Barley seed sensitivity to water stress at germination stage

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ABSTRACT

Barley seed sensitivity to water and anoxia was tested. Standard germination, mean time of germination (MTG), germination in sand wetted by water to 100% water capacity (anoxia) or by hydrogen peroxide (wet conditions without anoxia), germination in 0.75% hydrogen peroxide and laboratory emergence (15 and 20°C) were evaluated. Barley seed responds sensitively to stress conditions during germination. Significant germination decrease was found in abundance of water. Percentage of reduction depends on the variety and on the year of seed production. Extreme values of water sensitivity are in interval 4–90%. At wetted sand by 0.75%, solution of H_2O_2 the germination was significantly less reduced. That means that barley seed is very sensitive to oxygen deficiency above all and is less injured by quick imbibition. Heterogeneity in seed vigour was demonstrated in laboratory emergence tests. Quick test of germination in 0.75% hydrogen peroxide deserves attention for its high correlation coefficient with the seed laboratory emergence. The results significantly demonstrate a higher sensitivity of deteriorated seed to germination in abiotic stresses conditions. Variability in speed of germination is increasing, which unfavourably extends the mean time of germination.

Keywords: barley; seed; vigour; germination; laboratory emergence; stress conditions; anoxia; sensitivity

A relation between seed : water belongs to the most important factors of germination, in orthodox seeds it is the base for transition from the state of quiescence to active biochemical and physiological activity. The important role in seed sensitivity play the stresses from water deficiency and water abundance. Introduced stress situations in interaction seed : water commonly occur in field conditions, but they can occur also in some laboratory tests. It is caused not only by the direct influence of water on seeds, but also by the air availability for inhibition of germinated seeds (anoxia).

Seeds are sensitive to quick imbibition, cold and lack of oxygen. The critical period of germination is the first phase of water uptake by dry seeds (McDonald 1994). There is a quick and strong raise in tissue moisture and the seeds alter their volume. During the quick imbibition so-called injury from imbibition can occur. Therefore, it is possible to expect, that very dry seeds are exposed to injury from imbibition even in standard laboratory germination test (Ellis et al. 1990). They demonstrate various sensitivity in different lots of seed in a plant pea model. The essential substance of resistance to this stress conditions is attributed to seed vigour (Powell and Matthews 1980, Ganguli and Sen-Mandi 1990, Hosnedl and Honsová 1991). Mainly pea, soybean and vegetable seeds are the subject of this research concerning a relation between the seed vigour and sensitivity to injury from imbibition.

The term water sensitivity in barley seed was used by Matthews and Collins (1975). They found, that the percentage of emergence and emergence rate are in a negative correlation with water sensitivity to excessive moisture of environment (expressed by reduction to 100% bed wetting in comparison with the germination in optimal moisture to 60% of water capacity). Another demonstration of seed with greater water sensitivity is the elongation of emergence time. Less observation was yet dedicated to air substance, oxygen, respectively during germination. Oxygen deficit can demonstrate inhibition of root growth (Bewley and Black 1994). According to Crawford (cit. Halmer and Bewley 1984), the tolerant species react to anaerobic conditions during anoxia by decreasing the anaerobic respiration and lactate and ethanol production for the oxygen deficit balance. Intolerant species conversely mark out a great anaerobic respiration and a high level of ethanol. Perry and Ellerton (1983) observed this phenomenon in barley caryopsis with a lower vigour. While oxygen is necessary for respiration of imbibed seeds, CO_2 and ethylene, which are accumulated in the soil, can also influence the germination and dormancy.

Heydecker et al. (1969) practically demonstrated in their laboratory tests with *Spinacia oleracea* L. seeds and *Phaseolus vulgaris* L. the positive influence of oxygen to germination in saturation of environment by a weak solution of hydrogen peroxide. Application of calcium peroxide had a positive influence on barley (Perry and Ellerton 1983).

MATERIAL AND METHODS

Spring barley seed was obtained from four stations of variety testing ÚKZÚZ (Central Institute for Supervising and Testing in Agriculture Brno) in 1995–1998. Three genetically different varieties Akcent, Amulet and Forum were evaluated. Laboratory germination (optimal conditions for germination according to ISTA) was evaluated by the standard test. Stress conditions of germination

were created in the germination test by wetting of bed (sand) to 100% water capacity and in the test of laboratory emergence. For assessing oxygen significance during germination variants of 100% wetted sand of water capacity and method of germination in solution 0.75% H_2O_2 were integrated.

This research was conducted with fresh seed variants of spring barley and variants with decreased vigour after controlled deterioration. Adapted method of AOSA (TeKrony 1995) was used for the controlled seed deterioration. Reviser mechanism during this test was the last moisture of caryopsis 28–30%. After 48 hours of deterioration, every sample was kept in laboratory conditions to dry to standard moisture (AA).

Standard germination test (G_{st}) – standard method according ISTA in stacked filter paper at 100 caryopsis. Essential temperature for germination was 20°C. Percentage of germination was expressed by proportion of caryopsis germinated in 7 days.

Laboratory germination in wet conditions with water (G_{100}) – 100 caryopsis were germinated in boxes with fine – grained quartz sand (grains 0.05–0.8 mm) in 100% wetting with water. The evaluation after 4 and 7 days.

Water sensitivity (WS) – modified method of Matthews and Collins (1975) was used.

Water sensitivity (WS) = $(G_{st} - G_{100}/G_{st}).100$.

The identical test was executed earlier in 1990–1992 with five different malting barley varieties (Novum, Malvaz, Perun, Jaspis, Rubín).

Germination in wet conditions with hydrogen peroxide (G_{100PER}) – 100 caryopsis were germinated in boxes on the surface of 30 mm layer of fine – grained quartz sand wetted with 0.75% hydrogen peroxide to 100% water capacity. Temperature in the climatisation box was 20°C. The evaluation after 4 and 7 days.

Mean germination time (MTG) – during the standard laboratory germination test number of germinated caryopsis were evaluated every day. Total time of evaluation was 7 days. MTG was calculated from daily rise of germinated seeds (method Ellis and Roberts 1981).

MTG = S*n.d*/S*n*, where *n* marks number of germinated caryopsis in day *d* in order from beginning of germination.

Laboratory emergence (LE) – 100 caryopsis were tested on 30 mm layer of sand. 210 g of fine – grained sand wetted by water to 60% of water capacity were placed in the bottom of the box, then caryopsis were placed on the sand and covered up with 600 g of coarse – grained sand (grains 1–2 mm). Laboratory emergence was determined in two temperatures – 15 and 20°C. Evaluation occurred after 7 days (energy of emergence) and after 14 days by counting of emerged caryopsis.

Germination in hydrogen peroxide solution (PER) – 100 caryopsis were germinated at 20°C in 50 ml of 0.75% hydrogen peroxide for 4 days, with hydrogen peroxide exchange after 2 days. Germination was determined by counting germinated caryopsis.

All tests were executed in four replications.

RESULTS AND DISCUSSION

The barley seed germination evaluated by ISTA methods in standard conditions (G_{st}) is the basic criterion of seed viability. This study demonstrated a high sensitivity of barley grains to stress germination conditions. When the standard conditions were changed, the big importance of water and oxygen on germination was found (Figure 1). Research which took place in four years confirmed the biggest reduction of germination percentage under the conditions of sand seed bed with watering on 100% of water capacity, e.g. in anoxia conditions. Dependence of this sensitivity to anoxia between seed from different years of harvest was determined. G_{100} in 1997 and 1996 argue the higher sensitivity of barley seed to anoxia in comparison to seed harvested in 1995 and 1998. The percentage of germination in 100% watered sand was reduced to under 20% in 1997 while in the two another years it was above 80%. The variety differences in sensitivity to wet conditions were found, the most sensitive was Amulet.

The value of water sensitivity was used for explanation of the influence of some factors on seed quality comparison. This value derived from G_{st} and G_{100} was different in each year and shows the high influence of varieties (Figure 2). The values validate the results of germination in wet conditions under anoxia. The same results were obtained in earlier research, which was conducted in 1990–1992 with another barley variety groups by the same methods (Figure 3). In this research, influence of year condition and variety on anoxia was proved. The year variability of this value can have a big importance on the quality of barley production for malting purposes. The differences in variety sensitivity on quick water imbibition are very important from this case and from the seed point of view (Table 1, Figures 2 and 3). Water sensitivity recorded in years 1991, 1996 and 1997 values between 40.0–91.7. This extreme high water sensitivity argues the importance of this trait. The results from 7 years research indicate the highest water sensitivity of varieties with high malting quality.

Table 1. The share of variety on barley grain sensitivity to anoxia and MTG

Variety	Water sensitivity (ws)			
	1995	1996	1997	1998
Akcent	15.7 a	48.6 a	73.0 a	16.6 a
Forum	16.4 a	40.0 a	78.9 b	23.0 b
Amulet	40.9 b	70.4 b	91.7 c	31.2 c
F-test	23.87	150.92	80.78	320.41
Sign.l.	0.000	0.000	0.000	0.000

Significance levels: values with different letters significantly differ at 0.01 level according to Tukey's *HSD* test

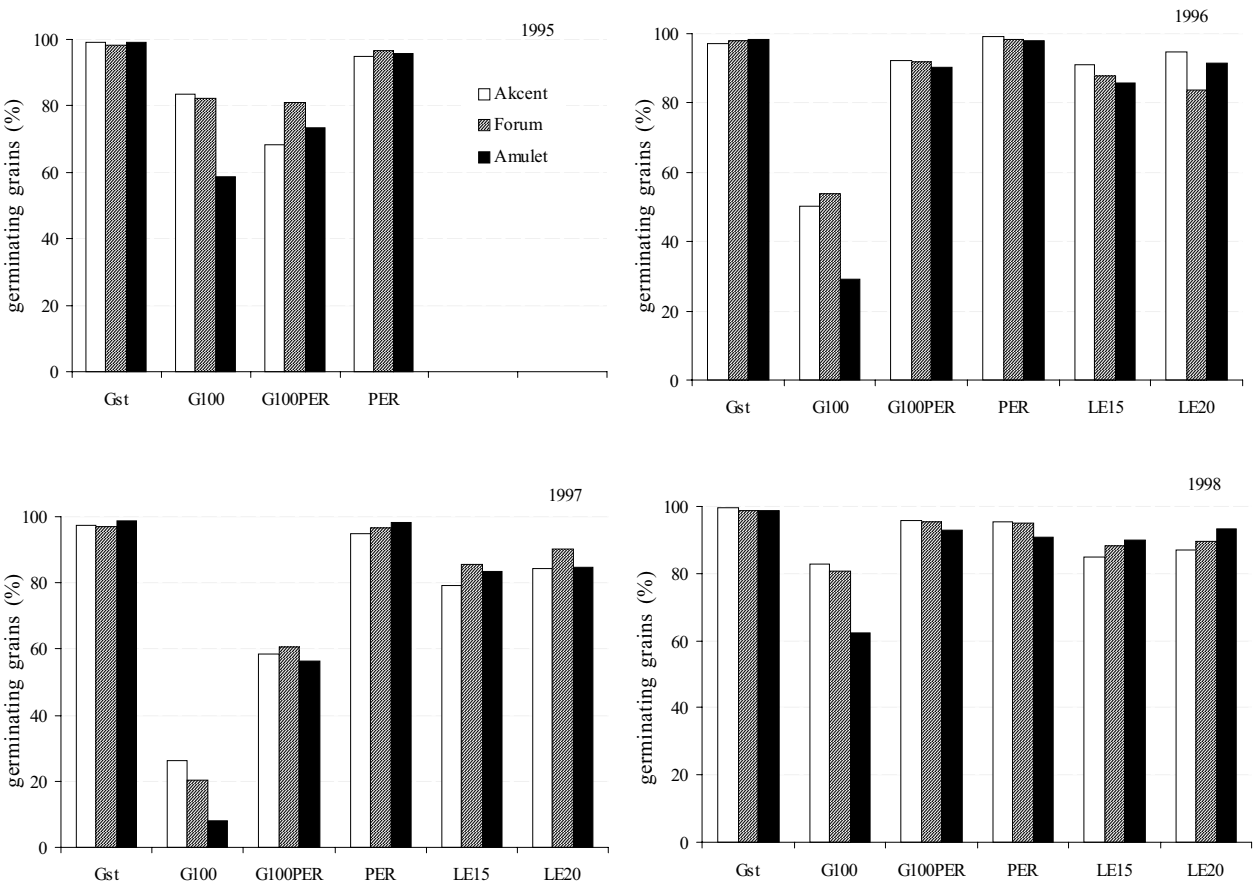

Figure 1. Germination and laboratory emergence of barley seed under standard and stress conditions – influence of varieties, year of harvest, water and oxygen conditions

The provenance of the seed has some influence too, but it was impossible to determine the best environmental conditions with relation to this value.

We demonstrate our results to water sensitivity by the used Matthews and Collins (1975) methods. Water directly influences a speed of swelling and there is coherence with tissue damages and cell membranes, but water has an indirect effect by the reduction of oxygen availability for germinating seeds. For proof of this factor proportion there were used the variants with sand wetting by 0.75% solution of hydrogen peroxide to 100% water capacity (G_{100PER}) and methods of germination in solution of hydrogen peroxide. G_{100PER} was reduced in comparison with the standard condition of germination, but losses in percentage germination were significantly lower against the conditions where only water was used (Figure 1). The all

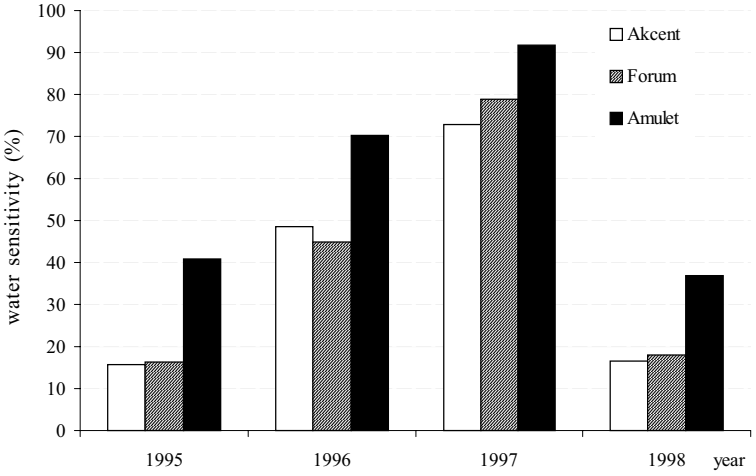

Figure 2. Water sensitivity of barley grains in different varieties in 1995–1998

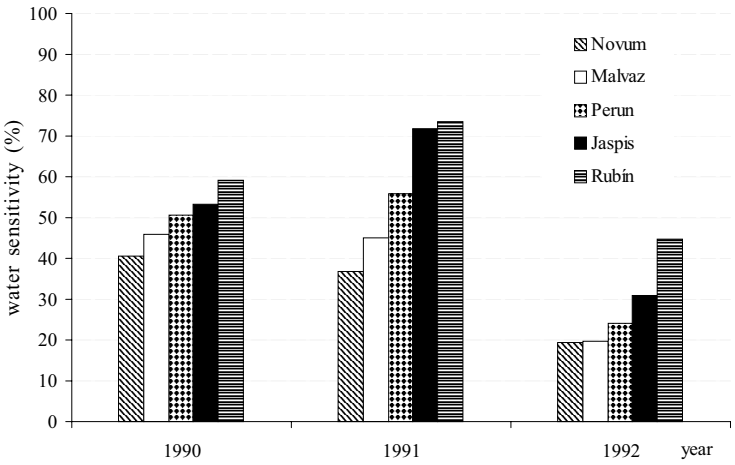

Figure 3. Water sensitivity of barley grains in different varieties in 1990–1992

results show the negative influence of anoxia on barley seed germination, if we count the hydrogen peroxide as a partial source of oxygen for seeds. Therefore, sensitivity to anoxia is more precise term than water sensitivity. This research also took place on winter wheat seed but the influence of anoxia was lower than that which was determined for barley seed. The importance of different kinds of seed, varieties and lots quality on anoxia is very high. Halmer and Bewley (1984) indicated these differences on the examples in field emergence conditions and as one of the reasons showed small oxygen content in the soil, which can be caused by the soil crust.

The seed vigour heterogeneousness in barley was evaluated by laboratory emergence tests in sand at two temperature conditions (15 and 20°C). These tests were suitable for a measure of the growth power of seedlings, which emerged from the 30 mm depth. These tests of laboratory emergence are very objective for grain seed vigour evaluation as the results of our research and of many other researches indicate.

The barley seeds are very good for the research of germination conditions sensitivity. Reactions of fresh seed and seed after their deterioration in controlled conditions were evaluated too. This research confirmed some conclusions of TeKrony (1995) in the case of relations between deterioration and results of some biological tests. The results from the stress conditions for deteriorated barley seed (AA) show a vigour decrease at the stage of germination and emergence. Seed with a lower vigour have the lower percentage of germination at optimal water, oxygen and temperature conditions (Table 2) as well as in anoxia. The best results gave the lab-

Table 2. Influence of barley seed deterioration on its sensitivity to water and anoxia stress

Test	G_{st} (%)	G_{100} (%)	G_{100PER} (%)	PER (%)	LE_{15} (%)	LE_{20} (%)	WS (%)	MTG (days)
1996								
F	98.0 b	44.4	91.5 b	98.5 b	88.3 b	90.0 b	54.7	3.18 a
AA	92.1 a	43.5	58.0 a	79.2 a	77.3 a	67.7 a	55.1	3.65 b
F-test	3.61	0.01 NS	16.42	9.39	4.79	13.43	0.02 NS	14.00
Sign.l.	0.022	0.915	0.001	0.001	0.039	0.001	0.964	0.001
1997								
F	97.8	18.4	58.5	96.5	82.8	86.4	81.2	3.05 a
AA	94.6	16.8	55.9	81.8	76.7	78.2	82.5	3.32 b
F-test	4.24 NS	0.09 NS	0.07 NS	3.87 NS	1.20 NS	1.95 NS	0.06 NS	44.93
Sign.l.	0.051	0.768	0.792	0.062	0.285	0.177	0.810	0.000
1998								
F	99.2 b	75.2 b	95.8 b	94.7 b	85.5 b	86.4b	24.2	2.03 a
AA	91.8 a	65.4 a	72.5 a	82.7 a	73.3 a	77.5 a	27.8	2.16 b
F-test	4.93	22.14	18.66	23.16	13.57	6.67	0.26 NS	16.25
Sign.l.	0.037	0.027	0.000	0.000	0.001	0.017	0.621	0.001

Significance levels: values with different letters significantly differ according to Tukey's *HSD* test
F – fresh seed from harvest of common year, AA – dry seed after accelerated ageing test
G_{st} – standard germination, G_{100} – germination in 100% wet sand by water, G_{100PER} – germination in 100% wet sand by H_2O_2, PER – germination in H_2O_2, LE_{15} – laboratory emergence at 15°C, LE_{20} – laboratory emergence at 20°C, WS – water sensitivity, MTG – mean time of germination

oratory emergence test. However, changes in water sensitivity were not found. Time of deteriorated germination was significantly longer and variability germination of individual grains was bigger. This value marks MTG (mean time of germination).

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ABSTRAKT

Citlivost osiva ječmene na vodní stres ve fázi klíčení

U osiva jarního ječmene byla testována citlivost obilek na vodu a anoxii ve fázi klíčení. Byla hodnocena laboratorní klíčivost, střední doba klíčení (MTG), klíčivost při zavlažení lůžka na 100 % vodní jímavosti vodou (anoxie) a peroxidem vodíku (vlhké prostředí bez anoxie), klíčivost v 0,75% peroxidu vodíku, laboratorní vzcházivost (15 a 20 °C). Obilky ječmene velmi citlivě reagují na stresové podmínky při klíčení. Průkazné snížení klíčivosti je vlastností odrůdy a závisí na roku pěstování. Extrémní hodnoty citlivosti na vodu jsou v intervalu 4–90 %. Při zavlažení lůžka na 100 % vodní jímavosti 0,75% roztokem H_2O_2 dochází průkazně k nižší redukci klíčivosti. To znamená, že ječmen je při klíčení velmi citlivý na nedostatek kyslíku. K vyjádření heterogenity ve vitalitě obilek jsou vhodné testy laboratorní vzcházivosti. Pozornost zaslouží rychlý test klíčivosti v 0,75% roztoku peroxidu, s vysokým koeficientem korelace s laboratorní vzcházivostí. Využití metod urychleného stárnutí prokázalo u deteriorovaných obilek zvýšenou citlivost na anoxii, větší variabilitu rychlosti klíčení a prodlužování střední doby klíčení (MTG).

Klíčová slova: ječmen; obilky; vitalita; klíčivost; laboratorní vzcházivost; stresové podmínky; anoxie; citlivost

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