., 2016). Although the variety Jessie had a higher chlorophyll content, which would be expected to have a higher photosynthetic activity than the variety Atlas, it was the variety Atlas which appeared to show

higher growth trait values (e.g. height and total above-ground biomass). Therefore, in addition to plant morphology, the differences between genotypes could also be attributed to a differential adaptability to the NE-England agroecological conditions.

There were also significant differences with respect to the duration of the growth cycle. The growth cycle of Jessie was shortened by up to 20% compared with the growth cycle of Atlas. As in the case of other quinoa genotypes, previous studies have also reported differences in growth cycle between genotypes (Curti *et al.*, 2016). Therefore, although Jessie had a short growth cycle compared with Atlas, it was Atlas which appeared to suit the local weather conditions because it showed higher growth trait values while Jessie appeared to be more negatively affected by the local weather conditions. These results also show that Atlas could be a genotype more sensitive to long photoperiods during reproductive stages than Jessie, thus requiring a longer growth cycle.

The results indicated that both genotypes required the same amount of time from seedling emergence to flowering, but from flowering to maturity Jessie required 30 – 45 days while Atlas required 60 – 75 days. Therefore, this confirms what was suggested in the literature (Curti *et al*., 2016) that flowering was the critical stage where genotype choice was the key factor affecting the duration of the entire growth cycle and determining the agroecological suitability. Moreover, although Jessie had higher chlorophyll content, it did not result in higher productivity nor better plant growth than Atlas, whereas the literature suggests that growth and productivity may increase with increasing chlorophyll content due to higher photosynthetic activity and photo-assimilation (Basra *et al*., 2014).

7.3.2. Yield and yield components

Grain yield was low with an average of 0.71 t/ha across all treatments and it was significantly affected by genotype but not by sowing date and foliar Zn application. On average across all treatments, the highest yield 0.96 t/ha was obtained from Block 4 and lowest 0.54 t/ha was obtained in Block 2. However, only the yield obtained from Block 4 could be correlated with the germination % for these blocks. Yield components were not affected by treatment except seed number which was significantly affected by genotype. The yield obtained in the present study was similar to the average yield 1.06 t/ha obtained in Europe in 1989 – 2000 (Gęsiński, 2008) with yields of 0.62 – 2.24 t/ha obtained in a number of other studies (Gonzalez *et al*., 2011; Basra *et al*., 2014; Garrido *et al*., 2014).

Sowing date

The effect of sowing date on yield and yield components was not statistically significant. Although early-May sowing resulted in shorter time from emergence to flowering than the mid-April sowing, it did not have the same effect on the time from flowering to seed setting and maturation. Most importantly, the effect of sowing date was not significant possibly because delayed sowing from mid-April to early-May did not result in significant differences in seed weight and seed number. This conclusion is supported by previous studies which showed a correlation between sowing date and improvements in seed number and seed weight (de Vasconcelos *et al*., 2012; De Santis *et al*., 2016), indicating that yields might increase when these components are significantly improved by variation in experimental factors.

Foliar Zn application

Zinc supply is generally related to grain yield especially in cereal crops grown on marginal or Zn-deficient soils, through the effect on translocation of assimilates, chlorophyll and biomass production (Alloway, 2009; Chattha *et al*., 2017). However, in the present study, all yield components remained unaffected by foliar Zn application. The most likely reason is that there was a condition of sufficient soil Zn availability for plant uptake (higher than 1.5 mg/L) which allowed crops to be of sufficient Zn nutritional status to sustain growth and development (Wang *et al.*, 2012; Boonchuay *et al.*, 2013; Olsen and Palmgren, 2014; Gupta *et al*., 2016). These results are consistent with the findings elsewhere in the literature (particularly on Zn biofortification of major cereals such as wheat and rice) that foliar Zn application did not affect yield and yield components under sufficient or marginal soil Zn availability (Wang *et al.*, 2012; Boonchuay *et al.*, 2013; Li *et al.*, 2015; Ram *et al.*, 2016). Another reason to consider could be that little or no Zn was transferred from leaves to developing seeds either because quinoa is a low-affinity Zn plant species due to physical or biochemical impediments (such as lack of yellow stripe-like transporters and zinc-regulated proteins), or because Zn was washed-off before being absorbed from the leaf surface (which could subsequently be available for uptake from the soil but dependent on plant uptake efficiency), or because the foliar application was too little and too late.

Genotype

The results of the present study indicated that Atlas and Jessie showed significantly different responses with respect to yield and yield components. Atlas showed a yield potential approximately 4 times higher than Jessie whereby on average Atlas produced 1.10 t/ha and

Jessie 0.31 t/ha. Thus, indicating that genotype choice caused significant improvements in grain yield of quinoa under the local weather conditions. Since seed weight was not significantly different between Atlas and Jessie, the difference in seed yield was explained by the fact that Atlas produced 2-3 times more seeds (per plant and per square meter) than Jessie despite that Jessie had higher plant number at harvest than Atlas. It could also be that the early canopy senescence of Jessie could have resulted in higher seed loss or flower abortion and therefore resulted in a much lower seed number per plant.

Yields produced by both genotypes are consistent with quinoa yields reported elsewhere such as 0.32 – 9.33 (India), 2.26 (Greece), 0.26 (Sweden), 0.34 (Denmark), 1.65 (Poland), 0.49 – 1.88 (Brazil) and 0.11 – 3.05 t/ha (Italy) (Bhargava *et al.*, 2007; Gęsiński, 2008; de Vasconcelos *et al.*, 2012; Shahzad *et al.*, 2014; De Santis *et al.*, 2016) with significant differences between genotypes. However, none of the studies used the genotypes used in the present study that could have enabled a more direct comparison between sites. Yield differences between Atlas and Jessie could also be associated with plant architecture. Atlas was a taller genotype (98.75 cm) with dense and broad panicles whereas Jessie was a shorter genotype (62.69 cm) with lax and narrow panicles. The taller the plants the more panicles they may produce and the more dense and broader the panicles the more seeds they produce (Bhargava and Srivastava, 2013; Tapia, 2015). Therefore, Atlas was genetically and morphologically more likely to produce a higher seed yield than Jessie. Contrary to De Santis *et al.* (2016), in the present study smaller plants with earlier panicle maturation did not produce more seeds than taller with later panicle maturation. Furthermore, although under different environmental conditions and using different genotypes, De Santis *et al.* (2016) in accordance with Casini and Proietti (2002) suggested that taller genotypes should not be considered for seed production because of the extended growth cycle, irregular flowering and possible abortion of flowers and delayed panicle maturation.

Both genotypes did not show significant differences in HI because differences in seed yield and total above-ground biomass were not statistically significant. Similar results were reported by De Santis *et al.* (2016) who studied 31 lines of quinoa grown in Italy and did not observe significant differences with respect to above-ground biomass and HI despite observing statistically significant differences in seed yield. Additionally, the values of HI (0.31 on average across all treatments and genotypes) observed in the present study were much higher than the average (0.14±0.01) reported by De Santis *et al*. (2016) but within the range 0.16 – 0.37 reported by Curti *et al*. (2014).

No significant interaction effects between sowing date, Zn application and genotype on yield, yield components and HI were detected in the present study, although significant interaction effects between sowing date and genotype were reported by Mariotti *et al*. (2016) and Siracusa *et al*. (2017).

7.3.3. Grain quality

The average crude protein was 12.7% across all treatments and genotypes which was approximately 3% below the average global value of 15% and just below the range 13 – 17% reported by several studies (Vega-Gálvez *et al*., 2010; Miranda *et al*., 2013; Vidueiros *et al*., 2015; Vicacundo and Hernandez-Ledesma, 2017). Nonetheless, similar results were published by Gonzalez *et al*. (2011) and Nascimento *et al*. (2014) who reported protein content of 12.5% and 12.1%, respectively.

All treatments did not significantly affect protein and mineral content except grain concentrations of Mo and Zn. Grain concentrations of Zn ranged between 31 and 37 mg/kg and was lower with delayed sowing date from mid-April to early-May. This difference is not likely attributed to the dilution effect because seed yield was not responsive to sowing date nor could it be explained by concentration of other minerals (divalent cations such as Ca, Fe and Mg) because neither were responsive to sowing date. However, since grain yield was on average higher but not statistically different in the early-May than mid-April sowing plots (0.79 and 0.62 t/ha, respectively), it could only be at least partly explained by the dilution effect. The mean value for grain Zn concentrations obtained in the present study was consistent with the literature for quinoa where several studies have reported grain Zn concentrations from 27 to 48 mg/kg (Ruales and Nair, 1993; Repo-Carrasco *et al*., 2003; Konishi *et al*., 2004; Bhargava *et al*., 2007; Sanders, 2009).

Grain concentrations of total polyphenols, antioxidants and flavonoids obtained in the present study were similar to those published by several previous studies. Alvarez-Jubete *et al*. (2010) reported 0.57 and 0.92 mg TE/g for total antioxidants determined by DPPH and FRAP assays, respectively; Repo-Carrasco-Valencia and Serna (2011) reported 2.35 – 3.68 mg TE/g for total antioxidants determined by ABTS assay; Pellegrini *et al*. (2018) reported 0.81 - 4.57 mg GA/g for total polyphenols determined by Folin-Ciolcateau. However, the present results were approximately six times lower than those published by Miranda *et al*. (2013) who reported concentrations of total polyphenols of 12.39 – 31.92 mg GA/100g determined by Folin-Ciocalteau. Nonetheless, one key factor to consider is the assay method and protocol, particularly the extraction method, type of solvent and time to reaction as these affect the final

results. Therefore, it was assumed that some of the differences between results obtained in the present study and those published in previous studies are attributable to differences in assay method and procedures.

Sowing date

Grain concentrations of total polyphenols, antioxidants and flavonoids were significantly affected by sowing date. The effect of sowing date was higher on total antioxidants than on total polyphenols. The highest concentration of total polyphenols was obtained in the early-May sown plots whereas the highest concentration of total antioxidants and total flavonoids was obtained from the mid-April sowing date. Concentration of total polyphenols was higher from the early-May sowing date possibly because growth rates and metabolic activities were higher in the early-May sowing date plots than mid-April sowing plots. However, this result should be interpreted with caution because of the inverse relationship between the concentration of total polyphenols and the concentrations of total antioxidants and total flavonoids observed in the present study. Nonetheless, it was clear that delaying sowing time from mid-April to early-May had positive effect on the concentration of total polyphenols but a negative effect on the concentrations of total antioxidants and total flavonoids.

Foliar Zn application

The effect of Zn application was not significant on grain concentrations of total polyphenols, antioxidants and flavonoids. Concentration of polyphenols, antioxidants and flavonoids is generally related to plant response to environmental stresses. Since Zn levels and other essential nutrients in the soil (and therefore in the plant) were not limiting, foliar Zn application did not affect the concentration of bioactive compounds such as polyphenols, antioxidants and flavonoids.

Genotype

Genotype choice did not affect the concentrations of total polyphenols, total antioxidants and total flavonoids although other studies on quinoa found significant differences between genotypes with respect to polyphenols, antioxidants and flavonoids (Miranda *et al*., 2013).

Variation in grain Zn concentration was mainly attributed to the interaction effect between sowing date and genotype. The interaction indicated that whilst grain Zn concentrations in Jessie were not different in both mid-April and early-May sowing plots, there was a 31% decrease in Atlas with delayed sowing time from mid-April to early-May. The decrease in grain

Zn concentration in Atlas from late sowing (i.e. early-May) could be explained by a dilution effect as it was associated with higher grain yield.

Significant interaction effects between sowing date × Zn application and between sowing date × genotype on total polyphenols and total flavonoids were detected. Whilst results show that there was an increment in total polyphenols with delayed sowing time from mid-April to early-May, the same effect was reversed by foliar Zn application, suggesting that the impact of sowing date on the concentration of total polyphenols in quinoa is not enhanced by extra Zn supply. However, both genotypes showed a large decrease of total flavonoids with delayed sowing date from mid-April to early-May, suggesting that the decrease in flavonoids (and antioxidants) in response to sowing time may be more than a simple effect of adverse environmental conditions as it should show a linear (or at least positive) correlation with variation in total polyphenols.

CHAPTER 8 – Effects of, Sowing Date, Nitrogen Fertilisation and Genotype on Growth, Yield and Quality of Quinoa (*Chenopodium quinoa* Willd.) in 2017 and 2018

8.1. Introduction

One of the key characteristics of quinoa is that it is a low-yielding crop, with an average seed yield around 0.9 t/ha worldwide with the major producers being Ecuador and Peru with average seed yield of around 1.5 t/ha (FAOSTAT, 2019).

As in the case of all field crops, sowing date and nitrogen fertilisation are important factors for crop production. Sowing date and nitrogen fertilisation affect production of crop biomass (vegetative development) and the canopy (development and senescence) by influencing photosynthetic efficiency of the crop. Therefore, sowing date and nitrogen fertilisation are often associated with improvement or decline of seed yield and quality. Within the context of the present study, quinoa has the potential to be a new spring grown low-input crop in NE-England.

Quinoa is a relatively new crop in the UK, and, therefore, there is limited knowledge and research associated with its cultivation. Although previous studies have shown that quinoa has high agroecological plasticity and variability in phenology and duration of the growth cycle, it is not known whether quinoa can be grown successfully in NE-England. Thus, there is the need to know the best spring sowing time of quinoa in NE-England because it will determine the agroecological suitability. There is also the need to know the most appropriate fertilisation practice because it will determine the potential for commercial production.

Most previous studies dealing with the effects of sowing date and nitrogen fertilisation growth, seed yield and quality of quinoa were carried out in countries with much higher temperatures and lower moisture than those observed in the UK. Therefore, the aim of this Chapter was to:

- Identify quinoa genotypes suited to NE-England, and
- Evaluate how the productivity and quality of quinoa can be affected by sowing date, and nitrogen fertilisation

8.2. Results

8.2.1. Weather data

The weather conditions were typical of NE-England which is defined as a cool temperate climate. Over the 2 years of the trial (2017 – 2018), the distribution of average temperature,

total rainfall and solar radiation varied during each growing season and between seasons (**Table 8.1, Table 8.2**). With respect to temperature, the average temperature over the entire growth cycle was 12.8º and 13.6ºC in 2017 and 2018, respectively; the average temperature over the germination period (i.e. assuming a germination time of two weeks after sowing) in 2017 was 7 and 10ºC for the mid-April and early-May sowing dates, respectively, whereas in 2018 it was warmer i.e. 9 and 11ºC for the corresponding sowing dates. With respect to rainfall, the total rainfall for the entire growth cycle (April – October) was 397.6 and 368.6 mm in 2017 and 2018, respectively. During the germination period in 2017, the total rainfall was 12 and 11 mm for the mid-April and early-May sowing dates, respectively, whereas in 2018 it was 8.6 and 25.2 mm for the mid-April and early-May sowing dates, respectively. The 2018 growing season was characterised by a high level i.e. 108.6 mm of rain in August during flowering (GS 50).

8.2.2. Crop growth

Overall, the growth cycle was 147 – 168 days long but it was two weeks shorter in 2018 than 2017. Delayed sowing from mid-April to early-May reduced the growth cycle by on average 20 days across both seasons and plants required 30-35 days from flowering to seed setting and from seed setting to maturity. There were significant differences among genotypes whereby Atlas had the longest growth duration and Jessie the shortest regardless of sowing date. Differences in duration of phenological phases with respect to nitrogen application rate were clear from GS50 to GS80, particularly between the 150 kg N/ha and zero-N treatments. Duration of crop development was extended with application of 150 kg N/ha relative to zero-N, with differences in duration of phenological phases up to 7 days from one growth stage to another. Nitrogen source (mineral N vs biogas digestate) did not significantly affect the duration of crop development.

Table 8.1 Summary of weather conditions (average temperature, cumulative monthly rainfall and monthly average solar radiation) from sowing to harvest of quinoa in 2017-18.

	2017			2018		
	Temp (°C)	Rain (mm)	Rad (W/m^2)	Temp (°C)	Rain (mm)	Rad (W/m^2)
April	8.0	14.8	147.3	8.1	67.6	123.5
May	11.9	19.8	189.7	12.0	31.0	216.4
June	14.4	127.2	179.5	10.9	38.6	225.0
July	14.4	68.4	160.2	16.9	25.2	208.2
August	14.7	31.6	152.3	15.3	108.6	136.5
September	12.3	84.4	88.5	12.4	53.0	113.3
October	11.5	51.4	45.1	9.5	44.6	56.5

Table 8.2 Weather conditions (minimum and maximum temperature and daily rainfall) over the germination period of quinoa for early and late sowing dates (white and shaded area, respectively) in 2017-18.

Date	2017			2018		
	Min (°C)	Max (°C)	Rain (mm)	Min (°C)	Max (°C)	Rain (mm)
13/04	4.5	11.3	0.0			
14/04	5.9	12.1	0.6			
15/04	3.7	10.9	0.0			
16/04	3.6	7.5	5.4			
17/04	1.3	9.2	0.6			
18/04	-1.4	9.7	0.0			
19/04	4.4	11.7	0.0			
20/04	9.6	15.5	0.0	8.3	16.8	0.0
21/04	6.9	13.5	1.8	4.3	17.9	0.0
22/04	4.5	10.1	0.0	9.1	15.6	0.2
23/04	2.6	13.1	0.0	8.1	12.8	1.0
24/04	0.5	10.4	2.0	6.3	12.1	5.4
25/04	-0.9	7.6	0.8	6.2	12.4	0.0
26/04	0.5	8.5	0.4	5.7	11.8	0.4
27/04	3.7	12.0	0.4	3.5	11.0	0.0
28/04				1.8	8.7	1.0
29/04				1.1	9.1	0.2
30/04				1.5	9.7	0.2
1/05				0.7	12.6	0.0
2/05	7.0	12.0	0.0	5.7	12.7	0.2
3/05	4.2	14.1	0.0	4.7	14.0	0.0
4/05	6.0	13.3	0.0	9.3	18.4	0.0
5/05	3.0	13.1	0.0			
6/05	3.6	12.0	0.2			
7/05	3.0	10.6	0.2			
8/05	6.1	10.3	0.0			
9/05	5.3	15.0	0.0	4.1	14.3	3.0
10/05	3.3	16.4	0.0	4.8	13.5	3.0
11/05	3.7	16.0	0.0	2.8	15.1	0.0
12/05	6.7	13.7	0.0	6.6	14.9	0.4
13/05	9.2	14.7	3.8	7.3	16.2	10.6
14/05	8.9	17.0	0.2	3.4	18.4	0.0
15/05	6.6	17.3	6.2	4.5	19.0	1.4
16/05	11.1	18.3	0.6	3.6	11.2	6.4
17/05				1.5	12.8	0.2
18/05				1.5	18.2	0.0
19/05				4.6	20.5	0.2
20/05				7.6	21.5	0.0
21/05				6.5	22.0	0.0
22/05				8.0	11.9	0.0
23/05				8.4	11.9	0.0

Seed germination of quinoa was significantly influenced by sowing date and genotype. Early-May sowing resulted in lower germination % than mid-April sowing and Duches had the highest germination % (**Table 8.3**). The average seed germination % was 53% across all genotypes and treatments. Significant year × sowing date interaction on seed germination % was detected, which showed that the highest seed germination % was observed in the mid-April sown plots in 2018. There was also a significant year × genotype interaction which showed that the highest % seed germination was obtained from Duches in both years (**Table 8.4, 8.5**).

Year, sowing date, nitrogen fertilisation (i.e. rate and source) and crop genotype influenced plant height significantly (**Table 8.3**). The average plant height was 80 cm across all treatments and genotypes. Plants were 25 cm taller in 2017 than 2018, 18 cm taller when sown early-May than mid-April, 10 cm taller at higher than lower nitrogen application rates, and 6 cm taller with application of mineral N than biogas digestate at the rate of 150 kg N/ha. There were also statistically significant differences between genotypes, with Atlas being up to 20 cm taller than Jessie. Significant year × nitrogen rate, year × nitrogen source and year × genotype interactions showed that 150 kg N/ha applied as mineral nitrogen resulted in the tallest plants for Atlas sown in 2017 (**Table 8.6 – 8.8**).

Except for nitrogen source, the effects of year, sowing date, nitrogen rate and crop genotype were statistically significant on total above-ground biomass (**Table 8.3**). Total above-ground biomass was two times higher in 2017 than 2018 and approximately 47% higher in the early-May than mid-April sown plots. Total above-ground biomass was also up to 76% higher with the application of 150 kg N/ha than lower rates. Atlas produced the highest total above-ground biomass which was approximately two times higher than that of Jessie. Significant year × nitrogen rate, year × nitrogen source, year × genotype and sowing date × genotype interactions showed that the highest total above-ground biomass was obtained from the variety Atlas in 2017 with application of mineral fertiliser at the rate of 150 kg N/ha whereas early-May sowing resulted in the highest total above-ground biomass obtained from the variety Duches (**Table 8.9 – 8.11**).

Overall, weed % of ground cover was less than 20% with significant differences between years and among genotypes but not with respect to sowing date nor nitrogen application. There was a higher weed cover in 2018 than 2017 and the genotype Atlas showed a higher ability to supress weeds than Jessie (and Duches to some extent) (**Table 8.3**). The weed population was dominated by chickweed in 2017 and oilseed rape in 2018. Significant year × sowing date and

year × genotype interactions showed that early-May sowing resulted in the lowest weed cover while the genotype Atlas supressed weed species the most in 2017 (**Table 8.12, 8.13**).

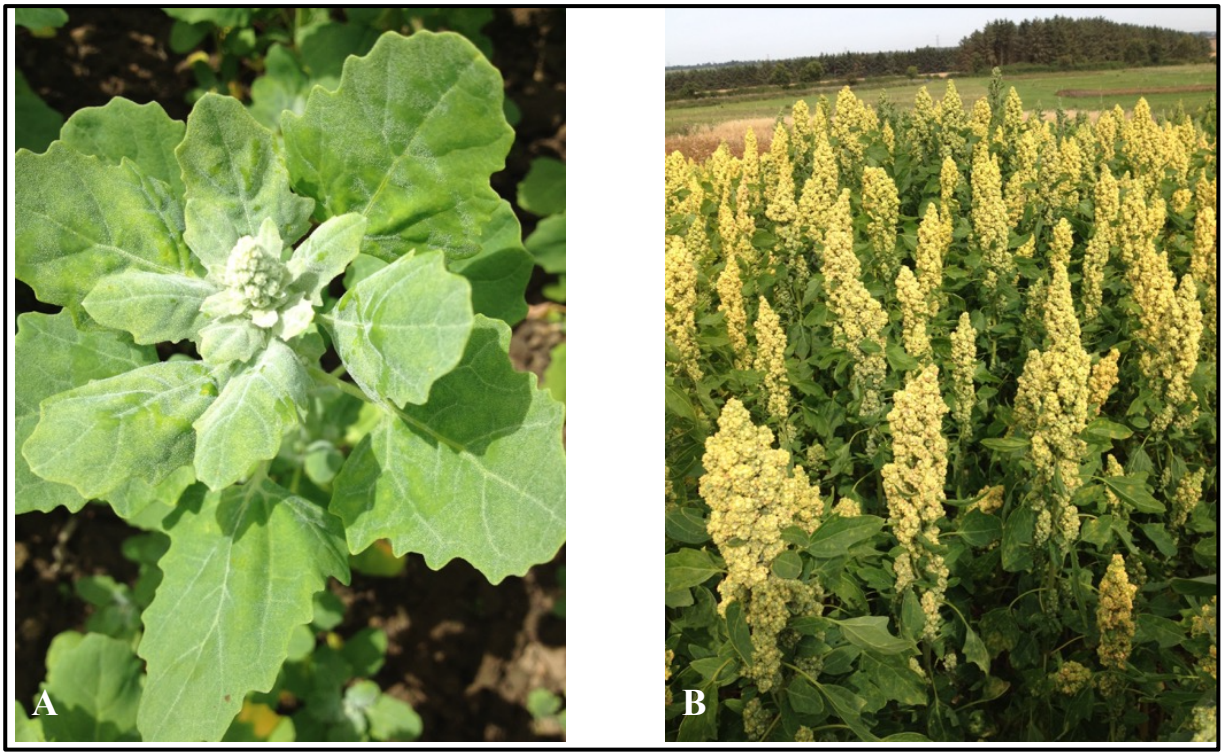

Fig. 8.1 Inflorescence emergence (A) and grain development (B) stages of *Chenopodium quinoa* Willd.

Crop biomass (NDVI) was not statistically different among genotypes but it was significantly affected by year, sowing date and nitrogen rate over the vegetative period GS30-50 (**Table 8.20**). Crop biomass was generally higher in 2017 than 2018 and in the early-May than mid-April sown plots. It was also significantly higher at 150 kg N/ha than at lower nitrogen rates. Significant year × sowing date, year × nitrogen rate and year × genotype interactions showed that the highest crop biomass (NDVI) of quinoa at GS50 was detected in Duches from the early-May sown plots combined with application of mineral nitrogen at the rate of 150 kg N/ha in 2017 (**Table 8.21 – 8.23**).

Overall, both genotypes were relatively clean with little foliar disease evident. Intensity and severity of infection by downy mildew (*Peronospora variabilis* Gaum, formerly called *Peronospora farinos*a Fr.) at GS60 was lower than 10% of the whole plant and approximately 5% of leaf area of the young fully expanded leaves in all plots and so data is not presented. Nonetheless, it was higher in 2017 than 2018 and higher in Jessie than Atlas and Duches.

Fig. 8.2 Ripening (A) and senescence (B) stages of Atlas and Jessie *Chenopodium quinoa* Willd. genotypes, respectively.

Year, sowing date, nitrogen fertilisation (i.e. rate and source) and crop genotype significantly affected chlorophyll content of quinoa particularly over the vegetative period GS40-50 (**Table 8.15**). Chlorophyll content of quinoa was generally higher in 2017 than 2018 and when sown early-May than mid-April at GS30 and GS50. Chlorophyll content of quinoa increased significantly with increasing nitrogen application rate at GS40 and GS50 and was significantly higher when mineral N was applied relatively to biogas digestate. Chlorophyll content was also generally higher in Jessie and Atlas than Duches. Significant year × sowing date, year × nitrogen rate, year × nitrogen source and sowing date × nitrogen rate interactions on chlorophyll content especially at GS50 showed that the highest chlorophyll content was obtained from early-May sown plots combined with the application of mineral nitrogen at the rate of 150 kg N/ha in 2017 (**Table 8.16 – 8.19**).

Table 8.3 Effects of year, sowing date, nitrogen rate, nitrogen source and genotype on % germination, plant height, total above-ground biomass of quinoa and % of ground cover by weeds in 2017-18. Means followed by the same letter within each column for each trait are not significantly different at p≤0.05.

	Germination (%)	Height (cm)	Biomass (g/m^2)	Weed (% of ground cover)
Year (Y)				
2017	53.1±1.38	92.9±1.28	734.7±21.62	3.33±0.18
2018	53.4±1.70	67.6±1.54	347.4±19.44	20.8±0.80
Sowing date (S)				
Mid-April	68.1±1.24	71.4±1.68	452.0±22.28	13.0±1.01
Early-May	41.0±1.17	89.1±1.42	630.1±25.69	11.2±0.66
Nitrogen rate (R)				
Zero		68.5±1.46c	390.0±18.45b	13.0±1.06a
75 kg/ha		79.4±1.45b	494.3±21.49b	11.2±0.74a
150 kg/ha		88.8±1.90a	651.8±27.54a	11.0±0.82a
Nitrogen source (T)				
Mineral N		88.8±1.90	651.8±27.54	11.0±0.82
Biogas digestate		84.2±1.54	628.2±26.59	13.1±0.77
Genotype (G)				
Atlas	44.4±1.42b	88.9±1.42a	662.9±22.66a	9.09±0.63b
Duches	60.6±1.42a	83.5±1.27a	588.0±25.13a	11.9±0.81ab
Jessie	54.1±1.59a	68.4±1.93b	372.4±22.01b	15.2±1.03a
ANOVA				
Year (Y)	ns	**<0.001**	**<0.001**	**<0.001**
Sowing date (S)	**<0.001**	**<0.001**	**<0.001**	*0.077*
Nitrogen rate (R)		**<0.001**	**<0.001**	ns
Nitrogen source (T)		*0.051*	ns	ns
Genotype (G)	**0.011**	**<0.001**	**<0.001**	**0.008**
Y*S	**0.005**	ns	ns	**0.025**
Y*R		**<0.001**	**0.003**	ns
Y*T		**0.002**	**0.038**	ns
Y*G	**<0.001**	**<0.001**	**0.005**	**0.027**
S*R		ns	ns	ns
S*T		ns	ns	ns

Table 8.3 ANOVA *continued...*

	Germination (%)	Height (cm)	Biomass (g/m^2)	Weed (% of ground cover)
S*G				
R*T		ns	ns	ns
R*G		ns	ns	ns
T*G		ns	ns	ns
Y*S*R		ns	ns	ns
Y*S*T		ns	ns	ns
Y*S*G	ns	*0.070*	*0.070*	ns
S*R*T		ns	ns	ns
S*R*G		ns	ns	ns
R*T*G		ns	ns	ns
Y*S*R*T		ns	ns	ns
Y*S*R*G		ns	ns	ns
Y*S*R*T*G		ns	ns	ns

Table 8.4 Interaction between year and sowing date on germination % of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	mid-April	early-May
2017	64.3±1.15bA	43.2±1.15aB
2018	72.0±1.29aA	39.1±1.19aB

Table 8.5 Interaction between year and genotype on germination % of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Duches	Jessie	Atlas
2017	57.7±1.15aA	51.6±1.15bB	49.3±1.15aB
2018	63.1±1.29aA	57.6±1.19aA	40.9±1.19aB

Table 8.6 Interaction between year and nitrogen rate on plant height (cm) of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Zero-N	75 kg N/ha	150 kg N/ha
2017	78.0±1.26aC	90.3±1.22aB	107.5±1.34aA
2018	59.0±1.70bB	68.4±1.59bA	70.1±1.74bA

Table 8.7 Interaction between year and nitrogen source on plant height (cm) of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Mineral N	Biogas digestate
2017	107.5±1.34aA	95.6±0.93aB
2018	70.1±1.74bA	72.9±1.94bA

Table 8.8 Interaction between year and genotype on plant height (cm) of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Duches	Jessie	Atlas
2017	94.0±1.13aA	88.5±1.29aA	96.1±1.38aA
2018	73.0±0.91bB	48.3±1.25bC	81.6±1.28bA

Table 8.9 Interaction between year and nitrogen rate on total above-ground biomass (g/m^2) of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Zero-N	75 kg N/ha	150 kg N/ha
2017	548.5±19.58aC	658.6±23.61aB	925.4±21.46aA
2018	231.5±13.43bB	330.1±17.61bA	378.3±22.83bA

Table 8.10 Interaction between year and nitrogen source on total above-ground biomass (g/m^2) of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Mineral N	Biogas digestate
2017	925.4±21.46aA	806.5±24.06aB
2018	378.3±22.83bA	449.9±29.77bA

Table 8.11 Interaction between year and genotype on total above-ground biomass (g/m^2) of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Duches	Jessie	Atlas
2017	784.5±20.96aA	622.2±17.45aB	797.5±24.21aA
2018	391.5±20.70bB	122.6±2.93bC	528.3±16.21bA

Table 8.12 Interaction between sowing date and genotype on total above-ground biomass (g/m^2) of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Duches	Jessie	Atlas
mid-April	397.3±21.36bB	347.4±19.16aB	611.5±22.05aA
early-May	778.7±20.87aA	397.4±24.71aB	714.3±23.00aA

Table 8.13 Interaction between year and sowing date on weed % of ground cover. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Mid-April	Early-May
2017	3.08±0.14bA	3.58±0.22bA
2018	22.8±1.01aA	18.7±0.46aB

Table 8.14 Interaction between year and genotype on weed % of ground cover. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Duches	Jessie	Atlas
2017	3.81±0.21bA	3.41±0.18bA	2.78±0.15bA
2018	20.1±0.76aB	26.9±0.81aA	15.4±0.59aC

Table 8.15 Effects of year, sowing date, nitrogen rate, nitrogen source and genotype on chlorophyll content (SPAD) of quinoa at GS30-50. Means followed by the same lowercase letter within each column for each trait and uppercase letter within each row are not significantly different at p≤0.05.

	SPAD (GS30)	SPAD (GS40)	SPAD (GS50)
Year (Y)			
2017	38.2±0.28B	37.5±0.64B	40.9±0.77A
2018	31.2±0.38C	39.4±0.58A	35.2±0.60B
Sowing date (S)			
Mid-April	33.4±0.54B	36.6±0.56A	33.5±0.70B
Early-May	36.0±0.20B	40.2±0.64A	42.6±0.58A
Nitrogen rate (R)			
Zero	34.4±0.46aA	31.0±0.47cB	33.2±0.43bA
75 kg N/ha	35.3±0.44aA	36.0±0.44bA	34.6±0.59bA
150 kg N/ha	34.7±0.42aB	45.4±0.61aA	44.1±0.90aA
Nitrogen source (T)			
Mineral N	34.7±0.42B	45.4±0.61A	44.1±0.90A
Biogas digestate	34.3±0.36B	41.3±0.32A	40.3±0.58A
Genotype (G)			
Atlas	36.9±0.44aA	38.9±0.57abA	38.3±0.67abA
Duches	33.5±0.36bA	36.0±0.55bA	35.7±0.68bA
Jessie	33.6±0.41bB	40.4±0.67aA	40.1±0.78aA
ANOVA			
Year (Y)	**<0.001**	**0.043**	**<0.001**
Sowing date (S)	**<0.001**	**<0.001**	**<0.001**
Nitrogen rate (R)	ns	**<.001**	**<0.001**
Nitrogen source (T)	ns	**<0.001**	**<0.001**
Genotype (G)	**<0.001**	**0.021**	**0.032**
Y*S	**<0.001**	ns	**<0.001**
Y*R	**<0.001**	**0.023**	**<0.001**
Y*T	**<0.001**	ns	**<0.001**
Y*G	ns	**<0.001**	*0.066*
S*R	ns	ns	**0.011**
S*T	ns	ns	ns
S*G	ns	ns	ns

Table 8.15 ANOVA *continued...*

	SPAD (GS30)	SPAD (GS40)	SPAD (GS50)
R*T	ns	ns	ns
R*G	ns	ns	ns
T*G	ns	ns	ns
Y*S*R	ns	ns	ns
Y*S*T	ns	**0.002**	ns
Y*S*G	**0.015**	**0.029**	ns
S*R*T	ns	ns	ns
S*R*G	ns	ns	ns
R*T*G	ns	ns	ns
Y*S*R*T	ns	ns	ns
Y*S*R*G	ns	ns	ns
Y*S*R*T*G	ns	ns	ns

Table 8.16 Interaction between year and sowing date on chlorophyll content (SPAD) of quinoa at GS50. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Mid-April	Early-May
2017	38.2±0.75aB	43.6±0.76aA
2018	28.8±0.43bB	41.6±0.31aA

Table 8.17 Interaction between year and nitrogen rate on chlorophyll content (SPAD) of quinoa at GS50. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Zero	75 kg N/ha	150 kg N/ha
2017	31.5±0.40aB	35.3±0.57aB	53.4±0.47aA
2018	34.8±0.43aA	33.9±0.63aA	34.8±0.71bA

Table 8.18 Interaction between year and nitrogen source on chlorophyll content (SPAD) of quinoa at GS50. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Mineral N	Biogas digestate
2017	53.4±0.47aA	43.3±0.49aA
2018	34.8±0.71bB	37.3±0.60bA

Table 8.19 Interaction between sowing date and nitrogen rate on chlorophyll content (SPAD) of quinoa at GS50. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Zero	75 kg N/ha	150 kg N/ha
Mid-April	30.7±0.47bB	29.0±0.55bC	38.8±1.14bA
Early-May	35.6±0.43aC	40.3±0.45aB	49.3±0.71aA

Table 8.20 Effects of year, sowing date, nitrogen rate, nitrogen source and genotype on crop biomass (NDVI) of quinoa at GS30-50. Means followed by the same lowercase letter within each column for each trait and uppercase letter within each row are not significantly different at $p \leq 0.05$.

	NDVI (GS30)	NDVI (GS40)	NDVI (GS50)
Year (Y)			
2017	0.53±0.01B	0.62±0.01A	0.60±0.01A
2018	0.51±0.01A	0.42±0.01B	0.42±0.00B
Sowing date (S)			
Mid-April	0.46±0.01B	0.52±0.01A	0.47±0.01B
Early-May	0.58±0.01A	0.56±0.01A	0.55±0.01A
Nitrogen rate (R)			
Zero – N	0.48±0.01aA	0.47±0.01cA	0.44±0.01cA
75 kg N/ha	0.53±0.01aAB	0.54±0.01bA	0.50±0.01bB
150 kg N/ha	0.55±0.01aA	0.58±0.01aA	0.56±0.01aA
Nitrogen source (T)			
Mineral N	0.55±0.01B	0.58±0.01A	0.56±0.01AB
Biogas digestate	0.52±0.01C	0.58±0.01A	0.55±0.01B
Genotype (G)			
Atlas	0.53±0.01aA	0.53±0.01aA	0.50±0.01aB
Duches	0.52±0.01aA	0.54±0.01aA	0.53±0.01aA
Jessie	0.52±0.01aB	0.55±0.01aA	0.51±0.01aB
ANOVA			
Year (Y)	**0.039**	**<0.001**	**<0.001**
Sowing date (S)	**<0.001**	**<0.001**	**<0.001**
Nitrogen rate (R)	ns	**<0.001**	**<0.001**
Nitrogen source (T)	ns	ns	ns

Table 8.20 ANOVA *continued...*

	NDVI (GS30)	NDVI (GS40)	NDVI (GS50)
Genotype (G)	ns	ns	ns
Y*S	**<0.001**	ns	**<0.001**
Y*R	ns	**0.001**	**0.002**
Y*T	ns	ns	ns
Y*G	ns	ns	**0.008**
S*R	ns	ns	ns
S*T	ns	ns	ns
S*G	**0.020**	ns	ns
R*T	ns	ns	ns
R*G	ns	ns	ns
T*G	ns	ns	ns
Y*S*R	ns	ns	ns
Y*S*T	ns	**0.002**	ns
Y*S*G	**0.010**	ns	*0.071*
S*R*T	ns	ns	ns
S*R*G	ns	ns	ns
R*T*G	ns	ns	ns
Y*S*R*T	ns	ns	ns
Y*S*R*G	ns	ns	ns
Y*S*R*T*G	ns	ns	ns

Table 8.21 Interaction between year and sowing date on crop biomass (NDVI) of quinoa at GS50. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Mid-April	Early-May
2017	0.58±0.01aB	0.66±0.01aA
2018	0.39±0.01bB	0.43±0.01bA

Table 8.22 Interaction between year and nitrogen rate on crop biomass (NDVI) of quinoa at GS50. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Zero-N	75 kg/ha	150 kg/ha
2017	0.52±0.01aC	0.57±0.01aB	0.70±0.00aA
2018	0.35±0.01bB	0.41±0.01bA	0.45±0.00bA

Table 8.23 Interaction between year and genotype on crop biomass (NDVI) of quinoa at GS50. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Duches	Jessie	Atlas
2017	0.64±0.01aA	0.60±0.01aA	0.60±0.01aA
2018	0.43±0.01bA	0.38±0.01bB	0.43±0.01bA

8.2.3. Yield and yield components

Grain yield

On average across all treatments, whilst combine seed yield ranged between 0.61 and 1.51 t/ha with an average of 1.02 t/ha, biomass seed yield ranged from 0.43 to 1.41 t/ha with an average of 0.96 t/ha. The main factors i.e. year, sowing date, nitrogen rate, nitrogen source and crop genotype significantly affected seed yield (**Table 8.24**). Overall, seed yield of quinoa was 2-4 times higher in 2017 than 2018 and at least 35% higher in the early-May than mid-April sowing date. Seed yield also increased significantly with increasing nitrogen rate. Combine seed yield increased with nitrogen rate by 39 and 149% at 75 and 150 kg N/ha, respectively, over the zero-N treatment. Atlas produced the highest seed yield (1.34 t/ha) and Jessie the lowest (0.67 t/ha). Significant year × nitrogen rate interaction showed that application of mineral nitrogen at the rate of 150 kg N/ha resulted in the highest combine seed yield of 2.24 t/ha whilst the year × genotype interaction showed that the highest seed yield of 1.71 t/ha was obtained from Duches in 2017 (**Tables 8.25 and 8.26**).

Harvest index

Harvest index was significantly affected by year, sowing date and genotype but not by nitrogen rate and source (**Table 8.24**). Quinoa HI was higher in 2018 than 2017 (i.e. 0.23 vs 0.18) and higher in the mid-April than early-May (i.e. 0.21 vs 0.19) sown plots. Duches had the highest HI. Significant year × sowing date, year × nitrogen rate, year × genotype and sowing date × genotype interactions showed that the highest HI was obtained from the genotype Duches in the mid-April sown plots combined with application of mineral nitrogen at the rate of 150 kg N/ha nitrogen in 2017 (**Table 8.27 – 8.30**).

Plant number

Plant number at harvest was significantly affected by year, sowing date and genotype but not by nitrogen rate and source (**Table 8.31**). Plant number at harvest was 59% higher in 2018 than

2017 and generally higher in the mid-April than early-May sown plots (153 vs 109 plants/m^2). Jessie had the highest number of plants/m^2 at harvest and Atlas the lowest. Significant year × sowing date, year × genotype and sowing date × genotype interactions showed that the highest numbers of plants/m^2 was obtained from Jessie in the mid-April sown plots in 2018 (**Table 8.32 – 8.34**).

Panicle number

Panicle number was significantly affected by year, sowing date and genotype but not by nitrogen rate and source (**Table 8.31**). On average across all treatments, there were 4487.4 panicles/m^2. Whilst number of panicles/m^2 was generally 26.4% higher in 2018 than 2017, number of panicles/plant was significantly higher in 2017 than 2018. Although number of cymes/plant was significantly higher in the early-May than mid-April sown plots, number of cymes/m^2 was at least 21.3% higher in the mid-April than early-May sown plots. While Atlas and Duches had more cymes/plant than Jessie, Atlas had fewer panicles/m^2 than Duches and Jessie. Significant year × genotype and year × sowing date interactions showed that whilst the genotype Duches had the highest number of cymes/plant in 2017, Jessie had the highest number of panicles/m^2 in 2018. The interactions also showed that the highest number of panicles/plant was obtained from the early-May sown plots in 2017 (**Table 8.35 – 8.37**).

Seed number

Seed number (i.e. seeds/plant and seeds/m^2) was significantly affected by year, sowing date, and genotype but not by nitrogen rate and source. However, nitrogen rate and source had a significant effect on number of seeds/plant (**Table 8.31**). Overall, quinoa seed number was approximately two times higher in 2017 than 2018 and 40% higher in the early-May than mid-April sown plots. Quinoa seed number (i.e. seeds/plant) increased by up to 69% at 150 kg N/ha compared to lower rates of nitrogen application. Atlas and Duches produced approximately two times more seeds/plant and seeds/m^2 than Jessie. Significant year × nitrogen rate, year × nitrogen source and year × genotype interactions showed that application of mineral N at the rate of 150 kg/ha in 2017 resulted in the highest seed number per plant obtained from the genotype Duches whereas the significant year × nitrogen source, year × genotype and sowing date × genotype interactions on number of seeds/m^2 showed that the highest seeds/m^2 was obtained from the genotype Atlas in the early-May sown plots combined with application of mineral nitrogen at the rate of 150 kg/ha in 2017 (**Table 8.38 – 8.43**).

Table 8.24 Effects of year, sowing date, nitrogen rate, nitrogen source and genotype on (combine and biomass harvest) seed yield and harvest index (HI) of quinoa. Means followed by the same lowercase letter within each column for each trait are not significantly different at $p \leq 0.05$.

	Biomass seed yield (t/ha)	Combine seed yield (t/ha)	HI
Year (Y)			
2017	1.37±0.09	1.36±0.09	0.18±0.01
2018	0.49±0.03	0.69±0.03	0.23±0.01
Sowing date (S)			
Mid-April	0.78±0.07	0.81±0.06	0.21±0.01
Early-May	1.06±0.08	1.24±0.07	0.19±0.01
Nitrogen rate (R)			
Zero	0.46±0.03b	0.61±0.04b	0.19±0.01a
75 kg N/ha	0.70±0.05b	0.84±0.05b	0.18±0.01a
150 kg N/ha	1.41±0.10a	1.51±0.10a	0.23±0.01a
Nitrogen source (T)			
Mineral N	1.41±0.10	1.51±0.10	0.23±0.01
Biogas digestate	1.08±0.06	1.12±0.06	0.21±0.01
Genotype (G)			
Atlas	1.21±0.31a	1.34±0.08a	0.22±0.01b
Duches	1.12±0.27a	1.05±0.08a	0.34±0.00a
Jessie	0.43±0.11b	0.67±0.03b	0.05±0.02c
ANOVA			
Year (Y)	**<0.001**	**<0.001**	**<0.001**
Sowing date (S)	**0.008**	**<0.001**	**<0.001**
Nitrogen rate (R)	**<.001**	**<.001**	ns
Nitrogen source (T)	ns	**0.024**	ns
Genotype (G)	**0.005**	**0.020**	**<0.001**
Y*S	ns	ns	**<0.001**
Y*R	ns	**<0.001**	*0.052*
Y*T	**0.015**	ns	ns
Y*G	*0.062*	**<0.001**	**<0.001**
S*R	ns	ns	ns
S*T	ns	ns	ns
S*G	ns	ns	**0.038**

Table 8.24 ANOVA *continued...*

R*T	ns	ns	ns
R*G	ns	ns	ns
T*G	ns	ns	ns
Y*S*R	ns	ns	ns
Y*S*T	ns	ns	ns
Y*S*G	ns	ns	**<0.001**
S*R*T	ns	ns	ns
S*R*G	ns	ns	ns
R*T*G	ns	ns	ns
Y*S*R*T	ns	ns	ns
Y*S*R*G	ns	ns	ns
Y*S*R*T*G	ns	ns	ns

Table 8.25 Interaction between year and nitrogen rate on combine seed yield (t/ha) of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Zero-N	75 kg/ha	150 kg/ha
2017	0.69±0.04aB	0.95±0.06aB	2.24±0.12aA
2018	0.53±0.03aA	0.73±0.03aA	0.79±0.03bA

Table 8.26 Interaction between year and genotype on combine seed yield (t/ha) of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Duches	Jessie	Atlas
2017	1.71±0.09aA	0.63±0.03aB	1.70±0.10aA
2018	0.40±0.02bB	0.71±0.02aAB	0.97±0.03bA

Table 8.27 Interaction between year and sowing date on HI of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Mid-April	Early-May
2017	0.43±0.00aA	0.43±0.01aA
2018	0.26±0.01bA	0.20±0.01bB

Table 8.28 Interaction between year and nitrogen rate on HI of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Zero-N	75 kg/ha	150 kg/ha
2017	0.41±0.01aA	0.43±0.00aA	0.43±0.00aA
2018	0.25±0.02bA	0.23±0.01bA	0.22±0.01bA

Table 8.29 Interaction between year and genotype on HI of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Duches	Jessie	Atlas
2017	0.44±0.00aA	0.40±0.01aB	0.43±0.00aAB
2018	0.44±0.01aA	0.21±0.00bC	0.24±0.01bB

Table 8.30 Interaction between sowing and genotype on HI of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Duches	Jessie	Atlas
mid-April	0.45±0.00aA	0.21±0.02aC	0.36±0.01aB
early-May	0.43±0.01aA	0.20±0.02aC	0.31±0.01aB

Thousand-grain weight

Thousand-grain weight (TGW) was significantly affected only by year and crop genotype but not by sowing date, nitrogen rate and source (**Table 8.31**). Thousand-grain weight was up to 58% higher in 2017 than 2018. The genotype Atlas showed highest TGW values. Significant year × sowing date, year × genotype and sowing date × genotype interactions showed that highest TGW values were obtained from Atlas and Duches in the early-May sown plots in 2017 (**Table 8.44 – 8.46**).

8.2.4. Grain quality

Protein and ash content

Seed protein content was significantly affected by year, sowing date, nitrogen source and genotype but not by nitrogen rate. The average seed protein content was 13.2% across all treatments (**Table 8.47**). Seed protein content of quinoa was about 5% higher in 2018 than 2017 and up to 7% higher in the early-May than the mid-April sown plots. Application of mineral nitrogen at the rate of 150 kg/ha significantly increased protein content by approximately 2%

compared with application of biogas digestate. On average, the genotype Atlas had the highest protein content of 13.8%. Significant year × genotype, sowing date × genotype and year × sowing date × genotype interactions showed that the highest protein content of 16.3% was obtained from Atlas in the early-May sown plots in 2018 (**Table 8.48 – 8.50**).

Seed ash content was significantly affected only by year of cultivation. The average ash content was 3.9% across all treatments (**Table 8.47**). Ash content was up to 17% higher in 2018 compared with 2017. Significant year × sowing date, year × nitrogen rate, sowing date × genotype and year × sowing date × genotype interactions indicated that the highest ash content of 4.9% was detected in Atlas from the early-May sown plots in 2018 (**Table 8.51 – 8.54**).

Minerals

The effect of main factors (i.e. year, sowing date, nitrogen rate, nitrogen source and genotype) on grain concentration of minerals was variable (**Table 8.55**). Grain concentration of all minerals except Ca and Zn was significantly affected by year of cultivation, whereby grain concentration of minerals (except Fe) was higher in 2017 than 2018. For example, whilst grain concentration of Fe was up to 44% higher in 2018 than 2017, grain concentration of Mn was up to 42% higher in 2017 than 2018.

Sowing date significantly affected grain concentration of minerals except for Fe and Ni. While grain concentration of Al and Mn was significantly higher in the mid-April than the early-May sown plots, the concentration of all other minerals was significantly higher in the early-May than mid-April treatment.

Table 8.31 Effects of year, sowing date, nitrogen rate, nitrogen source and genotype on plant, panicle and seed number and thousand-grain weight (TGW) of quinoa. Means followed by the same lowercase letter within each column for each trait are not significantly different at $p \leq 0.05$.

	Plants/m^2	Panicles/plant	Panicles/m^2	Seeds/plant	Seeds/m^2	TGW(g)
Year (Y)						
2017	101.3±2.89	38.8±0.44	3804.4±121.10	4167.6±295.54	363781.3±23433.21	3.66±0.02
2018	161.0±5.63	34.0±0.47	5170.4±154.52	1724.9±114.07	201046.8±9786.54	2.32±0.04
Sowing date (S)						
Mid-April	153.4±5.94	34.9±0.50	5021.0±166.54	2426.3±240.84	243363.0±17686.29	2.94±0.05
Early-May	108.9±2.99	38.0±0.45	3953.8±112.79	3436.2±235.65	321465.1±19639.89	3.03±0.05
Nitrogen rate (R)						
Zero	139.6±4.88a	33.4±0.45	4388.5±168.45a	1368.4±75.83	154788.8±8458.79a	2.90±0.06a
75 kg N/ha	130.6±5.10a	35.9±0.46	4455.9±151.93a	2479.9±218.99	224921.7±12938.73a	2.92±0.06a
150 kg N/ha	126.5±4.70a	37.9±0.53	4460.9±114.18a	4466.1±339.18	413957.5±26872.56a	3.05±0.06a
Nitrogen source (T)						
Mineral N	126.5±4.70	37.9±0.53	4460.9±114.18	4466.1±339.18	413957.5±26872.56	3.05±0.06
Biogas digestate	127.8±5.25	38.3±0.45	4644.2±152.13	3470.6±194.14	336888.2±16507.01	3.06±0.06
Genotype (G)						
Atlas	107.1±3.22c	38.6±0.46a	4034.0±117.78b	3848.8±240.25a	362029.3±21291.41a	3.03±0.06a
Duches	131.6±4.31b	37.6±0.46a	4673.8±135.35a	3429.9±292.24a	340347.4±20433.10a	2.79±0.06a
Jessie	154.7±6.29a	33.0±0.45b	4754.2±178.00a	1560.1±127.16b	144865.4±7717.42b	3.14±0.04b

Table 8.31 *continued...*

ANOVA						
Year (Y)	**<0.001**	**<0.001**	**<00.001**	**<0.001**	**<0.001**	**<0.001**
Sowing date (S)	**<0.001**	**<0.001**	**<0.001**	**0.015**	**0.006**	ns
Nitrogen rate (R)	ns	**<0.001**	ns	**<.001**	ns	ns
Nitrogen source (T)	ns	ns	ns	ns	ns	ns
Genotype (G)	**0.008**	**0.002**	**0.020**	**0.007**	**0.004**	**0.013**
Y*S	**<0.001**	**0.006**	ns	ns	*0.099*	**0.005**
Y*R	ns	*0.064*	**ns**	**<0.001**	ns	**ns**
Y*T	ns	**0.013**	ns	**0.024**	**0.025**	ns
Y*G	**<0.001**	**<0.001**	**<0.001**	**0.044**	**0.015**	**<0.001**
S*R	ns	ns	ns	ns	ns	ns
S*T	ns	**0.039**	ns	ns	ns	*0.099*
S*G	**<0.001**	ns	ns	ns	**0.038**	**0.002**
R*T	ns	ns	ns	ns	ns	ns
R*G	ns	ns	ns	ns	ns	ns
T*G	ns	ns	ns	ns	ns	ns
Y*S*R	ns	**0.018**	ns	ns	ns	ns
Y*S*T	ns	ns	ns	ns	ns	ns
Y*S*G	ns	ns	ns	ns	**0.017**	**0.024**
S*R*T	ns	ns	ns	ns	ns	ns
S*R*G	ns	ns	ns	ns	ns	ns
S*T*G	ns	ns	ns	ns	ns	*0.077*
R*T*G	ns	ns	ns	ns	ns	ns
Y*S*R*T	ns	ns	ns	ns	ns	ns
Y*S*R*G	ns	ns	ns	ns	ns	ns
Y*S*T*G	ns	ns	ns	ns	ns	*0.061*
Y*S*R*T*G	ns	ns	ns	ns	ns	ns

Table 8.32 Interaction between year and sowing date on plants/m^2 of quinoa at harvest. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Mid-April	Early-May
2017	96.8±2.92bA	105.8±2.85bA
2018	210.0±5.37aA	112.0±3.14bA

Table 8.33 Interaction between year and genotype on plants/m^2 of quinoa at harvest. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Duches	Jessie	Atlas
2017	98.8±2.63bA	99.9±3.19bA	105.3±2.88aA
2018	164.4±4.39aB	209.6±6.16aA	109.0±3.57aC

Table 8.34 Interaction between sowing date and genotype on plants/m^2 of quinoa at harvest. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Duches	Jessie	Atlas
mid-April	144.8±5.48aB	197.1±7.16aA	118.4±3.12aB
early-May	118.4±2.44aA	112.4±3.15bA	95.9±3.17aA

Table 8.35 Interaction between year and sowing date on panicles/plant of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	mid-April	early-May
2017	38.2±0.47aA	39.5±0.41aA
2018	31.5±0.42bB	36.5±0.46bA

Table 8.36 Interaction between year and genotype on panicles/plant of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Duches	Jessie	Atlas
2017	41.0±0.44aA	37.8±0.32aB	37.8±0.51aB
2018	34.3±0.34bB	28.4±0.29bC	39.4±0.41aA

Table 8.37 Interaction between year and genotype on panicles/m^2 of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Duches	Jessie	Atlas
2017	3851.0±116.34bA	3645.4±136.08bA	3916.6±112.59aA
2018	5496.6±128.12aA	5863.0±180.71aA	4151.5±123.95aB

Table 8.38 Interaction between year and nitrogen rate on seeds/plant of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Zero-N	75 kg/ha	150 kg/ha
2017	1642.0±102.84aC	3395.3±334.76aB	7039.1±449.42aA
2018	1094.9±63.29bA	1564.5±80.31bA	1893.1±124.64bA

Table 8.39 Interaction between year and nitrogen source on seeds/plant of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Mineral N	Biogas digestate
2017	7039.1±449.42aA	4593.8±259.23aB
2018	1893.1±124.64bA	2347.3±243.72bA

Table 8.40 Interaction between year and genotype on seeds/plant of quinoa at harvest. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Duches	Jessie	Atlas
2017	5320.5±361.80aA	2245.8±160.49aB	4936.3±284.31aA
2018	1539.2±65.05bB	874.4±45.28bC	2761.3±154.10bA

Table 8.41 Interaction between year and nitrogen source on seeds/m^2 of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Mineral N	Biogas digestate
2017	598157.9±44840.05aA	408102.4±25683.59aB
2018	227957.1±13995.49bA	265674.0±18539.78bA

Table 8.42 Interaction between year and genotype on seeds/m^2 of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Duches	Jessie	Atlas
2017	457614.2±25443.45aA	168854.1±8619.15aB	464875.5±25584.14aA
2018	223080.6±7297.68bAB	120876.7±6368.75aB	259183.1±12014.78bA

Table 8.43 Interaction between sowing date and genotype on seeds/m^2 of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Duches	Jessie	Atlas
Mid-April	296477.4±19021.98bA	105635.5±6592.50bB	327976.1±19941.33bA
Early-May	384217.4±21586.12aA	184095.3±7812.12aB	396082.5±22416.79aA

Table 8.44 Interaction between year and sowing date on TGW (g) of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Mid-April	Early-May
2017	3.55±0.03aB	3.77±0.01aA
2018	2.36±0.03bA	2.29±0.04bA

Table 8.45 Interaction between year and genotype on TGW (g) of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Duches	Jessie	Atlas
2017	3.72±0.02aA	3.64±0.03aA	3.62±0.02aA
2018	2.34±0.04bB	1.97±0.01bC	2.67±0.03bA

Table 8.46 Interaction between sowing date and genotype on TGW (g) of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Duches	Jessie	Atlas
mid-April	3.1±0.05aA	2.7±0.06aA	3.0±0.05aA
early-May	3.0±0.07aAB	2.8±0.07aB	3.3±0.04aA

Table 8.47 Effects of year, sowing date, nitrogen rate, nitrogen source and genotype on protein and ash content of quinoa. Means followed by the same lowercase letter within each column within each trait are not significantly different at $p \leq 0.05$.

	Protein (%)	Ash (%)
Year (Y)		
2017	12.9±0.12	3.6±0.03
2018	13.6±0.19	4.2±0.05
Sowing date (S)		
Mid-April	12.8±0.16	3.9±0.04
Early-May	13.7±0.14	3.9±0.06
Nitrogen rate (R)		
Zero	13.1±0.13a	3.9±0.04a
75 kg N/ha	13.0±0.20a	4.0±0.05a
150 kg N/ha	14.0±0.16a	3.8±0.05a
Nitrogen source (T)		
Mineral N	14.0±0.16	3.8±0.05
Biogas digestate	12.9±0.12	3.9±0.06
Genotype (G)		
Atlas	13.8±0.16a	4.0±0.05a
Duches	12.6±0.16b	3.9±0.05a
Jessie	13.4±0.14ab	3.8±0.04a
ANOVA		
Year (Y)	**0.023**	**<0.001**
Sowing date (S)	**0.002**	ns
Nitrogen rate (R)	ns	ns
Nitrogen source (T)	**<0.001**	ns
Genotype (G)	**0.062**	ns
Y*S	ns	**<0.001**
Y*R	ns	**0.046**
Y*T		ns
Y*G	**<0.001**	*0.070*
S*R	ns	ns
S*T	ns	ns
S*G	**0.002**	**<0.001**
R*T	ns	ns
R*G	ns	ns
T*G	ns	ns

Table 8.47 ANOVA *continued...*

	Protein (%)	Ash (%)
Y*S*R	ns	ns
Y*S*T	ns	ns
Y*S*G	**0.007**	**0.002**
Y*T*G	ns	*0.070*
S*R*T	ns	ns
S*R*G	ns	ns
R*T*G	ns	ns
Y*S*R*T	ns	ns
Y*S*R*G	ns	ns
Y*S*R*T*G	ns	ns

Table 8.48 Interaction between year and genotype on protein content (%) of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Duches	Jessie	Atlas
2017	12.1±0.08bA	14.0±0.12aA	12.7±0.09bA
2018	13.1±0.20aB	12.8±0.14bB	14.8±0.19aA

Table 8.49 Interaction between sowing date and genotype on protein content (%) of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Duches	Jessie	Atlas
Mid-April	11.9±0.19bA	13.7±0.15aA	12.9±0.12bA
Early-May	13.2±0.10aA	13.1±0.12aA	14.7±0.18aA

Table 8.50 Interaction between year, sowing date and genotype on protein content (%) of quinoa. Means followed by the same lowercase letter within a column for each year and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

		Duches	Jessie	Atlas
2017	Mid-April	11.7±0.08bA	13.7±0.14aA	12.3±0.08bA
	Early-May	12.5±0.08bA	14.3±0.11aA	13.2±0.10bA
2018	Mid-April	12.2±0.26bB	13.6±0.18aA	13.5±0.14bAB
	Early-May	13.9±0.09aA	11.9±0.05bB	16.3±0.18aA

Table 8.51 Interaction between year and sowing date on ash content (%) of quinoa at harvest. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at p≤0.05 by Tukey's test.

	Mid-April	Early-May
2017	3.55±0.03aB	3.77±0.01aA
2018	2.36±0.03bA	2.29±0.04bA

Table 8.52 Interaction between year and nitrogen rate on ash content (%) of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at *p*≤0.05 by Tukey's test.

	Zero	75 kg/ha	150 kg/ha
2017	3.6±0.03aA	3.6±0.04bA	3.5±0.04bA
2018	4.1±0.05aA	4.4±0.06aA	4.2±0.05aA

Table 8.53 Interaction between sowing date and genotype on ash content (%) of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at *p*≤0.05 by Tukey's test.

	Duches	Jessie	Atlas
Mid-April	3.8±0.03aA	3.9±0.05aA	3.9±0.03aA
Early-May	4.0±0.06aAB	3.6±0.03aB	4.1±0.07aA

Table 8.54 Interaction between year, sowing date and genotype on ash content (%) of quinoa. Means followed by the same lowercase letter within a column for each year and uppercase letter within each row are not significantly different at *p*≤0.05 by Tukey's test.

		Duches	Jessie	Atlas
2017	Mid-April	3.7±0.02bA	3.8±0.04aA	3.9±0.02bA
	Early-May	3.3±0.02cA	3.2±0.03bA	3.4±0.02cA
2018	Mid-April	3.9±0.04bA	4.1±0.05aA	3.9±0.03bA
	Early-May	4.8±0.03aA	3.9±0.02aB	4.9±0.06aA

For example, while grain concentration of Na and Zn increased by at least 42 and 32%, respectively, in the early-May compared with mid-April sown plots, grain concentration of Al and Mn decreased by 26 and 30%, respectively (**Table 8.55**).

The effect of nitrogen rate was not statistically significant on grain concentration of minerals whereas the effect of nitrogen source was only significant on grain concentration of Cu, Mg and P, showing that grain concentration of these minerals increased at least by 9% with

application of biogas digestate compared with application of mineral N at the rate of 150 kg/ha (**Table 8.55**). Only grain concentration of Ca and Mn varied significantly among genotypes whereby the genotype Duches had the highest concentration of Al and Jessie the highest concentration of Mn (118.8 and 145.3 mg/kg, respectively).

Significant year × sowing date, year × genotype and sowing date × genotype interactions on grain concentration of minerals showed that it was generally higher in the early-May sown plots in 2017 (**Table 8.56 – 8.60**). For example, with respect to grain concentration of Zn, the year × sowing date interaction showed that early sowing date resulted in the highest concentration of 64.2 mg/kg in 2017 whereas the year × genotype interaction showed that 54.2 mg/kg was obtained from Jessie in 2017. With respect to grain concentration of K, P and Cu, the sowing date × genotype interaction showed that the highest concentrations of 16.5, 8.4 and 0.01 mg/g, respectively, were obtained from Atlas sown early-May.

Total polyphenols, antioxidants and flavonoids

Concentration of total polyphenols was significantly affected only by sowing date and nitrogen rate, with an average of 1742 µg/g across all treatments (**Table 8.61**). Grain concentration of total polyphenols decreased by 9% in the early-May compared with mid-April treatment and was highest with application of mineral nitrogen at the rate of 75 kg/ha. No significant interaction effects on polyphenols was evident except for a trend ($p>0.05$ and<0.1) suggesting that the highest concentrations of polyphenols were obtained from Atlas sown mid-April in 2017 (**Table 8.62, 8.63**).

All main factors (i.e. year, sowing date, nitrogen rate, nitrogen source and genotype) significantly affected concentrations of total antioxidants in the grain (**Table 8.61**). The average grain concentration of total antioxidants was 1494.8 µg/g across all treatments; it was 26% higher in 2018 than 2017, 57% higher in the mid-April than early-May sown plots, 19% higher at 75 than 150 kg N/ha or zero-N and 18% higher with application of biogas digestate than mineral nitrogen at the rate of 150 kg/ha. Significant year × sowing date and year × genotype interactions showed that the highest concentration of total antioxidants was obtained from Jessie sown mid-April in 2017.

Only year and sowing date had significant effects on grain concentration of total flavonoids (**Table 8.61**). The average grain concentration of total flavonoids was 422.4 µg/g across all treatments; it was generally 24% higher in 2018 than 2017 and 60% higher in the mid-April than early-May sown plots.

Table 8.55 Effects of year, sowing date, nitrogen rate, nitrogen source and genotype on grain concentrations of minerals of quinoa. Means followed by the same lowercase letter within each column for each trait are not significantly different at $p \leq 0.05$.

	Al	Ca	Cd	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	P	S	Zn
	(mg/kg)					(mg/g)		(mg/kg)				(mg/g)		(mg/kg)
Year (Y)														
2017	13.2	112.9	0.09	13.7	96.4	13.6	3.35	114.1	0.33	59.2	2.54	7.99	2.40	47.9
2018	11.9	100.0	0.04	8.84	172.2	11.9	2.53	66.4	0.19	36.8	1.20	4.73	1.70	43.7
Sowing date (S)														
Mid-April	14.4	87.6	0.06	8.94	143.9	10.2	2.55	105.8	0.20	35.4	1.84	4.96	1.67	37.0
Early-May	10.6	126.1	0.07	13.7	124.0	15.3	3.34	74.4	0.32	60.6	1.82	7.90	2.45	55.0
Nitrogen rate (R)														
Zero – N	13.3a	109.0a	0.07a	11.4a	145.7a	12.3a	2.87a	58.9a	0.22a	42.3a	1.71a	6.49a	2.05a	46.8a
75 kg N/ha	13.2a	110.1a	0.06a	12.0a	139.5a	13.1a	2.38a	182.5a	0.25a	53.3a	1.80a	6.64a	2.10a	47.9a
150 kg N/ha	11.4a	103.8a	0.06a	10.4a	122.2a	12.9a	2.70a	57.4a	0.30a	45.5a	1.82a	6.16a	2.04a	43.6a
Nitrogen source (T)														
Mineral N	11.4	103.8	0.06	10.4	122.2	12.9	2.70	57.4	0.30	45.5	1.82	6.16	2.04	43.6
Biogas digestate	12.3	103.3	0.07	11.4	129.2	12.7	2.80	60.7	0.27	50.2	2.00	6.34	2.02	44.9
Genotype (G)														
Atlas	14.1a	104.0a	0.07a	11.5a	129.5a	13.4a	2.79a	61.1a	0.29a	44.7a	1.89a	6.59a	2.09	47.4a
Duches	13.1a	118.8a	0.06a	11.3a	141.2a	12.8a	2.88a	66.0a	0.26a	50.2a	1.82a	6.18a	2.07a	43.9a
Jessie	10.5a	96.4b	0.06a	11.0a	131.3a	12.0a	3.15a	145.3a	0.24a	48.8a	1.79a	6.46a	2.00a	46.3a

Table 8.55 *continued...*

	Al	Ca	Cd	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	P	S	Zn
ANOVA														
Year (Y)	ns	**<0.001**	**<0.001**	**<0.001**	**0.008**	**<0.001**	**0.005**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	ns
Sowing date (S)	**0.018**	**<0.001**	**0.014**	**<0.001**	ns	**<0.001**	**0.006**	**<0.001**	**0.002**	**<0.001**	ns	**<0.001**	**<0.001**	**<0.001**
Nitrogen rate (R)	ns	ns	ns	ns	ns	ns	Ns	ns	ns	ns	ns	ns	ns	ns
Nitrogen type (T)	ns	ns	*0.060*	**0.042**	ns	ns	**0.041**	ns	ns	ns	ns	**0.023**	ns	ns
Genotype (G)	ns	**0.010**	ns	ns	ns	ns	ns	**0.046**	ns	ns	ns	ns	*0.083*	ns
Y*S	**<0.001**	**<0.001**	**<0.001**	**<0.001**	*0.078*	**<0.001**	**<0.010**	ns	**0.051**	**<0.001**	**0.022**	**<0.001**	**<0.001**	**<0.001**
Y*R	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Y*T	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*0.098*	ns
Y*G	**0.026**	*0.084*	ns	**<0.001**	ns	ns	ns	ns	ns	**<0.001**	ns	**<0.001**	**<0.001**	**<0.001**
S*R	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
S*T	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*0.062*	ns	ns	ns
S*G	**0.040**	**<0.001**	**0.015**	**0.013**	ns	**<0.001**	ns	**0.001**	**0.057**	ns	ns	**0.002**	**<0.001**	ns
R*T	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
R*G	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
T*G	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Y*S*R	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Y*S*T	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Y*S*G	*0.060*	ns	ns	**<0.001**	*0.076*	ns	ns	ns	ns	*0.060*	ns	**0.036**	**0.015**	**<0.001**
S*R*T	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
S*R*G	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
S*T*G	ns	ns	**0.032**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
R*T*G	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Y*S*R*T	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Y*S*R*G	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Y*S*T*G	ns	ns	*0.089*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Y*S*R*T*G	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table 8.56 Interaction between year and sowing date on Zn concentration (mg/kg) of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Mid-April	Early-May
2017	31.9±0.68bB	64.2±1.35aA
2018	42.0±0.48aA	45.6±0.94bA

Table 8.57 Interaction between year and genotype on Zn concentration (mg/kg) of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Duches	Jessie	Atlas
2017	46.6±1.75aB	54.2±1.56aA	43.2±1.35bA
2018	41.2±0.49aB	38.4±0.45bB	51.7±0.87aA

Table 8.58 Interaction between sowing date and genotype on K concentration (mg/g) of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Duches	Jessie	Atlas
Mid-April	9.85±0.17bA	10.4±0.15bA	10.4±0.09bA
Early-May	15.7±0.23aA	13.7±0.22aB	16.5±0.21aA

Table 8.59 Interaction between sowing date and genotype on P concentration (mg/g) of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Duches	Jessie	Atlas
Mid-April	4.59±0.07bA	5.35±0.13bA	4.93±0.05bA
Early-May	7.73±0.23aA	7.64±0.24aA	8.36±0.19aA

Table 8.60 Interaction between sowing date and genotype on Cu concentration (mg/kg) of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Duches	Jessie	Atlas
Mid-April	8.53±0.00bA	9.48±0.00bA	8.80±0.00bA
Early-May	14.1±0.00aAB	12.7±0.00aB	14.3±0.00aA

Table 8.61 Effects of year, sowing date, nitrogen rate, nitrogen source and genotype on grain concentrations of total polyphenols, total antioxidants and total flavonoids of quinoa. Means followed by the same lowercase letter within each column for each trait are not significantly different at p≤0.05.

	Polyphenols (µg/g)	Antioxidants (µg/g)	Flavonoids (µg/g)
Year (Y)			
2017	1764.1±28.99	1268.7±87.29	366.1±29.35
2018	1719.9±34.88	1721.2±33.03	478.7±19.84
Sowing date (S)			
Mid-April	1827.0±29.94	2089.2±43.15	603.6±21.20
Early-May	1657.0±33.01	900.6±60.58	241.2±22.22
Nitrogen rate (R)			
Zero – N	1675.5±35.99b	1469.3±65.30b	422.9±21.95a
75 kg N/ha	1876.4±38.22a	1614.3±73.32a	445.0±28.08a
150 kg N/ha	1654.4±31.13b	1308.6±59.09b	373.6±23.11a
Nitrogen source (T)			
Mineral N	1654.4±31.13	1308.6±59.09	373.6±23.11
Biogas digestate	1761.7±16.74	1587.5±72.49	448.1±27.95
Genotype (G)			
Atlas	1780.0±30.80a	1506.7±67.43a	407.2±26.48a
Duches	1759.9±24.91a	1487.5±66.84a	435.6±24.72a
Jessie	1686.1±38.91a	1490.6±70.23a	424.3±25.07a
ANOVA			
Year (Y)	ns	**<0.001**	**0.002**
Sowing date (S)	**0.008**	**<0.001**	**<0.001**
Nitrogen rate (R)	**0.054**	**0.008**	ns
Nitrogen source (T)	ns	**0.006**	ns
Genotype (G)	ns	ns	ns
Y*S	*0.074*	**<0.001**	**<0.001**
Y*R	ns	ns	**0.048**
Y*T		ns	ns
Y*G	*0.062*	**<0.001**	**<0.001**
S*R	ns	ns	ns
S*T	ns	ns	ns
S*G	ns	ns	ns

Table 8.61 ANOVA *continued...*

	Polyphenols (µg/g)	Antioxidants (µg/g)	Flavonoids (µg/g)
R*T	ns	ns	ns
R*G	*0.071*	ns	*0.069*
T*G	ns	ns	ns
Y*S*R	ns	**0.016**	ns
Y*S*T	ns	**0.041**	ns
Y*S*G	ns	ns	ns
S*R*T	ns	ns	ns
S*R*G	ns	ns	ns
R*T*G	ns	ns	ns
Y*S*R*T	ns	ns	ns
Y*S*R*G	ns	ns	*0.093*
Y*S*R*T*G	ns	ns	ns

Significant year × sowing date and year × nitrogen fertiliser rate interactions showed that while mid-April resulted in 513.7 µg/g in 2017, a concentration of 527 µg/g was obtained at zero-N in 2018. Although ANOVA indicated that the year × genotype interaction was statistically significant (p<.000), Tukey's test showed that the interaction was not statistically significant (p = 0.318) (**Table 8.64 – 8.66**).

Table 8.62 Interaction between year and sowing date on the concentration of total antioxidants (µg/g) of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Mid-April	Early-May
2017	2366.2±50.83aA	171.3±2.40bB
2018	1812.3±19.06bA	1630.0±41.87aA

Table 8.63 Interaction between year and genotype on the concentration of total antioxidants (µg/g) of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Duches	Jessie	Atlas
2017	1250.4±1.75aA	1285.2±1.56aA	1270.6±1.35bA
2018	1724.6±0.49aA	1696.1±0.45aA	1742.9±0.87aA

Table 8.64 Interaction between year and sowing date on the concentration of total flavonoids (µg/g) of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Mid-April	Early-May
2017	714.0±21.28aA	18.2±1.00bB
2018	493.2±18.05bA	464.2±21.62aA

Table 8.65 Interaction between year and genotype on the concentration of total flavonoids (µg/g) of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Duches	Jessie	Atlas
2017	357.6±85.66aA	368.9±92.08aA	371.8±86.78aA
2018	513.7±33.72aA	479.8±33.04aA	442.6±33.29aA

Table 8.66 Interaction between year and nitrogen rate on the concentration of total flavonoids (µg/g) of quinoa. Means followed by the same lowercase letter within each column and uppercase letter within each row are not significantly different at $p \leq 0.05$ by Tukey's test.

	Zero-N	75 kg/ha	150 kg/ha
2017	318.5±26.45bA	439.7±43.62aA	350.2±32.95aA
2018	527.2±21.35aA	450.2±15.67aA	397.0±19.01aA

8.2.5. Correlation coefficients

Correlation tests indicated that plant height, chlorophyll content, crop and above-ground biomass showed a strong positive correlation with grain yield. The tests also indicated that grain Zn concentration showed a strong negative correlation with total antioxidants and flavonoids (**Table 8.67 – 8.69**).

Table 8.67 Correlation coefficients for growth traits of quinoa in 2017-18. Stars in the shaded area represent significance at p<0.05. Significance codes: 0.001 '***', 0.01 '**', 0.05 '*', ns 'not significant'.

	Height	SPAD-GS50	NDVI-GS50	Biomass	Yield	HI
Height	1.00	***	***	***	***	**
SPAD-GS50	**0.53**	1.00	***	***	**	ns
NDVI-GS50	**0.77**	**0.57**	1.00	***	***	ns
Biomass	**0.79**	**0.44**	**0.76**	1.00	***	ns
Yield	**0.58**	**0.42**	**0.57**	**0.54**	1.00	***
HI	**0.29**	-0.09	0.10	0.13	**0.32**	1.00

Table 8.68 Correlation coefficients for yield traits of quinoa in 2017-18. Stars in the shaded area represent significance at p<0.05. Significance codes:', 0.001 '***', 0.01 '**', 0.05 '*', ns 'not significant'.

	Plants/m^2	Panicles/m^2	Seeds/m^2	Biomass	TGW	Yield	HI
Plants/m^2	1.00	***	**	***	***	***	ns
Panicles/m^2	**0.90**	1.00	ns	**	***	ns	ns
Seeds/m^2	**-0.22**	0.04	1.00	***	***	***	***
Biomass	**-0.46**	**-0.21**	**0.60**	1.00	***	***	ns
TGW	**-0.41**	**-0.27**	**0.35**	**0.59**	1.00	***	ns
Yield	**-0.26**	-0.14	**0.91**	**0.54**	**0.38**	1.00	***
HI	-0.02	0.10	**0.49**	0.13	0.01	**0.32**	1.00

Table 8.69 Correlation coefficients for quality traits of quinoa in 2017-18. Stars in the shaded area represent significance at p<0.05. Significance codes: 0.001 '***', 0.01 '**', 0.05 '*', ns 'not significant'.

	Yield	Protein	Fe	Zn	Phenols	Antioxidants	Flavonoids
Yield	1.00	ns	ns	ns	ns	***	**
Protein	-0.03	1.00	ns	***	ns	*	*
Fe	-0.09	0.06	1.00	ns	ns	ns	ns
Zn	0.11	**0.31**	0.10	1.00	ns	***	***
Phenols	-0.02	0.03	-0.01	-0.03	1.00	***	***
Antioxidants	**-0.28**	**-0.13**	0.01	**-0.56**	**0.39**	1.00	***
Flavonoids	**-0.19**	**-0.14**	-0.08	**-0.51**	**0.26**	**0.68**	1.00

8.3. Discussion

8.3.1. Crop growth

As in the case of other crop species, quinoa crop growth was affected by environmental conditions and management practices. Although temperatures were higher over the germination period in 2018 (i.e. 9 and 11ºC for mid-April and early-May sowing date, respectively) compared with 2017 (i.e. 7 and 10ºC for mid-April and early-May sowing date, respectively), seed germination was not significantly different between years. There was a significant difference in germination % between sowing dates whereby delayed sowing from mid-April to early-May resulted in approximately 30% lower seed germination. This result is consistent with the previous experiment (**Chapter 7**) with the same genotypes where early-May sowing resulted in 35% lower seed germination compared with the mid-April sowing date. The reason

that delayed sowing resulted in lower seed germination was most likely due to lack of moisture (Bhargava and Srivastava, 2013) rather than temperature constraints, although the optimal temperature for quinoa seed germination is 18 – 35ºC (Boero *et al.,* 2000; Bois *et al.,* 2006; Jacobsen, 2015). Moreover, despite registering the lowest temperature over the 14-days germination period (with a minimal and maximal temperatures of -1.4 and 15.5ºC in 2017), mid-April sowing date still resulted in much higher seed germination than the early-May sowing (with a minimal and maximal temperatures of 3 and 18.3ºC in 2017). Therefore, under local weather conditions optimum seed germination of quinoa was obtained by sowing mid-April.

The present study showed that crop growth was significantly affected by year of cultivation. In 2017, the weather was wetter and with relatively lower temperature particularly in June – July (i.e. stem elongation and beginning of flowering). In contrast, in 2018, the same period was characterised by limited water availability (due to no rainfall for approximately 30 days from June 19th to July 17th) combined with relatively high temperatures. Thus, crop growth/development was negatively impacted in 2018 compared with 2017 due to reduced water availability which is likely to have been a factor contributing to the much lower yields in 2018 compared with 2017.

Despite lower seed germination, early-May sowing had a positive effect on the growth/development because it was correlated with higher yields compared to mid-April sowing. Thus, early-May sowing with higher temperatures shortened the length of the growth cycle (by reducing the time from sowing to seedling emergence, flowering and physiological maturity) due to rapid growth. For example, De Santis *et al*. (2016) found that 4-days increase in growth cycle (115 vs 111 days) resulted in approximately 5% increase in seed yield of quinoa (2.18 vs 2.30 t/ha) grown in Italy; Basra *et al*. (2014) observed that 1-day difference (113 vs 112 days) resulted in approximately 12% increase in seed yield of quinoa (1.78 vs 2.02 kg/ha) grown in India.

Early-May sowing resulted in a growth cycle of approximately 150 days, with a pre-flowering period of about 50 days with a further 100 days to physiological maturity. The length of the growth cycle was similar to, or shorter than, for quinoa grown in environments with similar temperature such as the cool-temperate climate of southern Chile (Miranda *et al*., 2013) or even in environments with much higher temperatures such as the Indo-Gangetic plains of north India (Bhargava *et al*., 2007). However, the growth cycle was up to 40 days longer than for quinoa grown in Italy (De Santis *et al*., 2016) and Morocco (Hirich *et al*., 2014). Therefore, it is

desirable that growth cycle of quinoa grown in NE-England should be shorter than 150 days long to maximise the potential for cultivation as a spring crop because extended growth cycles would hamper seed maturation later in the season due to low temperatures and relatively high rainfall and thus negatively affect yields.

The effect of nitrogen rate and source on duration of the phenological phases particularly during vegetative growth (stem elongation to flowering) was not statistically significant except at the reproductive stages (seed setting and maturation). While, nitrogen source (mineral N vs biogas digestate) did not affect crop development, higher nitrogen rate (i.e. 150 kg N/ha) extended the duration of seed setting and maturation by 1-2 weeks in both seasons. Similar results were published by Basra *et al*. (2014) who showed that nitrogen application had little effect on the duration of phenological phases particularly the earlier growth stages of quinoa.

The results also indicated that the effect of quinoa genotype on the duration of phenological phases was clearer from flowering to seed maturation. The genotype Jessie senesced earlier irrespective of sowing date and nitrogen application, whereas Atlas and Duches senesced much later. These differences in crop maturation among genotypes were attributed to differential phenology behaviour (Basra *et al*., 2014), thus indicating that Jessie can be classified as an early maturing and Atlas a late maturing genotype with Duches an intermediate maturing type.

Plant height varied significantly with year of cultivation. Plant height also increased significantly with delayed sowing and nitrogen fertilisation (i.e. rate and type) with significant differences among genotypes. Plant height decreased by 37% in 2018 compared with 2017. This was possibly due to reduced water availability over the vegetative period in 2018, which likely resulted in lower rates of cell division and expansion (Basra *et al*., 2014).

In accordance with Hirich *et al*. (2014), the present study found that quinoa height was significantly different between sowing dates. Early-May sowing increased plant height significantly in both years: 15 and 21 cm in 2017 and 2018, respectively. Such increase was also associated with higher chlorophyll concentration with potential for increased growth (Basra *et al*., 2014). The results also suggested a positive correlation between plant height and seed yield whereby the increase in plant height from the early-May sowing correlated with increasing seed yield, thus suggesting that the taller the crop the higher the yield. However, this is contrary to a previous study (De Santis *et al*., 2016) which showed significant negative correlations between seed yield and plant height in quinoa. The reason for this difference is most likely due to the response of different genotypes to the contrasting environments. So, when comparing different genotypes grown in different environments plant height should not be

taken as an indicator of productivity unless it is associated with panicle length in that the longer the panicle the more seeds it can produce (Bhargava and Srivastava, 2013; Tapia, 2015).

Plant height increased significantly with increasing nitrogen rate and with the use of mineral nitrogen due to higher nitrogen availability which is supported by the SPAD data in this and other studies (Basra *et al*., 2014; Alandia *et al*., 2016). The taller plants with increasing N rate might have also improved the yield potential by increasing the panicle length which is associated with higher seed production. In fact, the results of the current study showed that the taller the crop the higher the number of seeds as opposed to other studies which showed a negative correlation between seed number and plant height (De Santis *et al*., 2016).

On average across all treatments, total above-ground biomass (i.e. 541.1 g/m^2) was much higher than that of quinoa grown in NW-Argentina (i.e. 408 g/m^2) published by Curti *et al*. (2014). In the present study, the highest impact on total above-ground biomass was caused by year of cultivation with a 50% difference between years. However, the early-May sowing and higher nitrogen rate significantly increased total above-ground biomass with increased leaf area, stem diameter and plant height. Therefore, these results suggest that low temperature and mid-April sowing are the limiting factors for quinoa above-ground biomass production in NE-England.

The results also showed significant differences in above-ground biomass between genotypes with Duches and Jessie showing a more variable response to sowing date while Atlas was more stable and produced the highest total above-ground biomass. These differences in total above-ground biomass correlated with differences in seed yield and harvest index (HI), whereby Atlas produced the highest yields and Duches the highest HI.

8.3.2. Yield and yield components

The average yield obtained in the present study was 1.02 and 2.28 t/ha from the combine and biomass samples, respectively. The large differences could reflect the differences in harvest area (12.6 m^2 vs 0.25 m^2, respectively) but also the key challenge in combining this crop with very small seeds and therefore high potential losses at the cutter bar. These yields are higher than the current average quinoa seed yield of 0.9 t/ha worldwide but similar to those published in several previous studies (Hirich *et al*., 2014; Curt *et al*., 2014; Garrido *et al*., 2016; Alandia *et al*., 2016; De Santis *et al*., 2016).

As in the case of wheat and barley grown in the UK, quinoa yield was affected by weather conditions in 2018 (i.e. high rainfall in spring and a long dry spell with high temperatures in the summer), which caused decrease in yields relative to 2017. However, quinoa yields were

more affected by the weather conditions in 2018 than those of wheat and barley. For example, whilst yields of wheat and barley decreased by 5.1 and 7.9% (DEFRA, 2019) respectively, the present study showed that quinoa yields decreased approximately by 50% in comparison with yields in 2017.

The present study found that quinoa seed yield was improved by early-May sowing, nitrogen rate, nitrogen source and genotype. The results showed that early-May sowing, combined with application of 150 kg/ha of N not only maximised seed yield but also TGW and seed number (per panicle, plant and m^2) of quinoa. The results also showed that seed yield of quinoa was improved by genotype choice whereby Atlas and Duches produced higher TGW and total seed number relative to Jessie. Moreover, early-May sown Atlas and Duches combined with higher nitrogen application rate of 150 kg/ha resulted in increased panicle number/m^2. Therefore, these results indicate that early-May sowing dates, higher rates of nitrogen and genotype choice are key to increasing the productivity of quinoa in NE-England.

Seed yield of quinoa also reduced with year of cultivation despite improvements caused by late sowing, nitrogen fertilisation (i.e. rate and source) and choice of genotype. The results showed a 2-fold difference in seed yield associated with 1.5- and 2.3-fold reductions in TGW and total seed number (seeds/m^2), respectively. This difference in seed yield is consistent with the literature (Erley *et al*., 2005; De Santis *et al*., 2016; Noulas *et al*., 2017) which has showed significant variation in grain yield of quinoa between seasons. The most likely reason for the difference in the present study was the drought (or limited water availability) period combined with the relatively high temperatures that occurred from May through July in 2018. All three genotypes were unable to recover from impaired growth and sufficiently produce photoassimilates during and after the drought stress. Previous studies also showed that drought stress significantly reduced growth rates and seed yield of quinoa (Hirich *et al*., 2014, De Santis *et al*., 2016; Garrido *et al*., 2016; Noulas *et al*., 2017). Therefore, the results of the present study suggest that water availability was the key factor limiting growth, yield and nitrogen availability regardless of sowing date and genotype. Nonetheless, there is also evidence showing that seed yield of quinoa may not be affected significantly by drought stress (Martínez *et al*., 2009; Razzaghi *et al*., 2012; Alandia *et al*., 2016) which could be explained by the ability of the crop to cope with drought, depending on the growth stage and root system of the crops.

In the present study, early-May sowing improved seed yield of quinoa by up to 50% relatively to the early mid-April sowing. The improvement was due to increased seed production both per plant and m^2, with a 14 – 29% increase in seed number (i.e. per plant and m^2) in the early-May

compared with mid-April treatment. The increase in seed number in the early-May sowings was also associated with a reduced crop growth cycle with a positive correlation between time to physiological maturity and seed yield. This supports the data of de Vasconcelos *et al*. (2012) who found a 20-day difference in growth cycle resulted in a 3-fold increase of grain yield possibly because of environmental stresses such as extreme temperature and water availability (de Vasconcelos *et al*., 2012; Hirich *et al*., 2014; Noulas *et al*., 2017). These results show that early-May sowing of quinoa in NE-England is the optimum if this coincides with optimal weather conditions in terms of temperature and moisture.

Nitrogen rate and source improved seed yield significantly, especially when nitrogen rate interacted with the year of cultivation. The effects of nitrogen rate on seed yield of quinoa depended on the year of cultivation, whereby it decreased by 2-fold from one to another year. Seed yield increased by up to 149% at 150 kg N/ha relative to zero-N with largest increase of 88% from 75 to 150 kg N/ha. Similar results were also observed in previous studies: Erley *et al*. (2005) observed a 194% increase in yield over zero-N at 120 kg N/ha, with a 17% yield increase from 80 to 120 kg N/ha; Basra *et al*. (2014) observed a 145 and 166% increase in yield over zero-N at 75 and 100 kg N/ha but with 14 and 17% decrease, respectively, compared with 125 kg N/ha.

The effect of nitrogen rate on seed yield of quinoa in the present study was greater than in the studies of Erley *et al*. (2005) and Basra *et al*. (2014). These increments suggest that 150 kg N/ha could be the optimum rate for maximal yields. However, there is evidence showing that some quinoa genotypes grown in Argentina produced yields higher than 3 t/ha at a rate of 200 kg N/ha (De Santis *et al*., 2016), suggesting that the optimum nitrogen fertilisation rate may be different for different quinoa genotypes and in different environments. Therefore, much higher rates (e.g. 200 kg N/ha) should be tested for ascertaining with high degree of confidence about the optimum nitrogen fertilisation rate for optimising quinoa yield under the agroecological conditions of NE-England.

The increments caused by nitrogen application rate observed in the present study were also associated with improvements in TGW and seed number (per m^2) by 6% and 4-fold, respectively. Moreover, the year × nitrogen rate interaction showed that seed yield and TGW, for example, were highest (i.e. 2.24 t/ha and 3.8g, respectively) at the rate of 150 kg N/ha, thus highlighting the potential for optimising quinoa yield in NE-England with higher rates of nitrogen. These results are consistent with the general principle that the more readily available nitrogen, the higher the yield (Buchi *et al*., 2016).

Usually, addition of mineral N results in higher yields than the addition of organic fertilisers (Glaser *et al*., 2015), depending on the type of fertiliser and amount of available nitrogen in the fertiliser. In the present study, the effect of nitrogen source (mineral N vs. biogas digestate) on yield and yield components of quinoa was significant. However, the effect of nitrogen source was not significant with respect to seed number (i.e. seeds/m^2). Although application of mineral N resulted in 47 and 53% higher seed number per panicle and plant, respectively, than application of biogas digestate in 2017, it did not result in a significant difference in the overall seed yield. Nonetheless, the results showed that chlorophyll content (SPAD) was higher with application of mineral N rather than biogas digestate particularly at later growth stages (GS40-50).

Rózyto *et al*. (2017) found that fertilisation with mineral N resulted in higher grain yield of wheat grown in 2016 than using biogas digestate (3.98 vs 3.35 t/ha); Albuquerque et al. (2012) found that yield of watermelon and cauliflower was generally (up to 4 times) higher with application of mineral N than biogas digestate; Lopedota *et al*. (2013) found that yield of melon was not significantly different between mineral N and organic fertiliser treatments. Therefore, the present results suggest that biogas digestate could be an attractive alternative to mineral nitrogen fertilisers for maximising yield potential of quinoa because use of biogas digestate may be more cost-effective and environmentally sustainable.

The three genotypes Atlas, Duches and Jessie showed variation in seed yield in response to sowing date, fertiliser rate and fertiliser type. Overall, genotype choice generally interacted with year of cultivation but also, to a lesser extent, with sowing date. Seed yield of Atlas and Duches was lower in 2018 than 2017 whereas Jessie was not significantly affected by year of cultivation. Nonetheless, Atlas remained a high yielding genotype in comparison with Jessie in both years. These results indicated that, although low yielding, Jessie was the more stable genotype whereas Duches and Atlas were higher yielding genotypes but showed greater variation and required optimal growing conditions to attain the high yield. This points to a high genetic variability and adaptability to agroecological conditions in the UK, with considerable potential for improvement of quinoa lines (De Santis *et al*., 2016), suited to NE-England.

8.3.3. Grain quality

Most quality parameters were strongly influenced by both year of cultivation and sowing date. Overall, grain quality of quinoa improved with delayed sowing from mid-April to early-May. Nitrogen fertilisation rate and genotype choice had no effect on grain quality whereas nitrogen type significantly affected grain concentrations of protein, Cu, Mg and P.

Protein content ranged between 11 and 16% with an average of 14% across all treatments and was significantly influenced by year of cultivation and sowing date. The average protein content of 14% obtained in the present study was similar to those of the major cereals such as wheat and rice, which was also consistent with the worldwide range of 7 – 24% for quinoa (Alejandro *et al*., 2015; Geren, 2015; Navruz-Varli and Sanlier, 2016; Nowak *et al*., 2016) and particularly in the range 10 – 18% for Bolivian quinoa (Rojas *et al*., 2015). Generally, variations in protein content are explained by dilution effects caused by varying crop yields, for which low protein content is generally associated with high yield due to dilution effects (Biondi *et al*., 2015). However, variation in protein content in response to year of cultivation and sowing date (5% and 7% respectively) in the present study, could not be explained by the dilution effect caused by varying yields but it was more likely due to a low accumulation of carbohydrates (Alejandro *et al*., 2015) because high protein content remained positively correlated with high yield. Similar results were published by Bhargava (2007), showing a direct positive correlation between yield and protein content in quinoa.

On average, Atlas had the highest protein content and Duches the lowest but without statistically significant differences. The degree and nature of interaction effects between quinoa genotypes and environment are essential to improving crop quality (Alejandro *et al*., 2015; De Santis *et al*., 2016) as is the case for most arable crops. The year × genotype and sowing date × genotype interactions varied significantly with respect to protein content. Whilst protein content in Atlas and Duches was higher in 2018 than 2017, in Jessie it was the reverse. This could be due to dilution effects because while seed yield of Atlas and Duches decreased 4-fold from 2017 to 2018, seed yield of Jessie increased by 11%.

Grain mineral concentrations were generally influenced by year of cultivation and sowing date but not by nitrogen rate, nitrogen source and genotype. The concentration of minerals in the present study was relatively high compared with several previous studies (Stikic *et al*., 2012; Miranda *et a*l., 2013; Nascimento *et al*., 2014; Nowak *et al*., 2016; Ramzani *et a*l., 2017) most likely due to relatively lower yields.

Except for a few cases, the concentration of minerals showed a positive correlation with seed yield. Thus, suggesting that quinoa might have high nutrient use efficiency particularly zinc as the results indicated that higher yields did not necessarily result in lower concentrations of zinc attributable to higher internal dilution, which points to a selective biochemical mechanism for transport and storage of zinc in quinoa (Miranda *et al*., 2013).

Concentration of all macronutrients was strongly influenced by year of cultivation and sowing date but was not influenced by nitrogen rate and only Ca and Mn concentration was significantly different between genotypes. Overall, grain concentration of macronutrients decreased with the year of cultivation but increased as sowing was delayed. Furthermore, the variation in concentrations of macronutrients matched also the variation in protein content, considering that Ca^{2+}, K^{+}, Mg^{2+} and S^{2-} are essential for protein synthesis and carbohydrate metabolism.

Early-May sowing improved the concentration of macronutrients compared with the mid-April sowing, whereby K was the most abundant macronutrient and Ca the least abundant; which was similar to results of previous studies (Miranda *et al*., 2013; Vilcacundo and Hernadez-Ledesma, 2017). However, the concentrations detected in the present study were higher than the values published by others (Stikic *et al*., 2012; Miranda *et al*., 2013; Nascimento *et al*., 2014; Nowak *et al*., 2016; Ramzani *et al*., 2017). Considering the widespread micronutrient (i.e. Fe and Zn) malnutrition in humans worldwide, these results highlight the importance of early-May sowing in NE-England as an appropriate agronomic practice for improving the nutritional quality (i.e. in terms of grain Fe and Zn concentration) of quinoa under the local weather conditions.

Only grain concentrations of Mg and P were significantly higher with application of biogas digestate than mineral N possibly because biogas digestate (as in the case of other organic fertilisers) enhanced soil organic matter which directly improves P and Mg pools in soils and therefore increased P and Mg availability for plant uptake (Yang *et al*., 2019) especially when compared with mineral N.

Although the effect of genotype was not statistically significant on the concentration of macronutrients, except on Ca, it generally interacted with year of cultivation and sowing date. The results revealed that grain concentration of macronutrients was generally higher in Atlas and lower in Jessie. Differences in grain concentration of macronutrients among genotypes could not be attributed to dilution effects because although Atlas produced up to three times more seed yield than Jessie, Atlas had significantly higher concentration of macronutrients than Jessie. Therefore, these results suggest that Atlas could be used in breeding programs as a high-yielding genotype with the potential for increasing nutritional security as in the case of wheat (Zou *et al*., 2012).

Concentrations of all micronutrients except for Fe, Ni and Zn were affected by both year of cultivation and sowing date. Grain concentration of micronutrients detected in the present study was up to 3 – 4 times higher than the mean values published by previous studies (Stikic *et al*., 2012; Nascimento *et al*., 2014; Vidueiros *et al*., 2015; Nowak *et al*., 2016; Ramzani *et al*.,

2017). These results indicated that mid-April sowing reduced the concentration of micronutrients and resulted in a lower yield. In general, the present study showed that grain micronutrient concentration was not significantly affected by genotype possibly because the present study used three genotypes from the same breeder with the likelihood of little genetic variation in the varieties used.

Early-May sowing had a negative effect on the concentration of total polyphenols compared with mid-April sowing. The average concentration of total polyphenols of 175 mg GA/100g DW detected in the present study was similar to, or higher than, the values published by previous studies (Miranda *et al*., 2013; Tang *et al*., 2015; Pellegrini *et al*., 2018). Miranda *et al*. (2013) reported 12.39 – 31.92 mg GA/100g DW; Tang et al. (2015) reported 200 mg GAE/100g for a white quinoa genotype and Pellegrini *et al*. (2018) reported 75.30 – 87.58 mg/100g FW.

Quinoa showed a significant variation in total polyphenols in response to nitrogen rate. Concentration of polyphenols increased in response to N and was highest at 75 kg N/ha. This suggests that concentration of polyphenols is more dependent on environmental factors than nitrogen availability. Indeed, the results showed that it was not necessarily improved by increasing nitrogen rate in the present study.

Significant differences in total antioxidants were observed between years of cultivation (126.9 and 172.1 mg TE/100g DW, for 2017 and 2018, respectively) and between sowing dates (208.9 and 90.1 mg TE/100g DW, for mid-April and early-May sowing dates, respectively). These results are higher than those published by Hirose *et al*. (2010) who found 502 – 950 µmol TE/100g FW antioxidant capacity by DPPH assay.

Early-May sowing had a strong negative effect on the concentration of total antioxidants compared with the mid-April sowing. This was most likely due a relatively high concentrations of micronutrients, particularly Al, Fe and Mn, in the mid-April sown plots which increased the antioxidative defence mechanism against drought-induced oxidative cell damage (Zou *et al*., 2012) and enhanced the storage of antioxidative compounds in the developing seeds. As reported by previous studies, concentration of total antioxidants can be influenced by environmental conditions and it is regulated by the production and allocation of photoassimilates in the developing seeds. The contrasting weather conditions in both years especially regarding water availability and the higher temperatures in 2018, most likely triggered the differential response for grain antioxidant concentrations. Furthermore, this could be supported by the fact that the highest concentrations of total antioxidants as an antioxidative defence mechanism coincided with optimal environmental conditions for disease infection due

to relatively high temperatures accompanied by high rainfall during critical growth stages in 2017 compared with 2018.

The concentration of total flavonoids detected in the present study was similar to other published studies (Hirose *et al*., 2010; Tang *et al*., 2015). For example, Hirose *et al*. (2010) reported 3.3 – 113.3 mg/100g FW for flavonol glycosides of quinoa seeds grown in South America. Overall, the concentration of total flavonoids was 42.2 mg/100g DW on average across all treatments and it was significantly influenced by year of cultivation and sowing date. It showed a strong correlation with the concentration of total polyphenols and total antioxidants. Therefore, this indicates that concentration of total flavonoids in quinoa is primarily controlled by the same mechanisms and factors that control the concentration of total antioxidants However, it is described in the literature that concentrations of flavonoids may be more related to the concentration of total polyphenols than total antioxidants (Tang *et al*., 2015; Ma *et al*., 2015).

CHAPTER 9 – General Discussion

Considering that some genotypes were not cultivated in some years (i.e. Duches in 2016, Zita and Zamira in 2016 and 2018), the following discussion focuses mainly on results for the genotypes Bamby and Cebelica (buckwheat) Atlas and Jessie (Quinoa) cultivated over three consecutive years (2016, 2017 and 2018).

9.1 Crop growth

The effects of sowing date, nitrogen fertilisation and genotype on crop growth and development (i.e. time to physiological maturity, plant height and above-ground biomass) were generally similar to those of buckwheat and quinoa published from studies carried out in temperate and tropical environments (Basra *et al*., 2014; Hirich *et al*., 2014; Mariotti *et al*., 2016; Tummaramatti *et al*., 2016; Kasajima *et al*., 2017). The results showed that sowing date had a significant effect on growth, development, yield and quality of both crops. Temperature, particularly low temperature, was the key environmental factor influencing the growth and development of buckwheat and quinoa. However, water availability, particularly limited availability towards the end of seedling emergence, may have affected subsequent growth.

Seed germination of both buckwheat and quinoa was low (i.e. approximately 58% across the three years). Seed germination was significantly affected by sowing date although the effect was greater in quinoa than buckwheat. Seed germination % of both crops decreased with the late sowing i.e. early-May relatively to mid-April but the decrease was greater in quinoa than buckwheat. Hence, quinoa had lower plant population at harvest than buckwheat. But despite the lower plant population at harvest, quinoa still produced higher yields than buckwheat, suggesting that while increasing plant population could potentially increase yields of both crops, yet it was not the major factor for the higher yield of quinoa than buckwheat. Therefore, it appeared that higher seed germination % and plant population would be more beneficial to buckwheat than quinoa.

In the present study, poor seed germination and plant population of buckwheat and quinoa when sown early-May compared to mid-April was attributed to the low temperature and limitation in soil moisture during early growth particularly in 2016 and 2018. For example, the results showed that the average temperature during the germination period was lower than the optimal temperature for buckwheat germination i.e. 10°C (Farooq *et al*., 2016; Nurse *et al*., 2016). In accordance with Noulas *et al*. (2017) who investigated adaptation of quinoa under Mediterranean conditions, sowing in May resulted in poor seed germination and plant

population likely because of limited water availability causing the soil surface to dry out quickly hence imposing a water stress which was worse during early seedling growth due to the shallow root system.

Low seed germination and low plant population at harvest usually result in low yields (Stehno *et al*., 2007; Ghiselli *et al*., 2017). To our knowledge, while there is a general agreement on seed rate for buckwheat and quinoa (approximately 300 seeds/m^2), there is no clear evidence pointing to the optimum plant population in terms of yield but plant population at harvest varies in different countries (i.e. 50 – 200 plants/m^2) depending on agroecological conditions, crop species and genotype. For example, Stehno *et al*. (2007) evaluated common and tartary buckwheat genotypes grown in the Czech Republic in 2006 and found that plant population at maturity varied between 59 – 120 plants/m^2, whereby genotypes with lower plant population generally produced lower yields. Fang *et al*. (2018) evaluated the effect of plant population on agronomic traits of common buckwheat and found that higher plant population (90 vs 120 plants/m^2) resulted in slightly lower grain yield (1.3 vs 1.2 t/ha). In those previous studies (Stehno *et al*., 2007; Fang *et al*., 2018), though statistically significant, the effect of plant population on grain yield was not substantive. On the other hand, Bhargava and Srivastava (2013) indicated that while optimal seed rate of quinoa depends on various factors such as genotype, growth habit, sowing date, climatic conditions and soil fertility, plant population equal to or higher than 300 plants/m^2 usually results in relatively low yields. Nonetheless, Jacobsen (2015) acknowledged that plant density does not necessarily correlate with quinoa yields yet suggested that 100 plants/m^2 is recommended. Therefore, optimal plant population for optimal grain yield of buckwheat and quinoa can be different for different regions.

A low seed germination (hence low plant population) generally results in high weed proliferation and lower yield per plant due to increased inter-species competition (Stehno *et al*., 2007; Bhargava and Srivastava, 2013; Ghiselli *et al*., 2017). However, the present study showed that low seed germination and plant population of buckwheat and quinoa at harvest did not necessarily result in lower yields. For example, while seed germination and plant population of buckwheat and quinoa were higher when sown mid-April, grain yield was lower. Therefore, this raises the question of whether buckwheat yields could be significantly increased if seed rate is increased to account for plant losses and achieve a higher plant population at harvest (because of the strong positive correlation between plant population at harvest and seed number), or quinoa yields could be significantly increased if seed rate is reduced (because of the negative correlation between plant population at harvest and seed number).

Considering that a 90 and 10 kg/ha seed rate was used for buckwheat and quinoa, respectively, with target to 250-300 plants/m^2, yet both crops reached only half of that (150 and 120 plants/m^2, respectively), it would be important to determine the optimal seed rate and hence optimal plant population at harvest which could potentially result in higher yields under the local weather conditions. Nonetheless, considering that there was a pattern in the temperature and rainfall behaviour (i.e. warmer and drier conditions) towards the end of the germination period over the three years of the trial which probably affected seedling emergence and plant establishment, suggests a scope for improving seed germination (Sakata an Ohsawa, 2006) as well as plant population at harvest by trying wider sowing date intervals (perhaps mid-May based on the present study) combined with various seed rates which could also be key to increasing grain yield.

Life-cycle of buckwheat and quinoa was relatively long regardless of the harvest dates (5th – 27th October for Bamby, Cebelica and Atlas and 25th September – 27th October for Jessie), as both crops required 150 – 190 days, of which approximately 100 days were from pre-flowering (late-June) to maturity. The key difference between the present study and previous studies (Janovská *et al*., 2007; Stehno *et al*., 2007; Čepková *et al*., 2009; Noulas *et al*., 2017) with respect to the duration of life-cycle is that crops in the present study required more days from sowing to full emergence and from emergence to flowering most likely due to relatively low temperatures while the time from flowering to maturity was similar (approximately 100 days). In general, buckwheat genotypes tended to reach maturity earlier than quinoa genotypes (i.e. Atlas and Duches) which can also explain higher seed losses hence lower yields in the former as they were harvested on the same dates for technical reasons.

Crops were harvested at about 10 – 17% grain moisture content which became a key challenge to pass through the combine cutter bar and thus requiring further drying out of harvested seeds. This fact is likely to limit farmers' interest in growing these crops. However, it could be addressed by using pre-harvest treatment strategies such as swathing and desiccation. In fact, desiccation with glyphosate was carried out later in the season in 2018 but resulted in complete loss of buckwheat seeds (probably due to delayed harvest after desiccation than inappropriateness of the practice per se), thus raising the question regarding the efficiency and appropriateness of swathing and desiccation in protecting yields of buckwheat and quinoa. While swathing could have resulted in problems with drying of the crop due to high rainfall and declining temperatures, desiccation with glyphosate may negatively affect grain quality, with negative impacts on human health widely reported (Torretta *et al*., 2018). Since desiccation with glyphosate may have a negative effect on pesticide residues, it is not allowed

in organic production systems where market demand for organic buckwheat and quinoa is likely to be high. Therefore, the long life-cycle constitutes a major limitation for fitting buckwheat and quinoa into various crop rotations especially in northern England as late harvest dates (particularly in October) limit the sowing date of the next crop in the rotation. Weather conditions in summer in countries with much higher temperatures (such as Iran, Italy, Morocco and Japan) are more favourable for growth and yield as the temperatures are closer to the optimum temperatures of 18 - 25°C for the growth of buckwheat and quinoa (Sobhani *et al*., 2014; Hirich *et al*., 2014, Isobe *et al*., 2016).

Usually when relatively low temperature is not limiting, longer thermal time (and hence longer life-cycle) may increase grain yield (Silva *et al*., 2014, Curti *et al*., 2016). The results of the present study showed that low temperature was a key limiting factor contributing to a longer life-cycle and associated with limiting moisture conditions especially during the early stages of growth resulted in low grain yield. Therefore, this highlights the need to identify buckwheat and quinoa genotypes with faster development and shorter growth cycle to optimise crop growth under the local weather conditions. Since buckwheat has an indeterminate growth habit, it would be more important to identify genotypes with uniform seed maturation.

The quinoa, genotype Jessie had faster development and shorter growth cycle such that the variety senesced approximately three weeks earlier than Atlas with poor grain yield such that it is unsuitable for production in NE-England. Overall, Atlas (quinoa) and Cebelica (buckwheat) were the most suitable genotypes despite their relatively long life-cycle. Nonetheless, it would also be important to further test the genotypes Atlas and Cebelica under local weather conditions over multiple years to determine the stability of agronomic and quality traits such as yield, TGW, HI, protein and mineral concentrations.

9.2 Grain yield

On average across three years, grain yield of buckwheat and quinoa was low (0.77±0.39 and 0.87±0.43 t/ha, respectively). The poorest yields were obtained in 2016 and the best in 2017 most likely due to weather conditions. In particular, shortage of water accompanied by spells of relatively high temperatures in the summer of 2018 caused earlier senescence and significantly reduced the yield potential of buckwheat and quinoa as was the case for the yield of most crops in the UK (DEFRA 2019). Hence, it was assumed that the high rainfall combined with relatively low temperatures that occurred in August (which covered the full flowering and seed setting growth stages) were most likely factors leading to reduced grain yield in 2016 and 2018 compared to 2017 where the reverse (shortage of rainfall, allowing a drier condition for

seed formation) occurred. Nonetheless, it is also probable that weed proliferation (which was significantly higher in 2016 and 2018 than 2017, respectively) reduced yield performance in 2016 and 2018. Therefore, this indicates that even though buckwheat has been shown to have allelopathic activity in both field and laboratory studies with several allelochemical compounds identified (Falquet *et al*., 2015), the ability to suppress weeds very much depends on the weed species (Bulan *et al*., 2015).

The results showed that weed pressure was particularly high when yield was low. Indeed, while yields in 2016 and 2018 were similar and relatively low for each crop when % of ground cover by weeds was relatively high, yield in 2017 was 2 – 3 times higher than 2016 and 2018. Therefore, considering that the yield difference between years was up to 50%, an efficient weed control strategy (such as mechanical weed control in an organic production system) combined with an appropriate nitrogen fertilisation strategy would be important targets for improving grain yields (Jacobsen, 2015; Nurse *et al*., 2016).

Overall, buckwheat and quinoa produced relatively low yields and were responsive to sowing date. Grain yield of buckwheat and quinoa was higher when sown early-May than mid-April (0.87 vs 0.59 and 1.72 vs 0.84 t/ha, respectively). One of the reasons yields obtained from the late sowing could be greater is because of high potential seed losses with the early sowing as crops were harvested on the same date (except for the quinoa genotype Jessie in 2016 and 2018 which was harvested early due to premature senescence). However, the results also showed that all yield components, especially total panicle/cyme and seed number and to some extent TGW, were highest in the early-May sown plots which produced the best/highest yields over the three years of trials. In particular, panicle number (panicles/m^2) and seed number (seeds/m^2) were the yield components which correlated positively with grain yield obtained from the late sowing. Thus, despite low plant population (plants/m^2) at harvest, at least partly due to lower seed germination and seedling survival, early-May sowing optimises grain yield potential of buckwheat and quinoa grown in the NE-England. Grain yields obtained in the present study, especially those from the early-May sown plots, were consistent with yields published in the literature for buckwheat and quinoa grown in Europe, America and Asia even though temperature was more limiting. Most importantly, the yields obtained in the present study were similar to those obtained for some of the major global producers of buckwheat and quinoa (Popović *et al*., 2014; Nurse *et al*., 2016; FAOSTAT, 2019).

The present study showed that various factors including low seed set due to relatively low temperatures contributed to the overall low yields of buckwheat and quinoa. However, high

seed losses at harvest was also likely to have contributed towards the low yields and hence is a major limitation for management of these crops. Seed losses at harvest were associated with the small size of the seeds thus causing seeds to be blown out when passing through the combine or mixed with substantial amount of crop residue combined with high moisture reducing the efficiency of the separation and threshing process. Estimates of seed losses during cleaning (i.e. separating seeds from straw and leaves) were about 10 - 25% in 2017 and 50% or more in 2016 and 2018, which was higher in quinoa than buckwheat likely because of smaller seeds. Therefore, a key to increase yields of buckwheat and quinoa grown in the UK, particularly NE-England, would be to identify genotypes with bigger seeds (to reduce harvest losses) bred for intermediate maturity with a shorter life-cycle.

The results indicated that the difference between biomass and combine yield samples especially in 2016 (i.e. 5- and 4-fold for buckwheat and quinoa, respectively) appears to reflect seed losses in the present study. Therefore, these results also suggest that seed losses could be reduced substantially if technology allowed to harvest each crop species or genotype when they reached physiological maturity as opposed to harvest on the same dates.

Grain yield of buckwheat and quinoa was increased by nitrogen fertilisation but not by foliar zinc fertilisation. The response to the increase of nitrogen rate was consistent in both buckwheat and quinoa. This is likely due to the fact that nitrogen fertilisation increases the amount of plant available nitrogen for growth and development (Büchi *et al*., 2016). However, contrary to the typical nitrogen curve response whereby crops usually show the greatest response from the early increments of nitrogen (Fang *et al*., 2018), buckwheat and quinoa showed the greatest response from the final increment (i.e. 75 to 150 kg N/ha). In support, Bhargava and Srivastava (2013) suggested that quinoa responds strongly to nitrogen applications with highest responses at the highest nitrogen increments. This result raises the question of whether such response is due to environmental factors (such as temperature and rainfall) or whether it is simply due to crop genetics, and highlights that while it is widely agreed that buckwheat and quinoa can successfully grow under low nitrogen input, the optimum nitrogen rate for the greatest response remains unresolved. Therefore, since the highest rate in the present study was 150 kg N/ha, it would be important to test the performance of buckwheat and quinoa at higher rates of nitrogen (e.g. 200 kg/ha) for better understanding of the nitrogen response curve. However, although in the present study lodging was not observed, it could become an issue if higher nitrogen rates are used as especially buckwheat is prone to lodging at higher nitrogen fertilisation rates due to its weak stem (Wang *et al*., 2015b; Farooq *et al*., 2016).

Efficiency of biogas digestate matched that of mineral nitrogen. In fact, grain yield was comparable in 2017 and 2018, suggesting that the biogas digestate fertiliser was able to adequately match supply with demand. The biogas digestate fertiliser used in the present study is a readily available source of N which is supported by the leaf chlorophyll (SPAD reading) data which showed little difference between biogas digestate and mineral nitrogen fertiliser. A further improvement in uptake efficiency could be via the use of smaller and more frequent doses adjusted to soil requirements and crop needs (Rózyto *et al.*, 2017) from seedling emergence to the beginning of flowering but this has higher cost of application and is likely to cause greater crop damage due to the method of application. Nonetheless, these results suggest that where biogas digestate is widely available there is the potential for reducing the costs of crop production and improving environmental sustainability particularly in an organic farming system.

The results showed that there is the potential for improvements of buckwheat and quinoa yield in NE-England. For example, the genotypes Atlas (quinoa) and Cebelica (buckwheat) produced the highest yield (2.24 and 1.45 t/ha, respectively) when sown early-May with application of 150 kg N/ha. Considering that the yields obtained from Atlas and Cebelica were generally higher than the global average, this becomes particularly important taking into account that in NE-England crops had delayed maturity and harvest date. Additionally, with regard to buckwheat, the genotype Zamira from the Czech Republic showed a potential for higher yield (1.78 t/ha), so further evaluation of this genotype is highly recommended as it also has much bigger seed which may reduce potential cutter bar losses.

Although the results of the present study confirmed that buckwheat and quinoa are low-yielding species relative to the major cereal crops wheat and barley in the UK, there is also evidence elsewhere (Bazile *et al*., 2016; Siracusa *et al*., 2017; Kasajima *et al*., 2017; Guglielmini *et al*., 2019) showing that on average buckwheat and quinoa genotypes grown in temperate and tropical environments can produce yields up to 3 and 6 t/ha, respectively, which are similar to those of cereal crops. This indicates that the yields of buckwheat and quinoa grown in the NE-England could be increased by agronomic management (e.g. nitrogen fertilisation) and a wider screening for genetic variation in the growth cycle. In that regard, while quinoa shows a potential for yield improvements fundamentally because of uniform seed maturation and higher seed setting, buckwheat also shows a potential for much bigger seeds and hence lower seed losses at harvest.

Most importantly, if yields of buckwheat and quinoa grown in the UK, particularly NE-England, could be increased, not only is there an increasing local and global market for buckwheat and quinoa but also these crops give UK farmer advantages in terms of weed control (especially buckwheat because of the allelopathic properties) in the context of the increasing threat of black-grass and the ever-increasing levels of resistance to pesticides both globally and in the UK. Moreover, buckwheat and quinoa have potential as spring sown break-crops with high value for UK growers who currently operate very intensive winter cereal-based cropping rotations.

9.3 Grain quality

The results showed that while buckwheat had generally higher concentrations of total polyphenols, antioxidants and flavonoids than quinoa, the latter had higher concentration of protein and Zn. This is consistent with previous studies which showed that generally quinoa had higher concentration of protein and Zn than buckwheat (**Table 9.1**). Concentrations of Fe were relatively similar with highest concentrations of 281 and 172 mg/kg for buckwheat and quinoa, respectively. Moreover, quinoa had generally higher concentration of P, K, Mn and Ni whereas buckwheat had higher concentration of Al and Mo. In particular, in accordance with Cakmak and Kutman (2018) who indicated that Zn interacts with proteins in the grain of cereal crops, it is conceivable that quinoa grains had higher Zn concentration than buckwheat because of higher protein content considering that grain proteins constitute a physiological sink for Zn.

The large variability between buckwheat and quinoa in terms of concentration of secondary metabolites such as phenolics, antioxidants and flavonoids (**Table 9.1**) suggest that biosynthesis of these metabolites is lower in quinoa than buckwheat likely due to genetic differences between the species. Alternatively, local weather conditions may have caused a greater sensitivity on biosynthesis of secondary metabolites in quinoa, especially in relation to plant responses to nutrient availability and needs at each growth stage (Mazzoncini *et al*., 2015).

The effect of sowing date on grain quality was variable. Nonetheless, the results suggest that grain quality of buckwheat and quinoa was generally higher in the early-May than mid-April sowing, with an increase in grain protein content. This is similar to previous studies which also found a small increase in grain protein obtained from the May relatively to the September sown plots (14.1 vs 13.9%) of buckwheat grown in Italy (Siracusa *et al*., 2017) or July relative to June (14.8 vs 13. respectively) of buckwheat grown in Iran (Sobhani *et al*., 2014). Therefore, the present study confirmed that while the average concentration of protein in buckwheat and quinoa grains is generally lower than or similar to that published for some common wheat

varieties (Stehno *et al*., 2007; Filho *et al*., 2017; Mir *et al*., 2018), it also confirmed that it is much higher than that published for rice and maize (Joshi *et al*., 2019). These results highlight that buckwheat and quinoa grains can have a much higher nutritional value than the major cereal crops such as wheat, rice and maize because not only the protein content can be higher but buckwheat and quinoa grains have much higher concentrations of essential micronutrients such as Fe and Zn (**Table 9.1**).

The results showed that the lowest concentrations of minerals were observed in 2016 and 2018 when weather conditions were unfavourable for growth as opposed to highest concentrations observed in 2017 when yields were higher. Hence, it is conceivable that the negative impact of the local weather conditions in 2016 and 2018 may have reduced not only seed yield but also impaired the filling and translocation of minerals into the developing seeds, whereas, in contrast, the concentration of protein and secondary metabolites was likely due to dilution effects caused by variable yields. This hypothesis was supported by the correlation coefficients data which showed that concentration of secondary metabolites (total polyphenols, antioxidants and flavonoids) and protein was negatively correlated especially with Zn concentration.

Grain concentrations of minerals were generally higher from the early-May than mid-April sowing. While both buckwheat and quinoa were responsive to sowing date with respect to grain Zn concentration, only quinoa showed a significant response with respect to Fe concentrations. Quinoa Fe concentrations decreased by at least 19% when sown early-May relative to mid-April while buckwheat Fe concentrations did not change in response to sowing date. In fact, quinoa had consistently lower Fe concentrations than buckwheat in response to sowing date.

Grain concentrations of Zn (32.5 and 43.2 mg/kg, for buckwheat and quinoa respectively) decreased by 26 and 15% in the mid-April relatively to the early-May sown plots, likely due to dilution effects. Moreover, the results showed a strong negative correlation between grain Zn concentration and concentration of total antioxidants and flavonoids, suggesting that excess Zn may not necessarily stimulate lipoxygenase activities and lipid peroxidation in buckwheat and quinoa seeds contrary to the suggestion that excess Zn induces disruption of metabolic processes such as the antioxidant defence system or basic physiological functions of plants (Tsonev and Lidon, 2012). Therefore, considering that quinoa showed higher concentrations of minerals except for Al and Mo, the results suggest that quinoa has a higher mineral density than buckwheat, especially with respect to Zn with the potential for increasing the nutrition and health of the global population associated with micronutrient deficiencies such as Fe and Zn.

With respect to heavy metals (Cd, Cu, Mo and Ni), the results of the present study pointed to a moderate risk of toxic concentrations (with thresholds of 0.1, 20, 1, and 10 mg/kg, respectively), which appeared to be higher in quinoa than buckwheat and higher in the early-May than mid-April sowing particularly with respect to Cu and Ni concentrations. The relatively high concentrations of Cu and Ni in the present study were probably due to an overexpression of the zinc regulated ZRT/IRT-like transporter proteins (from the ZIP1-4 family) which mediate the uptake and transport of heavy metals (Vollmannová *et al*., 2013; Nishida *et al*., 2015). Since the ZIP1-4 family is the preferential pathway for Fe and Zn uptake, and there was a relatively high concentration of Fe and Zn, it was unclear why high concentrations of Zn and Fe due to overexpression of the zinc regulated ZRT/IRT-like transporter proteins would also induce increasing concentration of Cu and Ni because divalent heavy metal ions such as Cu^{2+} and Ni^{2+} compete for the same Zn^{2+}, Fe^{2+} and Mg^{2+} uptake system (Waters and Sankaran, 2011). Probably, buckwheat and quinoa may have additional pathways which also mediate uptake and transport of heavy metals.

This study showed that while grain concentrations of total polyphenols obtained from the early-May sowing increased by 7% relatively to the mid-April sowing, grain concentrations of total antioxidants and flavonoids decreased at least 4-fold. Variation in grain concentration of total antioxidants and flavonoids in response to sowing date was attributed to dilution effects caused by variable yields. Variation in grain concentration of total polyphenols in response to sowing date could be explained by chelation complexes between phenols and metals because phenols bind preferentially to trivalent cations such as Al^{3+} and Fe^{3+} or high charge density cations such as Cu^{2+} and Zn^{2+} rather than alkali and alkaline earth cations such as Ca^{2+}, K^{+} and Na^{2+} so that a high concentration of Al^{3+}, Fe^{3+}, Cu^{2+} or Zn^{2+} would be indicative of high concentration of polyphenols (Hider *et al*., 2001). Indeed, the results showed that grain concentrations of total polyphenols and metals such Cu and Zn were higher in the early-May than mid-April sown plots as well as that grain concentration of total antioxidants and flavonoids strongly correlated with grain Zn concentrations.

In comparison with cereal crops, the present study showed that grain quality (in terms of protein, bioactive compounds and minerals such as Fe and Zn) of buckwheat and quinoa was similar to or higher than that published for some wheat, rice and maize varieties. The results indicated that concentration of protein was higher than or similar to that published for wheat, spelt and rye (Cakmak *et al*., 2010a, b, c; Angiollini and Collar, 2011; Zhang *et al*., 2012a; Campiglia *et al*., 2015; Wang *et al*., 2015a; Ma *et al*., 2015) depending on the genotype and production system (conventional vs organic). Hence, considering the large prevalence of

protein malnutrition in the world (Cakmak and Kutman, 2018), buckwheat and quinoa may constitute valuable sources of protein (alongside legumes which have ~ 25% protein) for combating protein malnutrition especially in developing countries where sources of dietary protein are rather limited. The results also indicated that the concentrations of Zn obtained in the present study were at least similar or higher than those published for rice and maize (Marles, 2017; Garcia-Oliveira *et al*., 2018). Most importantly, the Zn concentrations of quinoa obtained from the early-May sowing in the present study was approximately two times higher than the current average concentration of Zn of modern wheat, rice and maize varieties (25, 16 and 25 mg/kg, respectively) (Murphy *et al*., 2008; Cakmak *et al*., 2017; Garcia-Oliveira *et al*., 2018). In addition, concentration of Fe in the grain of buckwheat and quinoa was much higher than the 89.5, 4, 23.5 mg/kg published for wheat, rice and maize, respectively (**Table 9.1**; Morgounov *et al*., 2006**)**, whereas concentration of Zn was two times higher than that of rice but similar to maize (Marles, 2017).

In accordance with Cakmak and Kutman (2018), network meta-analysis (**Chapter 3**) showed that current agronomic Zn fertilisation strategies increased grain Zn concentration of wheat, rice and maize by 23.9, 7.3 and 8.8 mg/kg, respectively, which was still lower than the concentrations obtained in the present study without Zn fertilisation. Additionally, the present results also showed that both buckwheat and quinoa had much higher concentrations of Fe than the major cereals. Hence, using buckwheat and quinoa as sources of Fe and Zn would be more cost-effective than the current agronomic Zn biofortification strategies of wheat, rice and maize, thus making buckwheat and quinoa attractive alternatives to combat Zn and Fe deficiencies in humans.

With respect to secondary metabolites, while concentration of total polyphenols obtained in the present study were generally higher than those published for buckwheat and quinoa, the reverse occurred for total antioxidant and flavonoid concentrations (**Table 9.1**). The results showed that concentration of total polyphenols, particularly in buckwheat, was similar to that published for wheat (Masisi *et al*., 2016) and much higher than 1250 – 3900 μg/g DW also published for wheat (Ma *et al*., 2014), spelt (Gawlik-Dziki *et al*., 2012), rice and maize (Das and Singh, 2015; Masisi *et al*., 2016). Moreover, while concentration of total flavonoids of quinoa was similar to that published for wheat, concentrations of total flavonoids in buckwheat were three times higher than that published for some common wheat varieties (Ma *et al*., 2014) and ten times higher than for maize (Das and Singh, 2015). Nonetheless, it would be important to measure the concentration of individual phenolic acids, antioxidants and flavonoids for a further understanding of the nutritional profile of buckwheat and quinoa grown in the UK.

Buckwheat genotypes (Bamby and Cebelica) did not show variation in grain quality in response to sowing date and zinc or nitrogen fertilisation. Both Bamby and Cebelica showed a strong positive relationship between grain yield and protein concentration. The results suggest that Bamby and Cebelica showed little to no genetic variation in nutritional quality. The same occurred with quinoa genotypes (Atlas and Jessie) possibly because they were obtained from the same breeder with the likelihood of little genetic variation.

Therefore, the present study showed that although buckwheat and quinoa produced relatively low yields (which can be increased by agronomic management), these crops may be of great economic value to UK farmers not only because they are gluten-free with increasing market demand but most importantly because they have a nutritional composition (in terms of protein, essential mineral micronutrients and bioactive compounds such as polyphenols, antioxidants and flavonoids) that can provide important health benefits to consumers beyond basic nutrition. The quinoa used in the UK was bred in Holland with low saponin content which avoids the need for washing prior to use which offers the potential to develop a UK supply chain for this crop with potential nutritional benefits beyond the high saponin content material imported material from Peru and Bolivia.

9.4 Conclusions and future work

Pseudocereal crops such as buckwheat and quinoa could be arguably the most attractive gluten-free species and provide cost-effective alternatives to cereal crops to help combat malnutrition and health issues in humans associated with coeliac disease, gluten sensitivity, IBS and Fe/Zn deficiencies because of their nutritional value in terms of protein, micronutrients and bioactive compounds composition. The need for biofortification of these crops should aim at wider genetic screening programmes with target to genotypes with large genetic variation in Fe and Zn concentrations which could be potential target crops for improving especially wheat-based diets.

The specific conclusions of this study may be summed up as follows:

- Overall, among current agronomic biofortification strategies for increasing grain Zn concentrations of the major cereals only soil+foliar Zn fertilisation was shown to be effective particularly on wheat species based on the upper and lower confidence intervals of the pooled effect sizes from various relevant studies.
- Sowing date had a significant effect on the growth, yield and quality of common buckwheat and quinoa genotypes grown in NE-England. Particularly, late sowing i.e.

early-May, resulted in better crop growth, higher yield and quality than mid-April sowing despite the relatively long life-cycle. This points to the need to identify buckwheat and quinoa genotypes with early or intermediate maturity and high yield potential by screening genetic variation in life-cycle duration, seed size and grain quality.

- Foliar Zn fertilisation did not affect grain yield and quality significantly of buckwheat and quinoa genotypes. However, it would be important to test the effects of foliar Zn application on buckwheat and quinoa over multiple years. Nonetheless, grain Zn concentrations obtained in the present study, especially those of quinoa, were similar to or higher than those of the major cereals subjected to soil+foliar Zn fertilisation strategies shown in the meta-analysis and elsewhere in the literature.
- The effect of source and rate of N fertilizer was significant only on the growth and yield of common buckwheat and quinoa genotypes grown in NE-England. Nitrogen fertilisation at the rate of 150 kg/ha has the potential to improve yields of common buckwheat and quinoa substantially beyond satisfactory yields of 1 t/ha. Moreover, use of biogas digestate as a nitrogen fertilisation strategy (where it is available) may be cost-effective and environmentally sustainable for crop production (not only buckwheat and quinoa but also other crops), especially in an organic farming system, because the efficiency of biogas digestate matched that of mineral nitrogen with respect to grain yield and crop growth.
- The key factors limiting production of common buckwheat and quinoa in NE-England are low temperatures and excess water (i.e. rainfall) during critical stages such as germination and fruit maturation. Specific agronomic requirements include use of photoperiod neutral and frost resistant large seed genotypes, spring sowing time, nitrogen fertilisation at 150 kg/ha, efficient weed control strategies, pre-harvest treatments and mechanised harvesting for satisfactory yields.
- Overall, grain quality of buckwheat and quinoa was approximately 2-3 times higher than that published for wheat, rice and maize in terms of protein, Fe, Zn, total polyphenols and total flavonoids. Thus, the results of the present study support the evidence for a nutritional value of buckwheat and quinoa higher than wheat, rice and maize.
- The genotypes Cebelica (buckwheat) and Atlas (quinoa) are best suited to the UK agroecological conditions as they produced the highest yields with relatively high grain quality.

Table 9.1 Comparison of the nutritional composition of buckwheat and quinoa (present study vs literature) and cereal crops (wheat, rice and maize). Superscripts indicate the reference source.

	Buckwheat		Quinoa		Major Cereal		
	Present study	Literature	Present study	Literature	Wheat	Rice	Maize
Protein (%)	11	12[cd]	13	14[bc]	14[b]	9[b]	11[b]
Fe (mg/kg)	130	61[g]	127	97[b]	30[a]	2[a]	30[a]
Zn (mg/kg)	28	26[g]	40	34[b]	25[a]	16[a]	25[a]
Phenols (µg/g)	5081	2126[e]	2050	1149[e]	5197[i]	1450[i]	3999[k]
Antioxidants (µg/g)	2868	3480[e]	1371	6330[e]	1560[i]	293[j]	535[k]
Flavonoids (µg/g)	1382	5780[f]	416	1739[h*]	500[l]	940[j]	101[k]

[a] Garcia-Oliveira *et al*. (2018); [b] Filho *et al*. (2017); [c] Mir *et al*. (2018); [d] Zhu (2016); [e] Vollmannová *et al*. (2013); [f] Zielińska *et al*. (2012); [g] Bonafaccia *et al*. (2003); [h] Hirose *et al*. (2010); [i] Masisi *et al*. (2016); [j] Gong *et al*. (2017); [k] Das and Singh (2015); [l] Ma *et al*. (2014); *fresh weight basis

Future work

To complement this study, it would be important to:

- Determine the effects of wider sowing date intervals (April – May) combined with various seed rates on yield of buckwheat and quinoa.
- Determine the photothermal quotient (i.e. radiation to temperature ratio) in relation to sowing date for optimisation of growth and yield potential of buckwheat and quinoa grown in NE-England.
- Evaluate the effects of pre-harvest treatments such as swathing and/or desiccation on grain yield and quality of buckwheat and quinoa.
- Evaluate the nitrogen response curve with respect to yield of buckwheat and quinoa grown in NE-England at higher nitrogen rates (e.g. 200 kg/ha).
- Evaluate genotype x environment interactions and yield stability of Cebelica and Atlas (including other promising high-yielding genotypes of buckwheat and quinoa) under different locations in NE England and over multiple years.
- Evaluate genetic variability in Fe and Zn concentrations in quinoa.
- Evaluate the allelopathic effect of buckwheat residues within a crop rotation.

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