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IN VITRO ANTHER CULTURE UTILIZATION IN HUNGARIAN TRITICALE BREEDING PROGRAM

[1]CSABA LANTOS, [2]LAJOS BÓNA, [3]ÁDÁM BORDÉ, [3]TAMÁS MONOSTORI, [1]JÁNOS PAUK

[1]Cereal Research Ltd., Department of Biotechnology
H-6726 Szeged, Alsókikötő sor 9
[2]Cereal Research Ltd., Department of Minor Cereals
H-6726 Szeged, Alsókikötő sor 9
[3]University of Szeged, Faculty of Agriculture
H-6800 Hódmezővásárhely, Andrássy Str. 15
janos.pauk@gabonakutato.hu

ABSTRACT
The doubled haploid plants can play a key role in applied research to map the agronomically, botanically important traits and acceleration of the breeding process in crop plants. The efficiency of triticale anther culture was tested by two field- and greenhouse grown winter triticale varieties (GK Idus and GK Szemes). The effect of growing conditions and genotype and their interaction were tested on four androgenic parameters (number of embryo-like structures, total, green and albino plantlets). The androgenesis was induced in each treatment of the two tested genotypes. Cultivar GK Szemes produced more embryo-like structures, total and albino plantlets, while the field grown GK Idus produced the most green plantlets/Petri dish. In the experiments of growing conditions, the efficiency of this tested method was higher in anther culture of field grown materials if compare to greenhouse origin materials. Based on this promising data, we can suggest the using of this method in applied research (production of mapping population) and practical breeding.

Keywords: androgenesis, anther culture, cereal breeding, genotype x environment interaction, triticale

INTRODUCTION

The doubled haploid (DH) plants can play a key role in i) applied research to map the agronomically, botanically important traits and ii) acceleration of the breeding process. Three different methods are known for the production of triticale DH plants; maize mediated haploid production, anther- and isolated microspore culture. The first one is an expensive technology because of parallel growing of two different species (maize and triticale); while albinism and genotype dependency reduce the practical efficiency of isolated microspore culture (PAUK ET AL. 2000, 2003 EUDES AND AMUNDSEN 2005, LANTOS ET AL. 2005, EUDES AND CHUG 2009). In 1973, the regeneration of the first anther culture derived plantlets was published (WANG ET AL. 1973, SUN ET AL. 1973). Despite of many efforts on androgenesis, some published data are available which mentioned the application of anther culture in practical breeding (THOMAS ET AL. 2003, OLESZCZUK ET AL. 2011). The main bottlenecks of triticale anther culture are genotype dependency, albinism and colchicine treatment of haploid regenerants because of low spontaneous rediploidization.
Genotype dependency is a well-known phenomenon in androgenesis of cereals including triticale. Many chromosomes and QTLs in the triticale genome were detected in connection with androgenic parameters such as number of embryo like structures (ELS), total and green plant regeneration (BALATERO ET AL. 1995, GONZÁLEZ ET AL. 2005, KRZEWSKA ET AL. 2012). Thus, significant differences were observed among the green

plant production of different triticale breeding materials (TUVESSON ET AL. 2000, 2003, WEDZONY 2003).

The phenomenon of albinism is another critical factor which hinders the production of large number of green plantlets for breeding (TUVESSON ET AL. 2000, 2003, WEDZONY 2003). Furthermore, the colchicine treatment of the regenerants is also an important step because of low spontaneous rediploidisation rate (OLESZCZUK ET AL. 2011).

The efficiency of anther culture is influenced by genotype (G) and environmental (E) factors such as growing conditions of donor materials, collection time of donor tillers, stress treatment, induction and regeneration media etc (CHIEN AND KAO 1983, HASSAWI ET AL. 1990, KARSAI ET AL. 1994, KARSAI AND BEDŐ 1997, PONITKA ET AL. 1999; IMMONEN AND ROBINSON 2000). In our experiment, the androgenic response of field- and greenhouse grown triticale genotypes was compared in anther culture based on four androgenic parameters (number of ELS, regenerated plantlets, green and albino plantlets). The effect of growing conditions and genotypes and their interaction were analysed (two-way ANOVA).

In this research, the focus was on the important parameters of triticale anther culture and comparison of two different origins of donor tillers for efficiency on the androgenic parameters.

MATERIAL AND METHOD

Plant material and growing conditions

In our experiments, two winter triticale (x *Triticosecale* Wittmack) varieties (GK Idus and GK Szemes) were used to test the efficiency of anther culture.

In the middle of October 2011, the genotypes were sown in nursery of Cereal Research Ltd. (Szeged). The fertilization of donor plants were carried out by an artificial manure of nitrogen, phosphorus and potassium (1:1:1) in autumn, in 60 kg/ha dose. The genotypes were also grown under greenhouse conditions where 16 h photoperiod and 20/15 ºC (day/night) was ensured for donor plants. In the greenhouse, the donor plants were fertilised once every two weeks with Volldünger (N:P:K:Mg/14:7:21:1, plus 1% microelements: B, Cu, Fe, Mn and Zn; Magyar Kwizda Ltd., Budapest, Hungary).

The donor plants were grown in the field and greenhouse until the collection of donor tillers.

Collection and pre-treatment of donor materials

Donor tillers containing early and mid uninucleated microspores were collected for anther culture. The harvested tillers were put into Erlenmeyer flask containing tap water and covered by PVC bags. The cool pre-treatment period was approximately two weeks at 3-4 °C.

Anther culture

The anthers of donor materials were isolated into 90 mm diameter glass Petri dish (250 anthers/Petri dish) containing modified W14 medium (OUYANG ET AL. 1989), namely W14mf (LANTOS ET AL. 2013). Three day heat shock was applied in the beginning of the culture, after the heat shock the Petri dishes were kept at 28 °C in dark thermostat.

Plant regeneration of microspore derived ELS

The microspore derived ELS with 2 mm diameter size were transferred onto 190-2Cu regeneration medium (PAUK ET AL. 2003). Approximately 40-50 ELS were placed on the

90 mm diameter plastic Petri dish (Sarstedt Ltd., Newton, Massachusetts, USA) containing the regeneration medium. The regenerated green plantlet with 20-30 mm length leaves were transferred individually into glass tubes containing the same regeneration medium.

Data collection and statistical analysis

The experiments were carried out at least five replications. Data of four androgenic parameters (number of ELS, total, green and albino plantlets) were collected from each treatment of anther cultures. The data were analysed by two-way ANOVA. The statistical analyses were carried out by Microsoft® Excel 2002 statistical software developed by Microsoft Ltd., Redmond, Washington, USA.

RESULTS

In triticale anther culture experiments, the effect of genotype and growing conditions were tested based on four androgenic parameters. The growing condition influenced all of tested parameters while the genotype influenced the ELS, total and albino plantlets production (*Table 1*). The statistical analysis revealed the interaction in connection with total, green and albino plantlets production.

Table 1. Statistical analysis of androgenic parameters based on data of anther culture of field grown and greenhouse grown triticale genotypes

Two-way ANOVA	*df*	*MS-ELS*	*MS-total plantlets*	*MS-green plantlets*	*MS-albinos*
E	1	15792.2***	3328.2***	369.8**	1479.2***
G	1	7683.2**	1008.2**	105.8	1767.2***
GxE Interaction	1	39.2	404.9*	245.0*	1280,0***
Error	16	612.65	67.15	30,225	25,05

*,**,*** Significant at the 0.05, 0.01 and 0.001 probability level, respectively

Androgenesis was induced in anther culture of each treatment and genotype. ELS were visible to the naked eye on the fifth week of culture (*Figure 1A*) which were transferred onto the regeneration medium. The ELS from each treatment produced green plantlets (*Figure 1B* and *Figure 1*C).

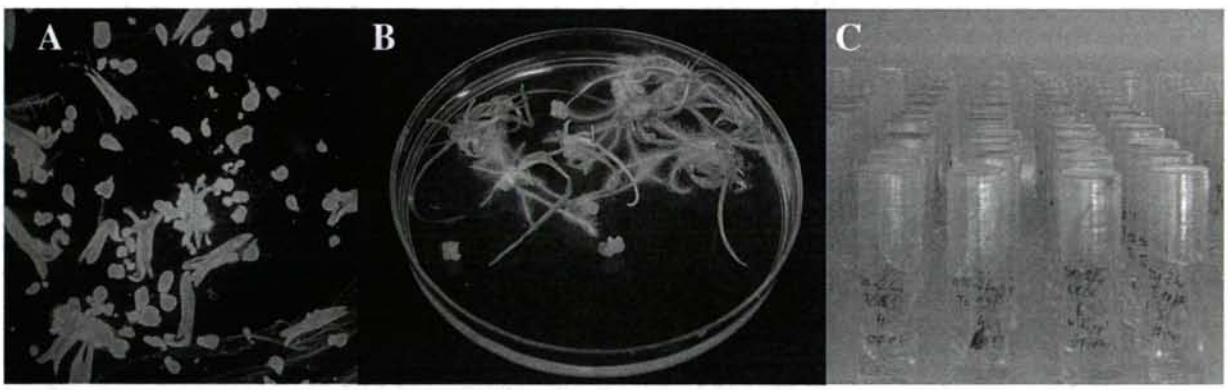

Figure 1. Important steps of anther culture in triticale. (A) The microspore derived ELS can be visible on the fifth week of anther culture. (B) The ELS regenerated plantlets on the first week of regeneration period. (C) The green plantlets were grown in individually glass tubes.

Based on the statistical analysis of data, significant differences were obtained between the two genotypes (*Table 2*). The ELS production and the number of total and albino plantlets was higher in anther culture of GK Szemes while the anther culture of field grown GK Idus achieved the highest green plantlets production (17.8 green plantlets/Petri dish).
In comparison of growing condition of donor materials, the field grown materials produced more ELS, total, green and albino plantlets in anther culture of the two tested genotype (*Table 2*). The differences of ELS production and total plant regeneration were significantly higher in anther culture of field grown genotypes. The ratio of green and albino plantlets ranged from 0.17 to 12.71 depending on genotype and origin of donor materials.

Table 2. Efficiency of triticale anther culture of field and greenhouse grown genotypes

Genotype	Origin of donor materials	ELS production/ Petri dish	Regenerated plantlets/ Petri dish	Green plantlets / Petri dish	Albino plantlets/ Petri dish	Ratio of green and albino plantlets
GK Szemes	field	102.6	42.4	6.2	36.2	0.17
GK Szemes	greenhouse	43.6	7.6	4.6	3.0	1.53
GK Idus	field	60.6	19.2	17.8	1.4	12.71
GK Idus	greenhouse	7.2	2.4	2.2	0.2	11
LSD5%=	-	23.467	7.769	14.84	33.92	-

DISCUSSION

Haploid plant production methods offer a good chance for breeding to accelerate the breeding process; these methods were used successful in the breeding of more crop plants (THOMAS ET AL. 2003). In our experiments, androgenesis was induced in anther culture of triticale genotypes to test the effect of growing conditions and genotype on androgenic response.
Our observation was in harmony with some published data that the genotype influenced the efficiency of anther culture response (BALATERO ET AL. 1995, IMMONEN AND ROBINSON 2000, TUVESSON ET AL. 2000, 2003, GONZÁLEZ ET AL. 2005, KRZEWSKA ET AL. 2012). These differences were enhanced among the numbers of ELS and mitigated among numbers of green and albino plantlets. So, other environmental factors (conditions of anther culture and plant regeneration) influenced also the regeneration of green and albino plantlets.
The origin of donor materials had a significant effect on response of triticale genotypes. The all tested androgenic parameters were higher in anther culture of field grown genotypes than data of greenhouse derived anther cultures. These results are in contrast with the earlier investigation of IMMONEN AND ROBINSON (2000). Based on this phenomenon, field grown materials are preferred in our anther culture protocol.
The production of green plantlets is the most critical and important parameter in DH plant production methods; regeneration of green plantlets is influenced not only by G but also the growing and culture conditions (CHIEN AND KAO 1983, HASSAWI ET AL. 1990, KARSAI ET AL. 1994, KARSAI AND BEDŐ 1997, PONITKA ET AL. 1999; IMMONEN AND ROBINSON 2000). In our investigations, the ratio of green and albino plantlets was also influenced by genotype and growing condition. Furthermore, strong G x E interaction was measured on

the number of albino plantlets. More experiments are required to clarify this phenomenon in triticale androgenesis.

Based on this promising data, we can suggest the using of this method in applied research (production of mapping population) and practical breeding. Following experiments should focus on the increasing the number of green plantlets among the regenerants and decreasing the effects of environmental factors.

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