



Germination of malting barley grains when using recycled steep-out water

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Abstract

The objective of the study was to quantify the inhibitory effect of repeated use of steep-out water on selected germination parameters. Ten malting barley genotypes from three sites were tested in this research. Statistically significant inhibitory effects of recycled steep-out water on germination rate GR (reduction by 22.8% on average compared to distilled water), germination index GI (reduction by 10.9% on average compared to distilled water), as well as germination energy GE (reduction by 2.4% on average compared to distilled water) were recorded. Compared to the average GI of all varieties in distilled water (4.6), GI was lower by 6.7% and 6.1% for Laudis 550, 5.0% and 6.3% for Manta, but 14.1% and 13.3% for Tango, and 17.2% and 15.2% for Vendela when water from the first and second steeps was used. The varietal differences identified, as well as the effect of provenience, indicated different levels of GI reduction depending on the vigour of the caryopsis. Caryopses of the genotypes with higher vigour are able to cope better with the inhibitory effect of the steep-out water on germination.

Keywords: germination; grain quality; grain vigour; variety; provenience

1 Introduction

In terms of abiotic stresses and their impacts, soil drought and high air temperatures represent serious issues for crop production. These abiotic stressors cause problems for a wide range of field crops, including spring barley (Klimešová et al., 2013; Středa et al., 2020). This has been evident at least in the last two decades in the Czech Republic as well. We need to take into account current issues of water availability, such as water withdrawal limits or increasing risks of dry episodes. At the same time, environmental aspects of wastewater management and economic context play also a fundamental role. Then we can argue that the possibility of resuing steep-out water in the malting process of barley is an important trend.

Breeding varieties with increased tolerance to abiotic stressors has been promoted as part of adaptation

measures to current climatic conditions (Frantová et al., 2022). Seed vigour is a promising criterion for selection of genotypes tolerant to certain abiotic stresses (Ullmannová et al., 2013). Seed vigour is generally defined as the ability to germinate in adverse conditions; it expresses the degree of tolerance of seeds to adverse conditions during germination and emergence (Vintrliková et al., 2015; Lazarova et al., 2016). The motivation behind breeding for greater vigour, when its heritability has been proven, was the assumption that genotypes with more vigorous seeds could have increased drought tolerance. In malting barley, high grain vigour is also an intensifying factor in the malting process because of the requirement for homogeneous and rapid germination in a wider range of conditions.

The main external factors that contribute to the biological value of seeds include the environmental conditions during their development and the storage. Maximum plant health and minimal stress during critical stages of plant development promote high germination. High-quality seeds are able to germinate under a greater range of environmental factors better than low-quality ones. The interaction of variety and environmental conditions during the seed development is then considered to be the main factor in quality variability. In this context, germination or generally the vigour, are strongly influenced by temperature and rainfall before full maturity of the seed. The internal quality of the seeds in this period is negatively affected by the combination of lower temperature and high rainfall. Chloupek et al. (1997) found a clear correlation between rainfall, vigour and germination of the grain in eight spring barley varieties grown at eight localities. Samarah and Alqudah (2011) studied the effect of late drought stress (during the caryopses formation stage) on germination and vigour of the barley grain. Drought stress during the stage of grain formation did not affect germination, but the vigour of the caryopses was lower. In addition to abiotic environmental conditions, germination is also affected by biotic and agronomic conditions. The provenience and seed age also influence tolerance to stress conditions.

Recently published studies have described the methods for objective, replicable, rapid and inexpensive determination of vigour applicable in research, including phenotyping prospective genotypes (Mavi et al., 2014; Anandan et al., 2020; Longlong et al., 2021). Prospectively, results from seed production research can be applied to other areas where vigour also plays an important role, such as malting. Testing the vigour with various factors involved may give a good prediction of germination parameters in malting plants. Grains/caryopses with higher vigour take up water rapidly at the beginning of steeping, but then the distribution of water in the grain is slowed down and steeping is accomplished later. Conversely, grains with lower vigour show slower, continuous uptake of water and earlier even steeping (Kunze, 2004).

Malting plants belong to important water consumers. Depending on the technological level of the plant, an average of 5,000 litres of water is used per tonne of malt (EUROMALT, 2022). Therefore, recycling of the steep-out water in the malting process must be considered, as investigated in a study by Guiga et al. (2008). Seeds of genotypes with higher vigour could cope better with the phenomenon of inhibition, and could possibly provide guidance on how to reduce the inhibitory effects. From a malting point of view, it is not necessary

to identify the specific biologically active substances in the steep-out water that inhibit barley germination, ranging from chemicals contained in dust from the husk surface to substances leached from the husks and metabolites of microorganisms (Kocková-Kratochvílová and Lukášová-Novotná, 1959; Kunze, 2004; Justé et al., 2011). It is essential to quantify the impacts of the use of the steep-out water, to determine the influence of variety and provenience when steep-out water is reused, and to assess the potential which reusing the steep-out water has.

The objectives of the study were:

- (i) to test the vigour of seeds stored in the exactly defined laboratory conditions after six years of storage,
- (ii) to compare parameters of germination using steep-out water and standard distilled water, and
- (iii) to verify and quantify the inhibitory effect of repeated use of steep-out water on malting barley germination.

Research hypothesis:

- (i) during the steeping process, substances with a certain degree of inhibitory effect on germination are released into the steep-out water and their content interacts with the vigour of the grains,
- (ii) varieties of malting barley are variable in vigour and thus show different capacity to germinate uniformly and rapidly under suboptimal conditions,
- (iii) the effect of the provenience demonstrably causes a different capacity to germinate uniformly and rapidly under suboptimal conditions.

2 Materials and methods

2.1 Plant material resources

Grain from the harvest of 2016 from three agro-ecologically different sites of the Central Institute for Supervising and Testing in Agriculture (CISTA) were used:

- Hradec nad Svitavou (Hradec): 49.7439069N, 16.5168358E, 450 m.a.s.l., long-term average annual temperature 7.4 °C, long-term average annual precipitation 616 mm,
- Věrovany: 49.4552808N, 17.2815244E, 207 m.a.s.l., long-term average annual temperature 8.7 °C, long-term average annual precipitation 502 mm,
- Vysoká: 49.6310619N, 13.9542983E, 585 m.a.s.l., long-term average annual temperature 7.1 °C, long-term average annual precipitation 611 mm.

The course of the weather in the harvest year of 2016 was normal in terms of temperature at all experimental sites. During the growing season (March to July), only June was different from normal (air temperature 1.3 to 1.7 °C above normal). Precipitation totals were different at the experimental sites. In Hradec nad Svitavou, precipitation totals were normal during the growing season. In Věrovany, precipitation totals were elevated in July. Precipitation totals in Vysoká were normal in the critical months. However, intense precipitation totals were recorded during a short period in some regions. This had an impact on the harvest date and potentially negatively affected the vigour of the caryopses. Psota (2017) reported up to 44.4% of samples damaged by overgrowth in some regions.

The experimental material comprised of ten spring barley varieties registered in the Czech Republic (Bojos, Francin, KWS Amadora, KWS Irina, Laudis 550, Man-ta, Overture, Sebastian, Tango, Vendela) (Horáková and Dvořáčková, 2017).

2.2 Germination tests

In 2016 as well as 2022, germination tests were carried out according to the European Brewery Convention (EBC) Analysis Committee methodology EBC 2010 – 3.7 (EBC Analysis Committee, 2010) and the germination rate (GR) was evaluated according to the formula $GR = (5 \times a + 3 \times b + c) / (5 \times 3)$ using three replicates, where: “a” is an average number of grains germinated after 24 hours from the beginning of the test, “b” is an average number of grains germinated 24 to 48 hours of the test duration, “c” is an average number of grains germinated from 48 to 72 hours of the test duration (Prokeš and Hartmann, 2005). In 2016 the grains were then placed in polythene bags and stored under vacuum at 4 °C. In 2022, these barley samples were subjected to repeated tests for germination energy (GE; GE corresponds to the speed and uniformity of seedling emergence – Zhang et al., 2020), and germination index (GI; GI is considered to be the best predictor of the dormancy depth – Frančáková and Lišková, 2009) according to the EBC 2010 – 3.7 (EBC Analysis Committee, 2010) and germination rate (GR; GR provides a measure of the time course of seed germination) according to Prokeš and Hartmann (2005). Tests were carried out as follows: 90 mm diameter plastic sterile Petri dishes, two layers of 85 mm diameter circle filter paper (“top of paper”), 4 ml of water per 1 Petri dish, 100 caryopses in 1 Petri dish, 3 replicates. Petri dishes were placed in a climatic test chamber at a temperature close to the test of vigour (Ullmannová et al., 2013), i.e. at 14 °C and relative humidity of 85% ± 5% in order to simulate the temperature conditions during germination

in the malt house. Visual evaluation was performed after 24, 48 and 72 h ± 1 h. Correctly germinating grains were identified according to the manual by Psota et al. (1998) and removed.

2.3 Variants of the experiment

- Control variant (DW): the grains were cultivated in distilled water.
- Fresh (steeping) water variant (FW): the grains were cultivated in fresh water taken from an underground borehole, commonly used by commercial maltsters for steeping malt. The water was analysed with a Jenway 4510 Conductivity Meter (Jenway™). High salt content, electrical conductivity (EC) 1577 $\mu\text{S}\cdot\text{cm}^{-1}$, classification based on EC according to Rhoades et al. (1992) – type II slightly saline, if EC rely between 700 and 2000 $\mu\text{S}\cdot\text{cm}^{-1}$; EC between 800 and 2 500 $\mu\text{S}\cdot\text{cm}^{-1}$ is still suitable for human consumption, while irrigation at these levels should be managed to minimise plant damage – Looock et al. (2015).
- Steep-out water 1 variant (SW1): the grains were cultivated in the water obtained after the first steep.
- Steep-out water 2 variant (SW2): the grains/caryopses were cultivated in the water obtained after the second steep.

2.4 Statistical analyses

The Shapiro-Wilk test was used to verify the normal distribution of the data. The data on germination energy (GE), germination rate (GR) and germination index (GI) deviate from a comparable normal distribution. For this reason, a non-parametric test, the Kruskal-Wallis H test, was used to evaluate the differences (the Kruskal-Wallis test is a non-parametric alternative to One Way ANOVA). The Mann-Whitney U test was used to compare differences between two independent groups (comparison of the results from 2016 and 2022). Pearson’s correlation coefficients were used to measure the strength of the linear relationship between the two variables. STATISTICA 12 software (StatSoft CR Ltd.) was used for statistical analyses.

3 Results and discussion

Testing of samples (GE, GR, GI) was carried out after harvest in 2016. In 2022, i.e. after six years of storage, the samples were used to test germination and vigour parameters at 14 °C. The reason for testing long-term stored grains was the assumption of greater variability in germination parameters as a function of vigour (influence of genotype, provenience and year) after the seeds had

aged. Seed aging is influenced by many factors, from plant emergence to storage, and affects germination (Tomer and Maguire, 1990; Nagel et al., 2016). Aging is modified by genetic characteristics of the variety, physiological, structural, chemical, and biochemical properties of the seed or its size (Kunze, 2004). It has been found that seed lots that do not differ in germination, can differ in emergence and storage potential (Matthews et al., 1986). Loss of vigour due to seed aging is mainly associated with the loss of cytoplasmic membrane integrity (Senaratna et al., 1988). This is associated with numerous cellular and biochemical alterations, mainly impaired RNA and protein synthesis as well as DNA degradation (Lehner et al., 2008).

The GI, GR, and GE values of all samples (10 varieties, 3 sites, n = 30) found in 2016 using distilled water were correlated with the GI, GR, and GE values of all samples (n = 30) found using distilled water in 2022. It was hypothesized that grains with high germination in 2016 would also have high germination in 2022. A statistically significant relationship (r = 0.539) was found only in the case of GE (Table 1). No statistically significant correlations were found between the 2016 GI and 2022 GI values, as well as between the 2016 GR and 2022 GR. Thus, a variable evolution of the internal grain quality during storage with an effect on germination parameters is evident. In the case of GE, a statistically significant correlation (r = 0.722) was found between the values of 2016 and 2022 grains from the Vysoká site (n = 10). This was influenced by the presence of samples with impaired quality already in 2016, which logically affected the statistical evaluation even after six years of storage. The effect of provenience on the germination parameters may be larger than varietal differences. Chloupek et al. (2008) used the vigour test for spring barley grain under

conditions of artificially induced water stress. The authors found that the lower the vigour of the caryopses, the lower the relative influence of the provenience and on the contrary the higher the influence of variety, the higher the interaction between varieties and sites.

3.1 Varietal differences in germination parameters (tests carried out in 2022)

After six years of storage, the Vendela variety (99.2%) was statistically significantly (p ≤ 0.05) different in the GE parameter (tested using distilled water) from the KWS and Amadora (96.7%), and Sebastian (97.0%) varieties.

Varietal difference in GI parameter was found (tested using distilled water). The group of the Laudis 550 (4.8), Manta (4.7), and Bojos (4.7) varieties was statistically significantly (p ≤ 0.05) different from KWS Amadora (4.4), Sebastian (4.5), and Vendela (4.5). The differences in GI of the caryopses (mean value 4.6) depending on provenience (site) after six years of storage were not significant.

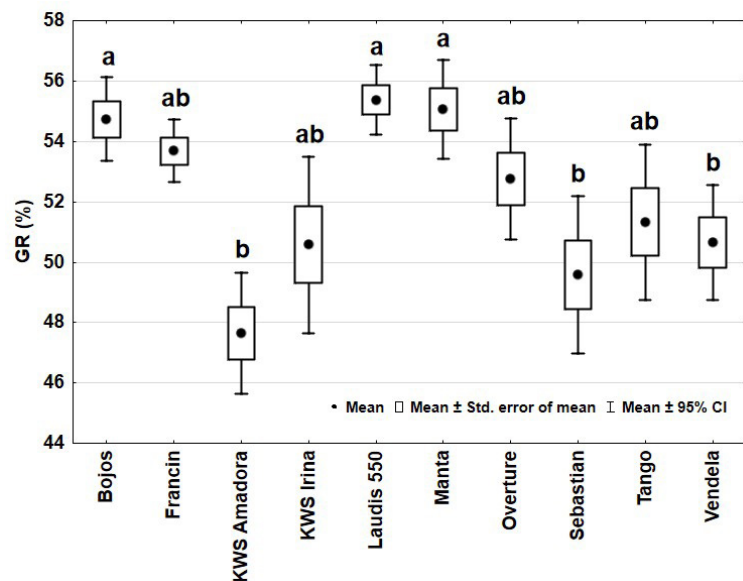


Figure 1 Barley varietal differences in GR - year 2022 (after six years of storage; tested using distilled water) Note: Kruskal-Wallis H test (df 9; n 90); p = 0.000. The values marked with different letters differ statistically significantly at p ≤ 0.05

Table 1 Relationship between germination parameters in 2016 and 2022 expressed by correlation coefficients

Site	Germination Parameters		
	Germination Energy GE	Germination Index GI	Germination Rate GR
Hradec (n = 10)	-0.225	-0.034	-0.127
Věrovany (n = 10)	-0.208	0.341	0.221
Vysoká (n = 10)	0.722	-0.020	0.055
All sites (n = 30)	0.539	-0.012	0.050

Note: Bold values denote statistical significance at the p ≤ 0.05

Similarly, varietal difference in GR (tested using distilled water) was found. The group of Laudis 550 (55.4%), Manta (55.1%), and Bojos (54.7%) varieties was statistically significantly ($p \leq 0.05$) different from KWS Amadora (47.6%), Sebastian (49.6%), and Vendela (50.6%) after six years of storage (Figure 1). The differences in GR of the caryopses (mean value 52.1%) depending on provenience were not significant in 2022.

3.2 Effect of the site on germination parameters (tests carried out in 2022)

The effect of the site on germination parameters (values across variants) is shown in Table 2. Grains from the Věrovany site had the best germination parameters.

3.3.1 Effect of steep-out water on GE

A statistically significant ($p \leq 0.05$) decrease in GE using SW1 and SW2 compared to the DW and FW variants was recorded (Figure 2). The GE value in the DW variant (98.1%) was reduced to 97.4% by the use of fresh water (FW). The GE in the SW1 and SW2 variants was reduced to 95.7%.

The results showed that the steep-out water had a different effect on the GE of caryopses of different provenience (site effect). When SW1 and SW2 were used, a statistically significant ($p \leq 0.05$) decrease in GE was found for the Věrovany and Hradec sites. In the case of Vysoká (the site with the lowest GE of 95.1%), a statistically significant decrease in GE was also observed in the FW variant. This indicates reduced vigour of the caryopses.

Table 2 Mean values and statistical differences of barley germination parameters – effect of the site in 2022

Site	Germination Parameters		
	Germination Energy GE [%]	Germination Index GI [-]	Germination Rate GR [%]
Hradec	96.6 a	4.2 a	43.7 a
Vysoká	96.1 a	4.3 b	45.1 b
Věrovany	97.4 b	4.3 b	46.0 b

Note: The values marked with different letters in the columns differ statistically significantly at $p \leq 0.05$

3.3 Germination parameters using recycled steep-out water (tests carried out in 2022)

In this section the research hypothesis (i) that “during the steeping process, substances with a certain degree of inhibitory effect on germination are released into the steep-out water and their content interacts with the vigour of the grains” was tested.

The values of GE, GI and GR of grain were statistically significantly lower in the FW variant (Table 3) than the results with distilled water (DW). This is most likely due to a high salt content in water electrical conductivity $1577 \mu\text{S}\cdot\text{cm}^{-1}$) with a negative effect of salinity on seed germination and early seedling stage (Uçarlı, 2020).

es from Vysoká and increased sensitivity to even moderately stressful conditions in the case of low GE.

The GE of the grains, depending on the steeping water used, was significantly variety specific. GE differences depending on the steeping water used were not statistically significant in the group of KWS Irina, Laudis 550, Overture, Sebastian, and Tango varieties (GE of the group 97.8% in the DW variant). SW1 and SW2 reduced statistically significant GE of Bojos, Francin, and Vendela (i.e. varieties with high GE averaged 98.9% in the control variant). SW2 reduced the GE of the KWS Amadora variety statistically significantly. Also SW1 reduced the GE of the Manta variety statistically significantly.

Table 3 Mean values and statistical differences of barley germination parameters – effect of the used water in 2022

Variant	Germination Parameters		
	Germination Energy GE [%]	Germination Index GI [-]	Germination Rate GR [%]
SW2 – Steep-out water 2	95.7 a	4.1 a	40.6 a
SW1 – Steep-out water 1	95.7 a	4.1 a	40.2 a
FW – Fresh water	97.4 b	4.3 b	46.3 b
DW – Distilled water	98.1 c	4.6 c	52.1 c

Note: The values marked with different letters in the columns differ statistically significantly at $p \leq 0.05$

Varieties that achieved a minimum GE value of 95%, met the requirements for malting purposes set up by the **EBC Analysis Committee (2010)**. KWS Amadora reached this GE limit only when germinated in distilled water.

3.3.2 Effect of steep-out water on GI

The effect of the tested water can also be expressed by GI, which is more indicative of barley grain behaviour (Guida et al., 2008) and is a suitable indicator of malting potential, according to Woonton et al. (2005). A statistically significant ($p \leq 0.05$) decrease in GI was found when using FW, SW1 and SW2 compared to the DW variant (Table 3). The reduction in GI (Table 3), compared to the DW control (4.6), was 6.5% in the FW variant (GI 4.3), and 10.9% in the SW1 and SW2 variants (GI 4.1).

The predominant effect on GI reduction was due to water salinity – FW variant (6.5% from 10.9%, i.e. 60%). Thus, the additive effect of water salinity and the grain (SW1 and SW2) would be significantly reduced by using water with optimal parameters.

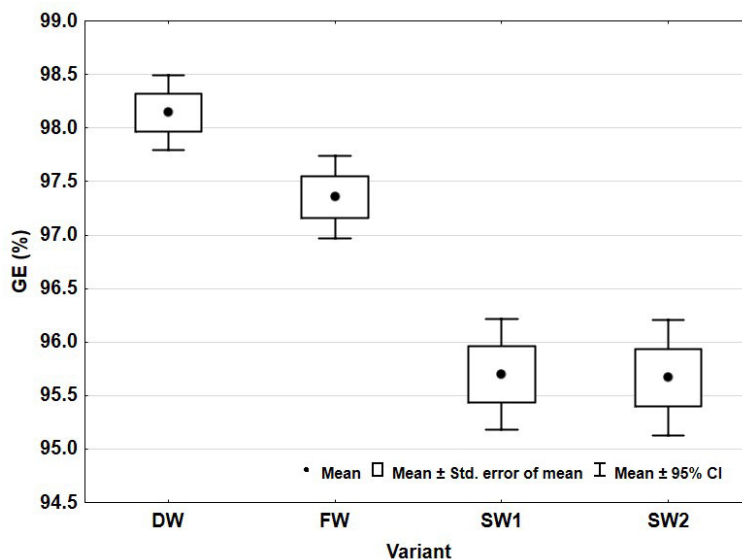


Figure 2 Effect of steep-out water on germination energy
 Note: Kruskal-Wallis H test (df 3; n 360); $p = 0.000$

The effect of the steep-out water on GI was the same for the grains from all sites, i.e. the use of FW, SW1 and SW2 statistically significantly reduced GI compared to the DW variant.

The GI response of the grains depending on the steeping water used was variety specific (Figure 3). However,

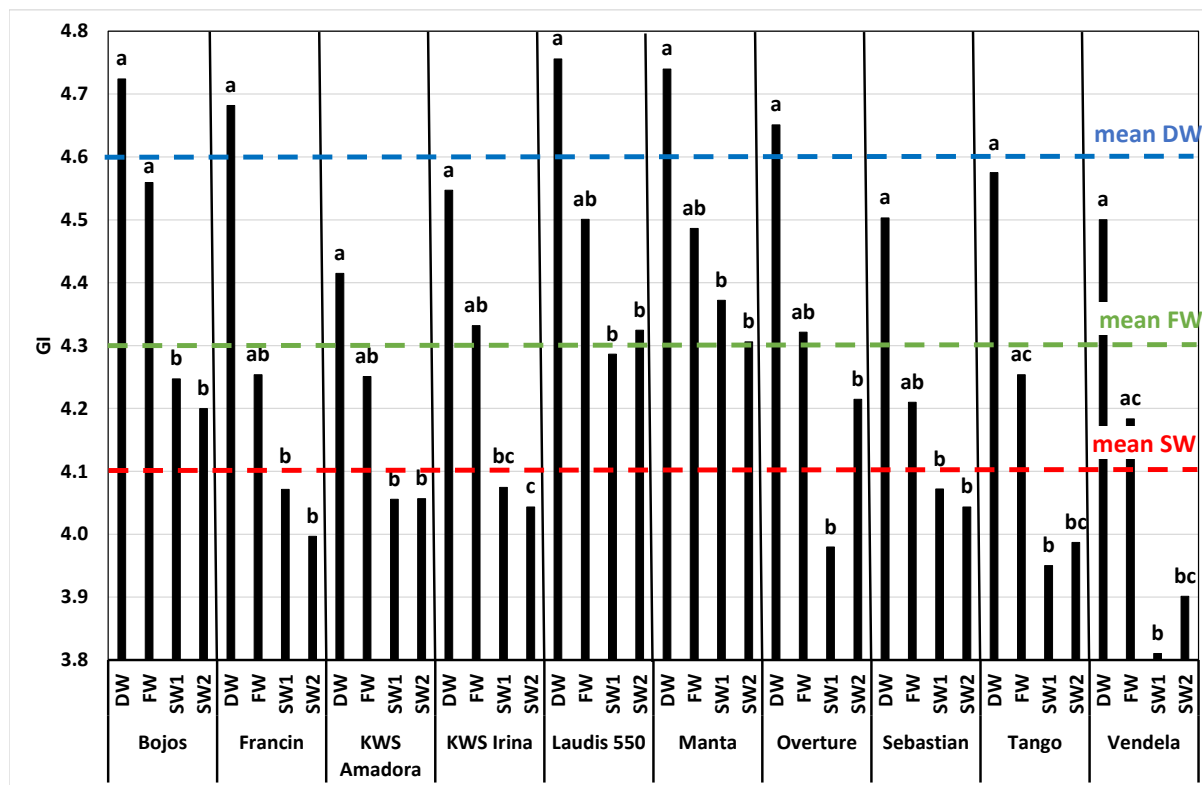


Figure 3 Effect of steep-out water on germination index of barley – varietal differences
 Note: Statistically significantly different values are indicated by different letters

SW1 and SW2 reduced GI statistically significantly in all varieties. In this section the research hypothesis (ii) that “varieties of malting barley are variable in vigour and thus show different capacity to germinate uniformly and rapidly under sub-optimal conditions” was tested.

3.3.3 Effect of steep-out water on GR

GR was also significantly modified. In the DW variant (distilled water), the mean GR across the samples from sites and varieties was 52.1% (Table 3). A statistically significant inhibitory effect ($p \leq 0.05$) on GR was observed in the FW (GR 46.3%, 11.1% reduction compared to DW, Table 3), SW1 (GR 40.2%, 22.8% reduction compared to DW), as well as SW2 (GR 40.6%, 22.1% reduction compared to DW) variants.

The effect of provenience (site) on GR was identical for all three sites. Across varieties, the same trend was identified in samples from all sites, with SW1 and SW2 variants differing from the other variants.

A detailed assessment of the effect of the steep-out water on GR by the site and variety is presented in Table 4. In this section the research hypothesis (iii) that “the effect of the provenience demonstrably causes a different capacity to germinate uniformly and rapidly under suboptimal conditions” was tested. GR was demonstrably affected only by the use of SW1 or SW2 in this case. Bojos (GR in the DW variant = 54.7 %, mean GR of all variants = 48.3 %), KWS Amadora (GR in the DW variant = 47.6%, mean GR of all variants = 42.0 %), and KWS Irina (GR in the DW variant = 50.6 %, mean GR of all variants = 44.2 %) varieties did not show

Table 4 Effect of steep-out water on germination rate of barley – varietal and site effects

Variety/site		Variant	
		DW versus SW1	DW versus SW2
Bojos	Hradec		
Francin	Hradec		
KWS Amadora	Hradec		
KWS Irina	Hradec		
Laudis 550	Hradec	SD	
Manta	Hradec	SD	
Overture	Hradec	SD	
Sebastian	Hradec		
Tango	Hradec	SD	
Vandela	Hradec	SD	
Bojos	Věrovany		
Francin	Věrovany		SD
KWS Amadora	Věrovany		
KWS Irina	Věrovany		
Laudis 550	Věrovany		
Manta	Věrovany		SD
Overture	Věrovany	SD	
Sebastian	Věrovany		SD
Tango	Věrovany	SD	
Vandela	Věrovany	SD	
Bojos	Vysoká		
Francin	Vysoká		
KWS Amadora	Vysoká		
KWS Irina	Vysoká		
Laudis 550	Vysoká		
Manta	Vysoká		
Overture	Vysoká	SD	
Sebastian	Vysoká		
Tango	Vysoká		SD

Note: Statistically significantly different values ($p \leq 0.05$) are indicated by SD

a statistically significant reduction in GR in any of the observations. This is due to the high vigour (germination rate) of Bojos and the low vigour of KWS Amadora, and KWS Irina.

4 Conclusion

The inhibitory effect of the repeated use of the steep-out water on germination of malting barley was verified and quantified. A significant inhibitory effect of both steep-out water and fresh steeping water used on the tested germination parameters was found. The identified varietal differences, as well as the effect of provenience, indicate different levels of reduction depending on seed vigour.

The paper offers a basis for practical decision-making which is at the same time considering economic and environmental aspects of the use of recycled steep-out water in relation to the inhibitory effects on germination. Some varieties showed greater tolerance to inhibitory effects when recycled steep-out water was used. The issue of steep-out water reuse is getting even more urgent with current and predicted water shortages.

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