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RESEARCH ARTICLE

Increasing grain selenium concentration via genetic and agronomic innovations

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Abstract
Aims To evaluate the potential to enhance grain Selenium (Se) concentration in wheat through agronomic innovation practices and exploitation of existing genetic variation.

Methods Grain samples from field experiments carried out as part of the EU projects Nitrogen Use Efficiency (NUE-CROPS), Healthy Minor Cereals (HMC) and Quality Low Input Food (QLIF) were analysed to identify the effects of wheat species/variety, fertiliser type and crop protection regime on grain yield, grain protein and selenium concentrations.

Results Fertiliser type significantly affected grain Se concentration. In the NUE-CROPS and QLIF trials the use of farm-yard manure (FYM) resulted in significantly higher grain Se concentration when compared with mineral fertiliser applied at the same N input level. Similarly, in the HMC trial, FYM and cattle slurry resulted in a significantly higher grain Se concentration compared with biogas digestate and mineral fertiliser. In the QLIF trials, organic crop protection resulted in significantly higher grain Se concentration when compared with conventional crop protection. The NUE-CROPS and HMC trials detected significant differences between varieties of both common wheat (*Triticum aestivum*) and spelt (*T. spelta*). Correlation analyses across the trials identified a negative correlation between yield and grain Se concentration for spelt and positive correlation

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between plant height and Se concentration for both species.

Conclusions Higher Se concentrations in the taller spelt varieties suggest that there is considerable potential to breed/select for high grain Se by exploiting traits/genetic variation present in older, traditional wheat species (e.g. spelt).

Keywords Cereals · Genetic variation · Agronomic management · Nutritional security

Abbreviations

BD	Biogas digestate
Ca	Calcium
CCC	Chlormequat
CON-CP	Conventional crop protection
CON-FM	Conventional fertility management
CS	Cattle slurry
Cu	Copper
Fe	Iron
FYM	Farm-yard manure
HMC	Healthy Minor Cereals
ICP-OES	Inductively coupled plasma optical emission spectropheter
K	Potassium
MN	Mineral nitrogen
N	Nitrogen
NUE-CROPS	Nitrogen Use Efficiency
ORG	Organic
ORG-CP	Organic crop protection
ORG-FM	Organic fertility management
pRDA	Partial redundancy analysis
P	Phosphorus
QLIF	Quality Low Input Food
QTL	Quantitative trait loci
RDA	Recommended daily allowance
RDI	Recommended daily intake
Se	Selenium
TGW	Thousand grain weight
UK	United Kingdom
US	United States of America
WHO	World Health Organisation
ZOR	Zurcher Oberländer Rotkorn

Introduction

Selenium (Se) is an essential micronutrient in both animals and humans for which wheat is a major dietary source (Gómez-Galera et al. 2010; Nelson et al. 2011; Vrček et al. 2014; Singh et al. 2016; Shewry 2018; Del Coco et al. 2019; Hlisnikovský et al. 2019). Although people can increase their selenium intake with food diversification from various types of main food sources including fish, meat, fruit and vegetables (Adams et al. 2002; Hawkesford and Zhao 2007), wheat has been shown to be a more efficient accumulator of bioavailable selenium than other crops (Poblaciones et al. 2014; Sharma et al. 2017). Selenium is relatively evenly distributed in the wheat grain, with the highest concentrations found in the embryo (Lyons et al. 2005a) which is in contrast to other minerals (Ca, Cu, Fe, Mg and Zn) which are concentrated in the outer layers of the grain and therefore largely lost during refining (Wang et al. 2020, 2021).

Selenium is an essential component of several enzymes (e.g. glutathionine peroxidase, thioredoxin reductase and deionase enzymes) in animals and Se-deficiency in humans and livestock is widespread, especially in regions with low soil Se-concentrations (e.g. many soils in Northern, Central and Eastern Europe, China and New Zealand) (Miller and Welch 2013; Alfthan et al. 2015; Reis et al. 2018). Soils have been defined as Se-deficient when concentrations are below 0.6 mg Se kg^{1} soil (Mora et al. 2015) or between 0.01 and 2.0 mg kg^{-1} soil (Govasmark et al. 2008). The UK and many other Northern European regions have been classified as Se-deficient regions (Zhao et al. 2005; Rahman et al. 2013; Wu et al. 2015). In contrast, regions with Se-rich soils include Canada, Colombia, Venezuela and the great plains of the US where soil Se concentrations of up to 10 mg kg^{-1}, have been recorded (Rahman et al. 2013).

It has been estimated that around one billion people are affected by Se deficiency globally (Mora et al. 2015; Jones et al. 2017; Reis et al. 2018). Se-deficiency has been linked to a range of negative health impacts including Keshan disease, Kashin-Beck disease, increased viral virulence, lower immune function, reduced fertility, thyroid autoimmune disease, cognitive decline/dementia, type-2 diabetes and certain cancers (Rayman 2005, 2020; Steinbrenner et al. 2015). Several organisations have published recommended daily intake values for Se. The World Health Organisation (WHO) suggests a daily intake of Se for adults of between 30 and

40 µg per day (Oliveira et al. 2015) and both the EU Recommended Dietary Allowance (RDA) and the US Recommended Daily Intake (RDI) for humans is ~ 55 µg Se day^{-1} (Poblaciones et al. 2014; Stoffaneller and Morse 2015). The Food and Nutrition Board of the US National Academy of Science recommends daily Se intakes of between 40 and 70 µg for men (40–70 µg), 45 and 55 µg for women and 15 and 20 µg for children (El-Bayoumy 2001).

Plants can take-up Se as selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) from the soil (Hawkesford and Zhao 2007; Carey et al. 2012; Deng et al. 2017) with selenate being more soluble, mobile and bioavailable than selenite (Poblaciones et al. 2014). Selenate is chemically similar to sulphate (SO_4^{2-}) and uptake from the rhizosphere occurs through the sulphur assimilation pathway via high-affinity sulphate transporters in the root cortex, root tip and lateral roots (Hawkesford and Zhao 2007; Carey et al. 2012; White 2016; Deng et al. 2017; El Mehdawi et al. 2018). High sulphur levels in soil therefore limit Se uptake by plants as both elements compete for the same transporter system (Malagoli et al. 2015). In this context, it is important to consider that sulphur fertilisers have been a regular input to wheat crops in the UK during the last two decades, especially milling wheat where it has been shown to increase grain yield and baking quality (Zhao et al. 2005).

One approach to address Se-deficiency has been to use Se-supplements for animal feeds and/or human diets (Pecoraro et al. 2022; Xia et al. 2005). Selenium is included in mineral supplements that are added to concentrate feeds used in both organic and conventional livestock production and this was shown to result in significant health benefits in animals (Pecoraro et al. 2022). Selenium is also an ingredient in a range of multivitamin supplements and infant formula products (Xia et al. 2005) but clinical trials have not identified clear health benefits of Se-supplements in humans (Myung et al. 2013; Djalalinia et al. 2021). Geographical differences in the availability of soil selenium account for most of the variation in the selenium content of foods. Selenium has a narrow range between deficiency and toxicity for both humans (Kieliszek 2019) and for animals (Schrauzer and Surai 2009).

Another approach used in some countries with low soil Se levels has been to include selenate often as sodium selenate (Na_2SeO_4) as a mineral fertiliser for wheat and other cereal crops (Gómez-Galera 2010; Singh et al. 2016). For example, in Finland, where most soils have a very low Se content and Se deficiency was widespread in both humans and livestock in the past, mineral nitrogen fertilisers are now routinely fortified with sodium selenate (Alfthan et al. 2015). This approach has been described as '*agronomic biofortification*' which is a relatively cheap method to increase concentrations of Se and other mineral micronutrients (e.g. Zn) in harvested products (Gómez-Galera et al. 2010; Fageria et al. 2012). Most research on selenium biofortification has been carried out on vegetables and cereals 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).

Globally, Se concentrations in wheat grain range between 0.001 and 30 mg kg^{-1} but most samples are within the range 0.020–0.600 mg kg^{-1} (Broadley et al. 2006). A number of studies have reported significant reductions in dietary Se-intake of the UK population over time. Adams et al. (2002) reported a decline from 60 µg Se day^{-1} in 1970 to 29–39 µg Se day^{-1} in 1997, and Broadley et al. (2010) reported a reduction from 60 to 32–34 µg day^{-1} between 1985 and 2000. This decline in Se intake is thought to have been primarily due to a reduction in Se concentration of wheat grain over time (Broadley et al. 2010; Stroud et al. 2010a, b; Sharma et al. 2017). More recent surveys by Broadley et al. (2010) and Hart et al. (2011) reported mean grain Se concentration in UK-wheat of 0.028 and 0.030 mg kg^{-1} respectively. Several factors may have contributed to the decline in Se-concentrations in wheat grain. An important contributory factor has been an increase in the use of home-grown wheat (which has a low Se content) for bread-making from ~ 50% in the mid-1970's (Hart et al. 2011) to about 85% today with a corresponding reduction in the use of wheat from the US and Canada which has a much higher grain Se concentration.

There is also evidence that the introduction of modern semi-dwarf varieties and changes in agronomic practices in the UK have also contributed to the decline in Se-concentrations in wheat grain. For example, a study in the US reported that concentrations of a range of mineral micronutrients (including Fe, Zn and Se) were significantly higher in grain of older, longer-straw varieties compared with modern semi-dwarf wheat (*T. aestivum*) varieties with the largest relative difference being recorded for Se (Murphy et al. 2007). Several other studies have suggested that the decline in concentrations of grain mineral concentrations may have been a negative consequence on wheat breeding/selection which has been focused primarily on improving grain yield and to a lesser extent protein content (varieties developed for bread-making) (Cakmak et al. 2000; Gómez-Galera et al. 2010; Ceseviciene et al. 2012; Hejcman et al. 2013; Singh et al. 2016). However, there is relatively limited published information on correlations between grain yield, protein content, straw length and mineral micronutrient concentrations in wheat and virtually no information on interactions between variety and agronomic practices (i.e. crop protection methods and fertilisation regimes) used in wheat production.

It is likely that the trend towards stockless arable production systems (with associated reduction in animal manure inputs) and an increase in the use of mineral NPK fertilisers has contributed to the decline in mineral concentrations in wheat grain. This is mainly because mineral fertilisers contain virtually no Se when compared with animal manures (Sager 2007). However, there is virtually no information on whether and to what extent changing from mineral NPK to livestock manure as fertiliser may increase Se-concentration in cereal grain.

Fageria et al. (2012) described that the cultivation of indigenous and traditional food crops provides a clear opportunity to enhance micronutrient concentrations in the human diet and for cereals this hypothesis is supported by studies which showed that ancient wheat species e.g. spelt (*Triticum spelta*), emmer (*Triticum dicoccum*) and einkorn (*Triticum monococcum*), have higher grain protein and mineral concentrations (Cakmak 2008; Kohajdová and Karovicova 2008; Zhao et al. 2009; Cakmak et al. 2010; Gomez-Becerra et al. 2010; Lachman et al. 2011) compared with modern common wheat (*Triticum aestivum*) varieties. However, the production of spelt, emmer and einkorn has declined due to the introduction of modern free-threshing wheats in the twentieth century which have higher grain yields (Hlisnikovský et al. 2019).

Singh et al. (2016) suggested that the combined use of innovative fertilisation together with exploitation of genetic variation at the wheat species/variety level may provide a more cost effective and sustainable approach to increase the dietary intake of Se and other mineral micronutrients than the use of food fortification and mineral supplements. The overall aims of this study were therefore to evaluate the potential to enhance Se-concentrations in wheat grain through agronomic innovation practices and exploitation of existing genetic variation in wheat and identify potential correlations/trade-offs between grain yield, other crop performance parameters (straw length, thousand grain weight) with grain protein and Se concentration. Our hypothesis is that organic fertilisers, crop protection and variety/species can be used to increase the grain Se concentration of cereals.

Materials and methods

All data presented in this study were collected from field trials which were part of 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). All trials were conducted using medium to long-term trial plots at Nafferton Farm, in north-east England (54:948490 N; 1.913180 W) between 2009 and 2016. The NUE-CROPS and HMC trials were carried out in different parts of the same field while the QLIF trial was located just over 500 m away. The mean soil selenium concentration of the topsoil (0–30 cm) was determined in the QLIF and NUE-CROPS projects immediately after drilling in the first season of each trial and analyses were carried out by NRM Ltd, Bracknell, Berkshire. Mean total soil selenium concentrations for topsoil (0–30 cm) in the QLIF and NUE-CROPS trials were 0.35 mg kg^{-1} and 0.38 mg kg^{-1} respectively.

Experimental design

The NUE-CROPS trial was conducted over two cropping seasons (2009/10 and 2011/12) and was designed to study the effects of fertiliser type, input level, crop protection protocol and variety on yield and resource use efficiency related gene, protein and metabolite expression patterns of winter wheat. 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 (24 m × 24 m) factor, crop protection protocol (conventional vs organic crop protection) as the sub-plot (24 × 12 m) factor and variety as the sub-sub-plot (3 m × 12 m) factor. Details of the crop management protocols are presented in Table S1 with soil data in S2 and a time-line of treatment applications in Fig. S1.

Composted FYM (compositional analysis is presented in Table S3) was applied at a rate equivalent to 170 kg N ha^{-1}, which also resulted in an input of 29.2 kg P ha^{-1}, 323.6 kg K ha^{-1} when applied on 8th April 2010 in the first growing season (2009/10) and 30.3 kg P ha^{-1} and 130.1 kg K ha^{-1} when applied on 11th October 2011 in the 2nd growing season (201,112). Mineral N fertiliser was applied as ammonium nitrate (34.5% N) and at the same total input level (170 kg N ha^{-1}), but as a split application (50:50) to the conventional fertilisation plots in each season.

Eight varieties of winter wheat including four short-straw (semi-dwarf) and four longer straw varieties were compared. This included four short straw varieties commonly grown in the UK i.e. Gallant, Cordiale, Grafton, and Solstice which are all modern varieties listed on the UK Recommended List for Winter Wheat (AHDB 2017). In addition, four longer strawed wheat varieties (Laurin, Scaro, Aszita, and Wima) were included in trials. These were developed for the organic and low-input farming sector in a Swiss breeding/selection programme led by Peter Kunz, which were supplied as organically certified seed by Sativa Biosaatgut GmbH (Keltenweg4, Jestetten, Germany; http://www.sativa.bio).

Crops were sown on 13th October 2009 and 15th October 2011. Conventional crop protection included two fungicide application timings, one autumn herbicide application and one application of the plant growth regulator chlormequat (at T1) in both seasons (Table S1). Organic crop protection was based on mechanical weed control which was carried out twice in spring of each season using an Einböck tine weeder (Einböck GmbH, 4751 Dorf an der Pram, Austria). Crops were harvested at maturity on 1st September 2010 and 4th September 2012.

The QLIF trial consisted of a series of four experiments established within four replicate blocks and each with a split-split-split-plot design and three factors i.e. rotation design (organic vs conventional), crop protection (organic vs conventional) and fertiliser type (cattle manure vs mineral NPK). A detailed description of the QLIF design has been published previously (Eyre et al. 2011; Bilsborrow et al. 2013) such that the trial design and treatments are only described briefly below. The relevant soil data for the trials is presented in Table S2.

The main plot factor was rotation and compared two 8-year crop rotations, specifically an intensive cereal dominated rotation typical for conventional mixed farms in Northern Britain versus a more diverse legume-rich rotation typically used in organic production. The sub-plot factor was crop protection and compared an organic protocol (ORG-CP) which complied with UK requirements for certified organic production (Soil Association 2010) with a conventional protocol (CON-CP) which followed the recommendations of the UK Red Tractor Farm Assurance Scheme (https://redtractor.org.uk/). Each crop protection sub-plot was further split into two fertility management sub-sub-plots i.e. organic (ORG-FM; using composted FYM) and conventional (CON-FM; using mineral NPK fertiliser). This design also allows the experiment to be analysed as four separate production systems: fully organic (ORG), organic crop protection with conventional fertility management (ORG-CP CON-FM), conventional crop protection with organic fertility management (CON-CP ORG-FM), and fully conventional (CON) within each crop rotation.

In the QLIF trial only the Group 2 winter wheat breadmaking variety Cordiale was used (NABIM 2016; AHDB 2017). Cordiale was sown using a commercial drill (3 m Lely combination drill; Lely UK Ltd, St Neots, UK) at a seed rate of 176 kg ha^{-1} on 2nd October 2014 and 180 kg ha^{-1} on 15th October 2015. Mineral N as ammonium nitrate (Yara UK Ltd) was applied at a rate of 180 kg N ha^{-1} in 2015 and 210 kg N ha^{-1} in 2016 with two split applications (Table S1). In the organic fertilisation plots no manure was added when grass/clover (G/C) was the pre-crop, while cattle manure at a total N-input level of 170 kg N ha^{-1} was applied when wheat was grown after winter-wheat or winter barley in the rotation. In the conventional crop protection plots standard herbicide, fungicide and plant growth regulators treatments were applied (Table S4), while the only crop protection treatment used in the organic crop protection plots was mechanical weed control using an Einböck tine weeder.

In the HMC trial spelt was grown in a split-split-split-plot factorial design with 4 replicate blocks in the 2014/15 and 2015/16 growing seasons. The main factors included in the HMC trial were (i) fertiliser type (24 m×6 m), with (ii) N rate i.e. a low and medium rate of 50 and 100 kg N ha^{-1} applied as mineral N, composted FYM, cattle slurry and biogas digestate and (iii) spelt variety (24 m×3 m) including modern varieties and landraces (Oberkulmer, Züricher Oberländer Rotkorn (ZOR), Rubiota and Filderstolz). The previous crop in both seasons was grass/clover. A full description of the experimental design and trial layout is provided in Magistrali et al. (2020) with soil data presented in Table S2, fertiliser compositional analyses in Table S3 and crop management details presented in Table S5 with a time-line of treatment applications presented in Fig. S1.

Plant measurements

Environmental conditions (including radiation, precipitation, air and soil temperature and relative humidity) during the growing season were recorded in all trials using an on-site automated weather station which was located within 500 m of each trial location. At maturity all crops were harvested using a plot combine harvester (Claas Dominator 38; Claas UK Ltd, Bury St Edmunds, UK). Grain samples were retained for moisture determination and a sub-sample oven dried at 45 °C for 72 h prior to cleaning with a grain cleaner (Lainchbury HC1/7W, Blair Engineering, Blairgowrie, UK). Thousand grain weight (TGW) was determined based on the mean weight of three replicates of 1000 grain samples using an electronic seed counter (Model Elmor C3, Switzerland). All grain yields are presented at 15% moisture content. All grain samples were stored under cool conditions prior to chemical analyses.

About 3.0–3.5 g of oven dried grain from all trials was ground with a Retsch SK300 mill (Retsch GmbH, 42781 Haan, Geremany) using a 0.25 mm mesh sieve. All milled grain samples were sent to Sabanci University for grain selenium analysis where approximately 0.2 g of milled grain 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, US). 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 used. All measurement for selenium were then cross-checked using a certified standard (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 Zou et al. (2019).

Grain protein content of all samples was determined using an infrared analyser (Foss, Infratec™ 1241, Foss A/S, Hillerød, Denmark). 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 NRM Ltd for soil selenium analysis.

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) fertiliser 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 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. The '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 Šmilauer 2012).

Results

Effects of harvest year, variety and fertiliser type on common wheat performance (NUE-CROPS trial)

In the NUE-CROPS trial the performance of eight common wheat varieties was compared on land which had been previously managed to conventional farming standards and had not received manure inputs and was not used for grazing livestock for more than 4 years. A two-year grass-clover ley used for silage production was the fertility building pre-crop. The main objective of the experiment was to assess the effect of replacing mineral N-fertilisers with manure at the same total N-input level on performance and quality parameters in contrasting wheat varieties in a conventional crop management background.

There was a significant effect of harvest year on grain Se concentration, TGW, protein concentration and plant height of winter wheat (Table 1). A much higher grain Se concentration was observed in the 2012 growing season (44.8 μg kg^{-1}) than in 2010 (19.6 μg kg^{-1}). The TGW, grain protein concentration and plant height were also significantly higher, but grain yield was much lower in 2012 (3.2 t ha^{-1}) than 2010 (4.9 t ha^{-1}) but not significantly. Fertiliser type exhibited a significant main effect for grain yield, protein concentration, grain Se concentration and plant height but not for TGW. Composted FYM gave a 60% higher grain Se concentration than mineral N. In contrast, protein concentration and plant height were 75%, 10% and 27% lower when FYM was used as fertiliser compared with mineral N.

There was a significant difference between varieties for grain Se concentration, grain yield, TGW, protein concentration and plant height (Table 1). Specifically, the four modern, UK short-straw varieties (Cordiale, Gallant, Grafton and Solstice) produced higher yields, but lower protein and Se concentrations when compared with the four longer straw varieties (Aszita, Laurin, Scaro and Wima). When TGW was compared the highest TGW was recoded for Wima and Grafton and the lowest for Aszita and Laurin (Table 1).

There was a significant harvest year × fertiliser type interaction for grain yield, TGW, protein and Se concentration, but not plant height (Table 1). When these interactions were further investigated, mineral N fertiliser resulted in significantly higher grain yield and protein concentration compared with FYM in the 2010 harvest year, while there was no significant difference between fertiliser types in 2012 (Table 2). In contrast, the TGW was significantly higher following the application of mineral-N in 2010 and following FYM in 2012. However, grain Se concentrations were significantly higher when FYM was used compared with mineral N in both harvest years (Table 2).

Correlation analysis (Table 3) identified significant negative correlations between grain yield and Se concentration ($p < 0.001$; $r = -0.36$), but significant positive correlations between Se concentrations and both TGW ($p < 0.001$; $r = 0.66$) and protein concentration ($p < 0.05$; $r = 0.18$).

Effects of harvest year, crop rotation, crop protection and fertilisation regime on wheat performance (QLIF trial)

The main objective of the QLIF trial was to assess the relative effects of rotation, fertilisation and crop protection used in organic and conventional farming systems on performance and grain quality parameters in the bread-making winter wheat variety Cordiale.

Table 1 Main effects (means ± SE and *p*-values) and interactions of harvest year, fertiliser type and variety on grain yield, thousand grain weight (TGW), grain protein and Se concentration and plant height of common wheat (*T. aestivum*) varieties recorded in the NUE-CROPS trial

Factor	Grain yield (t ha^{-1})	TGW (g)	Protein (%)	Grain Se (µg kg^{-1})	Plant height (cm)
Harvest year					
2010 (*n* = 64)	4.9 ± 0.3	35.9 ± 0.3	10.0 ± 0.2	19 ± 1	58 ± 2
2012 (*n* = 64)	3.2 ± 0.2	44.5 ± 0.3	10.7 ± 0.2	45 ± 2	69 ± 2
Fertiliser type					
FYM (*n* = 64)	2.9 ± 0.2	39.8 ± 0.7	9.8 ± 0.2	40 ± 3	56 ± 2
Mineral N (*n* = 64)	5.1 ± 0.3	40.7 ± 0.5	10.8 ± 0.2	25 ± 2	71 ± 2
Variety					
Cordiale (*n* = 16)	4.3 ± 0.7 ab	39.6 ± 1.3 cd	9.5 ± 0.2 d	30 ± 4 bc	52 ± 2 d
Gallant (*n* = 16)	4.6 ± 0.7 a	40.2 ± 1.0 bc	9.5 ± 0.2 d	30 ± 3 bc	56 ± 2 cd
Grafton (*n* = 16)	4.4 ± 0.7 ab	41.5 ± 1.7 ab	9.0 ± 0.2 e	32 ± 4 ac	51 ± 3 d
Solstice (*n* = 16)	4.4 ± 0.6 ab	39.6 ± 1.0 cd	9.9 ± 0.2 d	27 ± 3 c	61 ± 3 c
Aszita (*n* = 16)	3.5 ± 0.4 c	39.0 ± 1.3 cd	12.2 ± 0.4 a	33 ± 5 ac	73 ± 4 ab
Laurin (*n* = 16)	3.6 ± 0.5 c	38.7 ± 1.1 d	10.5 ± 0.2 c	35 ± 6 ab	69 ± 4 b
Scaro (*n* = 16)	3.6 ± 0.5 c	40.9 ± 1.2 b	10.6 ± 0.3 c	35 ± 5 ab	70 ± 4 b
Wima (*n* = 16)	3.8 ± 0.4 bc	42.5 ± 1.3 a	11.3 ± 0.3 b	37 ± 6 a	76 ± 4 a
ANOVA (*p*-values)					
Main effects					
Harvest year (YR)	NS	< 0.001	< 0.05	< 0.01	< 0.01
Fertilizer type (FT)	< 0.05	NS	< 0.01	0.0002	< 0.001
Variety (VR)	< 0.001	< 0.001	< 0.001	< 0.05	< 0.001
Interactions					
YR x FT	< 0.05	< 0.001	< 0.05	NS	NS
YR x VR	< 0.001	< 0.001	< 0.001	NS	< 0.05
FT x VR	< 0.001	NS	< 0.001	NS	NS
YR x VR x VR	NS	< 0.05	< 0.001	NS	< 0.05

Variety main effect mean/SE values followed by the same letter within each column are not significantly different (Tukey's general linear hypothesis test $p < 0.05$); *NS* Not significant

Grain yield and TGW were significantly higher in 2015 than 2016 but there was no difference between harvest years in grain Se and protein concentration (Table 4). Wheat produced significantly higher yield in the organic compared with the conventional rotation (7.4 vs 6.8 t ha^{-1}) but there was no significant effect on TGW, protein concentration and grain Se concentration. Conventional crop protection resulted in significantly higher grain yields and TGW, but lower grain protein and Se concentrations when compared with organic crop protection (Table 4). In contrast, conventional fertilisation resulted in higher grain yields and protein concentration, but lower TGW and grain Se concentrations compared with organic fertilisation (Table 4) therefore mimicking the trends for the effects of mineral N versus FYM use in the NUE-CROPS trial (Table 1).

There was a significant harvest year × crop protection interaction on grain yield; harvest year × fertiliser type interaction on grain Se concentration, grain yield and TGW and a crop protection x fertiliser type interaction on grain yield, TGW and protein concentration (Table 4). The conventional crop protection treatment produced significantly higher grain yield in both 2015 and 2016 than organic crop protection (Table S6). When the harvest year x fertilisation regime interactions were further investigated a significant effect of fertilisation was detected for (i) crop yield in 2015 only, (ii) grain Se concentrations in 2016 only and (ii) TGW in both years, although the relative difference between fertilisation regimes was larger in 2016 (Table 5). For the crop protection x fertilisation type interaction grain yields were lowest when organic crop

Table 2 Interaction means ± SE for the effect of harvest year (2010 versus 2012) and fertiliser type (Mineral N versus farmyard manure (FYM) applied at the same total N-input level) on grain yield, thousand grain weight (TGW), grain protein and grain Se concentration in eight common wheat (*T. aestivum*) varieties recorded in the NUE-CROPS trial

	Harvest year	Fertiliser type	
		Mineral N	FYM
Grain yield (t ha^{-1})	2010	7.0 ± 0.3 A a	2.8 ± 0.1 B b
	2012	3.3 ± 0.3 B b	3.1 ± 0.3 B b
TGW (g)	2010	37.6 ± 0.4 Ba	34.3 ± 0.3 B b
	2012	43.7 ± 0.4 Ab	45.3 ± 0.4 A a
Protein (%)	2010	10.8 ± 0.3 A a	9.2 ± 0.1 B b
	2012	10.9 ± 0.2 A a	10.4 ± 0.2 A a
Se concentration (μg kg^{-1})	2010	15 ± 3 B b	24 ± 1 B a
	2012	34 ± 2 A b	56 ± 3 A a

Means followed by different lowercase letters within each row and uppercase letters within each column are significantly different (Tukey's general linear hypothesis test $p < 0.05$)

protection was combined with conventional fertilisation and highest when conventional crop protection and fertilisation were used (Table S7). TGW was lowest when organic crop protection was used in combination with conventional fertilisation and highest when conventional crop protection was used with organic fertilisation. Protein concentrations were lowest when organic management (crop protection and fertilisation) was used and highest when organic crop protection was used in combination with conventional fertilisation regimes (Table S7).

Correlation analysis identified significant negative correlation between grain Se concentration and grain yield ($p < 0.01$; $r = -0.33$) but no significant correlation between grain Se concentration and both TGW and protein concentration (Table 3).

Effects of year, variety and fertiliser type on spelt performance (HMC trial)

The main objective of the HMC trial was to assess the effect of replacing mineral N-fertiliser with organic manure at the same total N-input level on the performance and quality of contrasting spelt varieties. Growing season had a significant effect on grain yield, protein concentration and plant height (Table 6). Specifically, grain yield was 31% higher in 2015, while protein concentration and plant height were 21 and 9% higher respectively in 2016 (Table 6).

Fertiliser type had a significant effect on all performance parameters assessed with the exception of plant height (Table 6). The use of mineral N fertiliser (MN) resulted in lower grain yield, TGW and grain Se concentration compared with the organic N sources, although it should be noted that the differences between MN and FYM were not significant for grain yield and TGW (Table 5). In contrast, the use of MN and biogas digestate

Table 3 Correlation coefficient (R-values; *p*-values) from Spearman-Rank correlation analysis testing the relationship between grain selenium (Se) concentration, grain yield, protein concentration, TGW and plant height in the NUE-CROPS, QLIF and HMC trials

Parameters correlated		NUE-CROPS		HMC		QLIF	
		Correlation coefficient	*p*-value	Correlation coefficient	*p*-value	Correlation coefficient	*p*-value
Grain Se (μg kg^{-1})	Yield (t ha^{-1})	−0.36	<0.001	0.15	NS	−0.33	<0.01
Grain Se (μg kg^{-1})	TGW (g)	0.66	<0.001	0.06	NS	−0.02	NS
Grain Se (μg kg^{-1})	Protein (%)	0.18	<0.05	−0.26	<0.01	0.07	NS
Grain Se (μg kg^{-1})	Plant height (cm)	0.15	NS	0.26	<0.01	ND	ND
Yield (t ha^{-1})	TGW (g)	−0.19	<0.05	0.32	<0.001	0.74	<0.001
Yield (t ha^{-1})	Plant height (cm)	0.02	NS	0.47	<0.001	ND	ND
Yield (t ha^{-1})	Protein (%)	0.05	NS	−0.32	<0.001	−0.54	<0.001
TGW (g)	Protein (%)	0.30	<0.001	−0.03	NS	−0.75	<0.001
TGW (g)	Plant height (cm)	0.46	<0.001	0.44	<0.001	ND	ND
Protein (%)	Plant height (cm)	0.70	<0.001	0.17	NS	ND	ND

Negative correlations are preceded by -, *NS* Not significant; *ND* Not determined i.e. plant height was not measured

Table 4 Main effects (means ± SE and *p*-values) and interactions of harvest year, crop rotation, crop protection and fertilisation regime on grain yield, TGW, protein and grain Se concentration in common wheat (*T. aestivum*) variety Cordiale recorded in the QLIF trial

Factor	Grain yield (t ha^{-1})	TGW (g)	Protein (%)	Grain Se (µg kg^{-1})
Harvest year (YR)				
2015 ($n = 32$)	9.8 ± 0.6	50.6 ± 1.0	10.2 ± 0.3	30 ± 1
2016 ($n = 32$)	4.4 ± 0.4	36.4 ± 1.1	11.1 ± 0.4	31 ± 2
Crop rotation (CR)				
Conventional ($n = 32$)	6.8 ± 0.6	43.3 ± 1.7	10.3 ± 0.4	31 ± 1
Organic ($n = 32$)	7.4 ± 0.7	43.7 ± 1.6	10.9 ± 0.4	30 ± 2
Crop protection (CP)				
Conventional ($n = 32$)	9.2 ± 0.7	46.3 ± 1.5	9.5 ± 0.2	27 ± 1
Organic ($n = 32$)	5.2 ± 0.5	40.7 ± 1.6	11.6 ± 0.4	34 ± 2
Fertilisation regime (FR)				
Conventional ($n = 32$)	7.9 ± 0.8	40.5 ± 1.9	11.9 ± 0.4	28 ± 1
Organic ($n = 32$)	6.3 ± 0.5	46.5 ± 1.2	9.3 ± 0.2	33 ± 2
ANOVA *p*-values				
Main effects				
YR	< 0.001	< 0.001	NS	NS
CR	< 0.05	NS	NS	NS
CP	< 0.001	< 0.001	< 0.001	< 0.001
FR	< 0.001	< 0.001	< 0.001	< 0.001
Interactions				
YR x CR	NS	NS	NS	NS
YR x CP	< 0.05	NS	NS	NS
CR x CP	< 0.001	NS	NS	NS
YR x FR	< 0.01	< 0.01	NS	< 0.01
CR x FR	NS	NS	NS	NS
CP x FR	< 0.001	< 0.001	<0.01	NS
YR x CR x CP	NS	NS	NS	NS
YR x CR x FR	< 0.05	NS	NS	NS
YR x CP X FR	< 0.01	NS	NS	NS
CR x CP x FR	NS	NS	NS	NS
YR x CR x CP x FR	NS	NS	NS	NS

NS Not significant

resulted in significantly higher protein concentration compared with FYM and cattle slurry treatments (Table 6).

Spelt variety had a significant effect on all performance parameters assessed (Table 6). The two shorter-straw varieties Filderstolz and Zürcher Oberländer Rotkorn (ZOR) produced lower grain yield, TGW, protein and grain Se concentrations compared to the two longer strawed varieties Oberkulmer and Rubiota, although it should be noted that there was no difference in grain yield between ZOR and Rubiota (Table 6).

There was a significant year × variety interaction on grain Se concentration and plant height. When this interaction for Se concentration and plant height was further investigated all four 4 varieties were taller and had higher grain higher Se concentrations in 2015 compared with 2016, although the difference in Se-concentrations between years was not significant for Oberkulmer (Table 7). There was also a significant year × variety interaction on TGW and protein concentration (Table 6). The two long-straw varieties Oberkulmer and Rubiota produced higher TGW in 2015, while the shorter straw varieties

Table 5 Interaction means ± SE for the effect of harvest year and fertilisation regime (conventional vs organic at the same total N-input level) on grain yield, thousand grain weight (TGW) and grain Se concentration in the common wheat (*T. aestivum*) variety Cordiale recorded in the QLIF trials

	Harvest year	Fertilisation regime	
		Conventional	Organic
Grain yield (t ha^{-1})	2015	10.9 ± 1.0 A a	8.6 ± 0.4 A b
	2016	5.0 ± 0.7 B a	3.9 ± 0.2 B a
TGW (g)	2015	48.8 ± 1.7 A b	52.4 ± 0.7 A a
	2016	32.2 ± 1.5 B b	40.6 ± 0.9 B a
Se concentration	2015	30 ± 2 A a	31 ± 2 A a
(µg kg^{-1})	2016	26 ± 2 A b	35 ± 3 A a

Means followed by different lowercase letters within each row and uppercase letters within each column are significantly different (Tukey's general linear hypothesis test $p < 0.05$)

Filderstolz and ZOR produced higher TGW in 2016, although the difference between years was not significant for Filderstolz (Table 7). Correlation analysis identified a significant negative correlation ($p < 0.01$; r = −0.26) between grain Se and protein concentration (Table 3).

Association between climatic, agronomic and genetic variables with performance parameters

Partial redundancy analysis (pRDA) was carried out (Fig. 1) using data from all three trials to investigate the associations between climatic (air temperature, radiation and precipitation), agronomic (fertiliser type) and genetic (wheat species *T. aestivum* and *T. spelta*) explanatory variables/drivers and the wheat performance parameters assessed (grain yield, TGW, protein and Se concentrations).

Wheat species (F = 92.5, $p < 0.002$), rainfall (F = 67.9, $p < 0.002$) and temperature (F = 68.2, $p < 0.002$) were identified as the strongest explanatory variables by pRDA and explained 27%, 16% and 12% of the variation, respectively. Solar radiation (F = 29; $p = 0.002$) and fertiliser type (F = 4.4; $p = 0.002$) were also identified as significant drivers, but each only explained 4% of the variation.

In the bi-plot (Fig. 1), axis 1 explained 49.3% of the total variation with temperature the main driver (F = 68.2, $p < 0.01$) with a further 7.4% explained by axis 2. Grain yield was positively associated with the use of common wheat and to a lesser extent mineral N-fertiliser use (along the negative axis 1). While TGW, grain protein content and Se concentrations were positively associated with air temperature, solar radiation, precipitation, the use of spelt and to a lesser extent the use of FYM as fertiliser (along the positive axis 1) (Fig. 1).

Discussion

Mineral versus organic fertilisers

Several studies have concluded that grain Se concentrations in wheat grain are strongly influenced by soil Se-supply, which differs by geographical location and seasonal conditions (Lyons et al. 2005b; Zhao et al. 2009; Manojlović et al. 2019; Lee et al. 2011). However, there is very limited information on the effects of different fertiliser types (e.g. mineral-N versus livestock manures) and/or contrasting fertilisation regimes (e.g. those used in organic and conventional farming) on grain Se-concentrations in wheat.

Recent studies in Poland and China which assessed the effects of increasing mineral N-fertiliser inputs reported that (i) grain yields of common wheat (*T. aesticum*) increased, while (ii) grain Se-concentrations either remain similar or increased, with increased mineral-N application up to total N-inputs of ~ 250 kg N ha^{-1} (Borowska et al. 2012; Chen et al. 2017; Klikocka et al. 2017). In Poland total soil Se varied from 0.108 mg kg^{-1} in the control treatment to 0.170 mg kg^{-1} where high rates of FYM had been used (Borowska et al. 2012) and from 0.162 – 0.167 mg kg^{-1} in the seasons 2009–11 (Klikocka et al. 2017) which were lower than the levels reported in the current study. However, the Polish study (Borowska et al. 2012) also showed that, in soils with very low Se content, both (i) selenate (VI) and selenite (IV) concentrations in the soil and (ii) grain Se concentrations in roots and above-ground parts of mineral N-fertilised spring barley crops increased with increasing input levels of manure to potato crops in the same crop rotation.

The significantly higher grain Se concentrations in FYM-fertilised crops in the NUE-CROPS and HMC trials may be due to the higher Se-content found in FYM compared with mineral N-fertiliser (which contains virtually no Se). Similar to the practice of

Table 6 Main effects (means ± SE and p-values) and interactions of harvest year, fertiliser type and variety on grain yield, thousand grain weight (TGW), grain protein and Se concentration and plant height of spelt (*T. spelta*) varieties recorded in the HMC trial

Factor	Grain yield (t ha^{-1})	TGW (g)	Protein (%)	Grain Se (µg kg^{-1})	Plant height (cm)
Harvest year					
2015 ($n=64$)	3.8 ± 0.1	46.1 ± 0.9	13.4 ± 0.2	57 ± 2	124 ± 3
2016 ($n=64$)	2.9 ± 0.1	44.1 ± 0.3	16.2 ± 0.2	47 ± 2	114 ± 2
Fertiliser type					
BD ($n=32$)	3.8 ± 0.2 a	46.1 ± 0.8 a	15.1 ± 0.3 a	44 ± 2 c	122 ± 4
FYM ($n=32$)	3.2 ± 0.1 bc	45.1 ± 0.8 ab	14.1 ± 0.3 b	58 ± 2 a	118 ± 4
CS ($n=32$)	3.4 ± 0.2 b	46.0 ± 0.7 a	14.5 ± 0.4 b	56 ± 3 a	117 ± 3
MN ($n=32$)	3.0 ± 0.2 c	43.3 ± 1.3 b	15.5 ± 0.3 a	51 ± 3 b	120 ± 4
Variety					
Filderstolz ($n=32$)	2.8 ± 0.1 c	42.8 ± 1.0 c	13.7 ± 0.2 c	51 ± 3 b	95 ± 1
Oberkulmer ($n=32$)	3.8 ± 0.2 a	48.7 ± 0.9 a	15.6 ± 0.4 a	56 ± 2 a	136 ± 2
Rubiota ($n=32$)	3.4 ± 0.2 b	45.6 ± 0.8 b	15.3 ± 0.4 a	57 ± 3 a	137 ± 2
ZOR ($n=32$)	3.4 ± 0.2 b	43.3 ± 0.8 bc	14.6 ± 0.3 b	45 ± 2 c	109 ± 2
ANOVA p-values					
Main effects					
YR	<0.05	NS	<0.001	NS	<0.05
FT	<0.01	<0.05	<0.001	<0.001	NS
VR	<0.001	<0.001	<0.001	<0.001	<0.001
Interactions					
YR x FT	NS	NS	NS	NS	NS
YR x VR	NS	<0.001	<0.05	<0.01	<0.001
FT x VR	NS	NS	NS	NS	NS
YRxFTxVR	NS	NS	NS	NS	NS

Fertiliser type and variety main effect mean/SE values followed by the same letter within each column are not significantly different (Tukey's general linear hypothesis test $p < 0.05$); *NS* Not significant; *BD* Biogas digestate; *FYM* Composted farmyard manure; *CS* Cattle slurry; *MN* Mineral N fertiliser; *ZOR* Zürcher Oberländer Rotkorn

fortifying N-fertilisers with Se used in countries like Finland (Alfthan et al. 2015), applications of cattle FYM and cattle manure slurry (CS) in this study may therefore have increased Se-availability in the soil, resulting in higher Se concentration in the grain. This hypothesis is supported by previous studies which showed (i) that FYM can contain high Se-levels especially where Se-supplementation of animal feeds is used (Saha 2017) and (ii) farms that provided the FYM and cattle slurry for the trials reported here, routinely use Se-supplemented concentrate feed. Øgaard et al. (2006) also reported an increase in Se accumulation from a pot based study when cattle slurry was applied to wheat with typical levels of Se in a peaty soil (0.23–0.28 mg Se kg^{-1} soil; pH of 6.8), but with no difference observed when a loam soil was used (0.26 mg Se kg^{-1} soil; pH of 6.0). Although Kao et al. (2023) showed that one time application of Se via sheep excreta did not increase the Se accumulation and concentrations of perennial ryegrass grown in pots and in some cases it was even decreased both the NUE-CROPS and HMC trials in this study were carried out in the same field and represented single applications of organic manures.

As grain yields were significantly higher in mineral-N fertilised crops, the lower Se concentrations may also have, at least partially, been due to a "dilution effect" resulting from Se-uptake and/or translocation into grain not increasing at the same rate as plant growth and biomass production during the growing season in mineral-N fertilised crops. The strong correlations between grain yield and Se-concentrations in common wheat (NUE-CROPS and QLIF trials) is consistent with the "dilution effect" hypothesis, while

Table 7 Interaction means ± SE for the effect of harvest year (2015 and 2016) and variety on TGW, grain Se concentration and plant height in spelt (*T. spelta*) recorded in the HMC trials

	Variety	Harvest year	
		2015	2016
TGW (g)	Filderstolz	41.9 ± 1.43 C a	44.0 ± 0.29 A a
	Oberkulmer	52.4 ± 0.96 A a	45.8 ± 0.44 A b
	Rubiota	46.3 ± 1.41 B a	43.3 ± 0.35 B b
	ZOR	42.5 ± 0.72 C b	45.5 ± 0.35 AB a
Se concentration (μg kg^{-1})	Filderstolz	58 ± 3 A a	44 ± 3 B b
	Oberkulmer	56 ± 3 A a	55 ± 3 A a
	Rubiota	63 ± 4 A a	51 ± 3 A b
	ZOR	51 ± 4 B a	39 ± 2 B b
Plant height (cm)	Filderstolz	99 ± 1 C a	90 ± 1 C b
	Oberkulmer	143 ± 1 A a	128 ± 1 A b
	Rubiota	146 ± 1 A a	126 ± 1 A b
	ZOR	111 ± 1 B a	105 ± 1 B b

ZOR, Zürcher Oberländer Rotkorn; Means followed by different lowercase letters within each row and uppercase letters within each column are significantly different (Tukey's general linear hypothesis test $p < 0.05$)

the trend towards a positive correlation in spelt wheat is not. The finding that, in 2012, average grain yields in the NUE-CROPS trial were not significantly different between mineral N and FYM fertilised common wheat (3.3 and 3.1 t ha^{-1} respectively), while grain Se-concentrations were significantly higher in FYM than mineral N fertilised crops (56 and 34 μg kg^{-1} respectively), also does not support a "dilution effect" hypothesis.

From the HMC trials the FYM and cattle slurry treatments resulted in significantly higher grain Se concentrations than the biogas digestate and mineral N treatments. Borowska et al. (2012) investigated the availability of total selenium content as influenced by FYM and nitrogen fertilisers in spring barley and also detected that selenium concentration in above-ground parts and roots of spring barley was improved by the application of FYM. The low grain Se-concentration from the biogas digestate (BD) treatment in the current study is likely because the digester feedstock was from energy crops only and would therefore be expected to have lower Se concentrations than cattle manure (although not measured in the current study). The higher yields obtained with BD when compared with FYM applied at the same total N-input level was also consistent with previously published studies on both spelt (Magistrali et al. 2020) and rye (Tupits et al. 2022). It confirmed that the use of BD as a fertiliser provides a sustainable option for both organic and conventional farmers to reduce their carbon footprint and fertiliser costs, while increasing/maintaining crop yields and overall economic performance. However, the current study also indicates that the use of BD may have some negative impacts on the nutritional quality of spelt grain (i.e. lower Se-concentration).

Crop protection

The effects of contrasting crop protection regimes used in organic and conventional wheat production could only be assessed in the QLIF trials, since the NUE-CROPS and HMC trials were only carried out with conventional crop protection regimes. The lower Se-concentration in wheat under conventional crop protection may be explained by a "dilution effect" since grain yields were significantly higher with conventional crop protection and correlation analyses identified a highly significant negative correlation between grain yield and Se-concentration.

There are, to our knowledge, no previous studies examining the effect of crop protection protocols on Se-concentration in cereal grains. However, a previous study, which compared grain yields together with Cu and Zn (but not Se) concentrations in winter wheat crops grown with organic versus conventional crop protection protocols reported higher yields, lower Cu and Zn concentrations, but similar Cd concentrations in

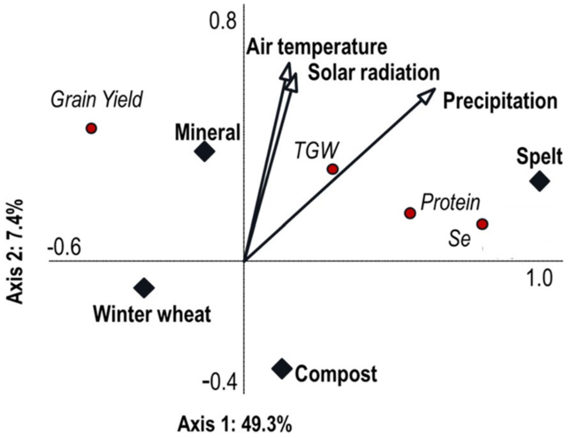

Fig. 1 Bi-plot derived from redundancy analysis (RDA) showing the relationship between the fertiliser (mineral and composted FYM), genetic (winter wheat and spelt) climatic factors (air temperature, solar radiation and precipitation) and grain yield, grain quality (protein concentration, TGW and Se concentration). Compost-Composted farmyard manure; Mineral–Mineral N

wheat grown with organic compared with conventional crop protection (Cooper et al. 2011), which is also consistent with a “dilution effect" on mineral micro-nutrients resulting from higher yields under conventional crop protection. It should be noted that the growth regulator chlormequat (CCC), which is used to reduce stem length and thereby prevent lodging in conventional wheat production, was shown to affect a wide range of morphological, physiological parameters in wheat, even in the absence of lodging (Green 1986; Naylor and Stephen 1993). It is therefore feasible that CCC may have had direct effects on plant micronutrient uptake and/or transport/incorporation into the grain.

Rotational position

Effects of rotational position on grain Se-concentrations could only be assessed in the QLIF trials, which had a unique factorial design that allowed the performance of winter wheat to be assessed in two rotational contexts in the same growing season (thus avoiding confounding effects of climatic conditions). However, in the harvest years assessed in this study winter wheat was grown after grass-clover leys in both the organic and conventional rotations, which is the most likely reason why no significant effects of rotation were detected.

Species/variety

The effect of genetics could only be assessed in the NUE-CROPS and HMC-trials, which compared the performance of eight contrasting common wheat and four contrasting spelt wheat varieties respectively. The finding of substantial differences in grain Se-concentrations and other performance parameters between varieties within each species and between the two species (common wheat varieties in the NUE-CROPS trial compared with spelt varieties in the HMC trial) is consistent with previous studies which have reported considerable variation in wheat for Se and other micronutrient concentrations (e.g. Zn) in wheat grain (Murphy et al. 2008; Souza et al. 2014). This, and the finding that wheat species was identified as a stronger driver than fertiliser type in the RDA, suggests that there is potential to improve grain Se-concentrations via crop breeding/selection without affecting yield which may lead to significant improvements in public health as suggested by Murphy et al. (2008). Specifically, Murphy et al. (2008) estimated that in order to achieve the recommended daily Se-intake would require the consumption of more than double the amount of bread (~ 124 slices) made from modern wheat cultivars compared with bread made form older/historic varieties with higher grain Se-content (55 slices). In terms of potential public health impacts this may not only reduce negative impacts of insufficient Se-intake, but also allows an adequate Se-intake to be achieved with half the calorie intake from bread and other cereal products, which may also contribute to a reduction in obesity.

The positive correlations between grain Se concentration and plant height in both spelt and common wheat varieties is also consistent with previous studies which reported that on average older/traditional, long-straw wheat species/varieties (which tend to have longer straw) produce lower grain yields, but higher grain concentrations of Se and other mineral micro-nutrients when compared with higher yielding, modern, short-straw wheat varieties (Garvin et al. 2006; Murphy et al. 2007, 2008; Gomez-Becerra et al. 2010; Lachman et al. 2011). This may indicate that physiological differences between long and short straw varieties that resulted in contrasting Se-uptake or relocation efficiency were responsible for the differences in grain Se-concentrations. However, this conclusion

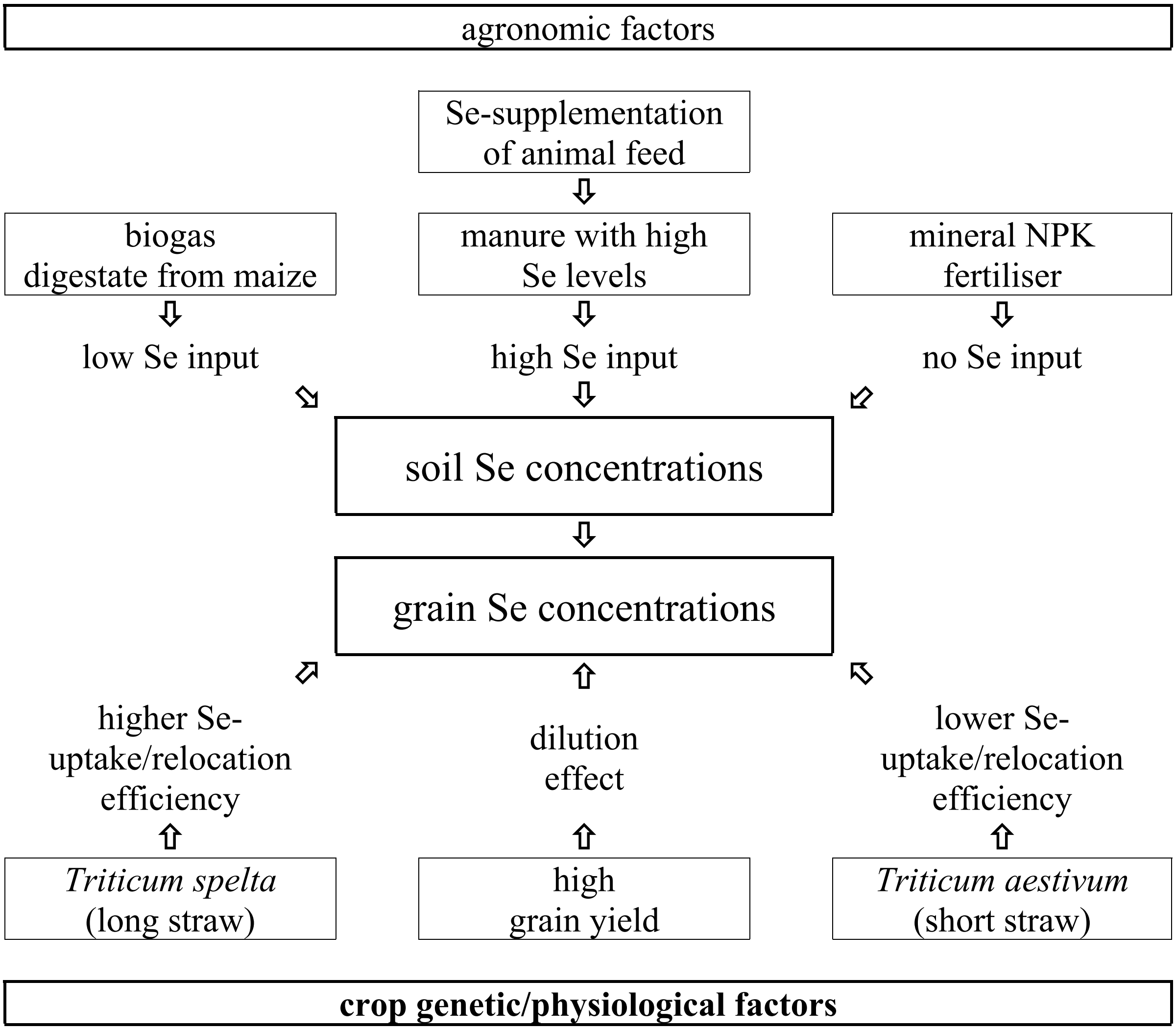

Fig. 2 Graphical summary of the agronomic and genetic factors thought to affect Se grain concentrations in winter wheat

needs to be treated with some caution as there was a relatively small difference i.e. 15 cm difference between the short and tall varieties in the NUE-CROPS trial and all spelt varieties including the semi-dwarf Filderstolz in the HMC trial were taller than the wheat varieties in the NUE-CROPS trial.

Future studies will have to confirm to what extent the physiological traits or QTLs responsible for high Se concentration are associated with stem length. However, if there are strong associations breeding and/or selection of new varieties with higher grain Se concentration and similar or higher grain yield or yield stability, is likely to be easier for the organic sector which often uses taller varieties to reduce competition from weeds and reduce disease levels. This hypothesis is based on results from both the NUE-CROPS and QLIF trials which showed that the use of FYM results in a lower risk of lodging in common wheat compared with mineral N or NPK fertiliser applied at the same total N-input level (Rempelos et al. 2020, 2023). The lower lodging risk is therefore thought to increase the feasibility of breeding and selecting longer-straw varieties for the organic farming sector, since this may also co-select for increased processing (e.g. grain protein concentration) and nutritional (e.g. grain phenolic concentrations) quality (Rempelos et al. 2023). The positive correlations between plant height and Se-concentrations identified in this study suggests that another important nutritional quality parameter may be improved in organic farming systems via the development and/or selection of longer straw varieties for the organic farming sector.

In contrast, intensive conventional arable farming relies on high mineral-N inputs which increase the risk of lodging unless short-straw varieties and growth regulators are used. Therefore, breeding high Se varieties for the conventional sector may require the identification of traits/QTLs for high grain Se that are not linked to stem length. This could for example focus on targeting root-based transporters that can discriminate between selenate and sulphate thereby providing an opportunity to enhance (Se) uptake and accumulation as suggested by Hawkesford and Zhao (2007).

Murphy et al. (2007) also reported a significant genotype x production system (organic vs conventional) interaction for yield in four of five locations in which they compared 35 soft white winter wheat genotypes. Analysis of these interactions demonstrated that variety selection in an organic system increased grain yield by 5- 31% depending on location compared with 'indirect selection' (i.e. selection based on yields obtained in conventional farming background). Given varieties for organic production in many countries are still primarily selected via 'indirect selection' there may also be a greater potential to select varieties for the organic sector with both improved grain yield and Se content. It is important to note that significant genetic variation for grain Se concentration in durum wheat (Rodríguez et al. 2011), rice (Zhang et al. 2006; Norton et al. 2012) and barley (Ilbas et al. 2012) exists which suggests that there is considerable potential to improve the grain Se concentration in other cereals by breeding/selection.

Conclusions

The NUE-CROPS, QLIF and HMC trial results reported showed that wheat species, variety, fertiliser type and crop protection can all influence grain Se concentration, yield and grain quality (protein concentration) with a graphical summary of the findings presented in Fig. 2. Results also provide evidence that farmyard manure can increase grain Se concentrations likely by increasing Se availability in soils especially when used annually e.g. in organic production systems and may provide an alternative to the use of Se-fortified mineral fertilisers, although this needs to be confirmed in future studies. The between and within species variation recorded for grain yield and Se in the NUE-CROPS and HMC-trials also confirmed previous studies which concluded that there is potential to breed/select varieties with higher grain Se concentrations while maintaining or increasing grain yield and/or processing quality. However, results suggest that this may need different breeding/selection strategies when targeting the organic and conventional sectors. Also, as long as agronomic practices which can result in higher Se and other micronutrient concentrations produce lower yield and do not receive a price premium in the market, it will remain difficult to persuade farmers to adopt innovations that deliver nutritional quality gains.

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Data Availability Data will be made available upon reasonable request to the corresponding author.

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