Increasing tolerance to abiotic stresses by haplodiploidization in *Hordeum vulgare* L.

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Intorduction

Barley (*Hordeum vulgare* L.) belongs to the most important cereal grains, due to its wide utilization in the malting and brewing industry, for animal feed and human consumption. However, every year its poor winter hardiness and low tolerance to drought at the generative phase of development result in significant yield losses. Therefore, the identification of factors that determine drought and freeze tolerance is very important, and could result in the improvement of valuable genotypes selection efficiency. Both, freeze tolerance and drought resistance, are polygenic traits resulting from multiple genes interactions, therefore a pyramiding approach of useful genes for resistance seems to be the most effective. Another beneficial tool is doubled haploid (DH) technology, which allows to instant production (within one generation) of totally homozygous lines from heterozygous donor material. Moreover, this haplodiploidization method could increase the genetic variation within progeny population by the expression of recessive allele based traits.

Material and methods

The plant material consisted of 75 F1 breeding lines of winter barley (*Hordeum vulgare* L.) kindly provided by the Polish and foreign breeding companies. The DH lines were obtained by androgenesis *via in vitro* anther culture according to Jacquard et al. (2003) and Cistué et al. (2003) with several modifications (Fig. 1). Shortly, for androgenesis induction the collected tillers were cold treated for 3-9 days at 4°C. Isolated anthers were pre-cultured on PreMn medium (Cistue et al. 2003) for 3-5 days at 26°C in the dark. Then, the anthers were passaged to the induction C3 medium (Jacquard at al. 2003) and kept at the same culture conditions. Androgenic structures (AS) were transferred to M1 regeneration medium (Jacquard et al. 2003) and cultured at 26°C and 16h/8h (day/night) photoperiod. Green regenerants were transferred to the rooting 190-2 medium (Zhuang and Xu 1983). At the 3-4-leaf stage, plants were planted into soil and grown under controlled conditions in a greenhouse.

Screening for drought tolerance

Drought tolerance was estimated by excised leaf water loss assay conducted at the 3-4 leaf stage in produced DH lines (Clarke and Mc Caig 1982). Sampled leaves were weighted and then incubated in hybridisation oven at 30°C for 2hrs. The water loss in excised leaves was calculated from the following formula:
%H2O/h = [((A-B))/((A-C)) × 100%]/2
where: A – fresh weight of leaf; B – fresh weight of leaf after 2h of desiccation at 30°C; C – dry weight of leaf after 48 h of drying at 70°C.

Freezing tolerance assessment

Freezing tolerance was estimated according to Rapacz et al. (2011). Plants were grown at 25/17°C (day/night) and 9/15h (day/night) photoperiod. At the five-leaf stage, plants were cold-acclimated for 20 days under controlled conditions (4/2°C day/night and 9/15h day/night photoperiod). After cold acclimation, the parameters of chlorophyll fluorescence were measured on leaves subjected to freeze-thawing (8 hrs at-15°C) using a portable chlorophyll meter (Handy-PEA, Hansatech Ltd. Kings Lynn, UK).

Results and discussion

In the study, 3004 green regenerants (GR) and 1787 albino plants (AR) were obtained, with mean regeneration effectiveness at 2.7 GR per one spike. Among them 57% occurred to be spontaneously diploidized.
Water loss in excised leaves of DHs plants ranged between 7.3 and 29.4% H_2O/h. Such high variation among tested genotypes allows for clear distinction between drought-susceptible and drought-resistant DH lines according to significantly various water loss rate (21.9 % versus 10.1 % H_2O/h, respectively). Moreover, drought resistance screening revealed that for some donor F1 lines, both highly-tolerant and highly-susceptible genotypes could be found among segregating DH progeny (Tab. 1).
According to selected parameters of chlorophyll a fluorescence produced DH lines segregated also in respect of freezing tolerance. For freeze-tolerant genotypes the mean value of maximum quantum yield of PSII photosystems (Fv/Fm) gained 0.812 with 722.8 active reaction centres (RC/CSm), whereas the same parameters for freeze susceptible genotypes gained respectively 0.477 (Fv/Fm) and 184.6 (RC/CSm). Similarly, for some donor F1 lines both highly-tolerant and highly-susceptible genotypes could be found among segregating DH progeny (Table 2).

Conclusions

Haplodiploidization through androgenesis increased the genetic variation within the produced DH line population of winter barley what confirms its usability as a tool for breeding progress acceleration.

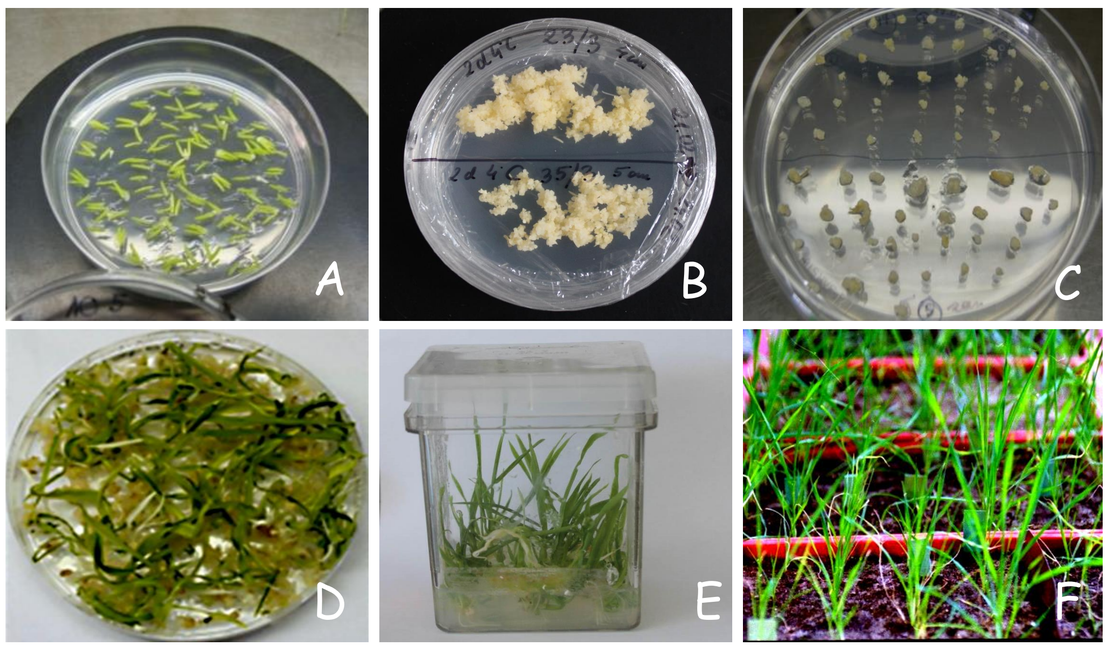

Figure 1. Haplodiploidization by anther cultures in barley. (A) Anthers pre-culture (0.7 mol l^{-1} mannitol in medium); (B) Anthers on induction medium; (C) Androgenic structures on regeneration medium; (D) Barley green and albino regenerants after 4 weeks of *in vitro* culture on regeneration medium; (E) Green regenerants on rooting medium (F) Green diplohaploids transfered to the soil.

Table 1. The variability among DH1 progeny of winter barley breeding lines with extremely different susceptibility to drought stress.

Breeding line	Resistant genotypes		Susceptible genotypes	
	No. of DH	Variability range	No. of DH	Variability range
5213	1	11.0	1	18.5
5216	5	8.5-11.0	2	20.4-20.7
5235	1	9.1	3	20.7-22.1
5237	2	10.1-10.2	9	18.8-25.1
5257	8	8.0-11.2	7	18.5-27.0
630	1	11.2	1	27.3

Table 2. The chlorophyll fluorescence parameters for the selected lines DH2 winter barley (*Hordeum vulgare* L.) the derived by inducing a process androgenesis in anther culture.

Breeding line	Resistant genotypes			Susceptible genotypes		
	No. of DH	Variability range		No. of DH	Variability range	
		Fv/Fm	RC/CSm		Fv/Fm	RC/CSm
5257	2	0.796-0.804	661.0-675.9	2	0.466-0.503	150.2-220.7
5237	1	0.806	678.0	2	0.466-0.503	150.2-220.7
630	1	0.820	731.4	2	0.347-0.544	103.6-233.6

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