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*Original scientific paper*

# ANDROGENIC RESPONSES OF WINTER WHEAT (*Triticum aestivum* L.) COMBINATIONS IN *IN VITRO* ANTHER CULTURE

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The androgenic parameters were investigated by *in vitro* anther culture (AC) on nine $F_{2-5}$ breeding combinations of winter wheat (*Triticum aestivium* L.). Each combination produced embryo-like structures (ELS), green plantlets, albino plantlets, transplanted plantlets and acclimatized plantlets, with respect to the number of anthers in AC. The number of AC-derived ELS was between 11.73 and 52.76 ELS/100 anthers with the mean of 26.22 ELS/100 anthers, out of which the number of regenerated green plantlets varied from 3.20 to 26.40 green plantlets/100 anthers and the mean was 9.76 green plantlets/100 anthers, while the number of transplanted plantlets ranged from 2.16 to 21.77 transplanted plantlets/100 anthers. Furthermore, the number of albinos/100 anthers was mitigated and varied between 0.72 and 6.20 albinos/100 anthers. We also studied the number of green and albino plantlets per 100 ELS. The rate of green plantlets per 100 ELS ranged between 14.81% and 64.01%, with the overall mean 33.59%, while the rate of albinos per 100 ELS ranged from 4.82% to 20.70% with the overall mean 11.93%. In our experiment, the rate of acclimatized plantlets (70.15–91.57%) depended mostly on the combination. This study asserted the importance of AC method in wheat for *in vitro* production of green plants. Although albinism was found in each combination, it did not hinder the production of green plantlets. The satisfying results were achieved in green plantlets production compared to the previously published data, but further improvement will be needed continuously,

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experiment by experiment. The generated acclimatized plantlets will be used in the wheat breeding program as doubled haploid (DH) lines.

*Keywords:* Androgenesis, anther culture, haploids, *Triticum aestivum* L., wheat

Abbreviations: AC Anther Culture; DH Doubled Haploid; ELS Embryo-Like Structures.

## INTRODUCTION

Recently, cereal crop breeders have adopted DH methods as a high-efficient requisite tool in their breeding programs (WEDZONY *et al*., 2009; CASTILLO *et al*., 2015; LANTOS and PAUK, 2016) to create homozygous pure-lines from the heterozygous plant material and save time taken for producing new varieties (EL-HENNAWY *et al*., 2011; YAN *et al*., 2017). Researchers have studied *in vitro* haploid tissue culture of several cereal crops (MALUSZYNSKI *et al*., 2003), which was applied in breeding programs (DUNWELL, 2010; NIU *et al*., 2014), and commercial varieties have been produced such as: wheat, triticale, barley, rice and maize (THOMAS *et al*., 2003).

Production of DH plants by AC leads to creation of new wheat varieties in less time compared to conventional breeding methods (DUNWELL, 2010; EL-HENNAWY *et al*., 2011) and gives a quick alternative in the development of homogeneity in different breeding programs (WEDZONY *et al*., 2009; LANTOS and PAUK, 2016). AC and isolated microspore culture are the most wide-used method in breeding programs of wheat (*T. aestivum* L.) crop for androgenic induction purpose (LANTOS *et al*., 2013; CASTILLO *et al*., 2015; NIELSEN *et al*., 2015; LANTOS and PAUK, 2016). Moreover, AC and wide hybridization were used repeatedly in breeding programs for DH production, and applied in: genetic studies for mapping of genes (HAO *et al*., 2013), mapping of quantitative trait loci (QTLs) (SHI *et al*., 2019), genomics and as a target for transformation (MUROVEC and BOHANEC, 2012), and marker-trait association studies (SORRELLS *et al*., 2011), whereas production of haploid and DH plants provide more accurate estimation of QTL × environment interactions (YAN *et al*., 2017).

Many factors influence the androgenic production efficiency by AC such as: genotypes of anthers donor (KONDIC-SPIKA *et al*., 2011), the timing of collection of the donor plants (HE and OUYANG, 1984), the physiological conditions of growth (EL-HENNAWY *et al*., 2011), different abiotic pre-treatment conditions (ISLAM and TUTEJA, 2012), physical factors in tissue culture (light, temperature), and compositions of AC medium (BROUGHTON, 2008; ZUR *et al*., 2015), where several studies have been carried out in recent decades to increase the efficiency of AC's induction medium. The most well-known media in AC are the $P_4$ (PAUK *et al*., 2003; RUBTSOVA *et al*., 2013), $W_{14}$ (LANTOS and PAUK, 2016), and $P_2$ (KONDIC-SPIKA *et al*., 2011).

Breeders have found that the phenomenon of albinism is one of the hindering factors of wheat androgenic production (WEDZONY *et al*., 2009; ISLAM, 2010; BROUGHTON, 2011; LANTOS *et al*., 2013), thus many trials were conducted to overcome the phenomenon of albinism. For example, the reduced number of albino plantlets and increased number of green plantlets during induction of DH plants by AC are due to the positive effects of the copper element (JACQUARD *et al*., 2009), polyamine treatments (REDHA and SULEMAN, 2011) and *n*-butanol treatment (BROUGHTON, 2011; SORIANO *et al*., 2008). Furthermore, genotype dependency is one of the important limiting-factors for AC-derived androgenic production of wheat (CHEN *et al*., 2011; KONDIC-SPIKA *et al*., 2011; DWIVEDI *et al*., 2015). The response of wheat for AC-derived

androgenic induction varies based on the genotype, among species, and within species. For instance, within hexaploid wheat, the winter genotypes are more responsive than spring genotypes (SHARMA *et al*., 2005). TUVESSON *et al*. (2000) presented one of the strategies to overcome the problem of genotype dependency and enhance wheat AC efficiency by using responsive breeding material in crossing programs. The represented-principle of this strategy is that one of the parental genotypes involved in crossing should induce at least one green plantlet/spike (TUVESSON *et al*., 2003). Application of responsive genotypes was also recommended in other breeding programs depending on AC (GONZALEZ *et al*., 2006; KONDIC-SPIKA *et al*., 2011). Consequently, the dependency on genotypes and the phenomenon of albinism are the main obstacle for AC-derived androgenic production of wheat (TUVESSON *et al*., 2000; BROUGHTON, 2011; CHEN *et al*., 2011; ISLAM and TUTEJA, 2012; NIU *et al*., 2014; DWIVEDI *et al*., 2015).

This investigation was conducted to produce DH winter wheat (*T. aestivum* L.) lines via *in vitro* androgenesis. The protocol of AC-wheat (*T. aestivum* L.) has been used (PAUK *et al*., 2003) to study and evaluate the response of androgenic parameters (the number of ELS, green plantlets, albinos, transplanted plantlets and acclimatized plantlets).

## MATERIALS AND METHODS

*Plant materials and growing conditions*

Nine winter wheat combinations (*T. aestivum* L.) were used for *in vitro* AC. The combinations were coded and listed in Table 1. and provided by the Cereal Research Non-Profit Ltd. in Szeged. Donor wheat plants were sown at the nursery in October 2018 at the Cereal Research Non-Profit Ltd. in Szeged, Hungary. The agricultural practices of the wheat crop have been carried out from fertilization to pest control depending on a standard protocol for small grain winter cereals. The required fertilizers of nitrogen, phosphorus and potassium (1:1:1) were added in autumn, followed by an application of ammonium nitrate in mid-April 2018 (18 g/m$^2$). Insect protection was executed by the application of Bulldock (Bayer Crop Science, Budapest, Hungary) as needed. In addition, weed control was carried out by the application of the herbicide Pointer star (DuPont Mo. Ltd., Budaors, Hungary) accompanied by mechanical treatment during the growing season.

*Table 1. List of winter wheat $F_{2\text{-}5}$ combinations tested in AC*

| No | Code number | Combination and its generation |
|---|---|---|
| 1 | 1440 | Komárom/Bani//Midas/Göncöl $F_4$ |
| 2 | 1516 | Toborzó//Tacitus/Körös $F_5$ |
| 3 | 2332 | Bodri/XJ5 $F_3$ |
| 4 | 2350 | Apród/XJ3 $F_3$ |
| 5 | 2566 | Beres/Robigus $F_5$ |
| 6 | 2610 | Nemere/Csillag $F_4$ |
| 7 | 2612 | Petur/Apród $F_4$ |
| 8 | 9118 | Kóló/331.15 $F_2$ |
| 9 | 9247 | Compass/Exotic $F_2$ |

*Collection and pre-treatment of donor spikes*

Prior to anthers isolation, the harvested-donor tillers which contained anthers at early- and mid uni-nucleate microspores stage were placed into an Erlenmeyer flask containing tap water, and enveloped with PVC bags for cold pre-treatment for 2 weeks at 3–4°C under continuous dim light in a cool chamber.

*Anthers isolation and ELS formation*

After the full cold pre-treatment, spikes were separated from tillers and checked under Olympus CK-2 inverted microscope (Olympus, Southernd-on-Sea, UK) to investigate the developmental stage of microspores, whereas the spikes containing anthers at early- and mid uni-nucleate microspores were selected for AC experiment. The selected spikes were sterilised inside Erlenmeyer flask containing 200 mL 2% NaOCl solution and one drop of Tween-80 with shaking for 20 minutes and washed three times in sterile distilled water (Millipore Elix 5).

Fine forceps were used to isolate anthers inside 90 mm diameter glass dishes (250 anthers/Petri dish) containing 15 mL of $W_{14}$mf induction medium (agar replaced with 10% Ficoll) (LANTOS and PAUK, 2016). Then, all Petri dishes that contained isolated-anthers were placed into thermostat at 32°C for three days (a heat shock). After this period, the Petri dishes were transferred into another thermostat at 28°C for 4–8 weeks in the dark to format ELS.

*Green plantlets regeneration*

Four weeks later, the AC- derived ELS with 1–2 mm diameter in size appeared and about 30–35 ELS were transferred onto separate plastic Petri dishes with a 90 mm diameter (Sarstedt, Budapest, Hungary) containing 30 mL of 190-2Cu regeneration medium solidified with 2.8 g/L Gelrite® (PAUK *et al*., 2003). We repeated transferring them every time when new ELS were found until 8 weeks (period of regeneration ELS).

After two weeks of transferring ELS into 90 mm Petri dishes, the green plantlets were regenerated. Then approximately 15–18 green regenerated plantlets with 20–30 mm long leaves were transferred onto separate plastic boxes (about 800 mL in size) containing the same regeneration medium (190-2Cu) for development of roots and shoot growths. We used small tubes (35 mL in size) to transfer green plantlets individually. The formed albinos were counted and discarded.

A growth room with temperature-controlled at 24°C under 16/8 h illuminated and dark conditions, respectively, was used for regenerating the green plantlets and developing the plantlets for more rooting and shoot growth.

*Acclimatization of plantlets*

Four weeks later, the well-rooted green plantlets were transferred to the glasshouse for acclimatization and were transplanted into 50 mm diameter plastic pots containing a mixture of 1:1 peat and sandy soils. The plantlets were covered with PVC for 3–5 days (acclimatization period) at 20–25°C, and after this period, the PVC was removed and acclimatized plantlets remained in the glasshouse under the same conditions for two weeks. In the period from July to September, the acclimatized plantlets were placed in a cool room at 8–12°C with continuous dim lighting. In October, the plantlets were transplanted in the nursery.

*Data collection and statistical analysis*

The experiment was carried out in 10 replications to study genotype factors. The collected data of androgenic parameters (Number of ELS, green plantlets, albino plantlets, transplanted plantlets) were analysed by R© software, version 3.6.1, 2019 (R CORE TEAM, 2019). Analysis of variance, one-way ANOVA function was used for each parameter. Multiple Comparisons of Means: Tukey Contrasts was conducted by using pairwise comparisons of means function. Least Significant differences ($LSD_{0.05}$), Mean Squares, (F-Value), and F Probabilities were calculated for all parameters. The percentage of acclimatized plantlets/transplanted plantlets was calculated for each combination.

## RESULTS

*Effect of Genotype on Parameters of Androgenesis in AC*

In this study, the steps of the AC method were tested on nine winter wheat $F_{2-5}$ combinations for androgenic production. All steps of androgenic production are presented in Figure 1.

The effect of genotype on androgenic production was shown in our experiment, where one-way ANOVA indicated that each androgenic parameter (ELS, green plantlets, albinos, and well-rooted transplanted plantlets) had highly significant differences among the investigated wheat combinations (Table 2).

*Table 2. One-way ANOVA of studied androgenic parameters per 100 anthers for nine winter wheat F2-5 combinations.*

| Androgenic parameter /100 anthers | Source of variance | DF | Sum of Squares SS | Mean Squares MS | F value | Pr (>F) |
|---|---|---|---|---|---|---|
| Number of ELS | Genotypes | 8 | 15418 | 1927.2 *** | 7.72 | <0.000 |
| | Error | 79 | 19721 | 249.6 | | |
| Number of green plantlets | Genotypes | 8 | 5554 | 694.3 *** | 11.03 | <0.000 |
| | Error | 79 | 4974 | 63.0 | | |
| Number of albino plantlets | Genotypes | 8 | 295.1 | 36.89 *** | 4.833 | <0.000 |
| | Error | 79 | 603.0 | 7.63 | | |
| Number of transplanted plantlets | Genotypes | 8 | 3418 | 427.2 *** | 9.155 | <0.000 |
| | Error | 79 | 3687 | 46.7 | | |

*** Significant at the 0.001 probability level.

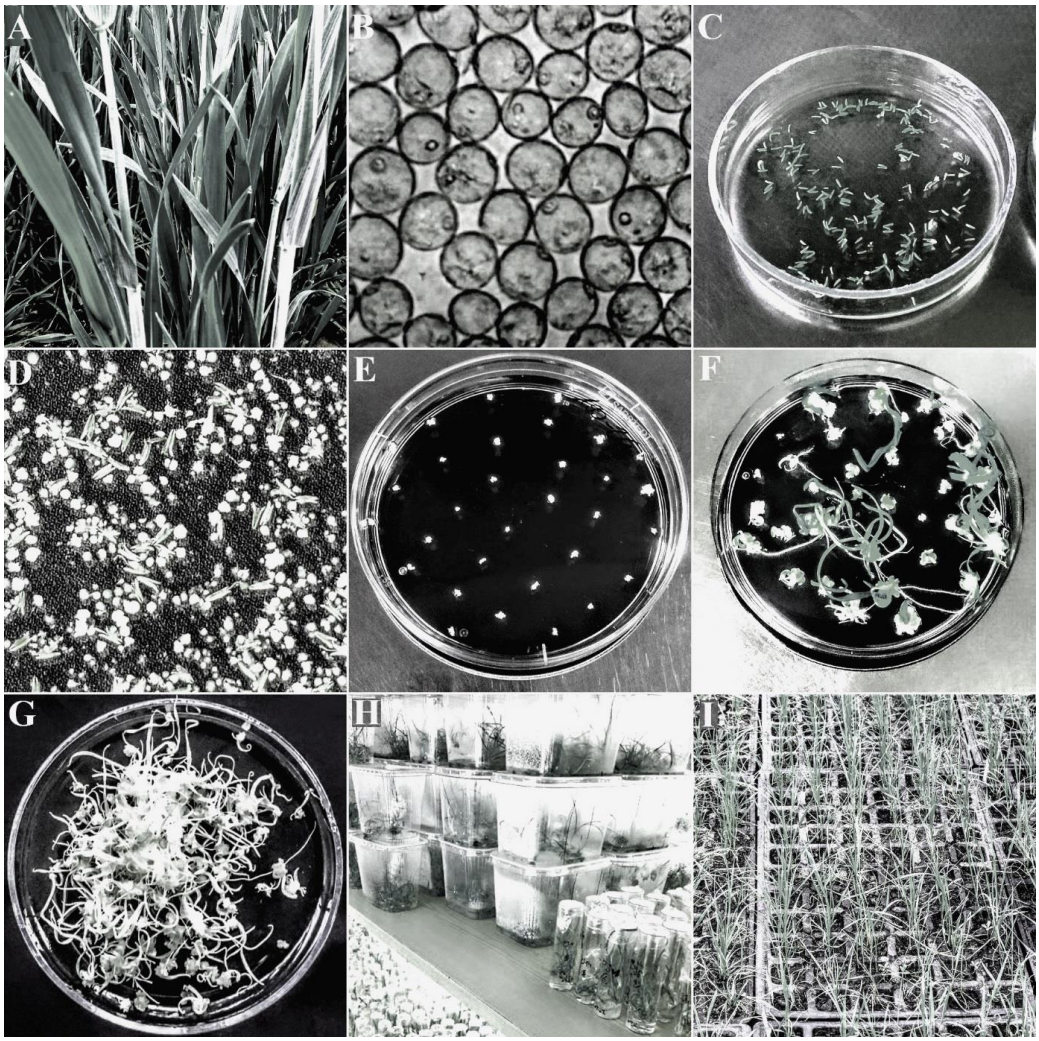


Figure 1. Main stages of winter wheat AC. (A+B) Collection of donor tillers in a uni-nucleated microspore stage. (C) Isolated anthers culture on $W_{14}$mf medium. (D) ELS stage developed from anthers after about 4–8 weeks of anthers isolation. (E) ELS with 1–2 mm transferred to 190-2Cu medium. (F) Green and albino plantlets formatted on the regeneration medium (190-2Cu). (G) Collected-counted albinos from Petri dishes. (H) The well-developed green plantlets transplanted into boxes and individual glass tubes. (I) the transplanted plantlets transferred into 50 mm diameter plastic pots for acclimatization in the glasshouse.

*Androgenic Efficiency of $F_{2-5}$ Winter Wheat Combinations in AC*

*The number of ELS/100 anthers*

A significant difference was found among the combinations for ELS/100 anthers (Table 2). The values of ELS/100 anthers were between 11.73 and 52.76 in "Petur/Apród" and ''Apród/XJ3" combinations, respectively. The overall mean of nine combinations was 26.22 ELS/100 anthers (Table 3).

*Table 3. Androgenic response per 100 anthers for nine winter wheat $F_{2-5}$ combinations in AC (means ± SD).*

| Combination code | ELS /100 anthers | Green plantlets /100 anthers | Albino plantlets /100 anthers | Transplanted plantlets /100 anthers |
|---|---|---|---|---|
| 1440 | $15.84 \pm 16.66^{cd}$ | $4.04 \pm 3.05^{c}$ | $0.72 \pm 0.86^{c}$ | $2.68 \pm 2.20^{c}$ |
| 1516 | $32.20 \pm 5.31^{ad}$ | $20.60 \pm 5.92^{ab}$ | $1.60 \pm 1.16^{bc}$ | $13.64 \pm 4.44^{ab}$ |
| 2332 | $15.84 \pm 11.57^{cd}$ | $3.28 \pm 4.17^{c}$ | $1.40 \pm 1.41^{bc}$ | $2.28 \pm 3.02^{c}$ |
| 2350 | $52.76 \pm 28.71^{a}$ | $26.40 \pm 17.36^{a}$ | $5.16 \pm 2.25^{ab}$ | $21.78 \pm 15.19^{a}$ |
| 2566 | $19.40 \pm 6.82^{bcd}$ | $3.20 \pm 2.78^{c}$ | $4.00 \pm 2.10^{ac}$ | $2.16 \pm 1.72^{c}$ |
| 2610 | $35.40 \pm 19.30^{ac}$ | $9.48 \pm 4.78^{bc}$ | $3.08 \pm 2.92^{ac}$ | $6.40 \pm 4.65^{bc}$ |
| 2612 | $11.73 \pm 7.87^{d}$ | $5.29 \pm 3.94^{c}$ | $0.84 \pm 0.90^{c}$ | $3.87 \pm 3.05^{bc}$ |
| 9118 | $14.04 \pm 8.03^{cd}$ | $3.44 \pm 5.52^{c}$ | $2.28 \pm 1.97^{ac}$ | $2.64 \pm 3.30^{c}$ |
| 9247 | $39.96 \pm 21.92^{ab}$ | $13.36 \pm 12.17^{bc}$ | $6.20 \pm 6.40^{a}$ | $9.96 \pm 11.28^{bc}$ |
| Mean | 26.22 | 9.76 | 2.80 | 7.14 |
| $LSD_{0.05}$ | 14.84 | 7.46 | 2.60 | 6.42 |

Values followed by the same letters within a column are not significantly different at the (P= 0.05) probability levels as determined by Pairwise comparison of means test (Tukey Contrasts).

*The number of green plantlets/100 anthers*

Each combination had green plantlets of AC-derived ELS, however, Table 2. shows a highly significant difference among combinations studied for green plantlets. The values of green plantlets/100 anthers varied between 3.20 in the "Beres/Robigus" combination and 26.40 in the "Apród/XJ3" combination. The overall mean of studied combinations was 9.76 green plantlets/100 anthers (Table 3).

*The number of albinos/100 anthers*

The phenomenon of albinism was found in each combination. However, the number of albino plantlets/100 anthers varied significantly among different combinations (Table 2). The values ranged from 0.72 albino plantlets/100 anthers in the "Komárom/Bani//Midas/Göncöl" combination to 6.20 albino plantlets/100 anthers in the "Compass/Exotic" combination. The mean of nine combinations was 2.80 albino plantlets/100 anthers (Table 3).

*The number of transplanted plantlets/100 anthers*

A significant difference was achieved among the combinations for transplanted plantlets (Table 2). The values of transplanted plantlets/100 differed between 2.16 in "Beres/Robigus" combination and 21.77 in "Apród/XJ3" combination. The overall mean of nine combinations was 7.14 transplanted plantlets/100 (Table 3).

The effect of genotype depending on the data of nine $F_{2-5}$ combinations was studied in AC experiment for each of green plantlets and albino plantlets per 100 ELS. The results of one-way ANOVA indicated that a significant effect of genotype was found among the investigated combinations for green plantlets/100 ELS at 0.001 probability level, while the significant difference at 0.01 probability level was in albinos/100 ELS (Table 4).

*Table 4. Statistical analysis of androgenic parameters (Green plantlets, and albinos) per100 ELS for nine winter wheat $F_{2-5}$ combinations by one-way ANOVA.*

| Androgenic parameter /100 ELS | Source of variance | DF | Sum of Squares SS | Mean Squares MS | F value | Pr (>F) |
|---|---|---|---|---|---|---|
| Number of green plantlets | Genotypes | 8 | 19661 | 2457.6*** | 10.23 | <0.000 |
| | Error | 79 | 18985 | 240.3 | | |
| Number of albino plantlets | Genotypes | 8 | 2431 | 303.9 ** | 2.765 | <0.009 |
| | Error | 79 | 8682 | 109.9 | | |

***, ** Significant at the 0.001, 0.01 probability levels, respectively.

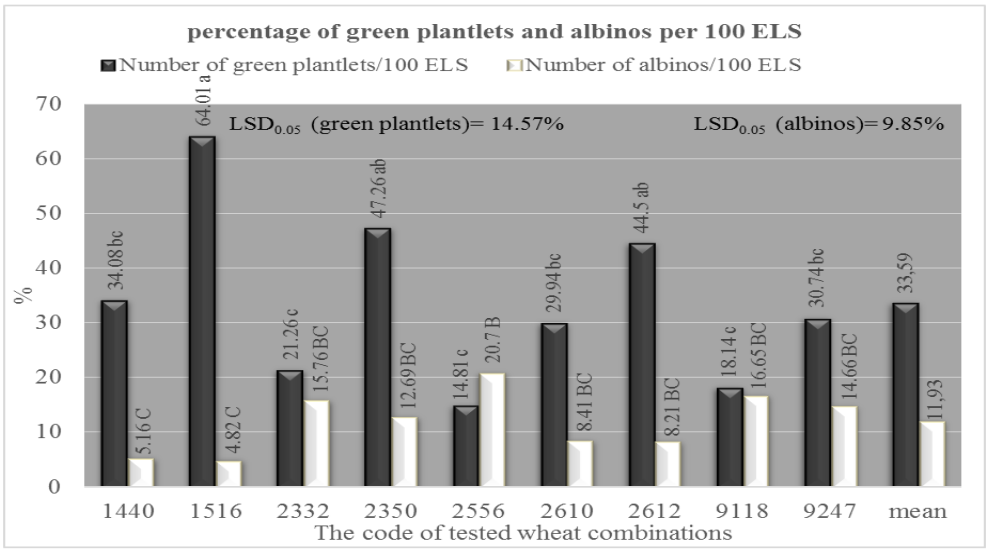


Figure 2. The rate of green plantlets and albinos per 100 ELS. Values followed by the same letters within green plantlets or albinos columns are not significantly different at the (P= 0.05) probability levels as determined by Pairwise comparison of means test (Tukey Contrasts).

However, the number of green plantlets/100 ELS varied significantly among the combinations (Table 4) and ranged from 14.81 green plantlets/100 ELS in the "Beres/Robigus" combination to 64.01 green plantlets/100 ELS in the "Toborzó//Tacitus/Körös" combination. The overall mean of nine $F_{2-5}$ combinations was 33.59 green plantlets/100 ELS (Figure 2).

Moreover, Table 4. illustrates that there was a significant difference between combinations for albino plantlets/100 ELS. The AC-derived ELS produced from 4.82 to 20.70 albino plantlets/100 ELS, with the overall mean for all $F_{2-5}$ combinations 11.93 albino plantlets/100 ELS (Figure 2).

*Percentage of acclimatized plantlets among AC-derived transplanted plantlets*

The transplanted plantlets were transferred into the glasshouse for acclimatization. In our experiment, the total number of transplanted plantlets was 1571, out of which the number of acclimatized plantlets was 1363, and the percentage of acclimatized plantlets ranged between 70.15 acclimatized plantlets/100 transplanted plantlets in "Komárom/Bani//Midas/Göncöl" and 91.57 acclimatized plantlets/100 transplanted plantlets in "Compass/Exotic" with respect to the combinations. The overall mean of acclimatized plantlets was 86.76 acclimatized plantlets/100 transplanted plantlets (Table 5).

*Table 5. Total number of haploid acclimatized plantlets for nine winter wheat combinations from AC.*

| Combination code | Total number of green plantlets | Total number and percentage of transplanted plantlets | | Total number and percentage of acclimatized plantlets | |
|---|---|---|---|---|---|
| | | No | % | No | % |
| 1440 | 101 | 67 | 66.34 | 47 | 70.15 |
| 1516 | 515 | 341 | 66.21 | 297 | 87.10 |
| 2332 | 82 | 57 | 69.51 | 51 | 89.47 |
| 2350 | 594 | 490 | 82.49 | 437 | 89.18 |
| 2566 | 80 | 54 | 67.50 | 40 | 74.07 |
| 2610 | 237 | 160 | 67.51 | 141 | 88.13 |
| 2612 | 119 | 87 | 73.11 | 74 | 85.06 |
| 9118 | 86 | 66 | 76.74 | 48 | 72.73 |
| 9247 | 334 | 249 | 74.55 | 228 | 91.57 |
| Total number | 2148 | 1571 | - | 1363 | - |
| Overall mean | - | - | 73.14 | - | 86.76 |

## DISCUSSION

Currently, most wheat breeding programs have been aimed at obtaining new desired varieties in less time (DUNWELL, 2010; EL-HENNAWY *et al*., 2011). Thus, the AC method is a highly efficient tool for this purpose (LANTOS *et al*., 2013; CASTILLO *et al*., 2015). In addition, the application of AC enables the production of homozygous pure-lines from heterozygous genotypes during only one generation (DUNWELL, 2010; EL-HENNAWY *et al*., 2011; YAN *et al*., 2017). Having in mind that these pure-lines are used in different genetic studies (MUROVEC and

BOHANEC, 2012; HAO *et al*., 2013; SHI *et al*., 2019), breeders have the responsibility to improve AC method efficiency according to the requirements of plant breeding programs and applied researches. Albinism and genotype dependency remain the most critical factors in AC- induced androgenic production (TUVESSON *et al*., 2000; BROUGHTON, 2011; CHEN *et al*., 2011; ISLAM and TUTEJA, 2012; DWIVEDI *et al*., 2015).

In our experiment, the effect of genotype on the *in vitro* androgenic traits in AC was investigated. The statistical analysis showed a significant difference among the nine wheat $F_{2\text{-}5}$ combinations for androgenic traits (ELS/100 anthers, green plantlets/100 anthers, and transplanted plantlets/100 anthers). The inconstancy in AC- induced androgenic responses among tested combinations has been attributed to genetic material; many researchers had found the same findings (TUVESSON *et al*., 2000; KONDIC-SPIKA *et al*., 2011; LANTOS and PAUK, 2016; KUTLU *et al*., 2019).

It has been found in our experiment that the ELS, green plantlets, albino plantlets, and transplanted plantlets were produced in each wheat $F_{2\text{-}5}$ combination, that was in accordance with the results achieved by (KONDIC-SPIKA *et al*., 2008; LANTOS *et al*., 2013; LANTOS and PAUK, 2016), but our findings were opposed to earlier results found by HOLME *et al*. (1999), TUVESSON *et al*. (2000), BROUGHTON (2008), and EL-HENNAWY *et al*. (2011), where their results revealed that some genotypes did not produce green plantlets.

The values of wheat combinations for each androgenic parameter have differed from each other. Consequently, the fluctuation is due to the genotypic dependency in *in vitro* AC. The values of ELS/100 anthers varied between 11.73 and 52.76 ELS/100 anthers. Some researches published maximum values less than the maximum values obtained in our experiment; 18% (EL-HENNAWY *et al*., 2011), and 42% (GRAUDA *et al*., 2014), while KIM and BAENZIGER (2005) and KHIABANI *et al*. (2008) published maximum values (52, 53 ELS/100 anthers, respectively), which were close to our results. But highly responding genotypes were found in studies published by KONDIC-SPIKA *et al*. (2008) (119%), and LANTOS *et al*. (2013) (169.40%, 190.40% in 2010, 2011), where the maximum value was more than 100 ELS/100 anthers in their researches.

With regard to the results of green plantlets/100 anthers in our experiments, the values varied from 3.20 to 26.40 green plantlets/100 anthers, where the maximum value was higher than the other maximum values provided by earlier studies (SADASIVAIAH *et al*., 1999; KIM and BAENZIGER, 2005; KONDIC-SPIKA *et al*., 2008; EL-HENNAWY *et al*., 2011; GRAUDA *et al*., 2014) (4.73, 22, 13.40, 10, and 13.10 green plantlets/100 anthers, respectively). Furthermore, some research reported values higher than 100 green plantlets/100 anthers such as; (BROUGHTON, 2011; LANTOS *et al*., 2013; CASTILLO *et al*., 2015). In this investigation, the overall mean was 9.76 green plantlets/100 anthers, which exceeded the means of green plantlets in previously published winter wheat breeding programs (HOLME *et al*., 1999; TUVESSON *et al*., 2000; KONDIC-SPIKA *et al*., 2008; EL-HENNAWY *et al*., 2011; LANTOS *et al*., 2013; GRAUDA *et al*., 2014), where the means ranged between 0.4 and 5.8 green plantlets/100 anthers.

Albinism is a hindering factor of wheat androgenic production (WEDZONY *et al*., 2009; ISLAM, 2010; BROUGHTON, 2011; LANTOS *et al*., 2013). In our study, the occurrence of albino plantlets of nine wheat combinations varied between 0.72 and 6.20 albinos/100 anthers. However, genetic dependency contributed to this variation, and 7 wheat combinations had low

values of albinos/100 anthers without significant differences among them; "Komárom/Bani//Midas/Göncöl", "Toborzó//Tacitus/Körös", "Bodri/XJ5", "Nemere/Csillag", "Petur/Apród", and "Kóló/331/15". This finding confirmed that the genetic dependency was restricted in albino plantlets parameter. Our maximum albinos/100 anther was lower than the other maximum values in early publications achieved by KIM and BAENZIGER (2005) 25%, EL-HENNAWY *et al*. (2011) 24%, (LANTOS *et al*., 2013) (14.60% and 4.50%) the maximum values of the high-responding genotype in the two years 2010 and 2011, respectively when they used in their study two control genotypes– high and low responding–and 93 $F_1$ crosses, LANTOS and PAUK (2016), 24.33% and 20.17% by using P4mf and W14mf media in AC, respectively. Consequently, the overall mean (2.80%) was lower compared to previous studies; 10.76% in (EL-HENNAWY *et al*., 2011), 9.55% (the overall mean of the high-responding genotype values in 2010 and 2011) and 18.29%, 23.98% (the overall means of 93 $F_1$ crosses in the two years 2010 and 2011, respectively) in (LANTOS *et al*., 2013).

The efficiency of androgenic production is affected by genetic background (KONDIC-SPIKA *et al*., 2011) in addition to many other factors such as: the collection timing of donor plants (HE and OUYANG, 1984), the physiological conditions of growth (EL-HENNAWY *et al*., 2011), different abiotic pre-treatment conditions (ISLAM and TUTEJA, 2012), physical factors in tissue culture (light, temperature), and the laboratory manual work. Thus, the efficiency of laboratory work is required for androgenic production in AC to ensure obtaining satisfying results for breeders and minimize the obstacles of AC- induced androgenic production in the future.

## CONCLUSIONS

This investigation has demonstrated the efficiency of AC in androgenic parameters of winter wheat breeding combinations. The production of ELS, green plantlets, and acclimatized plantlets were shown in all combinations. In our experiment, both maximum and mean values of green plantlets achieved desired results compared to the most values obtained in previous publications. Although albinism was found in each combination, it did not hinder the production of green plantlets in each combination.

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## ANDROGENI ODGOVOR KOMBINACIJA OZIME PŠENICE (*Triticum aestivum* L.) U *IN VITRO* KULTURI ANTERA

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Izvod

Androgeni parametri su ispitivani u *in vitro kulturi* antera (AC) kod devet $F_{2-5}$ kombinacija ozime pšenice (*Triticum aestivium* L.). Svaka kombinacija je proizvela strukture slične embrionu (ELS), zelene biljke, albino biljke, presađene biljke i aklimatizovane biljke, koje su prikazane u odnosu na broj izolovanih antera u kulturi. Broj ELS-a dobijenih u kulturi antera kretao se između 11,73 i 52,76 ELS/100 antera, sa srednjom vrednošću od 26,22 ELS/100 antera, od čega je broj regenerisanih zelenih biljaka varirao od 3,20 do 26,40 zelenih biljaka/100 antera i srednja vrednost je bila 9,76 zelenih biljaka/100 antera, dok se broj presađenih biljaka kretao u rasponu od 2,16 do 21,77 presađenih biljaka/100 antera. Pored toga, broj albino biljaka/100 antera je značajno smanjen, pa je varirao između 0,72 i 6,20 albino biljaka/100 antera. Proučavali smo i broj zelenih i albino biljaka na 100 ELS. Broj zelenih biljaka na 100 ELS kretao se u rasponu između 14,81% i 64,01%, sa ukupnom srednjom vrednošću od 33,59%, dok se broj albino biljaka na 100 ELS kretao u rasponu od 4,82% do 20,70%, sa prosečnom vrednošću od 11,93%. U našem eksperimentu, broj aklimatizovanih biljaka (70,15-91,57%) u velikoj meri je zavisio od kombinacije ukrštanja. Rezultati ovog rada potvrdili su značaj metode kulture antera kod pšenice za *in vitro* androgenu proizvodnju. Iako je albinizam bio prisutan u svakoj kombinaciji, to nije ometalo proizvodnju zelenih biljaka. Postigli smo zadovoljavajuće rezultate u pogledu proizvodnje zelenih biljaka, u poređenju s ranije objavljenim podacima, ali biće potrebno da se taj broj kontinuirano poboljšava u svakom budućem eksperimentu. Proizvedene aklimatizovane biljke biće korišćene u programu oplemenjivanja pšenice kao linije dvostrukih haploida (DH).

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