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Genetic analysis of anther culture response in wheat using aneuploid, chromosome substitution and translocation lines

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Summary. Marked effects of genotype on wheat anther culture response have been observed. Genetic factors have been recognised to be one of the major contributors to in vitro responses of cultured wheat tissues. In wheat anther culture, embryo induction, plant regeneration and albina/green ratio have been determined to be heritable traits. Using Chinese Spring (CS) monosomic 1D, single chromosome substitution lines of chromosome 5B or chromosome arm 5BL from Chinese Spring into six varieties, and F_1 hybrids heterozygous for the 1B chromosome structure (1BL-1BS/1BL-1RS), the anther culture response was studied: genes on CS1D chromosome and 5BL chromosome arm increases the embryo frequency; gene(s) involved in regeneration ability are located on the 1RS chromosome arm; a gene increasing albina frequency is located on Chinese Spring 5B chromosome. Our results support the fact that without gametic selection, a differential development occurred from the particular classes of microspores carrying genes for higher regeneration ability. Moreover, in some crosses, a few genes with major effects were involved in determination of anther culture response.

Key words: Wheat – Anther culture – Regeneration ability

Introduction

It was recognised from the very beginning of in vitro tissue culture studies that responses are affected by the genotype of the plant. For wheat, marked effects of genotype on anther culture response have been observed (De Buyser and Henry 1979; Wei 1982; He and Ouyang 1984; Henry and De Buyser 1985). It has also been demonstrated that overall wheat haploid plant production from anther culture is controlled by at least three different and independently inherited traits (Agache et al. 1988): embryo induction rate, embryo regeneration ability and the ratio of green to albino plants (Henry and De Buyser 1985).

Furthermore, genotype effects and genotype × environment interactions have been reported (Lazar et al. 1984; Charmet and Bernard 1984). However, studies of the inheritance of responsiveness to anther culture are difficult because of the large amount of uncontrollable and environmentally induced variations (Ockendon and Sutherland 1987). The first aim of such studies, thus, should be to separate the total variation into genetic and non-genetic components, the latter consisting of "variation between plants within a genotype and between occasions on which a plant is subjected to anther culture" (Ockendon and Sutherland 1987).

There is little information available on the genetic basis of responsiveness to anther culture in wheat, and only limited information on the number of loci involved. In our experiments, plants monosomic for chromosome 1D of Chinese Spring and Chinese Spring, several parental strains and their Chinese Spring-5B substitution lines, and F_1 hybrids heterozygous for 1B chromosome structure were examined. Comparisons were made for frequencies of embryo formation and regeneration ability.

Materials and methods

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

- Chinese Spring monosomic 1D (CSM1D). Seeds were kindly provided by Mme Cauderon (I.N.R.A., Versailles);
- single chromosome substitution lines of chromosome 5B from Chinese Spring into Highbury (Hg) and Sicco (S) (Snape et al. 1986);

- the varieties Aquila, Armada, Avalon and Hobbit "sib" and their substitution lines of chromosome arms 5BL and 7BL from Chinese Spring (Snape 1987);
- F_1 hybrids derived from crossing doubled haploid lines possessing a 1BL-1RS translocated chromosome (Henry and De Buyser 1985) with varieties such as Aquila or advanced pedigree lines having a normal 1B chromosome. These hybrids thus had different genetic backgrounds but were all heterozygous for the 1B chromosome containing a 1BL-1BS/1BL-1RS structure. They possessed only three satellited chromosomes (two from 6B and one from 1B), since for 1RS the appearance of the rye satellite is suppressed in wheat background (Miller 1984).

The production of haploid plants using anther culture was carried out on greenhouse-grown plants. The spikes were harvested at the late microspore uninucleated stage and subjected to a 1 week pretreatment at 3 °C. The anther culture medium (Henry and De Buyser 1985) contained 0.5–1 g l^{-1} glutamine, 70 ml potato extract and was gelled with 6 g l^{-1} agarose Sigma type I. After 20–40 days, the androgenetic embryos were removed and plated onto a regeneration MS medium similar to that developed by He and Ouyang (1984), gellified with 6 g l^{-1} agarose. Culture temperature was regulated at 28 °C by day and 24 °C at night with a 16 h 1,500 lux photoperiod.

In order to reduce uncontrollable environmental variation in genotypic comparisons, a minimum of 40 spikes were plated for each genotype, which were produced from a minimum of 12 plants per genotype. In the case of Sicco, Sicco CS5B and Highbury genotypes, additional anthers were cultured in order to allow a sufficient number of embryos for analysis of regeneration frequencies.

Results

Differences in embryo induction rate

When Chinese Spring monosomic 1D was compared to disomic plants produced from selfed CSM1D spikes (Table 1), there were differences for embryo yield although the regeneration abilities were similar. Monosomic 1 D was the least productive (1.1%) for embryos.

Experiments using substitution lines possessing Chinese Spring 5B chromosomes showed that there were significant differences (Table 2) from the recipient varieties. The substitution lines Hg-CS5B and S-CS5B produced more embryos than the parental recipient varieties, and also produced embryos with a higher regeneration ability in the case of Sicco-CS5B. Two main hypothesis can be proposed for this result: first, background genetic effects are responsible for the better performance, because only four backcrosses were used in developing the substitution lines; and secondly, the Chinese Spring 5B chromosome is involved in anther culture responsiveness.

In order to investigate further the genetic contribution to anther culture response in wheat, other CS5B substitute lines in other genetic backgrounds were tested. Four recipient varieties were compared with their Chinese Spring 5BL substitution lines for embryo frequency. The results (Table 3) confirm that the CS5BL chromosome arm increased the embryo production frequency.

Table 1. Performance in anther culture of the Chinese Spring monosomic 1D line compared to disomic controls

	Genotype	
	Monosomic 1 D	Disomic
No. of spikes excised	575	84
No. of anther cultured (A)	34,500	5040
No. of embryos produced (e)	368	75
e/A%	1.1 a*	1.5 b
No. of plants regenerated		
green (G)	28	5
G/e %	7.6 a	6.7 a
total (T)	74	17
T/e%	20.1 a	22.7 a

* Entries within lines followed by the same letter are not significantly different

Table 2. Comparison of CS5B substitution lines with their recipient varieties for embryo production during anther culture

	Genotype			
	Sicco	Sicco-CS5B	Highbury	Highbury-CS5B
No. of spikes excised	87	80	66	76
No. of cultivated anthers	5655	5200	4290	4940
No. of embryos obtained	176	266	7	59
%	3.1 c*	5.1 d	0.2 a	1.2 b

* Entries within lines followed by different letters are significantly different

Table 3. Overall performance in anther culture of varieties and their Chinese Spring 5BL substitution lines

	Genotype	
	Hobbit "sib" Avalon Armada Aquila	Hobbit "sib" CS5BL Avalon CS5BL Armada CS5BL Aquila CS5BL
No. of spikes excised	194	236
No. of embryos (E) produced	72	134
Embryo frequency (E/100 anthers)	0.57 a*	0.87 b

* Entries within lines followed by different letters are significantly different

A comparison of the mean performance of recombinant doubled haploid (DH) lines derived from the cross Hg(CS5B) × S (CS5B) with their original parental lines (Table 4) shows that the mean embryo frequency falls between the two parents and is, in fact, not significantly different from the mid-parent value. This implies the ad-

Table 4. Performance in anther culture of substitution lines and doubled haploids produced from the F_1 hybrid Hg-CS5B × S-CS5B

	Genotype		
	Sicco (CS5B)	Highbury (CS5B)	DH Mean
No. of cultivated anthers	5200	4940	44,135
Embryo frequency %	5.11 c*	1.19 a	3.07 b
Regeneration ability %	36.0 b	49.0 c	23.3 a

* Entries within lines followed by different letters are significantly different

Table 5. Comparison of CS5B substitution lines with their recipient varieties for regeneration ability

	Genotype			
	Sicco	Sicco-CS5B	Highbury	Highbury-CS5B
No. of embryos subcultured	189	270	10	59
No. of regenerated plants				
green	0	1	0	9
%	0	0.4	0	15.2
albina	35	97	3	20
%	18.5	35.6	30.0	33.8
total	35	98	3	29
%	18.5 a*	36.0 b	30.0	49.0 b
Albina frequency (%)	100	98.9	100	68.9

* Entries within lines followed by the same letter are not significantly different

Table 6. Frequency of translocated 1BL-1RS chromosomes in haploids or spontaneous diploids produced from F_1 hybrids 1BL-1RS/1BL-1BS

No. of satellited chromosomes		
Haploids	1	2
Diploids	2	4
No. of plants observed	66	32
%	67.3	32.7
Chromosome structure	1BL-1RS	1BL-1BS

ditive action of genes controlling this character and no epistasis (Agache et al. 1988).

Differences in regeneration ability

For green or total plant regeneration (green + albina), the Chinese Spring monosomic 1D equalled its disomic variety.

When compared to Sicco and Highbury, the embryos produced from the substitution lines S-CS5B and Hg-CS5B possessed a higher regeneration ability (Table 5). This suggests genes increasing regeneration ability located on CS5B chromosome, associated (linked) with a gene increasing albina frequency, However the number of embryos obtained was not sufficient in the case of Highbury and Hg-CS5B to confirm this fact. The regeneration ability from Aquila, Armada, Avalon, Hobbit "sib" and their substitution lines was also reduced to such an extent that a clear conclusion could not be reached.

None of the 38 tested DH exceeded the "Hg-CS5B × S-CS5B" F_1 hybrid (Snape et al. 1986) for regeneration ability, or equalled this F_1 for final green plant yield per spike. The mean regeneration ability of the total number of plants from the DH lines was significantly less than the lower scoring parent S-CS5B (Table 5). This occurred because of the ten DH lines with no regeneration, including four DH without any embryos (Agache et al. 1988).

From the F_1 hybrids heterozygous for the 1B chromosome structure, i.e. 1B/1RS, we have regenerated 67% of plants carrying the translocated 1BL-1RS chromosome (Table 6).

Discussion

Embryo induction and regeneration depends on nuclear genes and the induction frequency of green pollen plants is an heritable trait which is quantitatively controlled (Bullock et al. 1982). Embryo induction rate reflects the ability of genotypes to produce multicellular pollen grains and embryos from microspores. This embryogenic ability is environmentally modified (Charmet and Bernard 1984) and does not seem to be very simply inherited (Bullock et al. 1982). General and specific combining abilities were estimated to be highly significant (Lazar et al. 1984; Charmet and Bernard 1984). Most of the genotypic variance is due to general combining ability effects, and heritability estimates are in the range of 0.6–0.7 for embryogenesis (Lazar et al. 1984; Charmet and Bernard 1984). Our results clearly suggest that crosses such as Highbury CS5B × Sicco CS5B (Agache et al. 1988) probably reflect non-additive effects for the most part, whereas the Talent × Aurora cross shows predominantly additive gene action (Henry and De Buyser 1985).

Monosomic analysis has been performed by Zhang and Li (1984) in order to investigate which chromosomes are involved in wheat embryo production. Their analysis indicates that several chromosomes are involved in embryo production, with 2A and 2D possessing major genes and 5A, 5B, 4A and 2B having minor genes, which inhibits the embryo production frequency. Here, differences in embryo yields between monosomic 1D and disomic lines were observed. This probably derived from the positive effect of the 1D chromosome on embryo yield. However results from monosomic analysis should be considered with caution because of the reduced viability of nulli-haploid gametes (unpublished results). Analysis of substitution lines of the Chinese Spring 5B chromosome or 5BL chromosome arm into different recipient varieties indicates a stimulating effect of the Chinese Spring 5BL chromosome arm on embryo frequency. The embryo induction frequency was often lower in homozygous DH lines compared to F_1 parental hybrids, and several hypotheses can be proposed (Ouyang 1986): first, only specific combining ability effects are involved and pollen embryo induction frequency is controlled by genes of the haploid pollen cells; secondly, the heterosis for embryo yield is mainly influenced by the anther wall genotype and not by the microspore genotype (Raquin 1982); and thirdly, the dominant genes are dispersed between the parents.

The importance of the anther wall effect has been confirmed by isolated wheat microspore culture, which occurs only after preculture of the anthers for a few days (Henry and De Buyser 1981): Wei 1982; Datta and Wenzel 1987). As demonstrated by Prelletier and Ilami (1972), differences for in vitro culture responses of anthers not only arises from the different potentials of microspores to develop into embryos, but also from different anther wall inducing effects. Physiologically active substances contained in the anther wall cells have been shown to be involved in embryogenic induction (Xu and Sunderland 1981; Köhler and Wenzel 1985). The sporophytic control of embryo production (Raquin 1982) could be produced by an increased amount of efficient substances in the anther wall tissue of F_1 hybrids.

Several particular genotypes can have a genetic structure that appears to strongly modify the regeneration ability (Raquin 1982; Henry and De Buyser 1985). This can determine the mean quality of the embryos and therefore their regeneration ability.

Regeneration ability mostly depends upon the genotypes of the parental cross and appears inherited from both parents (Henry and De Buyser 1985). Varieties such as Clement or Aurora, which carry a 1BL-1RS translocated chromosome (Mettin et al. 1973; Miller 1984), have been observed to give a high regeneration rate up to double that of lines without this translocation. Thus gene(s) involved in regeneration ability are located on the 1RS chromosome arm (Henry and De Buyser 1985). Our results also support the conclusion that, without gametic selection, a differential development occurred for the particular classes of microspores carrying genes for higher embryo level or better regeneration ability.

From the F_1 cross of "Highbury CS5B × Sicco CS5B", it appears that at least two genes, and probably more, dispersed between the parents are acting on regeneration (Agache et al. 1988). No clear discountinuities are apparent in the distribution of the DH plants, so that a simple genetic control appears unlikely for regeneration ability (Agache et al. 1988). The mean number of plants regenerated from the DH lines is less than the inferior parent. This implies epistatic control (Agache et al. 1988).

Genetic factors have now been recognised to be one of the major contributors to in vitro responses of cultured wheat tissues. Genetic variation between lines was exhibited for high frequencies of competent spikes, embryo induction, embryo regeneration and albina/green ratio. As a general rule, the use of F_1 hybrid plants as anther-donors is an effective means of increasing the yield of pollen plants. The results suggest that complex gene actions are involved in the determination of anther culture yield, but in some tested crosses, a few genes with major effects are involved in determination of anther culture response, rather than many genes with minor effects.

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