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Effect of genotype on androgenesis in barley (*Hordeum vulgare L.*)

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Abstract. Current study was set up to determine the response of 12 Iranian winter diploid barley (*Hordeum vulgare* L.) cultivars to anther culture on solid FHG induction medium supplemented with 90 mg/l sucrose, 2 mg/l 2,4-D and 0.1 mg/l kinetin. For cold pretreatment each selected spike was kept in a refrigerator at 4 °C for 14 days. The experimental design consisted of a completely randomized design (CRD) with four replications. Embryogenesis and green plant regeneration occurred in all genotypes. The number of embryoids and total plant regeneration as well as the numbers of green and albino plants regenerated per 100 anthers, was recorded. The results showed that genotype affected significantly on embryo induction, total plant regeneration, green and albino regeneration. Genotype No. 9 gives the best results for embryo production, green and total plant regeneration (74.792%, 7.293% and 17.29%, respectively). Linear correlation analysis showed a positive and significant ($p<0.01$) relationship between embryo induction and green plant regeneration.

Key words: androgenesis, anther culture, genotype, barley (*Hordeum vulgare L.*)

Introduction

In the last decade anther culture, the induction of callus from cultured anthers and production of haploid plants from calli has been developed by many researchers (Kahrizi et al. 2000, Kahrizi & Mohammadi 2009, Kahrizi 2009). Methods of modern biotechnology allow to accelerate the process of breeding, and haploid production is one of the most widely used biotechnological methods in breeding of self-pollinating cereal crops. By doubling chromosomes in haploid plants it is possible to obtain completely homozygous lines in a short time thereby providing a method for speeding up and increasing the selection efficiency (Kruczkowska et al. 2002). Recessive mutations, important recombination and other genomic changes can be found in double haploids (DHs) more easily. The DHs can be used for genetic analysis, gene mapping and gene engineering. The DH material makes easier identification and stabilization of genetic variation. This method accelerates breeding by 3–5 years. The most important factor appears to be the genotype response to androgenesis in the development of callus from microspores. It appears that optimum media and pretreatment conditions vary from genotype to genotype. Nowadays, the three methods for obtaining haploids in barley breeding are anther culture, isolated microspore culture and chromosome elimination. According to Devaux (1995), both techniques are comparable in respect to their efficiency and the amount of required work. Considering the availability and number of pollen grains (around 2-4 thousand per anther), the potential efficiency of haploid production by induction of androgenesis could probably be several times higher. The mechanism which prompts the change in the developmental pathway of pollen is still unexplained; the limited percentage of microspores responding to the induction, the strong influence of the genotype, as well as formation of albino plants, reduce the efficiency of this technique and point to the need for further research on androgenic haploids. In order to change the developmental pathway of pollen from gametophytic to sporophytic, a proper stress should be applied during pretreatment of spikes or anthers. The most frequent methods are: chilling cut-off spikes (Huang & Sunderland 1982) and immersing spikes or anthers in mannitol solution (Roberts-Oehschlager & Dunwell 1990). Both techniques limit the intake of nutrients by microspores. In the case of chilling, the reason is the lack of synchronous development of the tapetum and microspores (Powell 1988). Pretreatment procedures differ in various laboratories. Chilling spikes at 4 °C may be effective with different treatment periods, that of 28 days being the most frequent. The optimum length of cold treatment depends presumably on the genotype (Powell, 1988) as well as on growth conditions and the physiological status of donor plants, affecting the endogenous hormone content (Jähne & Lörz 1995, Lezin et al. 1996, Ohnoutková et al. 2000). The experimental design consisted of a completely randomized design (CRD) with four replications. Analysis of variance was carried out in order to determine the significance of differences between the genotypes. Duncan's multiple range test was used to compare the mean performance of the genotypes for androgenetic embryogenesis and green plant regeneration. All data were normalized by transformation using the Arcsin $\sqrt{x}$ function.

Material and methods

The research was carried out at the Laboratory of Plant Physiology of the Faculty of Science, Razi University. 12 barley (*H. vulgare* L.) cultivars (2n=14)) were selected for this study. Plants were grown in a glasshouse. Each replication consisted of one pot with three plants. Seeds after vernalization were planted in pots containing a mixture of sand, fertilizer and soil (1:1:1). Donor plants were grown in a greenhouse with a photoperiod 16h / 8h (day / night) and temperature 25 °C / 15 °C (day / night). For cold pretreatment each selected spike was surface sterilized with 70% ethanol and was kept

in a refrigerator at 4˚C for 14 days. Stress such as pesticide treatment, water deficiency or temperature fluctuation was avoided during plant growth. Finally, they were taken out of the sheaths under sterile conditions, and the developmental stage of microspores was assessed in anthers from the central part of each spike. Experiments involved spikes in which a majority of microspores reached the mid- or late-uninucleate stage. Forty anthers were placed per 15 × 60 mm Petri dishes.

The study was set up to determine the response of 12 Iranian winter diploid barley cultivars to anther culture on solid FHG (Hunter, 1988) induction medium supplemented with 90 g/l sucrose, 2 mg/l 2,4-D and 0.1 mg/l kinetin. The pH of the media was adjusted using 1N NaOH and 1N HCl to 5.8 for induction and regeneration media. After 35 to 45d, all embryos and calli (hard structure) equal to or larger than 0.5 mm were collected and transferred to the MS regeneration medium with 20 g of sucrose. Petri dishes were sealed with parafilm, wrapped in aluminum foil and stored in the incubator at 28 °C in the dark for 21–28 days. Then green plantlets were transferred to small pots in a growth chamber and then to the greenhouse. After five weeks, the numbers of green and albino plants were recorded. The results were subjected to analysis of variance and Duncan's multiple range test. When the green regenerants reached the length of approximately 10 cm in coleoptiles they were removed from the culture tubes using pincers and transferred into pots containing soil. The covered pots were kept in a climate chamber or in the greenhouse under controlled plant growth conditions (the photoperiod 16/8 h).

Results

Embryoids were formed in the anther culture for all 12 winter barley cultivars (Table 1 and Fig. 1.A). Barley cultivars varied significantly according to their response in the anther culture. All cultivars produced plants after cold pretreatment and the highest green plant frequency was found in genotype No.9 (7.293).

Table 1. Comparison of barley cultivars androgenesis for embryo/callus induction and green plant regeneration.

Genotype	Embryo/callus induction (%)	Green plant regeneration (%)
1	5.418ij	0.415 f
2	21.872f	1.667 d
3	36.665d	3.332 c
4	52.292c	4.582 bc
5	15.625g	1.25 de
6	3.750j	0.622 ef
7	62.915b	5.832 ab
8	11.457h	1.25 de
9	74.792a	7.293 a
10	28.750e	3.332 c
11	5.625i	0.415 f
12	15.832g	1.667 d

Mean values followed by the same letter are not significantly different at P = 0.05 acc. to Duncan's test.

The highest Callus induction was determined for genotype No. 9 (74.792%) and genotype No.7 (62.915%). Genotypes No.1, 6 and 11 showed low callus formation in the anther culture (5.418, 3.750 and 5.625% respectively) (Fig. 1.A).

The highest plant regeneration efficiency was found in genotype No.9 (7.293%) and the lowest in genotypes No.1 (0.415%), 6 (0.622 %) and 11 (0.415%) respectively (Fig. 1 B and C). These results suggest that anther culture response is predetermined by the genotype.

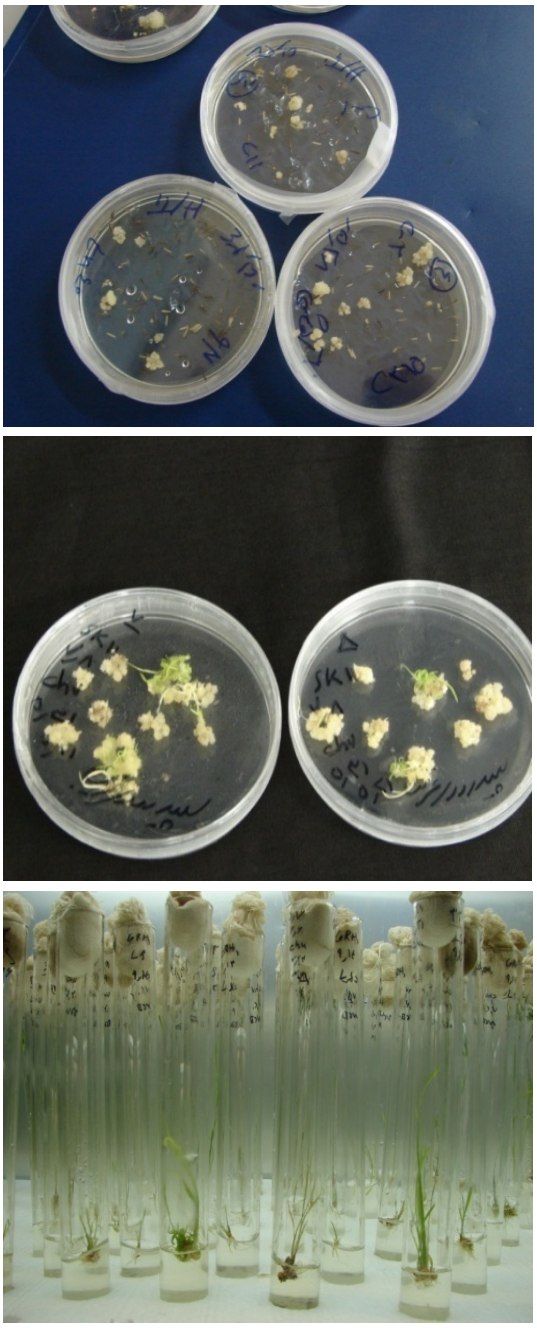

Figure 1. Androgenesis stages in barley. **A:** Callus induction, **B:** Plant regeneration (green and albino), **C:** plant elongation.

Discussion

The purpose of applying pretreatment before planting anthers in the medium is to bring about the stress needed to change the developmental pathway of immature pollen grains (Kruczkowska et al. 2002). The genotype No.9 produced highest frequency of calli after a 14 days cold pretreatment (74.792) (Table 1). Plant regeneration was observed in all cultivars (Table 1). Response to androgenesis for a number of crops including barley is known to be strongly genotype dependant and influenced by numerous

environmental factors. In our study, genotype No.9 produced the highest number of green plantlets. The elapsed time from anther planting to callus appearance varied between 25 and 60 days and 93% of the calli appeared in the first 30 days of culture in this study that is according to Savaskan et al. **(**1999) report. Franzone et al (1984) have reported that various barley varieties produced 85% of calli in the first 70 days of culture. A great obstacle in barley anther culture is a distinct manifestation of albinism. The reason for this is that the chloroplasts of microspores lose their inner membrane and become filled with lipids and globulins, and chlorophyll *a* is not protochlorophyllid *a* (Kahrizi et al. 2000). The DNA of microspore chloroplasts is damaged at the early stage of microspore synthesized from development. The efficiency of the anther culture method is largely dependent on plant genotype and cultivation conditions. Andersen has found that the genetic nature of donor plant affects the formation of embryoids by 20–40% and formation of green regenerates by 50–80% in wheat anther culture. Our results confirm that induction response in anther culture, embryoid formation, regeneration potential and the ratio of green regenerants to albino are controlled genetically as reported in literature (Jacquard et al. 2006, Asakaviciute & Pasakinskiene 2006). The *shd1* gene which is on the second chromosome of barley affects the formation of green plants from embryoids by 65.0% (Beck et al. 2000); therefore the main factor affecting the formation of green regenerants in anther culture is the genetic predetermination of a donor plant. The androgenic potential of Iranian winter barley cultivars in anther culture has been determined as follows: genotype No.9 has the best regeneration rate from callus. These results show that the ability to produce green androgenic plants is dependent upon the genotype and suggest that deeper genetic studies have to be undertaken in order to characterize this parameter.

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