Estimation of Barley Seed Vigour with Respect to Variety and Provenance Effects

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Abstract: The study was aimed at determining potential differences in seed quality of spring and winter barley in relation to varieties (four winter and two spring barley varieties) and growing conditions (six locations, two years). Three tests were performed on each seed sample, i.e. (1) the standard germination test; (2) the low temperature and drought stress germination test and (3) the germination test in a salt solution. The effects of the factors of variety, provenance and year on seed vigour were estimated by the analysis of variance and Tukey's post hoc test. The germination of winter varieties was influenced by provenance, both measures of vigour (2 and 3) by year, and significant differences between varieties were found in all three traits. Spring varieties differed only in germination and vigour (3) in one year when the influence of provenance prevailed. The results show different effects of variety and provenance on seed vigour. This could be promising for the breeding of high vigour barley varieties. Some preliminary results were obtained by using controlled deterioration (CD) as a vigour testing method for barley. The suitable conditions seem to be the combination of 45°C and 24% moisture content for the period of 24 hours.

Keywords: barley; germination; seed vigour; variety; provenance; controlled deterioration

Seed quality has traditionally been associated with germination and purity. Since high-germinating seeds can largely differ in their field emergence or in their ability to germinate after storage or transport, seed vigour as another important seed quality component was established. Seed vigour is defined by ISTA (International Seed Testing Association) as *"an index of the extent of the physiological deterioration and/or mechanical integrity of a high germinating seed lot which governs its ability to perform in a wide range of environments"*. Seed deterioration expresses itself in a reduction in germination rate and uniformity, reduced tolerance to environmental stresses and consequently inferior seedling emergence and growth. Most vigour tests are based on promoting seed deterioration or germination under stress conditions (Hampton & TeKrony 1995). Vigour is not a single measurable property but a concept describing several characteristics which include the rate and uniformity of germination and growth, tolerance to environmental stresses after sowing, and retention of performance after storage. Seeds that perform well in some or all of these aspects are termed high-vigour seeds (Black & Bewley 2000).

The vigour tests that involved the artificial ageing of the seed, i.e. accelerated ageing (AA) test and controlled deterioration (CD) test, are considered to be the most promising of all the tests based on some aspects of germination behaviour. The CD test was initially developed for evaluating the

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quality of small seeds of vegetable species such as brassicas, carrots and lettuce, but it also seems to be suitable for grasses (Hampton & TeKrony 1995). The CD test is based on exposing seeds to high temperature and moisture for a short period of time. The deterioration technique is in principle similar to the AA test with the exception that the seed moisture is artificially raised to the same level for all samples prior to the period of deterioration at high temperatures. Using AA tests on barley was reported by Hosnedl and Honsová (2002). Although we have not found any references to the CD test for barley seeds, the applications of this test to other grain crops were reported, e.g. wheat (Modarresi & Van Damme 2003) and maize (TeKrony *et al*. 2005).

The aim of the present work was to study a possibility of determining differences in seed quality of barley using two seed vigour tests in comparison with the standard germination test and to determine the relationship between these tests. Previous research indicated that in the case of unsuitable weather conditions during the seed development, seed vigour was affected by the cultivar to a larger extent than by the location. Under suitable conditions the location plays a more important role (Chloupek *et al*. 2003).

MATERIAL AND METHODS

Four winter and three spring barley varieties were chosen from the list of barley varieties tested in State Variety Registration Trials in the crop years 2001 and 2002. Winter barley varieties Luran, Luxor and Okal are six-rowed, non-malting; cv. Tiffany is a two-row winter barley with malting quality. The spring varieties Akcent and Olbram were used in 2001. In 2002 cv. Akcent was not available, therefore cv. Nordus was included. All the three spring varieties are two-rowed, grown mainly for malting production. The seed samples were obtained from six field trial stations throughout the Czech Republic, their characteristics are given in Table 1. Average temperatures in both years were higher than the long-term temperature and also total rainfalls were above the long-term mean, particularly in 2002. No pesticides were used during growing and only standard amounts of fertilizers were applied. Post-harvest treatment included cleaning with a 2.0 mm sieve for winter six-rowed varieties and a 2.2 mm sieve for two-rowed ones. Immediately after harvest barley seed may exhibit dormancy preventing germination. Therefore the seed samples had been stored for three months until they developed full germination capacity.

Each seed sample was evaluated by two methods for seed vigour determination, i.e. the low temperature and drought stress germination test developed by Chloupek *et al*. (1997) and germination in a salt solution. Drought was induced by a polyethylene glycol (PEG) solution, whose osmotic potential of –2 bars corresponded to 100 g of PEG 6000 in 1 litre of distilled water at 10°C (Burlyn & Kaufmann 1972). The different stress was set with 0.8% NaCl solution at a laboratory temperature (20°C). Each seed sample was tested in four replications of 50 caryopses. The analysis of variance and Tukey's multiple range test were used for evaluation, for analysis of variance the germination rates were transformed by the function $y = \arcsin\sqrt{p}/100$, where p is the percentage

Table 1. Main characteristics of six locations

Location	Altitude (m)	Sep. 2000–Aug. 2001		Sep. 2001–Aug. 2002		30-year means	
		sum of rainfall (mm)	mean temperature (°C)	sum of rainfall (mm)	mean temperature (°C)	sum of rainfall (mm)	temperature (°C)
Hradec n/Sv.	450	723	7.9	621	8.0	624	6.5
Chrastava	340	808	9.0	953	8.9	798	7.1
Jaroměřice	425	524	9.1	605	8.9	487	7.8
Staňkov	370	525	7.8	754	8.8	511	7.8
Vysoká	610	709	8.4	926	8.6	599	7.4
Žatec	285	472	9.7	637	10.0	451	8.3
Means		627	8.7	749	8.9	578	7.5

of germination. The relationships were estimated by correlation coefficients (software Statistica 7.0; StatSoft, Inc).

Seed samples of cvs. Nordus and Olbram from Hradec field station were selected for the CD test. The samples were divided into four replications (approx. 10 g) and according to seed moisture, time and temperature combinations suggested by Matthews (2003) the moisture of weighted samples was adjusted to 22% and 24% using the filter paper method. Each replication was immediately placed in an aluminium-coated polyethylene bag and heat sealed. The bags were kept at 10°C for 24 h and afterwards heat-treated at 45 ± 0.3°C for 24 or 48 h. After this treatment, the germination test was set up using 100 seed per replication, and the results were compared with germination before deterioration. To avoid fungal contamination, deteriorated seeds were disinfected prior to the germination test. The procedure is fully described in Hampton and TeKrony (1995).

RESULTS AND DISCUSSION

Table 2 and 3 present germination and vigour data of seven barley varieties from the crop years 2001 and 2002. Because of not having the same spring varieties in both years, the winter varieties were evaluated separately. The analysis of variance (Table 4) showed the high impact of the year on vigour regardless of the method of determination, and low on the germination rate. The interactions between all the factors for winter varieties were statistically significant with the exception of standard germination test. In the case of spring varieties no interactions were significant at the 0.01 probability level and only the interaction for germination in 2001 was significant at the 0.05 probability level. Although the mean germination rates of winter barley samples were very similar in both years (98.2% and 98.6%, respectively), significant differences were found between spring barley varieties (98.7% and 96.7%).

The germination capacity generally considered acceptable for malting barley is 98%, but some samples of the malting cultivar Nordus in particular were far below this level. The evaluation of vigour showed even larger statistically significant differences between winter varieties as well as between locations. The expectation that cv. Tiffany, which was the first winter malting barley registered in the Czech Republic, would maintain high germination and vigour was not fully confirmed. The germination rate of Tiffany seed samples was worse (97.9%) than that of most winter varieties in both years (mean value 98.4%). On the contrary, samples of cv. Tiffany reached an above-average germination rate in a salt solution, in particular in 2002 (94.9%;

Table 2. Comparisons between means using Tukey's procedure for germination, vigour and germination in NaCl solution (winter barley varieties)

		n	Germination (%)		Vigour (%)		Germination in NaCl solution (%)	
Year	2001	96	98.2	a	91.1	a	95.2	b
	2002	96	98.6	a	93.8	b	89.0	a
Variety	Luran	48	97.9	a	90.8	a	92.5	ab
	Luxor	48	99.0	b	93.6	b	88.8	a
	Okal	48	98.7	ab	93.4	ab	91.2	ab
	Tiffany	48	97.9	a	92.0	ab	95.9	b
Location	Hradec	32	98.6	ab	94.8	b	88.1	a
	Chrastava	32	98.0	ab	92.9	ab	94.7	bc
	Jaroměřice	32	97.2	a	91.8	ab	92.6	abc
	Staňkov	32	98.8	ab	92.9	ab	92.9	bc
	Vysoká	32	99.2	b	91.1	a	95.0	c
	Žatec	32	98.4	ab	91.1	ab	89.1	ab
Mean			98.4		92.4		92.1	

Different letters denote statistically significant differences at the 95% confidence level

Table 3. Comparisons between means using Tukey's procedure for germination, vigour and germination in NaCl solution (spring barley varieties)

		n	Germination (%)		Vigour (%)		Germination in NaCl solution (%)	
2001								
Variety	Akcent	24	99.2	b	94.9	a	97.7	a
	Olbram	24	98.2	a	95.5	a	95.5	b
Location	Hradec	8	100.0	b	97.5	a	95.0	a
	Chrastava	8	97.2	a	95.5	a	95.2	a
	Jaroměřice	8	99.7	b	93.0	a	98.0	a
	Staňkov	8	98.7	ab	93.0	a	96.0	a
	Vysoká	8	100.0	b	98.0	a	97.7	a
	Žatec	8	96.2	a	94.2	a	97.7	a
	Mean		98.7		95.2		96.6	
2002								
Variety	Nordus	24	96.3	a	92.6	a	91.3	a
	Olbram	24	97.2	a	92.4	a	91.3	a
Location	Hradec	8	99.5	c	96.5	b	98.5	c
	Chrastava	8	98.0	bc	93.2	ab	90.5	ab
	Jaroměřice	8	99.0	c	97.2	b	93.5	bc
	Staňkov	8	96.5	b	91.5	ab	91.7	ab
	Vysoká	8	88.7	a	86.2	a	88.2	ab
	Žatec	8	98.7	bc	90.2	ab	85.5	a
	Mean		96.7		92.5		91.3	

Different letters denote statistically significant differences at the 95% confidence level

mean value 89.0%). In spring varieties the impact of location on germination and vigour was higher in 2002 than in 2001. This fact can be explained by the adverse weather conditions during ripening in the crop year 2002, when heavy rains at the end of July and at the beginning of August delayed harvest, especially at the Vysoká field station (harvest date 18. 8.), where the total rainfall for the period of 20 days before harvest amounted to183 mm. The long-term sum of rainfall for the same period is only 40 mm. Winter varieties were already harvested at all stations (harvest dates from 3. 7. to 13. 7.), therefore their quality was not influenced. A genetic variation in seed vigour was confirmed in related research, e.g. by inheritance studies of winter barley and a screening programme for high vigour in spring barley (Riis *et al.* 1993).

The germination rate was not associated with vigour, the correlation coefficients between standard germination, germination in PEG solution and in NaCl solution were 0.24; 0.16 and 0.01 respectively. NaCl solution seems to induce different stress conditions than PEG. Vigour determination is generally applied for the detection of seeds that regardless of their high germination can cause some difficulties because of poorer field emergence or storage potential under environmental stresses. From this viewpoint both vigour tests used in this study could be applicable, the differences between seed samples with similar germination rates were found.

Varieties Nordus and Olbram from the Hradec station (2002) were used for the controlled deterioration test because of their high germination rates. The objective was to determine the optimum combination of temperature, moisture content and treatment time. The samples behaved differently upon the treatment (Table 5). For barley, the seed moisture level of 24% and temperature of 45°C for 24 h seems to be the most promising. Although Modarresi and Van Damme (2003) suggested the treatment period of 48 h as optimal for wheat, it showed to be too severe for barley, deterioration for 48 h decreased the germination rate to 4.2% at the moisture content of 22%; and almost all the seeds were killed at the moisture content of 24%.

Table 4. Analysis of variance for germination, vigour and germination in NaCl solution in the years 2001–2002

Source of variation	d.f.	MS					
		germination		vigour		NaCl germination	
Winter barley varieties							
Year	1	95.60		578.95	**	2959.94	**
Variety	3	77.18	*	108.90	*	284.92	**
Location	5	101.65	**	88.52	*	282.66	**
Interaction							
Year × Variety	3	41.78		166.04	**	256.33	**
Year × Location	5	97.85	**	310.08	**	593.35	**
Variety × Location	15	22.62		117.60	**	130.71	**
Residual	159	22.17		37.95		58.30	
Spring barley varieties							
2001							
Variety	1	73.15	*	1.96		239.08	**
Location	5	134.80	**	90.88		59.80	
Interaction	5	42.79	*	32.37		48.65	
Residual	36	13.44		44.63		25.60	
2002							
Variety	1	66.76		1.53		1.84	
Location	5	318.20	**	249.58	**	271.26	**
Interaction	5	46.80		37.56		29.20	
Residual	36	20.52		63.10		36.65	

*P = 0.05; **P = 0.01

Table 5. Comparison of standard germination with germination after deterioration at 45°C for moisture contents 22 and 24% and periods 24 and 48 h

Variety	Germination (%)	Germination after deterioration (%)			
		22%/24 h	24%/24 h	22%/48 h	24%/48 h
Nordus	99.0	86.7	77.7	4.2	0.0
Olbram	100.0	97.5	87.0	4.2	0.2

The high sensitivity of barley seeds to environmental stresses was described by Hosnedl and Honsová (2002). Further investigations will be done on a larger number of samples to test this method.

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