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Effect of Karyotype on Somatic Embryogenesis from Immature Triticale (× *Triticosecale* Wittmack) Embryos

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With 2 figures and 5 tables

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Abstract

To assess the tissue-culture response of different karyotypes of spring triticales, immature embryo explants of 20 secondary triticales with a complete karyotype (complete rye genome with or without a 6D/6A substitution) or substituted karyotype (incomplete rye genome with a 2D/2R substitution), two primary triticales with octoploid and two with hexaploid karyotypes, were cultured on MS and on Kao medium supplemented with 2,4-D. Complete triticales produced significantly more embryogenic callus than substituted types. Complete 6D/6A karyotypes showed the highest embryogenic response. Octoploid primary triticales showed high and uniform production of embryogenic callus on both media. These findings complement earlier results on the agronomic and quality differences between the karyotypes.

Key words: Triticale — karyotype — somatic embryogenesis — regeneration

Triticale (× *Triticosecale* Wittmack) includes primary types with varying ploidy levels and complete or substituted secondary forms (GUPTA and PRIYADARSHAN 1982). Commercially-grown secondary hexaploid triticales are complete with a full rye (*Secale cereale* L.) genome or substituted, with an incomplete R-genome. In the latter group, rye chromosome 2R is substituted by wheat chromosome 2D as the only stable rye/wheat substitution (LUKASZEWSKI 1988). LUKASZEWSKI (1988) reported stable 6D/6A substitutions, which have been favoured by phenotypical and empirical selection and become common in the complete triticales (GUSTAFSON et al. 1989, LUKASZEWSKI 1988).

Complete and substituted triticales differ from each other phenotypically. Complete karyotype triticales are broadly adapted and superior in marginal environments (FOX et al. 1990, VARUGHESE et al. 1987) whereas substituted types are strictly adapted to favourable environments (CIMMYT 1985). They are earlier, shorter and have better milling and baking quality (SKOVMAND et al. 1985). A number of the CIMMYT complete triticales carry a 6D/6A substitution. They are shorter with higher test weight in comparison with complete triticales having the 6A chromosome and they have sprouting resistance and tolerance of soil acidity (W. PFEIFFER, pers. comm.).

Triticale has a narrow gene base, which can be expanded using tissue culture methods. Somaclonal variation in triticale has been discussed by JORDAN and LARTER (1985). Tissue culture may enhance new introgressions from the D-genome or alien genomes into hexaploid triticale (LAPITAN et al. 1984) and it can be used in the primary cross to improve the production of wheat-rye hybrids (IMMONEN and VARUGHESE 1991). Plant regeneration through callus culture has been studied in octoploid and hexaploid triticales (STOLARZ 1991, STOLARZ and LÖRZ 1986, EAPEN and RAO 1984, NAKAMURA and KELLER 1982). Significant differences between triticale genotypes in the ability to form callus were reported by SHARMA et al. (1980).

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However, the effect of the karyotype, evident in the distinct phenotypic groups, has not been studied in relation to tissue-culture response.

The objective was to evaluate the importance of karyotype when defining tissue-culture methods for triticale primary production and breeding. The embryogenic response of primary and secondary karyotypes *in vitro* is discussed. A method to assess embryogenic capacity by visual scoring is presented.

Materials and Methods

Plant materials: The genotypes and respective karyotypes for Experiments 1 and 2 are given in Tables 1 and 2. Donor plants for immature embryo explants were grown in the field at the CIMMYT El Batan station (19 31′N 98 50′W 2249 masl) in the central Mexican highlands (Experiment 1) and at the CIMMYT Cd. Obregon station (27 20′N 109 54′W 39 masl) in the coastal region of north west Mexico (Experiment 2).

Immature caryopses from bagged heads were collected 16—18 days after anthesis. They were washed in 70 % ethanol for 1 min, and surface sterilized for 10 min in 20 % commercial bleach (6 % sodium hypochlorite) and 0.5 % polyoxyethylenesorbitan monooleate (Tween 80) followed by several rinsings in sterilized water. The embryos were placed in sterile plastic petri dishes, four per dish, scutellum side up with the embryo axis embedded in the medium. Embryos were classified as small (0.5 mm—0.6 mm dia.), medium (> 6 mm—0.8 mm dia.) and large (> 0.8 mm—1.2 mm dia.).

Callus induction: The basal media tested were MS (MURASHIGE and SKOOG 1962) with 2 % sucrose, and modified Kao medium (KAO 1977, NAKAMURA and KELLER 1982) without vitamins A, D_3 and B_{12} or coconut milk. The media were supplemented with 5 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) and 6 % agar and adjusted to pH 5.8. The vitamin, organic acid and sugar (except glucose and sucrose) component solutions were filter sterilized. All other components were autoclaved (15 min at +121 °C 20 psi). Cultures in Experiment 1 were incubated at 25 °C with a 16 h photoperiod (50 μmol/m^2/s^1, fluorescent light). In Experiment 2 cultures were incubated at 25 °C in darkness and a subsample of eight genotypes was cultured in a 16 h photoperiod. After five weeks of culture, an embryogenic section consisting of 1—10 embryoids of each embryogenic callus was subcultured to the respective hormone-free medium to check regeneration capacity.

Observations and statistical analysis: The frequency of embryos forming callus and calli with embryogenic sections was noted after five weeks of culture. Embryogenic callus was recognised by its smooth and compact appearance and formation of embryoids. In Experiment 2 the size of embryogenic sections in individual calli was scored visually on a weekly basis using a linear scale of 1—10 (1 = single or twin embryoid, 10 = > 45 embryoids) to investigate the development rate and intensity of somatic embryogenesis.

Table 1. Secondary triticale (TCL) varieties, advanced lines and primary triticales and their karyotypes. Experiment 1

Genotype		Karyotype		
No.	Name	Rye genome	Chromosome substitution	Ploidy level
Secondary TCL				
1	Eronga 83	Complete		6x
2	Rhino 3	Complete		6x
3	Gnu 4	Complete	6D/6A	6x
4	Stier 29	Complete	6D/6A	6x
5	Eda 5/Tapir	Substituted	2D/2R	6x
6	6TA876/6TB164//Panda/Rahum/3/Tesmo 2	Substituted	2D/2R	6x
Primary TCL				
7	Wuhan #3/Beredic	Primary		8x
8	Octo NV	Primary		8x
9	SNA 3/Moroc 54	Primary		6x
10	Karim/Snoopy resel.	Primary		6x

The frequency of calli forming plants with roots was calculated after six and eight weeks of subculture.

Chi-square analyses (INSTAT 1990) were used to analyse the frequency data. Data of the scores for embryogenesis were analyzed as a nested design using a generalized linear model analysis of variance [GLM ANOVA (SAS 1985)]. The average score for callus in each petri dish was considered a replicate. The data on the karyotypes were analyzed using the mean square for varieties within karyotypes as an error term. LSD was used to separate significantly different means for media and karyotypes. Mean separation for genotype × medium interactions was done using pair-wise t-tests.

In the analysis, callus induction frequency was based on the total number of plated embryos and frequencies of embryogenic calli on the total number of calli. Embryogenic calli were evaluated for the intensity of embryogenesis. The analysis of plant regeneration was based on the number of embryogenic calli subcultured. This was done to evaluate callus induction, embryogenic capacity and plant regeneration capacity separately for the karyotypes and media. The callus data including all calli were also analyzed with GLM ANOVA (SAS 1985) after transforming scores on a 0—10 scale according to LITTLE and HILLS (1978).

Results

Embryogenic areas were observed after one week of culture. Embryogenic callus either arose directly from the scutellum or was formed on top of transparent, watery non-embryogenic callus. Callus induction potential was similar with all triticale genotypes and media in both experiments. An average of 96 % of the plated embryos in Experiment 1 and 99 % in Experiment 2 produced callus. There were no differences in the frequencies of induced calli and embryogenic calli between the three embryo sizes ($P > 0.05$).

Experiment 1

More embryogenic calli were induced on MS medium than in Kao medium over all karyotypes ($P < 0.001$). The responses of the karyotypes on both media are shown in Table 3. The results for the genotypes and the genotype × medium interactions are illustrated in Figure 1. The frequencies of calli with plant regeneration over media were similar for all karyotypes. MS medium was superior to Kao medium for plant regeneration ($P < 0.05$).

Table 2. Secondary hexaploid triticale varieties and advanced lines and their karyotypes. Experiment 2

Genotype		Karyotype	
No.	Name	Rye genome	Chromosome substitution
1	Eronga 83	Complete	
2	Tarasca 87-1	Complete	
3	Lamb 4	Complete	
4	Fahad 8	Complete	
5	Rhino 3	Complete	
6	Nimir 4-1	Complete	6D/6A
7	Reh/Hare 212-10	Complete	6D/6A
8	Gnu 4	Complete	6D/6A
9	Stier 29	Complete	6D/6A
10	Gaur 1	Complete	6D/6A
11	Pika 2	Substituted	2D/2R
12	Esel	Substituted	2D/2R
13	Panda/Castor//Toro/3/Tapir	Substituted	2D/2R
14	Hippo 1/Panther/2*M2A	Substituted	2D/2R
15	Eda 5/Tapir	Substituted	2D/2R
16	Panther/Panda	Substituted	2D/2R
17	6TA876/6TB164//Panda/Rahum/3/Tesmo 2	Substituted	2D/2R
18	Dingo/Tesmo	Substituted	2D/2R
19	Cananea 79	Substituted	2D/2R
20	Alamos 83	Substituted	2D/2R

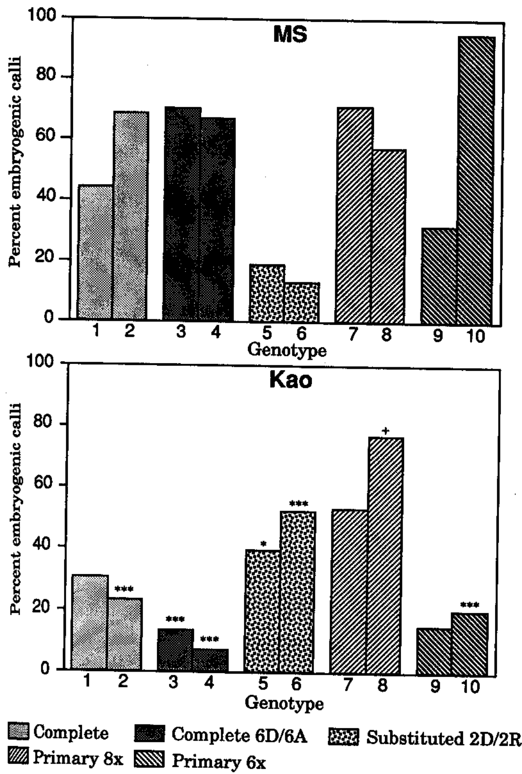

Fig. 1. Frequency of embryogenic calli in Experiment 1 as the percentage of total calli on MS and Kao media. Ten genotypes of five karyotypes. Asterisks in the lower figure indicate significant differences between media for marked genotypes (+ = 10 % level, * = 5 % level, ** = 1 % level, *** = 0.1 % level)

Table 3. Production of embryogenic callus on MS and Kao media of five triticale (TCL) karyotypes. Experiment 1

Karyotype	Medium			
	MS		Kao	
	n	EC %[1]	n	EC %
Secondary TCL				
complete	72	56.9 a	62	25.8 c
complete 6D/6A	63	68.3 a	58	10.3 d
substituted	76	15.8 b	61	44.3 b
Primary TCL				
octoploid	66	63.6 a	62	64.5 a
hexaploid	39	64.1 a	52	17.3 cd

[1] EC % = the frequency of embryogenic calli as a percentage of the total calli (n).

Numbers in columns with the same letter are not significantly different (χ^2, $P > 0.05$).

Experiment 2

There was no difference between MS and Kao medium in the frequencies of embryogenic calli over all genotypes. However, considering all calli, MS medium promoted a significantly higher intensity of somatic embryogenesis than Kao medium ($P < 0.05$). As a group, the substituted karyotypes were inferior to the complete karyotypes on MS medium and to the

Table 4. Production of embryogenic callus on MS and Kao media of three triticale (TCL) karyotypes. Experiment 2

Karyotype	Medium					
	MS			Kao		
	n	EC %[1]	Mean[2] score	n	EC %	Mean score
Secondary TCL						
complete	100	62.0 b	4.03 a	105	61.9 ab	3.92 a
complete 6D/6A	105	87.6 a	4.21 a	102	68.6 a	3.34 a
substituted	239	49.8 c	2.69 b	233	55.8 b	2.70 a

[1] EC % = the frequency of embryogenic calli as a percentage of the total calli (n).
[2] Mean score on a 1—10 scale indicates the size of the embryogenic part in individual calli.

Numbers in columns with the same letter are not significantly different (EC %: χ^2, $P > 0.05$; Score: LSD, $P > 0.05$).

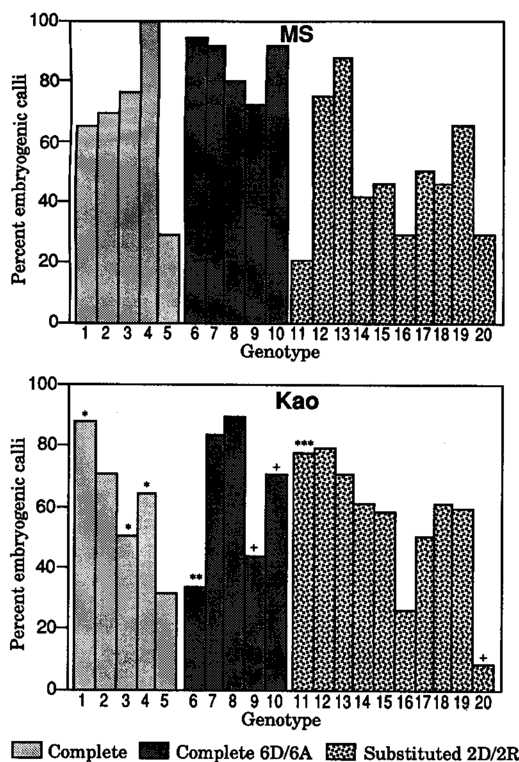

Fig. 2. Frequency of embryogenic calli in Experiment 2 as the percentage of total calli on MS and Kao media. Twenty genotypes of three karyotypes. For further legend see Figure 1

complete 6D/6A karyotype on Kao medium (Table 4). The complete 6D/6A karyotype showed high embryogenic capacity. However, as a group they produced significantly less embryogenic callus on Kao medium than on MS medium ($P < 0.001$). The embryogenic response of the genotypes and genotype × medium interactions is shown in Figure 2.

The frequency of plant regeneration (Table 5) was similar for all karyotypes on MS medium. On Kao medium, plant regeneration of the complete 6D/6A karyotype was significantly lower ($P < 0.05$) than on MS medium and inferior ($P < 0.05$) in comparison with the other karyotypes.

Cultures grown in a 16 h photoperiod produced on average more embryogenic callus than cultures in darkness ($P < 0.05$). The karyotypes showed no significant difference in response to darkness and light ($P > 0.05$). However, the substituted karyotypes tended to respond better in light.

Table 5. Plant regeneration on MS and Kao media of three triticale (TCL) karyotypes. Experiment 2

Karyotype	Medium			
	MS		Kao	
	n	R %[1]	n	R %
Secondary TCL				
complete	54	85.2 a	48	72.3 a
complete 6D/6A	84	76.2 a	53	41.5 b
substituted	108	76.9 a	97	76.3 a

[1] RC % = the frequency of calli that regenerated plants as the percentage of all subcultured calli (n). Numbers in columns with the same letter are not significantly different (χ^2, $P > 0.05$).

Discussion

There were significant differences in the tissue culture response of the spring triticale karyotypes tested. The complete 6D/6A karyotypes showed the highest embryogenic capacity, particularly on MS medium. This karyotype lacks chromosome 6A, which may carry genes that inhibit tissue culture response (KALEIKAU et al. 1989 a). However, there were significant genotype × medium interactions in both experiments with this karyotype; on Kao medium the production of embryogenic callus and plant regeneration were reduced.

The importance of the group 2 chromosomes (in particular the long arm of chromosome 2D), in controlling tissue culture response in wheat, in addition to chromosome 4B (HIGGINS and MATHIAS 1987), has been demonstrated by KALEIKAU et al. (1989 a, b). They suggest that modifier genes are present in other chromosomes. GALIBA et al. (1986) reported that a 2D substitution, among other substitution lines, from a non-responsive wheat line into 'Chinese Spring' showed reduced regeneration ability.

The substituted triticales and the octoploid primary triticales have chromosome 2D. These two karyotypes showed similar positive interaction with the Kao medium in Experiment 1 in contrast to complete hexaploid karyotypes. With the octoploid primary triticales the high *in vitro* response may have been due

to the positive effect of genes on chromosome 2D, and possibly other D-genome genes. In some of the substituted genotypes studied, chromosome 2D seems to have had an adverse effect. JUNG and LELLEY (1985) have suggested that a high proportion of the phenotypic expression of triticale originates from interaction between the wheat and the rye genomes. The expression of genes, that have been found to control tissue culture response in wheat or rye, may be altered or supressed in triticale.

STOLARZ (1991) compared octoploid and hexaploid triticales for their *in vitro* response and reported high response for three octoploid lines on MS medium, but low response on Kao medium, in contrast to the results from this study. The two octoploid lines studied showed high embryogenic response (average 64.1 % of all calli) on both media.

The results were consistent for the secondary karyotype groups between the contrasting donor plant growth environments. In Experiment 1 the differences between the two media were pronounced. MS medium gave more uniform results than Kao medium across environments and culture conditions.

In Experiment 2 the frequency of embryogenic calli and the intensity of somatic embryogenesis were highly correlated ($P < 0.001$) indicating that with genotypes showing a high frequency of embryogenic calli, embryogenesis is initially expressed in several scutellar cells. There was no significant correlation ($P > 0.05$) between embryogenesis and plant regeneration suggesting that these processes in triticale may be controlled in part by different genetic mechanisms, or that there are additional factors influencing the germination of somatic embryoids. AGACHE et al. (1988) made a similar observation in wheat androgenesis.

To exploit tissue culture techniques effectively, embryogenic callus, the starting material for cell suspension and protoplast cultures, needs to be efficiently produced from large numbers of triticale genotypes while retaining plant regeneration ability. Triticale is a diverse crop including karyotypically and phenotypically distinct types. Tissue culture response and growth requirements of these types appear to be different and should be considered when determining optimal culture protocol for triticale improvement.

Zusammenfassung

Einfluß verschiedener Karyotypen auf die somatische Embryogenese unreifer Triticale (× *Triticosecale* Wittmack) Embryonen

Fünf verschiedene Karyotypen von Sommertriticale (primäre oktoploide und primäre hexaploide Triticale, sekundäre hexaploide Triticale mit und ohne 6D/6A Weizen-Weizen Substitution und mit 2D/2R Weizen-Roggen Substitution) wurden auf folgende Merkmale untersucht: Embryogene Kapazität, gemessen an Häufigkeit embryogener Kalli und Intensität somatischer Embryogenese sowie Ausbeute regenerierter Pflanzen. Unreife Embryoexplantate wurden zur Kallusinduktion auf MS- und Kao-Medien mit 2 mg/l 2,4-D angezogen. Die Pflanzenregeneration erfolgte auf hormonfreien Medien. Für alle Merkmale wurden Unterschiede zwischen Karyotypen und signifikante Genotyp × Medium-Interaktionen gefunden. Für oktoploide primäre Triticale ergab sich eine hohe Rate an somatischer Embryogenese. Im Vergleich mit den anderen Karyotypen besaßen die 6D/6A Triticale eine höhere embryogene Kapazität auf MS-Medium. Für einige der 6D/6A Triticale war die Genotyp × Medium-Interaktion signifikant und die embryogene Kapazität auf Kao-Medium niedrig. Die 2D/2R Triticale wiesen im Durchschnitt niedrige embryogene Kapazität auf, erzeugten aber auf Kao-Medium vergleichsweise mehr embryogenen Kallus als auf MS-Medium. Die Merkmalsbeziehung zwischen embryogener Kapazität und Ausbeute regenerierter Pflanzen war nicht signifikant. Unterschiede im Karyotyp sollten bei der Auswahl der Medien und der Kulturmethode berücksichtigt werden, um den optimalen Einsatz von Gewebekulturtechniken in der Triticalezüchtung zu gewährleisten.

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