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Genetic analysis of anther culture response in wheat carrying alien translocations

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Abstract A bread wheat cultivar, 'Saratovskaya 29', (S29), its nearly isogenic lines carrying alien translocations [*Lr*9 from *Aegilops umbellulata* (Eg29) and *Lr19* from *Agropyron elongatum* (Ps29)] and two F_1 hybrids between three nearly isogenic lines of S29 that differed by the *Lr19* + *Rht1*, *Pro1* + *Pro2* and *Ppd1* + *Ppd2* gene complexes, namely the S29 (*Lr19* + *Rht1*)/S29 (*Ppd1* + *Ppd2*) F_1 and the S29 (*Pro1* + *Pro2*)/S29 (*Lr19* + *Rht1*) F_1, were studied for their culture response with the following results. (1) Translocations with *Lr9* and *Lr19* decreased embryo frequency and green plant regeneration. (2) Both F_1 hybrids showed a decrease in embryo frequency. One of the F_1 hybrids, S29 (*Lr19* + *Rht1*)/S29 (*Ppd1* + *Ppd2*) showed a decrease, with respect to S29 for green plant regeneration; the other F_1, S29 (*Pro1* + *Pro2*)/S29 (*Lr19* + *Rht1*), equalled S29 for green plant regeneration. (3) The gene complex of the F_1 hybrid S29 (*Pro1* + *Pro2*)/S29 (*Lr19* + *Rht1*) was better than that of the F_1 hybrid S29 (*Lr19* + *Rht1*)/S29 (*Ppd1* + *Ppd2*) for embryo induction and green plant regeneration. This effect was possibly induced by interactions between the *Pro1* + *Pro2* and *Lr19* + *Rht1* genes or was the result of direct actions of the *Pro1* + *Pro2* genes.

Key words Wheat-alien translocations · Gene complex · Anther culture · Regeneration ability

Introduction

It is well-known that anther culture response is affected by the genotype of the plant. In wheat this phenomenon has been observed by Henry and De Buyser (1985), Agache et al. (1988, 1989), De Buyser et al. (1989) and others and includes the effects elicited by nuclear genes and the cytoplasm (Sagi and Barnabas 1989; Ekiz Hassan and Konzak 1991a,b). It has been demonstrated that wheat haploid production from anther culture is controlled by at least three different inherited traits: embryo induction rate, embryo regeneration ability and the ratio of green to albino plants (Henry and De Buyser 1985; Agache et al. 1988, 1989).

The majority of these studies involved only the genotypes of bread wheat, and some of them the genotypes of bread wheat carrying alien translocations from rye, i.e. 1BL-1RS (Lane and Mornhinweg 1988; Muller et al. 1989, 1990). Unfortunately, there are few reports on the influence of alien translocations and chromosomes upon anther culture response in wheat. In this report the influence of alien translocations, *Lr9* and *Lr19* from *Ae. umbellulata* and *A. elongatum*, respectively, in bread wheat and the gene complexes in F_1 hybrids upon anther culture response are considered.

Materials and methods

The material examined consisted of spring wheat (*Triticum aestivum* L.) genotypes:

Ps29 = S29 × 7//Tc *Lr19*, the nearly isogenic line with *Lr19* into the genotype of cv 'Saratovskaya 29' (S29). The donor of *Lr19* is the nearly isogenic line 'Thatcher' with *Lr19* (Tc *Lr19*);
Eg29 = S29 × 8//Tc *Lr9*, the nearly isogenic line with *Lr9* into the genotype of cv S29. The donor of *Lr9* is Tc *Lr9*.
F_1 hybrid S29 (*Lr19* + *Rht1*)/S29 (*Ppd1* + *Ppd2*), derived from crossing lines L1063 and S29 P.I. L1063 is the nearly isogenic line of S29 possessing *Lr19* and *Rht1*. S29 P.I. is the nearly isogenic line of S29 with the *Ppd1* and *Ppd2* genes;
F_1 hybrid S29 (*Pro1* + *Pro2*)/S29 (*Lr19* + *Rht1*), derived from crossing lines ATS7 and L1063. ATS7 is the nearly isogenic line of S29 carrying the *Pro1* and *Pro2* genes.

Haploid plant production by means of anther culture was carried out on field- and greenhouse-grown plants during a 2-year period. In the first year comparisons were made between S29, Ps29 and Eg29 for two seasons. In the greenhouse season we made compared F_1 hybird S29 (*Lr19* + *Rht1*)/S29 (*Ppd1* + *Ppd2*), S29, Ps29 and Eg29; in the field season F_1 hybrid S29 (*Pro1* + *Pro2*)/S29 (*Lr19* + *Rht1*) was compared

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with S29, Ps29 and Eg29. In the second year the two F_1 hybrids were compared over two seasons.

The spikes of samples were cut at the late microspore uninucleated stage using the criterion suggested by Bernard (1980) and were subjected to a 2-week pretreatment at 4 °C. The anther culture medium N6 (Chu 1975) with 10% potato extract and 2 mg/l 2,4-D was used. The androgenetic embryos were removed and plated onto a regeneration medium (Ouyang 1986) with 0.2 mg/l indolyl acetic acid and 0.2 mg/l kinetin.

Results

Differences in embryo induction rate

Anthers of S29, Ps29 and Eg29 produced embryos at varying efficiencies (Table 1) with a significant variation among the lines in their frequency of embryo induction. The genotypes of Ps29 and Eg29 showed a significant suppressing effect on the embryogenic anther response when compared with S29. Moreover, a difference with respect to frequency of embryo induction between Ps29 and Eg29 in the greenhouse was obtained. In other words, the presence of translocations with *Lr9* and *Lr19* suppressed embryo induction. Data from the greenhouse season agreed with data from the field season, except that there was an absence of difference between Ps29 and Eg29 in the field season.

Comparison of F_1 hybrid S29 (*Lr19* + *Rht1*)/S29 (*Ppd1* + *Ppd2*) with S29 and Ps29 in the greenhouse season (Table 1) showed that embryo frequency was significantly lower in the former. When S29 and Ps29 were compared to F_1 hybrid S29 (*Pro1* + *Pro2*)/S29 (*Lr19* + *Rht1*), significant differences for embryo yield were observed. The embryo frequency of the F_1 hybrid fell between that of Ps29 and S29. Comparisons of the F_1 hybrids during two seasons showed significant differences (Table 2). F_1 hybrid S29 (*Pro1* + *Pro2*)/S29 (*Lr19* + *Rht1*) produced more embryos than F_1 hybrid S29 (*Lr19* + *Rht1*)/S29 (*Ppd1* + *Ppd2*). These data allow us to suppose that the presence of genes *Ppd1* and *Ppd2* in the former F_1 hybrid decreased embryo production frequency, and/or the presence of genes *Pro1* and *Pro2* in the latter F_1 hybrid increased it.

Table 1 Performance in anther culture of S29, Ps29 and Eg29 and two F_1 hybrids, S29 (*Lr19* + *Rht1*)/S29 (*Ppd1* + *Ppd2*) and S29 (*Pro1* + *Pro2*)/S29 (*Lr19* + *Rht1*)

Genotype	Number of anthers cultured	Number of embryos produced		Number of plants regenerated			
		Total (E)	E/1000 Anthers[a]	Green		Albino	
				Total (G)	G/E[a] (%)	Total (A)	A/E[a] (%)
Greenhouse season							
S29	2390	60	25.1d	17	28.3c	8	13.3a
Ps29	1731	28	16.8c	7	25.0b	4	14.3a
Eg29	1336	15	11.2b	4	26.7b	3	20.0b
S29 (*Lr19* + *Rht1*)/S29(*Ppd1* + *Ppd2*) F_1	1586	5	3.2a	1	20.0a	1	20.0b
Field season							
S29	2874	211	73.4c	81	37.0c	31	14.2b
Ps29	2729	50	18.3a	13	26.0b	5	10.0a
Eg29	3049	49	16.1a	6	12.3a	5	10.2a
S29 (*Pro1* + *Pro2*)/S29 (*Lr19* + *Rht1*) F_1	6773	325	48.0b	136	41.5c	70	21.2c

[a] Numbers within a column that are followed by different letters are significantly different

Table 2 Comparison of the two F_1 hybrids, S29 (*Lr19* + *Rht1*)/S29 (*Ppd1* + *Ppd2*) and S29 (*Pro1* + *Pro2*)/S29 (*Lr19* + *Rht1*), on embryo production and regeneration ability in anther culture

Genotype	Number of anthers cultured	Number of embryos produced		Number of plants regenerated			
		Total (E)	E/1000 Anthers[a]	Green		Albino	
				Total (G)	G/E[a] (%)	Total (A)	A/E[a] (%)
Field season							
S29 (*Lr19* + *Rht1*)/S29(*Ppd1* + *Ppd2*) F_1	2721	7	2.6a	1	14.3a	2	28.6b
S29 (*Pro1* + *Pro2*)/S29 (*Lr19* + *Rht1*) F_1	3742	27	7.2b	7	25.9b	2	7.4a
Greenhouse season							
S29 (*Lr19* + *Rht1*)/S29(*Ppd1* + *Ppd2*) F_1	6215	50	8.1a	7	14.0a	2	4.0b
S29 (*Pro1* + *Pro2*)/S29 (*Lr19* + *Rht1*) F_1	9329	199	21.3b	41	20.6b	4	2.0a

[a] Numbers within a column that are followed by different letters are significantly different

Differences in regeneration ability

With respect to green plant regeneration S29 was significantly higher than Ps29 and Eg29; thus, translocations with *Lr9* and *Lr19* suppressed green plant regeneration. When Ps29 and Eg29 were compared for green plant regeneration differences were obtained in field season but not in the greenhouse season. For albino plant regeneration no consistent results were obtained, but during two seasons Ps29 was significantly lower or equalled S29 and Eg29.

Comparisons of F_1 hybrid S29 (*Lr19* + *Rht1*)/S29 (*Ppd1* + *Ppd2*) with S29 and Ps29 showed that green plant regeneration was significantly lower in the F_1 hybrid. Conversely, albino plant regeneration was significantly higher in the F_1 hybrid than in S29 and Ps29.

The F_1 hybrid S29 (*Pro1* + *Pro2*)/S29 (*Lr19* + *Rht1*) showed significantly higher green plant regeneration than Ps29 and equalled S29. The former showed a significantly, higher albino plant regeneration than Ps29 and S29.

The F_1 hybrid S29 (*Lr19* + *Rht1*)/S29 (*Ppd1* + *Ppd2*) showed a significantly lower green plant regeneration but higher albino plant regeneration than F_1 hybrid S29 (*Pro1* + *Pro2*)/S29 (*Lr19* + *Rht1*). Thus, the presence of *Ppd1* + *Ppd2* in the former hybrid decreased both green plant regeneration and embryo induction while increasing albino plant regeneration. The presence of *Pro1* + *Pro2* in the latter hybrid increased green plant regeneration and decreased albino plant regeneration.

Discussion

Embryo induction and regeneration are heritable traits that are quantitatively controlled (Bullock et al. 1982), but embryogenic ability is environmentally modified (Charmet and Bernard 1984) and is not inherited in a simple way (Bullock et al. 1982). Our investigation on lines having the same genetic background as S29 but differing by genes *Lr9* and *Lr19* or other gene combinations, such as *Lr19* + *Rht1*, *Ppd1* + *Ppd2* and *Pro1* + *Pro2*, enabled us to determine the influence of those genes on embryogenic induction and regeneration ability. Errors due to environmental influences were avoided by conducting most of the studies over two seasons.

Monosomic analysis performed by Zhang and Li (1984) indicated that several chromosomes are involved in embryo production, with 2A and 2D possessing major genes and 5A, 5B, 4A and 2B having minor genes that inhibit the embryo production frequency. The positive effect of the 1D chromosome and 5BL chromosome arm from 'Chinese Spring' on embryo yield has been reported by Agache et al. (1989). We observed differences in embryo yields between S29, Ps29 and Eg29 that can be explained in two ways. In one case, the *Lr9* and *Lr19* genes, translocated from *Ae. umbellulata* and *A. elongatum* into the 6BL and 7DL chromosome arms of bread wheat, respectively, suppress embryo induction. These effects are possibly induced by other genes closely linked with *Lr9* and *Lr19*. The other explanation is that these effects are induced by the absence of wheat chromosome fragments that were substituted for by *Ae. umbellulata* and *A. elongatum* chromosome fragments by translocations.

As demonstrated by Henry and De Buyser (1985) regeneration ability primarily depends upon the genotypes of the parents and appears to be inherited from both parents. Cultivars such as 'Clement' and 'Aurora', which carry a 1BL-1RS translocated chromosome (Mettin et al. 1973; Miller 1984), have been observed to give a regeneration rate up to double that of lines without this translocation. This conclusion is also supported by the results of Agache et al. (1989). Thus, the gene(s) involved in regeneration ability is located on the 1RS chromosome arm. Our results show that the translocations from *Ae. umbellulata* with *Lr9* and *A. elongatum* with *Lr19* carry gene(s) which suppresses green plant regeneration.

Our investigation of the F_1 hybrids S29 (*Lr19* + *Rht1*)/S29 (*Ppd1* + *Ppd2*) and S29 (*Pro1* + *Pro2*)/S29 (*Lr19* + *Rht1*) allowed us to determine the influence of gene complexes (*Lr19* + *Rht1*)/ (*Ppd1* + *Ppd2*) and (*Pro1* + *Pro2*)/ (*Lr19* + *Rht1*) on embryo induction and green and albino plant regeneration. The gene complex (*Pro1* + *Pro2*)/ (*Lr19* + *Rht1*) showed a higher embryo induction and green plant regeneration than (*Lr19* + *Rht1*)/ (*Ppd1* + *Ppd2*), but its values were between those for S29 and Ps29 for embryo induction and higher or equal to those of Ps29 and S29 for green plant regeneration. These data show that gene interactions provided the higher or lower values for embryo induction and green plant regeneration than in found S29 with none of these genes or Ps29 with only the *Lr19* gene. Lowered values for embryo induction and green plant regeneration in the two F_1 hybrids can be explained by the presence of *Ppd1* + *Ppd2* genes or their interactions with *Lr19* + *Rht1*; increased values by the presence of genes *Pro1* + *Pro2* or their interactions with *Lr19* + *Rht1*.

Our results agree with the suggestion of Agache et al. (1989) that in some tested crosses a few genes with major effects are involved in the determination of anther culture response, rather than many genes with minor effects. These data are useful for wheat breeders because genes *Lr9*, *Lr19*, *Rht1*, *Pro1*, *Pro2* and *Ppd1*, *Ppd2* are very important and are often used in wheat breeding programs.

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