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Inheritance of anther culture derived green plantlet regeneration in wheat (*Triticum aestivum* L.)

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Abstract

A study was set up to determine the inheritance and combining ability of the factors anther culture response and green plant regeneration. Reciprocal crosses were made between cultivar 'Ringo Sztar', showing high anther culture response and the cultivars 'Ciano 067' and 'Benoist H77022', showing a high level of green plant regeneration. Averaged over all genotypes, 23.0% of the anthers responded and a callus induction frequency of 77.8% was observed. Of all the embryos, 43.0% developed into plantlets, 25.6% of the regenerants being green, the result being that 3.3 green plants per 100 anthers were formed. Genotypic effects accounted for 57.7%, 86.3% and 77.5% of the total variance of anther culture response, callus induction frequency and embryo induction frequency, respectively. Additive and dominant gene action was detected for all characteristics, including green plant regeneration. No reciprocal differences were found for anther culture response, embryo induction frequency and green plant regeneration, indicating no cytoplasmic effects. A small but significant reciprocal difference was found for callus induction frequency. Embryo production was primarily correlated with anther culture response and not with the number of embryos produced per plated anther or per responding anther. Possible mechanisms for the inheritance of green plant regeneration are discussed.

Abbreviations: CIRA – callus induction frequency per responding anther; ERA – embryo induction frequency per responding anther; FHB – fusarium head blight; MS-medium – Murashige & Skoog (1962) medium; REML – residual maximum likelihood

Introduction

Gramineous species have shown to be rather recalcitrant crops with regard to *in vitro* androgenesis techniques such as anther- and microspore culture. In general, many genotypes of most of the species respond poorly. In particular green plant regeneration is low, either because regeneration of albino plantlets occurs or no regeneration at all. The occurrence of high numbers of albino plants has been frequently reported. In wheat 97% albino plants for cultivar 'Edwall' were found by Zhou & Konzak (1989) and 88% averaged over four German spring wheat cultivars by Ziegler *et al.* (1990). High percentages of non-regenerating embryos (excluding albino's) were also reported in wheat, e.g. 90% by De Buyser & Henry (1979) and 80% by De Buyser *et al.* (1989). However, some wheat genotypes showed high levels of expression for certain other androgenic traits, e.g. anther culture response, callus and embryo induction frequencies, green plant regeneration and may therefore be used in crossing programmes to try to improve androgenesis in general in agronomical important cultivars. Wheat cultivars that are thought to express androgenic traits at high levels are e.g. 'Ciano 067', 'Pavon 076' and 'Dirkwin', which showed callus induction frequencies of 115, 334 and 479 calli per 100 anthers and a green plant yield of 70, 72 and 357 green plants per 100 anthers, respectively (Ouyang *et al.* 1983; Zhou *et al.* 1991; Orshinsky & Sadasivaiah 1994). Cultivar 'Gernard 81' produced up

to 1002 embryos per 100 anthers (Otani & Shimada 1993).

Optimization of anther culture conditions (i.e. growth conditions of the donor plants, culture medium and pretreatments of the anthers), has improved the efficiency of anther culture through the years. Foroughi-Wehr & Friedt (1984) reported in the barley genotype 'Igri' 2.4 green plants per 100 anthers. Cistué *et al.* (1994) reported ten years later in the same genotype up to 1800 green plants per 100 anthers. However, previous studies have indicated that androgenic traits are also under strong genetic control. Heritabilities were estimated in various studies. Lazar *et al.* (1984b) found in wheat narrow sense heritabilities for callus production frequency and regeneration frequency of 0.6–0.7, and Ekiz & Konzak (1994a) estimated in wheat narrow sense heritabilities of 0.68, 0.54 and 0.43 for callus induction, green plant percentage and green plant yield, respectively. A relatively high heritability indicates that introgression of that androgenic trait will show rapid progress. However, significant environmental variances (Lazar *et al.* 1984a) and relatively high error variances (Deaton *et al.* 1987) may slow down the introgression process.

Nuclear, as well as cytoplasmic or maternal effects are known to influence anther culture efficiency. In addition, interactions between several traits were found (Lazar *et al.* 1984b; Andersen *et al.* 1987).

Extensive deletions in the plastid genome are primarily suggested to be the cause for microspore derived albino plants in wheat and barley (Day & Ellis 1984, 1985).

This study was carried out to investigate the inheritance of green plant regeneration in relation to anther culture response in wheat. A wheat genotype with a high anther culture response, but low green plant regeneration was reciprocally crossed with two wheat genotypes with relatively low anther culture response, but high green plant regeneration. Genotypes that combine high values of both traits are presumed to be suitable genotypes for *in vitro* selection experiments (Bruins *et al.* 1993). For that reason, one of the genotypes in this study ('Ringo Sztar') was chosen for its high Fusarium head blight (FHB) resistance level in the field. *In vitro* selection for FHB resistance with highly responsive and regenerative genotypes at the haploid level would be an efficient way of producing homozygous FHB resistant genotypes.

Materials and methods

Three wheat cultivars ('Ringo Sztar', 'Ciano 067' and 'Benoist H77022'), known for their extreme response in anther culture and green plant regeneration were used in this study. 'Ringo Sztar' had previously shown to have a high anther culture response (Bruins *et al.* 1993). 18–20 Responding anthers and 60–70 calli per 100 anthers were found in several experiments. However, plant regeneration percentages were extremely low: 0.3 green plants and 1.1 albino plants per 100 embryos. For comparison: the means for plant regeneration, averaged over 23 genotypes from the same experiment, including 'Ringo Sztar', amounted to 3.4 green plants per 100 embryos and 33 green plants per 100 regenerated plants. Tuvesson *et al.* (1989) found in their experiments that the parents 'Ciano 067' and 'Benoist H77022' gave 19 and 24 embryos per 100 anthers, and had relatively high regeneration frequencies of 53 and 32 green plants per 100 regenerated plants, respectively.

Seeds of these three parent cultivars were sown and vernalized for eight weeks. Plants were grown in the greenhouse with a 14 h photoperiod and a temperature regime of 15 °C (light) and 10 °C (dark). Reciprocal crosses were made between cultivar 'Ringo Sztar' and the other two parents. Seeds of the four F_1-combinations were sown together with the parental cultivars and vernalized for eight weeks. Plants were grown in the greenhouse with a 14 h photoperiod for five weeks and a temperature regime of 15 °C (light) and 10 °C (dark). After that, plants were transferred to the field. At least ten F_1-plants per combination were tested. The first three heads of each plant were chosen for anther culture. Anther culture was carried out according to Bruins *et al.* (1993), in short: excision of the anthers at the mid-uninucleate stage, tillers were surface sterilized spraying with 70% ethanol, the anthers were plated on P2 medium and culture conditions were 28 °C in the dark. After six weeks of culture, the following factors were assessed: the number of responding anthers per spike that produced at least one embryogenic or non-embryogenic structure (watery callus) and the number of embryogenic structures and non-embryogenic structures per spike. The following calculations were made: number of responding anthers/number of plated anthers × 100% (= anther culture response), total number of embryogenic and non-embryogenic structures/number of plated anthers × 100% (= callus induction frequency), number of embryogenic structures/number of plated anthers ×

Table 1. Anther culture response of three wheat parents and four F_1-crosses*.

Genotype	Number of plated anthers	% Responding anthers	% Callus induction frequency	% Embryo induction frequency	Callus induction/ responding anther	Embryo induction/ responding anther
'Ciano 067'	2270	4.2a	19.9a	14.4a	3.25a	2.33a
'Ringo Sztar' × 'Ciano 067'	2598	18.4b	69.0c	45.7b	3.48a	2.29a
'Ciano 067' × 'Ringo Sztar'	3666	11.8b	37.8ab	22.7ab	2.89a	1.75a
'Ringo Sztar'	1726	32.3c	117.7d	72.9c	3.61a	2.24a
'Benoist H77022' × 'Ringo Sztar'	1959	36.0c	118.7d	72.6c	3.67a	2.21a
'Ringo Sztar' × 'Benoist H77022'	4014	33.1c	121.6d	74.3c	3.58a	2.18a
'Benoist H77022'	2211	13.8b	61.9bc	44.6b	2.88a	2.03a
Mean		23.0	77.8	48.9	3.38	2.13

*Treatment means not followed by the same letter are significantly different at the 0.05 level of probability as determined by REML Variance Component Analysis after square root transformation.

Table 2. Plant regeneration from anther derived embryos of three wheat parents and four F_1-crosses.

Genotype	Number of plated embryos	Green shoots (%)	Albino shoots (%)	Roots only (%)	Green plants per responding anther	Green plants per 100 anthers
'Ciano 067'	290	39.7	26.9	7.9	0.74	5.1
'Ringo Sztar' × 'Ciano 067'	759	20.4	32.5	8.3	0.30	6.0
'Ciano 067' × 'Ringo Sztar'	661	25.0	28.3	7.4	0.36	4.5
'Ringo Sztar'	601	2.8	36.9	7.5	0.03	1.0
'Benoist H77022' × 'Ringo Sztar'	809	1.2	34.9	8.5	0.01	0.5
'Ringo Sztar' × 'Benoist H77022'	1687	1.6	39.3	6.7	0.02	0.7
'Benoist H77022'	683	17.3	11.3	10.7	0.27	5.3
Mean		11.0	32.0	7.9	0.14	3.3

100% (= embryo induction frequency), total number of embryogenic and non-embryogenic structures per responding anther (CIRA), number of embryogenic structures per responding anther (ERA).

Embryos larger than 1 mm were transferred for regeneration to MS medium (Murashige & Skoog 1962), supplemented with 3% sucrose, 1 mg l^{-1} silver nitrate, 160 mg l^{-1} glutamine and 0.5 mg l^{-1} thiamine. After two to three weeks, regeneration of the embryos was assessed. Plated embryos were subdivided into four classes: green shoots, albino shoots, only roots or no regeneration. All data were transformed by taking the square root to improve the normality of the distribution. The transformed data were analyzed on the basis of predicted means from Residual Maximum Likelihood (REML) Variance Component Analysis (Genstat 5 Committee 1993).

Results

In vitro androgenic development could be induced in all parents and F_1-combinations (Table 1). Highly significant differences for anther culture response were found among entries. 'Ringo Sztar', which was chosen because of its good anther culture response, proved to have significantly the highest anther culture response of all three parents with 32.3% responding anthers. 'Ciano 067' had the lowest androgenic response (4.2%) and 'Benoist H77022' showed an intermediate response (13.8%). The F_1-combinations

Table 3. Correlation matrix of different traits. Presented are the values of r. $^{*}p < 0.05$, $^{**}p < 0.01$.

	Responding anthers	Calli/ responding anther	Embryos/ responding anther	Non-embryos	% Green shoots	% Albino shoots	%Roots only
Callus induction frequency	0.95**						
Embryo induction frequency	0.94**						
Calli per responding anther	0.17						
Embryos per responding anther	0.08	0.89**					
% Non-embryos	0.22	0.68	0.27				
% Green shoots	−0.98**	0.07	0.41	−0.49			
% Albino shoots	0.55	0.58	0.04	0.99**	−0.49		
% Roots only	−0.16	−0.57	−0.08	−0.91**	0.14	−0.86*	
% No regeneration	0.75	−0.47	−0.51	−0.08	−0.49	−0.10	0.39

'Ringo Sztar' × 'Ciano 067' and 'Ciano 067' × 'Ringo Sztar' showed intermediate anther culture responses (18.4% and 11.8%, respectively) between the two parents, indicating additive inheritance. The anther culture response values for the reciprocal were not significantly different from each other, implying no cytoplasmic effects. Anther culture response values of the F_1-combinations 'Benoist H77022' × 'Ringo Sztar' and 'Ringo Sztar' × 'Benoist H77022' were not significantly different from parent 'Ringo Sztar', indicating dominant inheritance, and were not significantly different from each other. Ranking for callus induction frequency and embryo induction frequency was similar to anther culture response (Table 1). For green plant regeneration, 'Ciano 067' showed the highest (39.7%), 'Benoist H77022' an intermediate (17.3%) and 'Ringo Sztar' the lowest frequency (2.8%) (Table 2), a ranking identical to previous results and literature (Tuvesson et al. 1989). The reciprocal F_1-combinations 'Ringo Sztar' × 'Ciano 067' and 'Ciano 067' × 'Ringo Sztar' were not different from each other. These combinations also showed intermediate green plant regeneration (20.4% and 25.0%, respectively) between the two parents, indicating additive inheritance. The reciprocal F_1-combinations 'Benoist H77022' × 'Ringo Sztar' and 'Ringo Sztar' × 'Benoist H77022' showed a comparable low green plant regeneration (1.2% and 1.6%, respectively) as parent 'Ringo Sztar'. The absence of reciprocal differences for anther culture response, embryo induction frequency and green plant regeneration indicates no role for cytoplasmic factors for these three traits. For callus induction frequency a low but significant reciprocal difference shows that 'Ringo Sztar' as a female parent favours callus induction more than as male parent. No large differences between parents and/or F_1-combinations for the percentage of embryos that regenerated only roots was found, and it varied from 6.7% to 10.7%.

Averaged over all genotypes, of the 18,444 anthers, 23.0% had responded with embryos or non-embryogenic structures. On these responding anthers 9028 embryos and 5322 non-embryogenic structures were found, most anthers producing more than one structure. The 5490 embryos that were larger than 1 mm were transferred to regeneration medium. Eleven percent of them developed into green plants, 32.0% into albino plants and 57.0% did not regenerate or developed only roots. Overall, of the embryos regenerated into plantlets, 25.6% was green. Per 100 anthers on average 3.3 green plants were formed.

Table 3 shows correlations between the different androgenic characters. Combinations with a significant high positive correlation were: percentage responding anthers with callus- and embryo induction frequency; callus induction frequency with embryo induction frequency (0.98; not shown); callus induction per responding anther with embryo induction per responding anther; and non-embryogenic structures with albino regenerants. Combinations with a significant high negative correlation were: anther culture response with green plant regeneration; callus- and embryo induction frequency with green plant regeneration (−0.96 and −0.96; not shown); and non-embryogenic structures and albino regenerants with root regeneration. Variance analysis showed that genotypic components for anther culture response, callus induction frequency

and embryo induction frequency accounted for 57.7%, 86.3% and 77.8% of the total variance, respectively.

Discussion

Average anther culture response and callus induction frequency values in this experiment of 23.0% and 77.8%, respectively, were higher than anther culture response and callus induction frequency values reported in most other publications on anther culture of wheat: 20.3% and 41.0% (Barnabas *et al.* 1991); 7.8% and 20.0% (Abd El-Maksoud & Bedö 1993); 18.0% and 57.4% (He *et al.* 1993), for anther culture response and callus induction frequency, respectively. The regeneration frequency was 12.8 plants per 100 plated anthers, of which 25.6% was green, whereas Tuvesson *et al.* (1989) reported a percentage of 23.4 plants per 100 plated anthers of which 15.3% was green. However, Ouyang *et al.* (1983) produced 72 green plants per 100 anthers in cultivar 'Ciano 067', whereas under our conditions with the same cultivar, only 5.1 green plants per 100 anthers were produced.

The majority of the total variance of the androgenic traits could be explained by genotypic effects. In this study, additive and dominant gene action were found for anther culture response and callus- and embryo induction frequency. Previous publications indicated that androgenic traits were mainly controlled by nuclear genes, with the additive gene action being predominant (Zhou & Konzak 1992). Dominant gene action was reported in wheat by Lazar *et al.* (1984b).

The absence of reciprocal differences for several androgenic traits, found in our experiments, is in agreement with other publications on wheat and barley where no indication for reciprocal effects (Bullock *et al.* 1982; Zhou & Konzak 1992) or small reciprocal effects (Foroughi-Wehr *et al.* 1982; Lazar *et al.* 1984b) were reported. However, other reports on barley and wheat indicated significant reciprocal effects for callus induction, green plant percentage and green plant yield (Powell 1988; Ekiz & Konzak 1994a). In the study of Ekiz and Konzak (1994a) the reciprocal effects were mainly caused by two cross combinations. Sagi & Barnabás (1989), using alloplasmic lines, found significant cytoplasmic effects for anther culture response. For plant regeneration no cytoplasmic effects could be detected. Ekiz & Konzak (1991 a,b,c) found significant reciprocal differences and explained the absence of such reciprocal differences in other publications by a narrow base of cytoplasm genetic variation or by relatively low levels of anther culture response, caused by the methods used. In our study a narrow base of cytoplasm genetic variation is unlikely because of the distant relationship between the three parents. 'Ringo Sztar' is a Hungarian cultivar and 'Ciano 067' and 'Benoist H77022' are CIMMYT and French cultivars, respectively. Besides this, the levels of callus induction frequency in the present study, up to 122 calli per 100 anthers, are similar to those found by Ekiz & Konzak (1991c) for genotypes 'Chris' and 'Edwall' (123 and 133 calli per 100 anthers, respectively).

Ouyang (1986), on the other hand, stated that pollen callus induction frequency is controlled mainly by genes of the diploid anther wall tissue and not by genes of haploid pollen cells. Such maternal effects occurred when pollen lines derived from F_1-hybrids with great heterosis for pollen callus yield were used again as anther donors. The pollen callus induction frequencies were much lower than the induction frequencies of the F_1-hybrids, showing the disappearance of heterosis.

As the pollen population in our study was formed in the anthers of F_1-plants, it consisted of a segregating F_2-population, and possible mechanisms of inheritance for green plant regeneration can be speculated upon, with the assumption that there is no gametic selection. The genetic constitution of the gametes is likely to play a role, however parental effects cannot be excluded. The intermediate percentages of green plant regeneration per 100 embryos or per responding anther in the F_1-combinations between 'Ringo Sztar' and 'Ciano 067' indicate segregation and that it is unclear whether the genetic constitution of the F_2-pollen population or of the F_1-maternal tissue caused the intermediate reaction. The low percentages of green plant regeneration per 100 embryos, per responding anther or per 100 anthers in the F_1-combinations between 'Ringo Sztar' and 'Benoist H77022', comparable to parent 'Ringo Sztar', is most likely to be caused by the genetic constitution of the F_1-plant, which could be caused by a maternal effect.

The number of embryogenic structures per responding anther (ERA) was not significantly different between the genotypes and varied from 1.81 to 2.75. This is in agreement with other publications were the ERA value varied not significantly from 1.9 to 2.4 (Barnabás *et al.* 1991) or from 2.2 to 2.7 (Takács *et al.* 1994). Using four tetraploid *Triticum turgidum* genotypes on nine different media combinations, the ERA value was found to vary from 1.2 to 1.9, with

one exception of a genotype producing 2.3 embryos per responding anther (Ghaemi *et al.* 1994). This ERA value appears to be an independent character of the medium used and which might be under genetic control.

The correlation between anther culture response and callus induction frequency or embryo induction frequency was high (r = 0.95 and 0.94, respectively), similar to the results that were found by Pauk *et al.* (1991). In their report 6 parents and 10 F_2-populations were tested on two media and the correlations between anther culture response and callus induction frequency were r = 0.92 and r = 0.91 for the two media, respectively. This correlation indicates that the production of embryos and non-embryogenic structures is mainly dependent on anther culture response and not on the number of structures per plated anther. No correlation was found between anther culture response and callus induction per responding anther or embryo induction per responding anther. This, combined with the fact that the number of embryogenic structures per responding anther was not significantly different between the genotypes, suggests that embryo production is predominantly related to anther culture response and not to the number of formed embryos per responding anther. In several previous reports anther culture efficiency was subdivided into three components: 1) Callus Induction Frequency; 2) Plantlet Regeneration Frequency (= number of structures producing green or albino plantlets/number of calli × 100%; 3) Green Plantlet Yield (= number of green plants produced/number of anthers cultured × 100%) (Ouyang *et al.* 1983; Konzak & Zhou 1991). Considering the strong correlation between anther culture response and callus induction frequency, found in our set of data and in previous publications where also both parameters were assessed (Pauk *et al.* 1991; Knudsen *et al.* 1989), while no reports of no correlation are known, and the absence of significant differences for calli or embryos per responding anther, suggests that in this experiment the genetic variance for anther culture response was larger than for callus induction frequency. Therefore, another component can be formulated which can be added to the above mentioned three, or in this study even replace the first component, namely: Anther culture response (the number of anthers giving one or more structures).

This set of genotypes showed a high negative correlation between anther culture response, callus induction frequency, or embryo induction frequency with green plant regeneration. This is not so surprising, as the genotypes in the present study were selected for their extreme response in green plant regeneration; 'Ringo Sztar' giving large numbers of embryos, but mainly albino regenerants and the other two parents giving less embryos but much higher percentages green regenerants. In contrast, the majority of other publications on the genetic basis of androgenic traits in wheat reported no significant correlation coefficients between different anther culture response components and regeneration components, indicating that these two groups of components may be controlled by different genetic factors (Agache *et al.* 1988; Ekiz and Konzak 1994b).

The F_1-combinations 'Ringo Sztar' × 'Ciano 067' and 'Ciano 067' × 'Ringo Sztar' combine a relatively high anther culture response with a relatively high percentage of green plant regeneration. The combination of these two traits in one genotype might provide a suitable genotype for *in vitro* selection experiments. The inclusion of a cultivar with a relatively high level of resistance against Fusarium head blight, 'Ringo Sztar', ensures the genetic variation needed for such *in vitro* selection experiments.

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