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VARIABILITY OF *IN VITRO* CULTURE RESPONSE IN WHEAT GENOTYPES, GENOTYPE AND ENVIRONMENTAL EFFECTS

Nevena MITIĆ [1], Dejan DODIG [2] and Radomirka NIKOLIĆ [1]

[1]Institute for Biological Research "Sinisa Stankovic", Belgrade,
[2]Agriculture Research Institute "Serbia", Center for Agricultural and Technological Research, Zajecar, Serbia

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The tissue culture response (TCR) of immature embryos, evaluated according to callus formation, percentage of regenerative green-spotted calli and the number of plants per embryo, was investigated in 96 wheat genotypes of worldwide origins. Immature embryos were collected 12-15 DAP from field-grown plants during three successive years 2003, 2004 and 2005. Year 2003 was with high air temperatures and tropical days during a period of vegetation, while the environmental conditions were more favorable for plant growth in the next two years, 2004 and 2005. Embryos were cultured on standard MS medium containing 2 mg l^{-1} 2, 4-D. In all genotypes calli were efficiently induced, ranging from 36.7 to 100% (2003), 68.4 to 100% (2004), and 94.3 to 100% (2005). The calli occasionally formed green spots, but frequencies markedly differed among genotypes that varied from 0 to 72.5% (2003), 0 to 97.9% (2004), and 0 to 94.0% (2005). Coefficient of variation was highest in term of

Corresponding author: Mitić Nevena, Institute for Biological Research "Sinisa Stankovic", Bul. Despota Stefana 142, 11000 Belgrade, Serbia ,email: mitic.nevena@ibiss.bg.ac.yu

percent of regenerative calli (66.7%) following by a number of plants per embryo (35.6%) and callus formation (5.1%). Components of phenotypic variance showed that factor year (71.4%) had the highest impact on expression of callus formation, genetic factor (47.1%) on percentage of regenerative green-spotted calli and interaction year/genotype (30.3%) on number of plants per embryo. The results indicated factor genotype as the most important for determining regeneration potential in wheat.

Key words: immature embryos, callus formation, regenerating calli, plant regeneration, genotype, environmental condition, wheat

INTRODUCTION

Wheat is one of the most important crops in the world. With the development of plant molecular biology and genetic transformation has become one of the central issues in molecular breeding (VASIL, 1994). Biotechnological approaches involving genetic transformation of wheat (PATNAIK and KHURANA, 2001) can be integrated into conventional breeding efforts to enlarge the germplasm pool and to enhance agronomic traits. For the practical application of these methods, efficient production of transgenic plants is required. Plant regeneration from target tissues or cells is an important step in transgenic plant production. Several tissues and cells have been examined as targets, such as immature embryos, immature inflorescences, microspores, mature embryos, shoot meristematic cultures, and protoplasts isolated from embryogenic cell suspensions (for review see MAHESHWARI *et al*., 1995). Among them calli derived from immature embryos appear to be easy to handle and to regenerate plants efficiently in wheat.

Many factors could affect tissue culture responses of wheat, particularly formation of embryogenic calli and plant regeneration. These factors include explant tissue (VASIL, 1994), culture medium and its supplements (MATHIAS and SIMPSON, 1986), donor plant growth conditions (HESS and CARMAN, 1998), among which genotype is often the dominant one. However, for a given cultivar, the cultural response cannot be known before being tested, because we have not a lot of data about the related mechanisms of tissue culture response control. In several cases, *in vitro* regeneration is shown to be a quantitative trait, (BHASKARAN and SMITH, 1990; BREGITZER and CAMPBELL, 2001), and the hexaploid nature of wheat makes the situation even more complex. To date, several studies have been described for mapping the genes that affect the plant regeneration ability from wheat immature embryos (BEN AMER *et al*., 1995, 1997).

As a part of long-term project on wheat biotechnology, we evaluated the tissue culture response in 96 wheat genotypes of worldwide origins. In tissue culture of cereals, including wheat, 2, 4-D is often used for callus induction (BHASKARAN and SMITH, 1990). This growth regulator is used to induce wheat calli, studied the callus formation and regeneration ability in wheat immature

embryos and influence of genotype and environmental condition factors on the culture response.

MATERIAL AND METHOD

Plant material

Ninety-six genotypes of 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). Seeds were sown in October of 2003, 2004 and 2005. Rows were 1 m long 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. Weeds were periodically removed by hand. The environmental conditions were not artificially controlled and growth conditions during vegetation were different among the years according to precipitation and air temperatures (Table 1).

Table 1: Precipitation and air temperature in the vegetative stage during 3 years at Zaječar

Year	Precipitations (mm)			Air temperature (°C)					
				April		May		June	
	April	May	June	Mean	Max*	Mean	Max*	Mean	Max*
2003	89.0	60.5	43.3	18.9	34.6 (1)	18.9	34.6 (7)	22.5	35.1 (15)
2004	46.4	28.6	81.3	14.8	28.8 (0)	14.8	28.8 (0)	19.5	32.2 (5)
2005	49.6	73.3	25.3	16.7	30.6 (0)	16.7	30.6 (0)	19.1	33.9 (4)

- *in parenthesis is given a number of tropical days (max. temperatures over 30°C)*

Immature embryo culture

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 was transferred to MS growth regulator-free medium for another two subcultures. The cultures were incubated at 25±2 ºC, in white fluorescent light, with irradiance of 47 µmol m-2 s^{-1}, and day/night regime of 16/8.

Evaluation of tissue culture response and statistical analysis

The *in vitro* response of ninety-six wheat genotypes was evaluated by measuring the following parameters: the percentage of immature embryos which developed a callus tissue, the percentage of calli with regenerative potential, and the average number of plants per regenerating embryo. The experiment was performed in three successive years 2003, 2004 and 2005. About 30 embryos in two replicates (about 60 embryos) per genotype were used in each experimental year. The callus formation 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. Callus regeneration was scored after six weeks of culture on 2, 4-D containing medium. The criterion used to determine regeneration was the formation of green spots or distinguishable shoots. Callus regeneration rate was defined as the percentage of callus that had regenerated shoots. Plants regenerated per regenerative embryo was scored 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.

The data were analyzed using two factorial ANOVA and the LSD value was employed for the mean comparisons. The components of phenotypic variability were analyzed in order to study the influence of the genotype and the environment on phenotype.

RESULTS AND DISCUSSION

Callus induction from immature embryos

Calli were induced from immature embryos of 96 wheat genotypes, isolated 13-15 days after anthesis, which was correspond to the embryo developmental stage III (TAKUMI and SHIMADA, 1997). All wheat genotypes induced calli while the induction rate varied depended on year and genotype (Table 2), and coefficient of variation for this trait was 5.1%.

In the year 2003 callus induction rate was in average 79.5%, and at 92 genotypes more than 70.0% of embryos formed calli. Only four genotypes showed a low rate of callus induction, ranged from 36.7% (Hira) to 46.7% (Brigand). Generally for all genotypes, the higher callus induction rate embryos displayed in the years (2004 and 2005) with suitable environmental conditions for the donor plant growth. In these years more than 90.0% of isolated embryos in all genotypes produced calli. A high frequency of callus induction from immature embryos of wheat was also observed earlier by BOMMINENI and JAUHAR, 1996; MITIĆ *et al*., 1999; MACHHII *et al*., 1998).

Table 2. Callus formation and regeneration from immature embryos of 96 wheat genotypes

Genotype - Origin	Callus formation, %				Regenerating calli, %			
	2003	2004	2005	Average	2003	2004	2005	Average
Tibet Dwarf - TIB	76.7	98.0	100.0	91.6	13.0	27.5	24.7	21.7
Ai-bian - JPN	38.3	98.0	96.0	77.4	17.7	10.3	14.9	14.3
Tom Thumb - TIB	91.7	98.0	100.0	96.6	1.7	3.3	0.0	1.7
HAYS 2 - USA	91.7	100.0	98.0	96.6	47.3	50.0	61.4	52.9
F 4 4687 - ROM	78.3	100.0	100.0	92.8	10.8	18.0	26.0	18.3
Min. Dwarf - AUS	71.7	93.5	100.0	88.4	32.9	30.5	34.0	32.5
Hira - IND	36.7	100.0	100.0	78.9	9.8	55.0	62.0	42.3
Mexico 120 - AUS	60.0	100.0	98.1	86.0	60.9	88.0	66.0	71.6
Norin 10 - JPN	93.3	100.0	100.0	97.8	12.4	18.0	18.0	16.1
L-1 - HUN	68.3	100.0	100.0	89.4	7.4	42.0	58.0	35.8
NS 602 - SER	63.3	100.0	95.8	86.4	15.4	88.0	36.2	46.5
NS 559 - SER	66.7	100.0	100.0	88.9	13.0	44.0	69.5	42.2
UPI-301 - IND	90.0	100.0	100.0	96.7	0.0	15.9	26.0	14.0
Saitama - 27 - JPN	81.7	98.0	100.0	93.2	46.9	65.5	78.9	63.8
Vireo S - MEX	43.3	100.0	100.0	81.1	17.3	40.0	43.2	33.5
L 1/91 - SER	85.0	100.0	100.0	95.0	13.7	44.0	36.7	31.4
L 1A/91 - SER	90.0	100.0	100.0	96.7	12.1	14.6	10.0	12.2
Nizija - SER	78.3	100.0	98.0	92.1	2.2	22.0	36.8	20.3
Rusalka - BGR	91.7	100.0	100.0	97.2	11.0	22.0	81.7	38.2
Suwwon 92 - IND	81.7	98.0	100.0	93.2	4.1	25.5	16.0	15.2
Sonalika - IND	85.0	100.0	100.0	95.0	2.1	5.9	4.0	4.0
Dons. polup. - RUS	95.0	100.0	100.0	98.3	68.0	84.0	81.7	77.9
NS 55-25 - SER	73.3	98.0	98.0	89.8	11.4	13.9	16.4	13.9
Slavija - SER	91.7	100.0	98.0	96.6	34.0	32.0	34.8	33.6
T. dirk S - AUS	86.7	100.0	98.0	94.9	4.6	30.0	20.4	18.3
Cajeme 71 - MEX	90.0	98.0	100.0	96.0	9.3	8.2	24.6	14.0
Acciaio - ITA	85.0	98.0	96.0	93.0	4.0	10.2	6.0	6.7
Szegedi 768 - HUN	81.7	100.0	100.0	93.9	63.4	74.0	72.0	69.8
Timson - AUS	71.7	100.0	100.0	90.6	0.0	36.4	12.3	16.2
Mex. 17 bb - MEX	80.0	100.0	100.0	93.3	50.0	68.0	53.1	57.0
Mexico. 3 - MEX	88.3	100.0	100.0	96.1	19.0	55.6	94.0	56.2
Cap. Despr. - FRA	85.0	68.4	100.0	84.5	38.9	90.7	64.4	64.6
Inia 66 - MEX	85.0	100.0	100.0	95.0	13.8	62.0	64.0	46.6
Durin - FRA	56.7	100.0	100.0	85.6	59.1	50.3	66.6	58.6
Avalon - GBR	66.7	96.0	100.0	87.6	36.0	97.9	40.9	58.2
Brigand - GBR	46.7	88.0	97.8	77.5	21.4	20.5	9.3	17.1
TJB 990-15 - GBR	83.3	98.0	100.0	93.8	33.8	26.6	32.0	30.8
Highbury - GBR	93.3	100.0	100.0	97.8	7.2	22.0	8.3	12.5
Pobeda - SER	80.0	100.0	100.0	93.3	14.6	18.0	18.0	16.9
NS 46/90 - SER	78.3	100.0	100.0	92.8	0.0	60.0	30.0	30.0
NS 63-24 - SER	88.3	100.0	100.0	96.1	7.5	10.0	24.3	13.9
WWMCB 2 - USA	76.7	100.0	98.0	91.6	19.6	38.3	75.6	44.5
NS 33/90 - SER	91.7	100.0	98.0	96.6	2.0	20.0	20.5	14.2
NS 79/90 - SER	85.0	95.9	100.0	93.6	7.9	48.9	52.0	36.2
Holly E - USA	80.0	100.0	100.0	93.3	10.7	32.0	24.0	22.2
Hope - USA	88.3	98.0	100.0	95.4	3.5	22.4	22.0	16.0
NS 66/92 - SER	86.7	100.0	100.0	95.6	17.0	77.4	80.0	58.1
Sofija - SER	85.0	95.9	100.0	93.6	0.0	0.0	8.0	2.7
NS 74/95 - SER	95.0	96.0	100.0	97.0	54.4	89.6	73.4	72.4
BCD 1302/83-MDA	93.3	100.0	100.0	97.8	0.0	36.0	24.0	20.0
Benni multifl.- USA	85.0	98.0	94.3	92.4	3.9	12.2	9.9	8.7
ZG 987/3 - CRO	73.3	93.9	100.0	89.1	0.0	24.0	8.0	10.7

ZG 1011 - CRO	61.7	100.0	100.0	87.2	0.0	12.4	16.0	9.5
ZGK 3/82 - CRO	88.3	96.0	100.0	94.8	0.0	22.5	13.0	11.8
ZGK 238/82 - CRO	55.0	96.0	100.0	83.7	15.1	22.9	16.0	18.0

Table 2 (continued)

Genotype - Origin	Callus formation, %				Regenerating calli, %			
	2003	2004	2005	Average	2003	2004	2005	Average
ZGKT 159/82-CRO	45.0	98.0	100.0	81.0	5.6	6.1	5.0	5.6
INTRO 615 - USA	88.3	100.0	100.0	96.1	5.8	20.0	12.0	12.6
Lambr. Inia - CHL	71.7	100.0	100.0	90.6	0.0	4.1	16.0	6.7
Magnif 41 - ARG	93.3	96.0	100.0	96.4	19.1	93.8	81.5	64.8
Vel - USA	81.7	98.0	100.0	93.2	41.0	93.8	54.0	62.9
Ana - CRO	85.0	100.0	100.0	95.0	3.9	50.0	59.6	37.8
Helios - USA	71.7	86.0	100.0	85.9	16.9	39.9	78.0	44.9
Renesansa - SER	93.3	94.0	98.0	95.1	15.5	35.9	25.1	25.5
Gala - ARG	88.3	96.0	100.0	94.8	16.0	43.5	36.0	31.8
S. Eligulata - USA	83.3	100.0	98.0	93.8	0.0	58.0	49.0	35.7
UC 65680 - USA	91.7	96.1	100.0	95.9	48.4	91.9	79.2	73.1
Tr. Sphaeroc.-USA	53.3	97.9	100.0	83.8	2.7	6.7	17.0	8.8
Tr. Compact.-SER	83.3	100.0	100.0	94.4	12.0	94.0	6.0	37.3
Sava - SER	78.3	90.0	100.0	89.4	4.3	24.2	36.9	21.8
Mina - SER	73.3	100.0	100.0	91.1	0.0	20.0	29.6	16.5
Nov. Crvena - SER	83.3	98.0	100.0	93.8	55.9	55.2	72.0	61.0
Florida - USA	68.3	98.0	100.0	88.8	72.5	57.0	56.0	61.8
Al Kan Tzao - CHN	65.0	100.0	98.0	87.7	54.0	60.0	57.9	57.3
Centurk - USA	90.0	98.0	100.0	96.0	7.3	73.4	50.0	43.6
T.dirk B/775- AUS	85.0	96.0	100.0	93.7	0.0	23.5	30.0	17.8
Ch.-Chang 6-CHN	68.3	100.0	100.0	89.4	65.6	54.0	89.9	69.8
Peking 11 - CHN	75.0	100.0	98.0	91.0	4.4	50.0	42.8	32.4
Cook - AUS	90.0	97.9	100.0	96.0	1.9	20.9	22.9	15.2
Kite - AUS	78.3	100.0	100.0	92.8	6.3	32.7	34.0	24.3
Phoenix - USA	83.3	93.7	100.0	92.3	12.0	43.2	40.9	32.0
Bezostaya 1 - RUS	88.3	100.0	96.0	94.8	10.1	28.0	20.9	19.7
Nova Banat. - SER	86.7	100.0	100.0	95.6	2.0	20.0	13.5	11.8
Mironov. 808-UKR	80.0	100.0	100.0	93.3	18.6	48.0	44.9	37.1
Bank. 1205 - HUN	85.0	91.9	100.0	92.3	7.5	11.1	10.0	9.5
Lr 12 - USA	83.3	100.0	100.0	94.4	11.8	21.4	34.0	22.4
Lr 10 - USA	100.0	100.0	100.0	100.0	19.2	16.4	42.0	25.8
Purd./Loras - USA	95.0	100.0	100.0	98.3	1.7	38.0	24.0	21.2
Red Coat - USA	80.0	100.0	100.0	93.3	15.1	26.0	0.0	13.7
Purd. 39120 - USA	73.3	100.0	100.0	91.1	22.8	31.0	16.0	23.2
Purdue 5392 - USA	85.0	92.0	100.0	92.3	15.6	37.5	16.0	23.0
Ivanka - SER	81.5	100.0	100.0	93.8	8.0	13.0	14.0	11.7
NS 22/92 - SER	84.5	100.0	98.0	94.2	8.4	24.5	17.6	16.8
PKB Krupna - SER	85.5	100.0	100.0	95.2	29.0	33.5	28.0	30.2
T. Dirk B/12 - AUS	87.5	100.0	100.0	95.8	8.0	18.0	23.0	16.3
Nor.10/Bre.14-USA	79.0	100.0	100.0	93.0	0.0	4.0	0.0	1.3
Siete Cerros - MEX	70.0	84.2	100.0	84.7	50.5	68.0	76.5	65.0
Average	79.5	97.9	99.5	92.3	17.5	38.0	36.3	30.6
CV (%)	16.4	4.4	1.2	5.1	108.4	67.4	68.7	66.6
LSD (0.05) for genotype			5.32					6.91
LSD (0.01) for genotype			7.01					9.08
LSD (0.05) for year			0.94					1.22
LSD (0.01) for year			1.24					1.61
LSD (0.05) for interaction			9.21					11.96
LSD (0.01) for interaction			10.14					15.76

Shoot and plant regeneration

The regeneration response was detected as the frequency of green spotted calli and it is presented in Table 2. Calli with green spots, so-called regenerative calli, were observed in most of the wheat genotypes examined in this work. However, a wide variation in frequency of calli containing green spots was observed (CV=66.7%), and a statistically significant differences in the mean value of this trait among cultivars and years was found. In the years 2003, 2004 and 2005, the variation ranged from 0 to 72.5% (17.5% on average), 0 to 97.9% (38.0%), and 0 to 94.0% (36.3%), respectively. The highest responsive genotypes with an average regeneration response in the three years over 60% were Donska polupatuljasta (77.9%), UC 65680 (73.1%), NS 74/95 (72.4%) and Mexico 120 (71.6%), followed by Szegedi 768 (69.8%), Ching-chang 6 (69.8%), Siete Cerros (65.0%), Magnif 41 (64.8%), Capelle Desprez (64.6%), Saitama 27 (63.8%), Vel (62.9%), Novosadska crvena (61.8%), and Mina (61.0%). According to the years 2003, 2004, and 2005, the highest regeneration potential was observed in the genotype Florida (72.5%), Avalon (97.9%) and Mexico 3 (94.0%), respectively. On the other hand, ten genotypes in the year 2003 (NS 63-24, Sofija, BCD 1302/83, ZG 987/3, ZG 1011, ZGK 3/82, Lambriego Inia, Mina, Tr. Dirk "B" (GK 775), Norin 10/Brevor 14), and in the year 2005 two cultivars (Norin 10/Brevor 14, Red Coat) did not produce regenerating calli. It is evident that unfavorable environmental conditions during vegetation season in 2003 influenced decreasing of regenerative response in most of the wheat genotypes. The higher difference in regeneration potential in 2003 compared to 2004 and 2005 was found in Magnif 41 (65.8%), NS 66/92 (61.7%) and Mexico 3 (55.8%). Contrary, eight genotypes displayed higher regeneration potential in 2003 then in 2004 and 2005. Among them Florida and Brigand had 16.0% and 6.5% better regeneration potential in the year 2003. Certain improvement of regeneration from calli in three Australian wheat cultivars after their exposure to dehidratation stress was also reported by KANNA and DAGARD (2001). These results suggested that although the plant genotype plays critical role affecting the efficiency of regeneration from explants (MATHIAS and SIMPSON, 1986; RAJYALAKSHMI *et al.*; 1987, TAKUMI and SHIMADA, 1997), while environmental factor can modify significantly regeneration potential of wheat calli. HESS and CARMAN (1998) reported that certain environmental conditions such as high donor plant temperature can eliminate embryogenic competence in small embryos. Their explanation for this phenomenon is hormone-based hypothesis that environmental factor decrease hormone (cytokinin/auxin) level and induces or prolongs embryogenic competence regardless of genotype.

Calli were regenerated green shoots. Number of plants obtained per one regenerative callus ranged from 0.3-10.7 (3.1 on average) in 2003, 2.0-7.7 (4.7) in 2004, and 1.5-14.3 (6.5) in 2005. Over the three years average number of plants obtained per one regenerative callus ranged from 1.1 in Lambriego Inia to 8.9 in Capelle Desprez (Figure 1). 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.

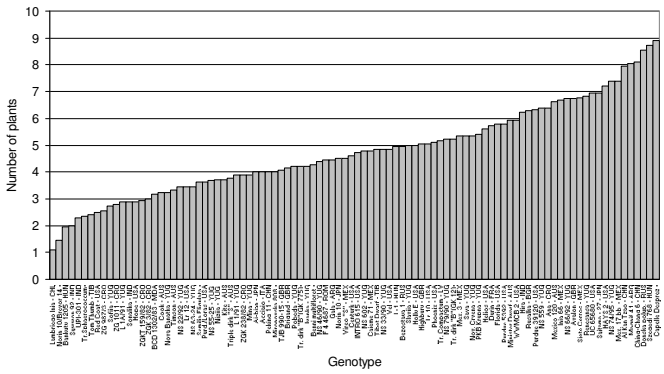

Fig. 1. Mean plant number per embryo (ascending ranked) for 100 genotypes trailed in 2003-2005 in Zaječar

Analysis of the components of phenotypic variance showed that factor year (71.4%) had the highest impact on expression of callus formation, genetic factor (47.1%) on percentage of regenerative green-spotted calli and interaction year/genotype (30.3%) on number of plants per embryo.

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. The wheat genotypes showed high ability for plant regeneration in immature embryo culture seem to be the most suitable for further work concerning *in vitro* wheat improvement and for genetic engineering.

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VARIRANJE *IN VITRO* ODGOVORA PŠENICE, UTICAJ GENOTIPA I SPOLJNE SREDINE

Nevena MITIĆ [1], Dejan DODIG [2] i Radomirka NIKOLIĆ [1]

[1]Institut za biološka istraživanja "Siniša Stanković", Beograd
[2]Institut za istraživanja u poljoprivredi SRBIJA, Centar za poljoprivredna i tehnološka istraživanja, Zaječar, Srbija

I z v o d

Nezreli embrioni 96 genotipova pšenice, poreklom iz različitih delova sveta, gajeni su u kulturi i proučavan je njihov *in vitro* odgovor na osnovu procenta formiranja kalusa, procenta regenerativnih kalusa i broja biljaka po embrionu. Nezreli embrioni sakupljeni su sa biljaka gajenih u polju u toku tri uzastopne godine (2003, 2004, 2005). Prva godina (2003) je bila sa relativno povoljnim rasporedom padavina, ali sa visokim prosečnim temperaturama vazduha i učestalom pojavom tropskih dana u vegetacionom periodu. Sledeće dve godine (2004 i 2005) bile su znatno povoljnije u pogledu temperatura i količine i rasporeda padavina. Ebrioni su gajeni na MS podlozi sa 2 mg l^{-1} 2,4-D. Svi genotipovi pokazali su visok potencijal za obrazovanje kalusa, koji je iznosio od 36.7 do 100% u 2003., 68.4 do 100% u 2004. i 94.3 do 100% u 2005. godini. Pojedini indukovani kalusi su formirali zelene tačke i potom pupoljke. Procenat regenerativnih kalusa je znatno varirao u zavisnosti od genotipa i godine i znosio je od 0 do 72.5% (2003), 0 do 97.9% (2004), i 0 to 94.0% (2005). Broj biljka po embrionu u proseku za sve genotipove po godinama je iznosio 3.1, 4.5 i 6.5, respektivno. Najveći koeficijent varijacije je zabeležen kod osobine procenat regenerativnih kalusa (66.7%), zatim kod broja biljaka po embrionu (35.6%) a najmanji za procenat formiranih kalusa (5.1%). Analiza komponenti fenotipske varijabilnosti je pokazala da je najveći uticaj na variranje procenta formiranih kalusa imala godina (71.4%), regenerativnih kalusa genotip (47.1%) a na broj biljaka po embrionu interakcija godine i genotipa (30.3%).

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