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Dodig D., Nikolic R., Mitic N.

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Tissue culture response of different wheat genotypes, environmental effect and association with plant traits

D. Dodig*, R. Nikolić and N. Mitić**[1]**
*ARI "Serbia", Center for Agricultural and Technological Research, Grljanski put bb, 19000 Zajecar, Serbia
**Institute for Biological Research "Sinisa Stankovic", Bul. Despota Stefana 142, 11000 Belgrade, Serbia

SUMMARY – The tissue culture response of immature embryos of 96 bread wheat genotypes was evaluated by measuring the percentage of callus formation (CF), the percentage of calli with regenerative potential (RC), and the average number of plants per regenerating embryo (PPE). The results showed great variability for RC among tested genotypes and strong genotype effect on this trait. Correlation analysis revealing positive and significant relationship between CF and early vigour, peduncle length, peduncle extrusion, spike index and yield per plant, as well as RC and PEE with chlorophyll content in flag leaf.

Introduction

Regeneration of cultured cells or tissues is important step for crop improvement through biotechnological approaches. These approaches, including genetic transformation of wheat, can be used into conventional breeding programs to increase genetic variability and to improve some of the agronomic traits. For the practical application of these methods, efficient regeneration of plants is required. Many factors could affect tissue culture responses of wheat, particularly formation of embryogenic calli and plant regeneration, such as explant tissue, culture medium and its supplements, donor plant growth conditions, while genetic factor is major contributor to the in vitro response of cereal tissues in culture (Hartmann *et al*., 1989). *In vitro* response of given cultivar cannot be known before being tested, because we have not a lot of data about the related mechanisms of its control. To date, some effort has been made on this way and Ben Amer *et al*. (1995, 1997) reported the potential genes that affect the plant regeneration ability from immature embryos of wheat. If the *in vitro* trait could be correlated to some of the agronomic traits, it would be possible to predict the tissue culture response in advance.

Plant material and experimental data

Ninety-six genotypes of bread wheat (cultivars, lines, local varieties), collected at the Institute for Field and Vegetable Crops Novi Sad, were grown in experimental field at Center for Agricultural and Technological Research in Zajecar, and used as embryo donor plants. These genotypes were chosen on the basis of contrasting expression for one or other of 26 traits important for wheat breeding program in Serbia (Kobiljski *et al*., 2002). Field experiments were carried out during 2002/03, 2003/04 and 2004/05 seasons. Seeds of each genotype were sown in October in single 1 m row at 20 cm spacing in two replications, with a sowing rate of 70 seeds per row. A complete randomized block design was used in the trials. Plants were scored for 25 various traits: agronomic, developmental and physiological. The traits measurement was done with 30 randomly selected plants of each genotype. Growing conditions were not artificially controlled and the seasons were different according to precipitation and air temperatures. The season 2002/03 was characterized by high air temperatures during anthesis and grain filling period and with smaller than usual amount of rainfalls during vegetative phase. The number of tropical days (max. temperature over 30°C) was 1 in April, 7 in May, and 15 in June. The environmental conditions were more favorable in the next two seasons, 2003/04 and 2004/05, when the tropical days (5 and 7, respectively) were detected only in June.

[1] Author for correspondence: Dr Nevena Mitić (mitic.nevena@ibbis.bg.ac.yu)

Immature seeds were collected 13-15 days after anthesis from field-grown donor plants and stored at 4 ºC for 24 h in refrigerator. They were surface sterilized using 70% ethanol (1 min), rinsed 3 times with sterile distilled water, disinfected in a solution containing commercial NaOCl bleach (8% active chlorine) with a few drops of the fungicide Captan (30 min), and rinsed four times with sterile water. Immature embryos, about 1.5-2 mm long, were isolated aseptically and cultured, with scutellar side up, on 20 ml solid nutrient medium in Petri dishes (30 embryos per one dish) in two replicates per genotype. The culture medium contained MS (Murashige and Skoog, 1962) mineral salts and vitamins, 100 mg l^{-1} casein hydrolysate, 30 g l^{-1} sucrose, 0.7% agar (Torlak), and was supplemented with 2 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) for callus initiation (two subcultures in 20 day-intervals). Regenerating calli were transferred to MS growth regulator-free medium for another two subcultures. The cultures were incubated at 25±2ºC, in white fluorescent light, with irradiance of 47 µmol m^{-2} s^{-1}, and day/night regime of 16/8.

The in vitro response of 96 wheat genotypes was evaluated by measuring the percentage of callus formation (CF), the percentage of calli with regenerative potential (RC), and the average number of plants per regenerating embryo (PPE). The experiment was performed in three successive years 2003, 2004 and 2005. About 60 embryos per genotype from two replicates were used in each experimental year. CF was scored four weeks after plating, and it was calculated as the number of embryos with induced callus over the total number of embryos plated x 100. RC was calculated after six weeks of culture on 2,4-D containing medium. PPE was measured two weeks after the regenerative calli were transferred to growth regulator-free medium and calculated as mean number of plants obtained per 10 regenerative calli.

Analysis of variance was done using a randomized block design with two factors, allowing the components of variance to be calculated. Relationships between tissue culture and some agronomic, developmental and physiological traits were determined by Pearson correlation analysis. Only significant ($p < 0.05$) and highly significant ($p < 0.01$) correlations were presented in this study.

Callus formation from immature embryos

All wheat genotypes formed calli while the induction rate varied depended on year. Coefficient of variation for this trait was 5.1%. In the year 2003 CF rate varied from 36.7% to 100 % (mean CF 79.5%). Four genotypes showed a low rate of CF fewer than 50.0%: Hira (36.7), Ai Bian (38.3), Vireo S (43.3) and Brigand (46.7). Higher CF rate embryos displayed in years 2004 and 2005 when mean CF was 97.9% (ranged from 68.4 to 100%) and 99.5% (ranged from 94.3 to 100%), respectively.

Shoot and plant regeneration

The regeneration response was detected as the frequency of green spotted calli, so-called regenerative calli (RC). A wide variation in frequency (CV=66.7%) of RC was observed, and a statistically significant differences in the mean value of this trait among genotypes and years were found. Generally, mean regeneration potential in 2003, compared to 2004 and 2005, was decreased and it's varied from 0 to 72.5% (mean 17.5%) in 2003, 0 to 97.9% (38.0%) in 2004, and 0 to 94.0% (36.3%) in 2005. The highest responsive genotypes with a mean regeneration response higher than 70% over tree years were: Donska semidwarf (77.9%), UC 65680 (73.1%), NS 74/95 (72.4%) and Mexico 120 (71.6%). According to the years the highest RC was observed in Florida (72.5%) in 2003, Avalon (97.9%) in 2004 and Mexico 3 (94.0%) in 2005. In 2003 RC was severe reduced in Magnif 41 (65.8%), NS 66/92 (61.7%) and Mexico 3 (55.8%) in comparison to their mean for 2004 and 2005. The highest difference in RC between 2003 (unfavorable year) and mean for 2004 and 2005 (favorable years) was found in Magnif 41 (65.8%), NS 66/92 (61.7%) and Mexico 3 (55.8%). Number of plants per embryo (PPE) ranged from 0.3-10.7 (mean 3.1) in 2003, 2.0-7.7 (4.7) in 2004, and 1.5-14.3 (6.5) in 2005. Over the 3 years PPE ranged from 1.1 in Lambriego Inia to 8.9 in Capelle Desprez. Coefficient of variation for this trait was 35.6%. Based on the results obtained, genotypes that produced higher percent of regenerative calli, produced also higher number of plants per regenerative callus.

Components of phenotypic variance

The analysis of the components of phenotypic variance is presented in Table 1. The highest percentage of the whole phenotypic variability for CF was assigned to year (71.4), while only 0.7 % was assigned to genotype. About 47% of total phenotypic variability for RC belonged to pure genetic variance. Even though the influence of the environment is evident, the greatest part of phenotypic variability for RC depended on genetic background of the examined genotypes, which makes the choosing genotypes for this trait more reliable. According to the results in Table 1, it is difficult to make a reliable judgment on PPE in a particular growth season. Changes of environmental conditions affect the phenotype considerably, especially having in mind the genotype x year interaction that appeared in the experiment.

Table 1. Components of phenotypic variance for regenerative calli (RC), callus formation (CF) and number of plants per embryo (PPE)

Source of variation	DF	Components of variance for CF		Components of variance for RC		DF	Components of variance for PPE	
		σ^2	%	σ^2	%		σ^2	%
Genotype (G)	95	1.7	0.7	173.0	47.1	95	1.7	17.6
Year (Y)	2	169.3	71.4	81.2	22.1	2	2.8	29.5
G x Y	190	29.2	12.3	90.9	24.8	190	2.9	30.4
Error	287	36.9	15.6	21.9	6.0	576	2.2	22.5

Association with plant traits

Pearson correlation analyses (Table 2) showed that CF was significantly positively related to spike index (r = 0.324**), yield per plant (r = 0.285**), peduncle extrusion (r = 0.269**), early vigour (r = 0.252*) and peduncle length (r = 0.240*). Negative and significant correlation was recorded between CF and sterile spikelets per spike (r = 0.326**). Association of RC and PPE was positive and significant only with chlorophyll content in flag leaf four weeks after flowering (r = 0.227* and 0.284**, respectively), while negative and significant correlation for both traits was observed with fertile spikelets per spike, days to heading and sterile spikelets per spike. Sterile spikelets per spike displayed negative and significant relationship with the all studied tissue culture response traits. The similar pattern of association between RC and PPE with traits presented in Table 2 is probably due to highly positive and significant relationship (r = 0.831**) between them.

Table 2. Simple correlation coefficients of regenerated calli (RC), callus formation (CF) and plants per embryo (PPE) with various plant traits for the 100 wheat genotypes (averaged over 3 years)

Character	CF	RC	PPE
Early vigour	0.252*	-0.006	-0.046
Days to heading	-0.078	-0.283**	-0.287**
Plant height	0.223*	-0.116	-0.140
Spike index	0.324**	0.122	0.110
Peduncle length	0.240*	-0.053	-0.045
Peduncle extrusion	0.269**	0.018	0.058
Fertile spikelets per spike	0.030	-0.236*	-0.299**
Sterile spikelets per spike	-0.326**	-0.201*	-0.212*
Chlorophyll content in flag leaf	0.080	0.227*	0.284**
Yield per plant	0.285**	-0.058	-0.053

*p < 0.05; ** p < 0.01.

In conclusion, the regenerative potential of calli varied significantly among tissue culture response traits tested, it was genotype depended, although environmental conditions can influence some modification of callus regeneration potential. Among 25 various plant traits only chlorophyll content in flag leaf showed positive and significant correlation with the regenerative potential of calli.

References

Ben Amer, I.M., Worland, A.J. and Börner, A. (1995). *Plant Breeding,* 114: 84-85.

Ben Amer, I.M., Korzun, V., Worland, A.J. and Börner, A. (1997). *Theor. Appl. Genet*., 94: 1047-1052.

Harttmann, C., Hery, Y., DeBuyser, J., Aubry, C. and Rode, A. (1989). *Theor. Appl. Genet*., 77: 169-175.

Kobiljski, B., Quarrie, S.A., Denčić, S., Kirby, J. and Ivegas, M. (2002). *Molecular Biology Letters,* 7: 685-694.

Murashige, T. and Skoog, F. (1962). *Physiol. Plant*., 15: 473-497.