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Genetics of *in vitro* organogenesis and precocious germination of wheat embryos

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ABSTRACT

The genetic bases of *in vitro* organogenesis and precocious germination of embryos in immature wheat embryo culture were investigated using six Brazilian genotypes and their F1, F2, BC1F1 and BC2F1 generations in a generation means analysis. Four parents and one set of F1s were also analyzed in a diallel experiment. The results indicated a complex gene action controlling both traits, with additive, dominant and epistatic effects. High broad sense heritability values were found, indicating genetic determination. Considering the complexity of gene control, genetic gain could be achieved by selecting for the traits in advanced generations of the segregating population. Low correlation values between organogenesis, precocious germination, regeneration and somatic embryogenesis (data shown in a previous report) indicated the possibility of obtaining recombinant genotypes.

INTRODUCTION

Plant regeneration from cultured cells and tissues is required for successful application of biotechnology in current plant breeding programs. Plant regeneration of many cultivars of wheat obtained from culture of immature embryos has been well documented (Maddock, 1985; Vasil and Vasil, 1986), including some Brazilian genotypes (Milach *et al.*, 1991; Handel *et al.*, 1995; Lange *et al.*, 1995).

In tissue culture, plant regeneration is preceded by other morphogenic processes, which in wheat anther culture involves callus formation followed by somatic embryo production (Wenzel *et al.*, 1977). Regeneration may occur in wheat immature embryo culture via organ differentiation or somatic embryogenesis (Ozias-Akins and Vasil, 1983; Maddock, 1985), while the same explant can be induced to follow both morphogenic pathways (Bhaskaran and Smith, 1990). Shoot-forming cultures are able to produce viable plants since subsequent rooting is obtained, but plants regenerated via somatic embryogenesis are preferred to organogenesis because embryoids usually arise from single cells (Vasil, 1988) and thus originate genetically uniform plants instead of chimeric ones.

Embryogenic sites of calli originated from immature wheat embryos can also show bud-like structures, which are precociously germinating embryoids (Vasil, 1988). Precocious germination is the development process of the somatic embryo before its complete maturation has taken place, and its occurrence varies extensively across genotypes (Bapat *et al.*, 1988). There is some controversy about the influence of precocious germination on other morphogenic processes. Although Bapat *et al.* (1988) reported a positive effect of precocious germination associated with embryoid production and plant regeneration, precocious germination is not a desirable trait, because its expression restrains the long-term morphogenic potential of the culture. Once precocious germination is initiated, somatic embryos cannot multiply to give rise to secondary embryogenic tissues.

Organogenesis and precocious germination of embryoids are not well documented, nor is their significance in tissue culture well understood. This report deals with the genetic control of both traits, *in vitro* organogenesis and precocious germination of embryoids, in immature embryo culture of six Brazilian wheat genotypes, using generation means and diallel analyses.

MATERIAL AND METHODS

Plant material

The Brazilian wheat varieties Maringá, Alondra, Palmeira, Nobre, BR 23 and CEP 14 were chosen based on their differential regeneration capabilities (Milach *et al.*, 1991). The first two and the latter two are good and poor regenerators, respectively, whereas Palmeira and Nobre have an intermediate response.

Laboratory procedures

Immature seeds were harvested 15 days after hand pollination or selfing. Seeds were disinfected in 70% ethanol for 2 min, 2.5% sodium hypochlorite for 10 min and 1% sodium hypochlorite for 10 min, and rinsed three times with sterilized distilled water. Immature embryos were cultivated in modified Murashige and Skoog (1962) medium according to Milach *et al.* (1991).

The characteristics were evaluated 42 days after inoculation. A visual score from zero to 100 was given to each callus considering the percentage of the callus surface presenting organ-like structures (organogenesis) or germinating embryoids. Each callus was considered to be a replication unit. Damaged explants and contaminated calli were disregarded.

Statistical procedure

Original data were transformed by using a $\sqrt{Y + 0.5}$ transformation (Steel and Torrie, 1980) to make means and variances independent, with the resulting

variances homogeneous. As each callus was considered to be a replication, and genotypes presented an unequal number of calli, analyses of variance for a complete randomized design were done using the general linear model. Since inoculation was conducted over several days in each experiment, covariance analysis using number of days after inoculation as the independent variable was carried out to correct organogenesis and precocious germination, which are dependent variables, for bias caused by variation in environmental conditions during the growing season of the donor plants. Covariance analyses using immature embryo length were also used to correct both traits evaluated, due to differences in embryo development rates observed among genotypes. Significance of the independent variables for correcting the dependent ones was tested by the *t*-test.

Genetic analysis

a) Diallel approach: 456 calli of genotypes Palmeira, Nobre, BR 23 and Maringá and a set of F1s were evaluated for both traits by method 2 (no reciprocals) model II (considering genotype fixed effects), as proposed by Griffing (1956).

b) Generation means: 1379 calli of the six genotypes and their F1, F2, BC1F1 and BC2F1 were used to fit the simple additive (d)-dominant (h) model in the generation means approach (Mather and Jinks, 1982). When non-allelic interactions were present, the six parameter model proposed by Hayman (1958) was used, which estimates the mean (m), additive (d) and dominant (h) effects, and those caused by their interactions, additive by additive (i), additive by dominant (j) and dominant by dominant (l). The fit of the model was tested by chi-square procedure, whereas the significance of the individual parameters was tested by *t*-test. Genetic (Vg), phenotypic (Vp) and environmental (Ve) variances and broad sense heritabilities (H) were estimated according to Allard (1960).

RESULTS

Covariate mean squares were significant, except for organogenesis. Neither organogenesis nor precocious germination was greatly affected by different inoculation dates, thus the estimated slope parameters were near zero. Organogenesis was also not greatly affect by variation in embryo length. However, precocious germination varied positively with embryo development. The estimated slope parameters showed that precocious germinations were more frequent in calli derived from large explants (Table I).

Table I - Analysis of variance and combining ability for organogenesis and precocious germination in wheat.

	MS				MS			
	d.f.	Organogenesis	Precocious germination		d.f.	Organogenesis	Precocious germination	
Genotype	35	5.58**	27.69**		9	9.00**	41.26**	
GCA	-	-	-		3	0.16**	1.01**	
SCA	-	-	-		6	0.33**	1.36**	
Inoculation date	1	24.76**	15.47**		1	3.66*	9.57*	
Embryo length	1	3.27 NS	230.22**		1	2.48**	66.70*	
Error	1341	1.39	3.67		444	1.92		
Total	1378				455			
Covariate	Estimate	*t*-test	Estimate	*t*-test	Estimate	*t*-test	Estimate	*t*-test
Inoculation date	0.02	3.92**	-0.01	0.80 NS	0.01	1.29 NS	0.01	1.47 NS
Embryo length	-0.15	1.53 NS	1.22	7.93**	0.25	1.14 NS	1.30	4.75**

**Significant at 1% probability level.
*Significant at 5% probability level.
NS = Not significant; MS = mean squares.

The genotype mean squares and general (GCA) and specific (SCA) combining abilities for diallel approach were also highly significant for organogenesis and

precocious germination. SCA mean squares were higher than those for GCA for both characters (Table I).

Combining abilities effects for organogenesis and precocious germination were all significant at the 1% probability level (Tables II and III). For organogenesis, Palmeira and Maringá presented the highest GCA values, while Nobre and BR 23 showed negative effects (Table II). All four genotypes presented positive GCA effects for precocious germination (Table III). The lowest SCA value was presented by BR 23, and the highest by the cross BR 23 x Maringá (Table III).

Table II - General (GCA) and specific (SCA) combining abilities for organogenesis in wheat.

	SCA				GCA
	Palmeira	Nobre	BR 23	Maringá	
Palmeira	-0.13**	0.18**	-0.67**	0.75**	0.19**
Nobre		0.22**	-0.29**	-0.31**	-0.02**
BR 23			0.17**	0.63**	-0.21**
Maringá				-0.53**	0.04**

**Significant at 1% probability level.

Table III - General (GCA) and specific (SCA) combining abilities for precocious germination in wheat.

	SCA				GCA
	Palmeira	Nobre	BR 23	Maringá	
Palmeira	0.55**	-0.51**	-0.29**	0.29**	0.40**
Nobre		0.18**	-1.17**	1.03**	0.04**

BR 23			-1.33**	1.77**	0.57**
Maringá				-0.22**	0.13**

**Significant at 1% probability level.

Except for the organogenesis of the cross Nobre x Alondra, interaction terms were added to the model for all crosses (Tables IV and V). Additive effects were all significant, except for precocious germination of Palmeira x Alondra. Considering dominance, only the crosses Nobre x Alondra, for organogenesis, and CEP 14 x Maringá and Palmeira x BR 23, for precocious germination, did not present significant effects (Tables IV and V). Dominance effects were generally equal or higher than the additive ones. Significant non-allelic interaction effects were detected, with highly significant additive x additive and additive x dominance interactions found in a large number of crosses for both traits (Tables IV and V).

Table IV - Genetic effects of eight crosses for *in vitro* organogenesis in wheat.

Crosses	m%	m	d	h	i	j	l	χ
Nobre x Alondra	7.76	2.96 ± 0.02**	0.30 ± 0.02**	0.03 ± 0.03 NS	-	-	-	3.51
Palmeira x Alondra	10.02	3.32 ± 0.04**	0.30 ± 0.02**	-4.78 ± 0.29**	-2.74 ± 0.19**	-	-	2.92
Palmeira x Maringá	11.49	3.39 ± 0.02**	-0.40 ± 0.01**	-1.07 ± 0.09**	-1.04 ± 0.05**	-	-	0.08
Palmeira x BR 23	7.88	2.98 ± 0.05**	-0.33 ± 0.04**	-1.13 ± 0.20**	-0.43 ± 0.20*	-0.64 ± 0.04**	-0.58 ± 0.24*	-
Palmeira x CEP 14	8.42	3.07 ± 0.03**	-0.50 ± 0.05**	0.46 ± 0.06**	-	-0.94 ± 0.05**	-	6.44
CEP 14 x	7.76	2.96 ± 0.03**	-0.48 - 0.06**	1.24 ± 0.07**	-	-0.54 ±	-	0.32

Alondra						0.07**		
BR 23 x Maringá	7.24	2.87 ± 0.04**	0.09 ± 0.02**	0.88 ± 0.20**	-0.13 ± 0.19 NS	-	1.11 ± 0.26**	2.91
CEP 14 x Maringá	10.42	3.38 ± 0.03**	0.71 ± 0.03**	0.50 ± 0.16**	0.66 ± 0.15**	0.66 ± 0.04**	-5.36 ± 0.22**	-

**Significant at 1% probability level.
*Significant at 5% probability level.
NS = not significant.
m% = mean data in % (nontransformed).

Table V - Genetic effects of eight crosses for *in vitro* precocious germination of embryos in wheat.

Crosses	m%	m	d	h	i	j	l	χ
Nobre x Alondra	22.14	4.87 ± 0.07**	-1.09 ± 0.32**	-7.59 ± 0.70**	-7.12 ± 0.69**	-1.39 ± 0.32**	9.00 ± 1.31**	-
Palmeira x Alondra	10.29	3.36 ± 0.07**	-	-3.12 ± 0.47**	-1.26 ± 0.30**	-0.43 ± 0.79**	-	2.17
Palmeira x Maringá	5.92	2.63 ± 0.07**	0.08 ± 0.02**	0.86 ± 0.28**	0.56 ± 0.20**	-	-	1.69
Palmeira x BR 23	6.56	2.75 ± 0.03**	1.16 ± 0.06**	-	-0.34 ± 0.05**	1.13 ± 0.06**	3.38 ± 0.17**	1.58
Palmeira x CEP 14	18.18	4.38 ± 0.10**	2.69 ± 0.09**	-2.32 ± 0.70**	-1.98 ± 0.44**	2.36 ± 0.09**	-	-
CEP 14 x Alondra	12.69	3.70 ± 0.05**	-1.07 ± 0.12**	2.18 ± 0.59**	1.38 ± 0.33**	-1.18 ± 0.12**	-	-
BR 23 x Maringá	15.40	4.05 ± 0.07**	2.70 ± 0.09**	2.89 ± 0.19**	0.57 ± 0.15**	2.59 ± 0.10**	-	0.01

CEP 14 x Maringá	6.56	4.10 ± 0.03**	1.23 ± 0.06**	0.25 ± 0.08**	-	0.82 ± 0.06**	-2.03 ± 0.19**	3.57

Symbols and abbreviations defined in Table IV.

Estimates of phenotypic and environmental variances were very similar for organogenesis and precocious germination in most crosses. Genetic variance estimatives and heritability in the broad sense had very distinct values among the crosses for the traits. The cross Palmeira x CEP 14 had the highest phenotypic and genetic variances and the highest heritability for both traits, whereas CEP 14 x Maringá showed the lowest values for the same parameters (Table VI).

Table VI - Phenotypic (Vp), genetic (Vg) and environmental (Ve) variances and broad sense heritabilities (H) in eight crosses for *in vitro* organogenesis and precocious germination of wheat.

Crosses	Organogenesis				Precocious germination			
	Vp	Vg	Ve	H	Vp	Vg	Ve	H
Nobre x Alondra	0.05	0.02	0.03	0.22	0.13	0.06	0.08	0.24
Palmeira x Alondra	0.05	0.03	0.02	0.56	0.14	0.08	0.06	0.59
Palmeira x Maringá	-	-	0.04	-	-	-	0.07	
Palmeira x BR 23	0.06	0.03	0.03	0.46	0.15	0.07	0.07	0.51
Palmeira x CEP 14	0.07	0.05	0.02	0.68	0.19	0.14	0.06	0.70
CEP 14 x Alondra	-	-	0.04	-	0.11	0.03	0.08	0.24
BR 23 x Maringá	0.05	0.01	0.04	0.24	0.13	0.05	0.09	0.35

CEP 14 x Maringá	0.0039	0.003	0.036	0.08	0.02	0.017	0.085	0.17

DISCUSSION

Little information is available concerning organogenesis and precocious germination of immature wheat embryo *in vitro* cultures, and their role on plant regeneration. Tissue culture responses have been shown to be under genetic control in several cereal crop species, such as maize (Beckert and Qing, 1984; Tomes and Smith, 1985; Hodges *et al.*, 1986) and wheat (Mathias *et. al.,* 1986; Ou *et al.*, 1989; Lange *et al,* 1995). Previous reports had already indicated that precocious germination is a genotype-dependant phenomenon (Bapat *et al.*, 1988), while diallel analysis on shoot and root formation capability in wheat culture shows that these traits are genetically controlled, and additive action plays a major role (Ou *et al.*, 1989). The present study indicates that both traits are under genetic determination and consequently their frequency in a population might be manipulated through breeding techniques.

Gene action parameters in both approaches were significant, and additive and non-additive actions were involved (Table I). Poor data fit to the six parameter model, although all parameters were highly significant, indicates a complex gene control with non-allelic interactions being involved (Tables IV and V). The genotypes showed more variability for precocious germination, which presented higher values than organogenesis for genotype, GCA and SCA mean squares (Table I), phenotypic, genetic and environmental variances and heritabilities (Table VI).

The two covariates, inoculation date and embryo size, were also important sources of variation. Organogenesis was largely affected by variation among the donor plants, mainly in generation means data, since inoculation date accounted for a large portion of the total variation (Table I). This behavior is in agreement with other studies, where morphogenic *in vitro* responses from the same

explants differed between well-nourished plants and nutrient-deficient ones (Bhaskaran and Smith, 1990), and between plants cultivated in the summer vs. during a cool season (Rines and McCoy, 1981; Hanzel *et al.*, 1985; Ma *et al.*, 1987). The inoculation date mean square in the diallel approach was lower, probably because of the shorter period that was necessary to carry out the experiment out (Table I).

The high variation accounted for by embryo length in both experiments shows a strong influence of the development stage of the explant on precocious germination (Table I). Short embryos should be preferred to initiate cultures with long-term morphogenetic potential. *In vitro* culture response of barley depends on size and location of the explant on the inflorescence (Bhaskaran and Smith, 1990).

Estimates of GCA indicate that additive gene action was an important component of the genetic control of these characteristics (Table I), and the additive effects and their interactions on generation means analysis give further evidence of this (Tables IV and V). Nonadditive genetic action was also important, as demonstrated by significant SCA and the significance of dominance effects, additive by dominance, and dominance by dominance interaction effects (Tables IV and V). SCA accounted for more variation than GCA for both traits (Table I), whereas Ou *et al.* (1989) found that GCA was more important than SCA for shoot-and root-forming ability. Thus, the results of both studies in a statistical sense are restricted to the varieties used, which have been chosen because of differences in the characteristics studied.

Additive and dominance effects in the generation means trial did not show any tendency to enhance or to diminish the characteristics (Tables IV and V), which indicates dispersion of favorable genes among the parents. Evidence of gene dispersion for *in vitro* morphogenic traits was also found by Afele and Kannenberg (1990) in maize and by Dunwell *et al.* (1987) in barley.

Genetic variance and heritability values varied among crosses, but higher values were found for Palmeira x CEP 14, showing great divergence between the parents. In contrast, CEP 14 and Maringá presented

the smallest divergence between genotypes for both traits.

Low correlation values between both characteristics and regeneration and somatic embryogenesis (Lange *et al.*, 1995) indicate that an efficient culture system can be obtained by breeding genotypes for this purpose, and our results support that, at least with this set of inbreds, progress can be made by selecting for low levels of organogenesis and precocious germination of embryoids. Selection should be made on advanced generations of segregating hybrid populations, F5 or beyond, to prevent the selection of superior progenies resulted from interaction, which will be lost in subsequent generations of selfing.

RESUMO

As bases genéticas da germinação precoce de embrióides de trigo e organogênese *in vitro* foram estudadas utilizando-se seis genótipos brasileiros e suas gerações F1, F2, BC1F1 e BC2F1, através da análise de média de gerações. Quatro pais e um conjunto de F1s também foram analisados em um experimento dialélico.

Os resultados indicam que ambos caracteres são determinados por uma complexa ação gênica, ocorrendo efeitos aditivos, de dominância e epistáticos. Foram obtidos valores altos para as estimativas de herdabilidade no sentido amplo, o que indica uma forte determinação genética, embora este parâmetro nada informe sobre a magnitude da ação aditiva. Considerando a complexidade do controle gênico, espera-se que maiores ganhos genéticos sejam obtidos selecionando para os caracteres em gerações avançadas de populações segregantes. Os baixos valores de correlação entre ambas características e embriogênese somática e regeneração de plantas (dados apresentados em trabalho anterior) indicam a possibilidade de obtenção de genótipos recombinantes.

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