

Hulless barley (*Hordeum vulgare* L.) resistance to pre-harvest sprouting: diversity and development of method for testing of breeding material

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Abstract. Pre-harvest sprouting significantly reduces grain yield and quality of hulless barley (HB). In field conditions pre-harvest sprouting resistance can be scored rarely. The objective of the research was to develop appropriate testing method for barley breeding material in laboratory conditions and to estimate genetic diversity in pre-harvest sprouting resistance among various HB genotypes. The amount of seeds with visible germination symptoms were determined in days 4, 7 and 10 after initiation of laboratory test; it was started in ripening stage and after 4 weeks of storage of harvested spikes. The average amount of germinated grains differed significantly between the three estimation days ($p < 0.001$) in all years and both if tested in ripening stage or during storage. The effect of genotype on the number of germinated grains was the highest in the 10th testing day. Germination was significantly higher if the test was started after 4-week storage of harvested spikes. The correlation between amounts of germinated grains with the sprouting scores obtained in field conditions was significant in most of the cases; the highest values of correlation coefficient were obtained in estimation days 7 and 10 if the test was started in ripening stage. As more suitable for performing laboratory test of pre-harvest sprouting in barley breeding program can be suggested testing in ripening stage with estimation day 10 or 7. A noticeable variation of amount of germinated grains among the genotypes was found (CV = 49.8%, 10th estimation day). It approves the possibility for improvement of this trait by breeding.

Key words: spring hulless barley, pre-harvest sprouting

INTRODUCTION

Pre-harvest sprouting significantly reduces grain yield and quality of hulless barley (HB). Due to climatic changes caused by global warming precipitation in northern Europe has increased 10–40% in the last century (McCarthy et al., 2001). Long-lasting precipitation periods can be expected and the need for HB resistance to pre-harvest sprouting becomes even more important.

Breeding barley for malt, breeders have put a selection pressure on early and uniform germination of grains – the acceptable quality for malting process is at least 95% of germinated grains – therefore barley has tendency to have low dormancy and thus grains may germinate in the ears (Fox et al., 2003; Gubler et al., 2008).

Seed dormancy and pre-harvest sprouting is subjected to multiple factors. It is controlled by multiple genes and influenced by environment and genotype by

environment interaction (Li et al., 2004). Several QTLs associated with pre-harvest sprouting have been mapped in barley in multiple mapping populations – Chebec/Harrington (Li et al., 2003), Steptoe/Morex (Ullrich et al., 2008) and Harrington/ TR306 and Triumph/Morex (Ullrich et al., 2009). Inheritance of the trait is complex and QTLs are spread over most of the chromosomes (Ullrich et al., 2009). Dormancy and release of dormancy are subjected to hormonal balance in the seed. Abscisic acid is necessary for induction of dormancy whether elevated levels of gibberellic acid trigger release of dormancy. (Adkinds et al., 2002; Gerjets et al., 2010). Majority of HB genotypes have a short dormancy period and ability to absorb water very rapidly (Box & Barr, 1997). According to resistance to pre-harvest sprouting HB genotypes can be divided in three groups: (1) resistant, (2) sprouting in small extent and (3) susceptible (Bihovec, 1949). The existence of such groups approves genetic diversity and selection possibilities for pre-harvest sprouting resistance.

In field conditions pre-harvest sprouting resistance can be estimated rarely (Li et al., 2004; Legzdina & Berzina, 2008). To be able to perform selection in breeding program it is essential to obtain the information about breeding material every season. Therefore indirect testing methods are required. Various testing methods have been used to access pre-harvest sprouting in cereals – keeping ears in wet chambers (Ullrich et al., 2008; Gerets et al., 2010), spraying ears regularly (Humphreys & Noll, 2002), keeping ears on moist filter paper on Petri dishes (Derycke et al., 2002; Groos et al., 2002), germinating threshed seeds on moist filter paper (Gerets et al., 2010).

The objective of the research was to develop appropriate testing method for barley breeding material in laboratory conditions and estimate genetic diversity in pre-harvest sprouting resistance among various HB genotypes.

MATERIALS AND METHODS

Trials were performed during 2007–2009 in Priekuli, Latvia. 61 HB genotypes were used, including 42 Latvian breeding lines and 19 varieties and lines from other countries. In addition 4 registered Latvian covered barley varieties and the variety ‘Samson’ (Canada) as resistant control were tested. Barley genotypes were grown on 2.3 m² plots.

Due to significant amount of rainfall in the grain ripening period in 2008 (Table 1) it was possible to score pre-harvest sprouting in field conditions. Sprouting was scored visually by assessing whole plot according to scale 0–4 (0 – no sprouted grains visible; 4 – practically all grains sprouted).

Table 1. Duration of vegetation and amount of precipitation before ripening.

Year	2007	2008	2009
Average duration of vegetation, days	101	104	101
Average date of ripening stage	8.08.	10.08.	9.08.
Precipitation, (mm) first decade of August	1.3	56.4	0.9

To assess the germination in laboratory conditions, three spikes per genotype were collected in grain ripening (growth stage 92 according to decimal code of growth stages of cereals); each spike was presumed as one replication. Spikes were placed on Petri dishes between moist filter paper layers and kept at 20°C with 16/8h photoperiod. The amount of seeds with visible germination symptoms were determined in days 4, 7 and 10 after initiation of the test. The testing method was adapted with modifications from Derycke et al. (2002). During 2008–2009 the test was initiated in two different times: in ripening stage and after 4 weeks storage of harvested spikes in 20°C.

Two factors ANOVA with replications was used for data statistical analysis and correlation coefficients were calculated using MS Excel.

RESULTS AND DISCUSSION

Development of testing method. The average amount of germinated grains differed significantly between the three estimation days ($p < 0.001$) (Fig. 1) in all years and both if tested in ripening stage or after storage. The effect of genotype on the number of germinated grains was significant in all cases (in days 4, 7 and 10 after starting the test and in ripening stage as well as after 4 weeks of storage of spikes, Table 2). The highest effect of genotype on amount of germinated grains (63.8–81.2%) was estimated in the 10th testing day in both testing terms.

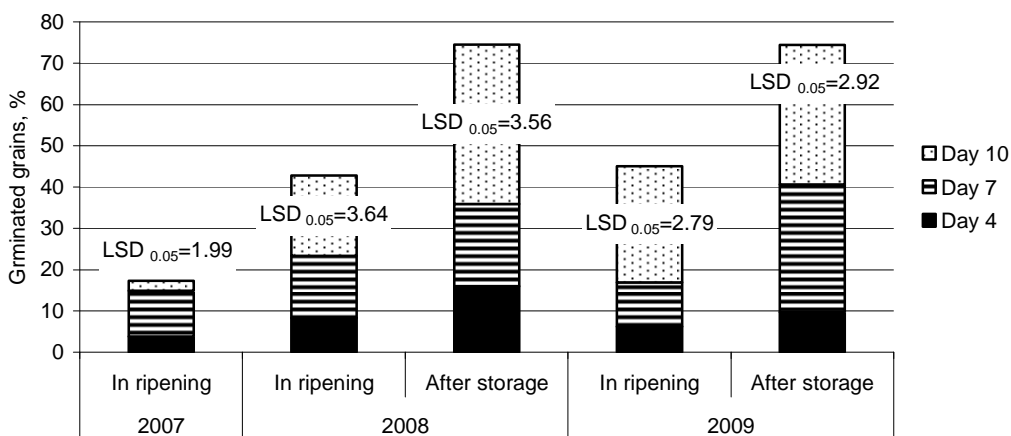


Figure 1. Average amount of germinated grains on day 4, 7 and 10 after initiation of test.

From the practical point of view it would be beneficial to postpone germination test to four weeks or even more after grain ripening time because during the harvest period usually there is a shortage of human resources for the laborious germination tests. It was the reason for inclusion in experiment the variant where test was started after storage of spikes. The time of initiation of test (in ripening or after storage)

significantly influenced average amount of germinated grains; germination was significantly higher if the test was started after 4 weeks of storage of harvested spikes (Fig.1). It can be explained by a gradual loss of dormancy. Because the aim of germination test in breeding program is to predict resistance to pre-harvest sprouting as close as possible to field conditions, a necessity to perform the test exactly in ripening stage was confirmed. Largest effect of time of initiation of test on the amount of germinated grains was observed in 10th testing day compared to days 4 and 7. Amount of germinated grains in 10th testing day was higher if test was started after storage of spikes. It suggests that if germination were assessed on 10th day after initiation of test, it would be even more important to perform the test in ripening stage but not after the storage of spikes.

If the effect of genotype on the average amount of germinated grains is compared among all the estimation days in both testing terms in 2008 and 2009 (Table 2), it was two times higher in ripening stage than after two weeks storage. It also comes out in favour to performing the test in ripening stage, when the differences between the genotypes were the largest. The results obtained by Derycke et al. (2002) with triticale showed opposite tendency: coefficient of concordance estimated from the rankings of laboratory tests and field observations was higher, if the spikes were stored at room temperature for 16 days in comparison to the test performed without storage.

The correlation coefficients between the amount of germinated grains and the sprouting scores obtained in field conditions was significant in all cases (except day 4 in 2007) (Table 2).

Table 2. Correlations among testing methods and effect of testing day on pre-harvest sprouting.

Parameter	Day after starting test	2007	2008		2009	
		In ripening	In ripening	After storage	In ripening	After storage
Correlation with score in field conditions in 2008 ($r_{0.05} = 0.244$, $r_{0.01} = 0.321$)	Day 4	0.16	0.35**	0.38**	0.27*	0.28*
	Day 7	0.27*	0.47**	0.28*	0.38**	0.25*
	Day 10	0.29*	0.51**	0.25*	0.45**	0.32*
Effect of genotype (η^2 , %)	Day 4	64.8**	65.8**	57.6**	68.3**	63.1**
	Day 7	79.8**	52.7**	43.9*	69.7**	55.6**
	Day 10	81.2**	63.8**	63.9**	72.4**	71.3**
Effect of genotype between estimation days (η^2 , %)	-	-	34.9**	17.5**	33.7**	16.0**
Effect of testing term, (η^2 , %)	Day 4	-	-	5.9**	-	2.5**
	Day 7	-	-	6.7**	-	19.7**
	Day 10	-	-	25.8**	-	24.2**

* $p < 0.05$, ** $p < 0.01$

The highest values of correlation coefficient were obtained in estimation days 7 and 10 if the test was started in ripening stage. If correlation coefficients in estimation days 7 and 10 were compared between both testing terms in 2008 and 2009, the significance level was higher if the test was started in ripening stage. In summary, testing in ripening stage and estimation of germination on days 10 or 7 can be suggested as the most suitable for performing laboratory testing of pre-harvest sprouting in barley breeding program.

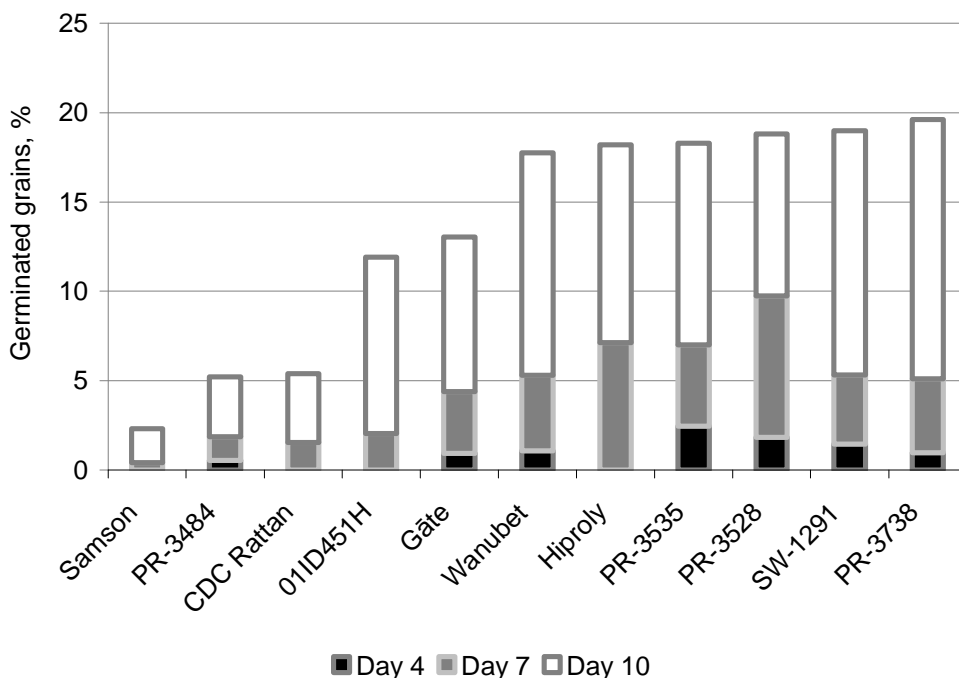


Figure 2. Average amount of germinated grains on day 4, 7 and 10 after initiation of test of the ten most resistant genotypes and resistant control ‘Samson’.

Estimation of genetic diversity. Histogram of germinated grains in the estimation day 10 over the three years (Fig. 3) shows a noticeable variation among the 66 tested genotypes ($CV = 49.8\%$). The highest resistance was registered for covered six-row barley variety ‘Samson’ (2.3%). ‘Samson’ is reported to have good sprouting resistance under wet-swath conditions (Nyachiro et al., 2002). The amount of germinated grains of all the ten genotypes (Fig. 2) significantly differed from ‘Samson’ in all the three test years ($p < 0.05$). The average values of germination for 10 most resistant genotypes ranged between 5.2–19.6%. From the tested Latvian covered barley varieties only ‘Gäte’ was among them with average of 13% germinated grains. Very good resistance to sprouting was found for HB breeding line PR-3484 (average 5.2%, Latvia) and ‘CDC Rattan’ (average 5.4%, Canada). Both genotypes can be

recommended for use in breeding for improving the pre-harvest sprouting resistance. Other genotypes with fairly good sprouting resistance were 01ID451H, ‘Wanubet’ (USA), PR-3535, PR-3528, PR-3738 (Latvia), SW-1291 (Sweden) and ‘Hiproly’.

High susceptibility to sprouting in all the three years was registered for covered barley ‘Idumeja’ (average amount of germinated grains 59%, Latvia) and HB genotypes HB 379 (68.9%, Canada), PR-3419 (69.1%, Latvia), L 13 (80.4%, Latvia) and ‘Jet’ (98%). The results approve that it is recommended to test sprouting resistance repeatedly, because there were genotypes with high variation among the years. Standard deviation for individual genotypes ranged between years from 1.9 to 43.6%, average 20.6%.

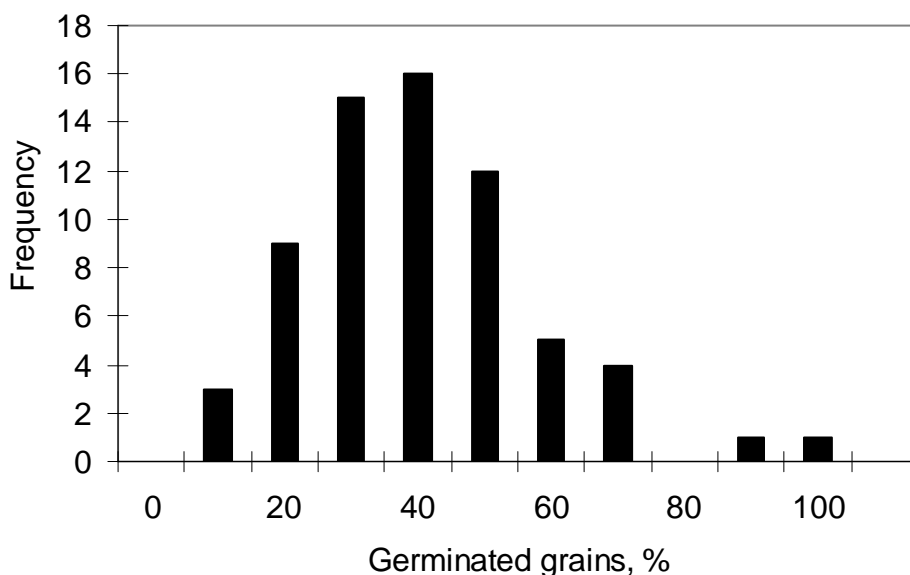


Figure 3. Descriptive histogram of observed average of germinated grains in estimation day 10.

CONCLUSIONS

As more suitable for performing laboratory testing of pre-harvest sprouting in barley breeding program can be suggested testing in ripening stage with estimation day 10 or 7.

There was notable variation of pre-harvest sprouting resistance among the tested genotypes. It approves the possibility for improvement of this trait by breeding; PR-3484 (Latvia) and ‘CDC Rattan’ (Canada) are suggested as resistance sources.

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