GENETIC ANALYSIS OF SOMATIC EMBRYOGENESIS IN WINTER WHEAT (*TRITICUM AESTIVUM* L.)

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ABSTRACT

Knowledge about the genetic control of somatic embryogenesis in 6x wheat is limited and rather contradictory, making difficult the use of tissue cultures for breeding and genetic manipulation. We analysed the embryogenic ability of 70 RIL's derived from a cross between two Romanian lines contrasting for tissue culture response, using scutellar callus cultures induced from immature embryos. A segregation ratio of 1 embryogenic (E) : 9 non embryogenic (Ne) was established, suggesting an oligogenic recessive control of embryogenic ability. The parent lines displayed polymorphism for several genetic markers (*Rht 1, Rht 8, Gli 1B, Gli 1D* and *β-Amy*), therefore we analysed the association between these markers and the embryogenic ability, using a χ^2 test. Significant positive effect of the 1B/1R translocation (as identified by alleles at *Gli 1B*) (P< 0.01) was detected in agreement with other reports. A significant association between the more complex zymogram type of *β-Amy* and embryogenic ability (P< 0.025) was also detected, suggesting the mapping of some genes controlling somatic embryogenesis in wheat on chromosome arms 4 BL, 4 DL or 5AL.

Key words: somatic embryogenesis, genetic control, wheat

INTRODUCTION

The use of tissue culture as an efficient tool for winter wheat (*Triticum aestivum* L.) breeding and genetic manipulation is limited by insufficient knowledge about the genetic and molecular control of somatic embryogenesis (SE) and plant regeneration.

Understanding the genetic basis of SE will facilitate the establishment of efficient regeneration protocols and also will allow some insight into early differentiation mechanisms in higher plants.

In hexaploid wheat, the first studies concerning the genetic control of tissue culture response (TCR) have been published since about ten years; some authors described the effect of several chromosomes/chromosomal arms (Mathias and Fukui, 1986; Felsenburg et al., 1987) and even genes, on TCR (Kaleikau et al., 1989).

More precisely, Mathias and Fukui (1986) reported a stimulatory effect of chromosome 4A from Cappelle Desprez, substituted into Chinese Spring (Ch. S.) both on callus growth and differentiation; Higgins and Mathias (1987) identified 4B chromosome as having a positive effect on regeneration ability.

In a subsequent study, Kaleikau et al. (1989) described the influence of the chromosomes from the homeologous group 2, mainly 2D, in stimulation of TCR and suggested a role for *Ppd* loci in controlling this trait.

By analysing the regeneration ability in several isogenic lines, Ben Amer and Worland (1992) reported a stimulatory effect of *ppd 1* allele.

De Buyser et al. (1992) established a segregation ratio of 1:9 for the embryogenic genotype, suggesting an oligogenic recessive control of the embryogenic ability. Recently, the results of a study concerning the regeneration ability in a set of aneuploids derived from Chinese Spring have allowed the location of major genes for TCR on chromosomal arms 1AL and 3DL (Henry et al., 1994a).

However, Ben Amer and Worland (1995) reported a major effect of chromosomes 2B and 6D on somatic embryogenesis and regeneration.

The most important data concerning the genetic control of somatic embryogenesis in hexaploid wheat (summarized in Table 1) are limited and rather contradictory.

The present study was designed to evaluate the regeneration ability of 70 recombinant inbred lines (RIL's) derived from a cross between two Romanian lines contrasting for TCR; these data have been used to establish the segregation ratio for the embryogenic genotype and to evaluate possible associations between this trait and several genetic markers available in our material.

MATERIALS AND METHODS

Plant material. Seventy RIL's derived from a cross between two Romanian lines (Sincron x F 1054) contrasting for TCR were

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analysed. The parental lines and RIL's have been produced at RICIC Fundulea, Dept. of Wheat Breeding, using a method similar to single seed descent (Săulescu et al., 1994).

Table l. Genetic control of somatic embryogenesis in hexaploid wheat (*T. aestivum* L.)

Genetic stocks	Results	Author(s)
I. SUBSTITUTION LINES		
Chinese Spring (Capp Dep. 4A)	stimulation of SE	Mathias and Fukui, 1986
Chinese Spring (T. spelta 1A, 1D, 3D, 6D); Ch. Spring (Marquis 2B)	major effect on SE: 2B, 6D	Ben Amer and Worland, 1995
II. ANEUPLOIDS		
Wichita (Ne) x ND 7532(E), monosomics (Rht 8, D1, D4)	major effect of 2D on callus developm. and regen.	Kaleikau et al., 1989
Ch. Spring - 36 Dt and 7 NT	major effect of 1 AL and 3 DL	Henry et al., 1994
Disomics (4H) and addition lines for 4A, 4D (Ch. Spring)	stimulation of SE	Ganeva et al., 1995
III. RECOMBINANT LINES		
Chinese Spring/ 1 RS	mapping 2 loci stimulating SE	Langridge et al., 1991
Cappele Dep. (2D), alleles Ppd , Rht	major effect :ppd 1, minor effect: Rht 8	Ben Amer and Worland, 1992
Isogenic lines- Rht 1, Rht 2, Rht 3/Ch. Spring (4AS, 4DS)	significant effect on callus growth and regeneration	Mathias and Atkinson, 1988
IV. RECIPROCAL CROSSES		
Ch. Spring (E) x Aquila (Ne) F1, F2	oligogenic recessive control	De Buyser et al., 1992

The parental lines expressed several genetic markers (*Rht1, Rht 8, Gli 1B, Gli 1D and β-Amy*), some of them being associated to embryogenic ability; the chromosomal location of these markers is well known (Table 2).

Table 2. Chromosomal location of genetic markers in the parental lines Sincron (P_1) and F 1054 (P_2)

Marker gene	Chromosomal location	Effect	P1 allele	P2 allele
Rht 1	4 AS	Reduced height, insens. to GA3	*rht1*	*Rht 1*
Rht 8	2 D	Reduced height, sens. to GA3	*Rht 8*	*rht 8*
Gli 1B	1 BS	Gliadinic fractions	*Gli 1B3*	*Gli 1Bl*
Gli 1D	1 DS	Gliadinic fractions	*Gli 1D2*	*Gli 1D4*
β-Amy	4 BL, 4 DL, 5 AL	Isozymes	2 isozymes	3 isozymes

In a previous experiment we evaluated the embryogenic ability of the parental lines, which was absent in Sincron (Ne).

Characterizations of the genetic markers in RIL's. The genes for reduced height (*Rht1* and *Rht8*) have been characterized on the basis of plant height and reaction to exogenous GA 3 (Săulescu et al., 1994).

The gliadinic fractions were analysed by electrophoresis in starch gels, using a method published in a previous paper (Hagima et al., 1989).

Three isoenzymes for *β-Amy* were detected in F-1054 (E) and only two isoenzymes in Sincron (Ne), the parental lines being differentiated by a slow band, with a high molecular weight.

***In vitro* culture.** The embryogenic ability was evaluated in scutellar callus cultures induced from immature embryos (15-18 days after pollination). The plants were grown in the field. Tissue culture response was analysed for 2 years (1994, 1995) using a medium-term regeneration protocol (six months).

Callus development was induced on Murashige-Skoog (1962) semisolid medium, supplemented with 3.0 mg/l 2,4- dichlorophenoxyacetic acid (2,4-D), 0.8 % agar and 3.0 % sucrose. For the next subcultures, the concentration of 2,4-D was gradually decreased. Details concerning the regeneration protocol have been published in a previous paper (Cealâcu et al., 1996).

For each genotype, a total of 40-50 explants were used; the cultures were scored monthly, recording two parameters: the percent of embryogenic calli (embryogenic ability) and plant regeneration.

The statistic analysis of the data was performed using a χ^2 test.

RESULTS AND DISCUSSIONS

Analysis of the segregation ratio. Among the 70 RIL's evaluated for the embryogenic ability, we identified eight genotypes (lines) which expressed this trait. These data were analysed using χ^2 test for the case of two, three or four complementary genes (Table 3).

Table 3. Segregation ratio for embryogenic ability in 70 RIL's

Genotypes tested	Experimental data	Theoretical data		
		1:3	1:7	1:15
Embryogenic	8	17.5	8.75	4.4
Nonembryogenic	62	52.5	61.25	65.6
Total	70	70	70	70
χ^2 value		6.76**	0.06	3.04
Probability		P<1	90<P<80	5<P<10

These results suggest a segregation ratio close to 1:7 for the analysed character (embryogenic ability).

Such a segregation is specific for an oligogenic recessive control exerted by complementary genes.

Similar results were reported by De Buyser et al. (1992) in a study of the F_2 population derived from a cross between Chinese Spring (E) and Aquilla (Ne).

Analysis of the association between embryogenic ability and several genetic markers. We analysed the distribution of the embryogenic lines as affected by the alleles of the genetic markers. According to the data summarized in figure 1, the percentage of embryogenic lines for different groups were between 8.3 and 20.

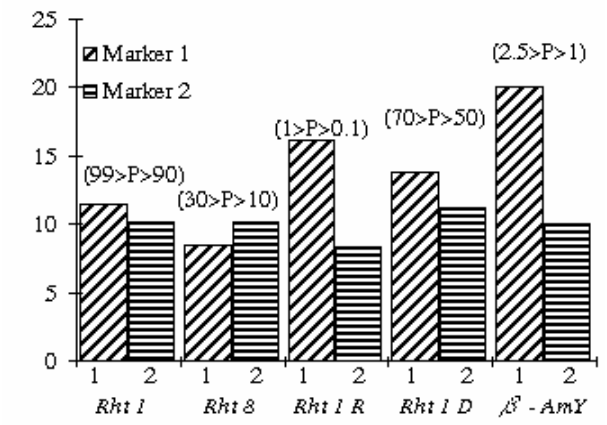

Figure 1. Frequencies of embryogenic lines according to genetic marker classes (70 RILs)

The data obtained using a χ^2 test are presented in table 4.

According to these data, there is no association between the embryogenic ability and the marker genes *Rht 1*, *Rht 8* and *Gli 1D*.

These results are in agreement with the recent data concerning the genetic control of SE in wheat. Although the stimulatory effect of allele *Rht 1* was reported by Mathias and Atkinson (1988) and Kaleikau et al. (1989) suggested a possible role of *Rht 8* allele for TCR in wheat, Ben Amer and Worland (1992) reported a minor effect of *Rht 8* for this trait.

Table 4. Evaluation of the association between embryogenic ability and several genetic markers in 70 RIL's of wheat

Marker gene	Allele	Analysed lines	Embryogenic lines	χ^2 value
Rht 1	*Rht 1*	34	4	0.019
	rht 1	35	4	
Rht 8	*Rht 8*	22	2	2.46
	rht 8	48	6	
Gli 1B	*Gli 1B3*	30	5	7.44**
	Gli 1Bl	36	3	
Gli 1D	*Gli 1 D2*	36	5	0.28
	Gli 1D4	26	3	
β-Amy	1	25	5	5.76*
	2	28	3	

For the marker gene *Gli 1B* a highly significant χ^2 value (P<0.01) was obtained, suggesting a strong positive effect of the allele *Gli1B3* on TCR in wheat. This effect is induced by the 1B/1R translocation from rye, which was reported also by other authors to stimulate the embryogenic ability (Langridge et al., 1991).

A significant χ^2 value (P<0.025) for the more complex zymogram of *β-Amy* was also obtained, suggesting the association between embryogenic ability and the high molecular weight isozyme present in F 1054.

As already established, the genes controlling β-amylase synthesis are located on chromosomal arms 4BL, 4DL and 5 AL (Forsythe and Koebner, 1992) which suggests the location in these regions of some genes controlling somatic embryogenesis in wheat.

Our data are partially in agreement with the previous results, suggesting a role for chromosome group 4 in stimulating TCR in wheat (Higgins and Mathias, 1987; Mathias and Atkinson, 1988; Ganeva et al., 1994). However, these data do not support the theory of Mathias and Atkinson (1988) , which suggested that the effect of chromosome 4B is induced by homeologous genes to *Rht* loci.

A possible explanation for the discrepancies between our results and other reports could be the type of genetic material studied. Most of the published data have been obtained

using aneuploids (monosomics, disomics, substitution lines) and isogenic lines. In the first case, the precision of the genetic analysis is limited to large chromatin blocks (chromosomal arms), and in the second one only a limited number of genes can be evaluated.

The study of recombinant inbred lines expressing polymorphism for several traits, including TCR, is more informative for evaluating segregation ratios and possible associations between the segregating traits.

Another particularity for our genotypes is their genealogy - both parental lines are Romanian genotypes, not related to Chinese Spring.

In a recent review concerning the genetic analysis of plant TCR, Henry et al. (1994b) assume that all genotypes possess the genetic information for zygotic embryogenesis and suggest that the intergenotypic variation for TCR is induced by: (a) differences in the regulation of genetic information or (b) differences in the ability of target cells to enter in a new developmental programme. Possible candidates for these functions are the genes involved in the phytohormones signals.

Somatic embryogenesis is considered a multistep process, controlled by the sequential expression of many genes (Zimmermann, 1993).

Possibly, the existing data concerning the genetic control of SE in wheat reflect the activity of several genes/genetic systems expressed in different stages of SE and plant regeneration.

Considering the results of mendelian analysis, there are a few regulatory genes; this idea is supported also by our data.

Further investigations - mainly molecular genetic analyses - will allow a more detailed characterizations of genes controlling SE in wheat.

CONCLUSIONS

Analysing the embryogenic ability of 70 RIL's derived from a cross between 2 Romanian lines contrasting for this trait we established a segregation ration of 1:9 for the embryogenic genotype. This result suggest an oligogenic recessive control of embryogenic ability in winter wheat.

The results of χ^2 test revealed a very significant effect of *Gli 1B* ($P<0.01$) and no effect of *Rht1*, *Rht8*, *Gli 1D*. We also obtained a significant positive effect of *β-Amy* ($P<0.025$), suggesting the mapping of some genes controlling TCR on chromosome arms 4BL, 4DL or 5AL.

The analysed genetic stocks could be an interesting material for further studies, aiming at the mapping and identification of genes controlling TCR in winter wheat.

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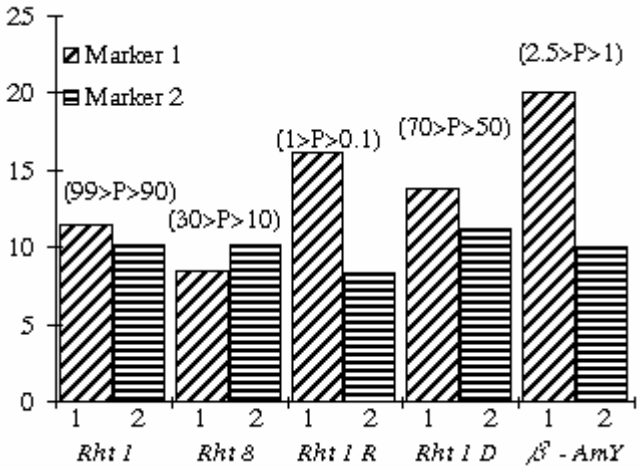

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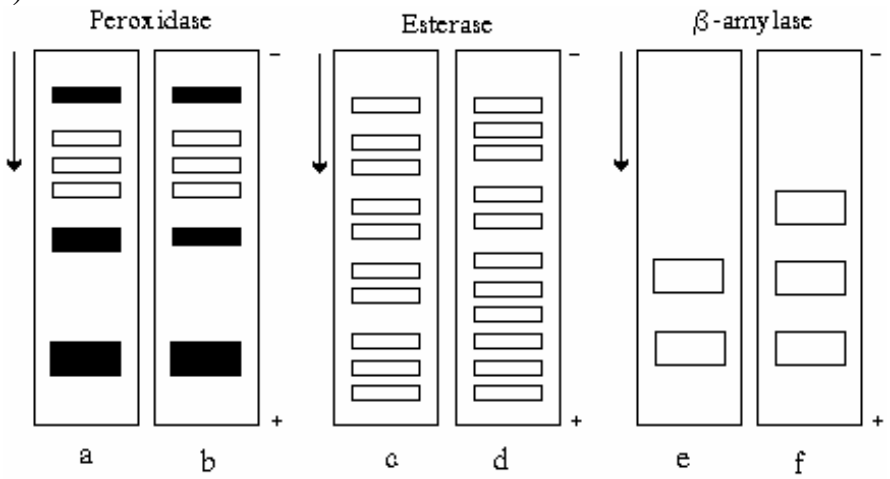

Figure 1. The electrophoretic patern of peroxidase, estarase and β-amylase for the parental lines (a, c, e = sincrom; b, d, f, = F 1054).