



RESEARCH ARTICLE

Sulfur uptake and distribution, grain yield, and quality of hybrid and inbred winter wheat (*Triticum aestivum* L.) varieties under early and late waterlogging

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Abstract

Background: Waterlogging at early and later growth stages could affect sulfur (S) uptake, distribution, yield, and quality of winter wheat. We hypothesized that the hybrid winter wheat variety (Hyvento), with a high degree of heterosis and genetic diversity, is likely to uptake and utilize S more efficiently and withstands waterlogging better than the inbred wheat variety (Kredo).

Aims: The objective of the present study was to investigate the effect of 2-week waterlogging at the end of tillering stage (Feekes Stage 6) and/or ear emergence stage (Feekes Stage 10.1) on grain yield, yield components, S-uptake and its partitioning, and composition of grain storage proteins (glutenins and gliadins) of hybrid (Hyvento) and inbred (Kredo) winter wheat varieties.

Methods: Plants were grown in large containers (120 L) in a semi-controlled greenhouse and harvested three times immediately after each waterlogging treatment and at agronomic maturity. Grain yield, S concentration in different plant organs, total protein content, and patterns of gluten proteins (gliadin and glutenin) and their subunits (by sodium dodecyl sulfate–polyacrylamide gel electrophoresis) in grains were evaluated.

Results: Grain yield of the inbred variety was more negatively affected under early and late waterlogging than the hybrid variety and was associated with reduced grain size. The decline in grain yield was significantly higher under late waterlogging (30%–31%) than early waterlogging (17%–18%). Compared to the hybrid variety Hyvento, the waterlogging-induced decline in S concentration in grains, husk, and straw was markedly lower in the inbred variety Kredo. While early waterlogging did not affect total grain protein content in either of the varieties, late waterlogging significantly reduced the total grain protein content (18%) in the inbred variety Kredo. Similarly, the grain gliadin content of the hybrid variety was not affected by both waterlogged conditions, whereas 32% and 23% reductions were recorded for the inbred variety under early and late waterlogging, respectively. Nevertheless, waterlogging significantly increased the proportion of S-poor high-molecular-weight glutenin subunits and ω -gliadin in the inbred variety.

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Conclusion: It could be inferred from the present study that hybrid winter wheat variety is relatively resistant to waterlogging than the inbred wheat variety, and thereby produces higher grain yield. Efficient uptake and metabolism of S could be one of the mechanisms conferring waterlogging resistance in winter wheat.

KEYWORDS

genetic diversity, hybrid and inbred wheat, sulfur uptake protein accumulation pattern, waterlogging resistance

1 | INTRODUCTION

Waterlogging (WL) is one of the major abiotic stresses that impede the production and quality of crops (Arata et al., 2019). Because of global climate change, the magnitude and frequency of WL risk are predicted to increase, especially in the high- and mid-latitude regions (Easterling et al., 2000; García et al., 2007; Jiang et al., 2008; Schumacher & Johnson, 2006). Moreover, the occurrence of flash floods is also expected to increase in Europe due to an intensified hydrological cycle by global warming (Feyen et al., 2012; IPCC, 2014). Annually, about 10% of the world's land area and 10–15 million ha of the world's wheat-growing regions, including 15–20% of wheat cultivated area, are affected by WL (Hossain & Uddin, 2011).

In Germany, heavy rainfall events have become more intense, and the average annual precipitation has been significantly higher over the last 20 years (Deumlich & Gericke, 2020). Accordingly, winter (December to February) and summer (June to August) precipitations in northern Europe have increased up to 70 mm (Maraun, 2013) and 18 mm per decade, respectively (Van den Besselaar et al., 2013). Therefore, wheat is likely to experience WL stress during the end of tillering (Feekes Stage 6) and ear emergence (Feekes Stage 10.1) stages, which are critical for crop establishment, grain yield, and quality.

Under WL conditions, ethylene production rapidly increases inside the plants as a primary signal for acclimation to this adverse condition (Pan et al., 2021). This stress can result in anaerobiosis, which may cause leaf senescence, reduced root growth, and tillering, ultimately affecting biomass and grain yield. In addition, WL restricts photosynthesis and carbohydrate synthesis both in the shoot and root (Xie et al., 2003; Yamauchi et al., 2013). These effects depend on the duration of WL, crop development stage, and the WL-resistance capacity of the crop species/variety (Arata et al., 2019; Arduini et al., 2016; De San Celedonio et al., 2014). Coincident with the susceptibility of wheat to WL, a grain yield decline ranging from 20% to 50% has been reported (Arduini et al., 2016; Belford, 1981; Cao et al., 1996; Ding & Musgrave, 1995; Zhang et al., 2006) depending upon the duration and timing of WL (Musgrave & Ding, 1998; Xie et al., 2003).

WL decreases the uptake and transport of ions through roots causing nutrient deficiencies (Setter et al., 2009; Wollmer et al., 2019; Zhang et al., 2011). Sulfur (S) deficiency is among the most probable nutrient deficiencies that might occur under WL conditions due to the reduction of sulfate into hydrogen sulfide and its subsequent volatilization or precipitation as sulfides of micronutrient cations (Fe,

Cu, Mn, and Zn) (McGrath et al., 1996; Ponnampereuma, 1972; Steffens et al., 2005). Apart from the precise effects on crop growth and grain yield, soil S deficiency can also exert a considerable influence on wheat quality due to the alteration of S-rich prolamins (α - and γ -gliadins, low-molecular-weight glutenin subunits [LMW-GS]) and S-low/S-poor prolamins (high-molecular-weight glutenin subunits [HMW-GS], ω -gliadins) (Wollmer et al., 2018). Thus, the proteins responsible for the breadmaking process, gliadins and glutenins, will be affected (Jiang et al., 2008). Glutenins are multimeric aggregates of HMW-GS and LMW-GS subunits. On the other hand, gliadins are mainly monomeric proteins and divided into four structural types, that is, α -, β -, γ -, and ω -gliadins. The quality of wheat grains is affected by the proportion of each protein fraction constituting that grain protein (Payne et al., 1987; Wieser, 2007).

Tolerance of the winter cereal to WL is related mainly to (1) the duration of the WL event, (2) the crop development stage at which WL occurs, and (3) the sensitivity of the species or variety (Brisson et al., 2002; de San Celedonio et al., 2014). For instance, WL for 20 days can reduce the grain yield of winter wheat by 32%–94% (de San Celedonio et al., 2014). Regarding the development stages of wheat, it was revealed that the order of susceptibility to WL at different stages was as follows: booting stage > jointing stage > tillering stage > grain filling stage (Bao, 1997). Furthermore, there are pieces of evidence of genotypic differences among wheat genotypes' intolerance to WL (Mano & Oyanagi, 2009). Therefore, hybrid wheat production is a potential strategy to alleviate yield depressions and fluctuations caused by weather extremes and associated plant stresses (Mühleisen et al., 2014). Hybrid genotypes showed more tolerance against different abiotic stresses, including WL, drought, frost, salinity, and heat stresses (Adee, 2016; Cao et al., 1992; Carretero et al., 2014; Collaku & Harrison, 2005; Longin et al., 2013; Ünay & Şimşek, 2020; Zhou et al., 2007). For example, hybrid wheat (Ibrahim et al., 2014; Jatoi et al., 2014) and maize (Adee et al., 2016; Singh et al., 2013) varieties were found more tolerant to drought stress and hybrid wheat to salinity and heat stresses (Yildirim et al., 2010).

The differences between the commercial high-yielding inbred and hybrid wheat genotypes regarding tolerance, yield, and yield quality under WL are still scarce, especially under west-European conditions. In addition, the WL-induced effects on inbred and hybrid wheat varieties, S uptake, and distribution and grain quality concerning protein composition have not so far been examined to draw a factual and sound inference (Ashraf, 2014). This justifies the integrated analysis

TABLE 1 pH, organic matter, and nutrient contents of the topsoil and subsoil used in the experiment.

Parameter	Topsoil	Subsoil
Texture	Loamy sand	Sandy loam
pH _{1:2.5} (CaCl ₂)	6.0	5.8.
Organic matter (g kg ⁻¹)	15.0	4.0
Total nitrogen (N, %)	0.09	0.03
Potassium (K, mg kg ⁻¹)	99.6	33.2
Phosphorus (P, mg kg ⁻¹)	48.0	21.8
Sulfur (SO ₄ ²⁻ , mg kg ⁻¹)	160.0	70.0

of these variables under WL stress. Therefore, we consider it a reasonable hypothesis that the hybrid wheat variety, with a high degree of heterosis and genetic diversity, uptakes and utilizes S more efficiently and withstands WL better than the inbred wheat variety at Feekes Stage 6 and/or Feekes Stage 10.1. Accordingly, the objective of the present study was to assess the comparative performance of commercial hybrid and inbred winter wheat varieties regarding grain yield, yield components, S-uptake and its partitioning, and grain storage proteins (glutenins and gliadins) composition under early and late WL conditions.

2 | MATERIALS AND METHODS

2.1 | Container experiment

Seeds of hybrid (Hyvento) and inbred (Kredo) winter wheat (*Triticum aestivum* L.) varieties were obtained from Nordsaat Saatzucht GmbH, Langenstein, Germany. The experiment was carried out in 120-L plastic containers (height: 0.9 m, area: 0.16 m²) with draining valves at the bottom (Hohmann et al., 2016), filled with the soil derived from the experimental farm of Kiel University, Germany. The soil is classified as a Cambisol following the criteria described by WRB (IUSS Working Group WRB, 2015). After drying and homogenization, the soil was filled in three steps in the containers: (1) gravel layer as drainage, (2) mixture of sandy loam subsoil and sand (1:1 [w/w]), and (3) mixture of loamy sand topsoil with sand (9:1 [w/w]). The pH, organic matter content, and nutrient contents of loamy sand topsoil and sandy loam subsoil are depicted in Table 1. Before seed sowing, each container was applied with the following fertilizers as basal dose (per kg of soil): 44 mg Ca(H₂PO₄)₂, 156 mg K₂SO₄, 23 mg MgSO₄·7H₂O, 150 mg urea (CH₄N₂O), and 25 mg (NH₄)₂SO₄. The experimental containers were placed in a nontemperature greenhouse for the whole growing period. The experiment consisted of the following three treatments: (1) control (moisture level equivalent to 60% soil water holding capacity [WHC]), (2) early WL (EWL; at first node visible stage [Feekes Stage 6; Large, 1954]), and (3) late WL (LWL; at beginning of ear emergence [Feekes Stage 10.1]). For both WL treatments, approximately 3-cm layer of water was kept above the soil surface for 14 days. The WL treatments were terminated by draining the water through the faucets at

the bottom of each container. After termination of WL treatments, the moisture level in the containers was maintained equivalent to the control, that is, 60% WHC. The water level in the containers was adjusted to 60% soil WHC by weighing the containers twice a week. The experimental treatments, each having four replicates, were arranged in a completely randomized design.

2.2 | Soil sampling and analysis

Soil samples (each of approximately 250–300 g) were collected from all the containers by digging three soil cores near the center to a depth of 50 cm before seed sowing and after the WL treatments have been implemented. The holes so formed in the pots were filled with the soil taken from the surrounding areas. After that, the soil samples were kept at –20°C, and later analyzed for SO₄²⁻-S contents by inductively coupled plasma optical emission spectrometry (ICP-OES) following their extraction with 0.0125 M CaCl₂ (soil to solution ratio of 1:4) as described by Bassler and Hoffmann (1997).

2.3 | Plant sampling and analysis

Plant samples were collected immediately after the termination of WL and at maturity. Five plants were randomly selected for the first sampling. From the top, three leaves were separated as younger leaves, while the rest of the leaves were collected as older leaves, and shoot samples were also collected separately. For sampling at maturity, plants in each container were harvested as one biological replicate and separated into straw, husk, and grains. The samples were oven-dried (65°C) to a constant weight, and their dry weights were recorded. For further analysis, samples were milled to a fine powder with a ball mill (MM 200, Retsch GmbH, Haan, Germany).

2.3.1 | Determination of total S and N

For determining S and N, the ground fine powdered samples were weighed (5–10 mg each) and mixed with 10 mg wolfram-(IV)-oxide. Subsequently, the samples were put into aluminum capsules, and total N and S contents were determined by a CNS elemental analyzer (Flash EA 1112 NCS, Thermo Fisher Scientific, Waltham, MA, USA) following the Dumas combustion method. Raw protein concentration of the wheat flour was obtained by multiplying N concentration by 5.81 (Fujihara et al., 2008).

2.3.2 | Extraction and quantification of storage protein fractions

Wheat grain storage proteins were extracted stepwise following a published protocol with modifications (Wollmer et al., 2018). In brief, 100 mg of whole flour was extracted with 1 mL extraction buffer

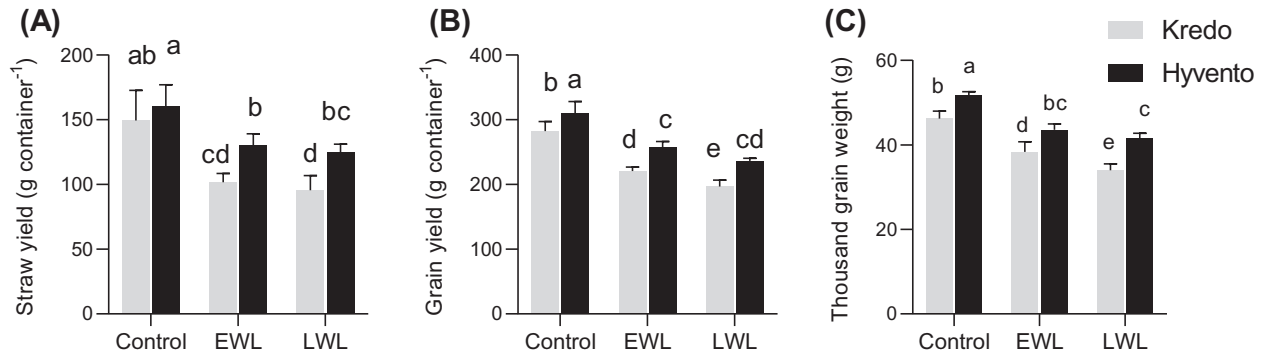


FIGURE 1 Effect of early waterlogging (EWL) and late waterlogging (LWL) on straw yield (A), grain yield (C), and thousand grain weight of the inbred (Kredo) and hybrid (Hyvento) varieties of winter wheat. Data are means of four replicates. Error bars represent standard deviations. Data with different letters are significantly different as measured by Duncan's multiple range (DMR) test ($p \leq 0.05$).

(pH 7.6) containing 0.4 M NaCl, 67 mM Na_2HPO_4 , and 7.7 mM KH_2PO_4 for obtaining the albumin-globulin fraction. Thereafter, gliadins were extracted from the residue with 0.5 mL 60% (v/v) ethanol and then glutenins with 1 mL extraction buffer containing 50% 1-propanol (v/v), 2 M urea, 1% dithiothreitol (DTT) (w/v), and 0.1 M Tris-HCl (pH 7.5). A chloroform/methanol solution was used to precipitate the proteins of each fraction. Finally, protein concentration was determined following the Bradford method (Bradford, 1976).

2.3.3 | Fractionation and determination of protein subunits

One-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (1D SDS-PAGE) was performed to quantify gliadin and glutenin subunits following the protocol described by Laemmli (1970). Based on molecular weights or S contents, the gliadin was separated into S-rich γ -gliadins and α/β -gliadins, and S-poor ω -gliadins. Similarly, glutenin was separated into LMW-GS, namely, D-type, B-type, and C-type, and HMW-GS (D'ovidio & Masci, 2004). Gels were prepared using 15% polyacrylamide gels (15% separating gels and 6.3% stacking gels) with a 7 cm \times 8.6 cm \times 0.1 cm in a Bio-Rad Mini-PROTEAN Vertical Electrophoresis Cell (Bio-Rad Laboratories, Hercules, USA). Each gel was loaded with 3 μg gliadin and glutenin per slot, with 70 volts for 120 min. A molecular weight standard (Rotimark 10–150, Roti-Mark, Roth, Crailsheim, Germany) was used for comparison. Proteins were fixed to the SDS-PAGE gels overnight using 40% ethanol and 10% acetic acid. After that, gel staining was done using “PhastGel™ Blue R” and then removed and stained with 10% acetic acid until the gel background was transparent. After destaining, all gels were scanned with an image scanner (Epson PerfectionV 700) at 300 dpi and 16 bits per pixel as TIF format. Gels were analyzed according to Wollmer et al. (2018) with modifications. In this study, all bands were calculated using Gel Analyzer 19a software. However, Gel lanes and bands were detected automatically and selected manually. The raw volumes of all bands in each lane were calculated with a different software than the above-referenced workers and adjusted manually where needed. Relative percentage of each band was used to equalize the differences

between each staining. In this process, the sum of all band's raw volumes was set to 100% and used to calculate the percentage of each band of a lane.

2.4 | Statistical analysis

The data were statistically analyzed by two-way ANOVA, and the significance of differences among means was determined by Duncan's multiple range (DMR) test at $p \leq 5\%$. The analytical software Statistix 10 (Tallahassee, Florida, USA) was used to perform the statistical analysis.

3 | RESULTS

3.1 | Plant growth and yield

The straw dry matter, thousand-grain weight, and grain yield of both varieties were significantly affected by WL treatments (Figure 1). WL-induced reduction in straw yield of Kredo was almost double (32%/37%) that of Hyvento (18%/22%) under both WL treatments (Figure 1A). Following the same trend, a significantly higher reduction in grain yield of Kredo (22%/44%) was recorded as compared to Hyvento (16%/31%) with both EWL and LWL treatments (Figure 1B). The reduction in grain yield was associated with thousand grain weight, which also decreased in the same proportion (Figure 1C). However, the negative effect of LWL on straw yield, grain yield, and thousand grain weight of both the varieties was markedly higher than the EWL treatment (Figure 1A–C).

3.2 | Changes in soil sulfate and plant S concentrations

The concentration of plant-available SO_4^{2-} in soil was significantly reduced by WL treatments, causing a 51%–60% reduction in SO_4^{2-} concentration in waterlogged soils compared to the control (Figure 2).

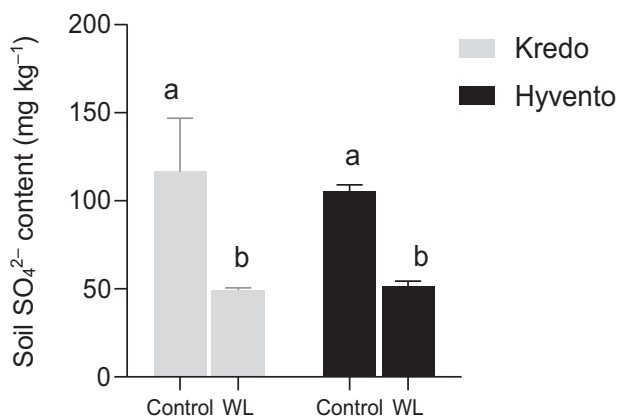


FIGURE 2 Effect of early waterlogging (EWL) and late waterlogging (LWL) on soil sulphate content. Data are means of four replicates. Error bars represent standard deviations. Data with different letters are significantly different as measured by Duncan's multiple range (DMR) test ($p \leq 0.05$).

Accordingly, WL had a drastic effect on S contents in different parts of waterlogged plants, collected immediately after the termination of WL treatments and at maturity. The highest significant differences were recorded in the younger leaves, showing reductions in S concentration by 47% and 50% in Kredo and 36% and 31% in Hyvento with EWL and LWL treatments, respectively, compared to the control (Figure 3A). Similarly, S concentration in the shoots decreased by 46% and 44% in Kredo and 27% and 37% in Hyvento under EWL and LWL, respectively (Figure 3B). WL at both stages caused significant changes in the S concentrations of grain and husk of both varieties. Sulfur concentration in the grains of Kredo and Hyvento reduced by 29% and 25% under EWL and 42% and 30% under LWL, respectively (Figure 4A). The highest reduction in S concentration was recorded in the husk for Kredo (60%–65%) and Hyvento (50%–57%) under both WL treatments (Figure 4C). Surprisingly, neither of the WL treatments affected S concentration in the straw of Hyvento (Figure 4B). In contrast, both WL treatments reduced S concentration in the

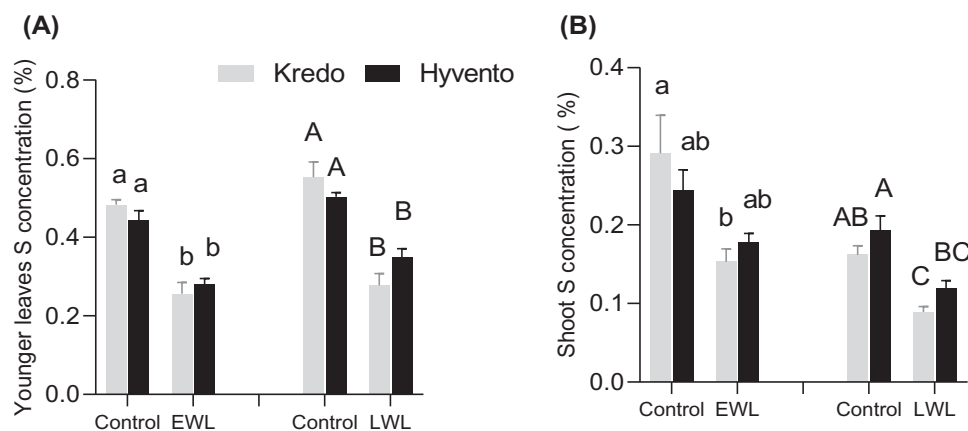


FIGURE 3 Effect of early waterlogging (EWL) and late waterlogging (LWL) on sulfur (S) concentration in younger leaves (A) and shoot (B) of the inbred (Kredo) and hybrid (Hyvento) varieties of winter wheat. Data are means of four replicates. Error bars represent standard deviations. Data with different letters are significantly different as measured by Duncan's multiple range (DMR) test ($p \leq 0.05$).

TABLE 2 Nitrogen to sulfur (N/S) ratio in grains of hybrid (Kredo) and inbred (Hyvento) varieties of winter bread wheat grown under control (no waterlogging), early waterlogging (EWL), and late waterlogging (LWL) treatments.

Treatment	Kredo	Hyvento
Control	14.28b	14.34b
EWL	19.40ab	17.94ab
LWL	21.21a	20.21ab

Note: Data presented are the means \pm SD of four replicates. Different letters indicate a significant difference from each other in all combinations (Duncan's multiple range test, $p \leq 0.05$).

straw of Kredo by 44% under EWL and 60% under LWL (Figure 4B). Though the N/S ratio of grains between varieties was unaffected at EWL, the N/S ratio of Kredo (inbred) significantly increased at LWL (Table 2).

3.3 | Grain total protein and protein compositions

WL at both growth stages tended to decrease the total grain protein concentration of Kredo (9%–10%) and Hyvento (15%–19%) (Figure 5A); however, the effect was significant only for Kredo with the LWL treatment (Figure 5A). Among the storage proteins, the amount of gliadin in Kredo was significantly reduced by both EWL (32%) and LWL (23%), but it remained unaffected in Hyvento (Figure 5C). The glutenin content in grains remained unchanged with EWL, but LWL significantly increased the amount of glutenin in grains of both varieties (Figure 5B). In Kredo, the ratio of gliadin to glutenin was reduced significantly by 35% and 38% compared with the control, respectively, whereas this response was absent in Hyvento (Figure 5F).

Nine protein bands were detected for the gliadin fractions, and their relative quantities were analyzed in both varieties. In Kredo, both WL treatments significantly increased S-poor ω -gliadins and decreased S-rich γ -gliadins compared to the control (Figure 5D).

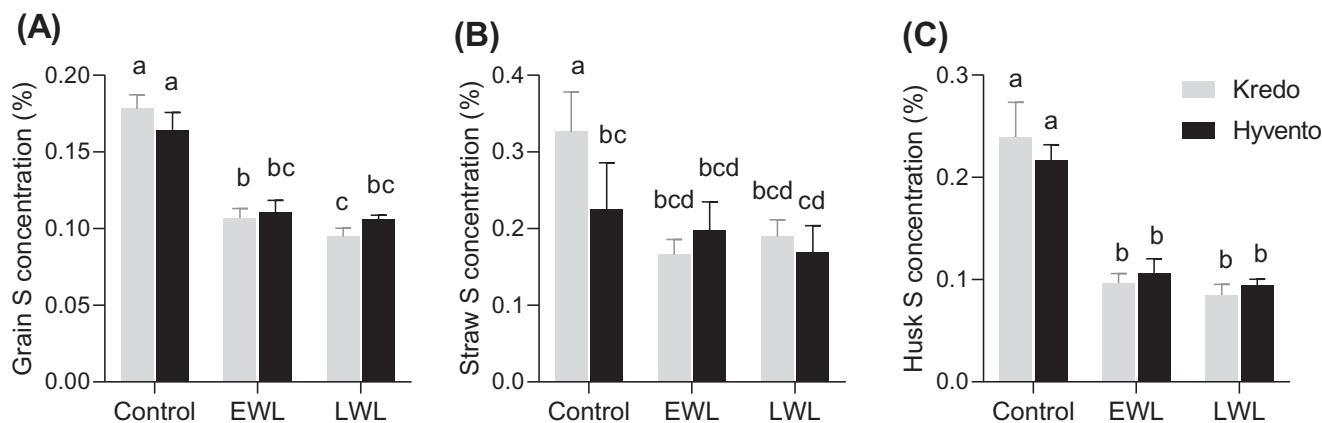


FIGURE 4 Effect of early waterlogging (EWL) and late waterlogging (LWL) on sulfur (S) concentration in postharvest grain (A), straw (B), and husk (C) of the inbred (Kredo) and hybrid (Hyvento) varieties of winter wheat. Data are means of four replicates. Error bars represent standard deviations. Data with different letters are significantly different as measured by Duncan's multiple range (DMR) test ($p \leq 0.05$).

TABLE 3 Relative abundance (%) of high molecular weight (HMW-GS) and subfractions (D-type, B-type, C-type) of LMW-GS glutenin in grains of inbred (Kredo) and hybrid (Hyvento) varieties of winter wheat grown under control (no waterlogging) early waterlogging (EWL) and late waterlogging (LWL) treatments.

Subunit/type	Kredo			Hyvento		
	Control	EWL	LWL	Control	EWL	LWL
HMW-GS	17.11de	21.25bc	23.15a	16.06e	17.66d	20.64c
D-type	13.67c	15.90b	16.61b	20.60a	21.67a	21.16a
B-type	26.67a	22.54b	20.89c	18.49d	15.54e	14.92e
C-type	45.90ab	42.01cd	39.43d	46.48a	43.74abc	43.58abc

Note: Data presented are the means \pm SD of four replicates. Different letters within a row indicate a significant difference from each other in all combinations (Duncan's multiple range test, $p \leq 0.05$).

However, these responses were not identified in the hybrid variety Hyvento (Figure 5E).

For the glutenin subfractions, 13 bands were detected in Kredo, and 12 bands were detected in Hyvento. In Kredo, S-low HMW-GS increased by 25% and 36% under EWL and LWL, respectively. However, in Hyvento, HMW-GS increased only by 10% and 28% under EWL and LWL, respectively (Table 3). Under EWL and LWL, there was a statistically significant and similar drop in the relative abundance of S-rich B-type LMW-GS subfraction both in Kredo (15% and 22%, respectively) and in Hyvento (16% and 19%, respectively) (Table 3). Nevertheless, the relative abundance of LMW-GS C-type subfraction in Kredo was decreased by 8% and 14% in EWL and LWL, respectively (Table 3). In contrast, Hyvento did not show a similar response under either of the WL treatment (Table 3).

4 | DISCUSSIONS

Although many researchers have evaluated the effects of WL on the growth, physiological behavior, and ionic homeostasis in different

crops, studies on the possible differences in WL tolerance between hybrid and inbred wheat varieties and the underlying mechanisms are lacking. To contribute to this platform, we evaluated the WL tolerance in two winter wheat varieties, a high-yielding inbred variety (Kredo) and a commercial hybrid variety (Hyvento), on the basis of growth, yield, yield quality as well as S uptake pattern. The present study revealed high genotypic differences for WL tolerance, whereby Hyvento (hybrid) wheat variety proved relatively tolerant to WL than Kredo (inbred) variety. These observations agree with previous work on other crops showing that hybrid varieties' tolerance to different abiotic stress is higher than inbred varieties (Adee, 2016; Carretero et al., 2014; Collaku & Harrison, 2005; Ünay & Şimşek, 2020; Yildirim et al., 2010). Other studies have shown that high-yielding wheat genotypes are more affected by WL than lower yielding one because they could not maintain high tillering and kernels per spike (Collaku & Harrison, 2002; Setter et al., 2003). However, in our study, the inbred variety Kredo showed a higher reduction in grain yield than the hybrid Hyvento, and the decline in yield was associated with a decrease in kernel size. Likewise, Sharma et al. (2018) noted substantial genotypic variation for grain yield and biomass under WL treatments. Compared to EWL for both varieties, grain yield decreased drastically at LWL. However, other published results also noted a substantially higher reduction in grain yield (92%) of wheat exposed to WL at the Feekes Stage 10.1 (de San Celedonio et al., 2014).

Our results showed that WL caused a significantly higher S decrease in all plant tissues (younger leaves, shoot, straw, husk, and grains) of the inbred variety Kredo than Hyvento (Figure 3). Moreover, the reduction in S concentration in plant tissues was higher in the case of EWL than in LWL treatment. Therefore, we assumed that WL induced a higher reduction of S concentrations in Kredo that would have more adversely affected the yield and grain quality than Hyvento. Similarly, Singh et al. (2017) noticed that average leaf S concentration was reduced by 61% relative to drained conditions, diminishing yield by 27%. Some other researchers also observed that S deficiency caused yield losses of up to 55% (De Ruiter & Martin, 2001; Flaete et al., 2005; Järvan et al., 2008; Sahota, 2006). The decline in tissue S concentration

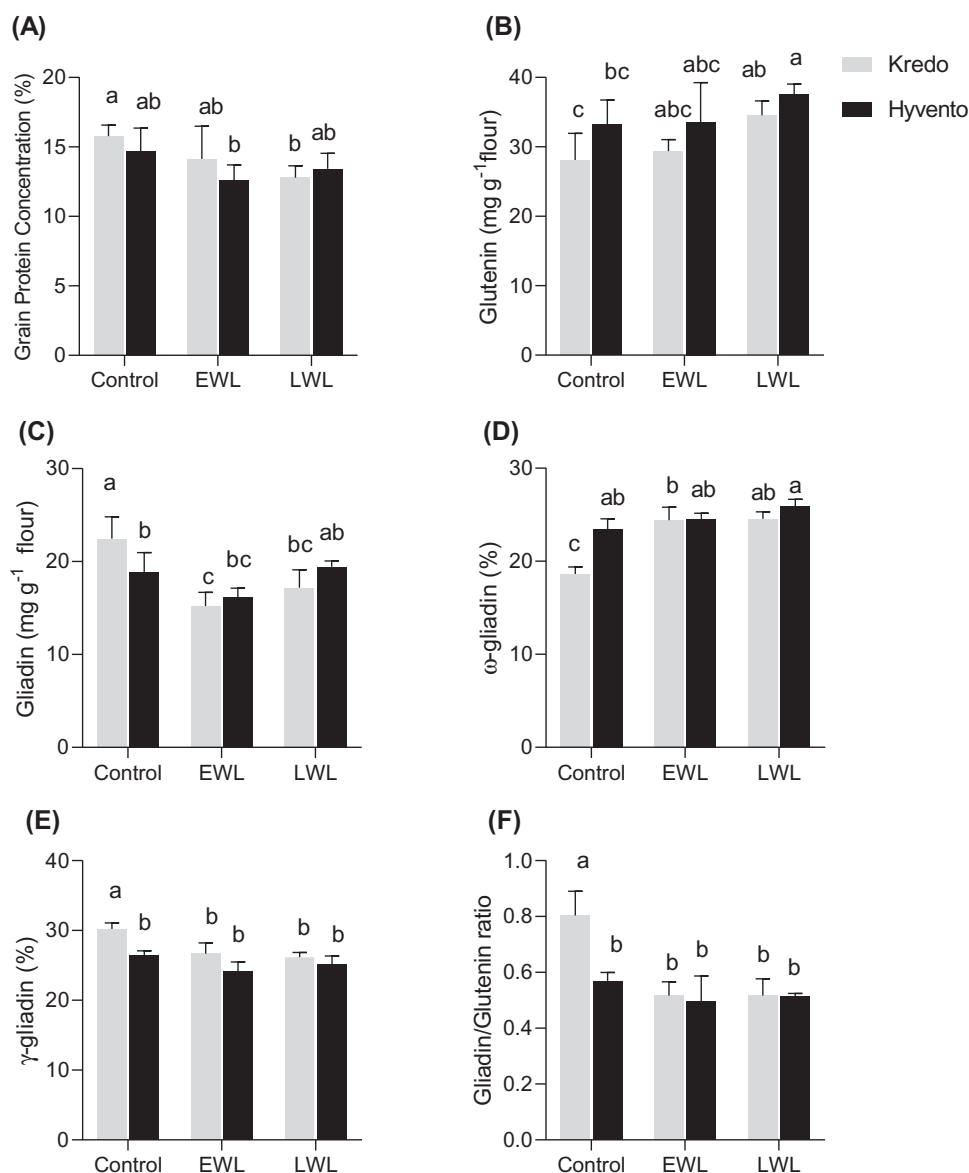


FIGURE 5 Effect of early waterlogging (EWL) and late waterlogging (LWL) on grain protein concentration (%) (A), glutenin (mg g⁻¹ flour) (B), gliadin (mg g⁻¹ flour) (C), gliadin/glutenin ratio (D), ω-gliadin (%) (E), and γ-gliadin (%) (F) of the inbred (Kredo) and hybrid (Hyvento) varieties of winter wheat. Data are means of four replicates. Error bars represent standard deviations. Data with different letters are significantly different as measured by Duncan's multiple range (DMR) test ($p \leq 0.05$).

was associated with a 54%–65% decline in SO_4^{2-} concentration in the WL soils (Figure 2).

WL-induced S deficiency in plants also affects the contents and composition of crude grain protein (Jiang et al., 2009; Zörb et al., 2009). Though recent studies demonstrate that WL affects the protein compositions, especially gliadin subunits of winter wheat varieties (Wollmer et al., 2018), the comparative effect of WL on inbred and hybrid varieties has not been studied so far. In this study, we observed that the grain protein concentrations and the gliadin and glutenin sub-fractions and their subunits pattern in the inbred variety Kredo were more severely affected than the hybrid variety Hyvento in response to EWL and LWL. Based on the criteria given by Randall et al. (1981), the grains of both varieties produced under EWL and LWL regimes could be

classified as S deficient ($S < 0.12\%$, N/S ratio $> 17:1$; Table 2). Accordingly, we can infer that this S deprivation, in turn, was underpinned by low grain gluten proteins as well as gliadin and glutenin subunits, thus, increasing the relative pools of S-low/poor HMW-GS and ω-gliadin. Indeed, it has been indicated that S deficiency was correlated with a significant increase in S-poor ω-gliadins and S-low HMW-GS as well as a substantial decrease in S-rich LMW-GS and γ-gliadin (Anderson et al., 1997; MacRitchie & Gupta, 1993; Moss et al., 1981; Shewry et al., 2001; Wieser et al., 2004; Zhao et al., 1999). Thus, this statement supports that WL negatively affected the S uptake, translocation, and remobilization from shoots and roots, which affects yield and storage proteins, because S is essential for the biosynthesis of wheat storage proteins (Yu et al., 2021). Under such circumstances, a remarkable shift

in grain storage protein composition is likely to occur because gliadins and glutenins comprise S-containing amino acids in different proportions (Shewry et al., 2000). A similar trend was reported under various abiotic stresses (DuPont et al., 2006; Zörb et al., 2017). Accordingly, gluten proteins and their subfractions were less affected in Hyvento under WL due to the lesser reductions in S concentration in grains, shoots, and leaves. We concluded that efficient uptake of S and its utilization into different grain proteins have partially conferred WL tolerance in hybrid winter wheat Hyvento.

5 | CONCLUSIONS

The results of this study proved that late water logging has more drastic negative effects on S uptake, grain yield, and protein patterns of both the varieties than early water logging. The hybrid variety Hyvento proved to be more efficient in S uptake and its utilization in different proteins than inbred variety Kredo. Moreover, unaffected total protein content and gliadin fractions in Hyvento under WL treatments, leading to a higher proportion of S-rich LMW-GS and γ -gliadin than Kredo, imply that this variety is also efficient in S utilization in different proteins. Thus, an efficient S uptake, its translocation to shoots, and subsequent metabolization could be among the key mechanisms conferring waterlogging tolerance in hybrid winter wheat.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

- Adee, E., Roozeboom, K., Balboa, G., Schlegel, A., & Ciampitti, I. (2016). Drought-tolerant corn hybrids yield more in drought-stressed environments with No Penalty in Non-stressed environments. *Frontiers in Plant Science*, 7, 1534. <https://doi.org/10.3389/fpls.2016.01534>
- Anderson, O., & Greene, F. (1997). The α -gliadin gene family. II. DNA and protein sequence variation, subfamily structure, and origins of pseudogenes. *Theoretical and Applied Genetics*, 95(1–2), 59–65.
- Arata, A. F., Dinolfo, M. I., Martínez, M., & Lázaro, L. (2019). Effects of waterlogging during grain filling on yield components, nitrogen uptake and grain quality in bread wheat. *Cereal Research Communications*, 47(1), 42–52.
- Arduini, I., Orlandi, C., Pampana, S., & Masoni, A. (2016). Waterlogging at tillering affects spike and spikelet formation in wheat. *Crop and Pasture Science*, 67(7), 703–711.
- Ashraf, M. (2014). Stress-induced changes in wheat grain composition and quality. *Critical Reviews in Food Science and Nutrition*, 54(12), 1576–1583.
- Bao, X. (1997). Study on identification stage and index of waterlogging tolerance in various wheat genotypes (*Triticum aestivum* L.). *Acta Agriculturae Shanghai*, 13(2), 32–38.
- Bassler, R., & Hoffmann, G. (1997). Bestimmung von mineralischem (Nitrat-) Stickstoff in Bodenprofilen, Nmin-Labormethode. *VDLUF A Methodenbuch*, 1, 4.
- Belford, R. K. (1981). Response of winter wheat to prolonged waterlogging under outdoor conditions. *The Journal of Agricultural Science*, 97(3), 557–568.
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1–2), 248–254.
- Brisson, N., Rebiere, B., Zimmer, D., & Renault, P. (2002). Response of the root system of a winter wheat crop to waterlogging. *Plant and Soil*, 243(1), 43–55.
- Cao, Y., Cai, S., Zhu, W., Fang, X., Bo, Y., & Wu, Z. (1996). Studies on identification of waterlogging tolerance in common wheat varieties and its inheritance. *Jiangsu Journal of Agricultural Sciences*, 12(1), 51.
- Cao, Y., Cai, S. B., Zhu, W., & Fang, X. W. (1992). Genetic evaluation of waterlogging resistance in the wheat variety Nonglin 46. *Crop Genetic Resources*, 4, 31–32.
- Carretero, R., Bert, F. E., & Podestá, G. (2014). Maize root architecture and water stress tolerance: An approximation from crop models. *Agronomy Journal*, 106(6), 2287–2295.
- Collaku, A., & Harrison, S. A. (2005). Heritability of waterlogging tolerance in wheat. *Crop Science*, 45(2), 722–727.
- Collaku, A., & Harrison, S. A. (2002). Losses in wheat due to waterlogging. *Crop Science*, 42(2), 444–450.
- De Ruiter, J. M., & Martin, R. J. (2001). Management of nitrogen and sulphur fertiliser for improved bread wheat (*Triticum aestivum*) quality. *New Zealand Journal of Crop and Horticultural Science*, 29(4), 287–299.
- De San Celedonio, R. P., Abeledo, L. G., & Miralles, D. J. (2014). Identifying the critical period for waterlogging on yield and its components in wheat and barley. *Plant and Soil*, 378(1), 265–277.
- Deumlich, D., & Gericke, A. (2020). Frequency trend analysis of heavy rainfall days for Germany. *Water*, 12(7), 1950.
- Ding, N., & Musgrave, M. E. (1995). Relationship between mineral coating on roots and yield performance of wheat under waterlogging stress. *Journal of Experimental Botany*, 46(8), 939–945.
- D'Ovidio, R., & Masci, S. (2004). The low-molecular-weight glutenin subunits of wheat gluten. *Journal of Cereal Science*, 39(3), 321–339.
- DuPont, F. M., Hurkman, W. J., Vensel, W. H., Chan, R., Lopez, R., Tanaka, C. K., & Altenbach, S. B. (2006). Differential accumulation of sulfur-rich and sulfur-poor wheat flour proteins is affected by temperature and mineral nutrition during grain development. *Journal of Cereal Science*, 44(1), 101–112.
- Easterling, D. R., Evans, J. L., Groisman, P. Y., Karl, T. R., Kunkel, K. E., & Ambenje, P. (2000). Observed variability and trends in extreme climate events: A brief review. *Bulletin of the American Meteorological Society*, 81(3), 417–426.
- Feyen, L., Dankers, R., Bódis, K., Salamon, P., & Barredo, J. I. (2012). Fluvial flood risk in Europe in present and future climates. *Climatic Change*, 112(1), 47–62.
- Flaete, N. E. S., Hollung, K., Ruud, L., Sogn, T., Færgestad, E. M., Skarpeid, H. J., Magnus, E. M., & Uhlen, A. K. (2005). Combined nitrogen and sulphur fertilisation and its effect on wheat quality and protein composition measured by SE-FPLC and proteomics. *Journal of Cereal Science*, 41(3), 357–369.

- Fujihara, S., Sasaki, H., Aoyagi, Y., & Sugahara, T. (2008). Nitrogen-to-protein conversion factors for some cereal products in Japan. *Journal of Food Science*, 73(3), C204–C209.
- García, J., Gallego, M. C., Serrano, A., & Vaquero, J. (2007). Trends in block-seasonal extreme rainfall over the Iberian Peninsula in the second half of the twentieth century. *Journal of Climate*, 20(1), 113–130.
- Hohmann, M., Stahl, A., Rudloff, J., Wittkop, B., & Snowdon, R. (2016). Not a load of rubbish: Simulated field trials in large-scale containers. *Plant, Cell and Environment*, 39(9), 2064–2073.
- Hossain, M. A., & Uddin, S. N. (2011). Mechanisms of waterlogging tolerance in wheat: Morphological and metabolic adaptations under hypoxia or anoxia. *Australian Journal of Crop Science*, 5(9), 1094–1101.
- Ibrahim, H. S., Mohamed, A. A., Ibrahim, K. I. M., & Sayed, A. N. (2014). Heterosis and combining ability for some agronomic and physiological traits in bread wheat crosses under water stress condition. *Egyptian Journal of Plant Breeding*, 18(3), 551–583.
- Intergovernmental Panel on Climate Change (IPCC). (2014). Climate change 2014: Synthesis report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Author.
- IUSS Working Group WRB. (2015). World reference base for soil resources 2014, update 2015. International soil classification system for naming soils and creating legends for soil maps. World Soil Resources Reports No. 106. FAO.
- Järvan, M., Edesi, L., Adamson, A., Lukme, L., & Akk, A. (2008). The effect of sulphur fertilization on yield, quality of protein and baking properties of winter wheat. *Agronomy Research*, 6(2), 459–469.
- Jatoi, W. A., Baloch, M. J., Khan, N. U., Munir, M., Khakwani, A. A., Vessar, N. F., Panhwar, S. A., & Gul, S. (2014). Heterosis for yield and physiological traits in wheat under water stress conditions. *Journal of Animal & Plant Sciences*, 24(1), 252–261.
- Jiang, D., Fan, X., Dai, T., & Cao, W. (2008). Nitrogen fertiliser rate and post-anthesis waterlogging effects on carbohydrate and nitrogen dynamics in wheat. *Plant and Soil*, 304(1), 301–314.
- Jiang, D., Yue, H., Wollenweber, B., Tan, W., Mu, H., Bo, Y., Dai, Q., & Cao, W. (2009). Effects of post-anthesis drought and waterlogging on accumulation of high-molecular-weight glutenin subunits and glutenin macropolymers content in wheat grain. *Journal of Agronomy and Crop Science*, 195(2), 89–97.
- Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227(5259), 680–685.
- Large, E. C. (1954). Growth stages in cereals. Illustration of the Feekes scale. *Plant Pathology*, 3, 128–129.
- Longin, C. F. H., Gowda, M., Mühleisen, J., Ebmeyer, E., Kazman, E., Schachschneider, R., Schacht, J., Kirchoff, M., Zhao, Y., & Reif, J. C. (2013). Hybrid wheat: Quantitative genetic parameters and consequences for the design of breeding programs. *Theoretical and Applied Genetics*, 126(11), 2791–2801.
- MacRitchie, F., & Gupta, R. B. (1993). Functionality-composition relationships of wheat flour as a result of variation in sulfur availability. *Australian Journal of Agricultural Research*, 44(8), 1767–1774.
- Mano, Y., & Oyanagi, A. (2009). Trends of waterlogging tolerance studies in the Poaceae. *Japanese Journal of Crop Science*, 78(4), 441–448.
- Maraun, D. (2013). When will trends in European mean and heavy daily precipitation emerge? *Environmental Research Letters*, 8(1), 014004.
- McGrath, S. P., Zhao, F. J., & Withers, P. J. A. (1996). *Development of sulphur deficiency in crops and its treatment*. Proceedings of the Fertiliser Society No. 379.
- Moss, H. J., Wrigley, C. W., MacRitchie, R., & Randall, P. J. (1981). Sulfur and nitrogen fertilizer effects on wheat. II. Influence on grain quality. *Australian Journal of Agricultural Research*, 32(2), 213–226.
- Mühleisen, J., Piepho, H. P., Maurer, H. P., Longin, C. F. H., & Reif, J. C. (2014). Yield stability of hybrids versus lines in wheat, barley, and triticale. *Theoretical and Applied Genetics*, 127(2), 309–316.
- Musgrave, M. E., & Ding, N. (1998). Evaluating wheat cultivars for waterlogging tolerance. *Crop Science*, 38(1), 90–97.
- Pan, J., Sharif, R., Xu, X., & Chen, X. (2021). Mechanisms of waterlogging tolerance in plants: Research progress and prospects. *Frontiers in Plant Science*, 11, 627331. <https://doi.org/10.3389/fpls.2020.627331>
- Payne, P. I. (1987). Genetics of wheat storage proteins and the effect of allelic variation on bread-making quality. *Annual Review of Plant Physiology*, 38(1), 141–153.
- Ponnamperuma, F. N. (1972). The chemistry of submerged soils. *Advances in Agronomy*, 24, 29–96.
- Randall, P. J., Spencer, K., & Freney, J. R. (1981). Sulfur and nitrogen fertilizer effects on wheat. I. Concentrations of sulfur and nitrogen and the nitrogen to sulfur ratio in grain, in relation to the yield response. *Australian Journal of Agricultural Research*, 32(2), 203–212.
- Sahota, T. S. (2006). Importance of sulphur in crop production. *Northwest Science*, 9, 10–12.
- Schumacher, R. S., & Johnson, R. H. (2006). Characteristics of US extreme rain events during 1999–2003. *Weather and Forecasting*, 21(1), 69–85.
- Setter, T. L., & Waters, I. (2003). Review of prospects for germplasm improvement for waterlogging tolerance in wheat, barley and oats. *Plant and Soil*, 253(1), 1–34.
- Setter, T. L., Waters, I., Sharma, S. K., Singh, K. N., Kulshreshtha, N., Yaduvanshi, N. P. S., Ram, P. C., Singh, B. N., Rane, J., McDonald, G., Khabaz-Saberi, H., Biddulph, T. B., Wilson, R., Barclay, I., McLean, R., & Cakir, M. (2009). Review of wheat improvement for waterlogging tolerance in Australia and India: The importance of anaerobiosis and element toxicities associated with different soils. *Annals of Botany*, 103(2), 221–235.
- Sharma, S. K., Kulshreshtha, N., Kumar, A., Yaduvanshi, N. P. S., Singh, M., Prasad, K. R. K., & Basak, N. (2018). Waterlogging effects on elemental composition of wheat genotypes in sodic soils. *Journal of Plant Nutrition*, 41(10), 1252–1262.
- Shewry, P. R., Popineau, Y., Lafiandra, D., & Belton, P. (2000). Wheat glutenin subunits and dough elasticity: Findings of the EUROWHEAT project. *Trends in Food Science & Technology*, 11(12), 433–441.
- Shewry, P. R., Tatham, A. S., & Halford, N. G. (2001). Nutritional control of storage protein synthesis in developing grain of wheat and barley. *Plant Growth Regulation*, 34(1), 105–111.
- Singh, S. P., & Setter, T. L. (2017). Effect of waterlogging on element concentrations, growth and yield of wheat varieties under farmer's sodic field conditions. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, 87(2), 513–520.
- Singh, V., Srivastava, A., Singh, R. K., & Bhatnagar, A. (2013). Performance of different maturity groups of maize (*Zea mays*) hybrids under temporary waterlogging condition in a Mollisol. *Indian Journal of Agricultural Sciences*, 83(6), 639–646.
- Steffens, D., Hütsch, B. W., Eschholz, T., Losak, T., & Schubert, S. (2005). Water logging may inhibit plant growth primarily by nutrient deficiency rather than nutrient toxicity. *Plant Soil and Environment*, 51(12), 545.
- Ünay, A., & Simsek, S. (2020). Heritability of waterlogging tolerance in wheat (*Triticum aestivum* L.). *Turkish Journal of Field Crops*, 156–160.
- Van den Besselaar, E. J. M., Klein Tank, A. M. G., & Buishand, T. A. (2013). Trends in European precipitation extremes over 1951–2010. *International Journal of Climatology*, 33(12), 2682–2689.
- Wieser, H. (2007). Chemistry of gluten proteins. *Food Microbiology*, 24(2), 115–119.
- Wieser, H., Gutscher, R., & Von Tucher, S. (2004). Influence of sulphur fertilisation on quantities and proportions of gluten protein types in wheat flour. *Journal of Cereal Science*, 40(3), 239–244.
- Wollmer, A. C., Pitann, B., & Mühling, K. H. (2019). Timing of waterlogging is crucial for the development of micronutrient deficiencies or toxicities in winter wheat and rapeseed. *Journal of Plant Growth Regulation*, 38(3), 824–830.
- Wollmer, A. C., Pitann, B., & Mühling, K. H. (2018). Grain storage protein concentration and composition of winter wheat (*Triticum aestivum*

- L.) as affected by waterlogging events during stem elongation or ear emergence. *Journal of Cereal Science*, 83, 9–15.
- Xie, Z., Jiang, D., Cao, W., & Dai, T. Q. J. (2003). Effects of post-anthesis soil water status on grain starch and protein accumulation in specialty wheat varieties. *Plant Growth Regulation*, 29, 309–316.
- Yamauchi, T., Shimamura, S., Nakazono, M., & Mochizuki, T. (2013). Aerenchyma formation in crop species: A review. *Field Crops Research*, 152, 8–16.
- Yildirim, M., & Bahar, B. (2010). Responses of some wheat genotypes and their F2 progenies to salinity and heat stress. *Scientific Research and Essays*, 5(13), 1734–1741.
- Yu, Z., She, M., Zheng, T., Diepeveen, D., Islam, S., Zhao, Y., Zhang, Y., Tang, G., Zhang, Y., Zhang, J., Blanchard, C. L., & Ma, W. (2021). Impact and mechanism of sulphur-deficiency on modern wheat farming nitrogen-related sustainability and gliadin content. *Communications Biology*, 4(1), 945.
- Zhang, H., Turner, N. C., Poole, M. L., & Simpson, N. (2006). Crop production in the high rainfall zones of southern Australia—Potential, constraints and opportunities. *Australian Journal of Experimental Agriculture*, 46(8), 1035–1049.
- Zhang, X., Jiang, D., Zheng, C., Dai, T., & Cao, W. (2011). Post-anthesis salt and combination of salt and waterlogging affect distributions of sugars, amino acids, Na⁺ and K⁺ in wheat. *Journal of Agronomy and Crop Science*, 197(1), 31–39.
- Zhao, F. J., Hawkesford, M. J., & McGrath, S. P. (1999). Sulphur assimilation and effects on yield and quality of wheat. *Journal of Cereal Science*, 30(1), 1–17.
- Zhou, M. X., Li, H. B., & Mendham, N. J. (2007). Combining ability of waterlogging tolerance in barley. *Crop Science*, 47(1), 278–284.
- Zörb, C., Becker, E., Merkt, N., Kafka, S., Schmidt, S., & Schmidhalter, U. (2017). Shift of grain protein composition in bread wheat under summer drought events. *Journal of Plant Nutrition and Soil Science*, 180(1), 49–55.
- Zörb, C., Steinfurth, D., Seling, S., Langenkaämper, G., Koehler, P., Wieser, H., Lindhauer, M., & Mühling, K. H. (2009). Quantitative protein composition and baking quality of winter wheat as affected by late sulfur fertilization. *Journal of Agricultural and Food Chemistry*, 57(9), 3877–3885.

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