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THE ANTHR-CULTURE RESPONSE OF TRITICALE LINE X TESTER PROGENIES

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Abstract: Seven triticale cultivars (Ampiac, Aubrac, Trinidad, Ticino, Lamberto, Pronto and Prado) and their F1 hybrids obtained after crossing in a line x tester scheme were examined with respect to their androgenetic effectiveness. The embryo induction rate (number of embryos per 100 anthers), green plant regeneration rate (number of green plantlets per 100 embryos), plant yield (number of green and albino plantlets per 100 anthers) and green plant yield (number of green plantlets per 100 anthers) were assessed. The multivariate and univariate effects of general (GCA) and specific (SCA) combining abilities for the studied traits were estimated and tested. Significant differences between the genotypes were found for individual traits as well as for all the traits treated jointly. Hybrids generally showed a better response in anther culture than their parental genotypes. Heterosis effects were observed in some hybrids for embryo induction rate and green plant yield. GCA and SCA variances were significant and a dominance of the GCA over the SCA variation was found. Among the examined cultivars, Ticino and Pronto were characterised by positive and significant GCA for embryo induction and green plant yield, and these cultivars may be recommended for the improvement of anther culture responsiveness in triticale.

Key Words: Anther Culture, Triticale, GCA, SCA, Multivariate Analysis

INTRODUCTION

Most cereal breeding programmes are focused on producing superior homozygous selections. In conventional breeding, a number of segregating

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Abbreviations used: 2-4D - dichlorophenoxyacetic acid; GCA - general combining ability; NAA - α -naphthaleneacetic acid; P - probability; SCA - specific combining ability.

generations must be grown before the plants reach homozygosity. For that reason, breeders are interested in methods that make it possible to reach homozygosity rapidly.

Anther culture is one of the methods enabling the production of homozygous lines from heterozygous genotypes (most often from F_1 hybrids) within a short time. The efficiency of this method depends on several factors, mainly on the genotypes of donor plants, the growth conditions of the source material, pretreatments, medium composition and culture conditions. Among these factors, the genotype of donor plants appears to be an important factor influencing the success of *in vitro* culture. The influence of genotypes on green plant yield was observed by many authors [1-4]. It has been shown that there are recalcitrant genotypes which do not respond in anther culture, and genotypes from which it is easy to obtain the required number of androgenetic plants. For this reason, more information on the combining ability of breeding material for anther culture response would be extremely useful for the improvement of anther culture effectiveness.

One of the basic questions in genetic experimentation is the choice of crossing system. This determines what kinds of genetic information can be obtained in a given experiment. Among the crossing systems that have been used most often are line \times tester crosses [5]. The line \times tester scheme involves crossing l parental lines with each of t testers. The crossing yields lt progenies, i.e. lt full-subfamilies. In the scheme, two different sets of parents (males and females) are used. In crosses of this type, well-known standard varieties are most frequently used as testers (it is assumed that lines may represent various levels of homozygosity). The line \times tester system permits the estimation of the effects of the general combining ability (GCA) of lines and testers and also the specific combining ability (SCA) of pairs of parental genotypes. There are many univariate statistical methods for the proper genetic analysis of data from line \times tester experiments (e.g. [5, 6]). In practice, however, the data taken from such experiments are most often multivariate, because they originate from observations taken on a set of traits, usually interrelated. The method that takes into account the relationships between traits is the multivariate analysis of variance (MANOVA). A general description of this method is given, e.g., in [7, 8]. A proposal/Some proposals of the multivariate analysis of line \times tester experiments is presented in Kaczmarek and Krajewski [9, 10].

This study work is focused on the assessment of individual and simultaneous GCA and SCA effects for anther-culture response characteristics in line \times tester progenies of hexaploid triticale.

MATERIAL AND METHODS

Four cultivars of hexaploid triticale, \times *Triticosecale* Wittmack, (Ampiac, Aubrac, Trinidad, Ticino) were crossed with three testers (Lamberto, Pronto and Prado) in a line \times tester scheme. Twelve F_1 hybrids along with their parental

genotypes were tested with respect to anther culture response. The experiment was carried out in 1999 in a complete randomised design with five replications. Spikes were removed from a plant just before heading, when microspores were at medium- to late-uninucleate stage. The collected spikes were stored at 4°C for 8 days before anther excision. In each replication 210-250 anthers of each genotype were plated on the same day. The anthers were cultured on a medium consisting of C17 basal salts [11] supplemented with 90 g/l maltose, 6g/l agarose, 2 mg/l 2,4-D (dichlorophenoxyacetic acid) and 0.5 g/l kinetin. The anthers were incubated at 28°C in the dark. After 4-7 weeks, the embryos were transferred to medium 190-2 [12] with 30 g/l sucrose, 0.5 mg/l NAA (α -naphthaleneacetic acid), 0.5 mg/l kinetin and 100 mg/l glutamine. During the first week, the regeneration process was performed at 22°C in the dark, and in the following weeks at 23°C under permanent illumination.

During the experiment, the number of anthers producing embryos and the number of green and albino plants were counted. The following traits were subjected to statistical calculations: the embryo induction rate (androgenetic embryos per 100 anthers plated), green plant regeneration rate (in % of embryos), plant yield (the number of albino and green plantlets per 100 anthers plated), and green plant yield (the number of green plantlets per 100 anthers

plated). Before statistical analyses, the data were transformed by $\arcsin \sqrt{x}$ to normalise the distribution. One-way multivariate analysis of variance was performed, and the differences between genotypes were tested using Newmann-Keuls's test. Univariate and multivariate analyses were performed, and the effects of general (GCA) and specific (SCA) combining abilities were calculated and tested for each trait individually and for all the traits treated simultaneously [6, 9, 10].

RESULTS

The mean values for traits concerning the anther-culture response of lines, testers and their F1 hybrids are presented in Tab. 1. Marked differences between genotypes were found for all the studied traits. A wide range of variation among genotypes was observed in the embryo induction rate: from 2.5% (cv. Prado) to 107.3% (Ticino x Pronto). On average, hybrids produced about 50 embryos per 100 anthers, and parental genotypes only about 35. Out of 12 hybrid progenies, only one hybrid (Aubrac x Prado) produced less than 20 embryos per 100 anthers, while the other hybrids produced more than 30. Among line x tester combinations the highest embryo induction rate was found for hybrids with Ticino as a common parent (78.78%), and the lowest for hybrids with Aubrac (26.38%). Among the studied hybrids, one (Ticino x Pronto) exhibited a heterosis effect in embryogenesis – the embryo induction rate for this hybrid amounted to 107.3% and was significantly higher than that for the best parent.

The mean value of the green plant regeneration rate for hybrids amounted to 12.75% and was similar to that for parental genotypes (11.62%), but variation among parents was found to be wider than among hybrids; the number of green plantlets regenerated from 100 embryos ranged from 1.1 to 54.5 in a set of parental cultivars, and from 3.5 to 26.4 in the hybrid progenies. The lowest green plant regeneration rates were observed for cultivars Ticino (1.1%), Trinidad (1.7%) and Lamberto (4.1%) and for hybrids with these cultivars as the parents (3.5-5.9%). It was noted that the progenies of cultivars exhibiting the highest green plant regeneration rate (Pronto 46.5 % and Prado 54.5%) gave the percentage of green plantlets on the level of mid-parent value or lower (Tab. 1).

Tab. 1. Embryo production rate, green plant regeneration rate and plant yields in the androgenesis of triticale line x tester progenies.

Genotype	Number of anthers plated	Embryo induction rate (per 100 anthers)	Green plant regeneration rate (per 100 embryos)	Plant yield (green and albino plantlets per 100 anthers)	Green plant yield (per 100 anthers)
Ampiac x	1075	50.5 bc	5.9 bx	15.0 bcd	3.1 cd
Ampiac x Pronto	1200	35.7 bc	10.5 ab	13.0 cdx	3.3 cd
Ampiac x Prado	1125	39.8 bc	10.8 ab	12.0 cdx	4.4 cd
Aubrac x Lamberto	1195	30.1 bc	4.0 bx	12.0 cdx	0.9 cd
Aubrac x Pronto	1230	32.0 bc	24.2 ab	21.7 bcx	9.4 cx
Aubrac x Prado	1165	15.3 cd	23.1 ab	6.9 cdx	3.1 cd
Trinidad x	1205	76.2 ab	3.5 bx	17.1 bcd	2.2 cd
Trinidad x Pronto	1125	44.0 bc	13.4 ab	18.7 bcd	6.5 cd
Trinidad x Prado	1230	49.7 bc	3.5 bx	11.2 cdx	2.0 cd
Ticino x Lamberto	1130	70.1 ab	4.2 bx	15.7 bcd	2.6 cd
Ticino x Pronto	1150	107.3 ax	26.4 ab	59.6 axx	27.9 ax
Ticino x Prado	1185	60.6 ab	14.3 ab	22.2 bcx	8.2 cx
Ampiac	1125	18.0 cd	27.1 ab	9.1 cdx	4.5 cd
Aubrac	1150	36.5 bc	10.6 ab	12.7 cdx	3.7 cd
Trinidad	1200	71.4 ab	1.7 bx	17.4 bcd	1.2 dx
Ticino	1140	55.5 bc	1.1 bx	12.9 cdx	0.3 dx
Lamberto	1205	16.1 cd	4.1 bx	6.8 cdx	1.4 dx
Pronto	1105	45.4 bc	46.5 ax	37.6 bxx	17.7 bx
Prado	1150	2.5 dx	54.5 ax	1.6 dxx	1.5 dx

The means for genotypes labelled with different letters (a, b, c, d) are significantly different, at least at P = 0.05.

Green and albino plant yield in the parental genotypes ranged from 1.6 (Prado) to 17.4 (Trinidad) per 100 anthers. A broader variation and higher values for this trait were observed in the hybrids – from 6.9 in Aubrac x Prado to 59.6 in Ticino x Pronto. In all the crosses and parents (with the exception of cv. Prado), the number of albino plants regenerated was higher than that of green plants, especially in the crosses with Lamberto as a common parent.

A wide range of variation among hybrids and parents was also observed for green plant yield: from 0.3 to 27.9 plantlets per 100 anthers plated. Hybrids, compared to parental genotypes, had a higher green plant yield (mean value for hybrids 6.19%, and 4.02% for parents). Among the line x tester progenies, two hybrids (Ticino x Pronto and Ticino x Prado) appeared to have significantly higher green plant yields than the better parent (Tab. 1).

Tab. 2. Analysis of variance for androgenetic traits in triticale.

Source of variation	Degrees of freedom	Mean square			Green plant yield	F-statistic value for all traits jointly
		Embryo induction rate	Green plant regeneration rate	Plant yield		
GCA lines	3	7761.03**	349.74**	279.60**	306.93**	7.76**
GCA testers	2	1407.81**	1033.30**	1370.20**	499.74**	8.54**
SCA lines x testers	6	1285.89**	147.51**	602.95**	195.70**	3.35**

* P<0.05, ** P<0.01

The multivariate analysis of variance was used to test the general and specific combining ability effects (Tab. 2). The GCA variances of lines and testers and SCA variance were significant for each trait individually and for all the traits treated jointly. Multidimensional GCA effects were significant for six out of seven parental genotypes (lines and testers) (Tab. 3). It was noted that in the case of cv. Ampiac GCA effects were significant for green and albino plant yield (at the level of P=0.05), but its multivariate effect was not important.

In terms of the embryo induction rate, only one cultivar (Ticino) was found to have a positive and significant GCA effect. Among the studied lines and testers, Aubrac and Pronto had a positive and significant GCA for green plant regeneration rate. In the case of cv. Aubrac, a negative, significant effect was noted for embryogenesis, and a positive effect for green plant regeneration rate. Two parental genotypes, Ticino and Pronto, were characterised by significantly high GCA for green plant yield (Tab. 3).

Tab. 3. The effects of general combining ability for triticale lines and testers

Genotype	Estimate of GCA				F-statistic value for all traits jointly
	Embryo induction rate	Green plant regeneration rate	Plant yield	Green plant yield	
<i>Lines:</i>					
Ampiac	-8.99*	-2.94***	-5.45*	-2.51**	1.72**
Aubrac	-25.16**	5.11**	-5.22*	-1.67**	11.88**
Trinidad	5.77	-5.13**	-3.10*	-2.57**	3.64*
Ticino	28.38**	2.97**	13.76**	6.75**	14.89**
<i>Testers:</i>					
Lamberto	5.83	-7.63**	-3.79**	-3.93**	8.73**
Pronto	3.79	6.64**	9.49**	5.63**	13.98**
Prado	-9.62*	0.99**	-5.71**	-1.70**	3.78**

* P<0.05, ** P<0.01

Tab. 4. The effects of specific combining ability for pairs of parental triticale genotypes

Pair of genotypes	Estimate of SCA				F-statistic value for all traits jointly
	Embryo induction rate	Green plant regeneration rate	Plant yield	Green plant yield	
Ampiac/Lamberto	2.72	4.46	5.47	3.43	1.76
Ampiac/Pronto	-10.11	-5.19	-9.81	-5.91**	4.83*
Ampiac/Prado	7.40	0.73	4.34	2.48	0.96
Aubrac/Lamberto	-1.45	-5.60*	2.27	0.39	1.88
Aubrac/Pronto	2.39	0.47	-1.30	-0.73	0.45
Aubrac/Prado	-0.94	5.14	-0.97	0.34	0.70
Trinidad/Lamberto	13.79	4.28	5.26	2.52	1.37
Trinidad/Pronto	-16.48	-0.08	-6.47	-2.73	1.76
Trinidad/Prado	2.68	-4.20	1.21	0.20	0.57
Ticino/Lamberto	-15.07	-3.13	-	-6.34**	7.15**
Ticino/Pronto	24.20*	4.79	-	9.37**	13.50**
Ticino/Prado	-9.13	-1.67	-4.58	-3.02	1.28

* P<0.05, ** P<0.01

The estimates of the SCA effects are presented in Tab. 4. Multivariate SCA effects appeared to be significant only in three crosses (Ampiac x Pronto, Ticino x Lamberto and Ticino x Pronto). Among these cross combinations, only Ticino x Pronto had a positive (and significant in two cases) SCA for all the individual

traits. In Ampiac x Pronto and Ticino x Lamberto, all the SCA effects appeared to be negative.

DISCUSSION

The experiment with line x tester progenies showed significant differences between genotypes for all the studied traits connected with androgenesis. This result clearly demonstrated that the main steps of androgenesis in triticale, i.e. embryo induction rate and green plant regeneration rate, as well as the effectiveness of androgenesis, measured as green plant yield per 100 anthers, are under genetic control. An important influence of genetic factors on androgenetic process in triticale, as in other cereal crops, has been frequently reported in the literature [1, 2, 13-17]. Charmet and Bernard [1] demonstrated that embryo production and green plant regeneration are independently controlled. Our results have confirmed this finding; in cv. Prado, for example, we observed the lowest number of embryos per 100 anthers (2.5) but the highest number of green plantlets regenerated per 100 embryos (54.5). The reverse situation occurred in the case of cvs. Trinidad and Ticino, for which the embryo induction rates were high (71.4% and 55.5%), and green plant regeneration rates were the lowest among the studied genotypes (1.7 and 1.1). Additionally, no cultivar was observed to have simultaneously a significantly positive GCA for embryo induction rate and green plant regeneration rate.

In this study, generally, hybrids were characterised by a higher effectiveness of androgenetic processes than their parental genotypes. That was especially visible in the higher number of embryos produced per 100 anthers and in the green plant yield. For these two traits, the effects of heterosis were also noted. In the case of green plants regenerated from 100 embryos, hybrids were only somewhat better than parents and no hybrid regenerated significantly more green plantlets than its parents did. The superiority of hybrids over breeding lines in embryogenesis and green plant regeneration was also observed by Charmet and Bernard [1] and Ślusarkiewicz-Jarzina *et al.* [16], which suggests the predominance of heterozygous over homozygous loci effects.

Statistical analysis of the line x tester experiment showed significant GCA and SCA variances both for all the traits treated simultaneously and for each trait individually. Higher values of GCA variance than SCA for all the studied traits indicate the predominance of contribution of additive over non-additive gene effects to observed variability in the androgenetic process. Charmet and Bernard [1] also revealed the predominance of GCA over SCA for embryogenesis, green plant regeneration rate and plant yield, but the results reported by Ślusarkiewicz-Jarzina *et al.* [16] showed a similar contribution of GCA and SCA. Traits connected with androgenesis are quantitatively inherited and their expression is different in various environments. The lack of concordance between our results and the results reported in the literature may be due to the different sets of genotypes under study and to the environmental conditions, both *in vivo* and *in*

vitro, under which those experiments were conducted. It is known that the effects of genes responsible for metrical traits exhibit interaction with environments (e.g. [18]). Similarly, estimates of GCA and SCA effects may also be influenced by environmental conditions [6, 19].

The results of this study showed that multidimensional analysis of GCA and SCA could help in the evaluation of genotypes with respect to their androgenetic ability, all the more because the particular steps of androgenesis are interrelated. It is especially promising in a situation where GCA or SCA effects for individual traits are insignificant or "nearly significant".

Among the studied parental genotypes, cv. Ticino appeared to be the best component because of its positive and high GCA both for embryogenesis and green plant yield. On the other hand, cvs. Aubrac and Prado may be distinguished as components with a negative GCA for embryo induction rate, and Trinidad and Lamberto for green plant regeneration rate. Anther culture responsiveness in crossing programmes could be genetically improved by using those lines which have a high GCA for the two main steps of the androgenetic process, i.e. for embryogenesis and green plant regeneration rate. Although in our experiment, no parental genotype with significantly positive GCA for these two traits was found, cvs. Ticino and Pronto seem to be promising because of their highly significant multivariate effects and positive GCA effects for all the studied traits. Additionally, those effects were significant in the case of three out of four traits.

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