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C. H. Balatero · N. L. Darvey · D. J. Luckett

Genetic analysis of anther-culture response in 6x triticale

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Abstract A quantitative genetic analysis was conducted to determine the inheritance of androgenetic response in hexaploid triticale. One highly-responsive genotype (Do 1 triticale) and three low-responding advanced CIMMYT lines (Rhino, Juanillo 97 and Ira Drira) were used as parents to produce a complete set of reciprocal F_1, F_2 and backcross generations. Estimates for genetic effects were determined using a generation-mean analysis following the method of Mather and Jinks. Both embryo induction and plant regeneration potential fitted well with the simple three-parameter additive-dominance (AD) model indicating the absence of any significant epistatic effects. Highly significant additive effects were detected for embryo induction, suggesting that breeding and selection can be effective in improving the induction response of triticale. The high $[d]/[h]$ ratio indicates dominance of the alleles causing high embryo induction. The production of regenerant plants from embryos appeared to be a more complex trait because of its high sensitivity to environmental factors.

Key words Tissue culture · Biometrical genetics Additive-dominance model

Introduction

The rapid development of homozygous lines from segregating populations is greatly facilitated by the anther-culture technique used to produce doubled haploids. The efficiency of anther-culture response is determined by both genetic and non-genetic (environmental) factors and their interactions. In triticale, most anther-culture experiments have focused on environmental factors, such as those determining the optimum culture conditions for induction and regeneration (Sozinov et al. 1981; Chien and Kao 1983; Wang and Hu 1984; Lukjanjuk and Ignatova 1986; Schumann 1990).

The genetic component of anther-culture response has received less attention despite being one of the most important factors limiting the widescale application of anther-culture technology in breeding programmes. The response of anthers *in vitro* is significantly influenced by the genotype and this has restricted the general applicability of the system.

Genetic analyses of anther-culture response have been carried out in other cereals such as wheat (Bullock et al. 1982; Lazar et al. 1984; Deaton et al. 1987; Agache et al. 1989; Becraft and Taylor 1992), barley (Foroughi-Wehr et al. 1982), rye (Wenzel et al. 1977), rice (Miah et al. 1985), and maize (Petolino and Thompson 1987). Embryo or callus induction and plant regeneration were found to be quantitatively and independently inherited (Foroughi-Wehr et al. 1982; Lazar et al. 1984).

In triticale, genetic studies of anther-culture response are limited. So far, only one extensive study has been made using the method of diallel analysis (Charmet and Bernard 1984). The present study was therefore conducted in order to further elucidate the genetics of anther-culture response in triticale through the analysis of different generation means derived from crosses between a highly-responding line and three low-responding genotypes.

Materials and methods

Experimental materials

Three advanced CIMMYT spring triticale lines (Rhi=Rhino, Jua=Juanillo 97, and Ira=Ira Drira) were reciprocally crossed to "Do 1 tri-

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C. H. Balatero (✉)[1] · N. L. Darvey
Plant Breeding Institute, The University of Sydney, Cobbitty Road, Cobbitty, NSW 2570, Australia

D. J. Luckett
NSW Agriculture, Agricultural Research Institute, Wagga Wagga, NSW 2650, Australia

Present address:
[1] Institute of Plant Breeding, University of the Philippines at Los Baños, Laguna 4031, The Philippines

ticale" from July to October 1990. The "Do 1 triticale" was synthesized from the cross "Do 1 (4x wheat)×H (2x rye)" and was provided by Dr. Lukaszewski of the University of California, Riverside, USA. The three F_1s (excluding reciprocals) were backcrossed to each of the original parents from January to May 1991 to produce two sets of backcross populations (B_1 and B_2). F_2 populations were also harvested from each of the three selfed F_1s. The complete set of: parents, F_1, reciprocal F_1, F_2 and backcross generations (B_1 and B_2) were used as donor plants for anther-culture. The Do 1 triticale was chosen as a high-responding parent while the three CIMMYT lines were chosen as low-responding parents.

The number of plants grown from each generation was: 15 plants from each of the four parental lines and the six reciprocal F_1s, 30 from each of the six backcross generations, and 45 from each of the three F_2 populations. The number of plants were equally divided over three sowing dates in order to spread the work. Plants were grown in 254-mm pots (five plants per pot) and completely randomized within each sowing date.

The donor plants were grown from August to December 1991 under glasshouse conditions with mean minimum and maximum air temperatures of 16 °C and 28 °C, respectively. Nutrient solutions (hydroponic or foliar fertilizers) were applied at weekly intervals. Only spikes with microspores at the uninucleate stage were cultured. The developmental stage was initially determined through cytological examination of different microspores from sample spikes and then correlating these stages with the spike's morphological appearance. Seventy-percent ethanol was used to surface-sterilize the spikes.

One spike was cultured from each donor plant to equally represent the variability present in each of the generations. Fifty to sixty anthers were taken from each spike for culture. However, the actual number of plants tested for each generation was affected by fungal contamination which destroyed some of the cultures taken from the second and third sowings. Consequently, a non-orthogonal analysis of deviance (ANODE) was carried out using generalized linear regression methods in the GENSTAT V computer package (Genstat V Reference Manual 1987) to determine differences among the generations.

The response of anthers in vitro was measured in terms of the number of embryos formed (induction) and green plants regenerated. The following variables were analyzed: (1) the proportion of anthers responding; (2) the number of embryos developed per 100 anthers; (3) the green plants regenerated per 100 embryos, and (4) the green plants regenerated per 100 anthers. The first variable was analyzed as a binomial distribution variate (i.e., $0 \leq x \leq 1$) while variables 2, 3 and 4 were analyzed as Poisson distribution variates (i.e., $0 \leq x \leq \infty$).

Media and culture conditions

Anthers at the uninucleate stage were plated using a semi-solid MC17 medium (Luckett et al. 1991) with minor modifications. Glutamine was used at 150 mg l^{-1}; kinetin was added at 0.5 mg l^{-1}; and Sigma Type 1A agarose (2.1 g l^{-1}) was used instead of Sea Plaque or Seakem LE agarose. Fifty to sixty anthers were plated onto 35-mm diameter disposable Petri dishes containing 3 ml of medium. The anthers were then incubated in the dark at 28±1 °C to promote embryogenesis. For plant regeneration, the embryos were transferred to a solid MS medium with vitamins (Murashige and Skoog 1962) supplemented with 1 mg l^{-1} IAA and 1 mg l^{-1} BAP. The transferred embryos were kept in a separate room (24–28°C) with a 16-h photoperiod (80–100 μE s^{-1} m^{-2}) provided by white fluorescent lamps.

Model for genetic analysis

Estimates for genetic effects were determined using a generation-mean analysis following the method of Mather and Jinks (1982). The model used can be expressed as:

$$Y=[m]+k_1[d]+k_2[h]$$

where "Y" is the generation mean, "m" is the mid-parent value, "$[d]$" is the additive genetic effect, "$[h]$" is the dominance genetic effect and "k_1" and "k_2" are coefficients specific for each generation.

In the simple three-parameter AD model, non-allelic interactions (epistasis), linkage, and genotype×environment interactions are assumed to have no significant effects. The validity of such assumptions is tested using individual (Mather 1949) and joint (Cavalli 1952) scaling tests. The scaling tests also determine the adequacy of the simple AD model within the limits of accuracy set by the sampling errors with which the various estimates of the generation means were obtained.

An attempt to use transformed values did not improve the precision of the estimates of the various parameters, hence the actual raw data were used throughout this analysis.

The joint scaling test was also used to estimate the parameters $[m]$, $[d]$, and $[h]$ using the weighted least-squares method. If the joint scaling test or one of the individual scaling tests showed a significant deviation from zero, then the AD model was extended to include estimates of non-allelic interactions (additive×additive, additive×dominance, and dominance×dominance). In this case, estimates of six genetic effects could be obtained using the perfect fit formulae of Jinks and Jones (1958).

Where applicable, an estimate of narrow-sense heritability was obtained following the method described by Warner (1952) as:

$$h^2_{ns} = [2V_{F2} - (V_{B1}+V_{B2})]/V_{F2},$$

where h^2_{ns} = narrow-sense heritability, and V_{F2}, V_{B1} and V_{B2}=variances of the F_2, B_1 and B_2 generations.

The inheritance of green-plant regeneration was determined from the proportion of green plants regenerated per 100 transferred embryos (instead of green plants regenerated per 100 anthers) since embryo induction and plant regeneration were found to be independently-inherited traits. The number of green plants regenerated per 100 anthers, however, was also taken since this represented the overall green-plant regeneration efficiences of the different generations.

Results

Embryo induction

Induction response in this study was expressed both in terms of the proportion of anthers responding and the mean number of embryos produced per 100 anthers. ANODE showed highly significant differences ($P< 0.001$) among the generations tested for both parameters. The performance of the different generations in terms of embryo induction is summarized in Table 1. The better parent (Do 1 triticale) resulted in 63.3% responding anthers compared to less than 30% responding anthers in the three CIMMYT lines. A similar trend was also observed in terms of the number of embryos produced per 100 plated anthers. The performance of the reciprocal F_1s, F_2 and backcross generations was generally close to or better than Do 1 triticale, indicating dominance and even over-dominance of the alleles causing high embryo induction.

The t-test showed no significant differences between the F_1s and their reciprocals in all three crosses in terms of the proportion of anthers responding, indicating the absence of maternal influence. The same trend was observed for the number of embryo per 100 anthers, except that in Jua×Do 1, the reciprocal cross gave considerably higher embryo than the F_1. The t-test, however, did not show any significant difference at the 5% level of probability.

Table 1 Mean (and standard error of mean) for embryo induction of parents, reciprocal F_1s, F_2, and backcross generations (B_1 and B_2) from three triticale crosses. Rhi, Rhino; Jua, Juanillo 97; Ira, Ira Drira; Do 1, Do 1 triticale

Cross ($P_1 \times P_2$)	Generation[a]						
	P_1	P_2	P_1	$F_1(R)$	B_1	B_2	F_2
Anthers responding (%)							
Rhi×Do 1	13.8 (3.2)	63.3 (8.3)	59.1 (2.8)	47.1 (6.5)	37.4 (3.7)	54.9 (8.5)	47.1 (5.6)
Jua×Do 1	25.0 (9.4)	63.3 (8.3)	61.2 (4.7)	71.3 (7.3)	36.6 (7.4)	60.7 (6.1)	55.4 (4.9)
Ira×Do 1	11.2 (6.3)	63.3 (8.3)	48.2 (6.2)	57.1 (6.2)	32.8 (7.0)	70.6 (5.1)	55.1 (5.2)
Embryos per 100 anthers							
Rhi×Do 1	14.5 (3.7)	112.2 (18.9)	90.1 (9.5)	81.5 (16.8)	48.8 (5.0)	88.9 (18.5)	79.1 (12.0)
Jua×Do 1	34.0 (16.3)	112.2 (18.9)	84.4 (15.4)	129.2 (18.8)	60.5 (15.2)	98.8 (10.8)	85.9 (9.5)
Ira×Do 1	18.0 (10.8)	112.2 (18.9)	70.0 (14.1)	82.4 (14.1)	35.5 (9.5)	105.6 (10.2)	81.4 (11.5)

[a] P_1=low-responding parent (female)
P_2=high-responding parent (male)
$F_1=P_1 \times P_2$
$F_1(R)=P_2 \times P_1$
F_2=selfed $(P_1 \times P_2)F_1$
$B_1=P_1 \times (P_1 \times P_2)F_1$
$B_2=P_2 \times (P_1 \times P_2)F_1$

Table 2 Results of individual and joint scaling tests and estimates of genetic effects for the proportion of anthers responding from three triticale crosses

Test	Cross		
	Rhi×Do 1	Jua×Do 1	Ira×Do 1
Scaling tests			
A	1.97± 8.58	−13.09±18.20	6.35±16.68
B	−12.65±19.15	−3.0 ±15.49	29.65±14.45
C	−7.00±24.93	10.81±24.96	49.73±26.52
Joint[a]	$\chi^2_{[3]}=0.54$	$\chi^2_{[3]}=1.09$	$\chi^2_{[3]}=5.61$
Genetic effects[b]			
m	37.27±3.85***	43.68±5.00***	43.47±4.45***
[*d*]	−23.19±3.91***	−20.27±5.24***	−30.79±4.37***
[*h*]	21.69±5.02***	17.29±7.65*	12.29±8.05[ns]
Heritability	0.45	+	+

[a] Critical value for χ^2 (3 *df*) at P=0.05=7.81
[b] Genetic effects: *m*, mid-parent value; [*d*], additive genetic effects; [*h*], dominance genetic effects
***=$P<0.001$; *=$0.05>P>0.01$; [ns]=$P>0.05$
[+] Heritability values could not be calculated

The results of both individual and joint scaling tests showed that the simple three-parameter additive-dominance (AD) model was adequate within the limits of the sampling error (Tables 2 and 3). The results of the individual scaling tests agreed very well with the results of the joint scaling test for both variables. Estimates of the different genetic effects showed the preponderance of highly significant additive genetic effects ([*d*]) over dominance genetic effects ([*h*]) (Tables 2 and 3). The relatively high [*h*]/[*d*] ratio, particularly in the cross "Rhi×Do 1", showed partial dominance of the alleles causing high embryo induction present in Do 1 triticale.

Narrow-sense heritability estimates ranged from 0.27 (Rhi×Do 1) to 0.58 (Ira×Do 1). No reliable heritability estimate was obtained from Jua×Do 1. The estimate obtained in this cross was outside the theoretical expectation, probably as a consequence of the reduction in the number of sample plants from the backcross generations due to fungal contamination.

Green plant regeneration

ANODE showed highly significant differences ($P<0.001$) for the number of green plants regenerated per 100 embryos among the different generations (Table 4).

Among the four parental lines used, Juanillo 97 gave the highest proportion of green plants per 100 anthers

Table 3 Results of individual and joint scaling tests and estimates of genetic effects for embryo production from three triticale crosses

Test	Cross		
	RhixDo 1	JuaxDo 1	IraxDo 1
Scaling tests			
A	−7.07 ± 14.30	2.60 ± 37.84	−17.12 ± 26.05
B	−24.52 ± 42.65	0.92 ± 32.59	28.82 ± 31.15
C	9.51 ± 54.93	28.58 ± 54.81	55.17 ± 58.21
Joint[a]	$\chi^2_{[3]}$=0.67	$\chi^2_{[3]}$=0.34	$\chi^2_{[3]}$=2.49
Genetic effects[b]			
m	62.11 ± 8.28***	75.14 ± 10.54***	71.16 ± 9.06***
[*d*]	−47.96 ± 8.22***	−38.66 ± 10.19***	−56.47 ± 8.43***
[*h*]	24.93 ± 11.80***	12.53 ± 19.51[ns]	1.79 ± 16.92[ns]
Heritability	0.27	+	0.58

[a] Critical value for χ^2 (3 *df*) at *P*=0.05=7.81
[b] Genetic effects: *m*, mid-parent value; [*d*], additive genetic effects; [*h*], dominance genetic effects
***=*P*< 0.001; [ns]=*P*>0.05
[+] Heritability value could not be calculated

Table 4 Mean (and standard error of mean) for green plant regeneration of parents, reciprocal F_1s, F_2 and backcross populations (B_1 and B_2) from three triticale crosses

Cross (P_1×P_2)	Generation						
	P_1	P_2	F_1	F_1(R)	B_1	B_2	F_2
Green plants/100 embryos							
RhixDo 1	2.59 (2.02)	1.13 (0.52)	8.80 (4.18)	15.64 (5.24)	7.47 (2.50)	3.82 (2.62)	4.65 (3.12)
JuaxDo 1	13.19 (6.96)	1.13 (0.52)	8.37 (3.22)	3.97 (1.77)	19.05 (9.66)	1.36 (0.97)	5.17 (1.47)
IraxDo 1	0.62 (0.62)	1.13 (0.52)	3.54 (1.56)	8.08 (4.83)	4.04 (2.67)	3.42 (1.80)	4.75 (2.07)
Green plants/100 anthers							
RhixDo 1	0.42 (0.28)	1.29 (0.69)	7.36 (3.19)	5.46 (1.64)	3.65 (1.23)	1.11 (0.69)	2.86 (1.71)
JuaxDo 1	2.07 (1.28)	1.29 (0.69)	5.00 (1.64)	3.41 (1.34)	6.45 (2.04)	0.63 (0.30)	3.93 (1.07)
IraxDo 1	0.50 (0.50)	1.29 (0.69)	2.14 (0.82)	3.83 (1.94)	1.01 (0.53)	3.44 (1.59)	2.24 (0.87)

Table 5 Results of individual and joint scaling tests and estimates of genetic effects for green-plant regeneration per 100 embryos from three triticale crosses

Test	Cross		
	RhixDo 1	JuaxDo 1	IraxDo 1
Scaling tests			
A	3.55 ± 6.83	16.53 ± 20.66	3.91 ± 5.59
B	−2.31 ± 6.72	−6.79 ± 2.97	2.18 ± 3.96
C	−2.75 ± 15.17	−10.39 ± 10.13	10.17 ± 8.88
Joint[a]	$\chi^2_{[3]}$=0.69	$\chi^2_{[3]}$=6.54	$\chi^2_{[3]}$=1.76
Genetic effects[b]			
m	2.06 ± 0.99*	6.89 ± 2.35**	0.95 ± 0.40*
[*d*]	0.95 ± 0.99[ns]	5.98 ± 2.34*	−0.24 ± 0.40[ns]
[*h*]	6.75 ± 3.00*	−2.37 ± 3.14*	3.61 ± 1.41*
Heritability	0.96	+	0.76

[a] Critical value for χ^2 (3 *df*) at *P*=0.05=7.81
[b] Genetic effects: *m*, mid-parent value; [*d*], additive genetic effects; [*h*], dominance genetic effects
**=0.01>*P*>0.001; *=0.05>*P*>0.01; [ns]=*P*>0.05
[+] Heritability value could not be calculated

(2.07) and green plants regenerated per 100 embryos (13.19). The two other CIMMYT lines produced only 0.42 (Rhino) and 0.50 (Ira Drira) green plants per 100 anthers while Do 1 triticale produced 1.29 green plants per 100 anthers. Green-plant regeneration efficiencies of the F_1 and subsequent generations (F_2 and backcrosses) clearly indicated overdominance effects for this character since the mean green-plant regeneration efficiency exceeded that of the parents.

There were no significant differences observed between the F_1s and their reciprocals in terms of green-plant regeneration for all three crosses as revealed by t-tests. This indicated the absence of maternal effects.

The individual and joint scaling tests showed no significant deviation from zero within the limits of the sampling error (Table 5), hence the simple AD model was adequate. Estimates of genetic parameters varied among the three crosses (Table 5). Significant additive and dominance genetic effects ($0.05>P>0.01$) were obtained from Jua×Do 1 while only the dominance genetic effect was significant ($0.05>P>0.01$) in Rhi×Do 1 and Ira×Do 1. Plant regeneration seems to be highly influenced by environmental factors making it difficult to obtain precise estimates of the genetic components of this trait.

Discussion

The genetic analysis of quantitatively inherited characters provides plant breeders with useful information for determining the applicability of various selection and breeding procedures. Estimates of the genetic components of variation will also lead to a better understanding of the mode of inheritance involved.

In cereals, embryo induction and plant regeneration from anthers shows continuous variation and the two traits were found to be independently inherited (Foroughi-Wehr et al. 1982; Lazar et al. 1984). Earlier work on the genetic analysis of anther-culture response showed the predominance of additive genetic effects for embryo production (Charmet and Bernard 1984; Lazar et al. 1984; Miah et al. 1985; Deaton et al. 1987).

In the present study, highly significant additive genetic effects were also greater than dominance genetic effects. These results indicated that embryo induction is a highly heritable character. The alleles causing high induction (present in Do 1 triticale) are dominant in their effects relative to alleles present in the three low-responding CIMMYT lines. These results, however, contradict the findings of Miah et al. (1985) who observed that "callus induction ability" in rice anther-culture was inherited as a recessive character.

The inheritance of embryo-production ability fits the simple three-parameter AD model suggesting that there were no significant effects due to non-allelic interactions and linkage. When the model was extended to include estimates of epistatic effects (six-parameter model), using the method of Jinks and Jones (1958), no significant epistatic effects were obtained confirming the adequacy of the three-parameter AD model. This indicated that the inheritance of embryo induction is relatively simple and does not involve significant digenic interactions.

Estimates of the genetic effects for green regenerants per 100 embryos did not yield highly significant additive and dominance effects in some crosses. This may be attributed to the compounding effects of the environment on plant regeneration, an observation also made by Charmet and Bernard (1984). However, the simple three-paramater AD model was adequate in the three crosses suggesting the absence of any significant non-allelic interactions. This implies that the inheritance of plant-regeneration ability in the present material may be simple and that alleles for high green-plant regeneration can be successfully transferred to lines with low-green plant regeneration capacity. Provided the non-genetic components of variation can be kept at a minimum, heritability of the trait can be improved and selection will be more effective.

The correlation between the level of embryo production and plant regeneration has been reported to be very low (Foroughi-Wehr et al. 1982; Deaton et al. 1987). Likewise, in the present study, no correlation was found between embryo induction and green-plant regeneration ($r=-0.108$; $P=0.660$; $n=19$). This clearly indicates that high embryo production does not necessarily result in high green-plant regeneration. This observation is relevant since the final measure of efficiency in anther-culture is the proportion of green plants that can be recovered per 100 anthers cultured.

The simple inheritance of embryo induction and green-plant regeneration, and the significance of both additive and dominance effects (particularly for embryo production), have two important implications for the usefulness of anther-culture in triticale breeding. First, the results suggest that high embryo induction and green-plant regeneration can be easily transferred and fixed in non-responsive or low-responsive lines through breeding and selection. Second, the anther-culture of F_1, F_2 and backcross populations, requires only that one parent be responsive to the culture system in order to obtain a reasonable level of response, since both characters show partial or complete dominance.

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References

Agache S, Bachelier B, de Buyser J, Henry Y, Snape, J (1989) Genetic analysis of anther-culture response in wheat using aneuploid, chromosome substitution and translocation lines. Theor Appl Genet 77:7–11

Becraft PW, Taylor GA (1992) Genetic variation for anther callus inducibility in crosses of highly culturable winter wheats. Plant Breed 108:19–25

Bullock WP, Baenziger PS, Schaeffer GW, Petolino PJ (1982) Anther-culture of wheat (Triticum aestivum L) F_1's and their reciprocal crosses. Theor Appl Genet 62:155–159

Cavalli LL (1952) An analysis of linkage in quantitative inheritance. In: Reeve ECR, Waddington CH (eds) Quantitative inheritance. HMSO, London, pp 135–144

Charmet G, Bernard S (1984) Diallel analysis of androgenetic plant production in hexaploid triticale (× *Triticosecale* Wittmack). Theor Appl Genet 69:55–61

Chien YC, Kao, KN (1983) Effects of osmolality, cytokinin and organic acids on pollen callus formation in triticale anthers. Can J Bot 61:639–641

Deaton WR, Metz SG, Armstrong TA, Mascia PN (1987) Genetic analysis of the anther-culture response of three spring wheat crosses. Theor Appl Genet 24:334–338

Foroughi-Wehr B, Friedt W, Wenzel G (1982) On the genetic improvement of androgenetic haploid formation in *Hordeum vulgare* L. Theor Appl Genet 62:233–239

Genstat V Reference Manual (1987) Oxford University Press, UK

Jinks JL, Jones RM (1958) Estimation of the components of heterosis. Genetics 42:223–234

Lazar MD, Baenziger PS, Schaeffer GW (1984) Combining abilities and heritability of callus formation and plantlet regeneration in wheat (*Triticum aestivum* L.) anther-cultures. Theor Appl Genet 68:131–134

Luckett DJ, Venkatanagappa S, Darvey NL, Smithard RA (1991) Anther-culture of Australian wheat germplasm using modified C17 medium and membrane rafts. Aust J Plant Physiol 18:352–367

Lukjanjuk SF, Ignatova SA (1986) Triticale: production of haploid and homozygous plants. In: Bajaj YPS (ed) Biotechonology in agriculture and forestry. Vol 2, Crops. Springer, Berlin Heidelberg New York Tokyo, pp 530–543

Mather K (1949) Biometrical Genetics 1st edn. Methuen, London

Mather K, Jinks JL (1982) Biometrical genetics: the study of continuous variation. Chapman and Hall, London New York

Miah AAA, Earle ED, Khush GS (1985) Inheritance of callus formation ability in anther-culture of rice, *Oryza sativa* L. Theor Appl Genet 70:113–116

Murashige T, Skoog S (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15: 473–497

Petolino JF, Thompson SA (1987) Genetic analysis of anther-culture response in maize. Theor Appl Genet 74:284–286

Schumann G (1990) *In-vitro* production of haploids in triticale. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry. Vol 13, Wheat. Springer,Berlin Heidelberg New Yorck Tokyo pp 382–402

Sozinov A, Lukjanjuk S, Ignatova S (1981) Anther cultivation and induction of haploid plants in triticale. Z. Pflanzenzucht 86: 272–285

Wang X, Hu H (1984) The effects of potato II medium for triticale anther-culture. Plant Sci Lett 36:237–239

Warner JN (1952) A method for estimating heritability. Agron J 44: 427–430

Wenzel GF, Hoffmann F, Thomas E (1977) Increased induction and chromosome doubling of androgenetic haploid rye. Theor Appl Genet 51:81–86