Enrichment of the selenium concentration of cereals under organic and conventional management practices

By

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Abstract

Selenium (Se) concentration of the major cereals has declined during recent times. At present, selenium malnutrition is a human dietary crisis which affects approximately 1 billion people worldwide. Key strategies are therefore urgently needed to increase the selenium content of major foods such as cereals. Therefore, the overall aim of this study was to evaluate the potential to enhance grain yield, quality and selenium content of wheat through agronomic management (crop protection, fertiliser type, crop species/variety) and selenium fertilisation.

The effects of soil and foliar applications of selenium fertiliser on grain selenium concentration and yield of cereals were studied using a systematic review and meta-analysis approach. Results showed that soil and foliar selenium application significantly increased grain selenium concentration without affecting yield, indicating that supplying selenium does have positive effects.

Field and glasshouse trials were conducted in 2016-2018. In 2016, four different fertiliser types (farmyard manure, mineral N, biogas digestate and cattle slurry) were studied with and without crop protection for their effect on the yield, quality and grain selenium concentration of the spring wheat variety Mulika. Results showed that the organic crop protection treatment significantly increased grain selenium concentration, but grain yield and grain quality (protein content, Hagberg falling number (HFN) and hectolitre weight) were significantly lower. Biogas digestate increased grain yield and protein content while grain selenium concentration was significantly higher in response to composted farmyard manure use than for the other organic fertiliser and mineral N treatments applied.

In 2018, a selenium fertiliser response field trial showed that there was no significant effect of selenium application method (soil vs foliar) or rate applied (0, 15 and 30 g Se/ha applied via the soil and 30 g Se/ha of foliar applied) on crop growth, grain yield and quality. However, there were significant effects of method of application and rate on selenium accumulation in different plant tissues, with the highest concentrations in the leaf at GS55 and GS70 and then in the grain at final harvest. A glasshouse trial was also conducted in 2018 to look at the effect of soil application rate at 0, 15 and 30 g Se/ha. It showed no effect on grain yield and protein content, but selenium application significantly increased plant tissue concentrations such that at final harvest highest concentrations were in response to 30 g Se/ha.

Grain samples were analysed from previous EU funded trials: Nitrogen Use Efficiency (NUE-CROPS), Healthy Minor Cereals (HMC) and Quality Low Input Food (QLIF) to evaluate

the effects of wheat species/variety, fertiliser type and crop protection on grain yield, quality (protein content), thousand grain weight and grain selenium concentration. Results in all trials indicated that fertiliser type significantly affected grain selenium concentration. Grain selenium concentration in the NUE-CROPS and QLIF trials was significantly higher in response to composted farmyard manure than to the mineral N treatment, while in the HMC trial, composted farmyard manure resulted in a significantly higher grain selenium concentration than biogas digestate, cattle slurry and mineral N treatments. Crop protection treatment also significantly influenced grain selenium concentration in the QLIF trial. In terms of wheat species, spelt wheat had greater grain selenium compared to common wheat, but this was across trials carried out in different years.

Data from the current study show that grain selenium concentrations can be improved through agronomic management of cereal crops via selection of fertiliser type, crop protection, crop species and variety. Pot and field-based studies also show that grain selenium concentration can be increased significantly via the use of selenium fertiliser, where a rate of 30 g Se/ha led to the greatest increases and soil-based application was more efficient than foliar.

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List of abbreviations

a.i.	Active ingredient
ANOVA	Analysis of variance
AUDPC	The Area Under the Disease Progress Curve
CI	Confidence interval
EU	European Union
FYM	Farmyard manure
GS#	Growth stage
GHG	Greenhouse gas
HI	Harvest index
HFN	Hagberg falling number
HMC	Health minor cereals
HNO_3	Nitric acid
H_2O_2	Hydrogen peroxide
N	Nitrogen
Na_2SeO_4	Selenate
Na_2O_3Se	Selenite
NDVI	Normalized difference vegetation index
NFSC	Nafferton Factorial Systems Comparison
NH_4^+	Ammonium
NO_3^-	Nitrate
NUE CROPS	Nitrogen use efficiency crops
PICO	Population, intervention, comparator, outcome
pRDA	Partial redundancy analysis
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analysis
QLIF	Quality low input food
RNI	Reference Nutrient Intake
RDA	The European Recommended Dietary Allowance
RDA analysis	The redundancy analysis
RDI	The US selenium daily recommendation intake
SE	Standard error
SD	Standard deviation
SMD	Standardised mean difference
TDM	Total dry matter
TGW	Thousand grain weight
UK	United Kingdom
USA	United States of America
WHO	The World Health Organisation

Chapter 1 : Introduction

1.1 Food and nutritional security

In 2050, the world's population is likely to reach 10 billion with the potential to reach 12 billion by 2100 (Cakmak, 2002; Gerland *et al.*, 2014; Reganold and Wachter, 2016; Khalil *et al.*, 2018). The rising population will significantly increase the demand for food (Tilman *et al*., 2002). The massive growth in world population in coming years may cause imbalances in supply and demand of the major food crops, wheat, rice and maize (Parry & Hawkesford, 2010), as most of these crops have shown modest yield increases over the last 20 years due to yield plateaus being observed in many developed countries (Olesen *et al*., 2011). It is a major challenge for food security in the 21st century as the trend of global crop yields consistently shows a declining trend (Cakmak, 2002). Increasing grain production in the future is a key requirement for global food security to ensure every individual has access to an adequate food supply that meets their dietary needs.

Food plays a vital role to fulfil the basic needs of humans and nutritional content of food is critical to maintain human health. Unfortunately, all agriculture production programmes, policy and practices implemented for major staple crops over the last 50 years have emphasised high grain production and food security (Miller and Welch, 2013) with little emphasis on nutritional security. The nutritional quality of cereal grains has often been overlooked, particularly in terms of protein and micronutrient content (Velu *et al.*, 2014). High protein content in cereal grain is crucial to manufacture high-end products like bread (Mader *et al*., 2007; Mason *et al.*, 2007), while humans require significant amounts of key micronutrients for a normal healthy life (White *et al*., 2012; Natasha *et al*., 2018). Several key mineral nutrients such as iodine, iron (Fe), zinc (Zn), calcium (Ca) and selenium (Se) have been found to have become lower in many cereal crop grains over recent decades (Gómez-Galera *et al.*, 2010; Hejcman *et al*., 2013; Cheema *et al.*, 2018). Continuous lack of these micronutrient elements in food sources ultimately has exposed humans to severe micronutrient malnutrition and health problems (Zou *et al*., 2019). Recently, more than two billion people worldwide have been suggested to be affected by micronutrient malnutrition (Miller and Welch, 2013; Souza *et al.*,

2014; Hefferon, 2015; Cheema *et al.*, 2018) with particular significance for children, old people and pregnant women (Reis *et al*., 2018). Severe micronutrient malnutrition has been attributed implicated in more than 50% of deaths related to various diet-related illnesses (Lyons, 2018). Therefore, combined strategies to enhance grain yields and nutritional quality of the major cereals are urgently needed. This can potentially be achieved by agronomic biofortification and genetic approaches.

1.2 Strategies to alter nutritional security

Lately, efforts to maintain grain yield and quality of staple crops have been linked to Sustainable Intensification based approaches with increasing interest from researchers in the United Kingdom (UK) and Europe (Cooper *et al*., 2011; Fagnano *et al*., 2012; De Ponti *et al*., 2012 ; Bilsborrow *et al.*, 2013; Palmer *et al*., 2013; Mazzoncini *et al*., 2015; Peigné *et al*., 2016). Sustainable intensification is an approach to achieve high yield through agronomic practices, crop variety improvements, innovations and increased input applications in a sustainable way (Tilman *et al.*, 2011). It is a key challenge in the future to sustain arable crop productivity without affecting environmental sustainability and nutritional security (Bilsborrow *et al.*, 2013). Excessive use of mineral N in the past has been a driving strategy to improve grain yields significantly, but this approach contributes a number of negative impacts to environments, particularly via eutrophication where mineral N fertiliser especially nitrate (NO_3^-), is instantly leached into surface and groundwaters (Vitousek *et al*., 1997). Added to that, the production of mineral N fertiliser is very costly, energy intensive and relies heavily on the use of fossil fuels where to manufacture 1 kg mineral N fertiliser involves the consumption of about 38,000 kJ of fossil energy (Refsgaard *et al.*, 1998). Application of mineral N in agriculture also significantly impacts greenhouse gas (GHG) emissions (IPCC, 2006). Negative impacts to environments from overuse of mineral N fertiliser combined with increasing fuel costs and an emphasis on sustainable agriculture have led many farmers to focus on alternative nitrogen and also phosphorus management strategies (Peoples and Craswell, 1992). Reserves of rock phosphate are limited, with increasing and urgent demand for efficient and sustainable supplies of phosphorus for use in agriculture (Bilsborrow *et al.*, 2013). Therefore, limited resources coupled with high costs of phosphorus fertiliser production definitely pose a risk to sustaining arable crop yields and to global food security (Cordell *et al*., 2009). As an alternative, organic fertiliser sources such as biogas digestate, composted food waste and composted farmyard manure (FYM) have the potential to maintain grain yield and quality and improve sustainability

compared to mineral N applications (Lošák *et al*., 2011; Šimon *et al*., 2015; Koszel & Lorencowicz, 2015; Riva *et al*., 2016; Madaras *et al*., 2018).

In terms of human mineral requirements, there are several intervention strategies to increase mineral concentrations in human diets and reduce the impact of micronutrient malnutrition. The strategies include dietary diversification with fish, meat, fruit and vegetables, food fortification, mineral supplementation, breeding programmes and agronomic biofortification (Gomez-Galera *et al*., 2010; White and Broadley, 2009; Broadley *et al*., 2010; Velu *et al*., 2014; Singh *et al*., 2016). Agronomic biofortification is a new option that has been added to the more traditional approaches (Cheema *et al*., 2018) to increase human micronutrient intake (Magallanes-López *et al*., 2017). Agronomic biofortification is a cheap method of applying fertilisers to increase micronutrient concentrations in harvested products (Gomez-Galera *et al*., 2009; Fageria *et al*., 2012). The present study is focused on the micronutrient selenium with considerable interest to increase selenium concentration in cereal grain through agronomic biofortification in the UK and elsewhere in Europe (Broadley *et al*., 2006; White & Broadley, 2009; Broadley *et al*., 2010; Mora *et al*., 2015; Oliveira *et al*., 2015). According to Singh *et al.* (2016) agronomic intervention (agronomic biofortification) combined with genetic variation (breeding varieties) has been more successful in promoting mineral acquisition in humans than food fortification, mineral supplementation and dietary diversification strategies. Enrichment of cereal grains is the most preferable option compared to food diversification and supplementation strategies and is a priority area as well as grain yield improvement (Żuk-Gołaszewska *et al.*, 2016). The successful enrichment of micronutrients in cereal grain through agronomic practices has been carried out under the Harvest Plus-Harvest Zinc programme using foliar Zn fertiliser application (Zou *et al*., 2019). The Harvest Plus programme also aims to develop varieties of crops such as wheat, rice, maize, sweet potato, common bean and cassava with improved nutritional and agronomic characteristics (Nestel *et al*., 2006).

Over the years, modern wheat varieties developed in breeding programmes have achieved high grain yields but with lower grain protein and mineral concentrations (Ceseviciene *et al*., 2012; Singh *et al*., 2016). Recently, ancient wheat species in particular spelt, emmer and einkorn, have been recognised as generally having higher grain protein content and mineral concentrations (Cakmak, 2008; Zhao *et al.*, 2009; Winterová *et al*., 2016). Cultivation of indigenous and traditional food crop species is another route to enhance micronutrient concentrations in the human diet (Fageria *et al*., 2012). Through genetic selection and exploiting available collections of germplasm of ancient wheat species such as spelt, human nutrition could be improved (Lyons *et al*., 2003). Many nations are dependent on cereal crops such as

wheat, maize and rice. As the most consumed food crop in the world, improving the grain quality of wheat is an important target to maintain food and nutritional security towards 2050.

1.3 Overall aim

The overall aim of this present study was to evaluate the potential to enhance grain yield, quality and selenium content of wheat through agronomic management (crop protection, fertiliser type, crop species and variety) and selenium fertilisation.

1.4 Specific objectives

- To evaluate the effects of selenium fertilisation methods (soil and foliar application) on grain selenium concentration and grain yield in cereals using a systematic review/meta-analysis.
- To evaluate the effects of N source and crop protection management on crop performance, grain yield and quality of spring wheat.
- To evaluate the effect of soil and foliar applications of selenium fertiliser on crop growth, yield and quality.
- To examine the accumulation and distribution of selenium in different plant tissues following selenium fertiliser application.
- To evaluate the effects of wheat species and variety, fertiliser type and crop protection on grain selenium concentrations, yield, quality (protein content) and thousand grain weight.

1.5 Hypotheses

- Grain selenium concentration, yield and quality of wheat can be increased through the application of organic N sources (biogas digestate & composted farmyard manure) and crop protection practices.
- Selenium fertilisation (method and rate) can be used to boost selenium concentrations in wheat grain.
- Exploiting available wheat species and variety can help increase grain selenium concentrations.

Chapter 2 : Literature review

2.1 Wheat

Wheat (*Triticum aestivum.* L) is one of the primary cereal crops and provides essential nourishment for billions of people globally (Sharma *et al.*, 2017; Cheema *et al.*, 2018). Annual world wheat production in 2018/2019 was 731 million tons and is projected to be 760 million tons in 2019/2020 (FAO, 2020). By 2050, global grain production is estimated to need to increase by 70% to meet rising population growth and increased food demands (Luo *et al*., 2018). In the UK and Europe, wheat is the largest cereal crop grown and dominates agricultural crop production (Fan *et al.*, 2008). The production of wheat in the UK was 14 million tonnes in 2018 (DEFRA, 2019).

Wheat is mainly consumed for the production of bread and bakery products and for animal feed. One of the high yielding wheat varieties recommended for bread making is Mulika spring wheat. Mulika is classified under the National Association of British and Irish Millers as a Group 1 variety suited to bread making and is on the UK Recommended List. Mulika is highly sought after by millers because of its milling and baking performance and provides the potential for high market premiums. In fact, Mulika displays high yield and excellent bread-making quality traits, including protein content, hectolitre weight and Hagberg falling number. Mulika also shows high resistance to mildew, yellow rust and orange wheat blossom midge (AHDB, 2019).

2.1.1 Bread making product quality

In modern agricultural production, enhancing grain quality of cereal crops is mainly driven by end-users' specification requirements, which are important in the manufacture of high quality baking products (Horvat *et al*., 2012). The three quality traits demanded by millers to produce high quality flour are protein content, hectolitre weight and Hagberg falling number (Mader *et al*., 2007). These three quality traits of bread making quality influence the price premium obtained by a grower above that of feed wheat. The price of wheat grain will be low

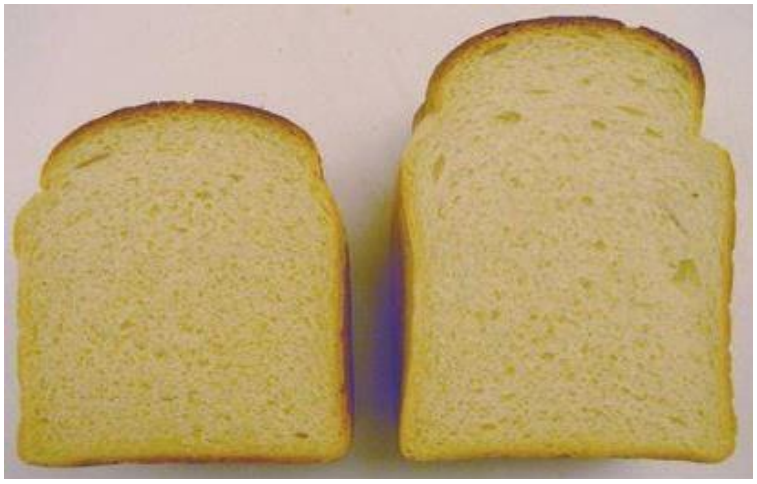
and not suitable for milling and baking if these three quality traits are below the threshold levels (Kettlewell *et al*., 1999).

Grain protein content is an important trait (Mason *et al*., 2007; Mader *et al*., 2007; Hlisnikovský *et al*., 2015) whereby high grain protein content provides high baking potential (Ceseviciene *et al*., 2012). In the UK, a minimum market specification for protein content required by flour millers for high quality bread making products is > 13% on a dry basis (Godfrey *et al*., 2010). In other countries such as Germany, flourmills offer a premium to farmers for >14% protein (Sieber *et al*., 2015). The quality of wheat flour depends on the nitrogen concentration of the grain (Fuertes-Mendizábal *et al*., 2010). The protein content in the grain is largely controlled by the rate and timing of N fertiliser application (HGCA, 2009).

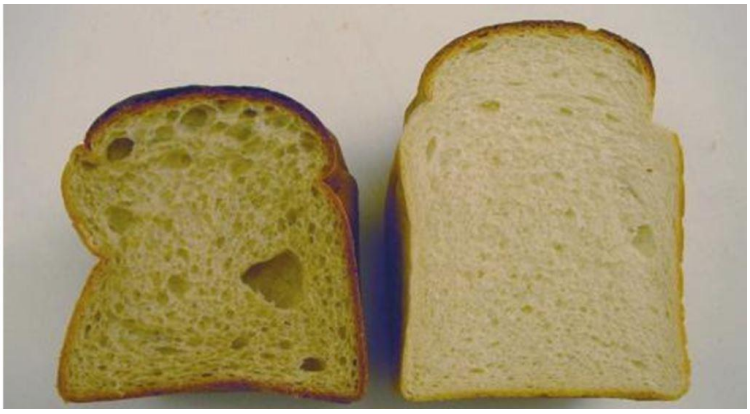
High hectolitre weight is important for flour mills in the marketing of grains as it signifies a high volume of extractable flour and is the relationship between weight and volume of the grains (Campiglia *et al*., 2015). In the UK, deductions in premium are offered for hectrolitre weight values below 76 kg/hl (Gooding *et al.*, 1999).

Hagberg falling number provides a measure of the viscosity (in seconds) of a heated suspension of flour in water and is a measure of the α-amylase enzyme activity (Dimmock & Gooding, 2002; Ceseviciene *et al*., 2012). α-amylase enzyme converts starch into simple sugars i.e. glucose and maltose during hydrolysis (Dimmock & Gooding, 2002). The activity of α-amylase enzyme should be low to avoid high starch breakdown and dough stickiness and is represented by a Hagberg falling number >350 seconds (Darby *et al*., 2013), while a HFN value <200s demonstrates high activity of α-amylase and poor bread-making quality. Fig. 2.1 demonstrates the influence of grain protein content and Hagberg falling number on bread-making.

Increasing grain protein content and loaf volume (left to right)



Low (left) and high (right) grain protein content of bread



Low (left) and high (right) Hagberg falling number of bread

Fig. 2.1 *Effect of grain protein content and Hagberg falling number on bread making quality (source: Yara 2019).*

2.1.2 Human nutrition and health

Wheat grain supplies the human population with calories and nutrients such as protein, vitamins, carbohydrate, amino acids, antioxidants, dietary fibre and minerals (Fan *et al.*, 2008; Velu *et al*., 2014; Vrček *et al.*, 2014; Shewry, 2018; Del Coco *et al.*, 2019; Hlisnikovský *et al.*, 2019). Wheat grain also supplies micronutrients which are important in human health including zinc (Zn), iron (Fe) and selenium (Se) (Gomez-Galera *et al*., 2009; Nelson *et al.*, 2011; Singh *et al.*, 2016). About 16 micronutrients are necessary for human nutrition and health; selenium is one of the most important nutrients in many food sources alongside zinc, iron, calcium (Ca) and iodine (I) (Singh *et al.*, 2016). Humans are likely to be exposed to severe selenium malnutrition if their diet is largely based on wheat grain with low selenium concentration (Miller and Welch, 2013; Reis *et al*., 2018). Currently, selenium malnutrition is one of the global human dietary crises (Dos Reis *et al*., 2017) as a billion people worldwide are affected by selenium malnutrition (Mora *et al*., 2015; Rahman *et al*., 2015; Reis *et al*., 2018; Lidon *et al*., 2018a). One in seven people in the world is diagnosed with low dietary selenium intake (Jones *et al*., 2017), which is generally associated with cardiovascular disease and cancers (Galinha *et al*., 2012; White, 2016). Selenium malnutrition occurs in many countries including the United Kingdom. There are several studies reported where British people have been shown to have low selenium intake. Stoffaneller and Morse (2015) showed a wide range age of the UK population with low selenium intake since the 1970s from national surveys. Rayman (2008) detected the average selenium intake in the UK was 35 µg per day, which is clearly below the Reference Nutrient Intake (RNI) recommendation level of 40 to 70 µg per day. Adams *et al.* (2002) noticed that British people have experienced a mean reduction of daily selenium intake from 60 µg Se/day in 1970 to 29-39 µg Se/day in 1997. Broadley *et al.* (2010) also showed that Se intake among UK people declined from 60 µg Se/day in 1985 to 32-34 µg Se/day in 2000. The constant decline in selenium intake by the UK community was clearly caused by lower wheat grain selenium concentration (Broadley *et al*., 2010; Stroud. *et al.*, 2010a and 2010b; Sharma *et al*., 2017). Ultimately, bread making wheat and its food-based products are the most important selenium source in the human diets of many people globally (Poblaciones *et al*., 2014ab; Alfthan *et al*., 2015; Gong *et al*., 2018).

2.2 Selenium (Se)

Selenium is a metalloid essential micronutrient (Malagoli *et al.*, 2015; White, 2016; De Vita *et al.*, 2017; Deng *et al.*, 2017). As a component of selenoprotein, selenium is critical for human health (Mao *et al*., 2016) to complete physiological functions and normal development (Eiche *et al*., 2015; El-Ramady *et al*., 2016; Ning *et al*., 2016). In a routine dietary intake, humans require selenium in trace amounts (Natasha *et al*., 2018). Selenate and selenite are two common selenium species which are soluble in soil and readily absorbed by crops (Ali *et al.*, 2017). In plants, selenium is not an essential element (Adam *et al*., 2002). A small quantity of selenium is sufficient to improve plant health (Pilon-Smits, 2015) but higher doses of selenium may be toxic to plants (Sharma *et al.*, 2017).

2.2.1 Selenium in soil

Soil is the primary source of selenium and a principal route for selenium intake for humans (López-Bellido *et al*., 2019). Selenium in food is mainly affected by selenium concentrations in soil, plant selenium uptake and distribution within the plant (White, 2016; Sharma *et al*., 2017; Grant *et al*., 2007; Bañuelos *et al*., 2017). Low uptake of selenium by plants results from low selenium bioavailability in soil (Poblaciones *et al.*, 2014b ; White, 2016; De Vita *et al.*, 2017; Reis *et al.*, 2018). Mora *et al.* (2015) defined selenium deficient soil as soil with a selenium concentration range between 0.01 and 2.0 mg Se/kg. Govasmark *et al.* (2008) considered with soil selenium concentration below 0.6 mg/kg as selenium deficient and this amount is very low to provide an adequate selenium concentration in the grain. Countries included in soil selenium deficient regions are: Australia, New Zealand, Denmark, Finland, Central Siberia, Central China, UK, Netherlands, Spain, India, France, Belgium, Serbia, Greece, Brazil, Italy, Ireland Slovenia, Turkey, China, Germany, Portugal, Sweden, Austria, Denmark, Slovakia, Nepal, Bangladesh, Siberia and Poland (Zhao *et al.*, 2005; Rahman *et al.*, 2013; Wu *et al*., 2015) (Fig. 2.2). A very low amount of soil selenium (< 0.5 mg/kg) was detected in Denmark, New Zealand, Finland and some parts of China (Sharma *et al.*, 2017). In the UK, about 95% of soil samples from the geochemical survey displayed soil selenium concentration less than 1 mg/kg (Tamás *et al*., 2010), while in Keshan China, Siberia and New Zealand, soil selenium concentrations are below 0.125 mg/kg (Broadley *et al*., 2007). Meanwhile, seleniferous soils are defined as those where vegetation had up to 5 mg Se/kg (Tamás *et al*., 2010) and the concentration may be higher than 1,200 mg Se/kg (Mora *et al.*, 2015). Some countries in the world have reported high soil selenium concentration up to 10,000

μg/kg, which are classed as seleniferous soil countries: Canada, Colombia, Venezuela, Great Plains of the USA, Hubei Province of China and some regions of Ireland (Rahman *et al*., 2013). The greater soil selenium bioavailability in wheat planting areas of the US and Canada is directly related to higher wheat grain selenium concentrations (Broadley *et al*., 2010).

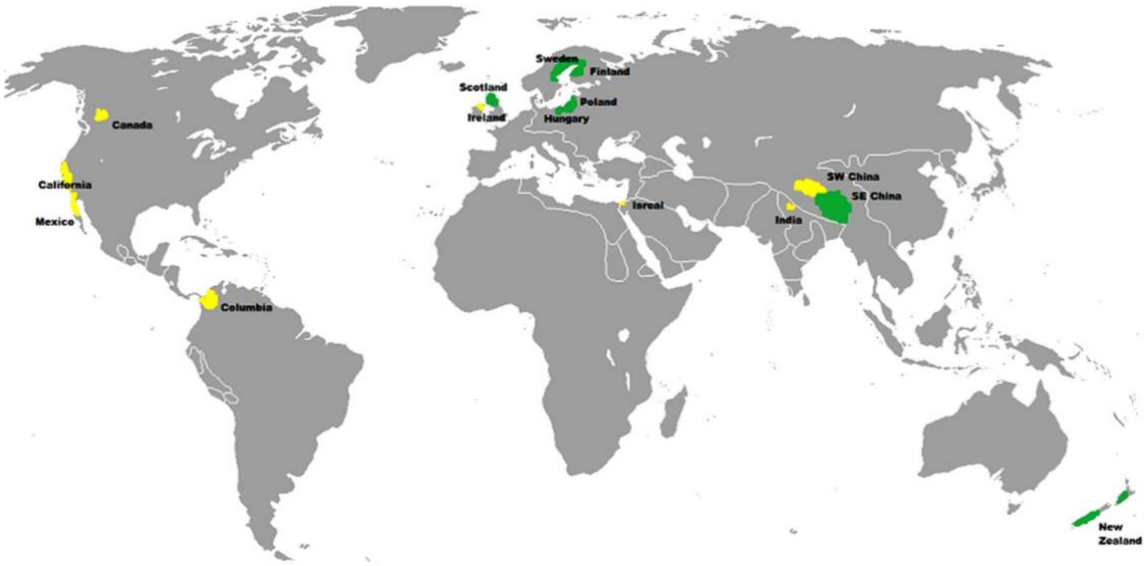


Fig. 2.2 *Distribution of selenium in soils of the world (Sharma et al., 2017). Green (Seleniferous area): South East China, Hungary, Poland, Finland, Sweden, Scotland, New Zealand. Yellow (Selenium deficient area): South West China, India, Ireland, Columbia, Mexico, California and Canada.*

In terms of selenium in the soil, plants can absorb selenate and selenite and translocate them to various plant tissues (Hawkesford and Zhao, 2007; Carey *et al*., 2012; Deng *et al*., 2017). Selenate is more soluble and highly mobile than selenite in many soils (Galinha *et al*., 2012; Oliveira *et al*., 2015; Ali *et al*., 2017). Selenate is more bioavailable and easily absorbed by plant roots than selenite (Poblaciones *et al*., 2014a ; Ros *et al*., 2016). The distribution of selenate by plant root cells from the rhizosphere to plant parts occurs through the sulphur assimilation pathway via high-affinity sulphate transporters (Keskinen *et al*., 2010;White, 2016; Deng *et al*., 2017; Jiang *et al*., 2018). The expression of high-affinity sulphate transporters occurs in the root cortex, root tip and lateral roots (Shibagaki *et al*., 2002; Hawkesford and Zhao, 2007). The high-affinity of sulphate transporters for selenate is the main reason for the difference in plant uptake between selenate and selenite (Hawkesford and Zhao, 2007; Ramos *et al*., 2010). The high-affinity sulphate transporters are regularly induced by sulphur deficiency

(Yoshimoto et al., 2002). The richness of selenate in the soil influences selenate influx from soil to plant root (Hawkesford and Zhao, 2007). Selenate in the root is then converted into selenite (White *et al*.,2007; White and Broadley, 2009), with selenite then being converted into selenide by glutathione (Carey *et al*., 2012). After a series of reactions, selenide is assimilated into organic selenium species such as selenocysteine (SeCys) and selenomethionine (SeMet) (Ellis and Salt, 2003; White and Broadley, 2009; Carey *et al*., 2012) and subsequently transported within plant parts such as leaves, and shoots (Eiche *et al*., 2015). SeMet is acknowledged as the major form of selenium in wheat grain with 56-83% compared to SeCys about 4-12% (Whanger, 2002). The uptake of selenite into plants from soil may also occur via phosphate transporters in plant roots (Hawkesford and Zhao, 2007; Li *et al*., 2008; Carey *et al*., 2012). However, root to shoot selenite transport has been found to be scarce (Hopper and Parker, 1999). Selenate is chemically similar to sulphate (SO_4^{2-}) (Adam, 2002), which enables it to use the sulphate transporter system (Carey *et al*., 2012). Sulphur fertiliser is regularly applied to wheat in the UK, especially milling wheat where it has been shown to increase grain yield and baking quality (Zhao *et al*., 2006). High sulphur levels in soil generally limit selenium uptake by plants as both elements compete to use the same transporter system (El Kassis *et al*., 2007; Malagoli *et al*., 2015).

The application of increasing rates of selenium to the soil results in higher selenium concentrations in grain, straw and roots of wheat (Ducsay *et al*., 2009). A study by Wang *et al.* (2020) ranked selenium distribution in plant parts as leaf> grain> glume> stem> root following selenate treatment and leaf> root> grain> glume>stem with selenite treatment. Meanwhile, Ducsay *et al*. (2009) found that selenium accumulation in wheat in individual parts was of the order of straw <grain <roots. During vegetative stages of growth selenium accumulation in young leaves while during reproductive growth high selenium concentrations are found in seeds, with reduced levels in leaves (Borowska *et al.*, 2012b). When selenate was applied to maize selenium accumulation occurred in the shoot (Longchamp *et al.*, 2015). In summary, the distribution of selenium in plant parts is influenced by form and rate of selenium application, sulphate levels in the soil and selenium fixation in the soil (Borowska *et al.*, 2012b).

2.2.2 Selenium in wheat grain

Globally, grain selenium concentration ranges between 0.001 and 30 mg/kg and mostly within the range 0.020–0.600 mg/kg (Broadley *et al.*, 2006). In the UK, British wheat grain has been found to be a poor source of selenium (Gómez-Galera *et al.*, 2010; Tamás *et al*., 2010). For instance, Adams *et al.* (2002) reported that about 88 % of wheat grain sampled from a survey had < 50 µg Se/kg of grain, which was suggested as insufficient for human and animal requirements. Stroud *et al*. (2010a) showed that wheat grain selenium concentration ranged from 15.5 µg/kg - 43.8 µg/kg in ten field trials following liquid and granular selenium fertilisation. Hawkesford and Zhao (2007) found the mean and median values of wheat grain selenium concentration in the UK were 27 µg Se/kg and 18 µg Se/kg on a fresh weight basis with a moisture content of around 15% in the grain. Meanwhile, Broadley *et al.*, (2010) and Hart *et al*. (2011) found the mean grain selenium concentration in the UK was about 28 µg/kg and 30 µg/kg. In the Slovak Republic, grain selenium concentration was 48 µg/kg (Ducsay *et al*., 2009). In Australia, Duncan *et al*. (2016) found the range of grain selenium concentration on a dry mass basis to be around ~ 22 to 70 µg/g.

2.2.3 Selenium in the human diet

There are several selenium daily intake recommendations, which generally depend on an individual country's regulatory authorities and the organisational body guidelines. In the UK, the Reference Nutrient Intake (RNI) suggests selenium level for adult men is 75 µg Se/day and for adult women is 60 µg Se/day (Broadley *et al.*, 2006; Hart *et al.*, 2011; Stoffaneller and Morse, 2015). The European Recommended Dietary Allowance (RDA) recommends a selenium intake dosage for humans of about 55 µg Se/day (Poblaciones *et al*., 2014a). Similarly to RDA, the US selenium recommended daily intake (RDI) indicates selenium intake for men and women is also about 55 µg Se/day (Stoffaneller and Morse, 2015). The World Health Organisation (WHO) suggested daily intake of selenium for adults in a range of 30-40 µg (Oliveira *et al*., 2015). The Food and Nutrition Board of the National Academy of Science classified the daily selenium intake dose for humans according to different groups: men (40–70 µg), women (45–55 µg) and children (15-20 µg) (El-Bayoumy, 2001). The minimum amount of selenium for each individual depends on their sex, body weight, age and health status (Pilon-Smits, 2015).

Notably, selenium deficiency is a global emerging problem because it has affected approximately 15% of people globally (Wang *et al.*, 2013; Idrees *et al.*, 2018). Few studies in

the past have reported that UK people historically have suffered with significant reductions in daily Se dietary intake. Adams *et al.* (2002) reported a decline in daily Se intake from 60 µg/day in 1970 to 29-39 µg/day in 1997, and Broadley *et al.* (2010) reported Se intake reduction from 60 µg/day in 1985 to 32–34 µg/day in 2000. The contributing factor of these reductions in intake is that most wheat grown in the UK is low in selenium (Broadley *et al.*, 2006, Broadley *et al.*, 2009; Li *et al.*, 2010; Stroud *et al.*, 2010a, b). Selenium deficiency arises when people consume major food sources such as wheat with low Se content in their daily diet (Gomez-Galera *et al.*, 2009; White and Broadley, 2009; Fageria *et al.*, 2012). Also, in previous years up until the mid-1970s, about 50% of bread products in the UK used flour imported from North America, mainly Canada, containing higher selenium concentrations (Hart *et al.*, 2011). UK people are likely to have greater exposure to a number of major chronic diseases and disorders such as cardiovascular disease (Malagoli *et al.*, 2015; Lidon *et al.*, 2018a), cancer, low male fertility, declining immune systems (Hatfield *et al.*, 2014), abnormal skin coloration, muscle weakness and inflammation and kidney damage (Dos Reis *et al.*, 2017) due to inadequate supply of Se contents in their routine diets. Sufficient Se intake in routine diets is critical to human health as pointed by Boldrin *et al.* (2013), who were concerned about ways to enhance Se concentration in major food sources.

2.3 Methods to boost selenium intake in the human diet

There are several strategies available to increase selenium intake in the human diet to lessen selenium malnutrition effects. In general, the most common methods practiced are food fortification, mineral supplementation, dietary diversification and biofortification of staple crops (Allen *et al.*, 2006; Longchamp *et al.*, 2015; Dos Reis *et al.*, 2017).

2.3.1 Food fortification

For many decades, food fortification has been recognised as the cost-effective and long-term strategy (Gómez-Galera *et al.*, 2010) to boost mineral intake. Manufactured food-based products like breakfast cereal enriched with folate, milk enriched with iron and margarine enriched with vitamin E have been successfully fortified using this approach. Food fortification through cereal crops is an attractive and productive method used in several developed countries to mitigate nutrient deficiencies in iron, iodine, vitamin A, D and several B vitamins (Allen *et al.*, 2006; Hurrell *et al.*, 2010). In developing countries, this method is widely accepted as being a major instrument to fortify iron and zinc in wheat flour which affects approximately 30% of

the global population (Akhtar *et al*., 2011). However, food fortification depends on the widespread distribution of fortified food products (Bañuelos *et al*., 2017). Food fortification is difficult to implement in developing countries because of a general lack of required infrastructure to enable product distribution (Gómez-Galera *et al.*, 2010).

2.3.2 Mineral supplementation

Mineral supplementation strategies, for example pills or mineral solutions are the most suggested immediate and short-term method to alleviate mineral deficiencies in humans (Gómez-Galera *et al.*, 2010). In developed countries mineral supplementation can be implemented relatively easily to small groups of people who are faced with a few mineral deficiencies. In the context of selenium supplementation, many individuals from western countries consume selenium supplements either in organic or inorganic form such as sodium selenite or selenate in tablet or fluid form to raise selenium levels in the body (Lyons *et al*., 2003). However, in developing countries, widen spread distribution of mineral supplements is more difficult and challenging (Gómez-Galera *et al.*, 2010; Cheema *et al*., 2018), due to limitations of funding, logistics, trained manpower, infrastructure and reliable supplies (Cheema *et al*., 2018). For instance, supplementation of iron in the form of syrups and tablets in developing countries has not been successful (Beinner & Lamounier, 2003). The success of mineral supplementation programmes basically depends on the level of coverage and compliance (Akhtar *et al*., 2011).

2.3.3 Food diversification

People can also enrich their selenium diet with food diversification from various types of main foods sources (Akhtar *et al*., 2011)**,** including fish, meat, fruit and vegetables. Meat and fish are alternative dietary sources for selenium instead of cereal products (Adams *et al*., 2002; Hawkesford & Zhao, 2007; López-Bellido *et al*., 2019). In the UK, cereals and bread products (27%) have become major sources of daily selenium intake with significant contributions also from meat (32%), fish (17%), eggs and dairy products (11%) (Geissler *et al*., 2017).

2.3.4 Biofortification of crops

Biofortification is a food-based technique to enrich bioavailability of micronutrients in the edible parts of staple crops (Broadley *et al.*, 2010; Rahman, 2014; Finkelstein *et al.*, 2017).

Minerals such as selenium, zinc and iodine in food crops can generally be elevated using this method (Hefferon, 2015). Biofortification of staple crops may occur through: (i) genetic biofortification (conventional plant breeding), (ii) modern biotechnology (transgenic biofortification) and (iii) fertilisation of crops, either soil or foliar, with appropriate fertiliser rates and sources (Fageria *et al*., 2012; Rahman, 2014; Garcia-Casal *et al.*, 2016; Cheema *et al*., 2018; Garg *et al.*, 2018). Genetic biofortification is a method used in conventional plant breeding to develop cereal crops with a high mineral concentration and encourage increased absorption of nutrients (Velu *et al*., 2014), but modern breeding programmes usually target yield, quality and disease resistance with little attenion paid to the mineral content of crops. New crop varieties established under a breeding programme through genetic biofortification showed an increased capacity to accumulate higher Se concentration in edible parts (Sharma *et al.*, 2017). Applying modern biotechnology via transgenic approaches also has the potential to biofortify cereal crops (Fageria *et al*., 2012; Garg *et al*., 2018). Transgenic biofortification is engaged when genetic diversity among crop varieties is limited or unavailable (Garg *et al*., 2018). An example of this transgenic approach (genetic modification) is Golden Rice which has been under development at the International Rice Research Institute (IRRI) (Zeigler, 2014). Biofortification through agronomic-based intervention complements other available approaches (Fageria *et al.*, 2012; Singh *et al.*, 2016; Cheema *et al.*, 2018). Recently, agronomic biofortification through fertiliser application has become globally accepted and progressively more popular because this method is sustainable and safe for the environment (Lyons *et al*., 2003; Broadley *et al.*, 2006; Mora *et al.*, 2015). Most agronomic biofortification work in the past has addressed Fe and Zn deficiency through the Harvest Plus programme (Velu *et al*., 2014). The Harvest Plus programme iniative involved people from various interdisciplinary research backgrounds particularly nutrition, plant breeding and genomics to develop biofortified cereal crops to benefit human health (Cheema *et al.*, 2018).

2.3.5 Selenium fertilisation of crops

Biofortification of crops to boost micronutrients content of foods through fertilisation is achievable and is also applicable to selenium (Lyons *et al.*, 2004). Studies on selenium biofortification have increased over recent years (Ducsay *et al*., 2016). The practice of selenium addition to the soil-plant system to increase selenium concentration in foods is termed biofortification (White and Broadley, 2005). Agronomic biofortification is a short term, reliable, cost-effective and safe method to reduce selenium deficiency in the food chain

(Broadley *et al*., 2006; Hawkesford and Zhao, 2007; Chilimba *et al.*, 2012; Carvalho and Vasconcelos, 2013; Mora *et al*., 2015; El-Ramady *et al*., 2016; Bouis & Saltzman, 2017; Li *et al*., 2017; Magallanes-López *et al*., 2017; Valença *et al*., 2017; Bañuelos *et al*., 2017; Jiang *et al*., 2018). Several authors have shown that selenium concentration of cereals can be improved by selenium fertilisation (Broadley *et al*., 2006; Hawkesford and Zhao, 2007; Chilimba *et al*., 2012). Most research on selenium biofortification has been carried out on vegetables and cereals (Li *et al*., 2018), with wheat, rice, maize, barley, oats, pearl millet, cassava, sweet potato and beans being primary targets for this approach (Miller and Welch, 2013; Sharma *et al.*, 2017). Wheat has been shown to be a more efficient accumulator of bioavailable selenium than other crops (Poblaciones *et al.*, 2014b; Sharma *et al.*, 2017).

In general, selenium fertilisation involves several techniques including seed dressing, seed soaking, seed priming as well as soil and foliar application (Wang *et al.*, 2013; Ducsay *et al*., 2016). Among these strategies, soil and foliar applications (Fig. 2.3) are the most commonly practised and notably successful (De Vita *et al.*, 2017). Fertilisation (soil and foliar application) with inorganic selenium sources has been widely practised in the United Kingdom, Europe, New Zealand, Africa and China (Grant *et al.*, 2007; Bañuelos *et al*., 2017; Dos Reis *et al*., 2017). Several countries like China, USA, Finland and several countries in Europe have reported successful biofortification programmes (Mora *et al*., 2015; Jiang *et al*., 2018). Biofortification of crops via fertilisation is used to complement existing conventional breeding strategies (Velu *et al*., 2014). Soil application is where selenium is added direct to soil in the form of granular fertiliser (Chu *et al*., 2013). Soil application is effective in uniform soil environments (Wu *et al*., 2015), whereby 5-30% of the applied selenium is taken up by the plant while the remainder is retained in the soil or lost via leaching to water bodies (Eich-Greatorex *et al.*, 2007; Li *et al.*, 2017). Soil application of selenium fertiliser can enhance selenium concentration in grain, fruits and vegetables several fold (Wu *et al*., 2015). Meanwhile, foliar application is a technique of spraying selenium-containing solution on the leaf surface of crops (Bañuelos *et al*., 2017). Selenium concentration in various vegetable types for example carrot, garlic, onion and radish, has been promoted through foliar application either using selenate or selenite (Wang *et al.*, 2013). Foliar application to wheat, rice and lettuce has been shown to successfully reduce selenium deficiency in the human population (Lidon *et al*., 2018a). Several studies in the literature have demonstrated that foliar application is more effective than soil selenium application. Lyons (2018) suggested foliar application is the most effective in both selenium and iodine biofortification. Wang *et al*. (2013) and Mao *et al*. (2014) agreed that foliar application is better than soil application in terms of effectiveness and cost of application. Foliar

application improved selenium concentrations in crop parts with almost double the effect of soil application (Ros *et al*., 2016).

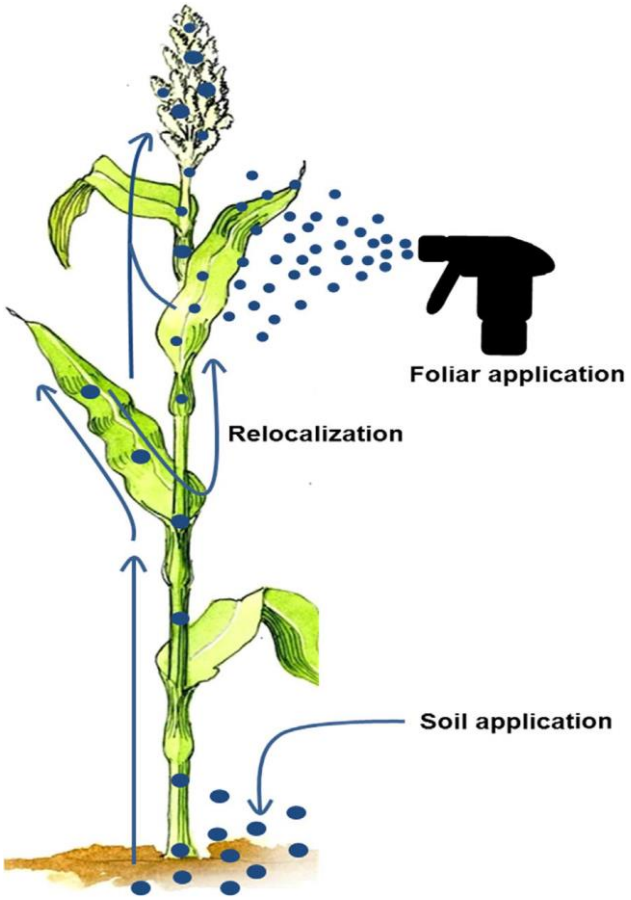


Fig. 2.3 *Foliar and soil application techniques (Valença et al., 2017).*

Timing of selenium application also influences grain selenium content of crops. Several studies have shown that application of selenium fertiliser at early growth stages can increase selenium concentration in edible parts of crops. According to Ducsay *et al*. (2016), selenium fertiliser application at growth stage GS32 (Zadoks, 1974) effectively increased selenium concentration of wheat. Curtin *et al.* (2006) found that selenium fertiliser applied at GS31 enhanced grain selenium concentration of wheat more than application at sowing. Likewise, Govasmark *et al*. (2008) also found selenium application at GS30 to GS70 to wheat gave higher concentrations in the grain than application at sowing or during early growth stages. Lyons *et al.* (2004) reported a study conducted in South Australia using wheat and observed that soil selenium application as selenate at seeding was better than foliar application at post-anthesis. Ros *et al.* (2016) observed that foliar application at the vegetative stage of crops encouraged faster selenium uptake. Lyons (2018) concluded that the best timing and effects of foliar

selenium application are normally achieved between booting and the early milk stage of grain growth in cereals. Boldrin *et al.* (2013) found that late foliar application at flowering was ineffective due to the time required to transfer selenium from the leaves to the grains through the phloem.

The effectiveness of selenium fertilisation is also affected by application rate. The effects on grain selenium concentration through soil and foliar selenium application generally increase progressively as selenium application rate is increased. Ros *et al.* (2016) observed that a low selenium application rate (<10 g Se/ha) in a field trial resulted in a crop selenium uptake increase by about 400%, while application of selenium at a rate more than 40 g Se/ha resulted in a 1500% increase in uptake. Selenium fertilisation at 20 g/ha using sodium selenate succeccfully achieved an increase in grain selenium to 374 µg Se/kg compared with 39 µg Se/kg in the control treatment without selenium fertiliser (Stroud *et al*., 2010b). In South Australia, grain selenium concentration increased about 20 to 133-fold and 6 to 20-fold with soil and foliar application in field trials on two soil types (Lyons *et al.*, 2004). Grain selenium concentration in the UK was increased about 10-fold from ambient levels following 10g Se/ha application (Broadley *et al*., 2010). Ducsay and Ložek (2006) recorded grain selenium concentration of 0.045 mg/kg in the control which was increased to 0.088 mg/kg and 0.145 mg/kg following the application of 10 g/ha and 20 g/ha of selenium. Vita *et al.* (2017) found grain selenium concentration increased by up to 35-fold with foliar selenium application rates of 120 g/ha. In a study conducted by Manojlović *et al.* (2019) in Serbia and Croatia, application rates of 5 g/ha and 10 g/ha of foliar selenium and 10 g/ha of soil selenium at Futog (Serbia) gave increases of 2.4, 4.4 and 3.3-fold and at Banovci (Croatia) 2.7, 4.7 and 3.5-fold. Selenium application rates between 4 and 120 g Se/ha applied to wheat enhanced selenium concentration in the grain up to 133-fold when sodium selenate was applied to the soil at seeding and 20-fold when applied as a foliar application after flowering (Lyons *et al.*, 2005a).

Several studies on selenium fertilisation of crops have reported that selenium application had no effect on grain yield, yield components, harvest index (HI %) and plant growth parameters for example growth, tiller production and biomass. In wheat, Lyons *et al.* (2005b) in field studies found no significant effect on grain yield either from soil or foliar applications of up to 120 g/ha as selenate. Broadley *et al.* (2010) and Sharma *et al.* (2017) reported grain yield and harvest index were not affected by selenium application. Application of selenate to durum wheat in high selenium soils in Canada also showed no effect on plant emergence, grain yield and biomass (Grant *et al*., 2007). Nawaz *et al.* (2017) conducted a study on selenium method and timing of application and showed that foliar application at tillering had

no effect on grain yield, yield components (spike length, number of grains per spike, thousand-grain weight) and other growth parameters such as tiller production. However, foliar application of both selenate and selenite had a negative effect ($p< 0.05$) on wheat grain yield and biomass production (Wang *et al*., 2019). In maize, Wang *et al.* (2013) found no significant effect on grain yield and biomass following soil and foliar application. In rice, selenium treatment applied also gave no significant effects on crop yield and growth (Chen *et al.*, 2002). In terms of grain quality, the most studied grain quality parameter for bread-making wheat in biofortification studies is protein content (Poblaciones *et al.*, 2014b). In field trials, selenium fertilisation had no effect on grain quality, particularly protein content (Broadley *et al.*, 2010; Poblaciones *et al.*, 2014b ; De Vita *et al.*, 2017). No significant variation was detected in total protein in genotypes of rice following foliar application of 60 g Se/ha (Lidon *et al.*, 2018a). Indeed, falling number of wheat grain also showed no effect following foliar application of 10 g and 20 g Se/ha (Ducsay *et al*., 2016). Similarly to falling number and grain protein content, hectolitre weight showed no response to applied selenite and selenate following foliar application (Poblaciones *et al.*, 2014b).

Selenium biofortification of crops also depends on the form of selenium used (Lyons, 2018). The two major forms of inorganic selenium available in soils are selenate (Na_2SeO_4) and selenite (Na_2O_3Se) (Guerrero *et al.*, 2014; Dos Reis *et al.*, 2017). Numerous studies on selenium fertilisation with soil or foliar application have generally reported that selenate is more efficient than selenite at increasing grain selenium concentration (Lyons *et al*., 2003). Plants more effectively take up selenate than selenite (Cartes *et al*., 2005; Rodrigo *et al*., 2013; Wang *et al*., 2013). A recent review by Ros *et al.* (2016) showed that selenate was 33 times more efficient than selenite in selenium fertilisation. Ali *et al.* (2017) found that grain selenium contents in wheat were significantly higher following selenate than selenite treatment when both were used at the same application rate. The authors further observed that selenium concentrations in grain and leaves were about 17-28 and 21-363 times higher following selenate treatment compared with selenite. Seppänen *et al.* (2010) found that foliar application of selenate contributed to higher Se accumulation compared to selenite. According to Deng *et al.* (2017), selenite adsorption by ferric soil minerals makes it less available in the soil and therefore reduces the availability to plants.

2.3.6 Nitrogen fertilisation

Nitrogen is a major limiting factor to plant growth, yield and quality of wheat (Hawkesford, 2014; Mandic *et al*., 2015; Madaras *et al*., 2018). Appropriate management of nitrogen fertilisation rate, timing and source positively influenced yield, grain protein content and bread-making quality traits of wheat (Garrido-Lestache *et al*., 2005; Fuertes-Mendizábal *et al*., 2010; Abedi *et al*., 2011). For instance, Garrido-Lestache *et al*. (2004) found that application of 200 kg N/ha increased grain protein content to 14.6%. Nitrogen fertiliser employed at the active tillering stage (GS22) optimised crop yield and grain protein content (Fageria, 2010; Nakano *et al*., 2008). The application of nitrogen fertiliser at anthesis (flowering) also had a significant effect on grain protein content in wheat (Malik *et al*., 2012). In fact, accumulation of micronutrient in wheat grain including iron (Fe), zinc (Zn), copper (Cu) (Shi *et al.*, 2010) and selenium (Premarathna *et al*., 2012), has been shown to be affected by nitrogen fertilisation. Micronutrient concentrations are greatly influenced by the nitrogen application rate (Kuppusamy *et al*., 2018). Shi *et al*. (2010) found that the concentration of iron, zinc and copper in wheat grain was positively increased by nitrogen application rate from 130 kg N/ha to 300 kg N/ha compared to the control. With respect to crop disease, several studies have shown increased level of the foliar diseases yellow rust, Septoria leaf spot, powdery mildew and tan spot in wheat linked to mineral nitrogen application (Olesen *et al.*, 2003; Bilsborrow *et al*., 2013; Devadas *et al.*, 2014; Fleitas *et al.*, 2018). In organic production systems, nitrogen supplied to crops is mainly in an organic form such as compost, farmyard manure, vermicompost (Litoriya *et al*., 2018) and biogas digestate (but often only under a Derogation) (Möller *et al*., 2008). Biogas digestate is the residue from biogas production by anaerobic digestion (Tambone *et al*., 2017)

2.3.6.1 Organic N fertiliser sources

Many forms of organic fertilisers including animal manures, composts, sewage sludge, farmyard manure (FYM) and digestate have been extensively studied in the past as alternative strategies towards sustainable production. In particular, biogas digestate and composted farmyard manure have the potential to reduce mineral N fertiliser application (El-Ghamry, 2009; Makádi *et al*., 2012; Koszel and Lorencowicz, 2015; Riva *et al*., 2016). Digestate is a final product of anaerobic digestion, rich with macro and micronutrients and organic matter (Tambone *et al*., 2010; Drosg *et al*., 2015). Farmyard manure is a product of cattle dung and other animal waste produced following aerobic fermentation (Khatab *et al*., 2015). Biogas

digestate and composted farmyard manure have demonstrated comparable grain yield to mineral N fertiliser application (Šimon *et al*., 2015; Riva *et al*., 2016; Tampio *et al*., 2016). Biogas digestate has high available N-content to meet crop N demand (Möller and Müller, 2012). Biogas digestate also aids the recycling of organic waste materials in an efficient and sustainable way (Tampio *et al*., 2016). Composted farmyard manure has been shown to improve cereal grain yield (El-Ghamry, 2009; Jan *et al*., 2011) and in some cases produce the same yield as mineral N when the dosage applied is at the same level (Gopinath *et al.*, 2008; Tétard-Jones *et al.*, 2013). In contrast, Rempelos *et al.* (2018) and Madaras *et al.* (2018) found that farmyard manure application significantly reduced grain yield compared with mineral N fertiliser application. Application of farmyard manure and organic fertiliser sources to the soil has the potential to supply a wide range of additional micronutrients (Miller and Welch, 2013). Application of farmyard manure was shown to increase the selenium concentration in above-ground plant parts and roots of spring barley (Borowska *et al*., 2012a). Overall, application of biogas digestate and composted farmyard manure benefits soil's physical, chemical, and biological properties, influencing nutrient availability (with reduced environmental impacts e.g. reduced N leaching and P run-off), improving water-holding capacity, soil fertility, soil organic matter, soil structure and soil infiltration rates (Gopinath *et al.*, 2008; Jan *et al*., 2011; Sharma *et al.*, 2011; Li *et al.*, 2017).

2.3.6.2 Soil N availability of organic inputs

Crop plants absorb nitrogen mainly from the soil in the form of nitrate (NO_3^-) and ammonium (NH_4^+) (Barunawati *et al*., 2013), with the former being the dominant form of N for plant uptake. The breakdown and availability of organic fertilisers is related to the dynamics of the mineralisation process in the soil, which is affected by temperature and other environmental conditions (Jones *et al.*, 2010; Palmer *et al.*, 2013; Buchi *et al.*, 2016). Under organic farming, nitrogen is considered as one of the key limiting factors for crop growth (Möller *et al*., 2008). Organic inputs such as composted manure, green manure and organic wastes are generally low in readily available ammonium and nitrate (Palmer *et al.*, 2013; Röös *et al.*, 2018). Palmer *et al.* (2013) and Gopinath *et al.* (2008) reported that the lower yield in organic compared with conventional production is due to reduced nutrient supply and the slower release of available nutrients via mineralisation. Organic nitrogen needs a longer time to be released and become available for crop uptake than mineral N (Berry *et al*., 2002). Low N availability at the time of crop demand generally limits productivity in organic production systems (Jones *et al*., 2010).

Sufficiency of N availability in organic production mostly depends on N synchronisation between the release of N from organic inputs and crop demand (Zuk-Golaszewska *et al*., 2015; Hazra *et al*., 2018).

2.3.7 Crop protection (conventional vs organic production)

Organic production is regularly considered as the most sustainable form of production due to a reduced use of inputs, in particular synthetic fertilisers and pesticides (Pang and Letey, 2000; Hazra *et al.*, 2018), which helps reduce the environmental impact of farming (Iannucci & Codianni, 2016). The Green Revolution achieved an increased output of conventional production but with a heavy reliance on high inputs of mineral N fertiliser and pesticides (insecticides, fungicides and herbicides) which have significant environmental impact (Mader, 2002; Iannucci and Codianni, 2016; Hazra *et al.*, 2018). In efforts to minimise the problems associated with intensive agriculture, organic production has become an option to produce food more sustainably (Cooper *et al*., 2011). This move to organic production is expanding across the globe (Petrenko *et al.*, 2018). Organic production is a technique to cultivate crops without the application of synthetic mineral N fertilisers and pesticides with increased emphasis on crop rotation, the use of organic fertilisers such as green and animal manure and non-use of synthetic pesticides to maintain soil health and productivity (Cooper *et al.*, 2011; Vrcek *et al*., 2014). Organic production optimises fertiliser recovery, reducing green-house (GHG) emissions, minimising N leaching and groundwater degradation (Pang & Letey, 2000), and providing foods free from pesticide residues (Zikeli *et al*., 2014; Mazzoncini *et al*., 2015).

2.3.7.1 Effect of crop protection on grain yield and quality

Most comparisons between organic and conventional production in previous studies have focused on yield and quality (particularly protein content) differences. Numerous authors in the literature have shown that yield in organic production is much lower than in conventional production. Reganold and Wachter (2016) indicated that many studies showed yield in organic production was 20-65% lower than conventional production. Rempelos *et al.*, (2018) and Iannucci and Codianni (2016) respectively found the yield in organic production was 45% and 40% lower compared to conventional production practices. Campiglia *et al*. (2015) in a study of long-term effects of cropping system and weather conditions found that the yield of durum wheat in organic compared to conventional production was lower by about 15%. Similarly, Knapp and van der Heijden (2018) found the average yield produced across all crops in

conventional production was 15% higher than under organic production. A recent review by De Ponti *et al*. (2012) concluded that the wheat yield gap between organic and conventional production was about 20%. Overall, the lower yield in organic than conventional production is primarily due to less efficient utilisation of crop protection and fertiliser (Rempelos *et al.*, 2018), difficulties in plant nutrient management, lack of pest management options (Hazra *et al.*, 2018) and nitrogen shortage during later growth stages (Mazzoncini *et al*., 2015; Iannucci & Codianni, 2016). Conventional agriculture has higher yield because this production relies on mineral N fertiliser, chemical inputs (pesticides and growth regulators), better pest, disease and weed control, with lower pressure on nutrient availability than in organic production (De Ponti *et al*, 2012).

In terms of grain quality, most earlier studies in cereal crops showed grain protein content was significantly lower in organic than conventional production (Gélinas *et al.*, 2009; Vrček *et al.*, 2014; Mazzoncini *et al.*, 2015; Röös *et al.*, 2018). In contrast, Mason *et al.* (2007) found that there was no variation in protein content between organic and conventional production systems. Mader *et al*. (2007) suggested that the significantly lower grain protein content in organic production was due to the absence of applying mineral N fertilisers. Hectolitre weight was reported to be higher in conventional than organic production (Przystalski *et al*., 2008; Mason *et al*., 2007). However in contrast, Iannucci & Codianni (2016) observed hectolitre weight in an organic production system was higher than under conventional production. Annicchiarico *et al*. (2010) and Mason *et al.* (2007) found little difference in hectolitre weight between the two production systems, whereas concentrations of micronutrients such as Ca, Mn and Fe were lower in organic production than conventional production of wheat (Vrcek *et al*., 2014).

2.3.8 Genetic variation of grain quality in common and ancient wheats

Wheat (*Triticum*) has five main species, classified as common or bread wheat (*Triticum aestivum*), durum wheat (*T. durum*), emmer (*T. dicoccum*), einkorn (*T. monococcum*) and spelt (*T. spelta*) (Jablonskytė-Raščė *et al*., 2013). Among these, common wheat is widely cultivated globally for a range of bread-making products, but its protein and nutrient content in grains is lower than in other species (Kohajdova & Karovicova, 2008). Recently, increased interest in the use of ancient cereals such as emmer, einkorn and spelt as alternatives to common wheat has occurred due to their increased protein content, nutrient density and perceived health benefits. In particular, spelt has received renewed interest during the last 20-30 years due to its

suitability for growing in a low-input environment (Gomez-Becerra *et al*., 2010). Spelt demonstrated greater grain protein content (16-17%), nutrient and micronutrient concentrations than common wheat even when cultivated under low fertilisation (Jablonskytė-Raščė *et al*., 2013; Biel *et al.*, 2016). Interestingly, spelt was also found to have greater grain selenium concentration than common wheat (Zhao *et al.*, 2009). Fageria *et al.* (2012) suggested crop species and genotype could be effectively exploited for increasing nutrient content of cereals. Exploitation of available germplasm is a principal strategy to improve concentration of micronutrients such as zinc and iron in staple crops (Cakmak *et al.*, 2004). At present, manufacturers and in particular artisan bakers have shown increasing interest in the use of spelt as a substitute for wheat in making various types of bread products. This is shown by the increased availability of various food spelt-based products in the market including bread, flour, crackers, pasta, breakfast cereals and flakes (Kohajdova and Karovicova, 2008; Jablonskytė-Raščė *et al.*, 2013). Spelt flour price in the market is also about 50% higher than wheat flour where spelt flour is regularly available in health-food stores (Kohajdova & Karovicova, 2008).

Chapter 3 : A systematic review & meta-analysis on grain selenium concentration in cereals

3.1 Background

Selenium deficiency has been a risk to human health for decades (Rahman *et al*., 2015). One feasible method to significantly increase grain selenium concentration is agronomic biofortification through the use of selenium fertiliser (Lyons *et al*., 2004; Stroud *et al*., 2010a, b; Mora *et al*., 2015). There are two general methods available for selenium fertilisation; soil and foliar application (Mora *et al*., 2015; Valença *et al*., 2017; Zhang *et al*., 2019), with both methods in the literature showing the potential to boost grain selenium concentration. Several studies have reported that foliar application is more efficient at increasing grain selenium concentration than soil application (Wang *et al*., 2013; Mao *et al*., 2014; Ros *et al*., 2016; Lyons, 2018; Ngigi *et al*., 2019). In contrast, Boldrin *et al*. (2013) found soil application resulted in grain selenium content higher than from foliar application. Scientific evidence is vital to examine which method may significantly influence grain selenium concentration of cereal crops more effectively. In this respect, one of the appropriate tools to assess the performance and effects of soil and foliar applications for grain selenium concentration accumulation and yield of cereal crops is through a systematic review and meta-analysis. To the best of our knowledge, a review assessing the effects of soil and foliar applications on grain selenium concentration and yield focusing on the major and minor cereals (wheat, rice, maize, barley, rye and oats) using a systematic review & meta-analysis approach is not available in the literature. Therefore, two main objectives are addressed in this review: (1) To evaluate the effects of selenium fertilisation methods in soil and foliar application on grain selenium concentration and yield (2) To explore the influence of soil properties (soil pH, soil selenium concentration and selenium form) on grain selenium concentration and yield under different selenium application methods (soil and foliar). Two key research questions have driven this review: (1) What are the effects of soil and foliar fertilisation on grain selenium concentration and yield of cereals? (2) How does soil heterogeneity influence the effectiveness of selenium biofortification on grain selenium concentration and yield?

3.2 Methodology

3.2.1 Eligibility criteria for the review

All studies included in the review were obtained from the electronic database searches and were selected based on the eligibility criteria outlined from the specific PICO(S) components outlined below (Haidich, 2010; Cuijpers, 2016; Ahn and Kang, 2018). PICO(S) stands for population, intervention, comparison/comparator, outcome, and study design (O'Connor *et al*., 2014). Specific PICO(S) components are necessary, lead to formulating good research questions, defining the inclusion and exclusion criteria for meta-analysis and help search strings for bibliographical databases (O'Connor *et al*., 2014; Cuijpers, 2016).

The eligibility criteria for the review as described in the PICO(S) are as follows:

3.2.1.1 Type of study design

All independent primary studies assessing the effects of soil and foliar selenium application on grain selenium concentration and yield were eligible for inclusion. Only studies carried out either in the field or in pots in the greenhouse which incorporated a control treatment, replication and randomization were selected to address the primary objectives and research questions.

3.2.1.2 Type of population

The type of population in this review focused on the major and minor cereal crops (wheat, rice, maize, barley, rye and oats). Other crop species found in the primary study were automatically excluded.

3.2.1.3 Type of interventions

The type of interventions in this review were soil and foliar selenium applications of cereals. Only primary studies which assessed the effects of soil and/or foliar on grain selenium concentration and/or yield were selected for inclusion in the study.

3.2.1.4 Type of comparator

In most agriculture field trials, control plots are used as a comparator to the applied treatments. The comparator in this study was the control without selenium fertiliser application. Primary studies without any control data (comparator) were excluded from the review.

3.2.1.5 Type of outcome

Data extracted for the primary and secondary outcomes from the primary studies were grain selenium concentration and yield.

3.2.2 Criteria for study selection (Inclusion and exclusion)

Inclusion criteria: All relevant studies which compared the effectiveness of soil and foliar selenium application (with a control) on grain selenium concentration and yield were included. Included articles were not restricted by date of publication, geographic location (countries and regional), number of experimental years, growing season, specific cultivars or rates and timing of selenium fertiliser application. Only primary studies written in English language were included.

Exclusion criteria: Primary studies with insufficient information, missing or lacking essential information to comply with all the eligible criteria for inclusion were automatically excluded from study selection. Also excluded were non-empirical studies such as review articles, abstracts or posters.

3.2.3 Search strategy

Relevant publications were searched using Web of Science, Scopus, Science Direct and Google Scholar electronic databases. Search terms were refined and reviewed with the aim to ensure the most successful outcomes and capture as many relevant primary studies as possible. The following search terms were used; “Selenium biofortification”, “Se biofortification”,“Selenium fertiliser”, “Selenium fertili*”, “Se fertili*”, “Agronomic biofortification”,“Selenium soil AND foliar application”, “Agronomic selenium biofortification”, “Selenium soil application”, “Selenium foliar application”, “Selenate biofortification” “Selenite biofortification” “Selenium concentration”. The Boolean operators “AND” or “OR” were applied to ensure the most successful search strategies. An asterisk “*” also was added where appropriate to any root term search used such as 'Selenium fertili* OR

Se fertili* and 'Selenium AND fertili*. Key search terms applied should appear either in the title section, abstracts, or keywords of each individual study. No restrictions were imposed regarding the date of study. All primary studies retrieved from the electronic database searches were then exported and merged into the EndNote referencing manager. Any duplications within the initial search were removed.

3.2.4 Search screening (study selection)

All titles and abstracts according to pre-determined eligibility criteria (PICO) were screened and identified separately by two reviewers: the thesis author (main reviewer) and Assoc. Prof. Ts. Dr. Fazleen Abd Fatah (econometrics & data analytics) as the second reviewer. The process of screening involved two stages. In stage 1, titles and abstracts were read and screened by the main reviewer. Only titles and abstracts matching the review objectives, research questions and eligibility criteria for study inclusion were used and subsequently searched for full text copies. In stage 2, the full text report was read and checked properly for inclusion by the main reviewer. The corresponding author was contacted by e-mail to request a full-text version if articles could not be retrieved electronically or were only available in abstract form. The second reviewer repeated stages 1 and 2 to confirm the eligibility criteria. Any disputes between the main reviewer and second reviewer were resolved through discussion.

The study selection process used in this review was in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) flow chart diagram (Moher *et al*., 2009). The PRISMA flow chart diagram (Fig. 3.1) shows the phases involved in the systematic review and meta-analysis protocol, which includes the total number of primary studies found, total number of duplicate studies which were screened, number of studies either included or excluded with reasons and the final total number of studies included in the meta-analysis.

3.2.5 Data collection and analysis

3.2.5.1 Data extraction and assessment by reviewers

Data extracted from the included studies consisted of study background, experimental details, moderator variables and outcomes (Table 3.1). Quantitative data, for example sample size (n), mean ($\bar{x}$), standard error (SE) or standard deviation (SD) reported as numerical values in the text or tables, were extracted and compiled into an Excel spreadsheet. Data extraction

from the included publications was conducted by the two reviewers (main and second reviewer) separately. The main reviewer was responsible for extracting all the relevant data while the second reviewer double-checked for any mistakes in the extracted data. Discrepancies detected by the second reviewer accounted for 10% of the extracted data from the main reviewer. Thus, data extraction was repeated and checked appropriately by the main reviewer in agreement with the second reviewer.

Table 3.1. *Information and data extracted from the studies selected for meta-analysis.*

Item	Information
Study background	• Study citation • Author name • Publication year • Country
Experiment details	• Cereal species • Variety • Experiment type (Field or pot-based) • Experimental site (study location) • Experimental year
Moderator variables	• Soil pH • Soil selenium • Selenium form (Selenate/Selenite) • Method of selenium application (soil and foliar) • Timing of selenium application • Rate (s) of selenium application
Outcome	• Grain selenium concentration (Primary outcome) • Yield (Secondary outcome)

3.2.5.2 Dealing with missing and incomplete data

The corresponding authors of included publications were contacted through e-mail where data was missing or unavailable. All studies where missing/unavailable data could not be recovered were excluded from the meta-analysis.

3.2.5.3 Quality assessment

Each included study after passing the screening process (study eligibility) underwent quality assessment to highlight the overall strength of evidence of the study. This assessment evaluated and critically appraised the clarity/quality of the methodology used in each included study associated with the study aims, appropriateness of methodology, findings, outcome reporting and any conflicts of interest. In this review, the quality assessment critically appraised the methodological criteria such as study overview, internal validity, analytical methods, results and general assessment. No single study was excluded from this assessment based on risk of bias.

The full quality assessment statements and overall rating descriptions are shown in Table 3.2. The overall methodological quality of each included study was scored using a three-level rating (low risk, high risk and unclear). The judgement answers were divided into three option categories: Yes, No and Unclear (Table 3.3). The rating quality of evidence and recommendations for grading strength for this review were adopted from the Grading of Recommendations Assessment, Development and Evaluation (GRADE) assessment guideline system (Guyatt *et al*., 2008; Moher *et al*., 2009).

The quality assessment was conducted by at least two reviewers to lower the risk of bias in the study inclusion. Any disagreements between reviewers was resolved by consensus and mutual agreement. The corresponding author of the study was subsequently contacted for clarity, if there were any disagreements between reviewers.

Table 3.2. *Quality assessment statements (Hasanaliyeva, 2018).*

Item	Quality assessment statement
Study overview	• The study addresses the agronomic question/hypothesis. • The type of study is clearly explained (field experiment or pot study).
Internal validity	• Comparison is made between appropriate agronomic systems in terms of question/hypothesis. • The number of replicates (sample size) is described. • The number of replicates (sample size) is sufficient for statistical evaluation. • The number of replicates (sample size) is the same or similar for all treatments used. • The season and cultivation conditions (e.g. climate, soil properties) are the same or similar for all treatments, except for factors used to test question/hypothesis. • The variety of the cereal used in each study is the same for all treatments.
Analytical methods	• Sample selection is described. • Sample selection is the same for all agronomic systems. • The post-sampling storage time and conditions are described. • The post-sampling storage time and conditions are the same for all agronomic systems. • Choice of statistical methods is appropriate.
Results	• Outcome measures are reliable and adequate to test the question/hypothesis. • Effect sizes are given as mean or median values for each agronomic system. • The measurement of variance is provided for each mean (as confidence intervals, standard error, etc.). • All outcome measures described in the methods section are reported (in tables, figures or text).
General assessment	• The limitations of the study design are discussed. • Authors discuss whether an effect found in the study can be seen in real life. • Study successfully minimises the risk of bias or confounding effects. • There is a clear evidence of an association between agronomic system and outcome. • Any sponsorship/conflict of interest is reported.
Overall rating	• *'Low risk'* when majority of criteria are met, there is a little or no risk of bias, and the results are complete and well described. • *'Some concerns medium risk'* when most criteria are met, there is low risk of bias but inadequate to invalid the results, results are complete and well described. • *'High risk'* when either most criteria are not met, or there is significant risk of bias relating to key aspects of the study design that possibly invalidate the results, results are incomplete. • *'Unclear'* Information in the paper does not reflect the assessment statement (Insufficient information).

Table 3.3. *The judgement answers for the quality assessment.*

Judgement category	Description
Yes	Content of the paper reflects the quality assessment statements.
No	No relevant information found in the paper.
Unclear	Information in the paper does not reflect the quality assessment statements (Insufficient information provided).

3.2.5.4 Assessment of heterogeneity and the risk of publication bias

Any kind of differences between included studies in terms of methodological factors such as variations in true treatments, variability in the intervention effects being evaluated in the different studies (statistical heterogeneity such as in standard errors or sample size) or if there are differences between studies in the way the outcomes are defined and measured, may lead to heterogeneity or differences in the observed intervention effects. Heterogeneity associated with methodological diversity would indicate that the studies suffer from different degrees of publication bias. The amount of variability (heterogeneity) between primary studies and the potential source of heterogeneity in the effect of the intervention were quantified statistically using the I^2 value from the I^2 test statistic (Higgins *et al*., 2003). I^2 index was calculated based on the formula below where Q is Cochran's heterogeneity statistic and df the degrees of freedom; -

$$I^2 = 100\% \times (Q - df)/Q$$

I^2 value, *p*-value and the degrees of freedom (df) are presented in the forest plots to show the heterogeneity of intervention effects. As a general guide, heterogeneity values $I^2 < 49\%$ (low heterogeneity), $I^2 = 50\%$-74% (moderate heterogeneity) and $I^2 > 75\%$-100% are considered as high heterogeneity. Meta-regressions were undertaken to assess the heterogeneity source if heterogeneity was identified as an issue.

Publication bias is one of the most common types of reporting bias in meta-analyses and may affect the validity and generalization of conclusions. Several lines of research reviewed by Dickersin (2005) have recognised that meta-analysis outcomes may be over-estimated due to a tendency of having greater preference for a higher likelihood of publication of statistically

significant studies rather than non-significant studies. All included studies were tested for publication bias (Bown and Sutton, 2010). In order to gauge the impact of publication selection bias or to identify the presence or absence of publication bias, a funnel plot method was used which includes visual examination of a funnel plot and the nonparametric trim and fill method (Bown and Sutton, 2010; Del Re, 2015; Rodney *et al*., 2015). Studies of soil and foliar selenium applications are plotted on a scatter plot with effect size on the x-axis and standard error on the y-axis (Russo, 2007; Bown and Sutton, 2010). There is no evidence of publication bias if the points or estimates form an inverted funnel shape, with a broad base that narrows towards the top of the plot where the most precise estimates at the top of this plot should be close to the true effect, and less precise ones at the bottom of the plot are more dispersed (Rodney *et al*., 2015) as shown in Fig. 3.5 (B) and Fig. 3.6 (B). Publication bias can be suspected if the plot shows an asymmetric shape with no points on one side of the graph or over-weighted on either one of the sides (Rodney *et al*., 2015; Ahn and Kang, 2018). In such a case when publication bias is detected, the trim-and-fill method can be used to correct the bias (Ahn and Kang, 2018). Trim-and-fill method is a diagnostic tool for meta-analysis that may identify and correct for funnel plot asymmetry when there is evidence of publication bias (Higgins and Green, 2008; Borenstein *et al*., 2009). This method can be used to trim any study that caused funnel plot asymmetry, where the remaining studies generated the overall effect estimate that may minimally be impacted by publication bias. Then, missing studies were imputed to fill in the funnel plot according to the bias-corrected (Higgins and Green, 2008; Shi and Lin, 2019). To confirm there is no evidence of publication bias in this meta-analysis, the trim and fill method was carried out for Fig. 3.5 (B) and Fig. 3.6 (B).

3.2.6 Meta-analysis

All pooled effect sizes from the included studies were analysed using subgroup analysis random-effects model and meta-regression (Carrick *et al*., 2018) in the R statistical environment version 3.6.3 (R Core Team, 2012). The random-effects model was performed using Meta and Metaphor analysis package (Viechtbauer, 2010) to observe the changes in each outcome. By the random-effects model, the assumption is made that the true effect size of an individual study is different from another study and the summary effect is considered as the mean of the distribution of the effect sizes (Borenstein *et al*, 2009).

Effect sizes are presented as standardised mean difference (SMD) and 95% CIs on the multiple outcomes which are grain selenium concentration and yield presented on non-

comparable scales derived from the number of foliar treatments (nf) and number of soil treatments (ns), mean of foliar treatment (mf) and mean of soil treatment (ms), standard deviation of foliar treatment (sdf) and standard deviation of soil treatment (sds), number of controls (nc), mean of controls (mc), standard deviation of control (sdc). A statistical significance level of $p<0.05$ was used for both outcomes. The standardised mean difference is a summary statistic and represents a common metric unit in meta-analysis which is used to compare and measure effect sizes of the studies which assess the same outcomes but uses different units across the trials (Takeshima *et al*., 2014). Standardised mean difference was calculated as the effect size of interventions across different studies which has variability observed (Higgins and Green, 2008; Barański *et al.*, 2014; Takeshima *et al*., 2014; Carrick *et al*., 2018). Formula calculation for a standardised mean difference in this study, which is also called Hedges' *g*, was according to Schwarzer *et al*. (2015).

DMETAR package analysis was used to perform subgroup analysis to see the changes in each outcome group based on soil and foliar application methods (control vs treatment) to generate two forest plots. Findings of meta-analysis were interpreted through construction of forest plots. Forest plots show summary results of pooled standardised mean difference values (effect sizes) and corresponding confidence intervals of 95% from the multiple primary studies (Barański *et al*., 2016).

In an effort to understand the sources of heterogeneity, multiple regression in meta-analysis was performed on soil and foliar application to see whether the predictors or covariates were statistically significant and had considerable unexplained heterogeneity (I^2). The multiple regression model was used to allow for the use of continuous covariates and to allow for the inclusion of more than one covariate at a time. Three covariates (soil pH, soil selenium concentration and selenium form) were chosen to be included in the multiple regression model for both soil and foliar application in the included studies which potentially could influence grain selenium concentration and yield. These covariates were altogether regressed in a random-effects regression model using the R software package.

3.3 Results

3.3.1 Study selection

The summary selection process to identify studies for the meta-analyses in this review is shown in Fig. 3.1. Out of 1919 studies identified in the initial literature search, 1288 duplicate studies were removed. The remaining 631 studies were screened based on title and abstract. Another 595 studies were further excluded at the screening stage as these screened title and abstracts were not matched with the review objectives, research questions and eligibility criteria for study inclusion. A total of 36 studies remained consisting of wheat (n=19), rice (n=8), maize (n=5), barley (n=3) and oat (n=1) which were searched for full-text studies and carefully read. No records were found for rye. At the eligibility stage, twenty-six studies were excluded from meta-analysis. Nine studies were found irrelevant due to non-compliance with eligibility criteria of the review, four pot-trial studies in the glasshouse were considered as an insufficient number of studies for meta-analysis (wheat, n=2 and rice, n=2) and 13 papers with missing data (without SE/SD information) and which failed to receive any feedback from corresponding authors when contacted via e-mail. Only one author replied to the e-mail and supplied the requested data. Overall, the final number of studies included in the meta-analysis was 10 field-based studies.

3.3.2 Description of included studies

Table 3.4 shows the general characteristics of the studies which met the eligibility criteria. Out of 10 studies, six were on wheat with the other four on rice (n=2) and maize (n=2). Most studies in this review appeared to practise foliar rather than soil application of selenium fertiliser in field trials and use selenate rather than selenite as the fertiliser source.

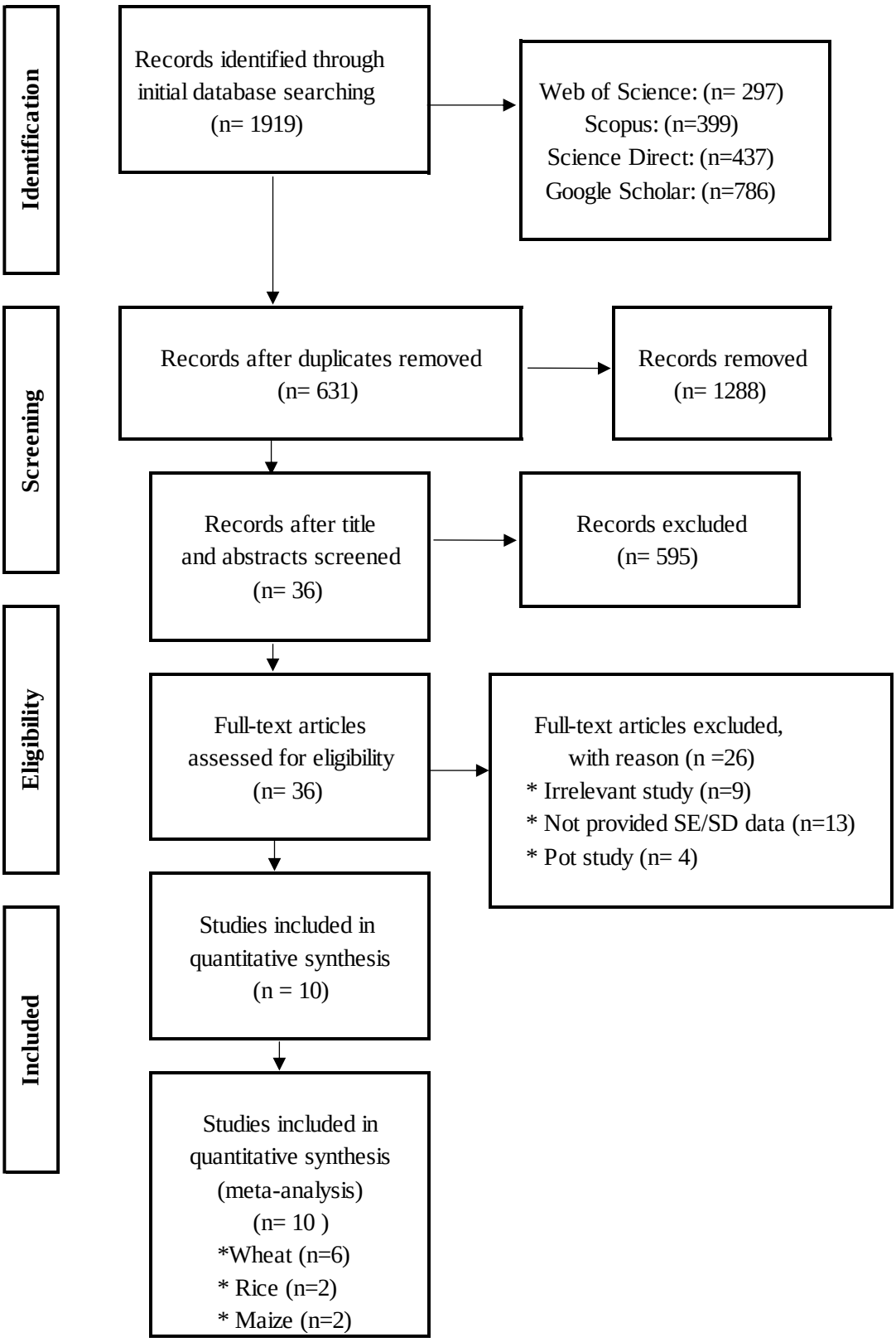


Fig. 3.1. *Summary of the searching and selection strategy to identify eligible studies for the meta-analyses based on PRISMA flow diagram (Moher et al., 2009). SD-Standard deviation; SE-Standard error.*

Table 3.4. *Primary author, country, study type, cereal species, method of application, and selenium form of the included studies in the meta-analysis.*

Primary author	Country	Study type	Species	Application method	Se form
De vita *et al*., 2017	Italy	Field trial	Wheat	Foliar	Selenate
Hefni *et al*., 2015	Egypt	Field trial	Wheat	Foliar	Selenate
Wang *et al*., 2019	China	Field trial	Wheat	Foliar	Selenite
Poblaciones *et al*., 2014a	Spain	Field trial	Wheat	Foliar	Seleneta/Selenite
Tveitnes *et al*., 1995	Norway	Field trial	Wheat	Soil	Selenate
Mao *et al*., 2014	China	Field trial	Wheat	Soil	Selenate
Mangueze *et al*., 2018	Mozambique	Field trial	Rice	Foliar	Selenite
Hu *et al*., 2002	China	Field trial	Rice	Foliar	Selenite
Chilimba *et al*., 2014	Malawi	Field trial	Maize	Foliar	Selenate
Ngigi *et al*., 2019	Kenya	Field trial	Maize	Soil & foliar	Selenate

3.3.3 Meta-analysis

Two separate meta-analyses were performed in this review. Fig. 3.2 and Fig. 3.3 show results of the meta-analysis presented in a forest plot for soil and foliar selenium application on grain selenium concentration and yield using random-effects meta-analysis.

3.3.3.1 Effect of soil and foliar applications on grain selenium concentration and yield

Fig. 3.2 shows the forest plot for the outcomes across all selected studies: grain selenium concentration and yield for the comparison between a group of studies where soil application with multiple selenium rates was compared to the control treatment. According to Fig. 3.2, the summary effect size of the group analysis for grain selenium concentration was SMD 2.03 (95% CI from 1.52 to 2.53). No heterogeneity was found between studies since the estimated I^2 was 0%, τ^2 was 0.72 and *p*-value was 0.48. Meanwhile, the summary effect size of the group analysis for grain yield was SMD -0.43 (95% CI from -1.15 to 0.30). No heterogeneity was found between studies since the estimated I^2 was 0%. Overall, the pooled analysis of effect size indicated that there was a significant difference ($p<0.01$) in pooled grain selenium concentration and grain yield under soil selenium application compared to control treatments. The summary effect size of pooled analysis was SMD 1.68 (95% CI from 1.08 to 2.29). Heterogeneity between studies was detected since the estimated I^2 was 48% (low heterogeneity).

Fig. 3.3 shows the forest plots for the outcomes (grain selenium concentration and grain yield) for the comparison between a group with foliar application (multiple selenium rates) compared with a control treatment without selenium added. According to Fig. 3.3, the summary effect size of the group analysis for grain selenium concentration was SMD 11.07 (95% CI from 7.69 to 14.45). Heterogeneity was found between studies since the estimated I^2 was 83% (high heterogeneity), τ^2 was 53.3 and $p < 0.01$. Meanwhile, the summary effect size of the group analysis for grain yield was SMD 0.00 (95% CI from -0.17 to 0.17). No heterogeneity was found between studies since the estimated I^2 was 0%, τ^2 was 0.04 and *p*-value was 1.00. Overall, the pooled analysis of effect size indicated that there was a significant difference ($p<0.01$) in pooled grain selenium concentration and grain yield under foliar selenium application compared to control treatments. The summary effect size of pooled analysis was SMD 4.28 (95% CI from 2.19 to 6.38). Heterogeneity between studies was detected since the estimated I^2 was 79% (high heterogeneity).

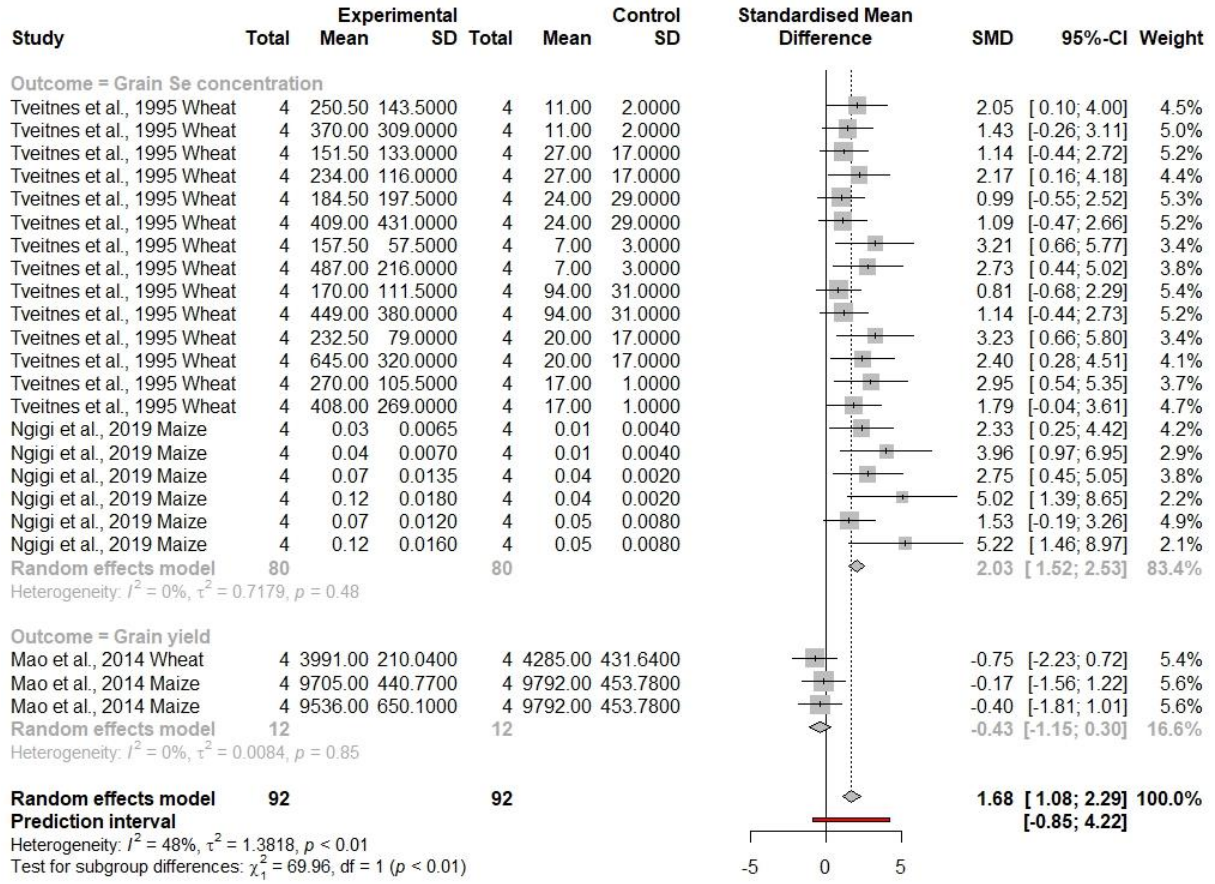


Fig. 3.2. *Forest plot presenting the results of two eligible studies examining the effects of soil selenium application with multiple rates on grain selenium concentration and one eligible study on grain yield of wheat and maize crops. The figure shows soil selenium application versus the control treatment using standardised mean differences (SMD) with corresponding 95% confidence intervals based on a random-effect model. Standardised mean difference is shown on the x-axis. Columns of figure represents; Study (All relevant study included for the meta-analysis); Experimental (selenium treatments), Total (number of soil treatment replication), Mean (mean of soil treatment), SD (standard deviation of soil treatment); Control (without selenium addition), Total (number of control treatment replication), Mean (mean of control), SD (standard deviation of control); SMD (Standardised mean difference) (Effect sizes for each study at multiple selenium rates); 95%-CI (95% confidence interval); Weight (The weight in percentage showed the influence an individual study has had on the pooled result). Rows of figure represents multiple selenium rates through soil application (experimental) which was compared to the control treatment (control) and computed for effect size.*

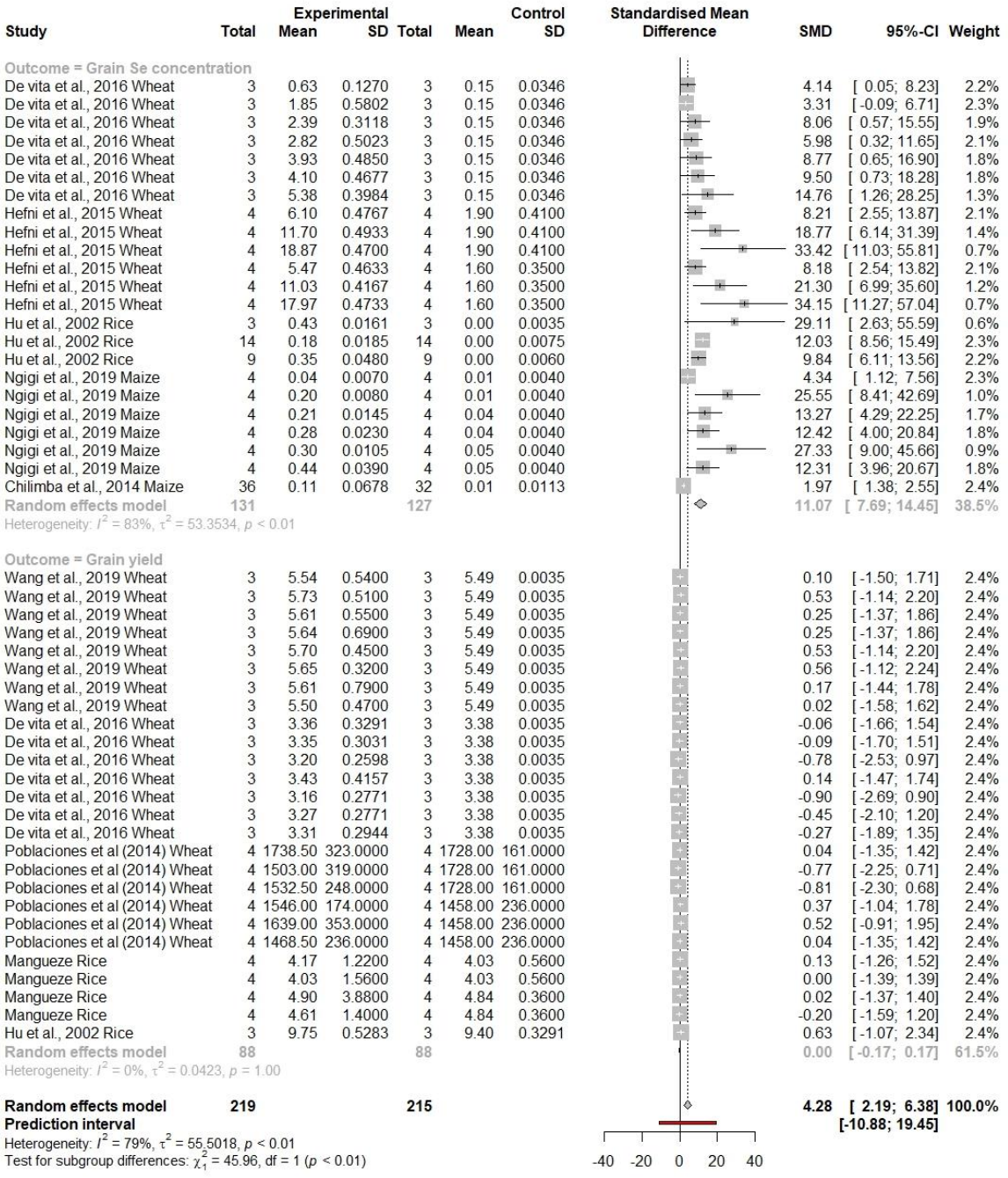


Fig. 3.3. *Forest plot presenting results of the five eligible studies examining the effects of foliar selenium application with multiple rates on grain selenium concentration and grain yield of wheat, rice and maize. The figure shows foliar selenium application treatment versus the control treatment using standardised mean differences (SMD) with corresponding 95% confidence intervals based on a random-effect model. Standardised mean difference is shown on the x-axis. Columns of figure represents; Study (All relevant study included for the meta-analysis); Experimental (selenium treatments); Total (number of foliar treatment replication), Mean (mean of foliar treatment), SD (standard deviation of foliar treatment); Control (without selenium addition); Total (number of control treatment replication), Mean (mean of control), SD (standard deviation of control); SMD (Standardised mean difference) (Effect sizes for each study at multiple selenium rates); 95%-CI (95% confidence interval);Weight (The weight in percentage showed the influence an individual study has had on the pooled result). Rows of figure represents multiple selenium rates through foliar application (experimental) which was compared to the control treatment (control) and computed for effect size.*

3.3.3.2 The risk of publication bias

According to the quality assessment summary (Fig. 3.4), all included studies for the meta-analyses in this review were judged to have low risk of bias. Besides that, examination of funnel plots in Fig. 3.5 and Fig. 3.6 showed that there was no publication bias detected in the included studies for foliar and soil selenium application as shown by the point forms of the inverted funnel shape in both funnel plots. Fig. 3.5 and Fig. 3.6. show visual inspection of publication bias using funnel plot, which was corrected using the trim-and-fill method for foliar and soil application using R software.

In Fig. 3.5, the funnel plot for foliar application (A) showed studies of foliar were plotted on a scatter plot with effect size on the x-axis and standard error on the y-axis. From the visual examination, the funnel plot for foliar application (A) showed no evidence of publication bias since the points or estimates form an inverted funnel shape, with a broad base that narrows towards the top of the plot where the most precise estimates at the top of this plot be close to the true effect, and less precise ones at the bottom of the plot are more dispersed. To confirm the absence of publication bias in this meta-analysis, the trim-and-fill method was carried out as shown in Fig. 3.5 (B)-trim and fill funnel plot. In the trim-and-fill method, the studies that cause a funnel plot's asymmetry were first trimmed, so that the overall effect estimate produced by the remaining studies has been considered minimally impacted by publication bias. Then, imputed missing studies were filled in the funnel plot based on the bias-corrected. However, that the trim-and-fill does not change the overall effect size much.

In Fig. 3.6, the funnel plot for soil application (A) showed studies of soil were plotted on a scatter plot with effect size on the x-axis and standard error on the y-axis. From the visual examination, the funnel plot for soil application (A) showed no evidence of publication bias since the points or estimates form an inverted funnel shape, with a broad base that narrows towards the top of the plot where the most precise estimates at the top of this plot be close to the true effect, and less precise ones at the bottom of the plot are more dispersed. To confirm the absence of publication bias in this meta-analysis, the-trim and-fill method was carried out as shown in Fig. 3.6 (B)-trim and fill funnel plot. However, the trim-and-fill does not change the overall effect size much.

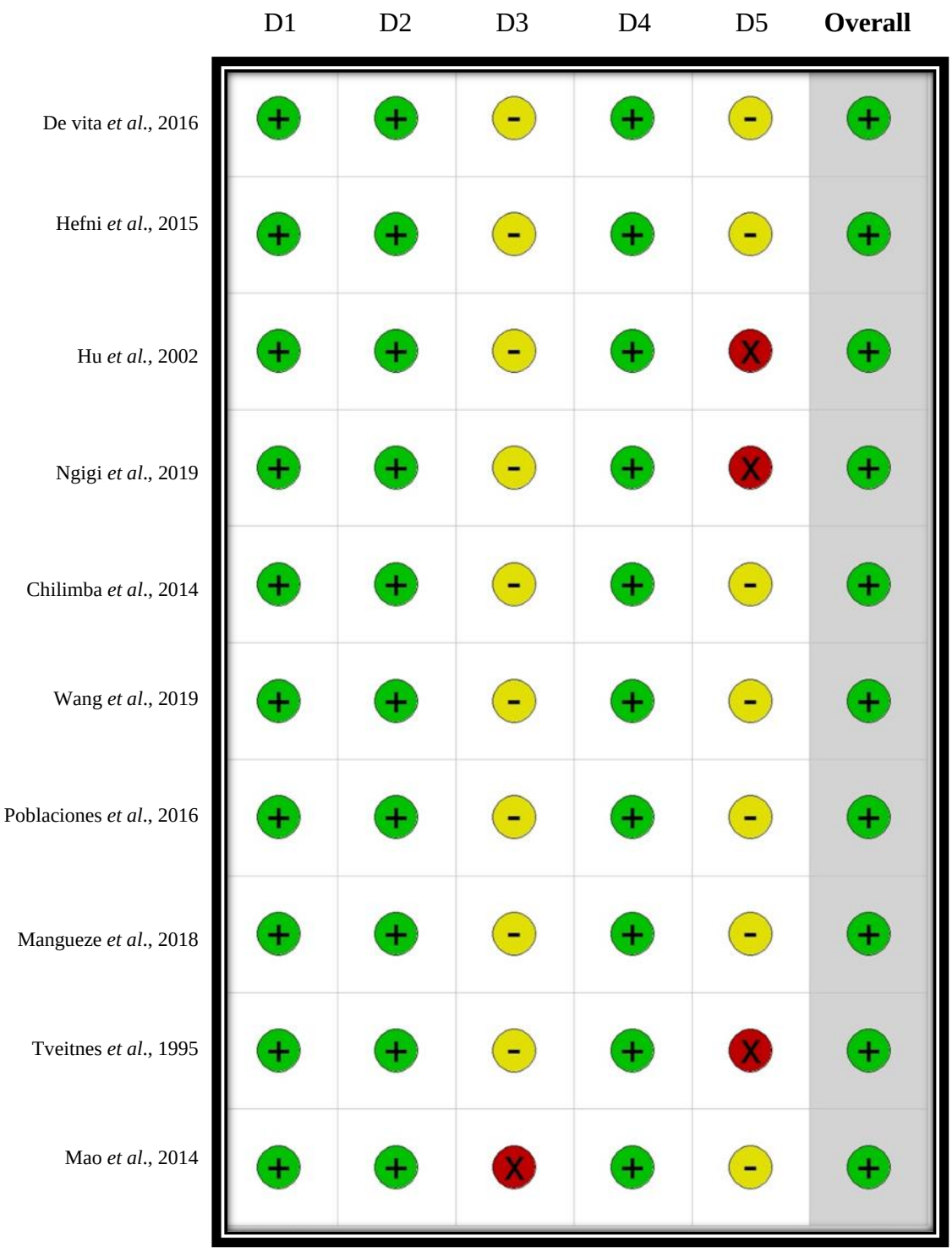
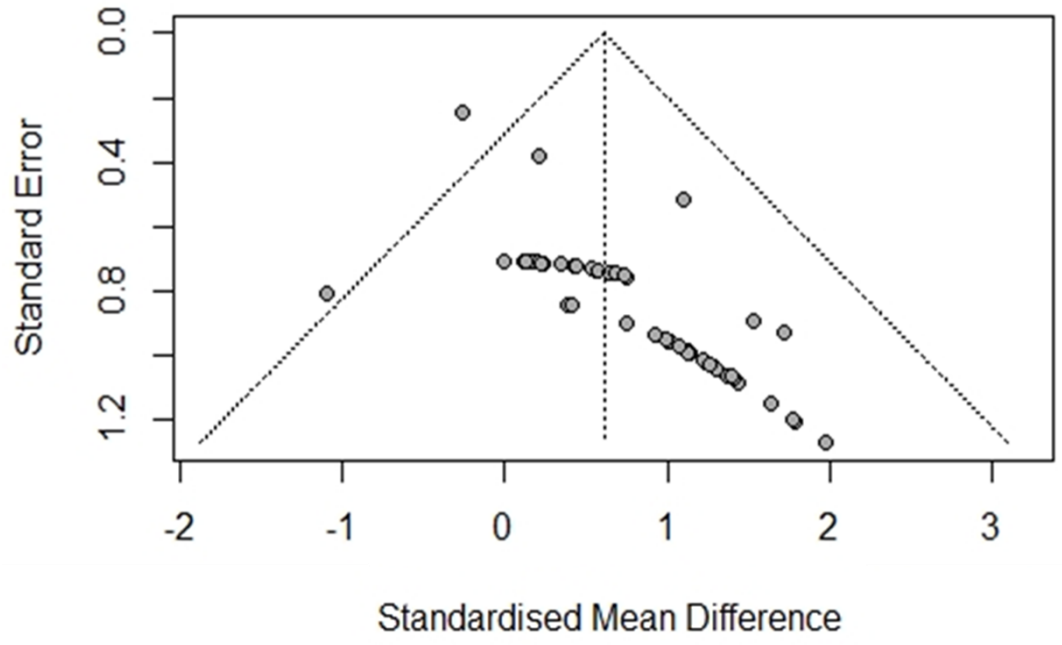


Fig. 3.4. *Quality assessment summary: review authors' judgement on the quality of methodology criteria e.g. study overview (D1), internal validity (D2), analytical method (D3), results (D4) and general assessment (D5) with overall rating results (Overall). Overall judgement result based on each study: Green button (Low risk), Red button (High risk) and yellow button (Some concerns risk/unclear)*

Foliar (A) – funnel plot.



Foliar (B)- trim and fill funnel plot

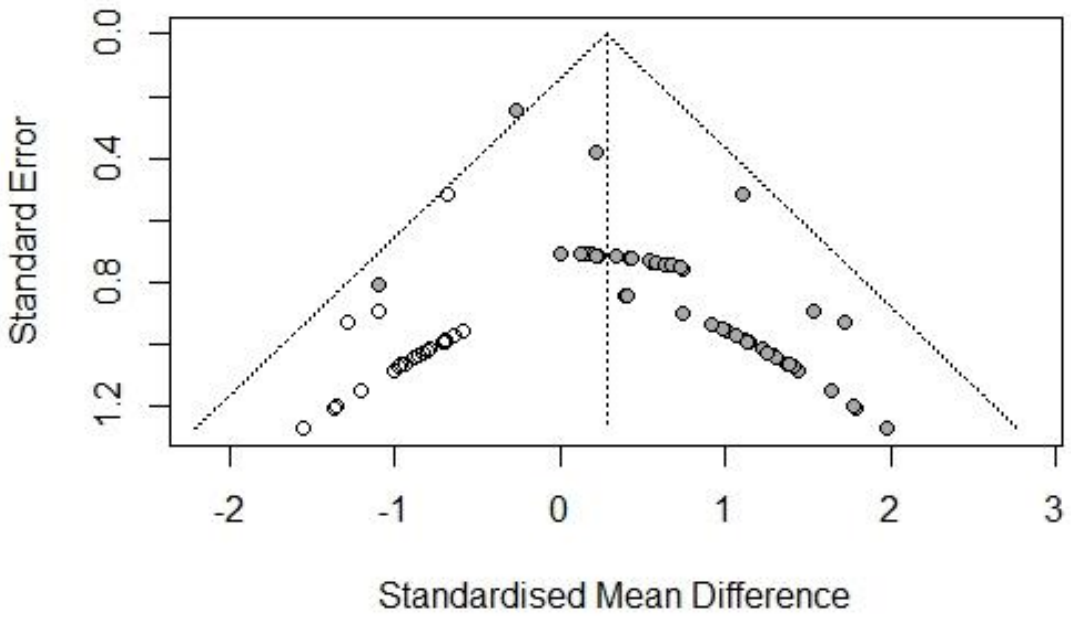
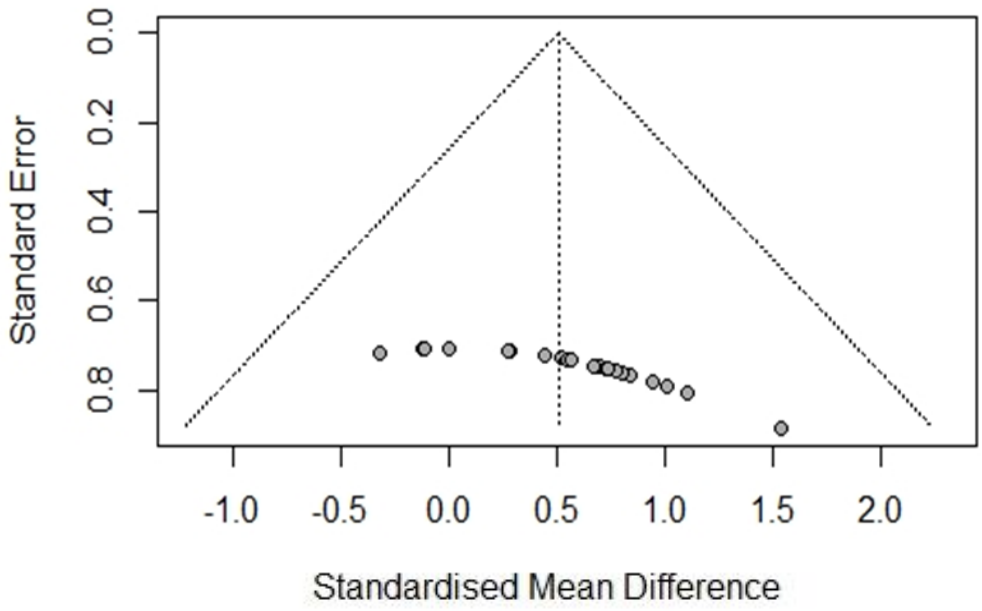


Fig. 3.5 *Funnel plot showing the effect size on the x-axis and standard error on the y-axis as a scatter plot for foliar application. (A) Funnel plot without publication bias (B) Funnel plot adjusted using the trim-and-fill method and the open circles are the imputed studies.*

Soil (A) – funnel plot.



Soil (B)- trim and fill funnel plot

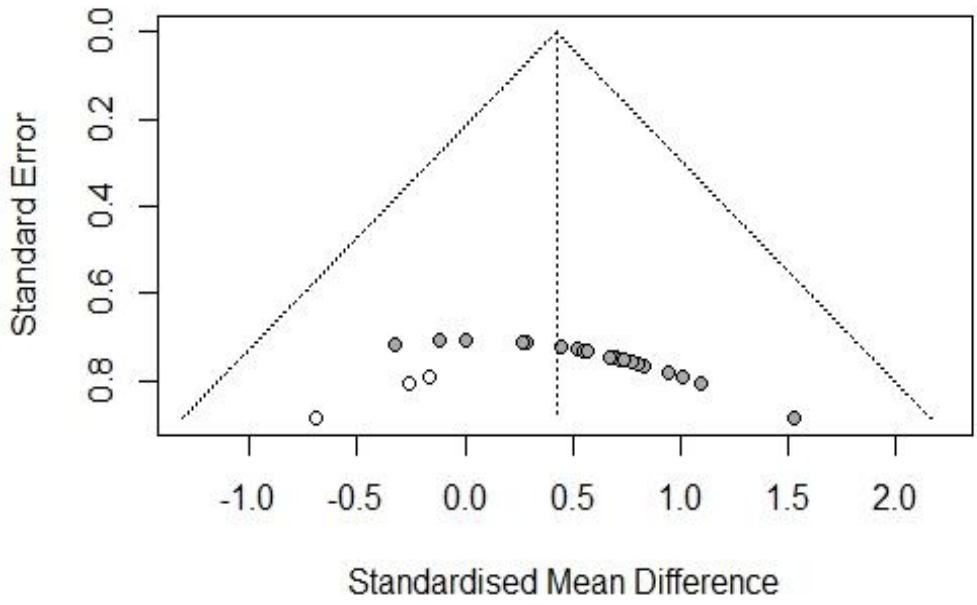


Fig.3.6 *Funnel plot showing the effect size on the x-axis and standard error on the y-axis as a scatter plot for soil application. (A) Funnel plot without publication bias (B) Funnel plot adjusted using the trim-and-fill method and the open circles are the imputed studies.*

3.3.4 Multiple regression

Four separate multiple regressions were performed. Table 3.5, Table 3.6, Table 3.7 and Table 3.8 show multiple regressions analysed separately for the three covariates (soil pH, selenium form, and soil selenium concentration) which are considered as confounding factors, which may have influenced the effectiveness of soil and foliar selenium application on grain selenium concentration and yield outcomes.

3.3.4.1 Effect of soil heterogeneity on grain selenium concentration and yield

Table 3.5 and Table 3.6 show the summary results of the multiple regression analysis for soil selenium application, which were analysed separately for grain selenium concentration and yield outcomes.

Table 3.5 shows the summary results of multiple regression analysis for effects of soil selenium application on grain selenium concentration. In Table 3.5, Se form coefficient showed NA (Not available) for estimate, standard error, t-value, and the *p*-value (Pr(>|t|)) due to singularities detected in the dataset. Initially, there was only one dataset for effect of selenate and no dataset for effect of selenite on grain selenium concentration. The soil pH coefficient indicated a very small positive estimate (1.296e-16) with non-significant *p*-value (0.58). Meanwhile, soil selenium concentration showed a very small negative estimate (-1.959e-15) with non-significant *p*-value (0.61) which would indicate that grain selenium concentration will decrease when soil selenium concentration increases. Overall, the summary result of the multiple regression analysis for soil selenium application on grain selenium concentration indicated that the result may be unreliable due to singularities detected in the dataset.

Table 3.6 shows the summary results of multiple regression analysis for soil selenium application on grain yield outcome. In Table 3.6, Se form, soil pH and soil selenium concentration coefficients displayed estimate, standard error, t-value, and the *p*-value (Pr(>|t|)) with zero (0) value, NaN (Not a number) and NA results. The summary result of the multiple regression analysis for soil selenium application on grain yield indicated that there are no residual degrees of freedom and coefficients cannot be defined because of singularities detected in the dataset. Initially grain yield outcome has only two selenate datasets while selenite has only one dataset, which leads to the NaN on 0 degrees of freedom.

Table 3.7 and Table 3.8 show the summary results of the multiple regression analysis for foliar selenium application, which were analysed separately for grain selenium concentration and yield outcomes.

Table 3.7 shows the summary results of multiple regression analysis for foliar selenium application on grain selenium concentration outcome. Soil form coefficient shows a very small positive estimate (1.864e-19) with non-significant correlation of *p*-value (1.00), indicating that foliar selenium application through application of Se form possibly increases grain yield. Soil pH coefficient indicates a very small negative estimate (-1.749e-19), with non-significant correlation of *p*-value (0.99), and suggests that foliar selenium application may increase grain selenium concentration with decreasing soil pH status. Meanwhile, the soil selenium concentration coefficient shows a very small positive estimate (3.522e-18) with a non-significant *p*-value (0.27), suggesting that foliar selenium application may increase grain selenium concentration when soil selenium concentration increases. The summary results of the multiple regression analysis for foliar selenium application on grain selenium concentration also indicate that the results may be unreliable, which is similar to the multiple regression analysis for soil selenium application on grain selenium concentration in Table 3.5.

Table 3.8 shows the summary results of multiple regression analysis for foliar selenium application on grain yield outcome. In Table 3.8, the summary result of the multiple regression analysis for foliar selenium application on grain yield presents almost a comparable result with the multiple regression analysis for soil selenium application on grain yield as shown in Table 3.6. Se form, soil pH and soil selenium concentration coefficient displayed estimate, standard error, t-value, and the *p*-value (Pr(>|t|)) with zero (0) value and NaN (Not a number) results, respectively.

Table 3.5. *Multiple regression analysis for soil selenium application (Outcome: Grain selenium concentration)*

Coefficient	Estimate	se	tval	Pr (>|t|)
Intercept	1.000e+00	4.921e-16	2.032e+15	<2e-16 ***
Se form	NA	NA	NA	NA
Soil pH	1.296e-16	2.307e-16	5.620e-01	0.582
Soil selenium concentration	-1.959e-15	3.770e-15	-5.190e-01	0.610

*Residual standard error: 6.978e-16 on 17 degrees of freedom, Multiple R-squared: 0.505, Adjusted R-squared: 0.4467, F-statistic: 8.67 on 2 and 17 degree of freedom, p-value: 0.002538, Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1, se (standard error), tval (T-value), NA (Not Available). The Pr(>|t|) is the p-value associated with the t-statistic for each coefficient.*

Table 3.6. *Multiple regression analysis for soil selenium application (Outcome: Grain yield)*

Coefficient	Estimate	se	tval	Pr (>|t|)
Intercept	0	NaN	NaN	NaN
Se form	0	NaN	NaN	NaN
Soil pH	0	NaN	NaN	NaN
Soil selenium concentration	NA	NA	NA	NA

Residual standard error: NaN on 0 degrees of freedom, Multiple R-squared:NaN, Adjusted R-squared: NaN, F-statistic: NaN on 2 and 0 degree of freedom, p-value: NA, se (standard error), tval (T-value). The Pr(>|t|) is the p-value associated with the t-statistic for each coefficient. NA (Not Available), NaN (Not a number).

Table 3.7. *Multiple regression analysis for foliar selenium application (Outcome: Grain selenium concentration)*

Coefficient	Estimate	se	tval	Pr (>|t|)
Intercept	1.000e+00	4.137e-16	2.417e+15	<2e-16 ***
Se form	1.864e-19	4.369e-16	0.000e+00	1.000
Soil pH	-1.749e-19	5.572e-17	-3.000e-03	0.998
Soil selenium concentration	3.522e-18	3.152e-18	1.118e+00	0.278

*Residual standard error: 6.785e-16 on 19 degrees of freedom, Multiple R-squared:0.5039, Adjusted R-squared:0.4256, F-statistic: 6.434 on 3 and 19 DF, p-value:0.003434. Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1, se (standard error), tval (T-value), The Pr(>|t|) is the p-value associated with the t-statistic for each coefficient.*

Table 3.8. *Multiple regression analysis for foliar selenium application (Outcome: Grain yield)*

Coefficient	Estimate	se	tval	Pr (>|t|)
Intercept	0	0	NaN	NaN
Se form	0	0	NaN	NaN
Soil pH	0	0	NaN	NaN
Soil selenium concentration	0	0	NaN	NaN

Residual standard error: 0 on 22 degrees of freedom, Multiple R-squared: NaN, Adjusted R-squared: NaN, F-statistic: NaN on 3 and 22 DF, p-value: NA, se (standard error), tval (T-value), The Pr(>|t|) is the p-value associated with the t-statistic for each coefficient, NaN (Not a number).

3.4 Discussion

3.4.1 Efficacy of soil and foliar applications on grain selenium concentration and yield

Selenium biofortification is one agronomy strategy to eradicate selenium malnutrition globally (Dos Reis *et al.*, 2017). In the literature, Ros *et al*. (2016) found that many authors highlighted application methods as one of the factors which can influence the efficacy of selenium fertilisation alongside form of selenium used, Se dose, timing and soil properties. In this case, soil and foliar application are the easiest and more effective methods to correct mineral deficiencies such as Se, Zn among humans (Khoshgoftarmanesh *et al.*, 2010). Notably, numerous studies have demonstrated the potential effects of soil and foliar application on grain selenium concentration and yield. Some studies concluded that foliar application was more effective than soil application (Ghasemi *et al*., 2013). No increases in crop yield as affected by selenium fertilisation have generally been reported in the literature (Broadley *et al*., 2010; Ros *et al*., 2016). As a transparent and objective statistical procedure, meta-analysis combines and summarises a large collection of results from individual primary studies (Del Re, 2015; Ahn & Kang, 2018). A meta-analysis has the power and precision to measure the overall significant effects of intervention by providing a total overall estimate of effect size which is not seen in any of the individual primary studies (Sedgwick, 2015). From the forest plot (Fig. 3.2 and Fig. 3.3), the summary effect size of meta-analysis of multiple selenium rates by soil and foliar application on grain selenium concentration and yield of cereals gave significant results for grain selenium concentration, but not for yield. These results indicated that grain selenium concentration of cereal crops were affected by rate of selenium and application method (soil and foliar) across all selected studies. In the aspects of heterogeneity, the statistical testing for soil selenium application showed I^2 = 48% (Fig. 3.2) which describes low variability associated with methodological diversity (research design) across all the included studies. Meanwhile, the statistical testing for foliar selenium application showed I^2 = 79% (Fig. 3.3) which describes high variability associated with methodological diversity (research design) across all the included studies.

The overall quality assessment scores in this review were low, which indicated the study results (true treatment effects) are considered acceptable (Viswanathan *et al*., 2017). The low risk of bias result in the present study shows that most included studies for the meta-analysis are reported to have clear aims, appropriate methodology, findings, and outcomes according to the quality assessment statements (Table 3.2). The strength of evidence also would be reduced if high publication bias was detected (Carrick *et al*., 2018). The existence of publication bias in

any dataset of included individual studies may affect the meta-analysis, as observed by Bown and Sutton (2010). Publication bias occurs when included studies selected in the meta-analysis were found to differ systematically from all studies (Borenstein *et al*., 2009). Examination of funnel plots for foliar and soil selenium application (Fig. 3.5 and Fig. 3.6) in this review showed the point forms of inverted funnel shape in both funnel plots which, indicated no publication bias was detected. Overall, the present review demonstrated high quality of evidence for all included primary studies according to the results of risk bias assessment, heterogeneity testing and publication bias examination.

To the best of our knowledge, most of the systematic reviews and meta-analyses on selenium studies in the literature are generally looking at the human health effects of selenium in the diet. Reviews assessing the efficacy of soil and foliar application on grain selenium and yield using systematic review and meta-analysis existed in the literature are limited. To date there is no study discussing the efficacy of selenium supplementation methods for cereal crops on grain selenium and yield using a systematic review and meta-analysis approach. Only a single review by Ros, *et al*. (2016) was available in the literature which studied the effects of selenium fertilisation strategy on crop performance and nutritional content applying a meta-analysis approach. In this review, about 94 studies were included with data extracted from a large range of crop species such as cereals (wheat, rice, maize, barley, rye, oats), grass, herbage, soybean. This review focused on the magnitude effect of crop selenium response and selenium uptake. Impact of methodology, management and environment on selenium response to selenium fertiliser form (selenate and selenite) were also observed. According to this review, selenium fertilisation practices e.g. timing and application, dose, and selenium form used significantly increased selenium uptake. In fact, the highest response (%) among crop parts was observed in grain, with root having the lowest. Selenate caused a greater crop response (%) than selenite fertiliser. Foliar application, which is mostly employed during the vegetative stage of growth, seemed more effective than soil application. Among cereal species, response (%) was greater in cereals than maize. In effect, the presence of sulphur fertiliser may reduce crop response to selenium. Furthermore, soil properties (pH, selenium content and organic carbon) had a minor influence on crop selenium responses. Overall, Ros, *et al*. (2016) concluded that selenium fertilisation of crops could boost uptake efficiency with effects of fertiliser dose, selenium speciation, application methodology and timing. Due to minor influence of total soil selenium, soil organic matter, soil pH this review also hinted other agro-ecosystem factors, i.e. climate linked variables, could to possibly be stronger drivers in crop response and selenium uptake.

3.4.2 Influence of soil heterogeneity on grain selenium concentrations and yield.

Selenium concentration in various foods might be linked to soil heterogeneity factors such as pH, microbial activity, plant type, soil, rainfall and biogeochemical parameters (Dos Reis *et al*., 2017). A number of studies in the literature showed the availability of selenium and the uptake efficiency generally are affected by prevailing soil properties such as soil selenium concentration and soil pH (Ros *et al*., 2016; Dos Reis *et al*., 2017; Manojlović *et al*., 2019). According to Rodrigo *et al*. (2013), soil selenium concentration is the key factor influencing grain selenium uptake and accumulation, but Rodriguez *et al*. (2005) highlighted that soil selenium concentration is not always a good indicator of the availability of plant selenium. Some studies have shown that soil pH could also affect soil selenium bioavailability and consequently control grain selenium concentration (Lee *et al*., 2011; Dos Reis *et al*., 2017). Hlušek *et al.* (2005) observed that soil pH could influence the concentration of selenium in potato tubers and observed that concentrations were higher in potato tubers at pH 6.9 than pH 5.9. Meanwhile Lee *et al.* (2011) reported that soil chemical properties such as pH showed non-significant correlations with grain selenium concentration. In several primary studies on selenium supplementation using selenium form (selenate or selenite), reported that foliar application was more efficient for grain selenium accumulation (Wang *et al*., 2013; Mao *et al*., 2014; Ros *et al*., 2016; Lyons, 2018; Ngigi *et al*., 2019). Similarly, Rodrigo *et al*. (2013) reported significantly higher grain yield under foliar application using selenate in the 2010/2011 growing season.

According to the present multiple regression analysis summary results (see Table 3.5, Table 3.6, Table 3.7 and Table 3.8) for soil and foliar selenium applications to evaluate whether the three covariates (soil pH, soil selenium concentration and selenium form) could potentially influence grain selenium concentration and yield indicated that the summary results may be unreliable due to singularities. The singularities detected in this study likely occurred when the collected datasets were too small or insufficient. Jenkins *et al*. (2020), highlighted that the biological and medicinal analysis probably use low sample size (N) which may affect reproducibility, whereby the author proposed that the most plausible sample size dataset for regressions is about $N \geq 25$, which is considered an appropriate sample size for the research based on regression or meta-regression. Therefore, running the multiple regression analysis separately in this study for grain selenium concentration and yield outcomes is not useful due to insufficient datasets.

Notably, dietary selenium intake of UK people has clearly shown a declining trend over more recent times. This declining trend has been linked to the low grain selenium concentration of staple crops such as wheat. The availability of selenium in the food chain is mainly dictated by the selenium levels in soil available for plant uptake (Ros *et al*., 2016). Besides three factors (soil selenium concentration, selenium form and soil pH) which likely influence the plant availability of selenium, the presence of sulphur in agriculture soil is likely to affect plant selenium uptake and accumulation. Stroud *et al*. (2010b) investigated the impact of sulphur fertilisation on grain yield and selenium uptake of winter wheat and discovered that sulphur fertilisation regularly applied to UK wheat grown could alter crop selenium uptake and ultimately cause low grain selenium concentration**.** Inostroza-Blancheteau *et al*. (2013) observed that the ability of plants to uptake selenium was reduced after sulphur addition to plant growth media. Other studies are in agreement that soil with rising sulphur doses may lessen crop responses to selenium fertilisation **(**Kikkert *et al.,* 2013; Ros *et al*., 2016). Selenium and sulphur are similar chemically and use the same absorption and transport mechanisms (Sharma *et al*., 2017; Lidon *et al*., 2018a). Interestingly, selenate is transported within plants by the sulphur assimilation pathway (Seppänen *et al*., 2010; Lidon *et al*., 2018a). Guerrero *et al*. (2014) mentioned that due to their similarity in chemical form, selenate competes with sulphur for plant uptake. The antagonistic effects of sulphur and selenate fertilisation are likely to have a considerable effect on the agronomic biofortification of selenium according to Hawkesford & Zhao (2007).

3.5 Conclusion

Overall, this present systematic review and meta-analysis study showed that soil and foliar application methods had significant effects on grain selenium concentration without affecting yield of cereal crops. In addition, the effectiveness of selenium biofortification in this study through soil and foliar application methods either on grain selenium concentration accumulation or yield was likely influenced by three covariates of soil properties (soil pH, soil selenium concentration and selenium form). However, these relationships cannot be further understood due to unreliable summary results since singularities were detected in the dataset contributed by low sample size (N).

Chapter 4 : The influence of nitrogen fertilisation and crop protection on grain yield and quality of spring wheat (*Triticum aestivum* L.)

4.1 Introduction

Nitrogen fertilisation and crop protection are two key agronomic practices in conventional wheat production which significantly influence grain yield and quality (Hawkesford, 2014; Żuk-Gołaszewska *et al*., 2016; Zhang *et al*., 2016). However, conventional agriculture is causing damage to the environment as a result of excessive application of mineral N fertiliser and crop protection inputs (Mader, 2002; Hazra *et al*., 2018; Litoriya *et al*., 2018). Therefore, ways to substitute for mineral NPK fertiliser with efficient recycling of nutrients from agricultural and urban waste streams into agricultural soils and reduce the application of pesticides are required to promote greater sustainability in food production. Currently, organic and low-input production are becoming increasingly popular globally (Petrenko *et al*., 2018) with improved environmental sustainability (Hazra *et al*., 2018). In many Europe on countries including the UK, organic production has progressively been adopted by local farmers due to increased demand for organic food (Cooper *et al*., 2011).

At present, since the world population is forecasted to expand approximately to 10 billion by 2050 (Meng *et al*., 2016), increasing grain production and quality in a sustainable way has become a key challenge. Numerous studies have compared cereal grain yield and quality in conventional and organic production systems, most findings observed that conventional production gave higher yield than organic production (Kitchen *et al*., 2003; Mason *et al*., 2007; Mader *et al*., 2007; Iannucci & Codianni, 2016). While, several studies have demonstrated that organic production has lower grain protein content than conventional production (Ceseviciene *et al.*, 2012; Fagnano *et al.*, 2012; Iannucci and Codianni, 2016), and suggested that it is likely due to insufficient soil N availability (nitrogen shortage) from organic fertiliser inputs (Fagnano *et al.*, 2012; Campiglia *et al.*, 2015; Mazzoncini *et al.*, 2015) especially later in the growing season. One of the recommended agronomic strategies to improve grain productivity and protein content in organic production is to apply organic fertilisers such as biogas digestate with a higher content of readily available N (Campiglia *et al.*, 2015). It is a major challenge to synchronize timing of N availability with crop nitrogen demand when using organic based N

fertilisers when compared with mineral N fertilisers. Many types of different organic fertilisers available to farmers such as biogas digestate, slurry, chicken manure or farmyard manure differ in their nutrient release patterns. Application of various forms of organic fertilizer sources as soil organic amendments has the potential to reduce the environmental impact of production.

Therefore, it is crucial to fully evaluate and optimise the potential of organic fertiliser regimes to increase sustainability and food security whilst protecting the environment. The number of studies on the influence of various fertiliser types on grain quality have been less frequently reported than yield of wheat in the literature (Litoriya *et al*., 2018). In keeping with the above views, this present work was undertaken using Mulika spring bread wheat, which has a high yield potential and excellent bread making qualities. The objective of the study was (i) to evaluate the effects to N source and crop protection management of spring wheat crop performance, disease severity, grain yield and quality, (ii) to determine the selenium status of spring wheat grown under differing fertility management and crop protection (organic vs conventional) practices.

4.2 Materials & methods

4.2.1 Description of the field trial

The present field trial was carried out during 2016 at the Nafferton Factorial Systems Comparison (NFSC) long-term field trial which was established in 2008 for the European Union (EU) funded project (EU FP6 NUE crops project). The trial site was in Quarry Field (Fig. 4.1) at Newcastle University's Nafferton Experimental Farm, Northumberland, United Kingdom (54:59:09 N latitude and 1:43:56W longitude)**.** The soil type in the area is sandy loam from the Stagnogley type with a mean organic matter level of 3.3% (Cooper *et al*., 2011). The preceding crops were winter wheat (2013), potatoes (2014) and spring wheat (2015). The meteorological data during the experiment period were recorded by an automated weather station located 1 km from the experimental site.

4.2.2 Experimental design and treatments

The experiment was conducted as a factorial design with crop protection and fertility types as the main effect factors. The trial was carried out on four 24 m x 24 m blocks. Each main block was divided into two sub-plots (12 m x 12 m) of crop protection management with synthetic fungicide and herbicides application (conventional crop protection) and without synthetic fungicide and herbicides application (organic crop protection).

The crop protection sub-plots were further separated into four different fertiliser treatment sub-sub-plots (6 m x 12 m) which were imposed at 90^0 to the direction of the plots. All treatments were arranged according to a randomized complete block design with four replicates (*n = 4*) with 32 individual plots in the whole trial (Fig. 4.2). The four different fertiliser treatments were applied in a single application at a rate of 150 kg N/ha as biogas digestate (BD), composted farmyard manure (CFYM), a combined treatment of 50 kg composted farmyard manure + 100 kg biogas digestate (CFYMBD) and mineral N (MN) fertiliser. Composted farmyard manure was added on 18th April 2016 prior to drilling of the spring wheat whilst the remaining fertiliser treatments were added on 9th June 2016 at the active tillering stage (GS22) (Zadoks *et al*., 1974). Mineral N fertiliser was applied as ammonium nitrate (33.5% N).

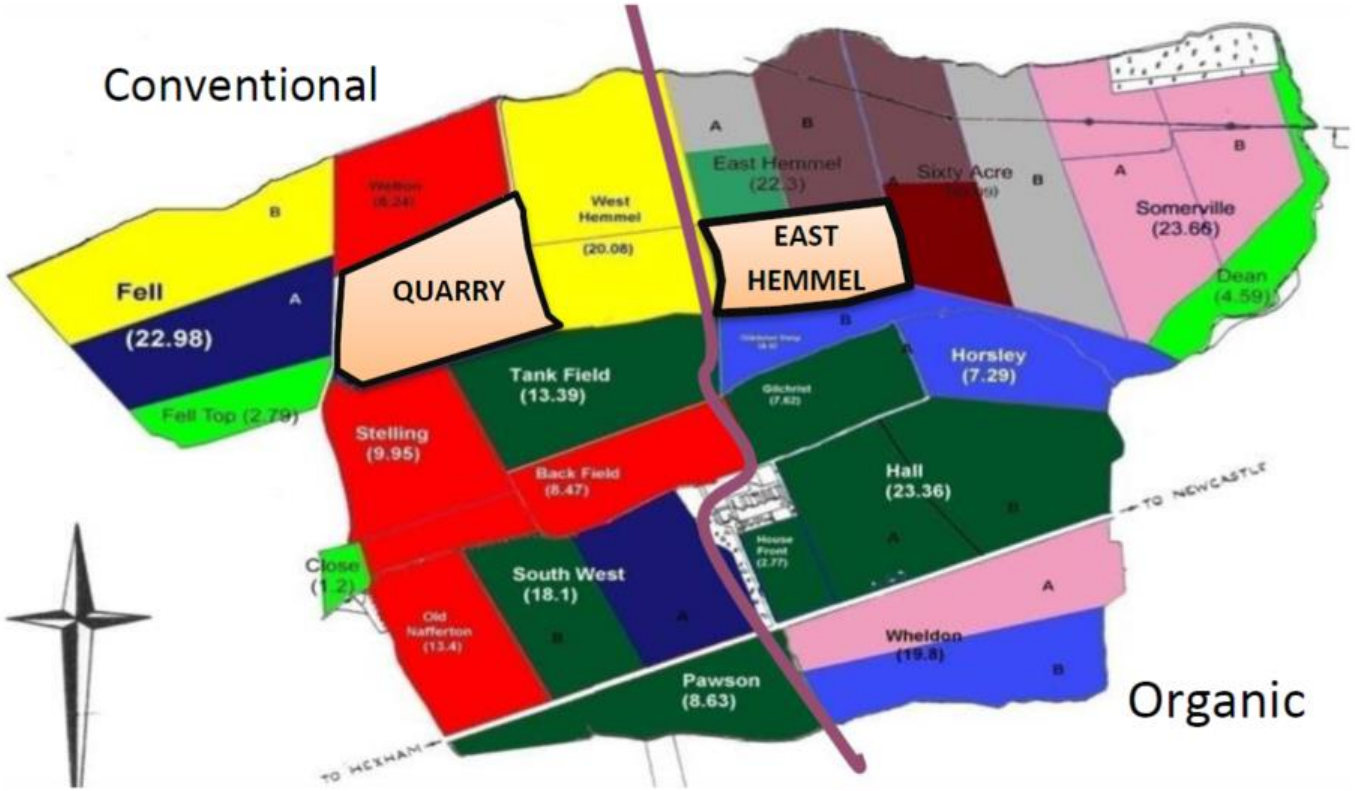


Fig. 4.1 *Location of Quarry field at Nafferton Farm.*

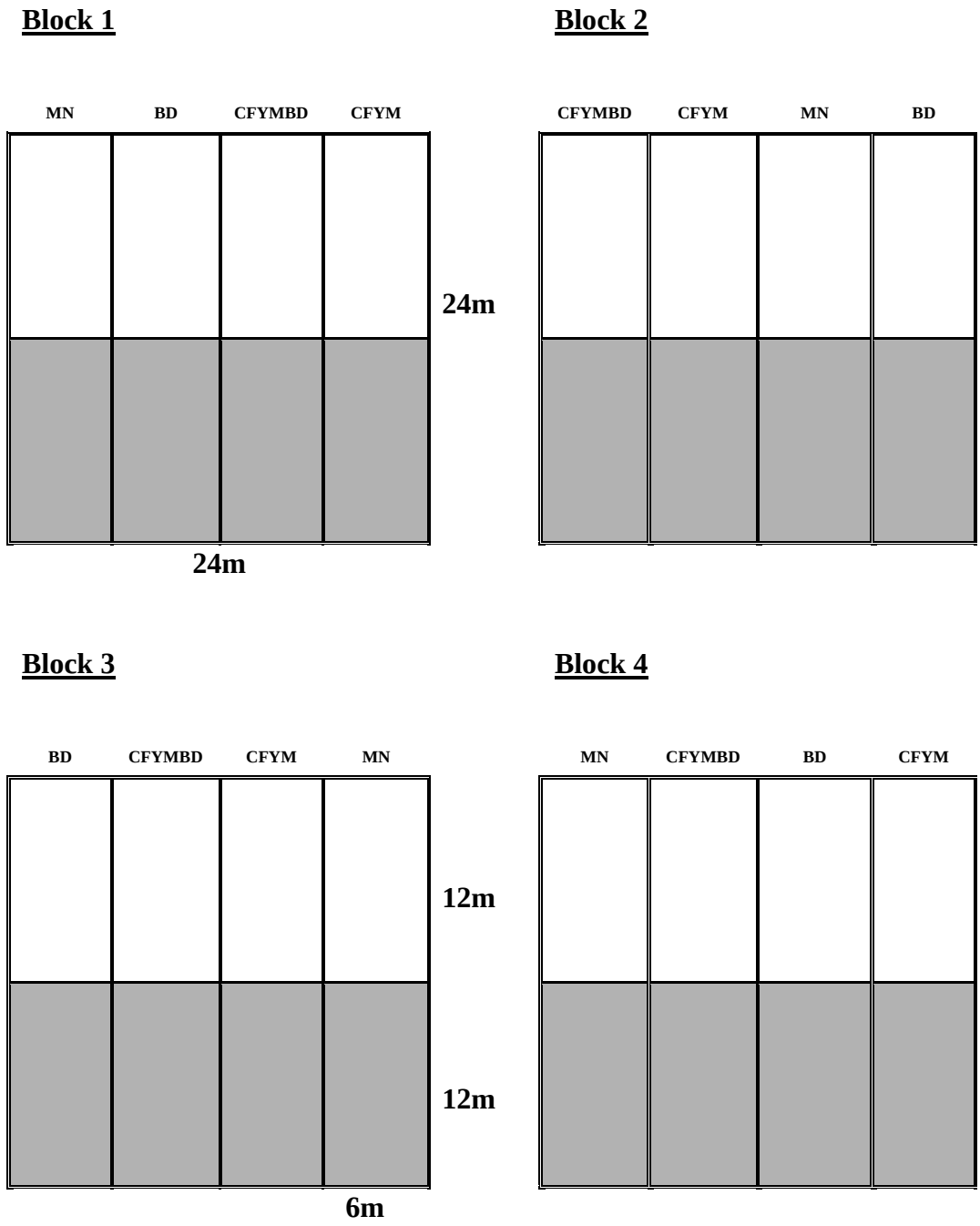


Fig. 4.2 *Layout of experimental trial*

4.2.3 Crop management and husbandry

The high yielding spring bread wheat (*Triticum aestivum* L.) variety Mulika was used. Mulika has a high yield potential, excellent disease resistance, short straw and good grain quality with high protein content, test weight and medium–high grain hardness (AHDB, 2017). The experimental site was ploughed prior to sowing, with Mulika seeds sown on 23rd April 2016 at a seed rate of 168 kg/ha (400 seeds/m^2). A commercial seed drill (3m Lely combination drill; Lely U.K. Ltd., St. Neots, U.K.) was used with a row spacing of 12 cm and a depth of 2-3 cm. Only sub-plots in the conventional crop protection were sprayed with fungicides and herbicides according to commercial practice to control foliar diseases (rusts, mildews and leaf blotch), annual grasses and broadleaf weeds. Active ingredients of fungicide and herbicide control consisted of chlorothalonil (1 litre/ha), epoxiconazole (0.5 litres/ha) and metsulfuron-methyl plus with thifensulfuron-methyl (70 grams/ha) at T1 (GS 31) and T2 (GS39) on 27th June 2016 and 7th July 2016 (Table 4.1). The organic crop protection plots did not receive any pesticide inputs.

Table 4.1 *Summary of crop management and treatment dates*

Activity	**Date**
Sowing date	23rd April 2016
Treatments application:	
i) Composted farmyard manure (CFYM).	18th April 2016
ii) Biogas digestate (BD), composted farmyard manure + biogas digestate (CFYMBD) and Mineral N (MN).	9th June 2016
Fungicide and herbicide applications at GS31 and GS39 (Conventional crop protection treatments only).	27th June 2016 and 7th July 2016
Biomass sampling prior to harvest.	26th September 2016
Combine harvesting date.	27th September 2016

4.2.4 Sampling and measurements

Zadoks's decimal code was used as a reference key scale of plant development for conducting field measurements and plant sampling activities (Appendix A). The full complete Zadoks development stage key scales is described in Zadoks *et al*. (1974).

4.2.4.1 Seedling establishment and crop N status

Wheat emergence was sampled using 0.5 m x 0.5 m (0.25 m^2) quadrats at GS12. Seedling establishment (%) was calculated based on the total plant number and the seed rate applied. A non-destructive portable chlorophyll meter (Minolta SPAD-502, Osaka, Japan) was used to estimate crop N status of wheat. Indirect SPAD measurements were conducted at stem elongation stage (GS30), flag leaf emergence (GS39) and beginning of anthesis (GS62). About 20 individual flag leaves per plot were randomly sampled.

4.2.4.2 Foliar and ear disease severity assessments

Foliar diseases were monitored visually (% infected leaf and ear area) for powdery mildew (*Blumeria graminis* f. sp. *tritici*), yellow stripe rust (*Puccinia striiformis*), brown leaf rust (*Puccinia recondita f. sp. tritici*) and Septoria leaf blotch (*Zymoseptoria tritici*). Visual foliar assessments were made at stem elongation (GS39) on 7th July 2016, half of inflorescence emerged (GS55) on 12th July 2016 and prior to and during anthesis (GS60 & GS65) on 21st July 2016 and 1st August 2016. Ten individual plants in each plot were visually assessed for disease randomly on the flag leaf (L1), second (L2), third (L3) and fourth leaves (L4). Foliar disease severity level was recorded as % infected area of 0%, 1%, 5%, 10%, 25%, 50%, 75% or senesced. Ear disease severity assessment for Fusarium head blight (*Fusarium graminearum*) and yellow rust (*Puccinia striiformis*) was monitored from GS70 to GS89 using a scale of % infected area (5%, 10%, 25% and 50%). Ten individual ears were randomly observed from each plot. The Area Under the Disease Progress Curve (AUDPC) method according to Shaner and Finney (1977) was carried out to calculate foliar and ear disease severity levels and AUDPC was reported as a single quantitative measure.

4.2.4.3 Above-ground biomass sampling

At physiological maturity (GS89), above-ground biomass samples based on a sampling area of 0.25 m^2 (0.5 m x 0.5 m) were collected on 26th September 2016. Data on plant height (cm), ears/m^2, grains/ear, total crop biomass (t/ha) and harvest index (%) were determined. Three individual plants were randomly measured in the field in each sub plot for plant height. Plant height was measured from the ground level to the base of the ear (Campiglia *et al*., 2015). Harvest index (%) was calculated as total grain weight divided by total above-ground dry weight. The plant above-ground biomass sampled was separated into stem, leaf, and ear after the roots were cut off. Fresh weight for stem, leaf and ear were recorded and samples were oven-dried at 60 °C for 72 h to constant weight and dry weight recorded. After oven-drying, ears were threshed by a laboratory thresher machine (Wintersteiger LD 350, Austria) to separate chaff and grains and weighed. All oven-dried biomass stem, leaf and grain samples were kept in sealed plastic bags and stored at room temperature prior to laboratory analysis.

4.2.4.4 Grain yield

All plots were combine harvested on 27th September 2016 (area of 2.1 m x 12 m) using a plot combine harvester (Claas Dominator 38; Class UK Ltd, Bury St Edmunds UK) to determine grain yields. Grain yields was expressed in t/ha and reported at 15% moisture content. Approximately 500 g of grain sub-samples were randomly taken from each plot at harvest, dried and stored at room temperature for subsequent 1000 grain weight (TGW), grain quality (protein content, HFN, hectolitre weight) and grain selenium analysis. TGW was calculated based on the mean weight of three replicates of 1000 grain samples using an electronic seed counter Model Elmor C3, Switzerland.

4.2.4.5 Nutrient analysis

One month prior to sowing, soil samples from four different locations within each block were collected at three different soil depths (0–30 cm, 30-60 cm and 60-90 cm) using a steel core auger to assess soil N residual status prior to fertiliser treatment application. Also, sub-samples of composted farmyard manure and biogas digestate fertilisers were sampled for Nitrate-N, Ammonium-N and Total nitrogen analysis. All soil, composted farmyard manure and biogas digestate fertiliser samples were sent to the NRM laboratory for analysis. The NRM laboratory is an accredited commercial analytical laboratory. The NRM laboratory has the

ability and offer services to check all aspects of soil, fertiliser, plant tissue, manures, and water samples.

Approximately 20-30 g of cleaned oven dried grain samples were milled with a miller model Retsch SK300 (+/- 3.4-4.0 speed rpm) with a 1.0 mm mesh sieve size. About 50 mg (0.05 g) sub-sample of milled grains was weighed into a tin foil cup. An elemental analyser (Elementar Vario Macro Cube) was used to determine Total N using the combustion method. Grain protein content (%) was calculated by multiplying Total N by 6.25 and expressed on a dry weight basis (Mariotti and Mirand, 2008). A reference method used for the grain protein content (%) determination was ISO/TS 16634-2:2009.

HFN analysis was determined by Perten Instruments Falling Number 1700 machine supplied with automatic stirring model and two viscometer tubes. The standard method according to the ICC 107/1, AACC 56-81B (ISO/DIS 3093) was used to conduct the analysis. HFN analysis was started with the determination of grain moisture content (%) using an infrared analyser machine (Foss, Infratec™ 1241). Approximately 50 g of grain was milled using the grain miller Model LM3100 with 0.8 mm mesh sieve size, weighed and poured carefully into each viscometer tube. Then, 25 ml of distilled water was added. Both viscometer tubes were immediately shaken 20-30 times vigorously to obtain a homogeneous suspension. The viscometer tubes were immediately transferred into a boiling water bath. The falling number was measured as the total time in seconds (s) taken by the stirrers to fall. Hectolitre weight was measured using a test weight-chondrometer (Fig. 4.3) and converted to hectolitre weight (kg/hl).

For selenium analysis, about 3.0-3.5 g of oven dried grain was milled using a Retsch ZM200 (speed 12,000 rpm) mill with 0.2 mm mesh sieve size at Nafferton Farm, Newcastle Upon Tyne and sent to Sabbanci University, Turkey in early February 2018. At Sabbanci, approximately 0.2 g of oven-dried and milled grain samples was subjected to acid-digestion with a mixture of HNO_3 (2 mL of 30% (v/v)) and H_2O_2 (5 mL of 65% (v/v)) using a closed-vessel microwave reaction system (Mars Express, CEM Corp., Matthews, NC, USA). For determination of selenium in the digested solution, an inductively coupled plasma optical emission spectrometer (ICP-OES; Vista-Pro Axial; Varian Pty Ltd., Mulgrave, Australia) was employed. The ICP-OES; Vista-Pro Axial was equipped with an autosampler SPS-5. Measurement conditions for all lines were: power 1.2 kW, plasma flow 15.0 l/min, auxillary flow 0.75 l/min, nebulizer flow 0.9 l/min and the detection limit for selenium is 2 µg/L. All measurement for selenium were then cross-checked using certified standard reference materials (SRM 1547 peach leaves) received from the National Institute of Standards and Technology

(Gaithersburg, MD, USA). The method for selenium analysis used in this study was based on Cakmak *et al*., (2020) and Grujcic *et al*. (2021).

Fig. 4.3 *Hectolitre weight measurement using the test weight-chondrometer, including filler, cutter bar and 0.25 L calibrator.*

4.3 Statistical analysis

Analysis of variance (ANOVA) was performed to assess the effect of fixed factors (fertiliser types and crop protection) on grain yield, yield components, grain quality, grain selenium concentration, SPAD, plant height and disease severity data which were all tested together. ANOVA tests were derived from the linear mixed-effects model procedure (Pinheiro and Bates, 2000) using *nlme* package with R statistical environment package (R Core Team, 2012). For pairwise comparison, Tukey contrasts in the general linear hypothesis testing (*glht*) function of the multcomp package in R were used to test the differences between treatment means and their interactions. Both means and SE values were calculated using 't apply'' function in R package. QQ-plots was performed to test the normality of the residuals. The square root transformation was employed to normalise the distribution of residuals for foliar disease severity (AUDPC values) prior to ANOVA tests.

4.4 Results

4.4.1 Weather

The overall weather data for monthly mean radiation, mean air temperature and monthly rainfall from March to October 2016 at Nafferton Farm is presented in Table 4.2. The highest monthly rainfall, mean solar radiation and mean air temperature occurred in June to August. Regarding rainfall distribution, the lowest rainfall occurred during seedling growth in May with a monthly total of 21.4 mm, while the wettest month was in June (88 mm) covering tillering and stem elongation. The crop received similar rainfall in July (63.6 mm) and August (66.4 mm) during the anthesis and grain filling periods. At the dough development and ripening stage, rainfall was lower (52.8 mm) in September prior to harvest. The total cumulative rainfall for the whole crop growth cycle was 428.2 mm. High solar radiation was received from the vegetative (tillering) to early anthesis stage in June (15.1 MJ/m^2) and July (15.5 MJ/m^2). Solar radiation reading then declined during grain filling (milk and dough development) in August (14.0 MJ/m^2) and September (9.1 MJ/m^2). The warmest month was August (mean 15.2 ^oC). The mean air temperature fell in September (14.7 ^oC) and October (9.8 ^oC).

4.4.2 Seedling establishment

Plant emergence recorded 248 plants/m^2 which represented 62 % establishment**.**

Table 4.2 *Monthly mean radiation, mean air temperature and cumulative monthly rainfall during the 2016 growing season at Nafferton Farm.*

Climatic data	Unit	Month							
		Mar	**Apr**	**May**	**June**	**Jul**	**Aug**	**Sept**	**Oct**
Mean solar radiation	MJ/m^2	7.1	11.5	13.5	15.1	15.5	14.0	9.1	4.4
Mean air temperature	^oC	5.5	6.1	7.6	12.7	14.9	15.2	14.7	9.8
Rainfall	mm	27.8	50.8	21.4	88.0	63.6	66.4	52.8	57.4

4.4.3 Soil mineral N

Soil nitrate nitrogen, ammonia nitrogen and total nitrogen (which were not tested statistically) were all highest in the topsoil (0-30 cm) with means of 1.00 mg/kg nitrate, 1.43 mg/kg ammonium and 9.08 kg total N/ha and decreased progressively with soil depth 30-60 cm and 60-90 cm (Table 4.3). It was observed that ammonia nitrogen was much higher than nitrate nitrogen at the 0-30 cm soil depth. However, nitrate nitrogen was greater than ammonia N at the 30-60 cm depth.

Table 4.3 *Mean values of nitrate (NO_3-N), ammonium (NH_4-N) and total nitrogen (NO_3-N + NH_4-N) at three different soil depths (cm) prior to fertiliser application.*

Soil depth (cm)	N residual status in soil		
	Nitrate nitrogen (mg/kg)	Ammonium nitrogen (mg/kg)	Total-N (kg/ha)
0-30	1.00	1.43	9.08
30-60	0.93	0.68	6.05
60-90	0.57	0.54	4.18

4.4.4 Nutrient compositional analysis

The results of the nutrient compositional analysis for Nitrate-N, Ammonium-N and Total nitrogen (which were not tested statistically) are presented in Table 4.4. Ammonium nitrogen concentration was much higher in biogas digestate (1453 mg/kg) than in composted farmyard manure (196 mg/kg). In contrast, composted farmyard manure contained higher total nitrogen (4.3 % w/w) and nitrate nitrogen (1378 mg/kg) than biogas digestate.

Table 4.4 *Nutrient composition of biogas digestate and composted farmyard manure used in the field trial.*

Property	Unit	Biogas digestate	Composted farmyard manure
Total Nitrogen	% w/w	0.19	4.3
Nitrate Nitrogen (NO_3–N)	mg/kg	< 10	1378
Ammonium Nitrogen (NH_4–N)	mg/kg	1453	196

4.4.5 SPAD content and plant height

The results regarding the main and interaction effects of crop protection and fertiliser type on leaf chlorophyll content (SPAD value) and plant height are shown in Table 4.5. Chlorophyll content at GS62 in conventional crop protection (42.2) was significantly higher than under the organic crop protection treatment (34.1). There was no significant difference in chlorophyll content between the two treatments at both GS30 and GS39. In conventional crop protection, SPAD values at GS30, GS39 and GS62 ranged from 42.2 to 43.1, while in organic crop protection, SPAD values declined from 42.3 and 42.6 at GS30 and GS39 to 34.1 at GS62.

Fertiliser type significantly influenced chlorophyll content at GS39. The biogas digestate treatment showed the highest SPAD value of 43.7 and the lowest was composted farmyard manure (37.9) among the organic fertiliser treatments. However, there was no significant difference between treatments for chlorophyll content at both GS30 and GS62. Biogas digestate gave higher SPAD at GS30 and composted farmyard manure + biogas digestate values were higher at GS62 when compared with all organic fertiliser types. In general, mineral N gave higher SPAD values at GS30, GS39 and GS62 than the organic fertiliser treatments used.

There was a significant effect of crop protection x fertiliser type interaction on chlorophyll content at GS62 (Table 4.6). Among fertiliser types applied under the conventional crop protection, the flag leaf SPAD reading was significantly higher from mineral N application than all organic fertiliser types, while in the organic crop protection, the SPAD reading was significantly higher in the composted farmyard manure + biogas digestate application than all other treatments. When compared between the conventional

crop protection and organic crop protection, mineral N, biogas digestate and composted farmyard manure gave significantly higher SPAD values in the conventional crop protection than organic crop protection while composted farmyard manure + biogas digestate was not significantly different between the two crop protection treatments. Plant height measured at GS89 was not significantly affected by fertiliser type or crop protection although the mineral N treatment produced the tallest plants.

Table 4.5 *Main effects (means and p-values) and interactions of crop protection and fertiliser type on SPAD readings at GS30, GS39, GS62 and plant height (GS89).*

Factor	Leaf chlorophyll content (SPAD value)			Plant height (GS89)
	GS30	**GS39**	**GS62**	**cm**
Crop protection (CP)				
Conventional	42.2±0.96	43.1±0.93	42.2±1.08 a	61.0±1.00
Organic	42.3±0.77	42.6±1.18	34.1±0.77 b	60.5±0.95
Fertiliser type (Ft)				
BD	42.9±0.82	43.7±1.22 b	38.6±1.84	61.3±1.35
CFYM	41.5±1.59	37.9±0.54 c	35.4±1.17	59.8±1.46
CFYMBD	41.0±0.91	42.4±0.97 b	38.9±1.29	59.5±1.59
MN	43.6±1.38	47.5±0.70 a	39.9±2.97	62.5±0.93
ANOVA *p-values*				
Main effects				
CP	0.971	0.534	**<0.001**	0.661
Ft	0.295	**<0.001**	0.074	0.410
Interaction				
CP x Ft	0.363	0.057	**<0.001**	0.959

Within columns, mean values followed by different letters are significantly different at p≤ 0.05. Values are the means and standard error (SE) of four replicates (n=4). Biogas digestate (BD), Composted farmyard manure (CFYM), Composted farmyard manure + biogas digestate (CFYMBD) and Mineral N (MN).

Table 4.6 *Interaction means ±SE for the effects of crop protection and fertiliser type on chlorophyll content at GS62.*

Fertiliser type	SPAD (GS62)			
	CCP		OCP	
BD	43.0±1.37	Ba	34.2±1.01	Bb
CFYM	38.1±0.83	Ca	32.7±0.92	Bb
CFYMBD	40.3±2.07	Ca	37.4±1.47	Aa
MN	47.5±0.65	Aa	32.3±1.50	Bb

Means ±SE values followed by different lower-case letters within each row and upper-case letters within each column are significantly different (Tukey's general linear hypothesis test p<0.05). CCP (Conventional crop protection), OCP (Organic crop protection). Biogas digestate (BD), Composted farmyard manure (CFYM), Composted farmyard manure + biogas digestate (CFYMBD) and Mineral N (MN).

4.4.6 Foliar and ear disease levels

Puccinia striiformis (yellow rust) was the only foliar disease detected throughout the study and the results shown in Table 4.7, Table 4.8 and Table 4.9 are presented in square root transformation values. In Table 4.7, crop protection had a significant effect on AUDPC of *Puccinia striiformis* infection on L1 and L2. The severity levels of *Puccinia striiformis* infection on L1 and L2 were significantly higher in the organic than conventional crop protection treatments**.** Fertiliser type had a significant effect on *Puccinia striiformis* infection on L1, but with no significant effect on L2 and L3. *Puccinia striiformis* infection was greater with mineral N (7.2) than the organic fertiliser types on L1. Among the organic fertiliser treatments, *Puccinia striiformis* infection was highest with biogas digestate (3.9) followed by composted farmyard manure (2.1) and composted farmyard manure + biogas digestate (1.2) on L1. Furthermore, *Puccinia striiformis* infection values were lower for L2 and L3 than for L1.

Table 4.7 *Main effects (means ± SE, p-values) and interactions of crop protection and fertiliser type on AUDPC for yellow rust (Puccinia striiformis) disease on flag leaf (L1), second leaf (L2) and third leaf (L3) in square root transformation values.*

Factor	*Yellow rust (Puccinia striiformis)*		
	L1	**L2**	**L3**
Crop protection (CP)			
CCP (Conventional)	0.5±0.50	0.0±0.00	0.0±0.00
OCP (Organic)	6.7±1.48	3.0±0.69	0.7±0.48
Fertility (Ft)			
BD	3.9±1.91 ab	1.1±0.66	1.2±0.89
CFYM	2.1±0.86 b	1.1±0.55	0.0±0.00
CFYMBD	1.2±0.69 b	0.7±0.34	0.1±0.12
MN	7.2±2.87 a	3.2±1.40	0.4±0.42
ANOVA *p-values*			
Main effects			
CP	**0.001**	**<0.001**	0.079
Ft	**0.025**	0.061	0.190
Interactions			
CPxFt	**0.001**	**0.014**	0.153

Within columns, mean values followed by different letters are significantly different at p≤ 0.05. Values are the means and standard errors (SE) of four replicates (n=4). CCP (Conventional crop protection), OCP (Organic crop protection). Biogas digestate (BD), Composted farmyard manure (CFYM), Composted farmyard manure + biogas digestate (CFYMBD) and Mineral N (MN).

There was a significant crop protection × fertiliser type interaction on *Puccinia striiformis* infections on the flag leaf (L1) and second leaf (L2) of Mulika spring wheat. For the flag leaf (L1) under conventional crop protection (Table 4.8), biogas digestate had higher *Puccinia striiformis* infection than the other fertiliser N sources. There were no significant differences in *Puccinia striiformis* infection between mineral N, composted farmyard manure and composted farmyard manure + biogas digestate fertilisers with no symptoms of infection observed.

In contrast, *Puccinia striiformis* infection was significantly higher in response to mineral N application under organic crop protection compared with the three organic fertiliser types. When conventional and organic crop protection were compared, mineral N had a higher significantly higher *Puccinia striiformis* in the organic than conventional crop protection but with no differences for the organic fertilisers used.

Table 4.8 *Interaction means ±SE for the effects of crop protection and fertiliser type on yellow rust (Puccinia striiformis) disease on the flag leaf (L1) in square root transformation values.*

Fertility type	*Puccinia striiformis* infection on L1			
	CCP		OCP	
BD	1.9±1.94	Bb	5.9±3.26	Ba
CFYM	0.0±0.00	Bb	4.3±0.67	Ba
FYMBD	0.0±0.00	Ba	2.5±1.08	Ba
MN	0.0±0.00	Bb	14.3±2.08	Aa

Means ±SE values followed by different lower-case letters within each row and upper-case letters within each column are significantly different (Tukey's general linear hypothesis test p<0.05). CCP (Conventional crop protection), OCP (Organic crop protection). Biogas digestate (BD), Composted farmyard manure (CFYM), Composted farmyard manure + biogas digestate (CFYMBD) and Mineral N (MN).

For L2 under conventional crop protection (Table 4.9), all organic and mineral N fertiliser types gave no significant difference in *Puccinia striiformis* leaf disease infection. Mineral N had significantly higher *Puccinia striiformis* infection in the organic crop protection treatment than the organic fertilisers used.

Table 4.9 *Interaction means ±SE for the effects of crop protection and fertiliser type on yellow rust (Puccinia striiformis) disease on the second leaf (L2) in square root transformation values.*

Fertiliser type	*Puccinia striiformis* infection on L2			
	CCP		OCP	
BD	0.0±0.00	Ab	2.3±1.08	Ba
CFYM	0.0±0.00	Ab	2.2±0.79	Ba
CFYMBD	0.0±0.00	Aa	1.3±0.48	Ba
MN	0.0±0.00	Ab	6.4±1.54	Aa

Means ±SE values followed by different lower-case letters within each row and upper-case letters within each column are significantly different (Tukey's general linear hypothesis test p<0.05). CCP (Conventional crop protection), OCP (Organic crop protection). Biogas digestate (BD), Composted farmyard manure (CFYM), Composted farmyard manure + biogas digestate (CFYMBD) and Mineral N (MN).

4.4.7 Grain yield, yield components, HI and total biomass

The results regarding main effects and interactions of crop protection and fertiliser type on grain yield, yield components, HI and total biomass are presented in Table 4.10. Crop protection had a significant effect on grain yield, yield components, HI and total biomass. The conventional crop protection treatment produced higher grain yield (3.8 t/ha) than organic crop protection (2.4 t/ha), with a yield gap of 1.4 t/ ha (58%). Differences in grain yield between conventional crop protection and organic crop protection were due to variation of yield components with significantly higher number of ears/m^2, grains/ear and TGW in the conventional than the organic crop protection treatments. Under conventional crop protection, the number of ears/m^2 was 403 with an average 28.4 grains/ear and TGW of 33.3 g, while for the organic crop protection treatment there was an average of 343.3 ears/m^2 with 25.6 grains/ear and a TGW of 30.7 g. HI was also higher at 46.9% in the conventional crop protection compared to 42.7% in the organic crop protection. Total crop biomass in the organic crop protection was significantly lower at 7.2 t/ha than in the conventional crop protection treatment (10 t/ha).

Significant main effects were observed on grain yield, yield components (number of ears /m^2 and TGW) and total biomass as influenced by fertiliser type. Biogas digestate produced the highest grain yield of 3.2 t/ha while the lowest was in composted farmyard manure (2.2 t/ha). The composted farmyard manure + biogas digestate (3.1 t/ha) and biogas digestate (3.2 t/ha) treatments showed similar values of grain yield. In terms of yield components, biogas digestate produced the highest ears/m^2, which was similar to the mineral N treatment. The lowest number of ears/m^2 was found with composted farmyard manure (295 ears/m^2) but composted farmyard manure + biogas digestate gave a greater number of ears/m^2 (383 ears/m^2) than composted farmyard manure. TGW was highest with composted farmyard manure (33.2 g) which was very close to composted farmyard manure + biogas digestate (33.1 g) and followed by biogas digestate (31.7 g). The lowest TGW value was in the mineral N treatment (30.1g). Among all fertiliser types the mineral N treatment gave the highest total crop biomass. The highest total crop biomass among organic fertiliser types was with composted farmyard manure + biogas digestate, with a value of 9.2 t/ha, followed by biogas digestate (9.1 t/ha). The lowest total crop biomass was with composted farmyard manure with 6.7 t/ha. In terms of grains per ear and HI, there was no significant difference between treatments. Composted farmyard manure + biogas digestate and mineral N both gave 28.0 grains/ear, while biogas digestate and composted

farmyard manure were very close with 26.3 and 25.6 grains/ear. Similar HI values were observed with mineral N (44.4%), biogas digestate (44.1%) and composted farmyard manure (44.2%), while the highest HI was observed with composted farmyard manure + biogas digestate (46.5%).

Table 4.10 *Main effects (means ± SE, p-values) and interactions of crop protection and fertiliser type effects on grain yield, yield components, harvest index and total biomass of spring wheat.*

Factors	Grain yield (t/ha)	Ears/m^2	Grains/ear	TGW (g)	HI (%)	Total crop biomass (t/ha)
Crop protection (CP)						
Conventional	3.8±0.39	403±19.9	28.4±0.82	33.3±0.43	46.9±1.13	10.0±0.6
Organic	2.4±0.13	343±23.7	25.6±0.67	30.7±0.53	42.7±0.55	7.2±0.41
Fertiliser type (Ft)						
BD	3.2±0.26 ab	407±34.2 a	26.3±1.22	31.7±0.81 ab	44.1±0.80	9.1±0.69 a
CFYM	2.2±0.18 b	295±21.9 b	25.6±1.19	33.2±0.47 a	44.2±0.78	6.7±0.60 b
CFYMBD	3.1±0.39 ab	383±31.8 a	28.0 ±1.06	33.1±0.52 a	46.5±2.43	9.2±1.06 a
MN	4.1±0.70 a	407±27.4 a	28.1±1.14	30.1±1.00 b	44.4±1.29	9.5±0.87 a
ANOVA *p-values*						
Main effects						
CP	**<0.001**	**0.013**	**0.002**	**<0.001**	**0.002**	**<0.001**
Ft	**0.014**	**0.006**	0.300	**0.004**	0.524	**0.007**
Interaction						
CP x Ft	**0.046**	0.537	0.169	0.227	0.551	0.442

Within columns, mean values followed by different letters are significantly different at p≤ 0.05. Values are the means and standard error (SE) of four replicates (n=4). Biogas digestate (BD), Composted farmyard manure (CFYM), Composted farmyard manure + biogas digestate (CFYMBD) and Mineral N (MN).

There was a significant crop protection x fertiliser type interaction on grain yield (Table 4.11). Within conventional crop protection (CCP), mineral N gave significantly higher grain yield than all other fertiliser types. However, under organic crop protection (OCP) all fertiliser types were not significantly different in grain yield. Mineral N, composted farmyard manure + biogas digestate and biogas digestate application gave significantly higher grain yield under conventional crop protection than organic crop protection, whereas composted farmyard manure there was no significant difference between crop protection treatments.

Table 4.11 *Interaction means ±SE for the effects of crop protection and fertiliser type on grain yield.*

Fertiliser type	Grain yield			
	CCP		OCP	
BD	3.6±0.37	Ba	2.8±0.27	Ab
CFYM	2.4±0.33	Ca	2.0±0.15	Aa
CFYMBD	3.8±0.54	Ba	2.4±0.28	Ab
MN	5.5±0.90	Aa	2.6±0.23	Ab

Means ±SE values followed by different lower-case letters within each row and upper-case letters within each column are significantly different (Tukey's general linear hypothesis test $p<0.05$). CCP (Conventional crop protection), OCP (Organic crop protection). Biogas digestate (BD), Composted farmyard manure (CFYM), Composted farmyard manure + biogas digestate (CFYMBD) and Mineral N (MN).

4.4.8 Grain quality and Se concentration

Hectolitre weight (Table 4.12) was significantly higher in the conventional crop protection (69.7 kg/hl) than in the organic crop protection treatment (68.3 kg/hl), while grain Se was significantly greater in the organic crop protection (21.0 µg/kg) than in the conventional crop protection treatment (16.8 µg/kg). However, protein content and HFN were not significantly affected by crop protection, with values of 10.8 % in conventional crop protection and 10.6 % in organic crop protection, while HFN was 667 s in the conventional crop protection and 627 s in the organic crop protection treatment. Overall, conventional crop protection gave higher values of protein, hectolitre weight and HFN than the organic crop protection treatment.

Fertiliser type significantly influenced protein content, hectolitre weight and HFN of wheat. The highest protein content was with mineral N (11.9%) and the lowest with composted farmyard manure (9.9%). Protein content was slightly higher in the biogas digestate (10.8 %)

than in composted farmyard manure + biogas digestate treatment (10.1 %). Hectolitre weights in the composted farmyard manure and composted farmyard manure + biogas digestate treatment were similar, with values just above 70.0 kg/hl, and significantly higher than in the biogas digestate (68.5 kg/hl) and mineral N (67.0 kg/hl) treatment. The lowest hectolitre weight was recorded in the mineral N treatment. HFN was highest in response to mineral N (749 s) and lowest in composted farmyard manure (574 s). Among the organic fertiliser treatments, the highest HFN was in biogas digestate (656 s). Grain Se concentration was not significantly influenced by fertiliser treatment with values which ranged from 16.0 to 21.8 µg/kg. The lowest value of grain Se concentration was found with mineral N whilst composted farmyard manure gave the highest grain Se concentration.

Table 4.12 *Main effects (means ± SE, p-values) and interactions of crop protection and fertiliser type on protein content, hectolitre weight, HFN and grain Se of spring wheat.*

Factors	Protein content	Hectolitre weight	HFN	Grain Se
	(%)	(kg/hl)	(s)	(µg/kg)
Crop protection (CP)				
Conventional	10.8±0.27	69.7±0.38	667.1±22.29	16.8±1.24
Organic	10.6±0.34	68.3±0.54	627.3±32.81	21.0±1.37
Fertiliser type (Ft)				
BD	10.8±0.38 b	68.5±0.59 b	656.9±37.98 ab	18.2±1.93
CFYM	9.9±0.15 c	70.2±0.41 a	574.8±30.87 b	21.8±2.20
CFYMBD	10.1±0.26 bc	70.2±0.43 a	607.9±27.69 b	19.6±1.54
MN	11.9±0.45 a	67.0±0.66 c	749.1±36.64 a	16.0±1.91
ANOVA *p-values*				
Main effects				
CP	0.598	**0.004**	0.148	**<0.001**
Ft	**<0.001**	**<0.001**	**0.008**	0.119
Interaction				
CP x Ft	0.395	0.125	0.961	0.949

Within columns, mean values followed by different letters are significantly different at p≤ 0.05. Values are the means and standard errors (SE) of four replicates (n=4). Biogas digestate (BD), Composted farmyard manure (CFYM), Composted farmyard manure + biogas digestate (CFYMBD) and Mineral N (MN).

4.5 Discussion

4.5.1 Grain yield

In the current study, crop protection had a significant effect on grain yield, yield components, HI and crop total biomass. A lower grain yield (58%) caused by lower values of yield components (ears/m^2, grains/ear and TGW) was found in the organic compared to conventional crop protection treatment. A lower yield with organic crop protection as found in this study is also supported by a number of other studies. Seufert *et al*. (2012) conducted a comprehensive meta-analysis to compare organic to conventional production yield and reported that organic production yields were 25% lower than conventional production. In another meta-analysis study, De Ponti *et al*. (2012) found that organic yield was 20 % lower than conventional through 362 yield comparisons. Fagnano *et al*. (2012) also highlighted that wheat yield in organic production was 21 % lower than conventional production. A higher yield gap in an organic production was reported by Jones *et al.* (2010) in a 3 seasons (2005-2007) comparison at organic and non-organic sites in the UK using 19 cultivars introduced to the market from 1934-2000. They observed that organic production had a 44 % lower grain yield than conventional production. Iannucci and Codianni (2016) concluded that the yield gap observed in different field trials is dependent on site, year and management system used. This study used the variety Mulika with a high yield potential, but a relatively low grain yield was detected in the trial, likely associated with a lower than expected crop establishment of 62 % and a later than ideal sowing date for spring wheat. In the current study HI and crop total biomass were significantly higher under conventional crop protection than organic crop protection which was also observed by Kitchen *et al.* (2003). Gevrek and Atasoy (2012) conducted a study to compare yield and agronomic performance under organic and conventional management in Turkey using twelve bread wheat genotypes during 2008-2010 and reported that higher total biomass was found under conventional production.

In general, many researchers relate lower grain yield in organic production with higher pest and disease levels and nutrient limitation (especially nitrogen). According to De Ponti *et al*. (2012), pests and diseases and nutrient limitations are generally acknowledged as major yield-limiting factors contributing to the yield gap between organic and conventional production. Mazzoncini *et al.* (2015) highlighted that nitrogen shortage is one of the main factors reducing grain yield and quality in organic wheat production. Hazra *et al.* (2018) found that the reasons for lower grain yield in organic production are primarily because of lack of pest management options and difficulties with plant nutrient management. Rempelos *et al.* (2018)

found that less efficient crop protection and fertilization resulted in lower grain yield (45 %) in organic than conventional wheat production. In the current study, *Puccinia striiformis* leaf infection was significantly higher on the flag leaf and second leaf in the organic than conventional treatment and likely contributed to the lower grain yield and quality. This was also observed by Röös (2018), who found that pests and disease may contributed to the yield gap between organic and conventional production. Devadas *et al.* (2014) agreed that yellow rust contributed to reduction in grain yield, protein content and biomass. Grain yield and TGW reductions in wheat were mostly associated with higher foliar disease levels (Serrago *et al*., 2011).

Significant main effects were observed on grain yield, number of ears/m^2, TGW and total crop biomass in response to fertiliser type. In this study, mineral N produced a significantly higher grain yield than biogas digestate, composted farmyard manure + biogas digestate and composted farmyard manure treatments. Campiglia *et al.* (2015) believed that the yield gap between organic and conventional production is closely linked to the quantity of available nitrogen supplied by organic fertiliser sources, which is significantly lower than from mineral fertiliser even when the same dosage of total N is applied**.** The mineralisation rate of organic fertiliser sources significantly contributes to the yield potential of crops (Pang & Letey, 2000). A longer period is required for release, supply and uptake of N from organic fertiliser sources (Buchi *et al*., 2016). Gopinath *et al.* (2008) observed that the slower release rate from organic materials and lower concentration of available nutrients resulted in a lower yield. In another study, Palmer *et al.* (2013) also suggested that the reason for yield gap between organic and conventional potato production is because of insufficient nutrient supply from organic fertiliser which is highly dependent on microbial mineralisation in the soil.

Results showed that biogas digestate and composted farmyard manure + biogas digestate produced significantly higher grain yield than composted farmyard manure. The nutrient composition (Table 4.4,) indicated that biogas digestate was higher in ammonium nitrogen than composted farmyard manure, while nitrate nitrogen was higher in composted farmyard manure than biogas digestate. Cavalli *et al.* (2016) reported that digestate has higher potential of N availability for crops because digestate has a high ratio of ammonium nitrogen to total N. Makádi *et al*. (2012) agreed that higher grain yields in winter wheat and spring wheat are because digestate has higher available nitrogen content than farmyard manure, in addition, the nitrogen mineralisation rate of digestate is greater (Diacono *et al*., 2018) than farmyard manure due to digestate having a ten times lower C:N ratio than farmyard manure (Šimon *et al*., 2015). Some studies have shown that digestate can be used as a possible substitute for

mineral N fertiliser (urea) because 90 % of its nitrogen content is highly available for plant growth (Alburquerque *et al*., 2012), similar to that of urea (Tambone and Adani, 2017). Röös *et al.* (2018) stated that organic waste, compost, manure and green manure are examples of general organic fertilisers with low plant-available N.

4.5.2 Disease severity

Puccinia striiformis leaf infection was significantly higher in the organic crop protection treatment. The lower infection of *Puccinia striiformis* in the conventional crop protection treatment in the present study is likely due to use of synthetic fungicides at GS31 and GS39. Hardwick *et al.* (2001) reported that grain yield in the UK could be optimised when fungicide control is applied to the flag leaf at GS39. As a result of fungicide use in the conventional crop protection treatment, the flag leaf and the second leaf maintained greater green area during the grain filling period. The flag leaf is the main source of assimilates for translocation to the grains. Delayed senescence would increase the grain filling and final grain yields (El Jarroudi *et al*., 2015) due to the longer period of photosynthesis. The current results are also in accordance with the report of Bilsborrow *et al*. (2013), who studied the effect of different production system components on the yield and quality of winter wheat and found that foliar disease such as Septoria tritici was significantly higher in the organic crop protection than the conventional crop protection management systems throughout trial years except for 2005. In their study, AUDPC values with conventional fertility management were significantly higher than with organic fertility management under the organic crop protection treatment for both Septoria tritici disease and powdery mildew (except for 2008, when the infection level of powdery mildew was low). The authors noticed that it is likely due to the response of wheat to increases of N-input level, as confirmed by several previous researchers who also compared the effects of organic and conventional management systems on powdery mildew and Septoria tritici for wheat.

There was a significant effect of fertiliser type on *Puccinia striiformis* infection of the flag leaf, where disease severity with mineral N was significantly higher than with all the organic fertiliser treatments. In the current trial, all fertiliser types received the same dosage of total N (150 kg N/ha) but varied in the available N. Notably, mineral N has the highest N availability and had the highest *Puccinia striiformis* leaf disease infection. According to Devadas *et al.* (2014), the severity of *Puccinia striiformis* disease is correlated to nitrogen nutrition where the infection of *Puccinia striiformis* disease increases with higher N. For the

organic fertilisers, biogas digestate has a slightly higher N availability than composted farmyard manure + biogas digestate and composted farmyard manure and higher *Puccinia striiformis* leaf disease infection was observed with biogas digestate than composted farmyard manure + biogas digestate and composted farmyard manure. Dordas and Christos (2008) reported that plants have the capacity to increase the synthesis of defence-related compounds for protection against pathogens when plants are grown in soil with low N availability. Interestingly, recent work carried out by Rempelos *et al*. (2018) showed that Septoria disease severity was significantly lowered by application of mineral NPK fertiliser, but there was significantly higher incidence of powdery mildew disease than in crops fertilised with composted farmyard manure. This finding is consistent with other studies that examined the impacts of different fertiliser types and level of N inputs on powdery mildew severity. The authors suggest that future studies should further examine the effect of differences in total N-availability or variation of the N-availability pattern throughout the growing season. Walters and Bingham (2007) found that the available information regarding the relationship between plant nutrition and disease in organically grown crops is still very limited.

4.5.3 Grain quality

Hectolitre weight was significantly higher under conventional crop protection than organic crop protection, which is in line with the findings of Mason *et al.* (2006), who examined the potential breadmaking quality of five Canadian Western Hard Red Spring wheat cultivars under conventional and organic management and found that hectolitre weight was higher in conventional (78 kg/hl) than organic (77 kg/hl) production. The lower value of hectolitre weight could be due to increased weed competition for water and nutrients but also due to higher disease levels which are likely to impact on grain filling. Results in this study showed that hectolitre weights in the conventional crop protection and organic crop protection treatments were below 70.0 kg/hl, which is well below the specification for top quality milling wheat of 76 kg/hl (Mason *et al*., 2007; Bilsborrow *et al*., 2013). Gooding *et al.* (1999) observed that hectolitre weight under organic production was > 75 kg/hl and sufficient for breadmaking, while Iannucci and Codianni (2016) observed hectolitre weights under organic production > 80 kg/hl using 10 durum wheat cultivars over a three year period (2012-2014) in a comparison of conventional and organic farming in Foggia, Southern Italy. Meanwhile, Mader *et al.* (2007) conducted a study to assess the quality of wheat grown in a 21 year comparison of organic and conventional systems in central Europe and found no significant difference in hectolitre weight

under these two production systems. L-Baeckström *et al*. (2004) also found similar results in comparison of hectolitre weight between organic and conventional production.

Grain protein content and HFN were not significantly different in response to the crop protection treatments used, with a slight difference in protein content. This small variation in protein content between organic and conventional sites was also observed by Mason *et al.* (2007) who concluded that this was probably due to sufficient supply of nutrients from the soil in the organic production. Some researchers have reported that protein content is lower in organic than conventional production (L-Baeckström *et al*., 2004; Ceseviciene *et al.*, 2012; Fagnano *et al*., 2012; Mazzoncini *et al.*, 2015; Iannucci and Codianni, 2016; Zörb *et al*., 2018). A reduction of protein content in grains in the organic crop protection treatment in the current study is possibly due to the increased disease pressure and reduced N availability (Mader *et al*., 2007; Rembiałkowska, 2007; Fagnano *et al*., 2012). Devadas *et al.* (2014) also highlighted that the protein content of wheat was increased by controlling rust with fungicides. Several researchers have confirmed little difference in HFN between production systems. For instance, Mazzoncini *et al.*(2015) concluded that HFN is not influenced by the cropping systems and found that HFN values were slightly lower in organic (335 s) than conventional system (339s). L-Baeckström *et al*. (2004) also observed that HFN in Swedish winter wheat cultivars was slightly lower in an organic (267.10 s) than a conventional system (270.70 s). However, the result of HFN from the current trial was much higher (574 s-749 s) than previous studies. Based on the HGCA Recommended List for cereals and oilseeds, HFN value for spring sown Mullika is recorded as 309 s (AHDB, 2016). This higher HFN value in the current trial is likely due to the temperature of 60 ^{o}C used when the grains were oven dried. At higher drying temperature i.e. 60 ^{o}C and above, α-amylase is inactivated due to denaturation of the enzyme's active site, which may cause low activity of α-amylase and negatively affects bread-making (Schirmer *et al*., 2006). This is agreed by Ugarčić-Hardi and Hackenberger (2001) who observed and suggested that the drying temperature of wheat should not exceed 50 °C to maintain biologically undamaged wheat grain. In general, the minimum HFN value for bread-making is 250 s (Lunn *et al*., 2001; Taylor *et al*., 2001).

Protein content, hectolitre weight and HFN were significantly different in response to fertiliser type. Mineral N gave the highest grain protein content and HFN but the lowest hectolitre weight. Among the organic fertilisers, biogas digestate gave a higher grain protein content and HFN than composted farmyard manure. The higher grain protein content and HFN from the use of biogas digestate is likely due to the increased available N content as shown by Diacono *et al.* (2018). The lowest grain protein content resulting from the use of composted

farmyard manure is likely due to the poor synchronisation of N supply from organic manures input with crop N demand (Möller *et al.*, 2008). It has been shown that composted farmyard manure has a very low content of readily plant available ammonium (NH_4–N) and nitrate nitrogen (NO_3–N), as observed by Palmer *et al.* (2013).

4.5.4 Grain Se concentration

Crop protection had a significant effect on Se concentration of wheat grain, with a higher level in response to organic crop protection than conventional crop protection. In contrast, Nelson *et al.* (2011) reported that grain Se levels were lower in organic than conventional production. However, studies on Se in organic vs conventionally grown grain are very limited in the literature. Limited information on the levels of macronutrient and trace elements in wheat flours from conventional and organic cultivation production has also been highlighted by Vrček *et al.* (2013). In other studies, mineral concentration in grains has been reported higher in organically than conventionally grown grain. According to Ryan *et al.* (2004), Zn and Cu were higher in organically than conventionally grown grain. Palmer *et al.* (2013) observed that minerals such as N, Ca and Mg were significantly higher in organic than conventional potato, most likely due to the dilution effect, while P, K and S were not significantly different between the management systems. The dilution effect can be explained by an association between yield and a particular mineral concentration in grain, for example decreased grain yield but higher grain mineral concentrations such as N, Ca and Mg and vice versa. The higher Se concentration in organically grown grain may be linked to the dilution effect, since it was observed that grain yield and total biomass production were higher in the conventional than organic treatment. This is also reported by Cooper *et al.* (2011), who stated that the lower Al and Cu concentration in grain from conventional crop protection is related with a dilution effect associated with higher yields and biomass production.

Fertiliser type had no significant effect on grain Se concentration, although organic fertiliser produced higher grain Se concentration than mineral N fertiliser. The highest grain Se concentration was in response to composted farmyard manure and was followed by composted farmyard manure + biogas digestate and biogas digestate. However, there is very limited information in the literature on the effects of biogas digestate and composted farmyard manure on grain Se status; an area which needs further investigation.

4.6 Conclusion

The present study has indicated that crop protection influenced the grain yield, quality and leaf disease of Mulika spring wheat, with grain yield being 58% for organic lower than for conventional crop protection. Protein content, hectolitre weight and HFN were all reduced in the organic crop protection but Se concentration was slightly higher. Organic crop protection has the potential to increase grain Se concentration of wheat, which is generally low in UK grown cereals. The infection of *Puccinia striiformis* disease on the flag leaf, second and third leaf also was particularly affected by the crop protection treatments, where higher severity infections were detected in the organic crop protection treatment. The reduction of grain yield and quality in organic production is likely related to the reduced N availability and supply and the mismatch between N supply and crop demand especially during grain filling. Limited availability of N from organic fertiliser sources is dependent on mineralisation and hence microbial activity in the soil, which is mainly influenced by soil temperature and weather conditions. Among the organic fertilisers tested in this study, biogas digestate showed potential in promoting grain yield and quality (protein content) of spring wheat while composted farmyard manure increased grain Se concentration but this needs to be explored further. The higher nutrient availability of N in biogas digestate than composted farmyard manure and composted farmyard manure + biogas digestate also has resulted in the higher severity of *Puccinia striiformis* leaf disease infection to Mulika spring wheat. The above study is only based on a single year's results, which means that any conclusions are severely limited. The trial was also drilled in both the 2017 and 2018 seasons but mainly due to weather conditions and a resulting poor-quality seedbed neither trial was taken to harvest although measurements and data were recorded in both seasons. Data from both 2017 and 2018 is not presented in this thesis because of the very low germination rates and plant establishment recorded.

Chapter 5 : Selenium biofortification of spring wheat

5.1 Introduction

Wheat is a staple crop for many countries globally with the potential to supply essential minerals such as selenium for human nutrition and health (Poblaciones *et al.*, 2014a, b). However, wheat grown in the UK generally has a relatively low grain selenium concentration (Broadley *et al.*, 2006, Broadley *et al.*, 2010; Stroud. *et al.*, 2010ab) due to low Se availability of British soils (White and Broadley, 2009; Gomez-Becerra *et al.*, 2010; Broadley, *et al.*, 2010; Vrcek *et al.*, 2014; White, 2016). One practical and easy method to enhance grain selenium concentration and to overcome nutritional deficiencies in humans is agronomic biofortification through fertilisation of crops (Broadley *et al.*, 2010; Mora *et al.*, 2015; Magallanes-López *et al.*, 2017; Dos Reis *et al*., 2017; Lyons, 2018). A recent meta-analysis study on selenium fertilisation suggested that this method has clear potential to enhance crop selenium uptake and human intake (Ros *et al.*, 2016). Selenium fertilisations consist of two approaches, soil and/or foliar application (Chu *et al.*, 2013; De Vita *et al.*, 2017) with both being used to improve food selenium concentration and contribute to human requirements (Nawaz *et al*., 2017; Idrees *et al*., 2018).

Numerous studies in the literature have studied the influence of soil and foliar selenium fertilisation applications on grain selenium concentrations and yield (Rodrigo *et al.*, 2013; De Vita *et al.*, 2017; Idrees *et al.*, 2018). However, Poblaciones *et al.* (2014b) highlighted that most agronomic biofortification studies on bread-making wheat have focused on grain protein content with less attention paid to other quality parameters such as HFN and hectolitre weight. Therefore, the current study was undertaken (i) to investigate the effect of soil and foliar applications of selenium fertiliser on crop growth, yield and quality, (ii) to examine the accumulation and distribution of selenium in different plant organs under field and greenhouse conditions.

5.2 Material & methods

Selenium fertilisation trials were carried out between March and October 2018 in the field at Nafferton Farm and in the glasshouse at Cockle Park Farm. Both trials examined the effect of selenium fertiliser application method and rate on grain selenium concentration, grain yield, yield components and quality. Soil and foliar selenium treatments were used in the field trial while the glasshouse study used soil application only.

5.2.1 Field trial

5.2.1.1 Site description

The field trial was conducted in Stelling field during 2018, located at Newcastle University's Nafferton Experimental Farm, Northumberland, United Kingdom (54:59:09 N latitude and 1:43:56W longitude). Stelling is adjacent to Quarry, which was used in previous trials in this thesis (Fig. 5.1). Stelling is a 10 ha field with a sandy loam soil of the Stagnogley type (Cooper *et al*., 2011). The previous cultivated crop was winter wheat.

5.2.1.2 Experimental design and selenium treatments

All treatments were laid out in a randomized complete block design with four replicates (Fig. 5.2). Each main block was 12 m x 24 m and divided into four sub-plots (6 m x 12 m), with a total of 16 experimental sub-plots. Four selenium rates equivalent to 0 g Se/ha (control), 15 g Se/ha and 30 g Se/ha (low and high Se rates) for selenium soil application and 30 g Se/ha (high Se rate) for selenium foliar application were applied to sub-plots. In the control treatment, the commercial granular ammonium nitrate-based fertiliser (Yara Bela Extran) containing total nitrogen (33.5 %) for nitric nitrogen (16.9 %) and ammoniacal nitrogen (16.5 %) was applied at a rate of 150 kg N/ha. Soil application treatments for low and high selenium were based on a mixture of Yara Bela Extran with the selenium-containing fertiliser Yara Bela Nutri-Booster with total nitrogen of 25.0 %, nitric nitrogen of 12.2 %, ammoniacal nitrogen 12.8 %, water soluble sulphur of 2.0 % and water-soluble selenium of 0.005 %. In the foliar application treatment, sodium selenate (Na_2SeO_4, 98 %, ACROS Organics) was dissolved in 25 litres of water to provide an application rate equivalent to 30 g Se/ha application. The selenate solution was sprayed on the plant leaf surface by a tractor mounted sprayer with a 6 m boom. Both selenium soil and foliar application treatments were applied as a single application at stem elongation (GS31) on 13th June 2018.

5.2.1.3 Crop management

The trial area was ploughed a month before sowing. Prior to sowing, soil samples were collected on 23rd March 2018 at different soil depths for determination of mineral N (0-30 cm, 30-60 cm and 60-90 cm) and soil selenium concentrations (0-30 cm, 30-60 cm). All climatic data were taken from an automatic meteorological station located 500 m from the trial location at Nafferton farm. The variety used was Mulika, a spring bread-making variety. The plots were sown on 19th April 2018 at a seed rate of 168 kg/ha (400 seeds/m^2) at a row spacing of 12 cm and depth of 2-3 cm using a commercial seed drill (3m Lely combination drill; Lely U.K. Ltd., St. Neots, U.K.). All plots were sprayed with herbicide and fungicide. The first pesticide application on 15th June 2018 at GS31 included a mixture of the fungicides Cortez (a.i. epoxiconazole): 500 ml/ha, Bravo 500 (a.i. chlorothalonil): 1 l/ha and the herbicide Chimera (a.i. thifensulfuron methyl and metsulfuron methyl) at 70 g/ha. The second application was sprayed on 22nd June 2018 at GS39 with the fungicides Bravo 500 (a.i. chlorothalonil) at 1 l/ha and Kestrel (a.i. prothioconazole and tebuconazole) at 300 ml/ha. Wheat was harvested at maturity on 5th Sept 2018 using a plot combine (Claas Dominator 38; Class UK Ltd, Bury St Edmunds UK). A summary of crop management and treatment application dates for the field trial is presented in Table 5.1.

Fig. 5.1 *Location of Stelling field trial at Nafferton Farm*

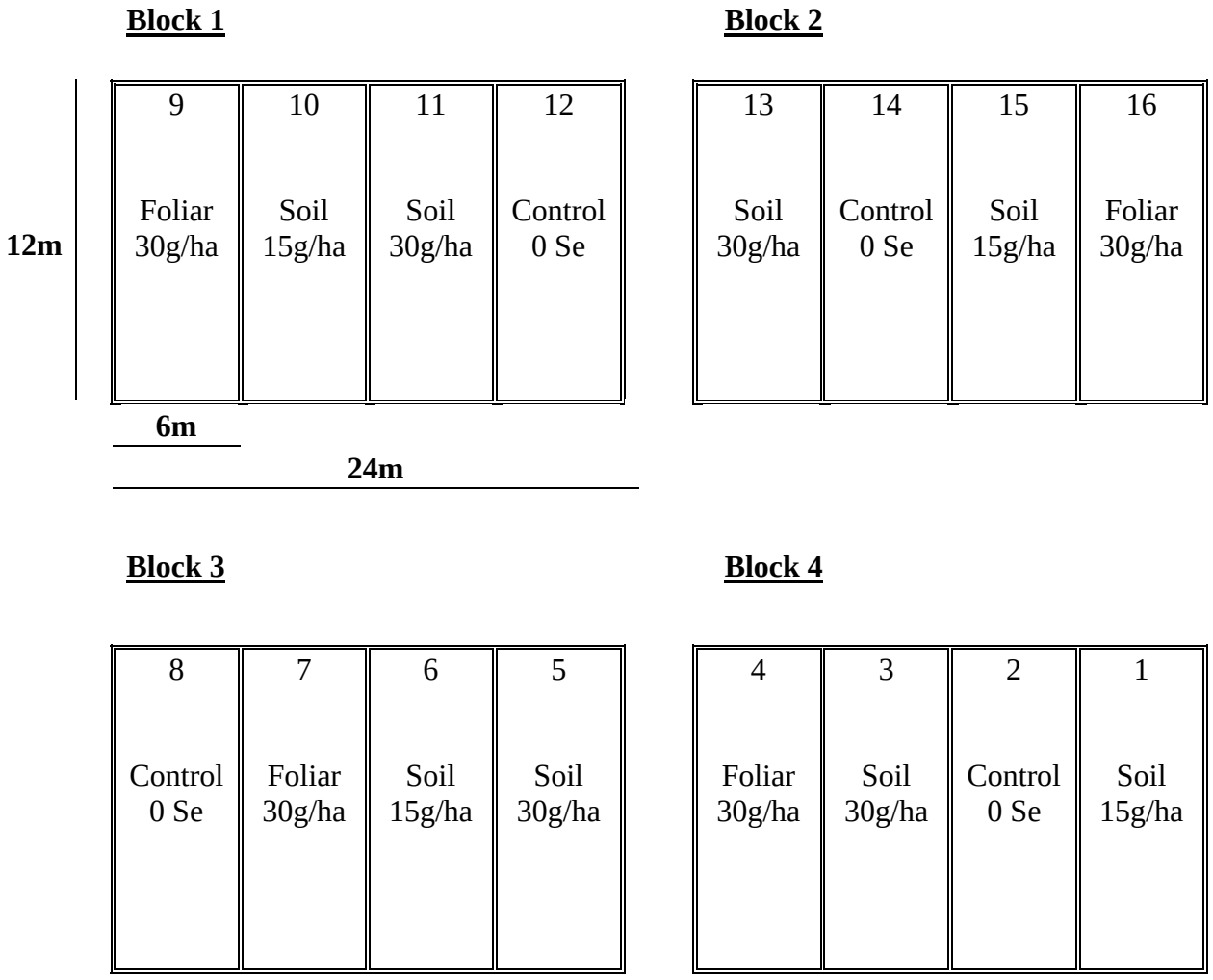


Fig. 5.2 *Layout of field trial at Nafferton Farm 2018.*

Table 5.1 *Summary of field trial crop management and treatment application dates in 2018.*

Crop management	**Date**
Soil sampling	23rd March
Sowing date	19th April
Selenium treatments applied at GS31	13th June
Fungicide and herbicide applications at GS31and GS39	15th June (GS31) and 22nd June (GS39)
Plant biomass sampling at GS55, GS70 and GS95	29th June (GS55)
	9th July (GS70)
	29th August (GS95)
Harvest	5th Sept (GS95)

5.2.1.4 Crop sampling and field measurements

All measurements and plant samplings were performed according to the Zadoks decimal code (Zadoks *et al*., 1974). Wheat emergence was measured at GS12 on 15th May 2018. SPAD and Normalized Difference Vegetation Index (NDVI) readings were conducted at flag leaf emergence (GS39) on 21st June 2018 and the beginning of anthesis (GS62) on 4th July 2018 using a SPAD meter (Minolta SPAD-502, Osaka, Japan) (20 individual flag leaves per plot randomly sampled) and the Greenseeker™ Handheld Crop Sensor (Model HSC-100), respectively, with 10 replicate measurements per plot.

To study selenium accumulation and distribution in different plant parts, above-ground biomass samples were taken at GS55 (29th June 2018), GS70 (9th July 2018) and GS95 (29th August 2018) using a 0.5 m x 0.5 m quadrat. After sampling, plant material was separated into stem and leaf (GS55), stem, leaf and ear (GS70) and stem, grain and chaff fractions (GS95). All separated plant parts were oven-dried at 60°C for 72 hours to constant weight, milled (Retsch SK300 equipped with a 0.25 mm mesh sieve size) and stored in ziplock bags before sub-samples were sent to Sabbanci University, Turkey for selenium analysis. All ear samples at GS95 were processed using a thresher machine (Wintersteiger LD 350, Austria) to separate grain and chaff parts.

At maturity, approximately 1 kg of grain sub-sample was taken from each plot of the combine. Grain yield was expressed in t/ha and reported at 15% moisture content. Grain sub-samples were taken and oven-dried for determination of grain moisture content. A separate sub-sample was oven-dried at 40 °C for 72 hours, kept in sealed plastic bags, and stored at room temperature prior to grain quality determination (protein content, HFN, hectolitre weight) and grain selenium concentration analysis. Data on plant height and above-ground biomass samples of grain yield, yield components, TGW (g), harvest index (HI %) and total crop biomass (DM t/ha) were recorded from a sampling area of 0.25 m^2 (0.5 m x 0.5 m) just prior to harvest. Plant height was measured from the ground level to the base of the ear at physiological maturity. All general procedures and methods conducted in the field measurements and laboratory work were as described in Chapter 4.

5.2.2 Glasshouse trial

5.2.2.1 Materials

A pot trial was carried out in the glasshouse at Cockle Park Farm, Newcastle University's Experimental Farm from May to September 2018. Mulika spring wheat (*Triticum aestivum* L.) seeds were sown in 5 L (22 cm) plastic pots filled with soil collected from the field trial site in Stelling field. Each pot was sown with 16 seeds (equivalent to a rate of 400 seeds/m^2) of wheat (variety Mulika) at 1-2 cm soil depth on 24th May 2018. A month after sowing, all pots were thinned to 13 individual plants to provide a uniform plant population prior to selenium application. Plants were watered regularly, and no disease was detected during the growing season.

5.2.2.2 Experimental design and selenium treatment

All treatment pots were laid out in a completely randomised design with five replications (Fig. 5.3). Only soil application of selenium was studied in the glasshouse trial with rates of 0 (control), 15 and 30 g Se/ha. These selenium treatment rates were the same as those used in the field trial (Section 5.2.1.2). All pots received selenium and nitrogen fertiliser from the commercial granular ammonium nitrate-based fertiliser Yara Bela Extran and selenium containing fertiliser Yara Bela Nutri-Booster equivalent at a rate of 150 kg N/ha in two split applications. The first application was at tillering (GS21) on 26th June 2018 and the second at stem elongation (GS37) on 5th July 2018. A summary of treatment dates and crop management for the glasshouse trial at Cockle Park Farm is presented in Table 5.2.

H1					H2					H3				
T3R1H1	**T1R2H1**	**T2R3H1**	**T3R4H1**	**T1R5H1**	**T2R1H2**	**T3R2H2**	**T1R3H2**	**T3R4H2**	**T2R5H2**	**T1R1H3**	**T3R2H3**	**T2R3H3**	**T1R4H3**	**T3R5H3**
T2R1H1	**T3R2H1**	**T1R3H1**	**T2R4H1**	**T3R5H1**	**T1R1H2**	**T2R2H2**	**T3R3H2**	**T2R4H2**	**T1R5H2**	**T3R1H3**	**T2R2H3**	**T1R3H3**	**T3R4H3**	**T2R5H3**
T1R1H1	**T2R2H1**	**T3R3H1**	**T1R4H1**	**T2R5H1**	**T3R1H2**	**T1R2H2**	**T2R3H2**	**T1R4H2**	**T3R5H2**	**T2R1H3**	**T1R2H3**	**T3R3H3**	**T2R4H3**	**T1R5H3**

Fig. 5.3 *Layout of glasshouse trial at Cockle Park Farm 2018 with three selenium treatment rates of T1 (0 g Se/ha), T2 (15 g Se/ha) and T3 (30 g Se/ha) with five replicates (R1-R5). H1: Plant harvesting at GS59 (12th July 2018), H2: Plant harvesting at GS72 (26th July 2018) and H3: Plant harvesting at GS95 (28th August 2018).*

Table 5.2 *Summary of crop management and treatment dates for the glasshouse trial in 2018.*

Crop management	Date
Sowing date	24th May
Plant thinning	26th June
Se treatment and mineral N applications	26th June (1st application)
	5th July (2nd application)
Plant biomass sampling	12th July (GS59)
	26th July (GS72)
	28th August (GS95)

5.2.2.3 Plant sampling and measurements

Plants were harvested at GS59 (12th July 2018), GS72 (26th July 2018) and GS95 (28th August 2018) to determine selenium accumulation and distribution in different plant parts. At each harvest, plants were separated into stem and leaf (GS59), stem, leaf and ears (GS72) and stem, chaff and grain (GS95). All plant fractions were oven-dried at 60 °C for 72 hours to constant weight. At GS95, data on wheat grain yield, ear numbers per plant, grains per ear, TGW (g), harvest index (%) and total biomass were determined at physiological maturity. Grain yield was presented as dry weight per pot at 15 % moisture content. TGW was calculated based on the mean of triplicates of 100 grains weighed using an electronic seed counter (Model Elmor C3, Switzerland). Harvest index was determined by the ratio of total grain weight divided by total above-ground dry weight. The dry weight of the separated plant fractions was summed to record total biomass (g/pot). All dried plant samples were milled (Retsch ZM200) with a 0.2 mm mesh sieve size and stored in sealed ziplock bags. Grain quality (nitrogen content) was determined by an elemental analyser (Elementar Vario Macro Cube) located in the 4th floor teaching laboratory, Agriculture School (see section 4.2.4.5) and converted to protein by multiplying total N by 6.25.

5.3 Statistical analysis

All results were subjected to analysis of variance (ANOVA) to assess the effect of selenium soil and foliar fertilisation application methods on grain yield, yield components, grain quality, grain selenium concentration, SPAD, NDVI, plant height, harvest index and total biomass, with the data all tested together. ANOVA was derived from linear mixed-effects model procedure *"lme"* (Pinheiro and Bates, 2000) using the "*nlme"* package in R software environment (R Core Team, 2017). When significant differences were detected, Tukey contrasts in the general linear hypothesis testing (*glht*) function of the "*multcomp*" package (Bretz *et al*., 2011) in R was used to test the differences between treatment means. Both means and standard errors of means (SE) were calculated using '*t apply''* function in R package. QQ-plots was performed to test the normality of the residuals and the data were transformed logarithmically prior to ANOVA.

5.4 Results

5.4.1 Field trial

5.4.1.1 Weather

The monthly rainfall was high in March (100.4 mm) and August (108.6 mm), whereas May, June and July were relatively dry with monthly rainfall being half of the long-term average for these months (Table 5.3). Total cumulative rainfall for the whole growing season (March to October) was 469 mm. Monthly mean air temperature varied between 3.7 ^{o}C and 16.9 ^{o}C.

Table 5.3 *Monthly mean radiation (MJ/m^2), air temperature (^{o}C) and cumulative monthly rainfall (mm) during the 2018 growing season at Nafferton Farm.*

Parameter	Unit	Month							
		Mar	**Apr**	**May**	**June**	**Jul**	**Aug**	**Sept**	**Oct**
Mean solar radiation	MJ/m^2	6.6	10.7	18.7	19.4	18.0	11.8	9.8	4.9
Mean air temperature	^{o}C	3.7	8.1	11.9	14.3	16.9	15.3	12.4	9.5
Rainfall	mm	100.4	67.6	31.0	38.6	25.2	108.6	53.0	44.6

5.4.1.2 Soil mineral N and total soil Se concentration

Results of soil analysis (which not tested statistically) indicated that soil NO_3^- N, NH_4^+-N and total nitrogen in the field trial were higher in the 0-30 cm than 30-60 cm and 60-90 cm soil depths (Table 5.4). Ammonium-N was greater than nitrate-N in the topsoil (0-30 cm) while nitrate-N was higher than ammonium-N in the lower soil depth (30-60 cm). However, the total soil selenium concentration in the 0-30 cm and 30-60 cm horizons was similar (0.35 mg/kg and 0.31 mg/kg).

Table 5.4 *Mean values of nitrate (NO_3-N), ammonium (NH_4-N), total nitrogen (NO_3-N + NH_4-N) at three different soil depths (cm) and selenium concentration at two different soil depths (cm) prior to treatment applications.*

Soil depth	N status in soil			Selenium (mg/kg)
	Nitrate nitrogen (mg/kg)	Ammonium nitrogen (mg/kg)	Total-N (kg/ha)	
0-30 cm	3.92	6.08	37.50	0.35
30-60 cm	1.90	1.17	11.50	0.31
60-90 cm	1.41	0.58	7.50	-

5.4.1.3 Seedling establishment

The mean for seedling emergence in the field trial was 326 plants/m^2, which represents 81% germination.

5.4.1.4 SPAD, NDVI & plant height

There was no significant difference detected in SPAD values at GS39 and GS62 as affected by either soil or foliar selenium application (Table 5.5). SPAD values at GS39 ranged from 40.5 to 41.7. SPAD values at GS39 were slightly lower than at GS62 (43.2 to 44.2) when averaged across all treatments. Plant height was not affected by selenium application method or rate**.** The mean plant height across all treatments at GS95 ranged from 45.0 cm to 48.0 cm. While NDVI showed no significant treatment effects at GS39 and GS62 (Table 5.6), with values ranging between 0.50 and 0.55.

Table 5.5 *Effects of selenium application method and rate on SPAD (GS39 and GS62) and plant height at maturity at Nafferton Farm 2018.*

Se application method	Se rate (g/ha)	SPAD		Plant height (cm)
		GS39	GS62	
Control	0	40.5±0.64	43.3±0.92	45.3±1.65
Soil (Low)	15	42.3±0.81	44.2±0.74	44.8±1.31
Soil (High)	30	41.2±0.32	43.8±0.59	48.0±0.82
Foliar (High)	30	41.7±0.45	43.2±0.06	45.0±1.68
ANOVA *p* value		0.225	0.555	0.376

Values are the means +/- standard error (SE) of four replicates (n=4).

Table 5.6 *Effects of selenium application method and rate on NDVI at GS39 and GS62 at Nafferton Farm in 2018.*

Se application method	Se rates (g/ha)	NDVI	
		GS39	GS62
Control	0	0.51±0.64	0.51±0.92
Soil (Low)	15	0.51±0.81	0.55±0.74
Soil (High)	30	0.55±0.32	0.51±0.59
Foliar (High)	30	0.51±0.45	0.50±0.06
ANOVA *p* value		0.431	0.517

Values are the means +/- standard error (SE) of four replicates (n=4).

5.4.1.5 Selenium accumulation and distribution

At GS55, selenium distribution in stem and leaf were significantly affected by method of selenium application and rate (Table 5.7a). Selenium concentration in the stem ranged from 42.3 µg/kg in the control treatment to 376.7 µg/kg for the high selenium soil application and 223.8 µg/kg for the high selenium foliar application. Leaf selenium concentration ranged from 177.0 µg/kg in the control treatment to 784.0 µg/kg for the high selenium soil application and 766.0 µg/kg with the high selenium foliar application. Application method had little effect on leaf selenium concentration, but concentration was significantly higher ($p<0.05$) in the stem following soil than foliar application. The differences in selenium accumulation between stem and leaf were generally double in all treatments.

Table 5.7 (a) *Effect of application method and rate on selenium concentration in stem and leaf at GS55 in the Nafferton Farm field trial 2018.*

Se application method	Se rate (g/ha)	GS55	
		Stem (µg/kg)	Leaf (µg/kg)
Control	0	42.3±11.54 c	177.0±9.10 c
Soil (Low)	15	181.3±37.39 b	367.5±59.75 b
Soil (High)	30	376.7±12.11 a	784.0±105.50 a
Foliar (High)	30	223.8±17.89 b	766.0±74.91 a
ANOVA *p*-value		**<0.001**	**<0.001**

Within columns, mean values followed by different letters are significantly different at $p \leq 0.05$. (Tukey's general linear hypothesis test). Values are the means +/- standard error (SE) of four replicates (n=4).

However, results in Table 5.7 (a) were found not to be normally distributed. Data in Table 5.7 (a) was therefore transformed logarithmically prior to ANOVA, as shown in Table 5.7 (b). After logarithmic transformation, selenium concentration in stem and leaf were both significantly affected by method of selenium application and rate (Table 5.7b). In stem, the natural logarithm of selenium concentration (µg/kg) in the control treatment was 3.6, with increases following low selenium soil application (5.1 ln µg/kg), high selenium soil application (6.3 ln µg/kg) and high selenium foliar application (5.4 ln µg/kg). In leaf tissue, the natural logarithm of selenium concentration (µg/kg) in the control treatment was 5.2, with subsequent increases following low selenium soil application (5.9 ln µg/kg), high selenium soil application (7.0 ln µg/kg) and high selenium foliar application (6.7 ln µg/kg). It was observed that selenium accumulation in stem and leaf (Table 5.7 b) showed increasing trends in response to selenium rate following soil application as was also indicated in Table 5.7 (a) before logarithmic transformation. High soil and foliar application (Table 5.7 b) were significantly different in stem.

Table 5.7 (b) *Effect of application method and rate on selenium concentration in stem and leaf at GS55 in the Nafferton Farm field trial 2018 (Transformed logarithmically*)

Se application method	Se rate (g/ha)	GS55	
		Stem (ln µg/kg)	Leaf (ln µg/kg)
Control	0	3.6±0.29 c	5.2±0.05 c
Soil (Low)	15	5.1±0.25 b	5.9±0.16 b
Soil (High)	30	6.3±0.38 a	7.0±0.37 a
Foliar (High)	30	5.4±0.08 b	6.7±0.13 a
ANOVA *p* value		**<0.001**	**<0.001**

Within columns, mean values followed by different letters are significantly different at p≤ 0.05 (Tukey's general linear hypothesis test). Values are the means +/- standard error (SE) of four replicates (n=4).

At GS70, selenium concentrations in stem, leaf and ear were also significantly affected by method and rate of selenium application (Table 5.8a). In stem, selenium concentration ranged from 58.3 µg/kg in the control treatment to 289.0 µg/kg for the high selenium soil application and 190.8 µg/kg for the high selenium foliar application. Leaf tissue selenium concentrations increased from 125.3 µg/kg in the control treatment to 823.5 µg/kg in response to the high selenium soil application, which was not significantly different from the foliar selenium application (784.8 µg/kg). Low selenium soil application (15 g/ha) produced much higher selenium concentrations in stem, leaf and ear than the control treatment and they were significantly different. The highest selenium concentration (546.3 µg/kg) in the ear occurred in response to the high selenium soil application. There was no significant difference between the low selenium soil application (240.3 µg/kg) and the high selenium foliar application (260.5 µg/kg). Ear selenium concentration was significantly higher in the low selenium soil application (15 g/ha) than the control treatment. Overall, the total plant selenium concentration at GS70 was higher in leaf compared to ear and stem. With respect to soil selenium application the second increment from 15 to 30 g/ha resulted in a greater response in tissue selenium concentration than the first increment from 0 to 15 g/ha.

Table 5.8 (a) *Effect of application method and rate on selenium concentration in stem, leaf and ear at GS70 in the Nafferton Farm field trial 2018.*

Se application method	Se rate (g/ha)	GS70		
		Stem (µg/kg)	**Leaf (µg/kg)**	**Ear (µg/kg)**
Control	0	58.3±9.30 d	125.3±10.48 c	51.0±9.10 c
Soil (Low)	15	147.5±12.63 c	341.5±33.05 b	240.3±21.78 b
Soil (High)	30	289.0±58.27 a	823.5±150.27 a	546.3±96.91 a
Foliar (High)	30	190.8±30.10 b	784.8±52.87 a	260.5±45.72 b
ANOVA *p*-value		**0.006**	**<0.001**	**<0.001**

Within columns, mean values followed by different letters are significantly different at p≤ 0.05 (Tukey's general linear hypothesis test). Values are the means +/- standard error (SE) of four replicates (n=4).

However, result in Table 5.8 (a) were found not to be normally distributed. Data in Table 5.8 (a) was therefore transformed logarithmically prior to ANOVA and is shown in Table 5.8 (b). After logarithmic transformation, selenium concentration in stem, leaf and ear were significantly affected by the method of selenium application and rate (Table 5.8b). In stem, the natural logarithm of selenium concentration (µg/kg) in the control treatment was 4.0, with increases following low selenium soil application (5.0 ln µg/kg), high selenium soil application (5.6 ln µg/kg) and high selenium foliar application (5.2 ln µg/kg). In leaf tissue, the natural logarithm of selenium concentration (µg/kg) in the control treatment was 4.8, with subsequent increases following low selenium soil application (5.8 ln µg/kg) high selenium soil application (6.7 ln µg/kg) and high selenium foliar application (6.7 ln µg/kg). In ear, the natural logarithm of selenium concentration (µg/kg) in the control treatment was 3.9, with subsequent increases following low selenium soil application (5.5 ln µg/kg) high selenium soil application (6.3 ln µg/kg) and high selenium foliar application (5.5 ln µg/kg). Table 5.8(b) showed that selenium accumulation in stem, leaf and ear exhibited rising trends according to the selenium rate applied following soil application as indicated in Table 5.8 (a) before logarithmic transformation. High soil and foliar application in Table 5.8 (b) were significantly different in stem and ear.

Table 5.8 (b) *Effect of application method and rate on selenium concentration in stem, leaf and ear at GS70 in the Nafferton Farm field trial 2018 (Transformed logarithmically*)

Se application method	**Se rate (g/ha)**	**GS70**					
		Stem (ln µg/kg)		**Leaf (ln µg/kg)**		**Ear (ln µg/kg)**	
Control	0	4.0±0.19	d	4.8±0.09	c	3.9±0.16	c
Soil (Low)	15	5.0±0.09	c	5.8±0.09	b	5.5±0.09	b
Soil (High)	30	5.6±0.21	a	6.7±0.19	a	6.3±0.19	a
Foliar (High)	30	5.2±0.15	b	6.7±0.07	a	5.5±0.17	b
ANOVA *p* value		**<0.001**		**<0.001**		**<0.001**	

Within columns, mean values followed by different letters are significantly different at p≤ 0.05 (Tukey's general linear hypothesis test). Values are the means +/- standard error (SE) of four replicates (n=4).

At maturity (GS95), selenium concentrations in stem, chaff and grain were also significantly affected by method and rate of selenium application (Table 5.9a). Selenium concentration in the stem ranged from 47.3 µg/kg for the control treatment to 353.3 µg/kg for the high selenium soil application (Table 5.9a). Selenium concentration was significantly higher in response to the soil application (353.3 µg/kg) than the foliar Se treatment (146.3 µg/kg). Grain showed the highest selenium concentrations of the plant tissues at maturity where again the soil application gave a significantly higher selenium concentration (907.5 µg/kg) than foliar application (389.8 µg/kg) at the high application rate of 30 g/ha. Similarly, for the residual chaff selenium concentration was also significantly higher in response to soil (346.5 µg/kg) than foliar application (140.5 µg/kg). As at GS70 for all plant tissues at maturity the increase in selenium concentration was greater in response to the 15 to 30 g/ha increment than from the 0 to 15 g/ha increment in selenium application. In general, selenium concentration in grain was higher than stem and chaff.

Table 5.9 (a) *Effect of method of selenium application and rate on selenium accumulation and distribution in stem, chaff and grain at final harvest in the Nafferton Farm field trial 2018*

Se application method	**Se rate (g/ha)**	**GS95**		
		Stem (µg/kg)	**Chaff (µg/kg)**	**Grain (µg/kg)**
Control	0	47.3±11.16 c	51.0±8.28 c	187.5±22.54 d
Soil (Low)	15	175.5±29.23 b	155.3±22.41 b	512.3±82.19 b
Soil (High)	30	353.3±88.57 a	346.5±66.94 a	907.5±129.57 a
Foliar (High)	30	146.3±26.16 b	140.5±19.76 b	389.8±91.62 c
ANOVA *p* value		**0.008**	**0.002**	**0.002**

Within columns, mean values followed by different letters are significantly different at p≤ 0.05 (Tukey's general linear hypothesis test). Values are the means +/- standard error (SE) of four replicates (n=4).

However, result in Table 5.9 (a) were found not to be normally distributed. Data in Table 5.9 (a) was therefore transformed logarithmically prior to ANOVA and is shown in Table 5.9 (b). After logarithmic transformation, selenium concentration in stem, chaff and grain were significantly affected by method of selenium application and rate (Table 5.9b). In stem, the natural logarithm of selenium concentration (µg/kg) in the control treatment was 3.8, with increases following low selenium soil application (5.1 ln µg/kg), high selenium soil application (5.8 ln µg/kg) and high selenium foliar application (4.9 ln µg/kg). In chaff, the natural logarithm of selenium concentration (µg/kg) in the control treatment was 3.9, with subsequent increases following low selenium soil application (5.0 ln µg/kg), high selenium soil application (5.8 ln µg/kg) and high selenium foliar application (4.9 ln µg/kg). In grain, the natural logarithm of selenium concentration (µg/kg) in the control treatment was 5.2, with subsequent increases following low selenium soil application (6.2 ln µg/kg), high selenium soil application (6.8 ln µg/kg) and high selenium foliar application (5.9 ln µg/kg). Table 5.9 (b) showed that selenium accumulation in stem, chaff and grain exhibited rising trends in response to selenium rate applied following soil application. High soil and foliar application in Table 5.9 (b) were significantly different in stem, chaff and grain.

Table 5.9 (b) *Effect of method of selenium application and rate on selenium accumulation and distribution in stem, chaff and grain at final harvest in the Nafferton Farm field trial 2018 (Transformed logarithmically*)

Se application method	**Se rate (g/ha)**	**GS95**					
		Stem (ln µg/kg)		**Chaff (ln µg/kg)**		**Grain (ln µg/kg)**	
Control	0	3.8±0.21	c	3.9±0.15	c	5.2±0.12	d
Soil (Low)	15	5.1±0.17	b	5.0±0.15	b	6.2±0.15	b
Soil (High)	30	5.8±0.23	a	5.8±0.21	a	6.8±0.16	a
Foliar (High)	30	4.9±0.23	b	4.9±0.16	b	5.9±0.24	c
ANOVA *p* value		**<0.001**		**<0.001**		**<0.001**	

Within columns, mean values followed by different letters are significantly different at p≤ 0.05 (Tukey's general linear hypothesis test). Values are the means +/- standard error (SE) of four replicates (n=4).

5.4.1.6 Grain yield, yield components, harvest index and total biomass

Selenium application had no significant effect on grain yield, yield components, harvest index and total biomass (Table 5.10).

Table 5.10 *Effects of selenium application method and rate on grain yield, yield components, harvest index and total biomass of spring wheat grown at Nafferton Farm in 2018.*

Se application method	Se rate (g/ha)	Grain yield (t/ha)	Ears (m^2)	Grain/ear	TGW (g)	HI (%)	Total biomass DM (t/ha)
Control	0	2.0±0.37	408±12.54	26.7±0.68	38.7±0.16	47.7±0.25	8.9±0.49
Soil (Low)	15	2.3±0.24	457±49.73	27.1±1.83	40.0±0.47	48.3±0.68	10.1±0.44
Soil (High)	30	1.9±0.23	440±29.89	26.2±1.54	39.5±0.72	46.9±1.16	9.7±0.95
Foliar (High)	30	1.8±0.40	438±34.74	26.7±1.98	39.5±0.26	47.2±0.86	9.9±1.40
ANOVA *p-value*		0.603	0.631	0.979	0.294	0.519	0.728

Values are the means +/- standard error (SE) of four replicates (n=4).

5.4.1.7 Grain quality

Selenium application had no significant effect on protein content, hectolitre weight and Hagberg falling number (Table 5.11) with very little variation between treatments.

Table 5.11 *Effects of selenium application method and rate on grain quality of spring wheat grown at Nafferton Farm in 2018.*

Se application method	Se rate (g/ha)	Protein content (%)	Hectolitre weight (kg/hl)	HFN (s)
Control	0	14.5±0.11	74.9±0.36	232±3.43
Soil (Low)	15	14.5±0.35	74.5±0.40	232±2.63
Soil (High)	30	14.8±0.53	74.7±0.09	225±7.11
Foliar (High)	30	14.4±0.33	74.3±0.41	224±10.96
ANOVA *p-value*		0.734	0.326	0.759

Values are the means +/- standard error (SE) of four replicates (n=4). HFN: Hagberg falling number

5.4.2 Glasshouse trial

5.4.2.1 Selenium plant tissue concentration

Selenium concentrations in stem and leaf sampled at GS59 were similar and increased significantly with selenium application rate (Table 5.12a). In stem, selenium concentration increased from 35.4 µg/kg in the control treatment to 367.2 µg/kg at 15 g/ha and to 748.0 µg/kg in response to 30 g/ha. In leaf tissue, selenium concentration ranged from 64.5 µg/kg in the control to 797.1 µg/kg at the high selenium rate.

Table 5.12 (a) *Total selenium concentration in stem and leaf at GS59 in response to Se application in the glasshouse trial at Cockle Park Farm.*

Se application method	Se rate (g/ha)	GS59	
		Stem (µg/kg)	Leaf (µg/kg)
Soil application	0	35.4±4.62 c	64.5±3.92 c
	15	367.2±58.80 b	361.6±47.20 b
	30	748.0±157.23 a	797.1±144.46 a
ANOVA *p* value		**0.001**	**0.001**

Within columns, mean values followed by different letters are significantly different at p≤ 0.05 (Tukey's general linear hypothesis test). Values are the means +/- standard error (SE) of five replicates (n=5).

However, result in Table 5.12 (a) were found not to be normally distributed. Data in Table 5.12 (a) was therefore transformed logarithmically prior to ANOVA and is shown in Table 5.12 (b). After logarithmic transformation, selenium concentration in stem and leaf were significantly affected by method of selenium application and rate (Table 5.12b). In stem, the natural logarithm of selenium concentration (µg/kg) in the control treatment was 3.5, with increases following low selenium soil application (5.8 ln µg/kg) and high selenium soil application (6.5 ln µg/kg). In leaf, the natural logarithm of selenium concentration (µg/kg) in the control treatment was 4.2, with subsequent increases following low selenium soil application (5.9 ln µg/kg) and high selenium soil application (6.6 ln µg/kg). It was observed that selenium accumulation in stem and leaf (Table 5.12 (b) showed increasing trends in response to selenium rate applied following soil application which also indicated selenium concentration in stem and leaf were significantly higher under high soil application than low soil application and the control treatment.

Table 5.12 (b) *Total selenium concentration in stem and leaf at GS59 in response to Se application in the glasshouse trial at Cockle Park Farm (Transformed logarithmically*)

Method application	Se rates (g/ha)	GS59			
		Stem (ln µg/kg)		Leaf (ln µg/kg)	
Soil application	0	3.5±0.16	c	4.2±0.09	c
	15	5.8±0.32	b	5.9±0.21	b
	30	6.5±0.50	a	6.6±0.37	a
ANOVA *p*-value		**0.001**		**0.001**	

Within columns, mean values followed by different letters are significantly different at p≤ 0.05 (Tukey's general linear hypothesis test). Values are the means +/- standard error (SE) of five replicates (n=5).

At GS72, selenium accumulation in stem, leaf and ear significantly increased with selenium rate (Table 5.13a). The response to selenium application was highest in the ear and lowest in the leaf. Selenium application increased the stem selenium concentration from 38.7 µg/kg in the control to 497.2 µg/kg at 15g Se/ha with and further increase to 684.8 µg/kg in response to 30g/ha. The response to selenium application was similar in the leaf and ear with significant increases in response to the first 15 g/ha of selenium. The stem and ear showed a significant increase in selenium concentration in response to the increase from 15 to 30 g Se/ha, whereas for the ear the increase was large, from 573.9 to 956.5 µg/kg.

Table 5.13 (a) *Selenium concentration in stem, leaf and ear at GS72 in response to Se application in the glasshouse trial at Cockle Park Farm.*

Se application method	Se rate (g/ha)	GS72					
		Stem (µg/kg)		Leaf (µg/kg)		Ear (µg/kg)	
Soil application	0	38.7±5.56	c	85.6±10.55	b	35.2±4.68	c
	15	497.2±39.66	b	414.9±48.34	a	573.9±59.96	b
	30	684.8±100.96	a	522.3±72.34	a	956.5±143.84	a
ANOVA *p*-value		**0.000**		**0.002**		**0.000**	

Within columns, mean values followed by different letters are significantly different at p≤ 0.05 (Tukey's general linear hypothesis test). Values are the means +/- standard error (SE) of five replicates (n=5).

However, results in Table 5.13 (a) were found not to be normally distributed. Data in Table 5.13 (a) were therefore transformed logarithmically prior to ANOVA, as shown in Table 5.13 (b). After logarithmic transformation, selenium concentration in stem, leaf and ear were significantly affected by selenium application rate (Table 5.13b). In stem, the natural logarithm of selenium concentration (µg/kg) in the control treatment was 3.7, with increases following low selenium soil application (6.2 ln µg/kg) and high selenium soil application (6.5 ln µg/kg). In leaf, the natural logarithm of selenium concentration (µg/kg) in the control treatment was 4.4, with increases following low selenium soil application (6.0 ln µg/kg) and high selenium soil application (6.2 ln µg/kg). In ear, the natural logarithm of selenium concentration (µg/kg) in the control treatment was 3.5, with subsequent increases following low selenium soil application (6.3 ln µg/kg) and high selenium soil application (6.8 ln µg/kg). Table 5.13 (b) showed that selenium accumulation in stem, leaf and ear exhibited rising trends in response to selenium rate applied following soil application. Low and high selenium soil application in Table 5.13 (b) were significantly different in ears.

Table 5.13 (b) *Selenium concentration in stem, leaf and ear at GS72 in response to Se application in the glasshouse trial at Cockle Park Farm (Transformed logarithmically*)

Se application method	**Se rates (g/ha)**	**GS72**		
		Stem (ln µg/kg)	**Leaf (ln µg/kg)**	**Ear (ln µg/kg)**
Soil application	0	3.7±0.20 b	4.4±0.12 b	3.5±0.20 c
	15	6.2±0.08 a	6.0±0.12 a	6.3±0.11 b
	30	6.5±0.17 a	6.2±0.16 a	6.8±0.27 a
ANOVA *p*-value		**<0.001**	**<0.001**	**<0.001**

Within columns, mean values followed by different letters are significantly different at p≤ 0.05 (Tukey's general linear hypothesis test). Values are the means +/- standard error (SE) of five replicates (n=5).

At maturity (GS95), selenium concentration of all plant fractions (stem, chaff and grain) was significantly increased by selenium application (Table 5.14a). In the absence of applied selenium, selenium concentration in the stem fraction was 56.2 µg/kg, but in response to selenium application the highest selenium concentrations (1043.2 µg/kg) was found at 30 g Se/ha of selenium rate**.** In treated plants, selenium concentrations were higher in grain than stem and chaff. At maturity, the response in terms of selenium concentration for all plant tissues was greatest to the first selenium increment, i.e. from 0 to 15 g/ha, but in all cases the second increment resulted in a significant increase in concentration. Chaff had much lower selenium concentrations than stem and grain but the responses to selenium application were similar. In grain, selenium concentration was increased from 33.2 µg/kg in the control treatment to 1374.6 µg/kg in response to 30 g Se/ha.

Table 5.14 (a) *Selenium concentration in stem, chaff and grain at maturity in response to Se application in the glasshouse trial at Cockle Park Farm.*

		GS95		
Se application method	**Se rate (g/ha)**	**Stem (µg/kg)**	**Chaff (µg/kg)**	**Grain (µg/kg)**
Soil application	0	56.2±11.77 c	24.1±1.17 c	33.2±4.33 c
	15	636.4±33.55 b	406.9±47.17 b	860.2±86.21 b
	30	1043.2±63.72 a	520.8±25.39 a	1374.6±75.20 a
ANOVA *p*-value		**<0.001**	**<0.001**	**<0.001**

Within columns, mean values followed by different letters are significantly different at p≤ 0.05 (Tukey's general linear hypothesis test). Values are the means +/- standard error (SE) of five replicates (n=5).

However, result in Table 5.14 (a) were not normally distributed. Data in Table 5.14 (a) was transformed logarithmically prior to ANOVA and is shown in Table 5.14 (b). After logarithmic transformation, selenium concentration in stem, chaff and grain were significantly affected by method of selenium application and rate (Table 5.14b). In stem, the natural logarithm of selenium concentration (µg/kg) in the control treatment was 3.8, with increases following low selenium soil application (6.4 ln µg/kg) and high selenium soil application (6.9 ln µg/kg). In chaff, the natural logarithm of selenium concentration (µg/kg) in the control treatment was 3.1, with subsequent increases following low selenium soil application (5.9 ln µg/kg) and high selenium soil application (6.2 ln µg/kg). In grain, the natural logarithm of selenium concentration (µg/kg) in the control treatment was 3.6, with subsequent increases following low selenium soil application (6.7 ln µg/kg) and high selenium soil application (7.2 ln µg/kg). It was observed that selenium accumulation in stem, chaff and grain (Table 5.14 b) showed increasing trends in response to selenium rate following soil application as well as Table 5.14 (a). Both low and high selenium soil application in Table 5.14 (b) were significantly different in stem, chaff and grain.

Table 5.14 (b) *Selenium concentration in stem, chaff and grain at maturity in response to Se application in the glasshouse trial at Cockle Park Farm (Transformed logarithmically*)

Se application method	**Se rates (g/ha)**	**GS95**		
		Stem (ln µg/kg)	**Chaff (ln µg/kg)**	**Grain (ln µg/kg)**
Soil application	0	3.8±0.05 c	3.1±0.04 c	3.6±0.12 c
	15	6.4±0.07 b	5.9±0.20 b	6.7±0.16 b
	30	6.9±0.05 a	6.2±0.09 a	7.2±0.09 a
ANOVA *p*-value		**<0.001**	**<0.001**	**<0.001**

Within columns, mean values followed by different letters are significantly different at p≤ 0.05 (Tukey's general linear hypothesis test). Values are the means +/- standard error (SE) of five replicates (n=5).

5.4.2.2 Grain yield, yield components and protein content

Grain yield increased in response to selenium application from 4.8 g/pot in the control treatment to 5.8 g/pot in response to 30 g/ha of soil applied selenium, but this was not significant (Table 5.15). There were corresponding increases in grains/ear, TGW, HI and total biomass in response to selenium application but again they were not significant except for TGW. Protein content was high but not significantly influenced by selenium application.

Table 5.15 *Effects of soil selenium application on wheat grain yield, yield components, HI, total biomass and protein content in the glasshouse trial at Cockle Park Farm.*

Se application method	Se rate (g/ha)	Grain yield (g/pot)	Ears/plant	Grains/ear	TGW (g)	HI (%)	Total biomass (g/pot)	Protein content (%)
Soil application	0	4.8±0.40	1.0±0.00	11.6±1.00	31.8±0.37 b	40.3±2.61	11.0±0.70	17.4±0.70
	15	5.0±0.16	1.0±0.00	12.0±0.24	32.3±0.79 b	41.4±0.56	11.7±0.33	17.3±0.33
	30	5.8±0.69	1.0±0.00	13.1±1.46	34.4±0.68 a	44.7±1.11	12.5±1.18	17.6±1.17
ANOVA *p*-value		0.298	1.000	0.454	**0.033**	0.216	0.370	0.781

Within columns, mean values followed by different letters are significantly different at p≤ 0.05 (Tukey's general linear hypothesis test). Values are the means +/- standard error (SE) of five replicates (n=5).

5.5 Discussion

5.5.1 Grain selenium concentrations

Wheat is one of the main dietary sources of selenium (Sharma *et al.*, 2017). As a significant staple crop in the world, wheat is considered as the best selenium accumulator among cereal crops (Lyons *et al*., 2003; Sharma *et al.*, 2017). The implementation of selenium supplementation using wheat has considerable promise for improving human selenium intake (Zhao *et al*., 2007). The implementation of selenium supplementation or selenium biofortification has been shown in several studies with significant effects on grain selenium concentration (Stroud *et al.*, 2010a; Galinha, 2014; Ning *et al.*, 2016).

In the current study, selenium supplementation in field and glasshouse trials had significant effects on wheat grain selenium concentration. From these results it can be observed that in both trials grain selenium concentration of wheat increased with selenium soil application rate (15 g/ha and 30 g/ha) and foliar application (30 g/ha) when compared to the control treatment without selenium application**.** These results are consistent with the study by Jiang *et al.* (2015), who discovered both soil and foliar application of selenium significantly increased Se accumulation in plant parts of common buckwheat. The authors also emphasised that selenium application rate was closely associated with selenium accumulation. Likewise, Lyons *et al.* (2004) carried out two field trials in South Australia and recorded a gradual increment of wheat grain selenium concentration with soil and foliar selenium application. Furthermore, current findings are consistent with those of by Grant *et al*. (2007), who observed that selenium concentration of durum wheat grain increased steadily with selenium rate, from a low of 195 µg/kg in the control treatment to a high of 1820 µg/kg in the selenium treatment of 40 g Se/ha in three years' field studies in Manitoba Canada.

With regards to the magnitude effects of selenium rates 30 g Se/ha applied at stem elongation (GS31) in the field trial significantly improved grain selenium concentration by 4.8-fold (soil application) and 2.1-fold (foliar application) compared to the control treatment, while in the glasshouse trial soil application significantly increased grain selenium concentration 41.4-fold compared with the control. The increase of grain selenium concentration was higher in the glasshouse than the field trial**.** In other work, a study by Zhao *et al*. (2007) in the UK employed selenium supplementation at stem extension stage of wheat with rates of 10-20 g Se/ha as selenate

and found that grain selenium concentration increased by 3 to 27-fold. Sharma *et al.* (2017) suggested that grain selenium concentrations are likely to increase by 10-fold from low ambient levels by employing selenium fertiliser at 10 g Se/ha. Lyons *et al*. (2003) found that selenium supplementation of wheat at 10 g Se/ha boosted grain selenium concentration to 200-500 µg/kg from a base level at 30-100 µg/kg. In the following year, Lyons *et al.* (2004) conducted a selenium biofortification study at Charlick, South of Adelaide with selenate fertiliser at selenium rate of 10 g/ha to the soil and improved grain selenium concentration more than four-fold (50 to 210 µg/kg). According to a regression model, Poblaciones *et al.* (2014a) concluded selenium accumulation in bread-making wheat grain could possibly achieve ~798 mg/kg in years with high rainfall and 363 mg/kg in dry years from an application of 10 g/ha using sodium selenate fertiliser. At a higher selenium rate, Curtin, *et al.* (2006) reported that 20 g Se/ha applied at the jointing stage can increase grain selenium concentration in wheat about 7-fold (to 0.39 mg/kg) compared to the control treatment (0.051 mg/kg). A maximum grain selenium concentration of 0.35 mg/kg was achieved using 25 g Se/ha as highlighted by Reis *et al.* (2018). Rodrigo *et al.* (2013) showed in an agronomic selenium biofortification study with barley that the application of 40 g Se/ha as selenite and selenate in the 2010/2011 growing season increased grain selenium concentration from 69 µg/kg to 520–2336 µg/kg and in 2011/2012 the increase was from 60 µg/kg to 316–1347 µg/kg respectively.

Numerous studies on selenium supplementation have resulted in different magnitude effects on grain selenium concentrations which basically depend on supplementation method and rate applied, selenium form and timing of applications. According to Lyons *et al*. (2003), the relative effectiveness of soil and foliar application of selenium is generally determined by several factors including selenium form and timing of application. In the current study selenium soil application had a greater effect on grain selenium concentration than foliar application in the field. Boldrin *et al.* (2013) also found that soil application of selenate produced greater selenium concentrations in wheat grain (about 450%) than foliar application. Furthermore, the author suggested that the greater response to soil application was related to the longer plant-Se contact time. In addition the authors suspected that with foliar application was at the flowering stage there was limited time for selenium transfer from leaves to the grain via the phloem to increase selenium in the grain. Wu *et al.* (2015) noticed that soil selenium fertiliser can be effective when soil conditions are uniform. Meanwhile, Lyons *et al.* (2004) found

that grain selenium concentration was higher following soil selenate application at seeding (20-133-fold) than following post-flowering foliar selenate application (6-20-fold) to a control plot in a field study conducted on two soil types in South Australia. De Lima Lessa *et al.* (2019) indicated that soil selenium application rates of 47 g/ha and 36 g/ha as sodium selenate effectively supplied rice grain with adequate selenium content.

Nevertheless, many selenium biofortification studies in the literature have shown foliar application to be more reliable and effective at increasing grain selenium concentration than soil application (Broadley *et al.*, 2010; Wang *et al.*, 2013; Poblaciones *et al.*, 2014a; Ducsay *et al*., 2016; Ning *et al.*, 2016). Foliar application of selenium has been shown to be a more effective method than soil application to enhance the selenium concentration of plants (Chu *et al*., 2013; Ros *et al*., 2016; El-Ramady *et al*., 2016; Deng *et al*., 2017) due to the limited mobility of selenium in plants (Ducsay and Ložek, 2006). A recent meta-analysis conducted by Ros *et al.* (2016) identified selenate at a rate of 30-60 g/ha for soil application and 4.5-10 g/ha for foliar application to increase selenium content in grain from 7 to 100 µg/kg. Mao *et al.* (2014) conducted a study on the effect of micronutrient biofortification in the edible parts of various crops such as cabbage, potato, maize, canola, soybean and winter wheat and reported that foliar selenium supplementation enhanced grain selenium concentration to 312 µg/kg at a rate of 60 g Se/ha and calculated that one gram of selenium could increase wheat grain concentration by 4.6 µg/kg. Based on this, foliar application is about eight times more efficient than soil application at increasing grain selenium concentration (Ros *et al*., 2016). Limited mobility of selenium in the phloem is one of the key reasons why selenium concentration is greater in wheat grain following foliar application (Boldrin *et al*., 2013).

In the case of selenium form, selenate was found to be about 33 times more effective than selenite in a meta-analysis review by Ros *et al.* (2016). For each gram of selenium supplementation as selenate, Chilimba *et al.* (2012) reported that grain selenium concentration was increased by 15-21 µg/kg when employed through a high-volume drench to maize under field conditions. In barley, Rodrigo *et al.* (2013) estimated that each g/ha of selenium in the form of selenate and selenite increased grain selenium accumulation by 55 and 33 µg/kg and by 10 and 6 µg/kg in 2010/2011 and 2011/2012 respectively. Mao *et al.* (2014) calculated that by using 1 g/ha of selenate, selenium concentration increased by 17.4 µg/kg (wheat grain), 8.6 µg/kg (maize grain),

17.3 µg/kg (soybean seed), 10.2 µg/kg (potato tuber), 4.1 µg/kg (canola seed) and 76.8 µg/kg (cabbage leaf). Lyons et al. (2005c) found wheat grain selenium concentration in both field and glasshouse trials were progressively increased up to 133-fold by using selenate application to the soil at seeding and up to 20-fold from foliar application after flowering at rates of 4-120 g/ha.

The low wheat grain selenium concentration in the UK is well understood to be associated with low soil selenium availability and has been confirmed in many selenium biofortification studies (Zhao *et al.*, 2007). The soil analysis results presented here which were not tested statistically have showed that selenium soil concentration at Nafferton Farm for 0-30 cm and 30-60 cm of soil depths was from 0.35 mg/kg to 0.31 mg/kg, which clearly confirms low Se bioavailability. These results are consistent with a study by Stroud *et al*. (2010a) who found the soil total selenium concentration from 10 field sites in the UK were between 0.2 and 0.6 mg/kg in the 0-30 cm layer (topsoil), which is considered generally low. According to Govasmark *et al.* (2008), soil selenium concentration <0.6 mg/kg in Scandinavian soils is classified as low content which makes it difficult to achieve the target concentration of selenium in food without the use of selenium fertilisers. Grain selenium concentrations for barley and wheat cultivated in Scandinavian soils ranged from 11 to 34 µg/kg (Govasmark *et al.*, 2008). More than 95 % of UK soils had soil selenium availability <1.0 mg/kg identified by the British Geological Survey (Broadley *et al.*, 2006). As described by Hawkesford and Zhao (2007), soil selenium concentration <150 µg Se/kg is classified as deficient-marginal. A study by Rodrigo *et al.* (2013) regarding agronomic selenium biofortification with two-row barley in Spain recorded total soil selenium without selenium application of 123.8 ± 15.4 µg/kg (2010/2011) and 134.4 ± 16.3 µg/kg (2011/12), which is therefore classified as deficient-marginal in total selenium. In Portugal, Galinha *et al.*, (2012) reported soil selenium concentration recorded under field conditions of about 0.1 mg/kg (118 ± 6 µg/kg) which is also considered as deficient-marginal in selenium. Selenium accumulation in grain and its distribution within the plant parts of wheat is also determined by selenium bioavailability in soil. According to Stroud *et al.* (2010a), soil selenium bioavailability is a main driving factor influencing selenium concentration in wheat grain. This is verified by Poblaciones *et al.* (2014b), who acknowledged soil selenium concentration as a major factor determining selenium uptake by plants and grain selenium accumulation.

Results obtained showed a large difference between selenium concentration in wheat grain of 187 µg/kg in the present field trial and 33 µg/kg in the glasshouse trial without Se fertiliser. Selenium concentration in wheat grain samples without selenium addition was reported at 59 ± 10 µg/kg by Galinha *et al.* (2012) and 35 µg/kg to 65 µg/kg by Poblaciones *et al.* (2014a), who observed lower grain selenium concentrations in barley and durum wheat (*Triticum durum* L.) from the same area of investigation, whilst Stroud *et al.* (2010a) found wheat grain selenium concentrations ranging between 15.5 and 43.8 µg/kg without selenium fertiliser. Previous studies have shown that most wheat grown in the UK contains grain selenium concentration <50 µg Se/kg, which is considered as inadequate for human requirements (Adams *et al.*, 2002; Zhao *et al.*, 2007; Stroud *et al.*, 2010a).

The higher grain selenium concentration found in the present field control plot would be linked to a low dilution effect from low yield. The link between grain selenium concentration and grain yield, i.e. the dilution effect, has been discussed in several studies in the literature such as Lyons *et al*. (2004), Poblaciones *et al.*(2014a), Ducsay *et al*. (2016), Nawaz *et al*. (2017) and Manojlović *et al*. (2019). While a study conducted by Lyons *et al.* (2004) in 2001-2002 at the Charlick experimental farm near Strathalbyn, south of Adelaide, Australia observed that grain yield of wheat in 2002 was half that in 2001 for two commercial bread wheats, Krichauff (1.83 t/ha and 3.66 t/ha) and Kukri (1.71 t/ha and 3.42 t/ha), with the grain selenium content reported as similar in both years (61 µg/kg in 2001 and 63 µg/kg in 2002). In this case, the authors concluded that there was no dilution effect detected due to higher yield in 2001. In other work, Lee *et al*. (2011) noted that plant nutrient concentrations generally decline as crop yield improves, but did not observe a dilution effect of reduced grain selenium concentration with increased grain yield from all tested winter wheat and spring wheat varieties in the study.

5.5.2 Selenium accumulation and distribution

Several studies have reported that soil and foliar application methods of selenium fertilisers with increasing rate of supplementation significantly affected selenium accumulation in wheat grain (Curtin *et al.*, 2006), improved Se uptake (Jiang *et al*., 2015) and translocation of selenium within crops (Jiang *et al*., 2015; Nawaz *et al*., 2017). A pot study conducted between 2000 to 2001 in Nitra, Slovakia by Ducsay *et al*. (2009)

showed increasing selenium rate significantly boosted selenium concentration of wheat in grain, straw and roots. Meanwhile, Cartes *et al.* (2011) verified translocation of selenium from root to shoots was determined by selenium rate. Also, Kahakachchi *et al.* (2004) indicated the accumulation of selenium in plants is largely active in developing tissues. With respect to the current results, accumulation of selenium in stem, leaf, ear, chaff and grain of spring wheat observed at GS55, GS70 and GS95 was also significantly increased by selenium rate.

Govasmark *et al.* (2008) reviewed earlier studies in the literature and noticed that supplementation with selenate boosted selenium concentration in grain, leaf, straw and ears. In addition to that, the authors recommended selenate as a good source to improve selenium concentration in plants. Similarly, Ali *et al.* (2017) conducted a study on selenium application to soil by using selenate and selenite and concluded that selenium concentration in root, straw, leaves, seed and glume of wheat was significantly improved with enhanced selenate and selenate in soil compared to the control treatment without selenium addition.

In potato, Turakainen (2007) demonstrated selenium soil supplementation increased selenium concentration in roots, upper leaves, stolons and tubers in a greenhouse experiment. Sun *et al.* (2010) suggested that determinating selenium concentration and distribution within plant tissues is vital to understand and improve the efficiency of selenium biofortification of crops. From the current study, the distribution pattern of selenium showed that at GS55 and GS70, a higher selenium concentration was observed in leaves of spring wheat than other plant parts. This finding concurs with results reported by Eiche *et al.* (2015), who studied selenium distribution in wheat and Indian mustard in a seleniferous area of Punjab India and detected greater selenium enrichment in upper plant parts. The authors showed selenium content in wheat stem and root was similar (191 mg/kg and 196 mg/kg) but concentration in leaves (387 mg/kg) was higher. In this case, the authors linked the higher selenium concentration in leaves with the high uptake and mobility of selenate inside plants. A study conducted by Ríos *et al.* (2008) on selenate and selenite suggested that selenate is more available in the soil and hence readily taken up by plant roots and translocated to the shoot tissues. Selenium in the form of selenate is transferred efficiently from plant roots to other plant organs in winter wheat, maize, soybean, potato, canola, and cabbage after selenate was applied to the soil at planting (Mao *et al*., 2014). In rice, Sun *et al.* (2010) observed a difference of selenium concentration in different plant parts ranked as

straw>bran>whole grain>polished rice>husk. As highlighted by Keskinen *et al*. (2010), several publications have reported the distribution and transport of selenium in the plant as greatly influenced by plant species, type of selenium absorbed from the soil and the development stage of the plant. In addition, Premarathna *et al.* (2012) stated that differences of selenium concentration in rice plants are driven by time and method of application, selenium species and also soil moisture levels. Keskinen *et al*. (2010) observed that selenium concentration in wheat roots, leaves and stems decreased as plants grew possibly due to dilution effect or increased selenium translocation to the grain. Added to that, the authors showed that the largest concentration of selenium was found in the young leaves and grain at harvest while the lowest selenium concentrations were in the roots and stems of mature plants. Premarathna *et al.* (2012) ranked the selenium concentration in rice plants in the following order; grains>leaves>culms and husks which is consistence with the results of the current study on spring wheat. Sun *et al.* (2010) noticed that selenium accumulation in rice grain which used the phloem was higher than husk, which may be linked to transport from the husk via the xylem. The author also further remarked that selenium compounds accumulated in the husk were subsequently transferred into rice grains due to the hydrostatic pressure differences which occur in the phloem, which are combined with proteins or starch granules in rice grains throughout grain development.

5.5.3 Grain yield

This study investigated the effect of soil and foliar applications of selenium fertiliser and rates on yield, yield components, grain quality, harvest index and total biomass of wheat under both field and glasshouse conditions in 2018. The grain yields in field trial were very low, between 1.8 t/ha to 2.3 t/ha, and much lower than the average of 6 t/ha generally achieved for spring wheat in the UK as reported by DEFRA (2019). This report also highlighted that weather conditions such as rainfall and temperature in 2018 with high rainfall in the spring and high temperatures in the summer with a long dry spell affected the 2018 harvest, which caused differing yields throughout region of the UK. A very low grain yields in the field trial 2018 is likely due to the very low rainfall received during grain development and growth (May-July), which was 94.8 mm which is 46% of the long-term average for the region. As one of the most important factors influencing crop yield, low rainfall was also reported by Poblaciones *et al.*

(2014a) who observed that severe reduction in rainfall during flowering and grain development may resulted in low grain yield of wheat. Also, our observations are in accordance with a study conducted by Lyons *et al.* (2004) in South Australia, who also linked low yield of wheat to low rainfall.

Current results also show that method and rate of selenium application had no significantly effect on the growth and yield of spring wheat in both trials. These results are consistent with the findings reported in many previous studies. For instance, Sharma *et al.* (2017) reported that grain yield of cereals was not affected by soil or foliar selenium application. Bañuelos *et al*. (2017) noticed that selenium application up to 100 g/ha did not affect grain yield of field-grown wheat. Likewise, Lyons *et al.* (2005b) showed no effect on yield of wheat following selenium application up to 120 g Se/ha using sodium selenate under field conditions. Several trials conducted by Grant *et al*. (2007), Poblaciones *et al.* (2014a, b) and Reis *et al.* (2018) consistently confirmed selenium fertilisation had no significant effect on grain yield of wheat. Ning *et al.* (2016) concluded that there were no significant effects on grain yield in foxtail millet with selenium application compared to an unfertilised control treatment. Selenium application at blooming-filling stage in wheat resulted in a non-significant effect on yield as found by Chu *et al.*, (2013). Selenium application did not affect yield of other crops such as rapeseed (Seppänen *et al*., 2010), maize (Chilimba *et al.*, 2012; Wang *et al.*, 2013), rice (De Lima Lessa *et al*., 2019) and soybean (Yang *et al.*, 2003).

In contrast, several studies in the literature have reported that selenium application improved grain yield. As observed by Idrees *et al.* (2018) from the findings of field experiments in Faisalabad, Pakistan, the grain yield of wheat was significantly improved via soil and foliar application of selenium at rates of 100 g/ha and 50 g/ha compared to the control treatment. Improved grain yield from selenium application has also been reported in other crops such as maize (Wang *et al*., 2012), rice (Zhang *et al*., 2014), citrus and garlic (Zahedi *et al*., 2019). Selenium application at regreening-jointing, jointing-heading, and heading-blooming stages significantly increased grain yield of winter wheat as reported by Ducsay *et al.* (2016). However, Reis *et al.* (2018) pointed out that the relationship between selenium and grain formation is low but is often linked with an interaction between Se and N.

Increases in grain yield are linked with increases in yield components such as the number of grains per ear, grain weight per ear and thousand grain weight (Żuk-Gołaszewska *et al.*, 2016). In the current field trial (soil and foliar selenium application)

and glasshouse studies (soil selenium application only) small but non-significant increases in yield components were observed, except that 30 g/ha Se significantly increased thousand grain weight in the glasshouse. In contrast Poblaciones *et al.* (2014a) found selenium application reduced thousand grain weight at the higher application rate (40 g/ha) in comparison with the control treatment.

With respect to harvest index and total biomass, the current study indicated no significantly effect of selenium application on harvest index in the field and glasshouse trial. Broadley *et al.* (2010) reported that the harvest index was unaffected with selenium fertiliser application at a rate of 100 g/ha in a field trial. Sharma *et al.*, (2017) also observed that selenium application did not influence the harvest index. In other studies, using garlic, no significant effects of selenium application on the harvest index were shown (Põldma *et al.*, 2011). Not only the harvest index, but also total biomass production in the present field and glasshouse trial was not affected by soil or foliar selenium application. Mao *et al.* (2014) investigated the effect of foliar application of sodium selenite at rates up to 60 g/ha to various crops including winter wheat and reported that biomass was unaffected by selenium application. Additionally, Ning *et al.* (2016) reviewed published studies from Longchamp *et al.* (2013), Pezzarossa *et al.* (2014) and Cartes *et al.* (2011) and acknowledged that there was no significant effect of selenium application on total biomass in crops such as maize, tomato and ryegrass. In a hydroponic study, biomass production of maize seedlings was not affected by the application of selenium either in the form of selenate or selenite (Longchamp *et al.*, 2013). However, Guerrero *et al.* (2014) noticed that selenium application under high selenium concentration reduced biomass production.

Chlorophyll content measured at GS39 and GS62 (Table 5.5) and presented as SPAD values was clearly not significantly influenced by selenium application in the field. A similar finding was reported by Reis *et al.* (2018), who suggested that chlorophyll content was not influenced by selenium application. A field trial assessing the influence of adding sodium selenate to the soil at rates of 12, 21, 38, 68 and 120 g/ha (De Lima Lessa *et al.*, 2019) showed a non-significant result of soil selenium application on SPAD.

5.5.4 Grain quality

Soil and foliar selenium application in the field and glasshouse trials in the present study had no significant effect on gain quality parameters (protein, HFN and specific weight), which is consistent with studies reported in the literature. De Vita *et al.* (2017) observed that selenium fertilisation under field conditions had no effect on grain quality. Poblaciones *et al.*, (2014a), showed that grain protein content in durum wheat was not affected by increasing selenate and selenite following foliar application. In a selenium biofortification study under Mediterranean conditions, Poblaciones *et al.* (2014b) suggested that selenium biofortification had a slight negative influence on grain protein content of bread-making wheat. Meanwhile, Lyons *et al.* (2004) reported selenium application ranging from 4-120 g/ha had no effect on protein content. In the current study, a higher but non-significant grain protein content was observed in both field and glasshouse trials in response to selenium fertilisation. The higher protein contents in wheat grain observed by Rodrigo *et al*. (2014) and Poblaciones *et al.* (2014b) were linked to dilution effects and the lower grain yield. Lyons *et al.* (2004) noticed a positive relationship between selenium level and protein in UK bread wheat, whereby grain selenium content was lower in soft wheat (0.02-0.13 mg/kg) than hard wheats (0.05-1.09 mg/kg) and associated this with the lower protein content of soft wheats.

Hectolitre weight was not significantly influenced by both soil and foliar selenium application in the current field trial. Poblaciones *et al.* (2014b) showed that selenium foliar application with increasing selenate and selenite did not affect hectolitre weight of durum wheat. However, Poblaciones *et al.* (2014a) in another selenium biofortification study using bread wheat noticed that hectolitre weight was significantly higher at a selenium application rate of 40 g/ha compared to the control. Ducsay *et al*. (2016) reported selenium application with selenate or selenite at rates of 10 g Se/ha and 20 g Se/ha using foliar application resulted in non-significant effects on falling number of winter wheat grain. However, to the best of my knowledge studies on hectolitre weight and falling number as affected by selenium application are very limited in the literature.

5.6 Conclusions

Overall, application of selenium fertiliser in the field and greenhouse trials had no effect on grain yield, yield components, growth, or grain quality. Selenium concentrations in plant tissue were higher in leaf than stem at GS55 in both field and glasshouse trials. The distribution pattern at maturity showed significantly higher selenium concentrations in the grain than in the stem and chaff, which suggests a preferential transport of selenium to the grain during grain filling. Regarding nutritional aspects, results from the present study clearly show that agronomic biofortification of UK-grown wheat is achievable, as the selenium concentration of grain was significantly improved following both soil and foliar application. Finally, this study is based on a single year trial in 2018, albeit under both field and glasshouse conditions where the results are very consistent under the different growing conditions. It was originally scheduled to run the field trial over two growing seasons (2017 and 2018), but in effect the whole data set from 2017 was unusable due to very poor germination and crop establishment in the difficult spring of 2017 because of “Beast from the East” and substantial rabbit and hare damage later in the season during ear emergence and grain filling. The data collected in the 2017 trial are unusable and not presented in this thesis because of the very low yield and poor crop performance. A glasshouse trial was therefore conducted in 2018 due to the lack of reliable data from 2017.

Chapter 6 : The effects of nitrogen fertilisation and crop protection on the yield, quality and grain selenium concentration of wheat and spelt

6.1 Introduction

Nitrogen fertiliser, crop protection (Kohajdová and Karovicova, 2008; Shi *et al*., 2010; Kraska *et al.*, 2012; Vrček *et al*., 2014; Zhang *et al*., 2016; Kuppusamy *et al*., 2018) and genetic variation (Mason *et al.*, 2007; Ceseviciene *et al.*, 2012; Shewry, 2018) have significantly increased wheat grain yield and caused changes in quality. In the case of N fertilisation, several studies have primarily focused on concentrations of micronutrients such as Fe and Zn (Shi *et al*., 2010; Svečnjak *et al*., 2013; Kuppusamy *et al*., 2018), but with far fewer studies conducted on selenium. Furthermore, Vrček *et al*. (2014) mentioned that comparative studies and discussing the influence of crop protection (organic vs conventional) on wheat grain yield, quality parameters and mineral concentrations in the literature have shown inconsistent conclusions.

Interestingly, differences in yield parameters and chemical composition of wheat grain also have been shown through genetic variation (Hlisnikovský *et al*., 2019). Recently, interest in ancient wheat species such as einkorn (*Triticum monococcum* L.), emmer (*T. dicoccum* L.) and spelt (*Triticum spelta* L.) has increased (Kohajdová and Karovicova, 2008; Gomez-Becerra *et al.*, 2010). Earlier studies have reported that ancient wheat species provide a significantly higher protein content and mineral concentrations (N, P, Mg, Cu, Fe, Mn and Zn) than modern bread wheat (Bálint *et al*., 2001; Bojňanská & Frančáková, 2002; Kraska *et al*., 2012; Hlisnikovský *et al*., 2019). Lachman *et al.*, (2011) and Zhao *et al.*, (2009) also highlighted that spelt, einkorn and emer varieties contain high selenium concentrations in the grain. Ancient wheat species production has declined (Hlisnikovský *et al*., 2019) due to the introduction of modern cultivars of free-threshing wheats in the 20th century which significantly improved grain yield (Winterová *et al*., 2016). However, investigations on the genetic variation in grain selenium concentration of wheat species and varieties are still scarce in the literature. Zhao *et al*. (2009) observed the existence of several reported trials on screening wheat

varieties for mineral nutrient concentration in grain and highlighted that these trials were performed with small numbers of wheat varieties with a limited geographical origin.

Therefore, with increasing interest in spelt wheat as a highly promising source of genetic diversity to increase grain protein content and mineral nutrient concentrations, particularly Zn and Fe (Gomez-Becerra *et al*., 2010), this current study was undertaken to look at selenium, one of the minerals which is most commonly deficient in the human diet. The influence of wheat species and variety, fertiliser type, crop protection and the relationships between grain selenium concentration, yield, protein content and seed size (thousand grain weight) were studied. The objectives of the study were: (i) to evaluate the effects of wheat species and variety, fertiliser type and crop protection on grain selenium concentrations, yield and quality (protein content); (ii) to determine the relationship between grain selenium concentration and grain yield, TGW and protein content; (iii) investigate the association between climatic data and effects of agronomic drivers on grain yield, quality, TGW and grain selenium concentration by multivariate analysis (RDA).

6.2 Material & methods

6.2.1 Site description

All data presented in this study were collected from field trials which were part of major European Union (EU) funded projects i.e. (i) Nitrogen Use Efficiency (NUE-CROPS) (ii) Healthy Minor Cereals (HMC) and (iii) Quality Low Input Food (QLIF). NUE-CROPS, HMC and QLIF trials were conducted using long-term trial plots at Nafferton Farm, Newcastle University between 2009 and 2016. NUE and HMC trials were carried out in Quarry field and the QLIF trial was in East Hemmel (Fig. 4.1, Chapter 4). A summary description of each trial is provided below.

6.2.1.1 NUE-CROPS trial

The NUE-CROPS trial was established in 2009 under the Nafferton Factorial Systems Comparison trial as part of an EU-FP7 funded project (grant number EU-FP7 222-645) (2009-2014). The NUE trial was conducted over two cropping seasons (2009/2010 and 2011/2012) to study the effects of fertilisation regime, crop protection and variety on yield and resource use efficiency (gene, protein and metabolite

expression) of winter wheat (Fig. 6.1). The trial was set up as a split-split-split plot factorial design with 4 replicate blocks consisting of fertiliser type (composted FYM, i.e. cattle manure, vs mineral N fertiliser) as the main plot factor (24 m x 24 m), crop protection as the sub-plot factor (conventional vs organic crop protection (24 x 12 m) and variety as the sub-sub-plot factor (3 m x 12 m). Composted FYM was applied with the equivalent of 170 kg N/ha, 29.2 kg P/ha, 323.6 kg K/ha on 8th April 2009 and 170 kg N/ha, 30.3 kg P/ha, 130.1 kg K/ha on 7th October 2011. Mineral N fertiliser was applied as ammonium nitrate (34.5 % N) in two split applications (85 kg N/ha in each application) with a total of 170 kg N/ha in both 2010 (6th April and 22ndApril) and 2012 (12th April and 27th April) growing seasons.

Eight varieties of winter wheat including four short-straw (semi-dwarf) and four longer straw varieties were tested. Short straw varieties commonly grown in the UK were used: Gallant, Cordiale, Grafton, and Solstice which are all modern varieties listed on the UK Recommended List for Winter Wheat (AHDB, 2017). Another four longer straw organically bred wheat varieties (Laurin, Scaro, Aszita, and Wima) were obtained from a Swiss organic breeding programme (Peter Kunz and marketed through Sativa). Sowing took place on 13th October 2009 and 15th October 2011. Two crop protection applications were applied to the conventional treatment plots. In early May 2010 and 2012 at T1 (GS31-33) plots were sprayed with chlorothalonil (1 L/ha), proquinazid (0.2 L/ha) and chlormequat (1 L/ha) and T2 (GS37-41) they were sprayed between 8-11 days after T1 (GS31-33) only with Spinosad (1 L/ha). The pre-emergence herbicides Prosulfocarb (2 L/ha) and Diflufenican (1 L/ha) were applied to the conventional plots in October of both seasons. Mechanical weeding (Einbock tine weeder) was carried out in the organic plots, with a minimum of two timings per season in early spring. Crops were harvested at maturity on 1st September 2010 and 4th September 2012 using a plot combine harvester (Claas Dominator 38; Claas UK Ltd, Bury St Edmunds, UK). After harvesting, grain samples were oven dried (45 ^{o}C) and cleaned with a grain cleaner (Lainchbury HC1/7W, Blair Engineering, Blairgowrie, UK) and grain yields are presented at 15% moisture content.

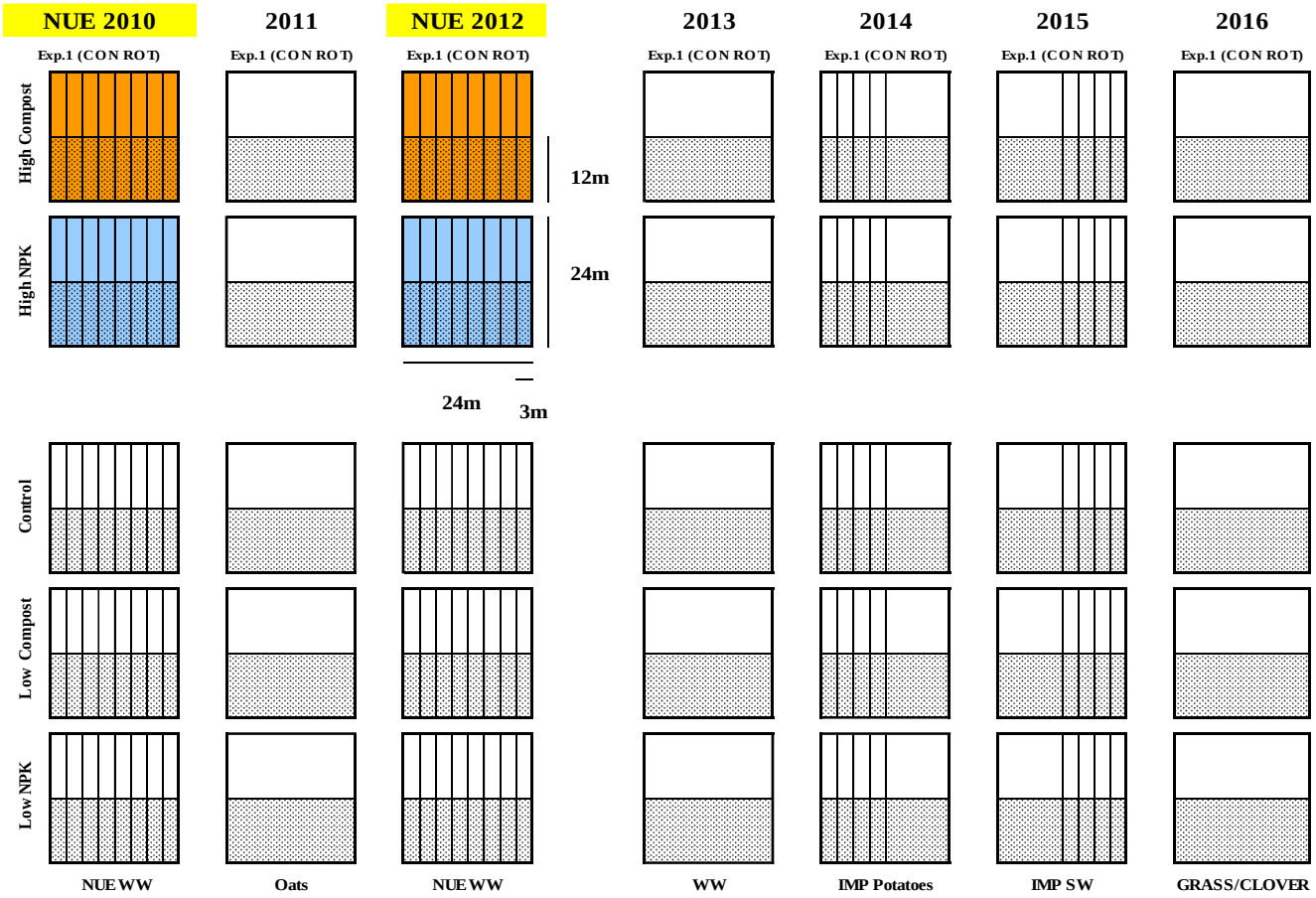


Fig. 6.1. *Experimental layout for the NUE-CROPS trials with rotational sequence from 2010-2016. Fertiliser treatment as a main plot (24 m x 24 m) was split with and without crop protection (12 m x 24 m) and winter wheat variety as sub-sub plots (3 m x 12 m). All grain samples were collected from high fertiliser rate of NPK and compost plots in the 2009/2010 and 2011/2012 seasons. NUE 2010/2012-NUE-Crops trial (2010 and 2012). CON ROT-Conventional rotation; NUE WW-NUE-Crops trial (Winter wheat); WW-Winter wheat; SW-Spring wheat.*

6.2.1.2 The QLIF trial

The QLIF trial was carried out in East Hemmel field under the Nafferton Factorial Systems Comparison long-term trial study. The main experimental factors for the QLIF study were (1) pre-crop (a diverse rotation representative of an organic system vs a less diverse conventional cereal dominated rotation) as the main plot factor, (2) crop protection (with and without the use of synthetic chemical inputs) as the sub-plot factor and fertiliser management (composted FYM vs Mineral N) as the sub-sub-plot factor. The NFSC was established in 2001 as a factorial field experiment as part of the European Union Integrated Project Quality Low Input Food (EU FP6 Contract CT- 2003- 506358).

The trial had four replicate main blocks separated by 10 m strips of grass and clover. Each main block consisted of four separate Experiments 1, 2, 3 and 4 (to represent different stages of the rotation), randomised within the trial design and arranged as a split−split−split−plot design (Bilsborrow *et al.,* 2013). Each main block was 122 m × 122 m and divided into 16 main plots. Each main plot size was 24 m x 24 m and further divided into 32 plots (24 m × 12 m) as sub-sub-plots. Data used in this study were collected and generated from winter wheat grown in Experiment 3 (2015) and Experiment 4 (2016). The full sequence of crop rotation (organic and conventional rotations) for Experiments 3 and 4 is presented in Table 6.1 and the experimental layouts are presented in Figs. 6.2 and 6.3. For crop protection, each of the four crop protection plots was further split with either conventional (mineral/inorganic) or organic (none or composted FYM) fertilisation regimes. This provided four combinations of crop protection and fertiliser (CPOF, CPCF, OPOF, OPCF i.e. Conventional crop protection-Organic fertiliser; Conventional crop protection-Conventional fertiliser; Organic crop protection–Organic fertiliser and Organic crop protection–conventional fertiliser). Further details of the experimental design are described in Eyre *et al.* (2011). Commercial insecticide, herbicide and fungicides were applied to the conventional crop protection plots, while permitted materials and methods according to the Organic Standards (Soil Association, 2010) were only used in the organic crop protection plots.

Table 6.1. *Sequence of crop rotation (organic and conventional) from 2003 to 2016 in Experiments 3 and 4 under the QLIF, Nafferton Factorial Systems Comparison long-term trial.*

Year	Exp. 3 (2015)		Exp. 4 (2016)	
	Organic Rotation (OR)	**Conventional Rotation (CR)**	**Organic Rotation (OR)**	**Conventional Rotation (CR)**
2003	G/C	G/C	Potato /veg	Potato
2004	Potato	Veg / Potato	Spring barley	Winter wheat
2005	G/C	Grass	G/C	Winter barley
2006	G/C	Grass	G/C	G/C
2007	Winter wheat	Winter wheat	G/C	G/C
2008	Veg / Potato	Winter wheat	Winter wheat	Winter wheat
2009	Beans	Winter barley	Veg / Potato	Winter wheat
2010	Potato / Veg	Potato / Veg	Beans	Winter barley
2011	Spring barley	Winter wheat	Potato / Veg	Potato / Veg
2012	G/C	Winter barley	Spring barley	Winter wheat
2013	G/C	G/C	G/C	Winter barley
2014	G/C	G/C	G/C	G/C
2015	**Winter wheat**	**Winter wheat**	G/C	G/C
2016	Veg / Potato	Winter wheat	**Winter wheat**	**Winter wheat**

Crop data and grain samples collected for use in this study are highlighted in bold by year and crop type. G/C=grass/clover ley.

Cordiale, a Group 2 winter wheat breadmaking variety (NABIM, 2016), was used in both the organic and conventional rotations in Experiment 3 and 4. Millers in the UK have high demand for Cordiale due its good performance in milling/baking studies (AHDB, 2017). Cordiale was sown using a commercial drill (3m Lely combination drill; Lely UK Ltd, St Neots, UK) at a seed rate of 176 kg/ha on 2nd October 2014 and 180 kg/ha on 15th October 2015. Mineral N as ammonium nitrate (Yara UK Ltd) was applied at a rate of 180 kg N/ha in 2015 and 210 kg N/ha in 2016 with two split applications. The first and second mineral N applications in 2015 were applied with 80 kg N/ha (13th March 2015) and 100 kg N/ha (20th April 2015) while in 2016, a split of 80 kg N/ha (17th March 2016) and 130 kg N/ha (20th April 2016) was applied. No P and K were applied to the first grown wheat crops after grass clover. No manure fertiliser application was used for Experiments 3 and 4 as the rotation of grass/clover (G/C) was

used for three years in the organic rotation and two years in the conventional rotation, which contributed to fertility build-up of the plots.

In the conventional crop protection treatment, herbicides, fungicides and growth regulators were used. Herbicide application was Axial (a.i. pinoxaden and cloquintocet-mexyl) at 0.6 l/ha, Fluroxypyr (a.i. fluroxpyr) at 0.6 l/ha and Mondial (a.i. metsulfuron-methyl) at 20 g/ha which was applied in Oct-Nov 2014 (GS31-33) and Gallifrey (a.i. fluroxypyr) at 0.25 l/ha, Atlantis (a.i. mesosulfuron-methyl and iodosulfuron-methyl-sodium) at 300 g/ha in Oct-Nov 2015 (GS31-33). Fungicide applications were used in 2015 and 2016. In 2015, fungicide application was carried out at two timings (T1 & T2). The first fungicide application (T1) was a tank mix of Kestrel (a.i. prothioconazole and tebuconazole) at 0.7 l/ha, Phoenix (a.i. folpet) at 1 l/ha and Boogie (a.i. bixafen, prothioconazole and spiroxamine) at 1.25 l/ha sprayed at GS31-GS33 in April. Whereas, the second fungicide application (T2) was a mix of Kestrel (a.i. prothioconazole and tebuconazole) at 0.25 l/ha, Phoenix (a.i. folpet) at 1 l/ha and Boogie (a.i. bixafen, prothioconazole and spiroxamine) at 1.25 l/ha sprayed at GS37-GS41 in May. In 2016, fungicide application also occurred with two spraying applications (T1 & T2). The first tank mix at T1 was Phoenix (a.i. folpet) at 1 l/ha, Boogie (a.i. bixafen, prothioconazole and spiroxamine) at 1.3 l/ha, Cortez (a.i. epoxiconazole) at 1 l/ha and Bravo (a.i. chlorothalonil) at 2 l/ha sprayed at GS31-GS33 in April. Meanwhile, the second application at T2 was Phoenix (a.i. folpet) at 1 l/ha, Boogie (a.i. bixafen, prothioconazole and spiroxamine) at 1.3 l/ha, Cortez (a.i. epoxiconazole) at 1 l/ha and Bravo (a.i. chlorothalonil) at 2 l/ha sprayed at GS37-GS41 in May. The growth regulator Chlormequat (a.i. chlormequat chloride) was used at 1.25 l/ha in Oct-Nov 2014 (GS31-33) and 1.0 l/ha in Oct-Nov 2015 (GS31-33).

At maturity, crops were harvested using a plot combine (Claas Dominator 38; Claas UK Ltd, Bury St Edmunds, UK) on 28th August 2015 and 6th September 2016. Harvested grain samples were oven dried and cleaned using a grain cleaner (Lainchbury HC1/7W, Blair Engineering, Blairgowrie, UK) for subsequent laboratory analysis.

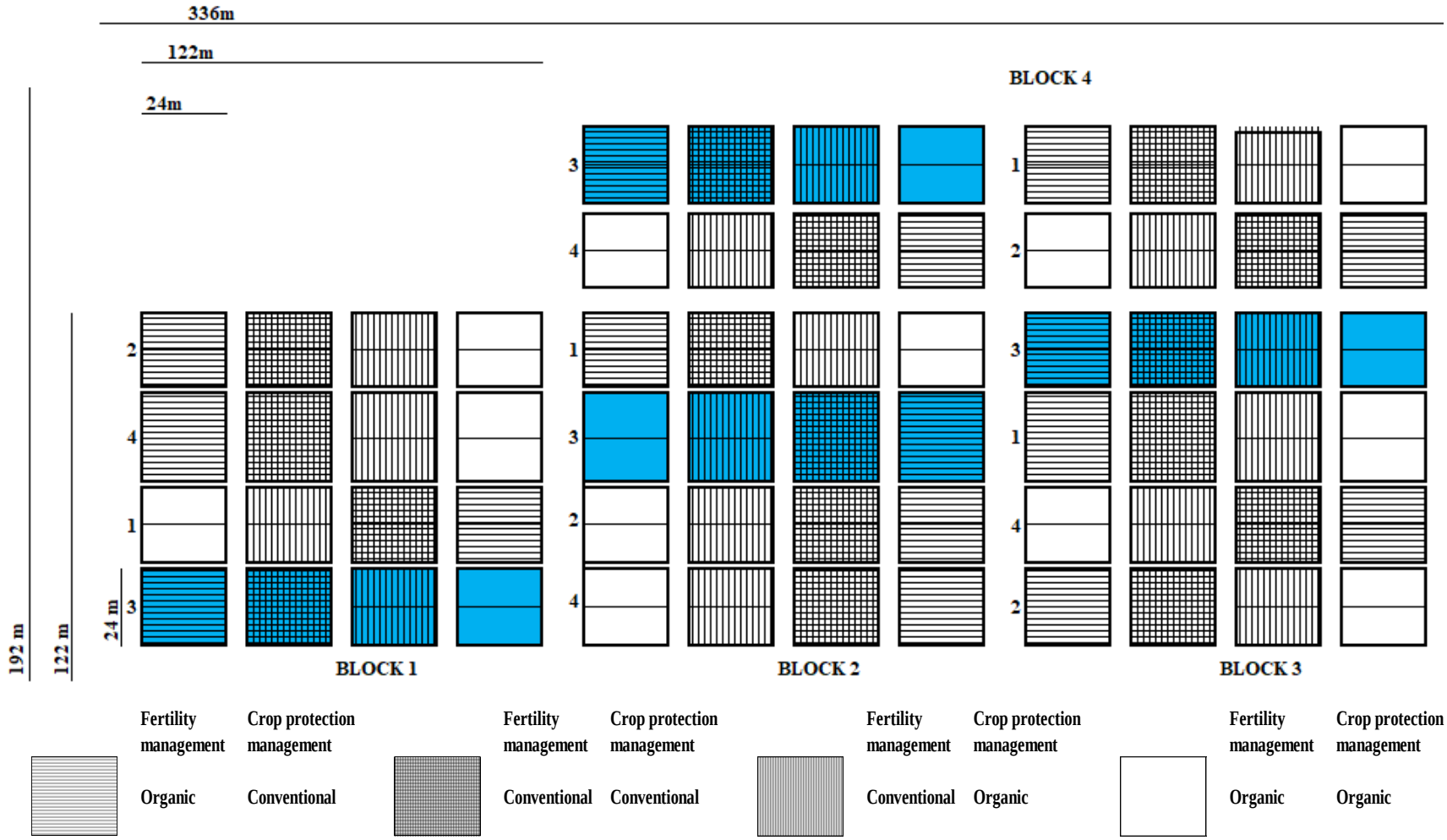


Fig. 6.2. *Experimental layout for the QLIF trial (Experiment 3, 2015, blue colour). Pre-crop as a main plot (24m x 24m) was split with and without crop protection (12 m x 24 m) and fertiliser type as sub-sub plots (3 m x 12 m). Each main block (1,2,3,4) consisted of four separate Experiments 1, 2, 3 and 4 (to represent different stages of the rotation).*

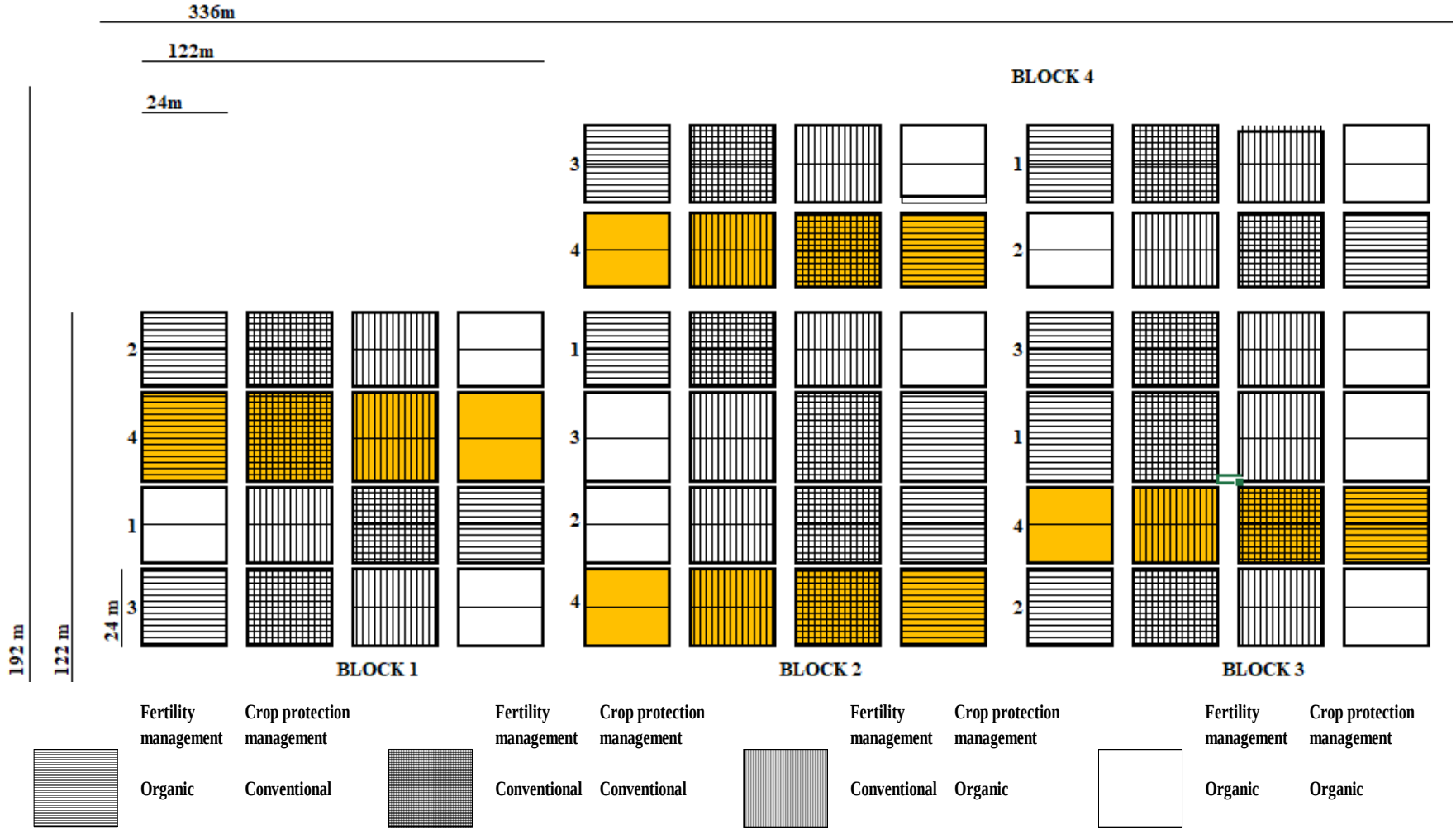


Fig. 6.3. *Experimental layout for the QLIF trial (Experiment 4, 2016, orange colour). Pre-crop as a main plot (24 m x 24 m) was split with and without crop protection (12 m x 24 m) and fertiliser type as sub-sub plots (3 m x 12 m). Each main block (1,2,3,4) consisted of four separate Experiments 1, 2, 3 and 4 (to represent different stages of the rotation).*

6.2.1.3 Healthy Minor Cereals trial

Trials for the EU-FP7 project Healthy Minor Cereals were conducted in the 2015/16 and 2016/17 growing seasons as part of the study funded by the European Union's Seventh Framework Programme (Healthy Minor Cereals grant number 613609). Spelt was grown in a split-split-split-plot factorial design with 4 replicate blocks (Fig. 6.4 and Fig. 6.5). The main factors for the HMC trial were (i) fertiliser type (24 m x 6 m) with a low and medium rate of 50 and 100 kg N/ ha applied as mineral N, composted FYM, cattle slurry and biogas digestate and (ii) spelt variety (24 m x 3 m) including modern varieties and landraces (Oberkulmer, Zurcher Oberlander Rotkorn (ZOR), Rubiota and Filderstolz) as the sub-plot factor. Filderstolz is a short straw spelt variety resulting from a cross between spelt and the high yielding common wheat variety (Maris Huntsman) under the spelt breeding programme of Hohenheim University. Zurcher Oberlander Rotkorn is a variety bred for organic growers by Peter Kunz in Switzerland. Rubiota and Oberkulmer are two long straw spelt varieties; Rubiota is from the Czech Republic while Oberkulmer is a Swiss landrace. The previous crop in both seasons was grass/clover. A full description of the experimental design and layout is provided in Magistrali *et al.* (2020). Seeds were drilled on 1st October 2014 and 5th October 2015 at a seed rate of 350 (2014/15) and 250 spikelets/m^2 (2015/16). Herbicide application was carried out in early November 2014/15 and 2015/16. In 2014/15, Fluroxypyr 200 (a.i. fluroxypyr) was sprayed at 0.6 l/ha. While Ultra (a.i. aminopyralid and triclopyr) and Galifrey (a.i. fluroxypyr) were sprayed respectively at 1.5 l/ha and 0.35 l/ha in 2015. Harvest took place at maturity on 9th September 2015 and 14th September 2016.

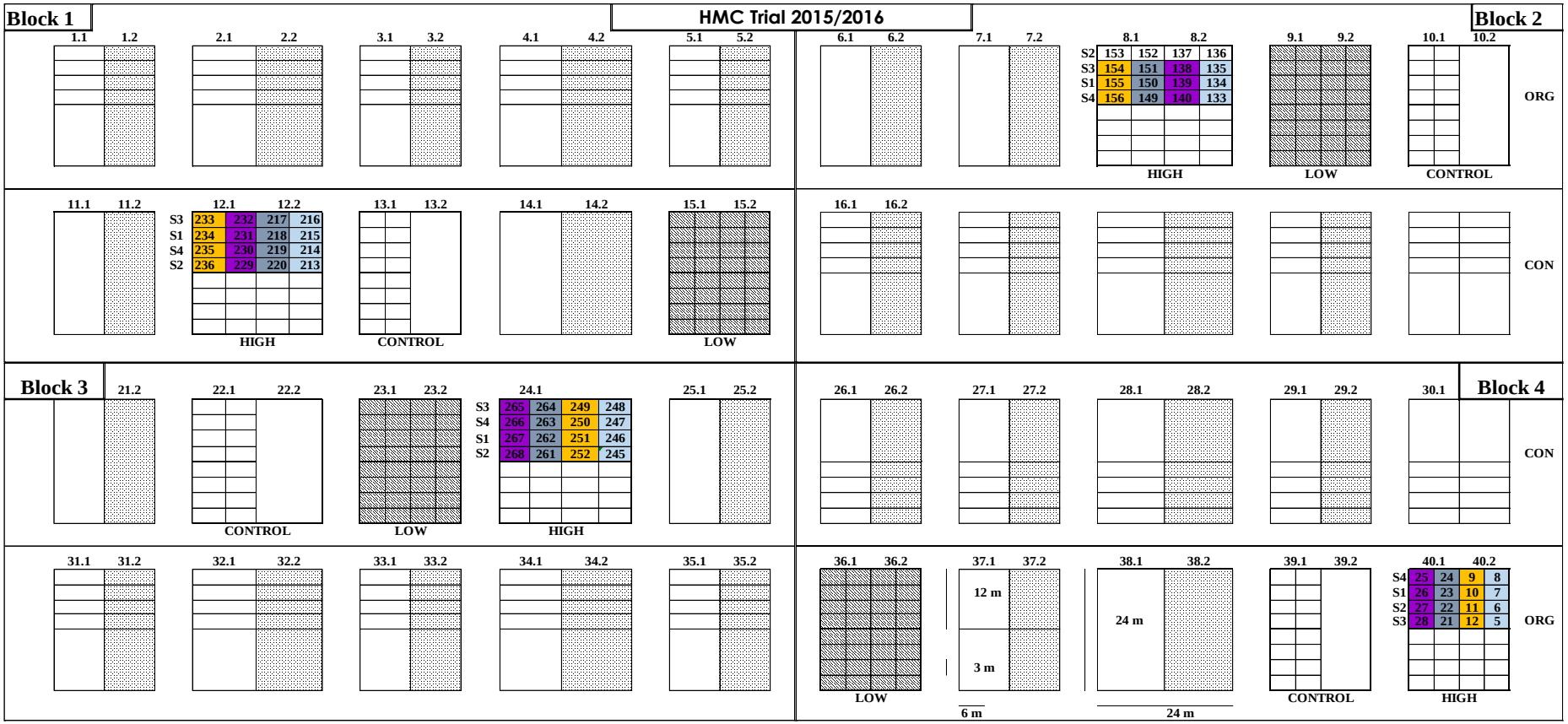


Fig. 6.4. *Experimental layout for the HMC trial* during the *2015/2016 growing seasons. Fertiliser treatment (Purple – Biogas digestate; Orange-Composted farmyard manure; Blue-Cattle slurry; Grey-Mineral N) as a main plot 24 m x 6 m and spelt variety (S1-Oberkulmer Rotkorn; S2-Zurcher Oberlander Rotkorn; S3-Rubiota; S4–Filderstolz) as sub-plots 24 m x 3 m. All grain samples were collected from high fertiliser rate (100 kg N/ha) plots.*

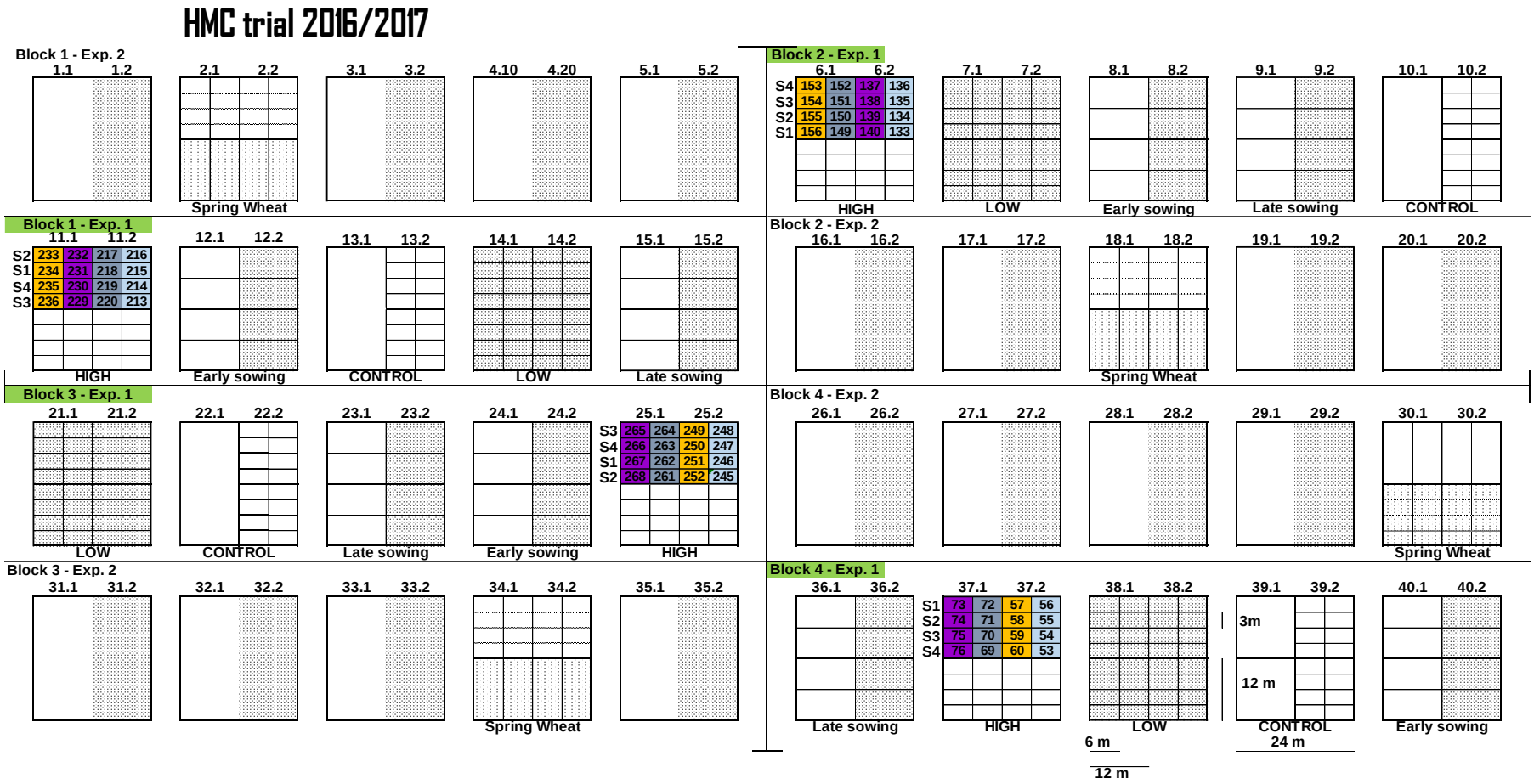


Fig. 6.5. *Experimental layout for the HMC trial* during the *2016/2017 growing seasons. Fertiliser treatment (Purple – Biogas digestate; Orange-Composted farmyard manure; Blue-Cattle slurry; Grey-Mineral N) as a main plot 24 m x 6 m and spelt variety (S1-Oberkulmer Rotkorn; S2- Zurcher Oberlander Rotkorn; S3-Rubiota; S4–Filderstolz) as sub-plots 24 m x 3 m. All grain samples were collected from high fertiliser rate (100 kg N/ha) plots.*

6.2.2 Data collection and nutrient analysis

Available data on grain yield, TGW, grain protein content and plant height were collected from the three trials (NUE-CROPS, HMC and QLIF). About 3.0-3.5 g of oven dried grain samples from all trials were ground with a Retsch SK300 mill (0.25 mm mesh sieve size) for large grain samples from the HMC and QLIF trials and a Retsch Cyclone Mill (0.2 mm mesh sieve size) for smaller grain samples from the NUE-CROPS trial. All milled grain samples were sent to Sabanci University on 15 February 2018, Turkey for grain selenium analysis. Selenium analysis was performed by an inductively coupled plasma optical emission spectrometer (ICP-OES; Vista-Pro Axial; Varian Pty Ltd., Mulgrave, Australia) as described in Chapter 4. Data on grain protein content from the QLIF trial was collected using an infrared analyser (Foss, Infratec™ 1241). Soil selenium concentration was determined for topsoil (0-30 cm) from collected soil sampled in the QLIF and NUE-CROPS trials prior to sowing and treatment application in December 2014 and mid-March 2010. Soil samples from both trials were stored at Nafferton farm and sent to the NRM laboratory for soil selenium analysis.

6.2.3 Statistical analysis

All data collected from the NUE-CROPS, QLIF and HMC trials were subjected to statistical analysis using the R package software (R Core Team, 2017). Analysis of variance (ANOVA) was derived from linear mixed-effects models, "*lme"* (Pinheiro and Bates, 2000) to produce ANOVA *p*-values for main effects and all interactions between (i) harvest year, fertiliser type and winter wheat variety for the NUE-CROPS trial; (ii) harvest year, crop rotation, crop protection and fertiliser type for the QLIF trial and (iii) harvest year, fertiliser type and spelt variety for the HMC trial by using the *"nlme"* (non-linear mixed effects) package in the R statistical environment. The hierarchical nature of the split-split-split-plot design was designated in the random error structures of the model as: (i) block/ harvest year/ fertiliser type for the NUE-CROPS trial; (ii) block/ harvest year/ crop rotation/ crop protection for the QLIF trial and (iii) block/ harvest year/ fertiliser type for the HMC trial. The random error structures that were specified in each trial were reflected by the hierarchical and the nested structure of the split-split-plot design. The normality of the residuals of all parameters was also checked by using the *"qqnorm"* function in R. In order to further investigate the significant main effects ($p < 0.05$) of (i) fertility type and variety for the NUE-CROPS trial; (ii) crop rotation, crop protection and fertiliser type for the QLIF trial and (iii) fertiliser type and variety for the HMC trial and/ or including significant interactions between those factors, general linear hypothesis

tests (Tukey contrasts) were performed using the *"glht"* function of the *"multcomp"* package (Bretz *et al*., 2011) in R. The split-split-split-plot design was reflected in the same random error structures used for the *"lme"* models. *'tapply'* function in R was used to generate both means and standard error of mean values for the main effect and interaction tables.

Pearson's correlation analyses were performed between grain selenium concentration and grain yield, protein content and TGW by using the *"cor"* function, while the significance of the correlation was tested using the *"cor.test"* function in R. The relationships between weather (air temperature, radiation and precipitation), fertiliser treatment (type and rate), wheat species (winter wheat and spelt) and grain yield/quality parameters were assessed on data from the NUE-CROPS, QLIF and HMC trials by using redundancy analysis (partial RDA), with trial replicates (blocks) used as covariates. The pRDA was performed using the CANOCO 5 package (Ter Braak and Smilauer, 2012).

6.3 Results

6.3.1 Total soil selenium concentration

Mean soil selenium concentrations for topsoil (0-30 cm) in the QLIF and NUE-CROPS trials were 0.35 mg/kg and 0.38 mg/kg respectively.

6.3.2 Effects of fertiliser type, crop protection, species and variety on grain selenium concentration, yield, quality, TGW, protein content and plant height

6.3.2.1 NUE-CROPS trial

Harvest year significantly affected grain selenium concentration, TGW, protein content and plant height of winter wheat (Table 6.2). A much higher concentration of grain selenium was observed in the 2012 growing season (44.8 µg/kg) than in 2010 (19.6 µg/kg). The TGW showed a similar pattern, where TGW was higher in 2012 (44.5 g) than 2010 (35.9 g). In 2012, grain protein content and plant height were significantly higher than 2010. However, there was no significant effect of harvest year on grain yield, although grain yield in 2012 was much lower (3.2 t/ha) than in to 2010 (4.9 t/ha).

Fertiliser type exhibited a significant main effect for grain yield, protein content, grain selenium concentration and plant height but not for TGW. Composted FYM gave a much higher grain selenium concentration (39.7 µg/kg) than mineral N (24.8 µg/kg). However, grain yield showed a contrasting result, where yield with mineral N (5.1 t/ha) was much higher than with composted FYM (2.9 t/ha). Protein content was significantly greater in the mineral N (10.8

%) than composted FYM treatment (9.8 %). Plant height was significantly higher following the application of mineral N (71.2 cm) compared to composted FYM (55.8 cm).

Variety showed a significant main effect for grain selenium concentration, grain yield, TGW, protein content and plant height. Grain selenium concentration was significantly different between varieties, where the range of selenium concentration in the grain of long straw wheat group was between 37.3-32.7 µg/kg, while the short straw wheat groups was 31.8–27.3 µg/kg. The highest grain selenium concentration among the long straw varieties was in Wima (37.3 µg/kg), while in the short straw group it was in Grafton (31.8 µg/kg). However, the short straw wheat varieties had significantly higher grain yields than the long straw varieties. TGW for short and long straw varieties was similar, with the highest TGW values detected in Wima (42.5 g) and Grafton (41.5 g). In general, the long straw wheat varieties had protein content significantly higher than the short straw varieties. The higher protein content detected in the long straw group was in the order of Aszita>Wima>Scaro=Laurin and in the short straw group Solstice >Gallant=Cordiale>Grafton. Meanwhile, the tallest long and short straw varieties were Wima (76.3 cm) and Solstice (60.8 cm).

Table 6.2. *Main effects (means ± SE and p-values) and interactions of harvest year, fertiliser type and variety on grain yield, TGW, protein content, grain Se concentration and plant height in the NUE-CROPS trial.*

NUE Factors	Grain yield (t /ha)		TGW (g)		Protein content (%)		Grain Se (µg/kg)		Plant height (cm)	
Harvest Year (YR)										
2010	4.9±0.31		35.9±0.33		10.0±0.19		19.6±0.82		57.7±1.66	
2012	3.2±0.20		44.5±0.30		10.7±0.16		44.8±2.10		69.3±1.90	
Fertiliser type (FT)										
Composted FYM	2.9±0.15		39.8±0.74		9.8±0.16		39.7±2.51		55.8±1.63	
Mineral N	5.1±0.31		40.7±0.48		10.8±0.17		24.8±1.45		71.2±1.70	
Variety (VR)										
Cordiale (S)	4.3±0.67	ab	39.6±1.34	cd	9.5±0.21	d	30.3±3.68	bc	51.6±2.23	d
Gallant (S)	4.6±0.67	a	40.2±0.95	bc	9.5±0.17	d	29.8±3.13	bc	56.2±1.66	cd
Grafton (S)	4.4±0.71	ab	41.5±1.65	ab	9.0±0.23	e	31.8±4.39	ac	51.2±2.77	d
Solstice (S)	4.4±0.61	ab	39.6±1.03	cd	9.9±0.24	d	27.3±3.09	c	60.8±2.77	c
Aszita (L)	3.5±0.41	c	39.0±1.33	cd	12.2±0.43	a	32.7±4.53	ac	72.7±3.66	ab
Laurin (L)	3.6±0.47	c	38.7±1.12	d	10.5±0.21	c	34.9±5.65	ab	68.9±3.54	b
Scaro (L)	3.6±0.52	c	40.9±1.19	b	10.6±0.25	c	34.5±5.17	ab	70.3±3.55	b
Wima (L)	3.8±0.43	bc	42.5±1.32	a	11.3±0.32	b	37.3±6.19	a	76.3±4.23	a
ANOVA *p-values*										
Main effects										
YR	0.073		**<0.001**		**0.045**		**0.002**		**0.003**	
FT	**0.012**		0.064		**0.002**		**0.000**		**<0.001**	
VR	**<0.001**		**<0.001**		**<0.001**		**0.029**		**<0.001**	
Interactions										
YR x FT	**0.019**		**<0.001**		**0.024**		**0.008**		0.204	
YR x VR	**<0.001**		**<0.001**		**<0.001**		0.128		**0.027**	
FT x VR	**<0.001**		0.238		**<0.001**		0.071		0.242	
YR x FT x VR	0.098		**0.016**		**<0.001**		0.547		**0.017**	

Letters were applied across all short (S) and long (L) straw varieties to show the difference between varieties in grain yield, TGW, protein content, grain Se and plant height. Means followed by the same letter within each column are not significantly different at $p \leq 0.05$. See Table 6.3-6.6 for interaction means ± SE.

There was a significant 2-way interaction of harvest year x fertiliser type for grain selenium concentration, grain yield, TGW and protein content (Tables 6.3-6.6). Table 6.3 shows selenium concentration in wheat grain under mineral N and composted FYM fertilisers application. Grain selenium concentration was significantly greater in harvest year 2012 than 2010. When compared between fertiliser types, grain selenium concentration was significantly higher in composted FYM than mineral N in both years.

Table 6.3. *Interaction means ±SE for the effect of harvest year × fertiliser type on grain Se concentration (µg/kg) in the NUE-CROPS trial.*

Year	Grain Se concentrations (µg/kg)	
	Fertiliser type	
	Mineral N	**Composted FYM**
2010	15.4±2.74 Bb	23.6±1.17 Ba
2012	33.9±1.63 Ab	55.8±2.74 Aa

Means ±SE values followed by different lower-case letters within each row and upper-case letters within each column are significantly different (Tukey's general linear hypothesis test p<0.05).

Table 6.4 shows mineral N application resulted in significantly higher grain yield in 2010 than 2012. In 2010, grain yield was significantly higher with mineral N than composted FYM application, but there was no difference between fertiliser types in the 2012 growing season.

Table 6.4. *Interaction means ±SE for the effects of harvest year × fertiliser type on grain yield (t/ha) in the NUE-CROPS trial.*

Year	Grain yield (t/ha)	
	Fertiliser type	
	Mineral N	**Composted FYM**
2010	7.0±0.32 Aa	2.8±0.08 Bb
2012	3.3±0.28 Bb	3.1±0.28 Bb

Means ±SE values followed by different lower-case letters within each row and upper-case letters within each column are significantly different (Tukey's general linear hypothesis test p<0.05).

Both fertiliser types gave significantly higher TGW in 2012 than 2010 (Table 6.5). It was observed that mineral N gave significantly higher TGW than composted FYM in 2010 but the result was the opposite in 2012.

Table 6.5. *Interaction means ±SE for the effects of harvest year × fertiliser type on TGW (g) in the NUE-CROPS trial.*

Year	TGW (g)	
	Fertiliser type	
	Mineral N	**Composted FYM**
2010	37.6±0.40 Ba	34.3±0.30 Bb
2012	43.7±0.40 Ab	45.3±0.41 Aa

Means ±SE values followed by different lower-case letters within each row and upper-case letters within each column are significantly different (Tukey's general linear hypothesis test p<0.05).

There was a significant interaction between harvest year and fertiliser type on grain protein content (Table 6.6). The effect of mineral N showed no significant difference between years. In contrast, composted FYM applied in 2012 had a significantly higher grain protein content compared to 2010. Grain protein content was significantly greater in response to mineral N than composted FYM application in 2010.

Table 6.6. *Interaction means ±SE for the effects of harvest year × fertiliser type on grain protein content (%) in the NUE-CROPS trial.*

Year	Grain protein content (%)	
	Fertiliser type	
	Mineral N	**Composted FYM**
2010	10.8±0.29 Aa	9.2±0.13 Bb
2012	10.9±0.20 Aa	10.4±0.24 Aa

Means ±SE values followed by different lower-case letters within each row and upper-case letters within each column are significantly different (Tukey's general linear hypothesis test p<0.05).

6.3.2.2 QLIF trial

Grain yield and TGW were affected significantly by harvest year (Table 6.7), where 2015 produced higher grain yield and TGW than 2016. However, grain selenium concentration and grain protein content were not affected by year. There was no significant effect on TGW, protein content and grain selenium concentration of the conventional vs organic rotation treatments except for grain yield. A significant effect of crop protection treatment was observed on grain yield, TGW, protein content and grain selenium concentration. It was observed that grain selenium concentration and protein content were significantly higher following organic crop protection when compared with the conventional crop protection treatment. In contrast, grain yield and TGW were significantly higher in conventional than the organic crop protection treatment. For fertilisation treatments, mineral N and composted FYM application significantly affected grain yield, TGW, protein content and grain selenium concentration. It was noticeable that composted FYM application gave significantly higher grain selenium concentration (33.3 µg/kg) and TGW (46.5 g) than mineral N application. Grain yield and protein content were also significantly higher in the mineral N than composted FYM application treatment, while TGW was significantly lower under mineral N compared to composted FYM application.

Table 6.7. *Main effects (means ± SE and p-values) and interactions of harvest year, crop rotation, crop protection and fertiliser type on grain yield, TGW, protein content and grain Se concentration in the QLIF trial.*

QLIF factors	Grain yield (t/ha)	TGW (g)	Protein content (%)	Grain Se (µg/kg)
Harvest year (YR)				
2015	9.8±0.58	50.6±0.96	10.2±0.33	30.4±1.25
2016	4.4±0.38	36.4±1.13	11.1±0.38	30.6±1.77
Crop rotation (CR)				
Conventional	6.8±0.64	43.3±1.73	10.3±0.35	31.2±1.33
Organic	7.4±0.73	43.7±1.57	10.9±0.37	29.8±1.69
Crop Protection (CP)				
Conventional	9.2±0.71	46.3±1.51	9.5±0.21	27.4±1.30
Organic	5.2±0.45	40.7±1.63	11.6±0.39	33.5±1.54
Fertiliser type (FT)				
Mineral N	7.9±0.81	40.5±1.86	11.9±0.35	27.7±1.29
Composted FYM	6.3±0.50	46.5±1.19	9.3±0.19	33.3±1.59
ANOVA *p-values*				
Main effects				
YR	**<0.001**	**<0.001**	0.054	0.943
CR	**0.021**	0.664	0.089	0.561
CP	**<0.001**	**<0.001**	**<0.001**	**<0.001**
FT	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Interactions				
YR x CR	0.322	0.393	0.794	0.730
YR x CP	**0.013**	0.269	0.338	0.059
CR x CP	**<0.001**	0.218	0.297	0.183
YR x FT	**0.009**	**0.007**	0.341	**0.009**
CR x FT	0.174	0.641	0.543	0.083
CP x FT	**<0.001**	**<0.001**	**0.002**	0.705
YR x CR x CP	0.059	0.458	0.976	0.896
YR x CR x FT	**0.034**	0.118	0.612	0.467
YR x CP X FT	**0.004**	0.886	0.322	0.077
CR x CP x FT	0.413	0.274	0.271	0.478
YR x CR x CP x FT	0.877	0.053	0.333	0.318

Mean values labelled with the same letter within each column are not significantly different at $p \leq 0.05$. See Tables 6.8-6.14 for interaction means ± SE .

The harvest year x crop protection interaction had a significant effect on grain yield (Table 6.8). Both conventional and organic crop protection treatments gave significantly higher grain yield in 2015 than 2016. It was observed that conventional crop protection produced significantly higher grain yield than organic crop protection in 2015 and 2016.

Table 6.8. *Interaction means ±SE for the effects of harvest year × crop protection (conventional vs organic) on grain yield (t/ha) in the QLIF trial.*

Year	Grain yield (t/ha)	
	Crop protection	
	Conventional	Organic
2015	12.0±0.79 Aa	7.5±0.30 Ab
2016	6.1±0.46 Ba	2.9±0.16 Bb

Means ±SE values followed by different lower-case letters within each row and upper-case letters within each column are significantly different (Tukey's general linear hypothesis test p<0.05).

There was also a significant harvest year x fertiliser type interaction detected for grain selenium concentration, grain yield and TGW (Tables 6.9-6.11). Application of mineral N and composted FYM in 2015 resulted in no significant difference in grain selenium concentration, but in 2016 grain selenium concentration was significantly greater with composted FYM than mineral N (Table 6.9).

Table 6.9. *Interaction means ±SE for the effects of harvest year × fertiliser type on Se grain concentration (µg/kg) in the QLIF trial.*

Year	Grain Se concentration (µg/kg)	
	Fertiliser type	
	Mineral N	Composted FYM
2015	29.6±1.97 Aa	31.2±1.56 Aa
2016	25.8±1.56 Ab	35.4±2.71 Aa

Means ±SE values followed by different lower-case letters within each row and upper-case letters within each column are significantly different (Tukey's general linear hypothesis test p<0.05).

When mineral N and composted FYM fertilisers were applied, there was a significantly higher grain yield in response to mineral N than composted FYM in 2015 but no significant difference between treatments in 2016 (Table 6.10).

Table 6.10. *Interaction means ±SE for the effects of harvest year × fertiliser type on grain yield (t/ha) in the QLIF trial.*

Year	Grain yield (t/ha)	
	Fertiliser type	
	Mineral N	Composted FYM
2015	10.9±1.02 Aa	8.6±0.41 Ab
2016	5.0±0.68 Ba	3.9±0.24 Ba

Means ±SE values followed by different lower-case letters within each row and upper-case letters within each column are significantly different (Tukey's general linear hypothesis test p<0.05).

Mineral N and composted FYM applications gave significantly higher TGW in 2015 compared to 2016 (Table 6.11). TGW following the application of composted FYM was significantly greater than mineral N in both years.

Table 6.11. *Interaction means ±SE for the effects of harvest year and fertiliser type on TGW (g) in the QLIF trial*

Year	TGW (g)	
	Fertiliser type	
	Mineral N	Composted FYM
2015	48.8±1.71 Ab	52.4±0.65 Aa
2016	32.2±1.49 Bb	40.6±0.87 Ba

Means ±SE values followed by different lower-case letters within each row and upper-case letters within each column are significantly different (Tukey's general linear hypothesis test p<0.05).

There were significant crop protection x fertiliser type interactions for grain yield, TGW and protein content (Table 6.12-6.14). Grain yield was significantly greater in response to conventional compared with organic crop protection under mineral N application with no significant difference between treatments when composted FYM fertiliser was applied (Table 6.12).

Table 6.12. *Interaction means ±SE for the effects of crop protection × fertiliser type on grain yield (t/ha) in the QLIF trial.*

Crop protection	Grain yield (t/ha)	
	Fertiliser type	
	Mineral N	Composted FYM
Conventional	11.0±0.99 Aa	7.2±0.73 Ab
Organic	4.9±0.65 Ba	5.5±0.63 Aa

Means ±SE values followed by different lower-case letters within each row and upper-case letters within each column are significantly different (Tukey's general linear hypothesis test p<0.05).

Table 6.13 shows that TGW was significantly higher in response to conventional than organic crop protection with mineral N but no significant difference was observed when composted FYM fertiliser was used. A significant difference between mineral N and composted FYM application was observed in the organic crop protection treatment with no significant difference when conventional crop protection was used.

Table 6.13. *Interaction means ±SE for the effects of crop protection × fertiliser type on TGW (g) in the QLIF trial.*

Crop protection	TGW (g)	
	Fertiliser type	
	Mineral N	Composted FYM
Conventional	45.8±2.43 Aa	46.9±1.87 Aa
Organic	35.3±2.17 Bb	46.1±1.52 Aa

Means ±SE values followed by different lower-case letters within each row and upper-case letters within each column are significantly different (Tukey's general linear hypothesis test p<0.05).

With both fertiliser types, the organic crop protection treatment gave a significantly higher grain protein content than conventional crop protection (Table 6.14). No significant difference was evident when mineral N and composted FYM were applied under conventional crop protection, but a significantly higher protein content was detected for the organic crop protection following the application of mineral N.

Table 6.14. *Interaction means ±SE for the effects of crop protection × fertiliser type on grain protein content (%) in the QLIF trial.*

Crop protection	Grain protein content (%)	
	Fertiliser type	
	Mineral N	Composted FYM
Conventional	10.5±0.24 Ba	8.7±0.18 Ba
Organic	13.4±0.42 Ab	10.0±0.26 Aa

Means ±SE values followed by different lower-case letters within each row and upper-case letters within each column are significantly different (Tukey's general linear hypothesis test p<0.05).

6.3.2.3 Healthy Minor Cereals trial

In the HMC trial, harvest year had a significant effect on grain yield, protein content and plant height (Table 6.15). Grain yield was higher in 2015 at 3.8 t/ha and was 0.9 t/ha lower in 2016. In contrast, protein content (%) was significantly greater in 2016 (16.2%) than 2015 (13.4%). Also, plant height was significantly less in 2016 (113.9 cm) than 2015 (124.6 cm) when averaged across the four varieties. Selenium concentration in grain was higher in 2015 (57.1 µg/kg) than 2016 (47.4 µg/kg) but not significantly different. TGW was similar between years at 46.1 g and 44.1 g in 2015 and 2016 respectively.

There were significant effects of fertiliser type on grain selenium concentration, yield, TGW and protein content but not on plant height. Grain selenium concentration was significantly higher in the order of composted FYM (57.9 µg /kg) > cattle slurry (55.9 µg/kg) > mineral N (50.6 µg/kg) > biogas digestate (44.1 µg/kg). Biogas digestate gave significantly higher grain yield (3.8 t/ha) than cattle slurry (3.4 t/ha), composted FYM (3.2 t/ha) and mineral N (3.0 t/ha) which was the lowest. Grain protein content was not significantly different between biogas digestate and mineral N. Biogas digestate produced significantly higher grain protein content than the other organic fertiliser types used (cattle slurry and FYM).

Spelt variety significantly influenced grain selenium concentration, grain yield, protein content, TGW and plant height. Grain selenium concentration varied from 45.3 µg/kg to 57.1 µg/kg, being higher in Oberkulmer and Rubiota with the lowest concentration recorded in Zurcher Oberlander Rotkorn (45.3 µg/kg). Spelt grain yield varied between 3.8 t/ha and 2.8 t/ha whereby Oberkulmer had the highest grain yield (3.8 t/ha) and Filderstolz the lowest (2.8 t/ha). Oberkulmer and Rubiota showed the highest protein content of 15.6 % and 15.3 % with

the lowest detected in Filderstolz (13.7 %). Oberkulmer had the highest TGW and the lowest TGW was detected in Filderstolz. The tallest varieties were Rubiota (136.6 cm) and Oberkulmer (135.5 cm) while the shortest was Filderstolz (95.3 cm). No significant difference in plant height was observed between Oberkulmer and Rubiota. Zurcher Oberlander Rotkorn plants were significantly shorter than Oberkulmer and Rubiota but taller than Filderstolz.

Table 6.15. *Main effects (means ± SE and p-values) and interactions of harvest year, fertiliser type and variety on grain yield, TGW, protein content, grain Se concentration and plant height in the HMC trial.*

HMC Factors	Grain yield (t/ha)		TGW (g)		Protein content (%)		Grain Se (µg/kg)		Plant height (cm)	
Harvest Year (YR)										
2015	3.8±0.09		46.1±0.90		13.4±0.16		57.1±1.85		124.6±2.80	
2016	2.9±0.11		44.1±0.29		16.2±0.18		47.4±1.61		113.9±2.14	
Fertiliser type (FT)										
BD	3.8±0.18	a	46.1±0.84	a	15.1±0.33	a	44.1±2.26	c	121.7±3.69	
CFYM	3.2±0.13	bc	45.1±0.79	ab	14.1±0.33	b	57.9±2.41	a	117.6±3.53	
CS	3.4±0.16	b	46.0±0.77	a	14.5±0.38	b	55.9±2.51	a	117.1±3.43	
MN	3.0±0.16	c	43.3±1.30	b	15.5±0.31	a	50.6±2.58	b	120.4±3.97	
Variety (VR)										
Filderstolz	2.8±0.13	c	42.8±0.97	c	13.7±0.24	c	50.8±2.54	b	95.4±1.47	c
Oberkulmer	3.8±0.15	a	48.7±0.91	a	15.6±0.38	a	55.6±2.39	a	135.5±1.99	a
Rubiota	3.4±0.17	b	45.6±0.83	b	15.3±0.37	a	57.1±2.55	a	136.6±2.30	a
ZOR	3.4±0.15	b	43.3±0.78	bc	14.6±0.30	b	45.3±2.47	c	109.4±1.69	b
ANOVA *p-values*										
Main effects										
YR	**0.018**		0.116		**<0.001**		0.123		**0.0429**	
FT	**0.005**		**0.048**		**<0.001**		**<0.001**		0.1781	
VR	**<0.001**		**<0.001**		**<0.001**		**<0.001**		**<0.001**	
Interactions										
YR x FT	0.362		0.515		0.204		0.721		0.097	
YR x VR	0.585		**<0.001**		**0.032**		**0.005**		**<0.001**	
FT x VR	0.764		0.302		0.180		0.252		0.684	
YRxFTxVR	0.827		0.385		0.920		0.544		0.193	

Mean values labelled with the same letter within each column are not significantly different at $p \leq 0.05$. See Table 6.16-6.18 for interaction means ± SE. Biogas digestate (BD), composted FYM (CFYM), cattle slurry (CS) and mineral N (MN).

Significant 2-way interaction between harvest year × variety were detected for grain selenium concentration, TGW and protein content (Table 6.16-6.18). There was a significant harvest year × variety interaction effect on grain selenium concentration (Table 6.16). In 2015, no significant difference between Filderstolz, Oberkulmer and Rubiota in grain selenium concentration was observed. However, the concentration of grain selenium was significantly lower in Zurcher Oberlander Rotkorn. In 2016, grain selenium concentration was not significantly different between Oberkulmer and Rubiota and also between Filderstolz and Zurcher Oberlander Rotkorn. The highest grain selenium concentration was found in Oberkulmer, with the lowest in ZOR. The only spelt varieties which contained grain selenium concentrations <50.0 µg/kg were Filderstolz and Zurcher Oberlander Rotkorn. When compared between harvest years, grain selenium concentration was significantly higher in 2015 than 2016 for Filderstolz, Rubiota and ZOR but not for Oberkulmer.

Table 6.16. *Interaction means ±SE for the effects of harvest year × variety on Se grain concentration (µg/kg) in the HMC trial.*

Variety	Grain Se concentration (µg/kg)	
	Year	
	2015	2016
Filderstolz	57.8±3.20 Aa	44.3±3.18 Bb
Oberkulmer	56.3±3.38 Aa	54.9±3.48 Aa
Rubiota	63.4±3.61 Aa	50.8±2.93 Ab
ZOR	51.1±4.15 Ba	39.5±1.84 Bb

Means ±SE values followed by different lower-case letters within each row and upper-case letters within each column are significantly different (Tukey's general linear hypothesis test p<0.05).

There was a significant harvest year × variety interaction effect on TGW (Table 6.17). In 2015, Oberkulmer gave the highest TGW value, while Filderstolz and ZOR had the lowest TGW. There was no significant difference in TGW detected between all varieties in 2016. There was a significantly higher TGW in 2015 than 2016 for Oberkulmer and Rubiota. In contrast, ZOR had a significantly higher TGW in 2016 than 2015 and Filderstolz showed no significant difference between growing seasons.

Table 6.17. *Interaction means ±SE for the effects of harvest year and variety on TGW (g) in the HMC trial.*

Variety	TGW (g)	
	Year	
	2015	2016
Filderstolz	41.7±1.89 Ca	43.8±0.43 Aa
Oberkulmer	52.7±0.86 Aa	44.7±0.70 Ab
Rubiota	48.3±1.52 Ba	42.9±0.55 Ab
ZOR	41.8±1.37 Cb	44.9±0.55 Aa

Means ±SE values followed by different lower-case letters within each row and upper-case letters within each column are significantly different (Tukey's general linear hypothesis test $p<0.05$).

There was a significant harvest year × variety interaction effect on grain protein content (Table 6.18). In 2015, Filderstolz had significantly lower protein content than the other spelt varieties with no significant difference between Oberkulmer, Rubiota and ZOR. In 2016, Oberkulmer and Rubiota had significantly higher protein content than ZOR and Filderstolz. All spelt varieties showed a higher grain protein content in 2016 than 2015.

Table 6.18. *Interaction means ±SE for the effects of harvest year and variety on grain protein content (%) in the HMC trial.*

Variety	Protein content (%)	
	2015	2016
Filderstolz	12.6±0.21 Bb	14.8±0.17 Ca
Oberkulmer	13.9±0.30 Ab	17.3±0.32 Aa
Rubiota	13.7±0.36 Ab	16.9±0.31 Aa
ZOR	13.3±0.27 Ab	16.0±0.19 Ba

Means ±SE values followed by different lower-case letters within each row and upper-case letters within each column are significantly different (Tukey's general linear hypothesis test $p<0.05$).

6.3.3 The relationships between grain selenium concentration, grain yield, protein content and TGW

Correlation analysis was conducted for the NUE-CROPS, QLIF and HMC trials to examine the relationships between grain selenium concentration and grain yield, protein content and TGW (Table 6.19-6.21).

In the NUE-CROPS trial (Table 6.19), there was a significant negative correlation between grain selenium concentration and grain yield showed (p<0.001; r = -0.37). Correlation analysis showed a weak significant positive correlation between grain selenium concentration and protein content (p=0.041; r = 0.18). Correlation analysis also showed a significant (p=0.000; r = 0.66) positive association between grain selenium concentration and TGW.

Table 6.19. *Correlation analysis (r values) for the relationship between grain selenium concentration, grain yield, protein content and TGW in the NUE-CROPS trial.*

Variable	Se (µg/kg)	Yield (t/ha)	Protein content (%)	TGW (g)
Se (µg/kg)	1.00	-0.37	0.18	0.66
Yield (t/ha)		1.00	0.05	-0.19
Protein content (%)			1.00	0.30
TGW (g)				1.00

In the QLIF trial (Table 6.20), correlation analysis between grain selenium concentration and grain yield showed a significant weak negative correlation (p=0.008; r = -0.33). This analysis indicated that as grain yield increased selenium concentration decreased. There was no clear relationship between grain selenium concentration and protein content (p=0.884; r = 0.02) and between grain selenium concentration and TGW (p=0.852; r = -0.02).

Table 6.20. *Correlation analysis (r values) for the relationships between grain selenium concentration, grain yield, protein content and TGW in the QLIF trial.*

Variable	Se (µg/kg)	Yield (t/ha)	Protein content (%)	TGW (g)
Se (µg/kg)	1.00	-0.33	0.02	-0.02
Yield (t/ha)		1.00	-0.39	0.74
Protein content (%)			1.00	-0.62
TGW (g)				1.00

In the HMC trial (Table 6.21), correlation analysis indicated that there was a non-significant relationship between grain Se concentration and grain yield (p=0.087; r = 0.15). A significant negative correlation was found between grain selenium concentration and protein content (p=0.003; r = -0.26), suggesting that as protein content increased grain selenium decreased. Correlation analysis between grain selenium concentration and TGW showed a non-significant correlation (p=0.493; r = 0.06).

Table 6.21. *Correlation analysis (r values) for the relationships between grain selenium concentration, grain yield, protein content and TGW in the HMC trial.*

Variable	Se (µg/kg)	Yield (t/ha)	Protein content (%)	TGW (g)
Se (µg/kg)	1.00	0.15	-0.26	0.06
Yield (t/ha)		1.00	-0.32	0.32
Protein content (%)			1.00	-0.03
TGW (g)				1.00

6.3.4 Effects of weather and agronomic variables on grain yield, protein content, TGW and selenium concentration

Partial redundancy analysis was carried out with the pooled data from all three trials (NUE-CROS, QLIF and HMC trials) to investigate how the combination of weather (air temperature, radiation and precipitation), fertiliser treatment (mineral N and composted FYM) and crop species (Winter wheat and spelt) used as explanatory variables (RDA drivers), affected grain yield, quality (protein content) and selenium concentration of cereals.

The biplot derived from the pRDA (Fig. 6.6), shows the relationship between the effects of weather (air temperature, radiation and precipitation), fertiliser treatment (mineral N and composted FYM) and crop species on grain yield, quality (protein content) and selenium concentration of cereals. Eigenvalues indicated that Axis 1 accounted for 49.3% of variability with a further 7.4% accounted for by Axis 2. In the present study, pRDA showed that wheat species ($F=92.5$, $p=0.002$), total rainfall ($F=67.9$, $p=0.002$) and temperature ($F=68.2$, $p=0.002$) explained most of the variation. From this study the pRDA suggested that grain protein and selenium concentration were strongly associated with spelt along the positive Axis 1. While grain yield was strongly associated with the use of mineral fertiliser than composted farmyard manure and to a lesser extent with winter wheat. Air temperature, solar radiation and precipitation were strongly related to the positive axis 1 and associated with thousand grain weight.

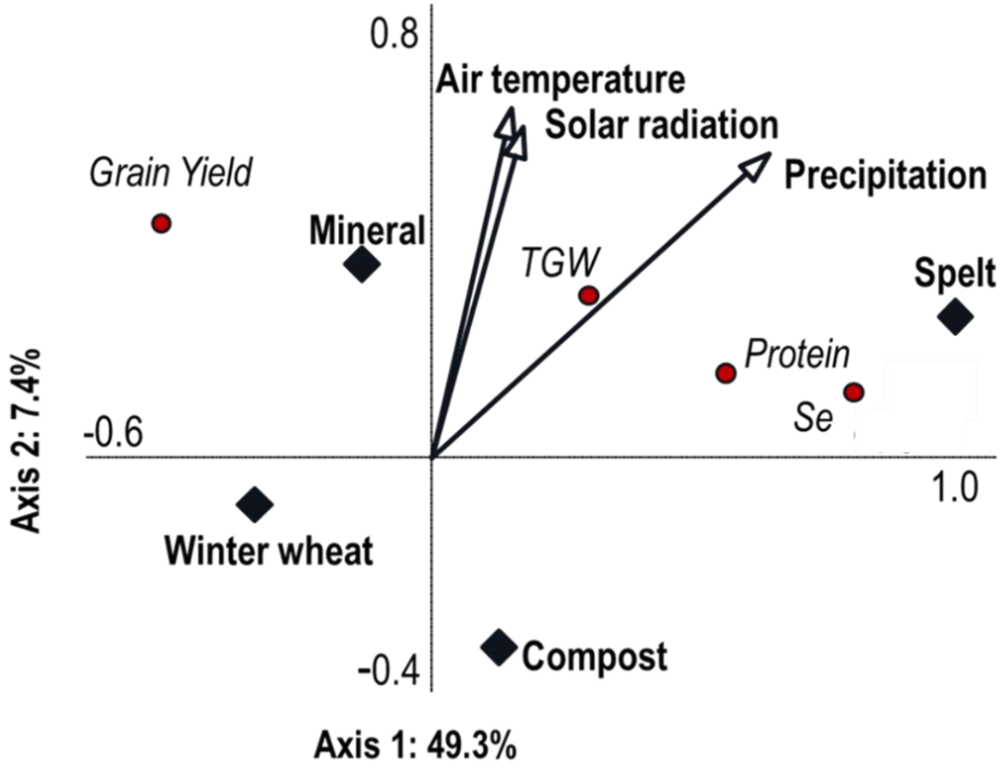


Fig. 6.6. *Bi-plot derived from redundancy analysis (RDA) showing the relationship between the agronomic (mineral and composted FYM fertiliser), genetic (winter wheat and spelt) and climatic factors (air temperature, solar radiation and precipitation) and grain yield, grain quality (protein content), TGW and mineral (Se) concentration of cereals.* Se-Selenium; Compost-Composted farmyard manure; Mineral-Mineral N. Table of % of variation explained, F and P values for all the explanatory variables are shown in Table 6.22.

Table 6.22. The main agronomic drivers (precipitation, temperature and wheat species) with F value and p-value explained most of the variation affected grain yield, protein content, TGW and selenium concentration of wheat investigated by redundancy analysis (RDA). Eigenvalues were accounted for 49.3 % of variability for axis 1 and with a further 7.4 % for axis 2 respectively.

RDA driver	F value	P value
Precipitation	67.9	0.002
Temperature	68.2	0.002
Wheat species	92.5	0.002

6.4 Discussion

6.4.1 Grain selenium concentration

Wheat is an important source of minerals which support human nutrition and health (Hussain *et al*., 2010). Undoubtedly, the introduction of modern high yielding semi-dwarf varieties resulting from the Green Revolution has had a major effect by increasing grain yields significantly. However concentrations of micronutrients such as zinc, magnesium, copper, iron (Fan *et al*., 2008; Cakmak *et al*., 2010) as well as selenium have generally declined. Crop genetics, soil, climate and management practices influence the variation of mineral concentrations of wheat (Hussain *et al*., 2010; Shewry, 2018). In particular, the significance of genetic variation of modern wheat and its wild relatives has been pointed out by Cakmak *et al.* (2000) using screening for concentrations of grain mineral such as iron and zinc. Furthermore, The International Maize and Wheat Improvement Centre (CIMMYT) gene bank has successfully screened about 3000 germplasm accessions for zinc and iron concentration to develop high yielding, disease-resistant wheat varieties with significantly improved iron and zinc concentrations (Monasterio & Graham, 2000; Velu *et al*., 2014).

Exploiting the available genetic variation of wheat through breeding programmes has the potential to enhance wheat varieties for higher micronutrient concentrations, and improve selenium intake among a majority of the world population (Haug *et al*., 2007; Souza *et al*., 2014). In the context of grain selenium concentration, significant genetic variation was observed in other cereal crops including durum wheat (Rodríguez *et al*., 2011), rice (Zhang *et al*., 2006; Norton *et al*., 2012), barley (Ilbas *et al*., 2012; Mangan *et al*., 2015)**,** wild barley (Yan *et al.*, 2011) and oat (Eurola *et al*., 2004). In contrast, there are a few studies which have found that bread wheat genotypes had no significant effect on grain selenium concentration. For instance, Manojlović *et al*., (2019) studied three different winter wheat cultivars (Simonida, Divana and Srpanjka) in Croatia and Serbia and showed no significant difference in selenium concentration and uptake**.** Lyons *et al.* (2005a) conducted a study using ancestral and wild relatives of wheat, wheat landrace accessions and commercial cultivars in Mexico and Australia. This study found no significant genotypic variation among modern bread, durum wheat and triticale in grain selenium concentration. Zhao *et al.* (2009) investigated about 150 lines of bread wheat from different origins and stated that grain selenium concentration was not affected by genotype. In barley, Lyons *et al*. (2005a) and Genc *et al*. (2005) found barley varieties had no significant difference in grain selenium concentration.

With regards to the current study, species and variety of wheat in the NUE-CROPS (winter wheat varieties) and HMC trials (spelt varieties) had significant differences in on grain selenium concentration. As observed in the NUE-CROPS trial without any input of selenium fertiliser, both longer and shorter straw winter wheat variety groups had grain selenium concentrations ranging from 0.027 mg/kg to 0.037 mg/kg. Grain selenium concentration in the longer straw group (32.7 µg/kg to 37.3 µg/kg) was much higher than in the shorter straw group (27.3 µg/kg to 31.8 µg/kg) and this could be due to the dilution effect as a result of lower grain yield. This was also found by Poblaciones *et al*., (2014a, b) and Manojlović *et al*. (2019), who found that lower yield was likely responsible for high grain selenium concentration in wheat through a dilution effect. Indeed, the selenium concentrations range reported here in grain are considered low for common wheat in the UK, likely due to low selenium concentration of the soil (Broadley *et al*., 2006). The low grain selenium concentration of common wheat, <0.03 mg/kg, observed in the NUE-CROPS and QLIF field trials also compares with the grain selenium concentrations of control treatments (without added selenium) from several selenium biofortification studies in the literature (Curtin *et al*., 2006; Curtin *et al*., 2008; Ducsay *et al*., 2009; Ducsay *et al*., 2016; Manojlović *et al*., 2019; Zou *et al*., 2019). Table 6.23 shows several selenium biofortification studies for bread wheat conducted in selenium deficient regions and consistently showing low selenium concentration in wheat grain without added selenium of below 0.5 mg Se/kg. Grain selenium concentration values in this table are close to results presented for the NUE-CROPS and QLIF field trials. The results are also in line with Hussain *et al*. (2010), who observed the concentration of grain selenium did not differ significantly among common wheat such as winter and spring wheat.

Table 6.23. Average mean grain selenium concentrations for *Triticum aestivum.* L in selenium biofortification studies carried out in field trials.

Wheat species	Country	Grain Se concentration (mg/kg)*	Reference
Wheat	New Zealand	0.03	Curtin *et al*., 2006
Wheat	New Zealand	0.01 - 0.03	Curtin *et al*., 2008
Wheat	China	0.03 - 0.45	Zou *et al*., 2019
Wheat	India	0.03 - 0.54	Zou *et al*., 2019
Wheat	Pakistan	0.03 - 0.26	Zou *et al*., 2019
Wheat	Mexico	0.02 - 0.03	Zou *et al*., 2019
Wheat	South Africa	0.02 - 0.09	Zou *et al*., 2019
Wheat	Turkey	0.004 - 0.07	Zou *et al*., 2019
Wheat	Brazil	0.018	Lara *et al*., 2019
Winter wheat	Slovak Republic	0.03	Ducsay *et al.*, 2016
Winter wheat	Croatia & Serbia	0.03 - 0.15	Manojlović *et al*., 2019
Spring wheat	Slovak Republic	0.05 - 0.05	Ducsay *et al*, 2009

** Average mean grain selenium concentration in the control plots i.e. without selenium supplementation.*

Meanwhile, in the HMC trial, the spelt varieties Rubiota, Oberkulmer and Filderstolz all had grain selenium concentrations > 0.05 mg/kg. The higher grain selenium concentration in spelt than common wheat is also supported by results of the RDA analysis in this study, which indicate a strong association a grain selenium concentration with spelt. This helps support recent interest in ancient wheat species, including spelt particularly in Europe and North America, because of its agronomic and nutritional attributes, particularly protein content and micronutrients such as selenium (Kohajdová and Karovicová, 2008; Konvalina *et al*., 2014). In addition to selenium, other minerals, for example magnesium and calcium, are also reported to be higher in spelt (Gomez-Becerra *et al*., 2010) and wild ancient wheat species than common wheat (Bálint *et al*., 2001). It is well reported that other ancient wheat species such as einkorn and emmer have been observed with high grain selenium concentration (Zhao *et al.*, 2009). Increasing interest in ancient species is also associated with increased demand for traditional food products, which is encouraged by the demand for increased genetic diversity and the need to preserve and grow suitable species in marginal areas (Lacko-Bartošová and Čurná, 2015). In general, selenium accumulation and uptake differs widely between plant species (Haug *et al*., 2007). Overall, it is clear from the HMC trial that spelt had greater grain selenium concentrations than common bread wheat. Based on this finding, the use of ancient species grain products rich

in selenium by the global population is likely to be an alternative way to reduce selenium deficiency, increase nutritional security and progressively eradicate selenium malnutrition in humans in the future. Some authors including Ros *et al*. (2016), Broadley *et al*. (2010) and Haug *et al*. (2007) have also suggested that finding alternative ways to increase selenium concentration in foodstuffs is essential for nutritional security of future generations.

Besides that, a review paper on exploiting genotypic variation in plant nutrient accumulation to alleviate micronutrient deficiency in populations (Genc *et al*., 2005) showed that with little genotypic variation in commercial wheat varieties soils with high Se concentration provide an alternative approach as shown by studies in Mexico and South Australia. Several authors including Zhao *et al*. (2009), Manojlović *et al.* (2019) and Lee *et al*. (2011), emphasised have that grain selenium concentration in wheat is less affected by genetic variation but is strongly influenced by soil selenium supply, which differs by geographical location and seasonal conditions. The UK and Europe are among the countries designated as selenium deficient regions which generally show low concentrations of grain selenium <0.05 mg/kg in cereals without supplementation (Broadley *et al*., 2010; Stroud *et al*., 2010ab). Based on soil selenium analysis results from QLIF and NUE-CROPS field trials, total soil selenium concentration was similar at 0.35 mg/kg and 0.38 mg/kg respectively. Borowska *et al*. (2012b) stated that soil selenium concentration below 0.5 mg Se/kg may result in selenium deficient crops and pasture. Ngigi *et al*. (2019) also noticed that the low selenium concentration in foodstuffs was associated with low soil selenium concentration as found in three different location in Kenya, Mbeu (0.37mg/kg) Kiaga (0.39 mg/kg), and Mbuyu (0.46 mg/kg) and related this to insufficient selenium intake in the diet. Therefore, Genc *et al*. (2005) considered that agronomic biofortification with selenium seems a more practical approach than modern crop breeding to effectively enrich the concentration of selenium of wheat grain over a short period.

During recent decades, demand for organic products has been growing (Vrcek *et al*., 2014). Consumers are preferring to choose food with improved quality, higher in minerals and phytochemicals for example organically produced food products (Rembiałkowska, 2007). A small number of former studies have the effects of compared organic and conventional crop protection on nutritional value of cereals, and the conclusions are inconsistent (Vrcek *et al*., 2014). In the literature, some trials have demonstrated that concentrations of minerals such as Al, Cu, Zn, K and Mo were significantly higher in wheat grown under organic than conventional crop protection (Ryan *et al*, 2004; Cooper *et al.*, 2011; Vrček *et al.*, 2014). In contrast, Ca, Mn and Fe were lower in organic than conventional crop protection (Vrcek *et al*., 2014). As well as wheat species and variety, crop protection in the QLIF trial significantly affected grain selenium

concentration of the variety Cordiale. The current study showed that grain selenium concentration was significantly higher following organic than conventional crop protection practices**.** Kwiatkowski *et al*., (2015) also found grain selenium concentration was significantly higher under organic crop protection. The higher grain selenium concentration in organic crop protection is likely at least partly associated with the dilution effect where grain yield was found to be significantly lower.

With regards to fertiliser type in the NUE-CROPS, QLIF and HMC trials**,** grain selenium concentration was significantly higher following application of composted FYM than mineral N fertiliser. Borowska *et al*. (2012a) investigated the availability of total selenium content as influenced by FYM and nitrogen fertilisers in spring barley and also detected that selenium concentration in plant above-ground parts and roots of spring barley was improved by the application of FYM. The higher grain selenium concentration in response to composted FYM application is possibly associated with the dilution effect, since grain yield was much lower than from mineral N fertiliser application. However, composted FYM can be rich in selenium because of selenium supplementation of animal feed (Saha *et al*., 2017), which leads to higher selenium amount in soil (Borowska *et al*., 2012a). Fertiliser application of farmyard manure has also been shown to increase the wheat grain concentration of other minerals such as Zn in organic farming (Helfenstein *et al*., 2016).

6.4.2 Grain yield, quality, TGW and plant height

Grain yield, protein content, TGW and plant height in the NUE-CROPS and HMC trials were significantly affected by wheat species and variety. In the case of yield, variation in the genetic background of wheat species and variety clearly influences grain yield (Hlisnikovský *et al*., 2019). As shown in the NUE-CROPS trial, all the modern short-strawed varieties displayed higher grain yield than the long-straw varieties**.** Between wheat species, common wheat has better grain yield than both durum wheat and spelt (Budzyński *et al.*, 2018). An inferior yield performance of the ancient wheat species spelt, einkorn and emmer compared to modern bread wheat varieties was also described by Hlisnikovský *et al.* (2019) and Konvalina *et al.* (2014). Interestingly, Konvalina *et al*. (2014) identified single accessions of *T. spelta* (such as Kew) with a comparable yield performance to modern varieties.

Wheat species and variety also strongly determine protein content and grain baking quality (Zhang *et al*., 2016; Hlisnikovský *et al*., 2019). The key indicators for bread-baking quality are grain protein quantity and quality (Hildermann *et al*., 2009; Ceseviciene *et al*., 2012).

The most desirable protein content for breadmaking is within the range 10.5 – 13.5% (Mason *et al*., 2007). The range of protein content found in *Triticum spelta* was 12%-19%, much higher than *Triticum aestivum* (Bojňanská & Frančáková, 2002; Winterová *et al*., 2016). In the HMC trial, spelt protein content was much higher (13.7%-15.6%) than that of the long and short straw wheat varieties in the NUE-CROPS trial (9.0% - 12.2%), although these trials were carried out in different seasons. In terms of TGW, spelt also demonstrated higher TGW value than common wheat. TGW value for spelt is between 39.0 g to 43.5 g (Warechowska *et al*., 2013) and for emmer 47.67 g (Lacko-Bartošová and Čurná, 2015). In the present study, TGW value in the HMC trials for spelt was higher (42.8 g - 48.7 g) than in the NUE-CROPS trial for the short (39.6 g – 41.5 g) and long (38.7 g - 42.5 g) straw groups. On the other hand, TGW value for two varieties spring of wheat in the Czech Republic was about 40 g and 41 g (Konvalina *et al*., 2008). TGW of wheat is also greatly affected by weather conditions according to Budzyński *et al.*, (2018).

Published work in previous trials clearly shows that organic crop production resulted in lower yield than conventional crop production (Bilsborrow *et al*., 2013; De Ponti *et al*., 2012; Mazzoncini *et al.,* 2015; Rempelos *et al.,* 2018; Knapp and Van der Heijden, 2018; Röös *et al.,* 2018). The lower yield in organic crop production is generally related to the use of organic forms of fertilisers and non-chemical crop protection practices (Gevrek & Atasoy, 2012). In the QLIF trial, crop protection practices significantly affected grain yield, protein content and TGW of the variety Cordiale. Similarly, environment, soil, variety, and differences in cultivation practices including organic production have been shown to influence grain composition and bread-making quality (Ceseviciene *et al*., 2012). Grain protein content in wheat at grain filling is mainly driven by the amount of available nitrogen in the soil, which is influenced by the amount of N fertiliser used and environmental conditions (Konvalina *et al*., 2014; Zhang *et al*., 2016). In organic crop production, wheat receives less nitrogen fertiliser (also with reduced availability) than in conventional crop production, which results in reduced grain protein content (David *et al*., 2005; Gélinas *et al*., 2009) and bread making quality (Mason *et al*., 2007). It was acknowledged that findings on grain protein content between organic and conventional crop production in past studies are inconsistent. For instance, when comparing organic to conventional, modern wheat varieties are regularly reported to have significantly lower grain protein content under organic crop management (Hildermann *et al*., 2009; Moudrý *et al*., 2011; Konvalina *et al*., 2014). Ceseviciene *et al*. (2012) found significantly lower protein content of winter wheat in organic than conventional production. In the present study, protein content in the QLIF trial using Cordiale, a winter breadmaking variety, was significantly higher under organic than conventional

crop protection. Similarly, research carried out by Bilsborrow *et al*. (2013) observed higher grain protein content under organic than conventional crop protection. David *et al*. (2005) suggested that organic wheat could encompass similar protein content to conventional production by selection of appropriate genotypes and management with organic fertilisers. TGW was found significantly higher under conventional than organic crop protection in the QLIF trial. Similarly, Bilsborrow *et al*. (2013) reported TGW significantly higher with conventional crop protection than with organic crop protection. Other work by Przystalski *et al*. (2008) also observed a higher TGW weight in conventional production than to organic production.

In general, fertiliser type applied in the NUE-CROPS, QLIF and HMC trials gave significant differences in grain yield and protein content. Fertiliser type also had a significant effect on TGW in the QLIF and HMC trials, but not in the NUE-CROPS trial. Černý *et al.* (2010) observed that FYM fertiliser produced lower grain yield of winter wheat than mineral N application. There were two main driving factors of yield reduction: numbers of ears per m^2 and TGW (Mayer *et al.*, 2015). Grain yield and protein content were both significantly higher in response to mineral N than composted FYM application in the NUE-CROPS and QLIF trials. The higher grain yields were also strongly associated with the use of mineral N as shown in the RDA analysis. According to Gopinath *et al*. (2008), mineral N greatly influences grain protein content. A higher grain yield and protein content following mineral N application is explained by the release of inorganic nitrogen which is highly available for plant uptake, and more readily available than in FYM (Bilsborrow *et al.*, 2013; Buchi *et al.*, 2016). It is acknowledged that FYM is low in readily available N (HGCA, 2009). Nutrient release by organic sources (e.g. FYM) rarely matches with peak crop demand over time, as the release of nutrients is highly dependent on temperature and biological activity of the soil (Jones *et al*., 2010).

6.4.3 The relationship between grain selenium concentration, grain yield, protein content and TGW

Correlation analysis in the NUE-CROPS and QLIF trials between grain selenium concentration and grain yield showed a very weak but significant negative correlation. In contrast, a very weak non-significant positive correlation between grain selenium concentration and grain yield was observed in the HMC trial. Morgounov *et al.* (2007) and Garvin, *et al.*(2006) found there was a significant but weak negative relationship between grain yield and mineral concentrations of mineral, for example Fe and Zn. The negative relationship between grain yield and micronutrient concentrations may be a consequence of the dilution effect (Oury *et al*., 2006; Morgounov *et al.*, 2007; Fan *et al.*, 2008). Correlation analysis between grain selenium concentration and protein content showed a significant but weak positive relationship in the NUE-CROPS trial and a significant but weak negative relationship in the HMC trial but with no clear correlation in the QLIF trial. Indeed, several previous studies in the literature have found a significant positive relationship between grain mineral concentration (Fe and Zn) and protein content (Morgounov *et al*., 2007; White and Broadley, 2009; Zhao *et al.*, 2009; Velu *et al*., 2014). According to Oury *et al*. (2006), a moderate correlation between protein content and mineral concentrations could be due to the dilution effect, which would influence both mineral and protein concentrations. Correlation analysis between grain selenium concentration and TGW detected a significant positive relationship in the NUE-CROPS trial, a very weak non-significant positive relationship in the HMC trial and a very weak non-significant negative relationship in the QLIF trial. A very weak or non-significant relationship between grain selenium concentration and TGW was also observed by Zhao *et al.* (2009), who investigated variation in grain mineral micronutrient concentrations of bread wheat, durum, spelt, einkorn and emmer.

6.5 Conclusions

As an overall conclusion, the HMC, NUE and QLIF trial results showed that wheat species and variety, fertiliser type and crop protection can all influence grain selenium concentration, grain yield, quality (protein content) and TGW. Spelt wheat had higher grain selenium than common wheat when concentrations were compared across the different trials, which offers the potential for increased production of spelt and other ancient cereals such as emmer and einkorn for increased nutritional security and human health. Long strawed common wheat varieties had consistently higher grain selenium concentrations than the semi-dwarf varieties grown in the NUE crops trial, with these varieties being more commonly used in organic production to

increase competitiveness against weeds and reduce fungal disease levels. Appropriate selection of wheat species and genotype for future breeding programmes might contribute to better nutrition of wheat for human use and possibly guarantee future nutritional security. Regarding crop protection, this present study has highlighted a significant higher grain selenium concentration of the variety Cordiale when grown under organic compared with conventional crop protection in the QLIF trial. The use of organic crop protection and of composted FYM fertiliser increased grain selenium concentration compared to conventional crop protection and the use of mineral fertiliser in all three trials. The significant effects of crop protection and fertiliser treatments are likely due to the dilution effect. In this study, there were very weak but significant correlation between grain selenium concentration and yield, protein content and TGW.

Chapter 7 : General discussion

7.1 Introduction

Globally, micronutrient malnutrition has become one of the major challenges in human nutrition. Enhancing wheat grain particularly for selenium concentration is crucial especially in western countries, where wheat is a major component of the diet.

7.2 Grain selenium concentration

According to results presented in field trials (2016 and QLIF), crop protection significantly influenced grain selenium concentration. In both trials the organic crop protection treatment consistently gave higher grain selenium concentrations than conventional crop protection but with lower yield. In agreement with our findings, concentrations of other micronutrient like Zn have also been reported to be higher in organic production with lower yield (Helfenstein *et al*., 2016). The negative relationship between higher of concentrations micronutrients such as Se and Zn and lower yield in organic crop protection is likely associated with the dilution effect (Oury *et al*., 2006; Morgounov *et al.,* 2007; Fan *et al.,* 2008). A dilution effect was also observed by Cooper *et al*. (2011) in conventional crop protection where lower concentrations of micronutrients such as Al and Cu were explained by higher yields.

With respect to organic fertilisers, the field trials (2016 and QLIF) showed that composted farmyard manure produced higher grain selenium concentration than cattle slurry, biogas digestate and mineral N fertiliser. According to Saha *et al*. (2017), animal manure contains of high selenium concentrations because selenium is regularly included in animal feeds as supplements, therefore application composted farmyard manure as organic fertiliser in the soil may increase selenium concentration directly with as well as due to the dilution effect.

In terms of wheat species and variety, results showed that grain selenium concentration was higher in spelt than common wheat, although this evidence came from separate trials in different years. In the HMC trial, the spelt varieties Rubiota and Oberkulmer showed particularly high grain selenium concentrations with values of 57.1 µg/kg and 55.6 µg/kg. All spelt varieties

studied in the HMC trial showed grain selenium concentrations >50.0 µg/kg, with the exception of the variety Zurcher Oberlander Rotkorn (45.3 µg/kg). As a modern spelt variety, Filderstolz (crossed with the high yielding old common wheat variety Maris Huntsman under the spelt breeding programme of Hohenheim University) also showed higher grain selenium concentration (50.8 µg/kg) than common wheat. The NUE-CROPS trial indicated small variation in the selenium concentration of short (27.3-31.8 µg/kg) and long-straw wheat (32.7–34.9 µg/kg) varieties, but these were much lower than in spelt. This result is also consistent with the work of Lyons *et al*. (2005a), who compared ten commercial bread wheats and found that there was little genetic variation in grain selenium concentration. Zhao *et al*. (2009) showed that the higher yielding common wheat varieties contained significantly lower Zn levels than old varieties or landraces which suggests that the dilution effect is a key factor. From these data, it appears as though spelt may have significant genetic variation for grain selenium concentration (but based on a small number of varieties evaluated) and higher than that for wheat. Also, spelt wheat has demonstrated the potential to supply more grain selenium than common wheat to meet human requirements (Zhao *et al*., 2009; Lachman *et al*., 2011). In fact, numerous spelt genotypes also exhibited high grain concentrations of other micronutrients, including Zn and Fe, with a high stability across different environments (Gomez-Becerra *et al*., 2010). Similarly, Cakmak (2008) clearly recommended that wild and primitive wheats provide a valuable genetic resource to supply high concentrations of micronutrients such as Zn compared to common wheat. Genetic diversity within ancient wheat species such as spelt may offer new genetic sources to enhance selenium concentration in grain. Currently, most crop breeding programmes, particularly of modern wheat varieties, focus on an increase in yield rather than quality (Dos Reis *et al*., 2017). Therefore, the genetic variation found within spelt has the potential to initiate new selenium-rich foods for human consumption and the potential to breed for high selenium concentration in common wheat.

Selenium biofortification in field and glasshouse trials resulted in a significant increase in grain selenium concentration with clear effects of both rate and source, which is consistent with many selenium biofortification studies (Curtin *et al*., 2008; Broadley *et al*., 2010; Boldrin *et al*., 2013; Poblaciones *et al*., 2014a, b; Idrees *et al*., 2018; De Lima Lessa *et al*., 2019). The relationship between selenium fertilisation rate and increased grain selenium concentration is linear (Broadley *et al*., 2010). In the field and glasshouse trials, the greatest grain selenium concentration was observed with the highest application rate (30 g/ha). The field trial also found that grain selenium concentration was greater following soil application than foliar. This result is similar with findings of Boldrin *et al*. (2013), who evaluated the influence of soil and foliar

application of the two selenium forms (selenate and selenite) on rice yield and grain selenium contents and further concluded that soil application was more effective than foliar application. In addition to method and rate of selenium application Curtin *et al*. (2006) and Manojlović *et al*. (2019) showed that increasing yield of crops affects grain selenium concentration through the dilution effect. In the glasshouse trial, soil application at the high rate of 30 g Se/ha boosted grain selenium concentration about 41-fold (1374.6 µg/kg) compared to the control treatment (33.2 µg/kg). In other work in the UK, Broadley *et al*. (2010) observed that the use of the Se-containing fertilisers Top Stock and Selcote Ultra raised grain selenium concentrations and reported that application of 10 g Se/ha improved grain selenium concentration by about 10-fold, compared with the ambient level (24.3 ng Se/g FW). Also, Broadley *et al*. (2010) stated that the application of 20 g Se/ha may achieve grain selenium concentrations of about 400 ng Se/g, which is equivalent to North American grain concentrations. The UK generally imports a significant amount of high protein content quality wheat from North America which contains higher selenium contents than wheat grown in the UK and this is generally blended in the final grist (Adams *et al*., 2002; Tamás *et al*., 2010). A reduction of wheat imported from North America has significantly exposed British and Europe people to increased risk of selenium malnutrition (Tamás *et al*., 2010) since locally grown wheat supplies about 85% of the flour used in many baking products (Adams *et al.*, 2002). Generally, the present study in the field and glasshouse trials demonstrated that grain selenium concentration of bread wheat can be enhanced by both soil and foliar Se application.

There are several granular selenium containing fertiliser products presently available commercially in selenate form for use in the UK, such as Top Stock (0.0012% Se, in Na_2SeO_4 form, Yara UK) and Selcote Ultra (1% Se; Nufarm NZ, Auckland, New Zealand, a 75:25 $BaSeO_4$:Na_2SeO_4 compound), and these products are currently applied to grass and other forage crops (Broadley *et al*., 2010; Ramkissoon *et al*., 2019). In addition, products from YARA including Yara Bela Nutri Booster, Yara Mila Stock Booster and Yara Mila Silage Booster, containing sodium selenate of about 0.0015 % are also available (YARA, 2020). This study used the Yara Bela Nutri Booster product in both the field and glasshouse trials in 2018. Soil application at the highest rate of 30 g Se/ha in the field trial resulted in grain selenium concentration of 907.5 µg/kg, which is four-fold higher than in control plots without selenium supplementation (187.5 µg/kg). In other studies, grain selenium concentration in UK wheat grown without selenium fertiliser was generally much lower at < 50 µg Se/kg (Adams *et al*., 2002; Hawkesford & Zhao, 2007; Stroud *et al.*, 2010a). In agreement, results observed in the QLIF, HMC and NUE-CROPS trials without selenium fertiliser were similar at <57 µg/kg. The

grain selenium concentration in the control plots of the 2018 field trial are high compared to other studies/trials and may have been due to the very low grain yield of 1.9 t/ha which is especially low compared to the UK national average yield for wheat of around 8 t/ha (DEFRA, 2016; AHDB, 2019). Grain selenium concentration can be grouped as deficient (25 µg/kg), marginal (25-40 µg/kg), moderate to high (40-1000 µg/kg) and excessive (>1000 µg/kg) (Tamás *et al*., 2010). As noticed in the current study, sufficient dietary intake by humans is 50–55 µg Se/day according to the Reference Nutrient Intake and the value of grain selenium concentration considered to be sufficient for human consumption is 0.1 mg/kg (100 µg/kg) (Ramkissoon *et al*., 2019). Therefore, this study has indicated that selenium fertilisation at a rate of 30 g Se/ha through soil application using Yara Bela Nutri Booster product to a high-yielding UK wheat grown variety (Mulika) could generate a grain selenium concentration in the field of 907.5 µg/kg, which is classified as moderate to high (40-1000 µg/kg) but with low yield (1.9 t/ha). However, this result is based on one growing season (2018).

In response to low yield recorded in the present field trial at Nafferton farm in 2018, numerous studies in the previous literature (Poblaciones *et al*., 2014a, Ducsay *et al*., 2016, Nawaz *et al*., 2017 and Manojlović *et al*., 2019) observed higher selenium concentration in grain wheat with low yield or vice versa, generally associated with a dilution effect. Nevertheless, Poblaciones *et al*., (2014a) observed that the higher grain yield of wheat in 2011-2012 compared to 2010-2011 contributed to lower selenium accumulation. Poblaciones *et al*., (2014a) suggested that if selenium accumulation in grain is presented in mg/ha i.e., grain yield multiplied by total selenium concentration (µg/kg) this would negate the dilution effect. In this case, Poblaciones *et al*., (2014a) found that grain selenium concentration still higher in the growing season (2010-2011) under severe and lengthy drought period which on that basis discounted the dilution effect. It might be worth calculating and presented selenium accumulation in g/ha to considerate the dilution effects. A simple prediction would be that if there is a dilution effect, there will be less variation in selenium content per hectare than per kg. In the recent field trial at Nafferton farm in 2018 (Chapter 5), grain yield was observed lower than 2.3 t/ha (Table 5.10), it could be that a possible dilution effect was resulted to higher grain selenium concentration (Table 5.9 (a)). Instead of dilution effect, Poblaciones *et al*., (2014a) also assumed that low water availability might influence selenium uptake and finally result in low grain selenium accumulation. The author also mentioned about variability and amount of rainfall distribution during the growing season might result in differences in selenium uptake and grain selenium accumulation after fertilisation. In order to maximise successful selenium biofortification, Poblaciones *et al.* (2014a) suggested that weather condition particularly rainfall must be given additional consideration

especially days prior to fertilisation application. This standpoint by Poblaciones *et al.* (2014a) also agreed by Rodrigo *et al*. (2014) who noticed that the amount of rainfall during the day before selenium fertilisation demonstrated the inverse correlation with selenium uptake and later accumulation. In the present study, the rainfall in the summer 2018 was recorded as low (DEFRA, 2019) and resulted in higher grain selenium concentration and low yield. Therefore, further investigation of dilution effect to see the response of grain selenium concentration is needed, to discover what selenium concentration is achieved when an average grain yield of 6 t/ha for spring wheat is achieved. Additional consideration about influence of local environmental conditions such as rainfall distribution on selenium uptake and grain selenium accumulation also need to be emphasised in future studies.

The effects of soil and foliar application methods on grain selenium concentration and yield were further studied using a systematic review and meta-analysis. According to Haidich (2010), meta-analysis may provide outcomes with a more precise estimate of the treatment effect than any individual study via the pooled analysis used, and that examination of variability (heterogeneity) in the study is a critical outcome. The results from the forest plot analysis (Fig.3.2 and Fig.3.3 in section 3.3.3.1) showed pooled meta-analysis of multiple selenium rates under soil and foliar applications from across studies gave significant results when compared to control treatments. This finding suggested that grain selenium concentration had significant effects by the multiple selenium rates under soil and foliar applications without affecting yield of cereal crops. This current review found a high quality of evidence in the included primary studies with low risk of bias and non-existence of publication bias. Even though there are various reviews associated with crop production using the systematic review and meta-analysis method, to the best of our knowledge, a review particularly about the effects of soil and foliar applications on grain selenium concentration and yield in cereal has not been done before. Noticeably, the majority of reviews on selenium have prioritised selenium dietary intake linked to human health effects.

Overall, it seems that the current findings may contribute to addressing and reducing selenium deficiency among UK people. Also, bread-making wheat would be a suitable candidate to be included in future selenium biofortification programme in the UK.

7.3 Grain yield

Selenium fertilisation in present field and glasshouse trials showed no significant effect on grain yield and yield components, which is consistent with findings from numerous selenium biofortification studies of wheat (Broadley *et al*., 2010; Chu *et al.*, 2013; Poblaciones *et al*., 2014a, b; Ducsay *et al*., 2016), rice (De Lima Lessa *et al*., 2019), maize (Wang *et al*., 2013), rapeseed (Seppänen *et al*., 2010) and buckwheat (Jiang *et al*., 2015). But, several studies also verified that selenium fertiliser may enhanced the grain yield of maize (Wang *et al*., 2012), wheat (Nawaz *et al*., 2015) and rice (Zhang *et al*., 2014). Therefore, based on the above findings, selenium supplementation of crops by either soil or foliar application has varying effects on yield.

Crop protection is one of the key areas of concern in sustaining long-term yield targets in sustainable arable crop production without compromising environmental impacts (Bilsborrow *et al*., 2013). Various positive effects on a range of environmental aspects offered by organic compared to conventional crop protection including soil quality, above and below-ground biodiversity, soil erosion, soil carbon stocks and reduction in global warming potential, are undeniable. Hence, strategies to improve yield and provide increased yield stability in the absence of or with reduced use of conventional pesticides is crucial (Knapp & van der Heijden, 2018). It is acknowledged that a yield gap exists between organic and conventional crop production. The lower yield in organic than conventional crop production is consistent and has been highlighted in many prior studies in the literature (Cooper *et al*., 2011; Bilsborrow *et al*., 2013; Palmer *et al*., 2013; Swain *et al*., 2014; Mazzoncini *et al*., 2015; Knapp and van der Heijden, 2018; Röös *et al*., 2018)**.** One of the most critical management areas in organic crop production is nutrient management because mineral N inputs are prohibited (Gopinath *et al*., 2008). Reduction of inputs of fertiliser and pesticides and applying longer crop rotations has contributed to the lower and variable yield under organic crop protection (Wolfe *et al*., 2008). Both the N source (2016) and QLIF field trials showed a significantly lower yield in organic than conventional crop production. The yield variation in previous studies is also influenced by experiment site, year and management system (Mader, 2002; Mason *et al*., 2007; Murphy *et al*., 2007; Przystalski *et al*., 2008; Annicchiarico *et al*., 2010; Jones *et al*., 2010). Palmer *et al.*, (2013) examined the effects of organic and conventional crop production of potato and found that the yield gap between these production systems is mainly caused by differences in fertilisation practices. Organic fertilisers used in the current study (biogas digestate, FYM and cattle slurry) might influence the grain yield of wheat. The RDA analysis across three trials (QLIF, HMC and NUE-CROPS) showed mineral N fertiliser produced higher yield than the organic fertilisers

used. Fertiliser timing in organic crop production is more difficult than conventional crop production as N is more readily available from mineral fertiliser than organic fertiliser sources (Knapp & van der Heijden, 2018). Notably, the release of mineral N from organic fertiliser inputs is delayed and mostly depends on the mineralisation rate, which is derived from microbial activity in the soil (Pang & Letey, 2000; Seufert *et al*., 2012). Also, the maximum rate for organic N fertiliser inputs in cereal and grass fertilisation in the UK and Europe according to the EU Environmental Legislation (EC No. 834/2007) is strictly limited to 170 kg N/ha/year, which seems inadequate to satisfy the requirements of modern short-straw wheat varieties (Tétard-Jones *et al*., 2013).

Among the organic fertiliser inputs employed in the N source and HMC trials, biogas digestate showed the potential for higher yield than the more commonly used organic fertilisers FYM and cattle slurry. The results presented in this study also agreed with findings by Makádi *et al.* (2012) that digestate application resulted in higher yields of spring and winter wheat than slurry and farmyard manure treatments. Surprisingly in the HMC trial, biogas digestate fertiliser displayed a significantly higher yield than the mineral N fertiliser application likely due to the highly available and higher NH_4-N concentration. Nkoa (2014) explained that digestate was similar in effectiveness to mineral N fertiliser according to findings from several studies worldwide. The comparable results of yield displayed by biogas digestate and mineral N fertiliser application in this study suggest that it is a very good substitute for mineral N fertiliser application for future more sustainable cereal production. Therefore, strategies to encourage the recycling and utilisation of biogas digestate should be encouraged to provide increased availability to farmers, and reduce the carbon footprint of crop production (Röös *et al*., 2018).

7.4 Grain quality

At present, cereal foods are not only essential for human basic needs but grain quality is critical for promoting and maintaining health and mental conditions as well as disease avoidance (Oliveira *et al*., 2015; De Vita *et al*., 2017). In this context, aspects of quality parameters in selenium biofortification studies have gained interest from a number of researchers in recent decades (Lyons *et al*., 2004; Rodrigo *et al*., 2014; Ducsay *et al*., 2016; Żuk-Gołaszewska *et al*., 2016; De Vita *et al*., 2017). However, the availability of information about the effects of selenium biofortification on grain quality parameters of bread-making wheat is scarce (Poblaciones *et al*., 2014a). Most previous selenium biofortification studies are more focused on protein content (Rodrigo *et al*., 2014; Poblaciones *et al*., 2014a, b; Lidon *et al*., 2018b; Manojlović *et al*., 2019),

which is commonly used as a quality parameter to assess the nutritional value of wheat, rice and legumes (Fang *et al*., 2008). In general, a few authors have considered the effect of selenium fertilisation on quality traits (hectolitre weight and falling number) and found non-significant effects (Lyons *et al*., 2004; Poblaciones *et al*., 2014b ; Ducsay *et al*., 2016; De Vita *et al*., 2017). This is also in accordance with the results of the present trials in the field and glasshouse. Selenium application however, has been found to improve crop quality parameters such as amino acid content (Xia *et al*., 2012), soluble sugar (Zhao *et al*., 2010) and protein (Hu *et al*., 2002).

Rembiałkowska (2007) and Lairon (2010) noticed that numerous studies concentrating on nutritional differences between organic and conventional production have led to various findings. In the current study, grain protein content from the observed trials were different. For instance, organic crop protection gave significantly higher grain protein contents than conventional crop protection in the QLIF trial. In contrast, grain protein content was reported to be significantly higher under conventional than organic crop protection by Iannucci & Codianni, (2016) and Rembiałkowska (2007). Meanwhile, the N source field trial showed no effect of crop protection on grain protein content. This result is in agreement with findings by both Mazzoncini *et al*., (2015) who observed non-significant effects of crop protection treatment on protein content, and Mason *et al*. (2007), who investigated the effects of organic and conventional wheat production on Canadian Western Hard Red Spring on breadmaking quality.

With regards to fertiliser type, it was observed that biogas digestate has the potential to improve grain protein content compared to mineral N fertiliser application. Weiland (2010) and Makádi *et al*. (2012) found that digestate was capable of improving protein content due to the short-term fertilisation effect, whereas composted farmyard manure fertiliser application increased grain selenium concentration of wheat as shown in Chapter 4 and Chapter 6 (HMC trial) but more likely by the dilution effect.

Genetic variation between wheat crop species and variety was associated with clear differences in grain protein content. In the HMC trial, spelt varieties produced significantly higher grain protein content (13.7%-15.6%), which is important for high quality bread making products. In the NUE-CROPS trial, the long-straw winter wheat varieties exhibited significantly higher grain protein content (10.5%-12.2%) than the short straw varieties (9.0%-9.5%). RDA analysis in this study showed protein content was more strongly associated with spelt than common wheat. Current findings demonstrated that spelt consistently produced higher grain protein content than common wheat, which is consistent with earlier studies (Konvalina *et al.*, 2010; Jablonskytė-Raščė *et al*., 2013; Warechowska *et al*., 2013; Winterová *et al*., 2016; Biel *et al*., 2016). Among the spelt varieties, the landrace Oberkulmer gave the highest grain protein

content. Oberkulmer is a well-known spelt landrace with excellent grain quality, particularly protein content (Biel *et al.,* 2016). Spelt has key advantages under low input and more-marginal planting conditions, requiring lower pesticide and fertiliser inputs than common wheat (Kohajdová and Karovicová, 2008). In Europe, a very interesting prospect for spelt has been the progressive increase in the organic farming area (Konvalina *et al*., 2010). Recently, the total land grown with spelt in Europe is approximately 60,000 ha with major areas now in Switzerland, Germany and Austria (Budzyński *et al*., 2018). Many countries including Austria, Belgium, Italy, Czech Republic, Germany, Spain, Slovenia and Switzerland consume spelt as a niche product and customers perceive that spelt grain is healthier than common wheat (Winterová *et al*., 2016). In the baking industry, spelt flour has become a valuable raw material for a wide range of food products: pasta products, muesli, breakfast cereal (flakes) and artisan bread (Bojňanská & Frančáková, 2002). As a minor cereal in the UK with high nutritional advantages, new market potential for spelt flour products in the baking industry exists. Spelt is now valued and gradually gaining the attention of organic growers with the potential for attracting a premium over common wheat. Also, there is active research collaboration with European partners in research projects including Healthy Minor Cereals (HMC) which has focused on the minor cereals spelt, rye, oat, einkorn and emmer.

7.5 Limitations of the study

This study has limitations associated with weather and field trial conditions at Nafferton Farm during the 2016-2018 growing seasons. Initially, the two year field trials at Quarry field were scheduled to run for the fertility trial (Chapter 4) in 2016 and 2017, and selenium biofortification trial (Chapter 5) in 2017 and 2018. However in 2017, we were faced with difficult spring conditions (e.g. Beast from the East) which resulted in poor germination and crop establishment, with later prolonged hare/rabbit damage during grain fill. All measured and recorded datasets for both trials in 2017 were unusable and data is not presented in this thesis. The failure of the fertility trial in 2017 resulted in the trial being repeated in the growing season 2018. Unfortunately, due to very low germination rates caused by poor quality seedbed and plant establishment, all measured and collected data for the fertility trial in 2018 are also not presented in this thesis. With the lack of good quality data in 2017, a glasshouse trial at Cockle Park farm was conducted in 2018 to run alongside the field trial. Both field and glasshouse trials showed consistent results even though they were conducted under different growing conditions. Results

shown in Chapter 4 and Chapter 5 of this thesis are therefore based on single year trials in 2016 and 2018. Therefore, conclusions made in both chapters are limited.

Chapter 8 : Conclusions and future work

8.1 Conclusions

Overall, the findings from this study showed a clear potential to increase grain selenium concentration in wheat through agronomic management and selenium fertilisation. Organic crop protection with lower yield also gave higher grain selenium concentration and protein content. This relationship is associated with dilution effects. Application of organic N fertilisers such as biogas digestate and composted farmyard manure can help increase grain selenium concentration sustainably. Biogas digestate and composted farmyard manure use also aids agricultural recycling. Importantly, sustainable intensification through adoption of biogas digestate and composted farmyard manure as novel plant nutrient sources in fertilisation practices may improve environment quality. Selection and exploiting of available genetic variation in selenium concentration in the production of spelt (as an alternative to common wheat) and the presence of varietal variation in Se concentration, for example in the landrace Oberkulmer, provides the potential to increase Se supply for human nutrition and health. Selenium fertilisation may improve grain selenium concentration without affecting yield and bread making quality of wheat. Method and rate of selenium application influenced the magnitude of effects of interventions on grain selenium accumulation. This method would provide a short-term solution and safe method to enrich selenium content of wheat rather than the longer-term route via plant breeding. As a major food crop consumed intensively by the global population, wheat is considered as the most efficient accumulator for humans to acquire selenium in the diet.

8.2 Future work

- Evaluate other ancient wheat species such as einkorn and emmer for their selenium concentration.
- Screen spelt genotypes for Se concentration to try and identify varieties with the ability to maintain a high Se concentration in the grain.

- Carry out feeding trials to look at Se bioavailability to humans, for example assessing different human Se daily dietary intake levels to achieve optimal blood plasma or serum Se concentrations and correlate with human health status.
- Investigate further the dilution effect to see to what extent high grain Se correlates with low grain yield.

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Appendix A:A decimal code for the growth stages of cereals (Zadoks, 1974)

2-digit code	General description	2-digit code	General description
	Germination		***Stem elongation***
00	Dry seed	30	Pseudo stem erection
01	Start of imbibition	31	1st node detectable
02	—	32	2nd node detectable
03	Imbibition complete	33	3rd node detectable
04	—	34	4th node detectable
05	Radicle emerged from caryopsis	35	5th node detectable
06	—	36	6th node detectable
07	Coleoptile emerged from caryopsis	37	7th node detectable
08	—	38	—
09	Leaf just at coleoptile tip Seedling	39	Flag leaf ligule/collar just visible
	Seedling growth		***Booting***
10	First leaf through coleoptile	40	—
11	First leaf unfolded	41	Booting Flag leaf sheath extending
12	2 leaves unfolded	42	—
13	3 leaves unfolded	43	Boots just visibly swollen
14	4 leaves unfolded	44	—
15	5 leaves unfolded	45	Boots swollen
16	6 leaves unfolded	46	—
17	7 leaves unfolded	47	Flag leaf sheath opening
18	8 leaves unfolded	48	—
19	9 leaves unfolded	49	First awns visible
	Tillering		***Inflorescence emergence***
20	Main shoot only	50-51	First spikelet of inflorescence just visible
21	Main shoot and 1 tillers	52-53	1/4 of inflorescence emerged
22	Main shoot and 2 tillers	54-55	1/2 of inflorescence emerged
23	Main shoot and 3 tillers	56-57	3/4 of inflorescence emerged
24	Main shoot and 4 tillers	58-59	Emergence of inflorescence completed
25	Main shoot and 5 tillers		
26	Main shoot and 6 tillers		
27	Main shoot and 7 tillers		
28	Main shoot and 8 tillers		
29	Main shoot and 9 or more tillers		

2-digit code	General description	2-digit code	General description
	Anthesis		***Dough development***
60-61	Beginning of anthesis	80	—
62	—	81	—
63	—	82	—
64-65	Anthesis half-way	83	Early dough
66	—	84	—
67	—	85	Soft dough
68-69	Anthesis complete	86	—
	Milk development	87	Hard dough
70	—	88	—
71	Caryopsis water ripe	89	—
72	—		***Ripening***
73	Early milk	90	—
74	—	91	Caryopsis hard (difficult to divide by thumb-nail)
75	Medium milk	92	Caryopsis hard (can no longer be dented by thumb-nail)
76	—	93	Caryopsis loosening in daytime
77	Late milk	94	Over-ripe, straw dead and collapsing
78	—	95	Seed dormant
79	—	96	Secondary dormancy induced
		97	Seed not dormant
		98	Secondary dormancy induced Secondary dormancy lost
		99	Secondary dormancy lost