Anther culture response in heterozygous triticale (x *Triticosecale* Wittmack) populations

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Abstract

The subject of research was the anther culture response of 186 lines of winter crop triticale coming from two types of crossings: AxB and (AxB)xC. The number of embryoids and green regenerants obtained from particular lines were adopted as property indicators. The examined lines were characterised by a relatively good ability to create either single embryoids or conglomerated embryoids, but the number of green regenerants obtained later on from such structures was generally low. It was found that there were no significant differences between these objects both with regard to the ability to create embryoids and green regenerants in the case of anthers collected from hybrids from simple crossings, whilst there were significant differences (with p=0.05) in the case of hybrids originated from crossings of the type (AxB)xC. The coefficients of heritability of the ability to create embryoids was h^2 =0,682 for anthers coming from hybrids of the type: AxB and h^2=0,371 for anthers collected from hybrids of the (AxB)xC type. However genetic conditioning of the ability to regenerate green plants was 0.699 and 0.522 respectively. It may thus be supposed that the ability to create embryoids passed onto the next generation would be better with the forms originating from crossing of the AxB type, and the heritability of the ability to create green plants would be similar with both types of hybrids.
For both of the groups compared, also the coefficients of variability CV(p) and CV(g) were calculated as well as the coefficients of correlation between the number of embryoids and the number of green regenerants obtained from them at a later phase.
Key words: androgenesis, heritability, winter triticale

Introduction

In vitro androgenesis is an efficient system of homozygote line production. Doubled haploid (DH) plants derived from haploid microspores provide excellent material for research, plant breeding, and plant transformation. Via androgenesis, fertile homozygous progeny from a heterozygous parent can be obtained in a single generation, thus significantly reducing time required in breeding programs and providing a major advantage in preparing F_1 hybrid seeds as well. By combining anther culture with chromosome engineering techniques, the production of stable doubled-haploid lines containing alien chromosomes carrying agronomically desirable genes can be greatly accelerated (Zhang et al. 2001). Possibilities for studying basic processes in plant development and the economic importance have motivated numerous research groups to investigate androgenesis in different crops. The exploitation of this potentially powerful method for crop improvement is limited by its low efficiency in the DH production. Several methods of haploid production have been investigated and reported in the literature (see Liu et al. 2002, Wang et al. 2000) but protocols that can be applied to

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breeding programs have become available only recently. Anther culture ability is a heritable trait and can be transferred by crossing (Bullock et al. 1982, Foroughi Wehr et al. 1982)
Significant differences between triticale genotypes in anther culture response have been reported by many authors (Schumann 1990, Gonzales et al. 1997, Immonen & Robinson 2000). Therefore the results of microspore regeneration experiments are highly dependent on both the variety used and the growth condition of the donor material (Schumann 1990). Arzani and Darvey (2002) demonstrated that due to the observed significant cytoplasmic differences in anther culture response the direction of a cross between genotypes is also an important factor in determining microspore development under *in vitro* conditions.
The currently grown cultivars and strains of triticale are derived from widespread genetic sources, so there is a great probability of finding well responding genotypes directly in unselected lines or hybrids. Most investigations were carried out with the homozygous genotypes whereas the heterozygous ones are particularly important for the use in plant breeding (Arzani & Darvey 2002). From the breeding practice (unpublished work of the Szelejewo Plant Breeding) it occurs that A×B and A×B×C crossings are most advantageous for many practically important traits. So the haploidization of hybrids derived from crossing of two or three forms may give most interesting results from the breeders' point of view. For this reason the aim of this paper was to compare adrogenesis potential of triticale lines containing two or three parental components and to infer the degree of the genetic determination of this trait in triticale hybrids obtained in result of A×B and (A×B)×C crossing of two and three parental components, respectively.

Materials and methods

The plant material chosen for this investigation consisted of 105 winter triticale hybrid lines type A×B and 81 hybrid lines type A×B×C. These hybrids were obtained at Plant Breeding Station at Szelejewo, by means of crossing, in the patterns mentioned above, Polish local strains of triticale (Table1). with the Stations' own lines and families characterised with favourable agronomical traits. The origin of these lines and families is the ownership of the Breeding Station, but it is possible however to make them available for the interested researchers and breeders.

Table 1

Polish strains of triticale with hybrid origin of the donor forms

Year	Females (A)	Males (B)	Males (C)
2001	Kazo, Pronto, Tornado, Ugo	Angus, Bogo, Boreas, Kazo, Lamberto Marko, Prego, Presto, Tornado, Ugo, Vision,	Bogo, Lamberto
2002	Fidelio, Kazo, Lamberto, Lasko, Sekundo, Ugo	Ego, Fidelio , Flair, Kazo, Kitaro, Lamberto, Woltario	Kazo, Kitaro, Lamberto, Woltario
2003	Lamberto, Lasko, Moreno, Presto -"Valdy", Ugo	Fidelio, Kazo, Kitaro, Lamberto, Prado, Woltario	Kazo, Woltario

The donor plants were grown on 10 m^2 plots with standard fertilization. Anthers were collected and the developmental stage of pollen was observed just before tillering, when spikes were still covered with the leaf sheath. Pollen development was tested with the use of acetocarmine stain. The stage in which pollen grains had one, peripheral nucleus and a large vacuole was regarded to be optimal. Shoots with anthers in the optimal stage were harvested, kept at +4°C and placed in solution containing micro- and macro elements based on N6 medium (Chu et al. 1975). Spikes were sterilized with 5% $Ca(OCl)_2$ solution containing a drop of Tween 20 for 5 min. and then washed 5 times with sterile water. Anthers were extracted from spikes and placed on 6 cm Petri dishes with induction medium C17 (Wang and Chen 1983). Anthers were incubated in darkness at +30°C until macroscopically visible androgenic embryos were obtained (after about 30 days). Embryos were transferred onto regenerating medium 190-2 (Zhuang and Jia 1983) and grown in controlled environment chambers (16 hours daylight of 4000 lux, +22°C) until green plants were formed.
Plants with developed roots were planted in pots with soil and the ploidy level in root tip meristems was recorded with the use of Feulgen's method and in leaf cells using flow cytometer. The haploid plants were collected for the duplication of the chromosome number. For this purpose plants were removed from soil, shoots and roots were cut to 10 and 2 cm length, respectively, and treated with 0.1% colchicin solution in water containing 4% of DMSO, 1 drop of Tween 20 mg/l and 25 mg/l of GA_3 at +25°C with intensive light. Plants were thoroughly washed with running water (about 2 hours), placed in pots with soil, vernalized according to standard procedures and planted on field.
To compare the androgenetic response of studied populations the number of embryoids (single and/or cluster) obtained per 1000 anthers and the number of green regenerants per 1000 anthers were used.
Variability coefficients: total variability coefficient, phenotypic and genotypic variability coefficient (CV %, CVp and CVg, respectively) as well as the genetic determination coefficient h^2 were calculated for each of the compared groups of hybrids when the numbers of embryos and green regenerants were used as independent parameters. The coefficients of genotype determination (h^2) of the traits (equivalent to heritability - H), were marked from the mean square values for genotypes and genotype-environment interaction, according to the following formula:

$$h^2=(m_1-m_2)/m_1,$$

where: h^2 - the coefficient of genetic determination,
m_1 and m_2 - mean squares for genotype and genotype-environment interaction respectively (Baker et al. 1968)

Result and discussion

Numbers of embryos as well as regenerated haploid plants obtained each year in both groups of triticale hybrid populations presented in Table 2 show great differences in anther reactivity in different seasons. A good ability of studied hybrids to form single embryos or clusters of embryos may be concluded on the basis of the data presented. However, generally the number of plants regenerated from these structures was small. A similar phenomenon was previously observed in different cereal species (Van Bergen et al. 1999) despite of different methods of plant regeneration used by other authors (Marciniak 2001; Wędzony 2003). Some

new developments in this area (Liu et al. 2002) suggest a possibility to achieve a quite high yield of regenerated plants, however, the described methods are not yet commonly used.

Table 2.

The numbers of embryoids and green regenerants per 1000 anthers obtained in different years from triticale hybrid plants depending on their crossing origin

Year	AxB crossing type				(AxB)xC crossing type			
	Number of lines	Mean	Minimum	Maximum	Number of lines	Mean	Minimum	Maximum
Embryoids								
2001	64	208,6	16,0	583,3	30	356,0	44,9	1476,7
2002	19	113,0	10,3	312,2	30	90,7	7,9	440,9
2003	22	144,2	5,0	290,7	21	220,6	9,1	618,3
Green regenerants								
2001	32	4,9	1,3	15,6	21	9,4	0,4	43,3
2002	14	7,9	0,5	59,1	18	9,5	0,4	26,3
2003	16	8,2	1,0	40,0	20	9,3	1,1	20,0

According to data presented in Table 2. much more embryos were obtained from hybrids derived from AxBxC crossing, whereas (with the exception of 2001) the mean number as well as the minimal number of green plants obtained in both groups were similar. Large differences in the maximal number of regenerated green plants were observed between the groups of hybrids. However, in 2001 more plants were obtained from triple crosses and in following years higher yields were obtained using double crosses. Most probably genotypes of the parental components of respective crosses were decisive in this respect as many authors reported the genetic determination of this process (Picard and Buyser 1977, Ślusarkiewicz-Jarzina et al. 1996). Some other factors such as environment (Bullock et al. 1982, Foroughi Wehr et al. 1982, Schumann 1990) as well as physiological state of the donor plants (Luckett and Darvey 1992) may significantly influence the processes leading to obtaining regenerated plants.

Table 3.

Analysis of variance of embryoids and green regenerants number in compared types of crossing in years 2001-2003

Source of variation	Mean squares for crossing			
	AxB		AxBxC	
	Embryoids	Green regenerants	Embryoids	Green regenerants
Years	82660.786**	76.643	527641.014**	0.137
Strains	20612.175**	124.412**	56801.328	109.910*
	6546.710	37.480	35723.012	52.488

**, * - significant at P=0,01 and 0,05 respectively

The analysis of variance performed for the number of embryos and regenerated plants per 1000 anthers in culture revealed highly significant differences in the embryo formation efficiency in both groups of hybrids (Table 3.). In most cases, significant differences were found between individual hybrids as well. No significant differences in the ability to produce embryos and green regenerated plants were found only between the hybrids derived from AxBxC crossings. In this case a high error including interaction between treatments and years and random error of experiment was obtained. Highly significant differences between years in the number of regenerated green plants in both types of hybrids may suggest that the environmental factors have a higher influence on this trait. The significant differences between hybrids of AxB type in the numbers of formed embryos and regenerated plants indicate that this type of hybrids should be chosen for obtaining forms with anthers giving a high productivity of regenerated plants. On the other hand, the coefficient of genetic determination of green plants regeneration, h^2 equal to 0.699 and 0.522 for double and triple crosses, respectively (Table 4.) suggests that the progeny of AxB crosses should inherit this trait to a higher extent.

Table 4.

Means and coefficients of variability: CV %, CV(p), CV(g) and heritability (h^2) of investigated traits

	Type of crossing			
	AxB		AxBxC	
	Embryoids	Green regenerants	Embryoids	Green regenerants
Mean	177.812	6.409	222.632	9.389
CV%	45.504	95.528	84.896	77.166
h^2	0.682	0.699	0.371	0.522
CV(p)	46.617	99.90	61.806	64.469
CV(g)	38.508	83.997	37.650	46.598

In this type of crosses the selection of parental forms has a particularly high effect so the androgenesis ability of the parental forms should be addressed in the crossing programs along with their agronomical traits.

The significant effects of general (GCA) and specific (SCA) combination ability values according to numbers of derived embryos and regenerated plants observed by Ślusarkiewicz-Jarzina et al. (1996) support such an approach.

A high coefficient of total variability (84%) calculated for ability to embryoids formation suggest the presence of this interaction which may be the reason for the lack of significant differences between genotypes. The highest differentiation was found for regenerated green haploid plants and the lowest for embryiods formation in AxB hybrids. Coefficients of genetic determination (h^2) had similar values in the range 0.52-0.70 for all the studied traits with the exception of the embryo formation ability of AxBxC hybrids (h^2=0.37 in this case).

In addition, coefficients of genotypic CV(g) and phenotypic variability CV(p) were calculated. The highest values of these coefficients were obtained for the regenerated green haploid plants derived from AxB hybrids. This suggests a higher practical value of this type

of hybrids as well as also provide some information concerning genetic phenomena occurring in the analyzed population.

The number of formed embryos or embryoid structures, total number of regenerants (albino and green) and the final number of green plants are the parameters most commonly used to characterize the androgenetic potential of cultivars and lines. A similar approach was adopted in this paper, however the albino plants were not taken into account. As it can be seen from Table 1. the obtained values differ to a large extent. The phenotypic correlation coefficients for the numbers of embryos and regenerated green plants calculated separately for each type of hybrids (Table 5.) show a lack of dependence between these traits for A×B hybrids as well as significant, repeatedly observed in different years, correlations for (A×B)×C hybrids. Similar dependence was previously observed in barley (Foroughi-Wehr et al. 1982).

Table 5.

Coefficients of correlation between numbers of embryos formed and green plants regenerated depending on the crossing type of hybrid

Years	Correlations	
	A×B	A×B×C
2001	0.010	**0.786****
2002	-0.254	**0.493***
2003	0.217	**0.601***

**, * - significant at P=0,01 and 0,05 respectively

The lack of interdependence of these traits observed in simple hybrids seems to confirm the hypothesis of Gonzalez et al. (1997) claiming that the embryoid induction and plant regeneration are independent traits. The results obtained for hybrids derived from triple crosses are somewhat contradictory. The coefficients of genetic determination (Table 3) suggest independence of these traits, whereas the phenotypic correlation coefficients suggest their close relation. These results are difficult to explain at the moment; however they are in some agreement with findings of Lazar et al. (1984), who similarly found no correlation between the number of calli formed and the number of green plants regenerated from them. It seems that these results confirm hypothesis of Foroughi-Wehr et al. (1982), who proposed existence of four independent mechanisms of haploid formation: callus induction, callus stabilization, induction of plant growth and finally the formation of green plants.

Conclusions

1. The ability of hybrids derived from double (A×B) and triple ((A×B)×C) crosses to regenerate green plants significantly differs between years which suggests a major influence of environmental factors on this trait.
2. The lack of significant differences between (A×B) hybrids in the ability to produce androgenic embryos and regenerate green plants and also significant differences between the A×B×C hybrids indicate that forms with a good performance of anther cultures should be chosen in the latter group.

3. The higher genetic determination of the ability to regenerate green plants (h^2=0.699) in single-cross hybrids compared to triple-cross hybrids (h^2=0.522) suggests a higher inheritance of this trait in lines derived from A×B hybrids.
4. Phenotypic correlations coefficients between the number of embryos formed and green plants regenerated show a lack of dependence between these traits in A×B hybrids and a significant correlation in (A×B)×C) hybrids, observed in different years.
5. Coefficients of genetic determination calculated for triple-cross hybrids suggest independent inheritance of abilities to form androgenic embryos and regenerate green plants whereas coefficients of phenotypic correlations show a significant dependence of these traits. Explanation of this phenomenon needs additional studies.

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