Ciência Rural, Santa Maria, v.38, n.1, p. 240-242, jan-fev, 2008
ISSN 0103-8478

Genotypic differences in proembryoid development and green plantlets regeneration through androgenesis in barley varieties

Diferenças genotípicas no desenvolvimento de pró-embrióides e regeneração de plântulas verdes via androgênese em genótipo de cevada

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- NOTE -

ABSTRACT

The development of in vitro haploid plants followed by spontaneous or induced genome duplication allows to achieve, in one generation, the recovery of total homozygosis. The efficiency of the haplodiploidization process through in vitro anther culture of barley is variable among genotypes. This study was aimed at determining the androgenetic response of nine barley genotypes from the breeding program of Embrapa Trigo, analysing proembryoid development and green plantlets regeneration in anthers cultivated in vitro. Cultivar 'BR2' presented the highest average of proembryoids (104/anther) and 'MN698' presented the highest average of green plantlets (0,41/anther). There was a significant variation among the average values of barley genotypes for embryo formation and green plantlets regeneration, making possible the selection to combine androgenetic capacity and good agronomic traits.

Key words: *haploid, in vitro androgenesis, androgenetic capacity,* ***Hordeum vulgare*** *L.*

RESUMO

A obtenção, na cevada, de plantas haplóides in vitro *e a posterior duplicação natural ou artificial do genoma permitem alcançar a homozigose completa, em uma geração. A eficiência da haplodiploidização pela cultura de anteras é variável entre os genótipos. Foi avaliada a resposta androgenética através da formação de pró-embrióides e da regeneração de plântulas verdes em nove cultivares do programa de melhoramento de cevada da Embrapa Trigo, em anteras cultivadas* in vitro. *A cultivar "BR2" apresentou maior média de pró-embrióides (104/antera), enquanto "MN698" mostrou a maior média de plântulas verdes (0,41/antera). Houve variação significativa entre os valores médios dos genótipos em relação à formação de pró-embrióides e à regeneração de plântulas verdes, indicando a possibilidade de seleção para combinar a capacidade androgenética com boas características agronômicas.*

Palavras-chave: *haplóides, androgênese in vitro, capacidade androgenética,* ***Hordeum vulgare*** *L.*

After GUHA & MAHESHAWARI (1966) discovered *in vitro* androgenesis in cultured anthers of ***Datura innoxia***, it become possible, in responsive crops, to obtain, in one step, fully homozygous lines, saving time and resources in breeding programs. Androgenetic capacity is defined as the ability of the male gametic cell to reverse its development, giving rise to haploid green plantlets that become completely homozygous and fully fertile after spontaneous or colchicine induced genome duplication (PICARD et al., 1990; PETERS et al., 1999).

Microspores of androgenetic genotypes are able to develop haploid proembryoids which may be regenerated in green haploid plantlets. In barley, as well as other responsive species, inherited factors have been reported as involved in successful haploid plant development (FAROUGHI-WEHR et al., 1982; LARSEN et al., 1991; HENRY et al., 1994). But androgenetic response is possible only when androgenetic genotypes have appropriate environmental conditions that include physiological state of the donor plant, culturing anthers at the right stage of development of microspore and the right culture medium composition (OLSEN, 1987; MORAES-FERNANDES, 1990;

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Received 12.05.05 Approved 04.18.07

HOEKSTRA, 1996; GRANDO & MORAES-FERNANDES, 1997). If some of these factors are inadequate, the anther response may be absent even in androgenetic genotypes. The androgenetic ability may be introduced by crossing in genotypes with good agronomic traits speeding up breeders selection that can be made directly in uniform progenies (FAROUGHI-WEHR & FRIEDT, 1984; QUÉDRAOGO et al., 1998; MORAES-FERNANDES et al., 1999; SMYKAL, 2000). The purpose of this research was the analysis of *in vitro* proembryoid development and green plantlets regeneration, in *in vitro* cultivated anthers, in order to investigate the androgenetic capacity of barley cultivars belonging to crossing block of Embrapa Trigo Barley Breeding Program that had good agronomic traits.

The experiment was carried out at the Biotechnology Laboratory of Embrapa Trigo. The cultivars used were 'BR2', 'BRS180', 'BRS195', 'EMBRAPA127', 'FM404', 'GIMPEL', 'MN684', 'MN698', and 'SCARLETT'. To minimize environmental effects, donor plants were cultivated in a growth chamber with controlled temperature, humidity and photoperiod. A complete randomized experimental design was used. Three plants of each genotype were grown per pot, with three replications (nine plants per genotype). When the main spikes reached the size of development in which uninucleated microspores could be cytologically identified in the anthers of central flowers, these spikes were asseptically cleaned with a 70% ethanol and a 10 days pretreatment at 4ºC, in dark, was made; 36 anthers from 12 central flowers of each spike (three anthers/flower, 324 anthers/genotype), were cultured on modified Hunter medium (HUNTER, 1985). The Petry dishes were wrapped in aluminum foil and placed in a growth chamber at 25ºC. After 18 days of *in vitro* culture, six main anthers of six central flowers were fixed in an acetic acid - ethanol solution (3:1). For optical microscope cytological studies, the anthers content was stained with acetic-carmine and cultured microspores were analyzed. After that, the remaining 24 anthers from each Petry dish were left 12 days more, covered with aluminum foil (total of 30 days in dark) and then left in light for monitoring green plantlets emergence. As soon as green plantlets were formed they were transferred to Potato II regeneration media (MORAES FERNANDES & PICARD, 1983). Therefore, from a total of 2916 anthers cultured *in vitro* proembryoids were counted in 486 anthers and green plantlets in 1944 anthers that remained in the medium. Proembryoids identification was made when three or more identical nuclei were observed in microspores with non disrupted membrane or clear embryo like structures. Data were submitted to statistical analysis and means were compared by using the Duncan test at 1% probability, using the Statistical Analysis System (SAS).

A very large variation in proembryoid occurrence was observed among cultured anthers of the same spike and among spikes of the same genotype (Table 1). Genotypes BR2 and BRS195 presented the highest value of proembryoids/anther and FM404, BRS180 and SCARLETT presented the lowest value. The statistical analysis of the regenerated green plantlets data showed that the barley cultivar 'MN698' was the best one for this trait, presenting a significantly higher number of regenerated plantlets: 41 green plantlets per each 100 cultured anthers (Table 1). There was no significant correlation between frequencies of

Table 1 - Results of microspores proembryoids induction and green plantlets regeneration in nine barley genotypes.

Genotype	NAResp	Proembryoids			Green plantlets	
		NPE	Mean/Anther and SD/Anther	Range	NPG	GP/100AC
BR2	97	5643	104 ± 108 A	0 - 446	24	11 B
BRS195	53	3407	63 ± 62 AB	0 - 230	15	7 B
GIMPEL	35	2711	50 ± 63 BC	0 - 317	9	4 B
MN684	50	1777	33 ± 58 BCD	0 - 356	14	6 B
EMBRAPA127	34	1220	23 ±32 CD	0 - 108	6	3 B
MN698	66	961	18 ± 42 DE	0 - 239	88	41A
SCARLETT	40	821	15 ± 55 DEF	0 - 398	0	0 B
BRS180	5	140	3 ± 8 EF	0 - 49	0	0 B
FM404	59	97	2 ± 6 F	0 - 32	19	9 B
TOTAL	439	16777	-	-	148	-

NAResp: number of responsive anthers; NPE: number of pro-embryoids; Mean/Anther and SD/Anther: mean and standard deviation by anther; NGP: number of green plantlets; GP/A: mean of green plantlets by 100 anthers. *Means followed by the same letter are not statistically different according to the Duncan test at 1 % probability.

proembryoids and frequencies of regenerated plantlets. But, BRS195, GIMPEL and MN684 had statistically similar means of proembryoids and green plantlets regeneration. However, MN684, EMBRAPA127, MN698 and SCARLETT, although having statistically similar means as far as proembryoid development, were different in relation to green plantlet regeneration.

QUÉDRAOGO et al. (1998), evaluating the androgenetic capacity of agronomic lines of barley and their hybrids, observed that Igri was less responsive than Léger in relation to proembryoid development, but similar in green plantlet regeneration. However, Cadette was similar to Igri in proembryoid development, but produced fewer green plantlets. STIVAL et al. (1997) studied heterozygous populations resulting from crosses between barley lines and found two groups of independent genotypes, one presenting high proembryoid induction and another group with high green plantlets regeneration. All genotypes presenting high regeneration capacity were descendent from line PFC9104, which is descendent from Igri which is referred as an androgenetic genotype, confirming the transmission of androgenetic ability. Therefore, the literature reports as well as the results observed here among cultivars of barley crossing block makes possible the recommendation for use as breeding strategy the simultaneous selection for androgenetic capacity and agronomic traits in order to increase genetic progress.

ACKNOWLEDGEMENTS

We would like to thank Embrapa Trigo for making possible the development of this research and also, thanks to Gilney Müller, Adrimara Radin, and Regina Fontaneli for their help.

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