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Genomic influence on somatic embryogenesis in the Triticeae

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Abstract Somatic embryogenesis is generally accepted to be under genetic control. The influence of genome interactions is not well understood given the difficulties of obtaining the appropriate vegetal material. Synthetic alloploids in which genomes from two species are fused together are suitable subject material to analyse this factor. In the Triticeae tribe amphiploids can be easily synthesised, which provides the opportunity to carry on this type of study. Crossing three autotetraploids, *Hordeum chilense* (HHHH), *Triticum tauschii* (DDDD) and *Secale cereale* (RRRR), we obtained three tetraploid amphiploids, ×*Tritordeum* (HHDD), ×*Triticale* (DDRR) and ×*Hordecale* (HHRR). Somatic embryogenesis from immature inflorescence and flag leaves were scored on parents and amphiploids. Immature inflorescences had a higher embryogenic response than the flag leaves. The amphiploids showed higher regeneration ability than their parents. The best genomic combination was the tetraploid triticale DDRR for every inflorescence and flag leaf size tested, followed by HHDD and HHRR. Heterosis was found to be the main genetic factor affecting the in vitro culture response although there are clear differences among the three amphiploids tested.

Key words Somatic embryogenesis · Triticeae · Amphiploidy

Introduction

While in vitro culture has traditionally been a powerful tool for breeding purposes, it has gained additional importance as the first step in plant transformation protocols. For this reason there is renewed interest in unravelling the genetic basis of this process.

In vitro clonal propagation through somatic embryogenesis is a routine method for the vegetative propagation of some crops (e.g. oil palm, pine) or sterile genotypes (Nakamura et al. 1981; Fedak, 1985). It is well-documented that somatic embryogenesis is the result of hereditary, developmental and environmental factors (Evans et al. 1981). The genotype dependence of the embryogenic response of in vitro culture is widely accepted at the species level, while at the interspecific or intergeneric levels the existence of recalcitrant groups also supports this assumption.

Literature on attempts to unravel the genetic basis of somatic embryogenesis is abundant and, in the Triticeae, in which chromosomal variants are available thereby enabling chromosomal analysis of the embryogenic response this approach has been widely used with different explants, e.g. anther culture (Szakács et al. 1988). However, the information available on the influence of intergenomic interaction is scanty. The easiness of obtaining synthetic alloploids (amphiploids) in the Triticeae after chromosome doubling of hybrids or direct crossing of two autotetraploids offers a unique experimental system for obtaining information on the influence of "heterocygosis" at the genomic level.

The aim of the experiments reported in this paper was to analyse the influence on somatic embryogenesis of the interaction of distant genomes when working together. In other words, alloploidy itself, which is associated with heterosis (Stebbins 1971), is tested against autoploidy.

Materials and methods

Three induced tetraploids *Triticum tauschii* (DDDD, 2n = 4x = 28), *Hordeum chilense* ($H^{ch}H^{ch}H^{ch}H^{ch}$, 2n = 4x = 28) and *Secale cereale* (RRRR, 2n = 4x = 28) and the amphiploids×*Tritordeum* ($H^{ch}H^{ch}$DD, 2n = 4x = 28, Cabrera and Martin 1991), ×*Triticale* (DDRR, 2n = 4x = 28, Cabrera et al. 1996) and ×*Hordecale* ($H^{ch}H^{ch}$RR, 2n = 4x = 28, Martin et al. 1988) were used in this investigation.

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Table 1 Inflorescence and flag-leaf developmental stages

Developmental stage	Inflorescence size (mm)	Description
0	<0.5	
1	0.5–1.5	Awns not visible
2	1.5–5.0	Awns begin to develop
3	5.0–10.0	Awns well-developed
4	10.0–30.0	Small spike
5	>30.0	Spike well-developed

Plants were grown in a glasshouse under a 16-h photoperiod and 25°/15 °C day/night temperature cycle. Inflorescences and the surrounding flag leaves at different developmental stages (Table 1) were used as explants for in vitro culture response. Tillers containing inflorescences and flag leaves were harvested, sterilised in 70% ethanol for 1 min and 0.05% sodium hypochloride plus two drops of Tween-20 for 20 min and washed twice with sterile distilled water. Explants were dissected individually, cut in 0.5- to 1.5-mm pieces and placed on petri dishes containing induction medium. They were cultured at 25 °C under a photoperiod of 16 h, and after 5 weeks proliferation capacity was scored. The inflorescence and flag-leaf segments which were able to grow as a result of the mitotic activity of their cells were considered as "proliferative". Consequently, "no-proliferative" were those not able to proliferate at all or those able to grow only for a limited period of time, mostly by the elongation of pre-existing cells. Proliferating explants were transferred to regeneration medium and regeneration capacity was scored after 5 weeks of culturing as the number of explants producing shoots per total number of proliferating explants transferred.

Basal medium used for the induction of embryogenesis was L2 (Lazzeri et al. 1991) supplemented with 30 g l^{-1} maltose and 2 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D). For regeneration, L2 medium was supplemented with 30 g l^{-1} sucrose.

Results

Table 2 shows the percentage of proliferation and regeneration from inflorescence tissue cultured at different developmental stages for three cereals and their corresponding hybrid combinations. All three non-hybrid species showed a proliferation capacity from all of the inflorescence developmental stages tested (Table 2 A). However, tetraploid *S. cereale* exhibited a higher proliferation capacity than tetraploid *T. tauschii*, and in tetraploid *H. chilense* the percentage of proliferation was higher when young inflorescences were used, declining as the inflorescence matured. The percentage of regeneration from parental genotypes was lower than proliferation at all of the developmental stages tested (Table 2 B). Both *S. cereale* and *T. tauschii* showed regeneration only when the inflorescence was longer than 0.5 mm (Stage 1 or higher, Table 1) and, in both genotypes inflorescences at the oldest developmental stage did not show regeneration at all. In contrast, *H. chilense* only regenerated when the youngest inflorescence stage was used as the explant for tissue culture. With respect to hybrid combinations, all three amphiploids showed a proliferation capacity equal to or higher than that of their corresponding parents (Table 2 A). Plant regeneration in the hybrids was achieved from a wide range of inflorescence developmental stages, and for all three hybrids the percentage of plant regeneration was higher than that of their parents. Both HHDD and DDRR amphiploids showed different optimal regeneration capacities at different developmental stages. Thus, the inflorescences of HHDD exhibited the highest percentage of regeneration at the youngest developmental stage and declined with maturity, whereas in DDRR, the percentage of regeneration increased with inflorescence maturity and the oldest developmental stage demonstrated the highest percentage of regeneration. Number of plants per explant was higher in the hybrid combinations, giving an average of 5 plants per explant for HHRR and HHDD and more than 15 plants per explant for DDRR (Table 2 C).

Table 2 Effect of inflorescence developmental stage on tissue culture response from different cereal genomic combinations. Values are average of two isolations, each with 15 explants except for RRRR for which 6 explants per isolation were used

Developmental stage	Genomic combination					
	Non-hybrids			Hybrids		
	HHHH	RRRR	DDDD	HHRR	HHDD	DDRR
A) Percentage of proliferation						
0	100	100	50	ND[a]	100	100
1	90	100	80	100	65	100
2	100	100	87	100	100	100
3	11	100	77	100	100	100
4	13	100	67	100	80	92
5	11	100	75	100	47	89
B) Percentage of regeneration						
0	11	0	0	ND	100	25
1	0	0	42	33	64	50
2	0	18	31	46	62	77
3	0	25	30	25	50	82
4	0	17	25	21	33	91
5	0	0	0	15	0	94
C) Plants per explant regenerated from different genomic combinations						
Plants per explant ± SE	0	2 ± 1.0	3 ± 0.8	5 ± 1.1	6 ± 1.3	17 ± 2.5

[a] ND, Not done

Tissue culture response from flag leaves at different developmental stages of the three autotetraploids and their corresponding hybrids is shown in Table 3. When flag-leaf explants were cultured, the younger ones of all three parental genotypes showed a higher proliferation capacity. Of the three genotypes, only *S. cereale* was able to proliferate at all of the developmental stages tested (Table 3 A). Plant regeneration was only achieved in *T. tauschii*, but only in stages 1 and 2 (Table 3 B). With respect to hybrid combinations all three showed a higher proliferation capacity than their corresponding parentals, and this was true for all of the developmental stages tested. The HHRR and DDRR amphiploids showed a higher proliferation capacity than HHDD. Plant regeneration was also obtained from all three hybrid combinations. However, only DDRR showed a high regeneration capacity at all developmental

Table 3 Effect of flag-leaf developmental stage on tissue culture response from different cereal genomic combinations. Values are average of two isolations, each with 15 explants except for RRRR for which 6 explants per isolation were used

Developmental stage	Genomic combination					
	Non-hybrids			Hybrids		
	HHHH	RRRR	DDDD	HHRR	HHDD	DDRR
A) Percentage of proliferation						
0	89	70	50	ND[a]	79	100
1	40	91	64	100	67	100
2	33	100	60	100	80	100
3	0	75	50	100	38	100
4	0	50	0	100	27	91
5	0	50	0	89	22	77
B) Percentage of regeneration						
0	0	0	0	ND	55	75
1	0	0	14	11	0	73
2	0	0	11	9	17	86
3	0	0	0	0	0	80
4	0	0	0	0	0	70
5	0	0	0	0	0	60
C) Plants per explant regenerated from the different genomic combinations						
Plants per explant ± SE	0	0	1 ± 0.5	2 ± 0.6	3 ± 0.9	15 ± 1.9

[a] ND, Not done

stages, giving an average of 15 plants per explant (Table 3C).

When the tissue culture response of the non-hybrid and hybrid combinations for both explants (inflorescences and flag leaves) was compared, regeneration capacity was higher in the hybrids than in their parents. Even stronger, the response of the hybrids was in general higher than that of the non-hybrids, except for the regeneration capacity of DDDD from the flag-leaf, which was similar to that of HHRR. With respect to regeneration response from the two explants used, for hybrid and non-hybrid genomic combinations, the immature inflorescence always showed a higher response than flag-leaf explants. In addition to this, regeneration capacity from inflorescences was obtained from a wider range of developmental stages.

Discussion

The aim of our investigation was to test the in vitro culture response of three intergeneric amphiploids and their corresponding tetraploid parents in order to comprehend the influence of different Triticeae genomes and their interaction on this response. As presented in Tables 2 and 3 tissue culture response among parental genomes showed significant differences both in proliferation and regeneration capacities. This is not surprising as somatic embryogenesis and plant regeneration are complex morphogenetic processes and have been shown to be genotype-dependent (Mathias and Simpson 1986; Fennell et al. 1996). The fact that for both explants and genomic combinations (parental and hybrid) proliferation capacity was not correlated with regeneration and that highly proliferative genotypes showed a low regeneration capacity or did not regenerate at all suggests that there are genetic components controlling embryogenesis and plant regeneration. For both explants plant regeneration was enhanced in hybrid combinations compared with their parents, and this enhancement was specially important when inflorescences were used as the explant for tissue culture. This hybrid response is outstanding in tetraploid triticale and may be based on the fact that the pattern of development is altered by genome interactions in synthetic combinations. However, previously a low frequency was reported for in vitro regeneration of hexaploid (Nakamura and Keller 1982; Eapen and Rao 1985; Stolarz and Lörz 1986; Stolarz 1991) and octoploid (Stolarz 1991; Immonen 1992) triticales. The discrepancy between the results with hexa- and octoploid triticale and our results with tetraploid triticale could mean that not only genome interactions but also ploidy levels are important factors determining the in vitro culture response in Triticeae. In fact, Barcelo et al. (1989) compared regeneration capacity of hexaploid and octoploid tritordeum, showing that hexaploids exhibited a higher regeneration capacity than octoploids. This relation is not surprising because the negative relationship between DNA content and rate of development is well known (Bennett 1996). In a parallel work Millán (1989) analysed the relationship between mitotic index (MI), vegetative vigour and autoploidy or amphiploidy, finding that for similar DNA content (similar ploidy level) the MI and vigour are higher for amphiploids. In addition, the MI increases with ploidy but there is an optimum between the tetraploid and hexaploid level.

In cereals it has been proposed that the D genome or some of the chromosomes from this genome could specifically play an important role in determining in vitro culture response (Nakamura and Keller 1982; Fedak 1985; Kaleikau et al. 1989; Immonen 1992). In agreement with this hyphotesis, our results suggest that those hybrid combinations containing the D genome had a higher morphogenetic capability. However, the fact that both triticale (DDRR) and tritordeum ($H^{ch}H^{ch}DD$) amphiploids showed a higher response than *T. tauschii* (DDDD) itself also indicates that the heterotic effect at the inter-genomic has to be taken into consideration.

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