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IN VITRO ASSESSMENT OF WHEAT TOLERANCE TO DROUGHT

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Analyzed in this paper were the *in vitro* effects of drought stress in 13 genotypes of winter wheat, one genotype of spring wheat, and three Triticale genotypes of different geographic origin. Callus tissue was induced from immature zygotic embryoes (10-15 days after pollination) on a modified MS nutrient medium. After two weeks, callus tissue was transplanted onto the same medium enriched with 5% high-molecular polyethylene glycol (PEG 6000), which was used as the stress agent to produce the effect of drought chemically. A control group of calluses was grown on an identical medium but without PEG. After four weeks of growing calluses on these mediums, we assessed callus mass survival ability of the genotypes before the transplantation as well as percentage reduction of callus fresh weight after the transplantation onto the nutrient medium with 5% PEG. Statistically significant differences were found among the genotypes in their response to the induced stress. The best survival ability before the transplantation was found in the genotype Mexico120 (83%), while the lowest was recorded in Slavija (11.3%). Culture growing under stress conditions significantly reduced callus fresh weight in all of the genotypes. The lowest decrease of the callus mass relative to control was recorded in Rozofskaja (14.4%) and the highest in Miranovska (58.4%), indicating the genotypes' tolerance levels towards drought stress.

Key words: whear, drought, embryo culture

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INTRODUCTION

Drought is one of the most important and earliest studied abiotic stresses and one of the major limiting environmental factors for plant development and, hence, plant mass production. Plant defense against a water deficit is a complex endeavour that the plant undertakes to protect itself. In order to overcome the stressful state of drought, plants have developed different adaptation mechanisms to mitigate the effects of water deficits, such as the reduction of leaf area; leaf senescence, desiccation and abscission; elongation of the root system; closure of stomata controlled by abscisic acid (ABA); restriction of photosynthetic activity within the chloroplasts; intensification of wax production; thickening of the cuticular layer; and so on.

Besides the biophysical processes mentioned above, another thing of particular interest are the chemical processes that take place at a lightning pace within plant tissue exposed to stress. In lower organisms, the ability to survive desiccation manifests itself in the accumulation of trehalose, a non-reducing glucose disaccharide (MADIN and CROWE, 1975), whereas in higher plants the same role is played by saccharose, which interacts with lipids inside the cell membrane to form hydrogen bonds between hydroxyl groups of sugars and phosphate groups of phospholipids. This way sugars play the role of water surrogates and maintain the hydrophobic-hydrophilic orientation of membrane phospholipids under water deficiency conditions. Sugars not only protect membrane integrity in organisms exposed to drought, but they also provide protection for proteins (DARBYSHIRE, 1974), because by forming hydrogen bonds with specifically charged groups on proteins they prevent the formation of hydrogen bonds within proteins, which would otherwise change their three-dimensional structure irrevocably. Also, by vitrification, or the formation of a glassy structure within the cytoplasm, the plant avoids the fatal effects of extreme desiccation at the cellular level that crystalizes proteins and solutions in the cytoplasm (LEOPOLD, 1990).

Man and plants are inextricably intertwined and mutually reliant. In the last few decades, great efforts have been made by breeders to improve plant tolerance to drought stress. These efforts have been focused mostly on exploiting high-yield potential and genotype selection for morphological, physiological and agronomic traits indicative of drought tolerance in field conditions (DENČIĆ *et al*., 1995; DENČIĆ *et al*., 2000; DHANDA *et al*., 2004). In addition to the classical method of breeding, modern technologies such as biotechnology and genetic engineering have been developed in support of the classical breeding method in research on plant tolerance to drought.

The objective of this paper was to investigate the drought tolerance of different wheat genotypes in *in vitro* conditions.

MATERIALS AND METHODS

One spring and 16 winter wheats of different geographic origin were used as materials for immature embryo isolation. The materials were prepared and sterilized according to ŠESEK (1988). Isolated immature embryos were grown on a modified MS (MURASHIGE and SKOOG, 1962) nutrient medium. After two weeks, half of the induced embryogenic calluses were transplanted onto a fresh MS medium without PEG (polyethilene glycol) to be used as the control group. For the other half, PEG was added to the MS medium at a concentration of 5% and used as the stress agent to induce chemical drought effects in the embryos. After four weeks of *in vitro* growing, callus fresh weight was measured and a reduction in this weight was determined in the embryo group with PEG.

RESULTS AND DISCUSSION

The results showed that the genotypes had significant variability with respect to their ability to form callus fresh weight in a very short time. The highest callus survival percentage (83%) was recorded in the Mexico120 genotype and the lowest (11.3%) in Slavija (Table 1), confirming that all of the genotypes studied have a different capacity for callus mass formation and hence different potential for plant regeneration. This piece data is very important for in the context of the possible use of these genotypes in other areas of biotechnology research.

Table 1. Callus survival percentage on a modified MS nutrient medium before transplantation into experimental conditions

Genotype	No. of embryos inoculated	No. surviving calluses	% survival
Mexico120 (MEX)	106	88	83
Stephens (SAD)	80	66	82.5
Odisej (SCG)	106	73	68.9
Stepnjačka30 (RUS)	100	66	66
Purdue53/92 (SAD)	100	75	75
ChineseSpring(CHN)	96	57	59.4
Fahad	74	40	54.1
NS55/25 (SCG)	104	52	50
Pesma (SCG)	60	30	50
Venera (SCG)	100	47	47
Miranovska (UKR)	80	37	46.3
Rapsodija (SCG)	100	45	45
Koštana (SCG)	100	43	43
Hira (IND)	76	28	36.8
Košuta (SCG)	60	17	28.3
Rozofskaja (UKR)	80	22	27.5
Slavija (SCG)	80	9	11.3

The introduction of 5% PEG to induce drough stress had an inhibiting effect on the growth of callus fresh weight in all of the genotypes. Differences observed in callus mass morphology further confirmed this effect (Fig. 1). The in-

hibitory effect of the stress agent manifested itself in a lower total mean value of callus fresh weight relative to control (33 mg), which was observed (20.3 mg) under stress conditions in all of the genotypes, in agreement with DHANDA *et al*. (2004). Significant variability was recorded among all the genotypes with regard to

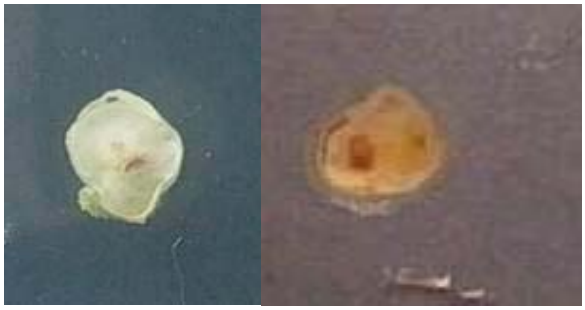

Fig.1 Appearance of callus fresh mass in the control group (C) and on the nutrient medium with PEG

Table 2 Callus fresh weight content and reduction on the nutrient medium with 5% PEG

Genotype	Callus fresh weight (mg)		Decrease of callus fresh weight (%)
	Control	PEG	PEG
Mexico120 (MEX)	30.6	15.7**	46.7
Stephens (SAD)	28.3	12.9**	54.4
Odisej (SCG)	33.2	14.0**	57.8
Stepnjačka30 (RUS)	33.4	18.5**	44.6
Purdue53/92 (SAD)	23.4	18.8	19.7
Chinese Spring (CHN)	24.2	11.7**	51.6
Fahad	32.5	23.4*	28.0
NS55/25 (SCG)	25.2	14.6**	42.1
Pesma (SCG)	17.2	9.9*	42.4
Venera (SCG)	31.9	19.8**	37.9
Miranovska (UKR)	35.0	14.6**	58.3
Rapsodija (SCG)	37.1	24.8**	33.1
Koštana (SCG)	23.3	13.2**	43.3
Hira (IND)	36.2	15.4**	57.5
Košuta (SCG)	32.9	23.7*	27.9
Rozofskaja (UKR)	84.5	72.3**	14.4
Slavija (SCG)	29.3	21.5*	26.6
Average	33.0	20.3	40.4
LSD 0.05	6.8815		
0.01	9.2675		

callus fresh weight reduction in response to stress conditions caused by PEG presence in the nutrient medium (Tab. 2). The only genotype whose callus fresh weight did not decrease significantly under induced stress relative to control was Purdue53/92. This genotype did not react to the water deficit conditions and can there-

fore be said to be the most tolerant of induced stress of all the genotypes concerned. Relative to the control group, the largest reduction of callus fresh weight (58%) was recorded in the Miranovska genotype and the lowest (14.4%) in Rozofskaja. Percentage decrease of callus fresh weight found in the Novi Sad genotypes can be considered medium, ranging between 26.6% (Slavija), 27.9% (Košuta), 43.3% (Koštana), 33.1 (Rapsodija), 37.9% (Venera), 42% (Pesma and NS55/25), and 57.8% (Odisej). Several other genotypes had a callus mass reduction of over 50%, namely Miranovska, Chinese Spring, Hira and Stephens.

Studying the effects of different PEG concentrations (10%, 20% and 30%) on the survival of the cultivars Košuta and Renesansa in *in vitro* conditions, KONDIĆ and ŠESEK (2000) determined that these concentrations had a lethal effect on wheat callus tissue. They also found that the PEG concentrations were not selective, since they caused callus growth inhibition of over 50%. The present study, by contrast, showed the 5% concentration to have been selective enough, as significant differences were identified among the genotypes in their tolerance to drought stress and in their reduction of callus fresh weight of 50% higher.

CONCLUSION

The results of the paper showed that drought stress induced by PEG presence at 5% concentration had an inhibitory effect and reduced callus fresh weight by as much as 50% and more. The significant differences in callus fresh weight reduction found among the genotypes confirmed the selective nature of the stress agent applied. In conclusion, the genotypes Rozofskaja (a callus fresh weight reduction of 14.4%) and Purdue53/92 (a 19.7% reduction) exhibited the greatest tolerance to drought stress, while the Miranovska genotype proved to be the least tolerant (a reduction of 58.3%). The Novi Sad genotypes exhibited medium tolerance of drought stress induced in *in vitro* conditions. The genotypes Slavija, Košuta and Rapsodija were somewhat more tolerant than Venera, NS55/25 and Odisej.

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IN VITRO ISPITIVANJE TOLERANTNOSTI PŠENICE NA USLOVE SUŠE

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I z v o d

U radu je analiziran *in vitro* efekat stresa suše na 17 različitih genotipova pšenice, različitog geografskog porekla. Kalusno tkivo je indukovano iz nezrelih zigotnih embriona (10-15 dana posle polinacije) na modifikovanoj MS (MURASHIGE and SKOOG, 1962) hranljivoj podlozi. Nakon dve nedelje kalusno tkivo je presađeno na istu hranljivu podlogu obogaćenu 5% visokomolekularnim polyethylene glycol-om (PEG 6000) koji je upotrebljen kao agens stresa za postizanje hemijskog efekta suše. Kontrolna grupa kalusa gajena je na istoj hranljivoj podlozi bez PEG-a. Nakon četiri nedelje gajenja na ovim podlogama ocenjena je sposobnost preživljavanja kalusne mase kod posmatranih genotipova do presađivanja kao i procenat smanjenja sveže kalusne mase nakon presađivanja na hranljivu podlogu sa 5% PEG-om. Utvrđeno je postojanje statistički značajne razlike između genotipova u odgovoru na indukovani stres. Minimalno sniženje vrednosti sveže mase kalusa u odnosu na kontrolu ocenjeno je kod genotipa Rozofskaja (14,4%) a maksimalno kod genotipa Miranovska (58,4%) ukazujući na nivo tolerantnosti posmatranih genotipova na stres suše. Rezultati su pokazali da se novosadski genotipovi odlikuju srednjom tolerantnošću na vodni deficit indukovan u *in vitro* uslovima. Genotipovi Slavija, Košuta i Rapsodija su nešto tolerantniji na stres suše od genotipova Venera, NS55/25 i Odisej

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