Journal of Agriculture and Food Research 16 (2024) 101118

Contents lists available at ScienceDirect

Journal of Agriculture and Food Research

journal homepage: www.sciencedirect.com/journal/journal-of-agriculture-and-food-research

Combating heavy metals in wheat grains under drought – is alien or ancient germplasm a solution to secure food and health?

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A R T I C L E I N F O

Keywords:
Wheat-rye introgression
Heavy metal concentration
Drought
Food security and toxicity

A B S T R A C T

Alien and ancient wheat germplasms have been utilized to combat diseases and improve yield performance under climate change. However, the potential risk of excessive heavy metal uptake with these germplasms has been less studied. In order to ensure food security, this study aimed to evaluate the levels of cadmium (Cd), lead (Pb) and mercury (Hg) in 30 wheat lines, including modern, old and wheat-rye introgression genotypes grown under three conditions i.e., control, early drought and late drought. The results of this study revealed a generally higher Cd grain accumulation in old and 1R genotypes than in the other genotype groups evaluated here, while old genotypes also showed an excess Pb grain concentration. The induced late drought resulted in an increased Cd uptake in wheat, leading to significantly elevated grain Cd concentration in modern, 1R, 1RS and 2R genotypes, while similar results were not obtained for the other heavy metals e.g. Pb or Hg. Specifically, an old genotype, 207, showed an extremely high Cd value across control and drought conditions. There was a greater genotypic variation in Pb concentration compared to Cd, while consistently high Hg concentrations were observed in several genotypes carrying 1R or 1RS. Some wheat-rye introgression genotypes, particularly those with the 3R chromosome, showed a low Cd accumulation across all treatments. The results from the present study pin-point the necessity of a rigorous assessment of heavy metal accumulation in wheat grain when utilizing ancient and alien genetic resources in breeding for disease resistance, and wheat resilience to environmental stress and climate change. Furthermore, the specific lines identified in this study with elevated heavy metal accumulation should be avoided in breeding programs. Additionally, mechanisms for the found differences in heavy metals accumulation among genotypes and treatments should be further revealed.

1. Introduction

Heavy metals such as cadmium (Cd), lead (Pb) and mercury (Hg), are metallic elements that are extremely harmful to all living organisms [1]. These elements are naturally present at various concentrations in soils throughout the earth but human industrial activities such as mining and smelting have significantly increased the environmental presence of these heavy metal pollutants [2]. Crops are known to absorb and accumulate heavy metals from the soil, posing a threat to human health, especially if staple crops consumed in large quantities by humans and animals have high levels of accumulation [3]. Wheat is one of the three major crops in the world and is primarily used as a staple food crop [4]. Field-grown wheat from various regions has been reported to accumulate heavy metals in the grains from contaminated soils [5–8]. However, significant differences in heavy metal accumulation have been observed across wheat lines indicating opportunities to breed low accumulating wheat that can thrive even in somewhat polluted soils [9,10]

Despite the fact that various novel approaches and technologies have been utilized to secure food production [11–13], climate change will significantly alter crop cultivation patterns globally. This will affect food security and the accumulation of heavy metals in food crops [14]. Drought, a common abiotic stress affecting wheat due to global warming is the most prevalent of the stresses [15,16]. Understanding the interactions between drought stress and heavy metal accumulation is crucial for safe wheat cultivation in the face of climate change [17]. Old and alien wheat material have in previous studies been found as useful resources for disease resistance [18,19], drought tolerance [20], end-use quality [21,22] and nutrition and minerals accumulation in the wheat grain [17,23]. Accumulation of heavy metals in these materials have been less studied. This study evaluates the potential of using old and alien wheat germplasm material to mitigate heavy metal accumulation (Cd, Pb and Hg) under non-drought and drought conditions using a

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https://doi.org/10.1016/j.jafr.2024.101118
Received 21 February 2024; Received in revised form 14 March 2024; Accepted 17 March 2024
Available online 21 March 2024

diverse set of wheat genotypes. The goal of this study was to determine whether alien wheat-rye germplasm source can contribute to improved food security and production of wheat with acceptable levels of Cd, Pb and Hg. The study highlights significant variation in heavy metal accumulation in the wheat material evaluated and emphasizes the importance of avoiding 1R and ancient/old genotypes as they are potential high accumulators of heavy metals. Breeding wheat for human consumption should steer clear of such lines to prevent adverse effects on human health.

2. Materials and methods

To assess the risk of excessive heavy metal accumulation in wheat grains of different origin (modern, old and introgressed), wheat genotypes of different genetic sources were grown in climate-controlled conditions under controlled and drought stress conditions according to lan et al. (2022). Seeds were then harvested from well-watered and drought treated plants, which were subjected to heavy metal content analysis.

2.1. Plant materials and growing conditions

A total of 30 spring wheat (*Triticum aestivum* L.) genotypes grouped into modern wheat cultivars and breeding lines (n = 5), old Swedish cultivars released between 1928 and 1990 (n = 5), wheat-rye introgression lines with chromosome 1R (n = 5), 1RS (n = 5), 2R (n = 5) and 3R (n = 5) were utilized in this study (Table S1).

Plants were cultivated as previously described [20], with three biological replicates under hourly-regulated temperature and humidity based on the average climate data of Malmö, Sweden recorded from 2010 to 2019 (Swedish Meteorological and Hydrological Institute, SMHI). Three growing conditions namely control (C), early drought stress (EDS) and late drought stress (LDS) were applied.

2.2. Sample preparation

Grain samples collected at maturity from each growing condition were oven-dried (40 °C, 24 h) and milled (30 s; mixer mill 400 MM, RETSCH) into flour. All flour samples were then digested (121 °C, 200 Kpa, 30 min) with nitric acid following a similar protocol to the one previously described [24].

2.3. Heavy metal determination

All prepared sample solutions (10 ml each) underwent inductively coupled plasma mass spectrometry (ICP-MS; Aurora Elite, Bruker, U.S.) for Cd, Pb and Hg determination, with concentrations presented in mg/kg. The average amount of heavy metal contained in the grains of a single plant (μg/plant) was calculated by multiplying the concentration by the previously obtained grain yield.

2.4. Data analysis

All statistical analyses were conducted in RStudio [25]. The Cd and Pb concentrations of each genotype were visualized using R packages 'ggplot 2' and 'ggbreak'. Mean comparison (LSD post-hoc test) were performed between genotype groups (modern, old, 1R, 1RS, 2R and 3R) and between treatments (C, EDS and LDS) separately using the R package 'agricolae'. To identify genotypes with a tendency to accumulate low levels of heavy metals across the three treatments, stability analyses were performed using the additive main effects and multiplicative interaction (AMMI) with the R package 'metan'.

3. Results and discussion

3.1. Concentration and yield of Cd, Pb and Hg

With the exception of modern genotypes under EDS, modern, 1RS, 2R and 3R genotypes, all exhibited low levels of Cd concentration in their grains. In contrast, 1R genotypes consistently maintained the highest Cd concentration among introgression genotypes across the three cultivation conditions (Fig. 1A). For the total amount of Cd provided by a single plant, modern, 1RS, 2R and 3R genotypes had significantly lower Cd yields than old and 1R genotypes under C, particularly with 1RS and 3R genotypes maintaining a low Cd yield across the three conditions (Fig. 1D). The robust and fast-developed root system conferred by the 1R chromosome likely plays a role in the higher Cd accumulation as the 1R genotypes in this study have demonstrated a more vigorous early root system than 2R and 3R genotypes [20]. Similarly, the lower Cd concentration in modern genotypes compared to old genotypes can be attributed to the smaller root system of the former. This finding alerts breeders and the food industry to the risk of elevated Cd levels in wheat products due to a larger root system. A larger root system is generally seen as a positive trait for high yield in a changing climate with drought spells. Although 1R introgression has been primarily used to enhance disease resistance [26,27] and yield performance [28] of wheat, few studies have explored the relationship between chromosome 1R and heavy metal accumulation. Therefore, the risk of excessive Cd content in grain with 1R introgression needs careful consideration in modern breeding programs and further studies are urgently needed to investigate alternatives such as transferring 1R in combination with other rye chromosomes contributing to low Cd accumulation such as 3R. Moreover, the mechanisms behind the high Cd accumulation of 1R genotypes need evaluation to avoid such behavior in drought stressed wheat varieties. Generally, it needs to be further evaluated wether a large root system such as the one in the 1R genotypes always contribute a higher uptake of everything including water, nutrients, minerals and heavy metals or if there is mechanisms that can come with the larger root system that can regulate different part of the uptake mechanisms. Such types of studies may facilitate the breeding for larger root systems and more recilient crops without the hampering effects of e.g. a high cadmium uptake.

Modern genotypes exhibited lower Pb concentration and yield than most other genotype groups under C, while, old genotypes showed the highest Pb concentration and yield under EDS (Fig. 1B and E). Significant differences in Hg concentration and yield were only observed under LDS with modern genotypes versus 1R and 1RS genotypes (Fig. 1C), and modern genotypes versus 1R (Fig. 1F), respectively. The generally low heavy metal accumulation in modern wheat aligns with the low mineral uptake of this wheat material [24]. In contrast, the high Pb accumulation in old genotypes corresponds well with a previous study that found higher Pb concentration in primitive wheat than in modern cultivars [9]. Therefore, the human-influenced evolution from old-to-modern wheat successfully reduced food toxic compounds, even though this reduction is likely due to an overall compromise in the mineral accumulation of modern wheat.

Regarding the effect of drought stress on concentrations, only LDS was found to increase Cd concentration in modern, 1R, 1RS and 2R genotypes compared to C, with no effect observed from EDS (Fig. 1A). Neither EDS nor LDS affected Pb or Hg concentrations (Fig. 1B and C). The different effects between EDS and LDS on heavy metal concentration are likely due to their different impacts on grain yield, as LDS was reported to severely reduce final yield while EDS showed no effect [20]. As for the effect on heavy metal yield, EDS decreased the Cd yield of 1R genotypes while LDS further decreased the Cd yield of modern, 1R, 1RS and 3R genotypes compared to the C condition (Fig. 1D). EDS decreased the Pb yield of 1R, 1RS and 2R genotypes and the Hg yield of old and 3R genotypes while LDS induced reductions in the Pb yield of 1R and 1RS genotypes and Hg yield of old, 1R, 1RS and 3R genotypes (Fig. 1E and F).

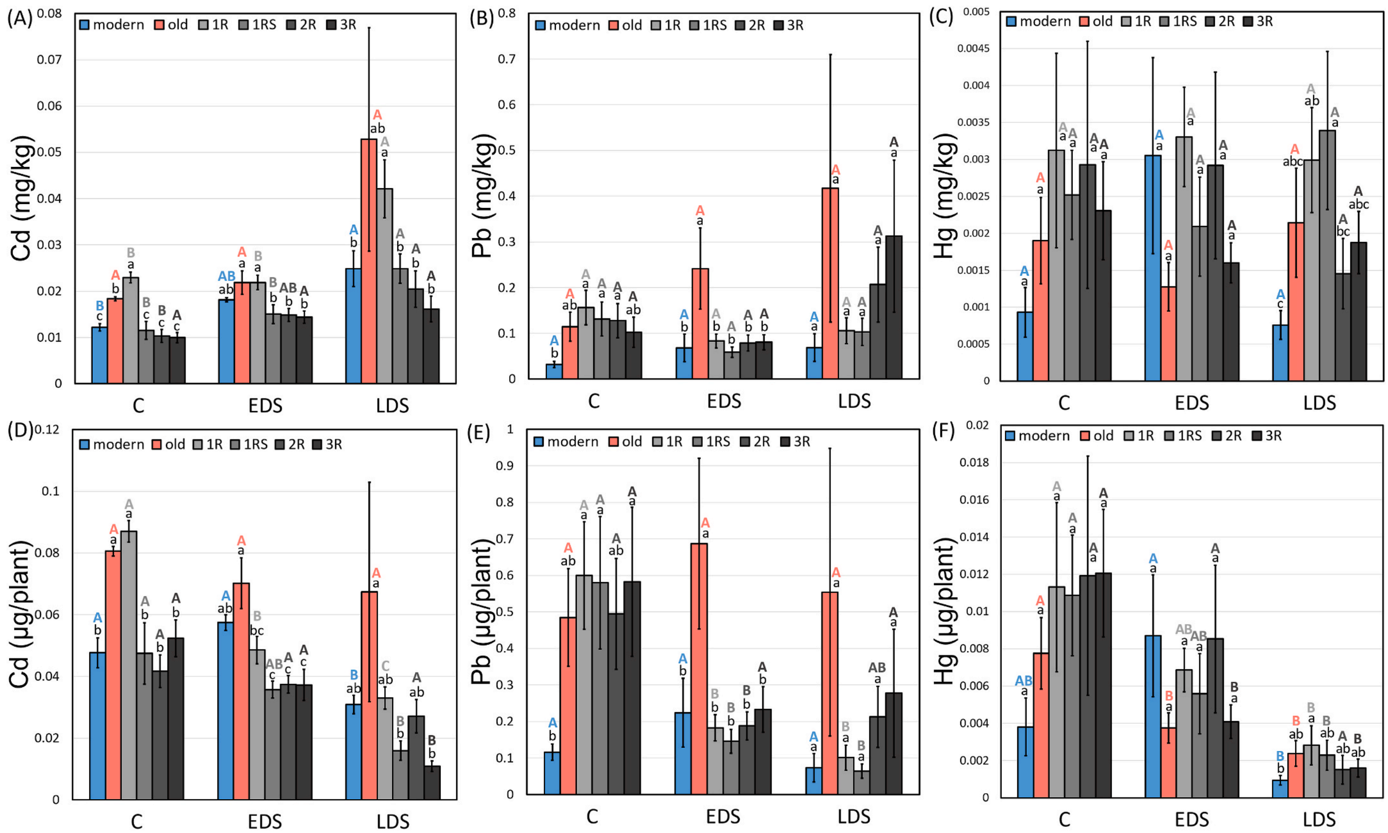

Fig. 1. The mean concentration of (A) Cd, (B) Pb and (C) Hg, and mean yield of (D) Cd, (E) Pb and (F) Hg of each genotype group under control (C), early drought stress (EDS) and late drought stress (LDS). Modern = approved cultivars and breeding lines received from company Lantmännen; old = old Swedish cultivars released from 1928 to 1990; 1R, 1RS, 2R and 3R = introgressions of chromosome 1R, 1RS, 2R and 3R. Means of the same genotype group between treatments marked by the same capital letters do not differ significantly. Means between different genotype groups within each treatment marked by the same smaller letters do not differ significantly (LSD post-hoc test at $p < 0.05$).

Unlike the unaffected Pb and Hg concentration, the increase in Cd concentration under LDS highlights the risk of elevated grain Cd content in the future climate. This rising trend of Cd concentration is consistent with various wheat seeds [29] and other species like peanut [30]. Thus, individually evaluating grain Cd concentration of wheat genotypes grown under different conditions becomes the major focus of this study.

3.2. Large variation and extreme values for Cd and Pb concentration

An extremely low Cd concentration was found in the 1RS genotype 235 (0.0040 mg/kg), and several other introgression genotypes; 238 (2R), 245 (2R), 250 (3R) and 256 (3R), also showed low grain Cd under C condition (Fig. 2A). The genotypes 235 (1RS), 238 (2R) and 251 (3R) had low Cd concentration under EDS while genotypes 234 (1RS), 238 (2R), 251 (3R) and 256 (3R) exhibited low Cd concentration under LDS (Fig. 2B and C). Old genotype 207 consistently showed the highest Cd concentration across C (0.077 mg/kg), EDS (0.078 mg/kg) and LDS (0.22 mg/kg) conditions, contributing to the high mean Cd concentration of the old genotype group. This highlighted several 2R and 3R genotypes as potential germplasms for reducing Cd content in wheat. In a previous study, a combination of low Cd and high Zn concentration was detected in several genotypes carrying 2R and 3R [21] and previous studies have also reported variation in Cd accumulation in various genotypes [31]. However, the potential combination of high minerals content and low heavy metals accumulation, as reported for some of the 2R and 3R genotypes evaluated here, is extremely interesting for the future breeding of highly nutritious wheat. Mechanisms behind the selected uptake of these different compounds should be further evaluated and determined in order to further elucidate breeding goals for health promoting wheat genotypes.

Unlike Cd, there was more significant genotypic variation in Pb concentration within each genotype group. A low Pb concentration was found in an old genotype, 202 (0.0047 mg/kg), and also in 235 (1RS), 248 (3R) and 279 (modern) under C conditions (Fig. 2D). Low Pb concentration was found in genotypes 202 (old), 235 (1RS), 248 (3R), 275 (modern) and 277 (modern) under EDS, while 197 (old), 202 (old), 210 (1R), 215 (1R), 238 (2R) and 279 (modern) showed low Pb concentration under LDS (Fig. 2E and F). Genotype 207 (old) with high Cd concentration also had high Pb concentration under C (Fig. 2D). Genotype 203 (old) with relatively high Cd concentration also exhibited high Pb concentration under EDS and LDS (Fig. 2E and F), suggesting a risk of elevated Cd and Pb contents under climate change. This more contrasting genotypic variation in grain Pb concentration than Cd concentration aligns well with other studies [32,33]. The low and stable Pb concentration among modern genotypes under C conditions suggested selections made in years of wheat breeding programs focusing on low Pb lines. However, those modern low-Pb genotypes did not show good consistency under early or late drought, pointing out the need for increased stability of the low-grain Pb in modern wheat to combat climate change.

3.3. Stability of concentration and yield of Cd, Pb and Hg

The additive main effects and multiplicative interaction (AMMI) clearly demonstrated different genotype × environment interactions for Cd, Pb and Hg accumulation in grains (Fig. 3). Genotypes 238 (2R), 251 (3R) and 256 (3R) were identified with low and stable Cd concentration while genotypes 235 (1RS), 250 (3R) and 251 (3R) showed low and

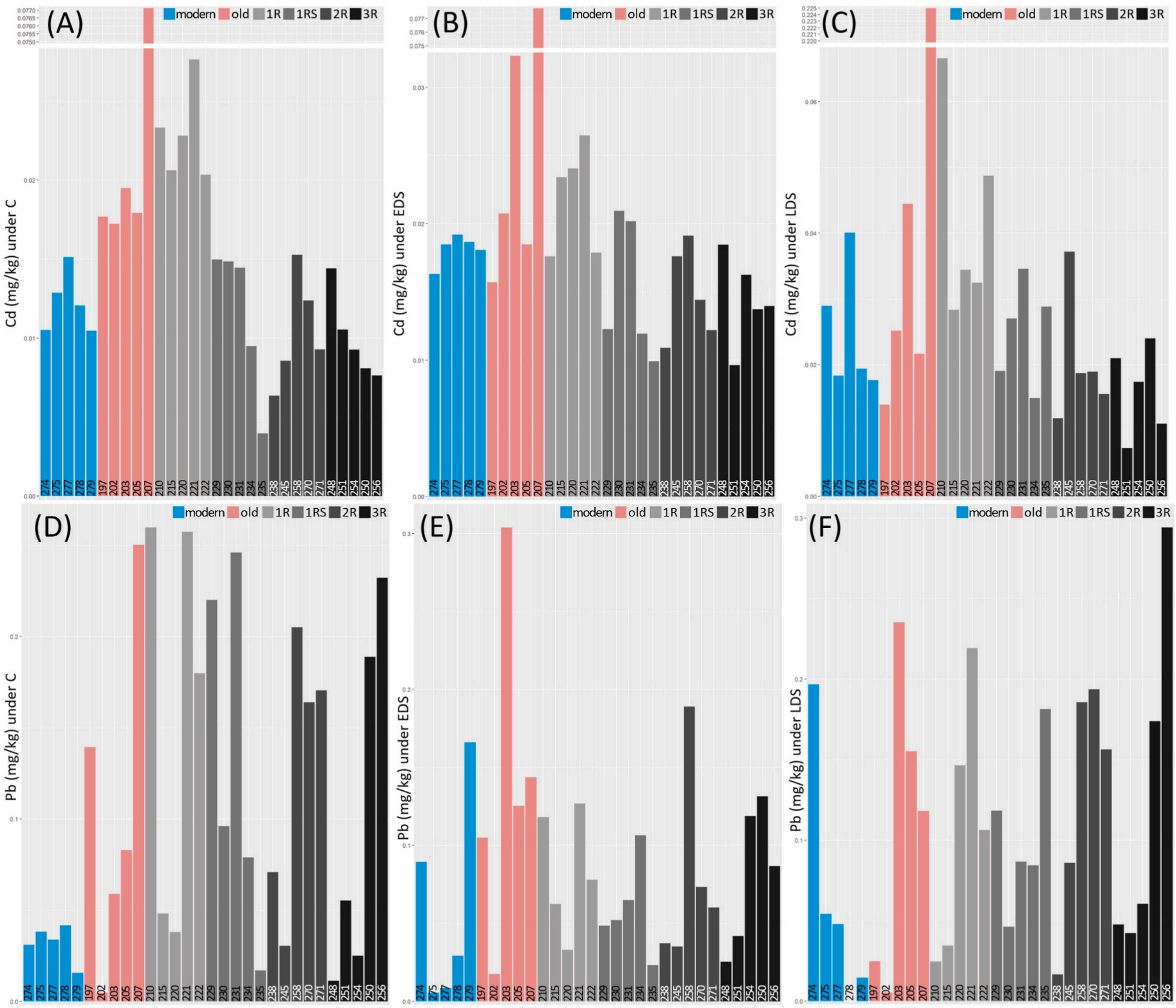

Fig. 2. Cadmium (A–C) and Pb (D–E) concentration of each genotype under control (abbreviated as C), early drought stress (EDS) and late drought stress (LDS). Modern = approved cultivars and breeding lines received from company Lantmännen; old = old Swedish cultivars released from 1928 to 1990; 1R, 1RS, 2R and 3R = Introgressions of chromosome 1R, 1RS, 2R and 3R. The value of each genotype was generated from the mean of three biological replicates. The Pb concentration of genotype 278 is missing under LDS.

stable Cd yield, suggesting genes on chromosome 3R contributing to a lowered and stable grain Cd level (Fig. 3A and D). Meanwhile, old genotypes and genotypes with 1R that appeared in the above-average area suggested high Cd accumulation in their grains (Fig. 3A and D). Furthermore, the stability of this high-Cd trait for those two genotype groups across different conditions was also observed. The rapid development of an early root system in old and 1R genotypes might contribute to the high and stable Cd uptake [20], which is supported by another observation of higher Cd content in the root of an old genotype [34]. Generally, the predicted climate change will force breeders to use a wide array of novel and less adapted plant material in their strive to produce novel cultivars that will produce enough food for the growing world population. As related to the Cd results presented here, the 2R and 3R lines are promising and has the potential to contribute novel beneficial genes to the breeding gene pool, while 1R lines should be avoided. However, understanding of uptake mechanism connected to the different lines is also important for further successful use of these genes.

Considerable diversity in genetic background was found among genotypes highlighted for low and stable Pb and Hg accumulation. Two modern genotypes (275 and 277), one old genotype (202) and one 3R genotypes (248) were identified for low and stable Pb concentration (Fig. 3B). Additionally, three modern genotypes (275, 277 and 278), one old genotype (202), one 1R genotype (220), one 1RS genotype (235) and one 3R genotype genotypes (248) were identified for low and stable Pb yield (Fig. 3E). Two modern genotypes (278 and 279), one old genotype (202) and one 2R genotype (270) were identified for low and stable Hg concentration (Fig. 3C), while genotypes with low and stable Hg yield were found to be 278 (modern), 202 (old) and 270 (2R; Fig. 3F). Furthermore, genotypes with chromosome 1R (221 and 222) and 1RS (229) showed a risk of a high level of Hg (Fig. 3C). However, due to the limited scale of the current study, the low-and-stable heavy metal accumulating genotypes identified here need to be subjected to field trials of a larger scale to confirm the beneficial effects of those rye chromosomes.

4. Conclusion

The predicted climate change will have a tremendous effect on crop cultivation. To cope with the changing growing conditions with a general increase in temperature and unpridcted spells of extreme weather, currently not utilized germplasm will need to be introduced to widen the genetic base for breeding. However, introduction of novel germplasm into conventional plant breeding of food crops migh also pose human health risks at consumption cultivars developed. Human health is significantly impacted by the nutritional value and toxicity of the foods consumed daily. Intake of heavy metals from food products contribute risk of toxicity and should be avoided. Increased levels of Cd in wheat during late drought conditions suggest that wheat-based food will

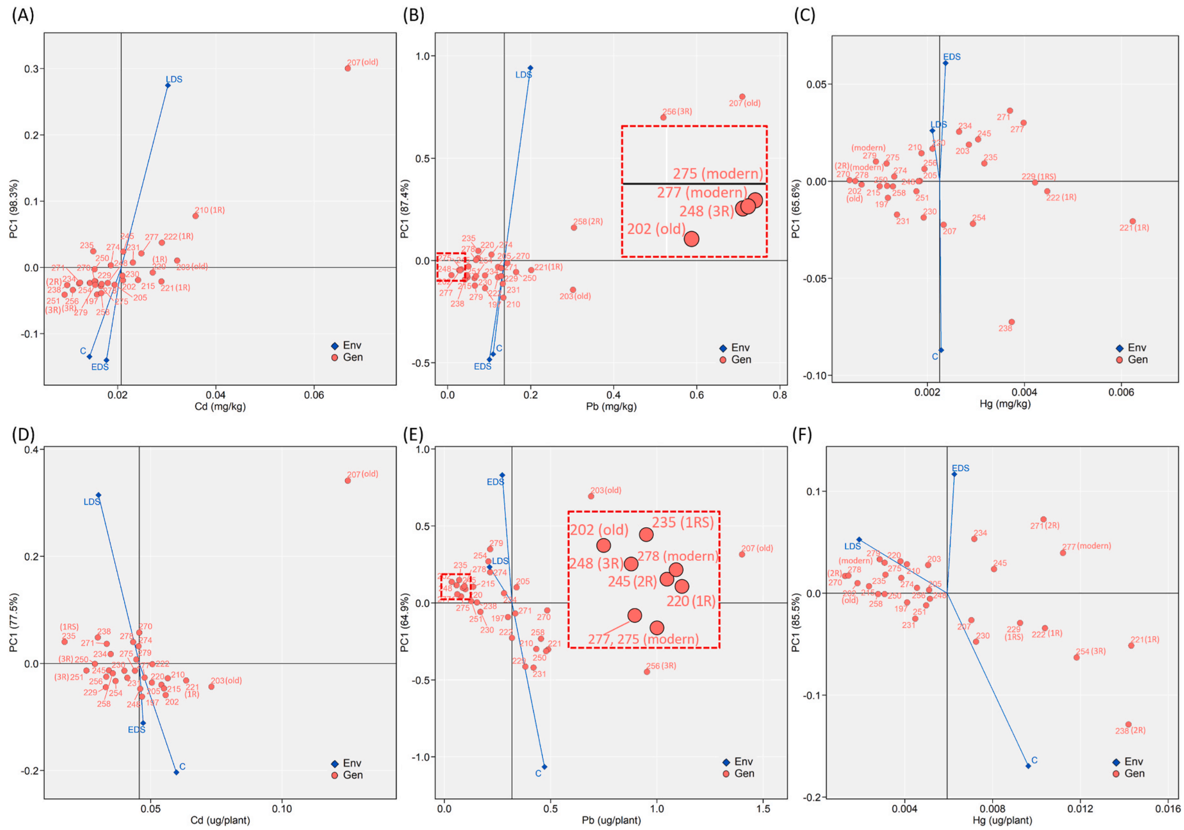

Fig. 3. Additive main effects and multiplicative interaction (AMMI) biplots showing (A) Cd, (B) Pb, (C) Hg concentration and (D) Cd, (E) Pb and (F) Hg versus the first principal component (PC1) score of 30 genotypes (Gen) and three growing conditions (Env) including control (abbreviated as C), early drought stress (EDS) and late drought stress (LDS). Genotypes located closer to the horizontal axis (score 0 on PC1) had relatively higher stability across three growing conditions. The vertical line in each figure indicates the average values of 30 genotypes. The red dashed-line square boxes indicate a zoomed-in view of the corresponding area.

potentially be higher in Cd due to climate change. This study also highlights concern of elevated Cd and Pb levels in certain wheat genotypes with chromosome 1R as well as older genotypes, such as 203, 207, 210 and 221. These genotypes are identified as potential sources of heavy metal accumulation and should be avoided in breeding programs. Some wheat-rye introgression genotypes with 1RS, 2R and 3R show promise in reducing wheat grain Cd content under both well-watered and drought conditions. The gene complexes found in the 3R lines are particularly interesting as they not only exhibited low Cd uptake, but have also shown high yield [20], high mineral content [24] and good and stable baking performance under drought conditions, which indicate their potential to be used in breeding future wheat for food security in a changing climate. The high accumulation of Cd and other heavy metals, especially in drought conditions, in the 1R genotypes, calls for more careful selection when incorporating 1R lines in breeding programs, despite their contribution to early vigor and high yield. Despite the generally high Pb content in older genotypes, specific lines like 202 with consistently low Pb levels should be further utilised as a genetic resource with low Pb content.

CRediT authorship contribution statement

Yuzhou Lan: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ramune Kuktaite:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Aakash Chawade:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Eva Johansson:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This research was funded by Trees and Crops for the Future (TC4F) and SLU Grogrund.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jafr.2024.101118.

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