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Anther culture response of winter barley (Hordeum vulgare L.)

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Abstract. The potential of anther culture to produce double-haploid lines was evaluated for Bulgarian winter barley (Hordeum vulgare L.) breeding applications. Two 6-row and two 2-row F1 hybrids were studied. Anthers were culture on two different induction media – N6 and MS, both supplemented with 60 g/L maltose, 40 g/L starch, 3 g/L Gelrite. Composition of the culture media, genotype and their interaction showed significant effect on the frequency of responsive anthers and regenerated plants. The response ranged from 1.33 to 60.67 green regenerants per 100 anthers cultured. The number of green plants regenerated on N6 based media was significantly higher than on MS based media in the genotypes – F1 Lambic x KT 287 and F1 PG 4348 x KT 2168. Changes in the composition of the culture media and the identification of responsive genotypes makes anther culture an efficient procedure for Bulgarian barley breeding program.

Keywords: anther culture, doubled haploids, Hordeum vulgare L.

Introduction

After the first protocol for barley anther culture was developed by Clapham (1973), plant breeders paid attention to anther culture and its possible application in breeding. The advantages of anther culture in barley breeding are: shortening the breeding cycle by immediate fixation of homozygosity, increased selection efficiency and allowing the early expression of recessive genes. Numerous doubled haploid lines have been produced and field tested, including some resistant to barley yellow mosaic virus (Foroughi-Wehr and Friedt, 1984; Gheorghe, 2010). Nevertheless, most of the studies have been done using a small number of responsive cultivars which have an extraordinary androgenetic capacity. The use of highly responsive genotypes may adapt the technique to genotypes which are not very useful for practical purposes and restrict the genetic variability available to the breeder (Luckett and Smithard, 1992). In order to prevent that, it is recommended to develop protocols and culture media applicable to a broader spectrum of genotypes.

The objective of this study was to evaluate androgenetic capacity of hybrid genotypes in two culture media for obtaining doubled-haploids for Bulgarian winter barley breeding program.

Material and methods

The work was performed by using F1 PG 4365 x K-99/23-2, PG 4348 x KT 2168, Lambic x KT287, Merian x KT 287 population derived from crosses made at the Institute of Agriculture, Karnobat. Donor plants were grown in the field. Spikes were collected when anthers were in the early-mid and mid-uninucleate stage. They were preselected on the basis of interligule length between the flag and the uppermost leaf. Before removing spikes they were surface-sterilized with an aerosol of 96% ethanol. One or two anthers from the middle floret were detached, stained with 4% acetocarmine and examined under the microscope to check the initial stage.

Anthers were cultured in two different culture media: modified MS (Murashtige and Skoog, 1962) or N6 (Chu, 1981). Both media contained 60 g/L maltose, 40 g/L wheat starch, 3 g/L Gelgite, 2 mg/L naphthaleneacetic acid (NAA) and 1 mg/L 6-benzylaminopurine (BA). Anthers were incubated in the dark, at 25 ± 1°C, for 30 days and the Petri dishes were then transferred to the light (16 h). The frequency of responsive anthers, green and albino plantlets was assessed. Green plantlets were rooted in test tubes containing modified MS medium (Olsen, 1987), with 30 g/L sucrose, 7 g/L agar, and 0.5 mg/L indole-3-acetic acid (IAA).

Plantlets with well-developed roots were transferred to pots containing standard potting mix. Pots were covered with a beaker which was gradually removed until exposing the plants to ambient conditions. Self-fertilization and formation of grains were attained in the greenhouse. Seeds of spontaneously doubled barley plants were collected.

The parameters observed were number of responsive anthers, number of total plants regenerated and number of green and albino plants regenerated per 100 anthers cultured. Statistical analysis was performed using Generalized Linear Model (GLM) procedure in the SPSS. Mean separations were tested by Duncan’s Multiple Range Test at 5% probability level.

Results and discussion

Variance analysis showed a significant effect of culture media and genotype on the frequency of responsive anthers and regenerated plants (Table 1). The effects of the two factors are not independent, which means that genotypes respond differently to the culture medium and both culture media induce different effects depending on the genotype.

Percentage of responsive anthers on MS medium ranged between 7.00 and 51.00%, with an average of 26.17% (Table 2). An average of 38.67% of the anthers cultured on N6 medium were responsive, with a minimum of 5.00% and a maximum of 74.00%. The superiority of N6 medium was statistically significant for 3 of 4 genotypes by the rate of responsive anthers. Only the hybrid F1 PG 4348 x KT 2168 produced a similar number of responding anthers on
cytokinin biosynthesis (Weissman, 1972a, 1972b; Darral and Lörz (1993)

F Lambic x KT 287 – 51.33% on MS media and to Logue et al. (1993), the ability to regenerate a great number of

phenotypes manifested by the two media. The highest rate of responding anthers was manifested by F1 Lambic x KT 287 – 51.33% on MS media and 74.00% on N6 media. The higher plant regeneration rate on N6 media was recorded for two of four hybrids. The frequency of green plants per 100 anthers plated in F1 Lambic x KT 287 was almost 13-fold higher on N6 based medium than on MS based medium – 60.67% on N6 medium compared with 4.67% on MS medium. The F1 hybrid PG 4348 x KT 2168 showed 6.00 in contrast with 3.33 green plants regenerated per 100 anthers for the media N6 and MS, respectively.

Compared with N6, MS medium has a greater amount of total nitrogen and ammonium (Grimes and Hodges, 1990). Modhorst and Lörz (1993) found that the poor development of microspores in media containing just NH4+ or with a high ratio NH4+/NO3- is caused by ammonium toxicity. It is also possible that the effects of the addition of ammonium to the culture medium may be caused by a change in cytokinin biosynthesis (Weissman, 1972a, 1972b; Darral and Wareing, 1981; Mercier and Kerbauy, 1991).

No relationship between rate of anther responded and rate of plant regeneration in some of hybrids was observed. On the medium MS F1 Lambic x KT 287 produced 51.33 responsive anthers and 18.67 total plants per 100 anthers and F1 Merian x KT 287 produced 32.00 responsive anthers and 22.67 total plants per 100 anthers. These results confirm the hypothesis that androgenetic capacity may result from two independent processes: induction and regeneration. These processes are controlled by different genetic mechanisms that may not be present together. The best genotypes are those combining high frequency of induction with a reasonable frequency of regeneration.

The greater capacity of some genotypes to develop green plantlets could be caused by differences in the ratio of green/total plantlets. All hybrids on N6 medium showed higher frequency of green plantlets of the total number of plants regenerated. According to Logue et al. (1993), the ability to regenerate a great number of green plants depends on the reduction of albinism. Knudsen et al. (1989) reported that the proportion of green plants and the total frequency of regeneration are determined by different genetic traits. This explains why regeneration of green plants is rather influenced by the ratio green/albino than by the total frequency of regeneration. According to Knudsen et al. (1989) and Larsen et al. (1991), the proportion of green plants is strongly genotype dependent and has great heritability. Logue et al. (1993) showed that the ratio green/albino remained constant even when donor plants were cultivated under completely different environments.

The two hybrids F1 Merian x KT 287 and F1 Lambic x KT 287, which had a common parent demonstrated different androgenetic potential. According to Larsen et al. (1991) it is common for hybrids to present levels of response equal or superior to their parents. Therefore, the higher androgenetic capacity of F1 Lambic x KT 287 is probably due to the cultivar Lambic.

In our study the frequency of spontaneous diploids was 72.67%. This result confirmed that barley anther culture usually shows a high percentage of spontaneous doubling of the chromosomes making it unnecessary to treat plants with colchicine in order to obtain doubled haploids (Foroughi-Wehr and Friedt, 1981; Foroughi-Wehr and Friedt, 1989; Forget and Friedt, 1984; Olsen, 1987; Luckett and Smithard, 1992). A total of 117 doubled haploid lines were obtained from different crosses. These lines set seed and were multiplied for agronomic testing in the field.

The efficiency of the anther culture method is largely dependent on plant genotype and cultivation conditions (Bojanova and Gramatikova, 1996; Jacquier et al., 2006). This usually makes comparison of the work of various laboratories difficult as genotypes, media and culture conditions differ considerably. The results of the current study indicate the importance of considering such

### Table 1. Mean square from analysis of variance for frequency of responsive anthers, green and albino plant regenerated per 100 anthers cultured in barley anther culture.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>RA</th>
<th>GP</th>
<th>AP</th>
<th>TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>11385.000***</td>
<td>3858.333***</td>
<td>2768.657***</td>
<td>12469.028***</td>
</tr>
<tr>
<td>Culture medium</td>
<td>3125.000***</td>
<td>4400.556***</td>
<td>823.472***</td>
<td>9031.250***</td>
</tr>
<tr>
<td>Genotype X medium</td>
<td>610.926*</td>
<td>3781.286***</td>
<td>816.065***</td>
<td>8085.324***</td>
</tr>
<tr>
<td>Error</td>
<td>160.679</td>
<td>225.802</td>
<td>88.966</td>
<td>346.065</td>
</tr>
</tbody>
</table>

*, ** - significant at p = 0.05 and p = 0.001; RA – percentage of responsive anthers; GP – percentage of green plants regenerated; AP – percentage of albino plants regenerated; TP – percentage of total plants regenerated.

The frequency of regeneration are determined by different genetic traits. The greater capacity of some genotypes to develop green plantlets could be caused by differences in the ratio of green/total plantlets. All hybrids on N6 medium showed higher frequency of green plantlets of the total number of plants regenerated. According to Logue et al. (1993), the ability to regenerate a great number of green plants depends on the reduction of albinism. Knudsen et al. (1989) reported that the proportion of green plants and the total frequency of regeneration are determined by different genetic traits. This explains why regeneration of green plants is rather influenced by the ratio green/albino than by the total frequency of regeneration. According to Knudsen et al. (1989) and Larsen et al. (1991), the proportion of green plants is strongly genotype dependent and has great heritability. Logue et al. (1993) showed that the ratio green/albino remained constant even when donor plants were cultivated under completely different environments.

### Table 2. Percentage of responsive anthers, percentage of total, green and albino plant regeneration in F2 hybrids of winter barley

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Media</th>
<th>Responsive anthers, %</th>
<th>Total plant regeneration, %</th>
<th>Green plant regeneration, %</th>
<th>Albino plant regeneration, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>F2 PG 4365 x K-99/23-2</td>
<td>MS</td>
<td>14.33*</td>
<td>5.00*</td>
<td>1.33*</td>
<td>3.67*</td>
</tr>
<tr>
<td></td>
<td>N6</td>
<td>33.66*</td>
<td>9.67*</td>
<td>3.33*</td>
<td>6.33bc</td>
</tr>
<tr>
<td>F2 PG 4348 x KT 2168</td>
<td>MS</td>
<td>7.00*</td>
<td>4.33*</td>
<td>3.33*</td>
<td>1.00*</td>
</tr>
<tr>
<td></td>
<td>N6</td>
<td>5.00*</td>
<td>7.00*</td>
<td>6.00bc</td>
<td>1.00*</td>
</tr>
<tr>
<td>F2 Lambic x KT 287</td>
<td>MS</td>
<td>51.33*</td>
<td>18.67*</td>
<td>4.67*</td>
<td>14.00*</td>
</tr>
<tr>
<td></td>
<td>N6</td>
<td>74.00*</td>
<td>100.00*</td>
<td>60.67*</td>
<td>39.33*</td>
</tr>
<tr>
<td>F2 Merian x KT 287</td>
<td>MS</td>
<td>32.00*</td>
<td>22.67*</td>
<td>17.33*</td>
<td>5.33bc</td>
</tr>
<tr>
<td></td>
<td>N6</td>
<td>42.00bc</td>
<td>19.00*</td>
<td>16.00*</td>
<td>3.00*</td>
</tr>
</tbody>
</table>

a, b, c, d – Values followed by the same letter in columns do not differ significantly (p < 0.05)
interactions in the development of protocols with locally adopted genotypes and methodology.

According to Luckett and Smithard (1992), a productivity of 10 plants/100 plated anthers allows the production of 100 doubled haploid lines from 50 different crosses by a single worker during one year. In our study using different F1 populations an average of 14.08 and a maximum of 60.67 green plants/100 plated anthers were obtained. These results are similar or superior to those obtained by Finnie et al. (1989), Knudsen et al. (1989), Kühlmann and Foroughi-Wehr (1989), Luckett and Smithard (1992). Obtaining a reasonable number of doubled haploids makes anther culture an useful tool for the Bulgarian barley breeding program.

Conclusion
Composition of the culture media (induction media – N6 and MS), genotype (two 6-row and two 2-row F1 hybrids) and their interaction showed a significant effect on the frequency of responsive anthers and regenerated plants. The number of green plants regenerated on N6 based media was significantly higher than on MS induction media in the genotypes – F1 Lambic x KT 287 and F2 PG 4348 x KT 2168. The response in our study ranged from 1.33 to 60.67 green regenerants per 100 anthers cultured. Changes in the composition of the culture media and the identification of responsive genotypes makes anther culture an efficient procedure for Bulgarian barley breeding program.

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