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APPLICATION OF A LOW DOSE OF GAMMA RAYS IN WHEAT ANDROGENESIS

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Abstract - Gamma-rays of dose of 5 Gy using a ^{60}Co source was applied in a single wheat cross to study the effect on anther culture (AC) parameters. The female parent was a 6U^{g} /6D mono-substitution line of cv. Trakia with resistance to powdery mildew and leaf rust, while the male parent was cv. Aglika, characterized as one of the best local varieties with high productivity and grain quality. Callus induction, plant regeneration, green and albino plants from the control (normal anther culture, 0 Gy) designated C, were compared with parameters studied in three other groups, derived from anthers irradiated with 5 Gy (designated AI), calli irradiated with 5 Gy (designated CI) and anthers and calli irradiated consecutively with 5+5 Gy (combined treatment group, designated ACI). Anther culture response of the four F_1 group of plants was intermediate between the parents, with a slightly depressive effect of the dose irradiation in the AI and ACI groups. In general, the effect of the 5 Gy dose of irradiation on androgenesis was estimated to be negative for at least one group of haploid plants (AI), derived from irradiated anthers. Green plants were mostly reduced in the combined treatment group originating from irradiated calli and anthers.

Key words: Anther culture, gamma irradiation, wheat, substitution line

INTRODUCTION

Doubled haploid production (DHP) technology can greatly reduce the time and cost of cultivar development. In plant breeding programs, an *in vitro* anther culture procedure has been utilized to obtain haploid plants from F_1 hybrids. DHP in wheat has been reported by microspore or anther culture (androgenesis), ovule culture (gynogenesis), alien species chromosome elimination (using maize and *H. bulbosum* L. crosses) and the effect of an alien cytoplasm. Microspore and anther culture methods have the potential to produce more than a thousand haploid plants per cultured anther. The production of wheat doubled haploids (DH) from anther culture (AC) is limited due to low calli/embryoid formation frequency, genotype-dependant response and poor regeneration of green plantlets. A large number of regenerated plants remained albino and did not survive. The chromosome doubling was also very dependent on the environment in which the plantlets are cultured and matured (Weyen, 2009; Jauhar et al., 2009).

Genetic factors have been recognized to be one of the major contributors to *in vitro* responses of cultured wheat tissues (Agache et al., 1989; Chaudhary et al., 2003). The success of the anther culture technique can be improved by using stress treatments such as cold pretreatment, osmotic stress, starvation, medium concentration and low dose of irradiation. Ding et al. (1991) and Belchev and Kostov (2003) demonstrated that a low dose of gamma rays (up to 7 Gy) could enhance anther culture response in wheat. A positive effect was also found in potato (Al-Safadi

et al., 2000) and barley (Arabi et al., 2005). Stresses and the mechanisms of their actions in inducing microspore embryogenesis have been completely described (Shariatpanahi et al., 2006).

The practical aspects of doubled-haploid breeding through anther culture were manifested by increasing the Al tolerance in wheat (Bakos et al., 2008), enhancing freezing tolerance (Humphreys et al., 2007), grain yield (Sadasivaiah et al., 2004), earliness, disease resistance and moisture stress tolerance in DH-mutants (Khan et al., 2001). Several AC wheat varieties were produced in Hungary and other countries (Barnabas et al., 2000; Pauk et al., 2003). The advantages of DHP have long been recognized by breeders and have resulted in more than 280 varieties produced in several crops (Szarejko and Forster, 2007). The combination of anther culture and conventional breeding methods has resulted in released commercial wheat cultivars (Sadasivaiah et al., 2004; Weyen, 2009).

There are rare cases when aneuploids are included in anther culture. Zhang et al. (2001) produced a DH line (2n=44) with one rye chromosome pair added (1R) and a homozygous 6R(6D) substitution chromosome. A '6x triticale' and 'Chinese Spring' nulli-tetrasomic N6DT6A were the parents of this cross, bearing several lines with high resistance to powdery mildew and moderate resistance to yellow rust.

The aim of this study was to apply the anther culture technique in a single cross with a $6U^g$(6D) monosubstitution line as a female parent, and a wheat variety 'Aglika' as a pollen parent, investigating the effect of low-dose irradiation on the AC parameters in F_1. A dose of 5 Gy gamma rays was differently involved in anther culture to compare its efficiency on androgenic capacity: callus yield, plant regeneration, frequency of green and albino regenerants.

MATERIALS AND METHODS

Hybrid plants were produced from a 'Line 12-z' x 'Aglika' cross. The female parent was selected from a BC_2F_2 population ('Trakia' x *Aegilops geniculata*) where $6U^g$ was monosubstituted for the wheat 6D chromosome (Stoilova and Spetsov, 2006). The alien chromosome bears a gene(s) for resistance to powdery mildew in wheat and to leaf rust in the adult plant stage. The pollen parent was 'Aglika', which served as a standard variety in State Official Wheat Trials for A group in the country. It is also a high yielding variety expressing the highest plant regeneration ability among several other cultivars studied after spike treatment with gamma rays (Belchev and Kostov, 2003).

DHP follows the method as previously described (Plamenov et al., 2009). The donor plants were vernalized and grown primarily in a cold greenhouse (5-15°C) for three months; then the temperature was increased to 16-24°C. Suitable spikes with anthers containing microspores at the mid-to-late uninucleate stage were cut from plants with powdery mildew resistance, put in a vessel with water and stored at 4°C for 7 days. Forty spikes for each variety/line were collected and after cold pretreatment they were sterilized with 70% ethanol. Thirty anthers from each spike were plated in test tubes with 9 ml Potato 2 medium. Anthers were incubated at 28°C in the dark. Embryogenic structures (calli and embryoids) produced from the microspores were transferred to 190-2 regeneration medium (Zhang et al., 2001) and cultured at 25°C under illumination 3000 lx (16/8 h photoperiod). Green and albino regenerants were calculated after 30 days.

Anther culture response was characterized by the following parameters: callus induction: the number and percentage of embryogenic structures induced per 100 cultured anthers; plant regeneration: the number and percentage of green and albino plants regenerated from calli transferred to the regeneration medium; albino and green plants: calculated the total number and albino/green plants produced per 100 plated anthers.

Gamma-rays in a dose of 5 Gy using a ^{60}Co source were applied three times during the anther culture: on spikes (anthers treated), on a part of calli (calli

Table 1. Two-way ANOVA for the androgenic ability of the 'Line12-z' x 'Aglika' cross and its parents.

Source of variation	D.F.	MS	F
Variants	5	375.7	28.2 **
Parameters	3	5866.1	440.6 **
Interaction	15	107.9	8.1 **
Treatments	23	917.2	68.9 **
Error	24	13.3	

**, significant differences at P=0.01

Table 2. Anther culture response of parents and F_1 hybrid plants ('Line 12-z' x 'Aglika'), divided in four groups after application of a 5 Gy dose of gamma rays.

Variant	Cul-tured anthers	Callus induction		Plant regeneration		Green plants		Albino plants		Mean
		No	%	No	%	No	%	No	%	
'Aglika'	510	126	24.7	93	74.0	58	11.4	30	5.9	27.9 a
'Line12-z'	720	45	6.3	9	20.0	2	0.3	7	1.0	7.4 d
C	4260	499	11.7	239	47.9	130	3.1	109	2.6	16.4 b
AI	4440	227	5.1	98	43.2	65	1.5	33	0.7	12.6 c
CI	4470	509	11.4	272	53.4	136	3.0	136	3.0	18.1 b
ACI	4380	235	5.4	123	52.3	49	1.1	74	1.7	14.9 bc
Mean			10.3 b		49.0 a		3.0 c		2.7 c	

C, control, anthers cultured, 0 Gy; AI, anthers irradiated with 5 Gy; CI, calli irradiated with 5 Gy; ACI, anthers and calli irradiated consecutively with 5 + 5 Gy (combined treatment group).
Means followed by the same letter are not significantly different (p=0.05).

treated), and on a part of calli produced from treated anthers (anthers and calli treated). Thus, DH plants from four groups were produced and designated as follows: C (control, 0 Gy), AI (anthers irradiated, 5 Gy), CI (calli irradiated, 5 Gy), and ACI (anthers and calli irradiated consecutively, 5+5=10 Gy).

The experiment was conducted with two replicates. Two-way ANOVA was used to determine the main effects, interaction between them and the significance between factorial means. Statistical analysis was carried out using an appropriate statistical package (version 5.0 for Word 2003).

RESULTS

Analysis of variance showed that all the main effects were significantly different, including the interaction between treatment groups and androgenic parameters (Table 1). The female parent expressed a poor response in androgenesis, producing the least plant regenerants and green plantlets among all investigated genotypes. On the contrary, the pollen parent variety 'Aglika' responded in the best way with the highest ability in all parameters studied (Table 2). The treatment groups (C, AI, CI and ACI) behaved differently in anther culture as seen from the factorial means. Three of them, except the AI group, showed almost equal means of variants (16.4, 18.1 and 14.9), without significant difference in between. The low dose of irradiation did not enhance the androgenesis in the CI group (calli irradiated with 5 Gy, mean=18.1) because all anther culture (AC) parameters were equal or slightly positive to those of the control (group C, 0 Gy, mean=16.4) (Table 2).

The effect of low-dose irradiation was prominent in the group AI (anthers irradiated) as a negative in-

fluence on callus induction, plant regeneration and green plants. This tendency was strengthened in the ACI group for callus induction and green plants, but less pronounced for the other two parameters. Overall, the effect of a 5 Gy dose of irradiation was found as negative for anther culture in the AI group of plants and to a lesser extent, for a number of green plants produced in the combined treatment group ACI (anthers and calli irradiated consecutively with 5+5 Gy). The overall AC mean of the CI group was the highest (18.1), except for the pollen parent, but the difference compared to C group (16.4) was not significant.

DISCUSSION

Anther culture ability in wheat can be divided into three independently inherited components: callus induction, plant regeneration and green plant formation. Each of them has been governed by more than one gene (Lazar et al., 1984; Szakacs et al., 1988; Chaudhary et al., 2003). The production of DH lines of wheat from anther culture is limited by a relatively low callus/embryoid induction frequency, genotype-dependent response, poor regeneration and large number of albino plants.

In the present study, genotype seemed to be one of the major determinants of callus and embryoid production, since a wide range of variations in callus initiation (5.1-24.7%) and haploid green plantlet formation (0.3-11.4%) were observed. The gamma irradiation did not show any stimulatory effect on AC parameters. The present findings are in contrast to the results obtained (Ding et al. 1991; Belchev and Kostov, 2003) for the enhancement of anther culture response in wheat through the use of a low dose of irradiation (LDI). Our data did not indicate that gamma irradiation in low dose could be an essential key point of androgenic induction in this single cross.

The use of one parent as male or female could lead to a change in the production of green plants from F_1 hybrids (Yildirim et al., 2008). The resulting hybrid plants from a '12-z' x 'Aglika' cross share the AC traits between the parents. It follows that a population of androgenic plants derived from different treatment groups will have different gene combinations or induced mutations. It has been demonstrated conclusively that within such a population it is possible to select plants of different genotypes with contrasting phenotypes for a range of features expressed in parents or new combinations (Humphreys et al., 2007). Ionizing radiation was first used for the induction of mutants in haploid cells, i.e. microspores (Sangwan and Sangwan, 1986; Pechan and Keller, 1989). ϒ-irradiation with 10 Gy significantly increased the induction of embryogenesis in cultured *Nicotiana*, *Datura*, *Brassica* and *Lycopersicum* (Mac Donald et al., 1988; Shtereva et al., 1998; Shariatpanahi et al., 2006). Sarr et al. (2012) pointed that female gametes were better suited for transmission of the alien chromosome in cotton than male gametes. Gao and Jung (2002) reported a higher alien transmission of *Beta corolliflora* in *B. vulgaris* at a rate of 60%. Information is not available for the use of a wheat line with a 6U chromosome upon anther culture in the genus *Triticum*.

In wheat, the results are different. Some authors documented that gamma irradiation (2 and 4 Gy) did not show a stimulatory effect on embryoid production and frequency of plant regeneration in anther culture (Khiabani et al., 2008). Conversely, the LDI was found to promote the anther culture response in wheat (Ding et al., 1991; Belchev and Kostov, 2003). The role of LDI on wheat androgenesis is still unclear. At least three questions arise from such experiments: (i) whether the stimulatory effect on androgenesis, if present, correlates with the DH plants produced; (ii) how could the doubled-haploid plants be used in breeding if the effect of LDI is neutral or negative? (iii) what happens with the alien $6U^g$ chromosome – will it occur in selected DH progenies? To give a precise answer, we intend to grow and develop the resulting DH plants to select the best DH lines in field conditions for a conclusion on the effect of LDI and its potential use in genetics and wheat breeding.

In conclusion, the effect of a 5 Gy dose of gamma irradiation on androgenesis in one wheat cross

was estimated as negative for at least one group of haploid plants derived from irradiated anthers. Among the AC parameters, green and albino plants were slightly reduced by the irradiation applied, and this action occurred mostly in the combined treatment group, originating from irradiated calli and anthers.

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