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Diallel analysis of androgenetic plant production in hexaploid Triticale (*X. triticosecale*, Wittmack)

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Summary. Studies were made on the genetic determination of androgenetic plant yield and its two components: embryo production and green plant regeneration. This involved the analysis of a complete 7×7 diallel cross of 4 androgenetic lines and 3 lines obtained by pedigree selection, one of them having the *Triticum timopheevi* cytoplasm. The three traits analysed are both heritable and environmentally influenced (by season and culture medium composition). The analysis of embryo production shows a mainly nuclear inheritance, with predominantly additive gene action, but also a favourable effect of *Triticum timopheevi* cytoplasm. Green plant regeneration has a more complex genetic determination, with additive as well as non-additive gene action and cytoplasmic influences. Hybrids appear superior to inbred lines for embryogenesis and green plant yield, but not for green plant regeneration. Androgenetic lines used as parents did not show superiority over other parents either in their own value or in the transmission of androgenetic abilities. Genetic improvement seems to be possible by recombination in progenies of hybrids between lines having complementary abilities.

Key words: Hexaploid Triticale – Anther culture – Androgenetic responsiveness – Diallel analysis – Genetic control

Introduction

The production of homozygous lines by haplo-diploidisation could be very helpful for plant breeders, for instance to improve the efficiency of breeding programmes using recurrent selection (Griffing 1975; Gallais 1978) or to save time in the creation of commercial lines in self pollinated species.

In cereal crops haploids are now intensively used in barley breeding (Kasha 1980), and are mainly produced by interspecific hybridization with *Hordeum bulbosum*, followed by chromosome elimination (Lange 1971). Although this *"bulbosum"* technique could be used on wheat (Barclay 1975), it is successful only with genotypes having the recessive alleles *kr 1* and *kr 2* (Falk and Kasha 1981), and has not proved to be efficient in other species.

In contrast, in vitro androgenesis via anther or pollen culture is now successful in more than one hundred species (Maheshwari 1980) and is used in breeding programmes of tobacco, potato (Wenzel and Uhrig 1981) rape (Hoffman et al. 1982) wheat (De Buyser and Henry 1981) and barley (Foroughi-Wehr and Friedt 1984). This method could have another advantage if positive gametophetic selection occurs during anther culture (Friedt and Foroughi-Wehr 1983). Unfortunately, in many other species, especially in the *Graminacae* (forage grasses or cereal crops with the exception of wheat and barley), androgenesis is not yet of practical value in breeding because of its relatively low yield of haploid plants. Investigations have been carried out in two directions to improve the yield of this method.

The first is the study of physiological and environmental factors involved in the yield of haploid plants and its two components: embryo production and green plant regeneration. Thus, progress has been made, for instance, by using cold pretreatment of anthers (Picard and De Buyser 1975); by adding potato extract (Research group 301 1976), glutathione (Wenzel et al. 1977) and amino acids (Sozinov et al. 1981) into the culture medium; by substituting liquid medium (Henry and De Buyser 1981) or Ficoll® medium (Kao 1981) to the solid agar medium; by controlling temperature and photoperiodism during the embryogenetic (Ouyang et al. 1983) or during the regeneration process (Bernard 1980).

The second is genetic improvement, requiring a better understanding of the genotypic factors involved at each step of the androgenetic process and the study of their heritability. Many authors have related genotypic influences on in vitro culture success of somatic callus (Buiatti 1974; Nesticky et al. 1983). Wenzel et al. (1977) in rye, Bullock et al. (1982) in wheat, Foroughi-Wehr et al. (1982) in spring barley and Foroughi-Wehr et Friedt (1984) in winter barley have reported variations among lines or hybrids in the abilities for both embryo production and plant regeneration. These two com-

ponents could be transmitted from a line into F_1 hybrids and seem to be inherited independantly. They are controlled mainly by nuclear genes, but some maternal influences have also been found in barley reciprocal F_1's (Foroughi-Wehr et al. 1982). Picard et al. (1978), using isogenic lines, have shown a favourable effect of *Triticum timopheevi* cytoplasm in wheat, and Picard and De Buyser (1977) have also indicated an increased ability for embryogenesis in doubled haploid lines obtained from a preceeding cycle of anther culture.

In hexaploid triticale, we have previously pointed out an important heterotic effect on embryogenesis (Bernard 1977).

The aim of this work is to provide further information about the genetic control involved in androgenetic abilities and their inheritance in order to predict the possibility of increasing the yield of haploid production via anther culture by genetic improvement in hexaploid triticale.

Materials and methods

All crosses of a complete diallel set, including reciprocals, were made in 1982, using the seven following lines of hexaploid triticale (*X. Triticosecale* Wittmack, 2n=6x=42, A, B, R genomes):

- AN 1, AN 2, AN 3 and AN 5 are androgenetic doubled haploid lines previously obtained by S. Bernard from F_1 hybrids, and have been sampled for their diversified values of agronomic traits (Table 1). AN 1 and AN 3 come from the same F_1.
- GE 6 is an F_7 line selected in the progeny from a cross between an octoploid (2n=8x=56, A, B, D, R genomes) and a hexaploid triticale chosen for its high embryogenic capacity.
- GE 7 is an F_6 line resulting from an interspecific hexaploid triticale × bread wheat cross followed by gamma irradiation.

These 6 lines were obtained in our Institute and possess a *Triticum aestivum* cytoplasm.

- GE 8 has the nuclear background of an American line 'Fas Gro' transferred to the *Triticum timopheevi* cytoplasm by five successive backcrosses and was provided by Dr. Y. Cauderon from the plant breeding institute of Versailles.

GE 6 is known to have a high yield of embryo production in anther culture, but a low grain yield, and GE 7 to have a good grain yield, but to be nonresponsive in anther culture (Table 1).

The 49 combinations, including the selfed parental lines, were tested in a factorial design in 1983, with two treatments and three replications per treatment. Anther donor plants, after a 7 week vernalization period at 5 °C, were grown in the greenhouse:

- from December 15, 1982 to end February 1983 for the first replication, with an artificial light supply of 40 W/m², an average temperature of 18±3 °C and a 16 h-day/8 h night photoperiod.
- from March 15 to end May 1983 for the second and third replications, without a light supply and a temperature limited to a maximum of 30 °C by a cooling system.

Forty-nine plants per replication (one from each genotype) were disposed randomly in the greenhouse with wide spacing. Only the first four appearing spikes were removed just before heading (this phenologic stage is well correlated with the medium uninucleate stage of microspores) and these were stored at 3 °C for 14 days as a cold pretreatment before anther excision.

The two treatments differed by the anther culture medium: the MB medium previously described (Bernard 1977) supplemented with 8 g/l agar in treatment 1 and 100 g/l Ficoll 400[1] in treatment 2. The anthers from half a spike were plated onto the agar medium while those of the other half were put onto the Ficoll medium, according to Kao (1981). For each treatment and each replication, an average of 500 anthers per genotype was used.

In vitro culture conditions and medium composition have been previously described (Bernard 1977, 1980), except that 500 mg/l glutamine were added to the regeneration medium. In these conditions, embryos appear 5 to 7 weeks after anther plating and plant regeneration can be observed about 5 weeks after the transfer of the embryos to the regeneration medium.

Statistical analyses were made for the three following traits:

- embryo production rate, expressed as the number of embryos per 100 anthers plated;
- green plant regeneration rate, as the number of green plantlets per 100 embryos transferred to the regeneration medium;
- androgenetic plant yield, as the number of green plantlets per 1,000 anthers plated.

In order to normalize the distribution, data were transformed by the arc sin $\sqrt{x}$ function before variance analysis, but original data were used for parental ability calculation.

For the embryo production rate, the variance analysis was based on a factorial design with two treatments. Only the results obtained on the Ficoll medium were then studied by a Griffing's Model I (fixed parents), Method 1 (complete diallel) analysis giving General Combining Ability (GCA), Specific Combining Ability (SCA) and Reciprocal Effects (RE) significance and values (Griffing 1956).

Because of the small number of embryos obtained on agar medium, we have regrouped the two treatments and replications 2 and 3 for the study of green plant regeneration and androgenetic plant yield. Thus, only two replications remained available for a one-way variance analysis and Griffing's analysis.

Results and discussion

Original data concerning each of the three traits studied are summarized in Tables 2–4 (mean value for each genotype in 2 or 3 replications). We have also indicated the hybrids showing a positive, significant SCA and underlined the combinations exhibiting significant differences between reciprocals.

First we can note the wide range of variation among genotypes for all three characters: from 1.1 to 109.6% for embryogenesis on Ficoll® medium, with three combinations yielding more than 50 embryos per 100 anthers and 16 out of 49 having more than 20 embryos per 100 anthers; from 0 to 23.7% for the green regeneration rate and from 0 to 54.9% for green plant yield with 19 out of the 49 combinations having more than 10 plants per 1,000 anthers cultivated.

[1] Pharmacia chemical product

Table 1. Main agronomic characteristics of the parental lines used in the diallel cross (in 1983)

Line	Code	Height (cm)	Heading date in May	Grain yield 1,000 g wt		Test wt (g/100 ml)
				(q/ha)	(g)	
A 89-30	AN 1	115	28	47.6	37.5	62.9
A 114-3	AN 2	100	30	34.5	29.5	57.9
A 89-36	AN 3	95	26	32.3	36.5	53.5
A 78-1	AN 5	120	29	71.6	46.0	66.7
86 042-1	GE 6	115	30	35.8	48.0	65.8
532 D 28	GE 7	125	26	57.4	39.0	61.9
T 764	GE 8	145	28	23.6	42.0	61.6

Table 2. Embryo production rate (embryos/100 anthers) on Ficoll medium

♀ \ ♂	AN 1	AN 2	AN 3	AN 5	GE 6	GE 7	GE 8
AN 1	2.8	6.0	28.7*	5.0	8.4	5.5	34.2*
AN 2	4.7	3.2	14.2	1.2	21.8*	4.6	2.4
AN 3	22.0*	8.0	21.4	8.2	19.2	13.1	7.4
AN 5	2.6	1.5	4.8	1.1	8.7	3.6	5.1
GE 6	11.2	24.7*	9.3	9.0	52.3	50.6*	29.7*
GE 7	3.6	5.8	21.9	4.2	44.9*	3.1	9.8
GE 8	29.1*	14.0	24.2	20.9	109.6*	23.5	2.4

* Positive specific combining ability significant at the 5% level (greater than 4.10)
_ Reciprocal effects significant at the 5% level (greater than 4.72)
Average value for hybrid lines: 12.3
Average value for F_1 hybrids: 16.4

Table 3. Green plant regeneration rate (% of embryos)

♀ \ ♂	AN 1	AN 2	AN 3	AN 5	GE 6	GE 7	GE 8
AN 1	5.70	8.35	3.60*	5.80	3.00	8.10	8.75
AN 2	16.60	24.55	12.45	0.00	9.35*	4.00	0.00
AN 3	23.70*	12.50	7.75	19.20*	0.75	14.75	11.15
AN 5	8.55	0.00	17.80*	0.00	2.90	0.00	4.75
GE 6	4.60	8.80*	1.00	3.00	0.55	2.05	0.95
GE 7	0.00	7.55	2.85	0.00	2.90	0.00	3.75
GE 8	8.10	13.35	4.15	9.45	4.40	5.45	21.55

* Positivie SCA significant at the 5% level (> 2.32)
_ Significant reciprocal effects (> 2.66)
Mean value of inbred lines: 8.58
Mean value of F_1 hybrids: 6.63

Table 4. Androgenetic plant yield (per 1,000 anthers)

♀ \ ♂	AN 1	AN 2	AN 3	AN 5	GE 6	GE 7	GE 8
AN 1	1.80	6.70	11.50*	3.00	2.70	4.50	30.45*
AN 2	10.55	7.65	18.55	0.00	20.60*	2.45	0.00
AN 3	53.55*	10.35	16.50	16.30	1.50	20.40	8.60
AN 5	2.25	0.00	9.45	0.00	3.20	0.00	2.15
GE 6	6.10	23.75*	1.20	4.10	2.80	11.35	7.60*
GE 7	0.00	5.85	6.85	0.00	14.05	0.00	4.20
GE 8	24.00*	21.35	10.65	19.85	54.90*	14.00	5.65

* Positive significant SCA (> 8.98)
_ Significant reciprocal differences (> 8.03)
Mean value of inbred lines: 4.91
Mean value of hybrids: 11.15

In addition, we have calculated the mean heterotic effect on the three traits: +33% for embryogenesis (mean value of parents 12.3 compared to the mean value of hybrids 16.4), according to our previous results, and +127% for green plants yield (mean value of hybrids 11.15 compared to 4.91 for parents) but it is negative for green plant regeneration (6.63 for hybrids instead of 8.58 for parents).

Regarding the green plant yield, the superiority of hybrids can be mainly explained by the multiplication of complementary parental yield components (for example hybrid GE 6×AN 2). However, true heterosis is also evident, especially for embryogenesis: some hybrids (such as AN 1×GE 8 or GE 8×GE 6) have a much higher embryo production rate than the best parent, whereas in barley the responsiveness of hybrids is closely related to that of the most productive parent (Foroughi-Wehr and Friedt 1984).

Figure 1 illustrates the relationships between the two yield components: embryo production and green plant regeneration. There is no linear correlation ($r = -0.14$ N.S.) but all the projections except one (AN 1× AN 3) are on the left of the line between AN 2 (higher regeneration rate) and GE 6 (line having the higher embryo reproduction rate). Thus, these two components of androgenetic yield seem to vary in inverse relation, but not very closely. This agrees with the hypothesis of

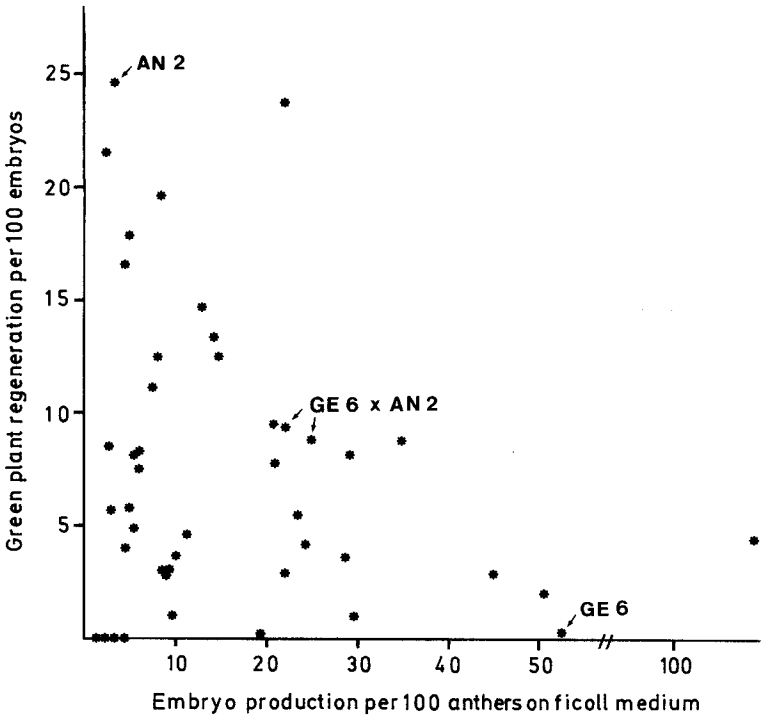

Fig. 1. Relationship between embryo formation and green plant regeneration for the 49 combinations of the diallel cross

two different systems of quantitative genes for the determination of androgenetic yield components (Wenzel et al. 1977). Moreover, it means that both must be taken into account for the genetic improvement of androgenetic plant yield, and not only the embryogenetic ability.

For each of the three characters, all the factors tested through variance analysis appear to be significant: genotype, replication (covering a season effect) and, for embryogenesis, treatment (culture medium effect) and genotype × treatment interaction (Table 5).

We particularly point out the considerable increase in embryo production by using Ficoll medium: the mean rate for all genotypes reaches 14.36 embryos per 100 anthers on Ficoll instead of the 5.79 obtained on solid agar medium – an increase by a factor of 2.5. Thus, Ficoll seems to be as efficient in Triticale as in barley (Kao 1981). Moreover, Ficoll medium allows embryo production from all the 49 combinations while only 38 are responsive on solid agar medium.

Another surprising fact is the season effect found on embryogenesis (16.6% in February compared to 12.8% in May) on both total (albino + green) regeneration and on green/albino ratio, resulting in a higher green regeneration rate (7.45% in February instead of 3.75% in May) and green plant yield (8.95 in February and 3.28 in May) (Table 6).

This is not in agreement with our previous results (Bernard 1977) reporting an increasing success in late spring, but the better supply of artificial lighting may account for this phenomenon.

Although such environmental factors are involved in androgenetic success, genotypic factors are also significant: the broad sense heritability (h^2) reaches 0.66 for embryogenesis on Ficoll medium, 0.54 for green plant regeneration and 0.27 for green plant production.

Results of Griffing's variance analysis are summarized in Table 7. The three components of the variance, GCA, SCA and RE are highly significant in each case, indicating that complex gene actions are involved in the determination of androgenetic plant yield

Table 5. Analysis of variance (mean squares) factorial design (embryogenesis) or block design

Source of variation	Embryogenesis	Green regeneration	Plant yield
Total	138.8	78.8	12.7
Replication	213.0**	95.8*	49.8**
Treatment	5,372.1**	–	–
Genotype	581.1**	143.3**	22.1**
T × G Interaction	111.1**	–	–
Error	8.5	14.5	2.7
Heritability (h^2)	0.66[a]	0.54	0.27
Coef of variation	17.4%	30.2%	35.7%

*, ** Significant at the 5% and 1% level, respectively
[a] Calcul of h^2 on Ficoll medium only
Data submitted to Arcsin $\sqrt{x}$ transformation

Table 6. Environmental influences on androgenetic plant yield and yield components

Season	February 83		May – June 83	
Culture medium	Agar	Ficoll	Agar	Ficoll
No of cult. anthers	13,015	13,015	19,080	19,020
No of embryos	970	2,156	885	2,443
%	7.45%	16.56%	4.65%	12.83%
Interval of confidence at 5%	(7.00–7.90)	(16.92–17.20)	(4.35–4.95)	(12.46–13.22)
No of embryos	3,126		3,328	
No of albino plantlets	465		427	
No of green plantlets	233		125	
% total regeneration	22.3%		16.5%	
Interval of confidence at 5%	(20.8–23.8)		(15.2–17.8)	
Green regeneration	7.45%		3.75%	
Interval of confidence at 5%	(6.53–8.39)		(3.10–4.40)	
Green/albino + green	33%		23%	
	(29.5–36.5)		(14.9–31.1)	
Green plant yield	8.95%		3.28%	

Table 7. Griffing's variance analysis (mean squares)

Source of variation	D.F.	Embryogenesis on Ficoll medium	Green regeneration	Plant yield
GCA	6	680.7**	204.1**	25.4**
SCA	21	140.7**	69.3**	10.1**
Reciprocal	21	72.7**	36.7**	7.9**
Error	94 or 46	4.1*	7.2	1.3
GCA/SCA		4.8**	3*	2.5

* Significant at the 5% level; ** Significant at the 1% level

Table 8. GCA and maternal (MAT) effect values (original data)

Parent	Embryogenesis on Ficoll		Green regeneration		Green plant yield	
	GCA	MAT	GCA	MAT	GCA	MAT
AN 1	– 3.89*	1.05	0.99*	–1.71	–1.09	–2.69
AN 2	– 7.53*	–0.80	3.24*	–0.58	–0.59	–1.13
AN 3	0.21	–1.80	3.05*	2.87*	4.16*	3.75*
AN 5	–10.28*	–1.57	–1.81*	–0.25	–5.96*	–1.87
GE 6	16.48*	–5.58	–3.71*	–0.21	0.93	–3.06
GE 7	– 1.68*	–0.76	–3.24*	–1.24	–4.29*	–1.55
GE 8	6.70*	9.46*	1.4*	1.11	4.67*	6.55*

* Significant at the 5% level

and their yield components. The value of G.C.A./S.C.A. mean square ratio is significant for green regeneration rate (greater than $F^6_{21} = 2.51$ at the 5% level) and highly significant for embryo production (greater than $F^6_{21} = 3.81$ at the 1% level), but it is not significant for androgenetic plant yield. This means a predominance of additive gene action in the genetic control of androgenetic yield components, especially for embryogenesis, in agreement with results of in vitro callus growth of maize (Nesticky et al. 1983). However, non-additive gene action and cytoplasmic influences or nucleo-cytoplasmic interaction are also involved, as both S.C.A. and reciprocal effects are significant.

The mean parental values of GCA and maternal effect for the three characters are shown in Table 8.

For embryo production, two parents have a positive, significant G.C.A. and come from classical breeding. There does not appear to be any favourable and inheritable effect of androgenetic doubled haploid lines on androgenesis. Only one line, GE 8, shows a significant maternal effect, confirming the favourable effect of *Triticum timopheevi* cytoplasm already pointed out by Picard (1978). If data concerning GE 8 line are excluded from the calculations, the reciprocal effect become non significant.

Four out of the seven parental lines have a positive G.C.A. value for green plant regeneration, the higher being those of the doubled haploids AN 2 and AN 3 – the less vigorous lines (Table 1). A relation might exist between reduced vigour and ability to green shoot regeneration, but further studies are necessary to clearly establish this fact. Two lines show a significant maternal value: GE 8 on *Timopheevi* cytoplasm, but also AN 3 on *T. aestivum* cytoplasm. For the plant production yield character, we again found GE 8 and AN 3 lines to show both the higher GCA value and maternal effect.

Five hybrid combinations and their reciprocals exhibit a positive, significant SCA for embryogenesis, three for green regeneration and four for androgenetic plant yield, indicating that dominance and gene interaction are involved, as well as additive gene action, in the genetic control of androgenetic abilities (indicated

in Tables 2–4). Significant differences between reciprocals in embryo production rate are principally found in the line and column involving the GE 8 line (underlined in Table 2) these confirm the role of *Triticum timopheevi* cytoplasm. In the same way, reciprocal differences for androgenetic plant yield are only found in data involving the AN 3 and GE 8 lines, having a positive, maternal effect. Thus, it appears that maternal effects are more important than specific reciprocal effects, leading to the conclusion that cytoplasmic influences are involved in the abilities for embryo production (*timopheevi* cytoplasm) and green plant regeneration (AN 3 cytoplasm).

Conclusion

Our results clearly demonstrate that genetic factors are involved at both steps of the androgenetic process; they are heritable and seem to be, at least partially, independently controlled. The first of them, embryo production, is the most reliable and appears to be controlled mainly by additive gene action. The favourable effect of *Triticum timopheevi* cytoplasm has been demonstrated in hexaploid Triticale using reciprocal F_1's. However, we did not find any particular behaviour of the androgenetic doubled haploid lines in the transmission of their embryogenic abilities: neither their GCA nor their maternal effect values are systematically higher than those of conventional inbred lines, as was noted by Picard and De Buyser (1977).

Green plant regeneration rate is a complex character involving the ability to regenerate into plantlet and the green/albino shoots ratio: these components are environmentally controlled (both are decreasing from February to May, Table 2) and also inheritable, although the green/albino ratio explains the greatest part of the variation. For practical reasons, we have considered the unit "green plant regeneration" as a whole: it seems to have a more complex genetic control system, involving both additive and non-additive gene action, as well as cytoplasmic influences.

The GCA additive part and the possible cytoplasmic maternal effect part of inheritable abilities differ from one line to another for each of the two yield components, with a cumulative effect on the global yield. In addition, the fact that both yield components are not closely related allows the conclusion that independant systems of polygenic control are involved, in agreement with Wenzel et al. (1977).

Thus, it may be possible to genetically improve anther culture responsiveness by using lines having high GCA values, and especially by cumulating the abilities for embryogenesis and for green plant regeneration. We are searching for such recombinant lines in, for example, the progeny of the F_1 between GE 6 (highest GCA value for embryogenesis) and AN 2 (highest GCA value for green regeneration).

We have pointed out in Fig. 1 the projections of GE 6, AN 2 and their reciprocal F_1's. These are in an intermediate position and we hope to observe transgression to higher androgenetic yield in the lines extracted from these F_1's. In this case, genetic improvement of both androgenetic abilities and high agronomic performance could take place in a population breeding scheme using AN 2, AN 3 and GE 8 as a source of genetic variability for androgenetic success and some selected lines as a source of variability for interesting agronomic traits.

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