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SULFONYLUREA TOLERANCE OF WHEAT GENOTYPES IN ZYGOTIC EMBRYO CULTURE

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Abstract — Tolerance of wheat genotypes to the sulfonylurea herbicide metsulfuron-methyl was studied using *in vitro* culture. Six randomly selected wheat genotypes were used for isolation. Mature embryos were inoculated onto a modified MS medium to which three concentrations of metsulfuron-methyl were added: I-30 g l-1, II-60 g l-1, and III-90 g l-1. The control group of embryos was cultivated on a herbicide-free medium. After one month of cultivation, callus fresh weight was measured. There were significant differences among the genotypes regarding their tolerance to metsulfuron-methyl. The Pobeda cultivar expressed the highest and the Lr-12 genotype had the lowest level of herbicide tolerance.

Key words: *Triticum aestivum*, wheat genotype, herbicide tolerance, metsulfuron-methyl, *in vitro* culture

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INTRODUCTION

Herbicides generally function by disrupting unique and essential processes in plants. Both crops and weeds share these processes. Consequently, selectivity at present is mostly based on different herbicide uptake between weeds and crops, controlled timing and site of application, or rapid detoxification of the herbicide by crop plants. Reliance on these natural selective processes limits the effective use of potent herbicides. Because of that, the development of crop cultivars with tolerance to effective broad-spectrum herbicides is a very important goal for breeding programs worldwide (Mulwa and Mwanza, 2006).

In addition to conventional breeding methods, there are also several biotechnology methods, such as *in vitro* culture (Bozorgipour and Snape, 1997; Jain, 2001), genetic engineering (Jauhar and Chibbar, 1999; Sharma et al., 2003), and molecular markers (Jauhar and Chibbar, 1999), which can be used to develop herbicide-tolerant crops. Plant tissue culture represents the simplest of the biotechnologies available to plant scientists today.

In wheat (*Triticum aestivum* L.), tolerance to chlortoluron and difenzoquat (Bozorgipour and Snape, 1991), sulfonylurea (Kondić and Šesek, 1998), tribenuron (Kondić-Špika et al., 2007), and dicamba (Kilinc, 2004) were tested using callus culture.

Metsulfuron-methyl is a residual sulfonylurea, a chemical compound whose herbicide activity was discovered in 1966 by Koog. It was first commercialized for wheat and barley crops in 1982. Metsulfuron-methyl is a selective systemic herbicide that can be absorbed through the roots or leaves. It moves very fast acropetally and basipetally in plants. Metsulfuron-methyl stops cell division by inhibiting biosynthesis of the essential amino acids, valine and isoleucine (Janjić, 2005).

The aim of this study was to determine the reaction of six wheat genotypes to different metsulfuron-methyl concentrations in order to select the most tolerant genotype and the best selective concentration for future *in vitro* screening studies.

MATERIALS AND METHODS

Preparation of plant material

Six randomly selected wheat (*Triticum aestivum* L.) genotypes (Lr-12, Durin, NS 55-25, Pobeda, Florida,

and Vel) were used as the material for this study. Sterilization and isolation of embryos were done as described by Kondić and Šesek (1998).

Herbicide treatments

Isolated embryos were inoculated on modified MS medium (Murashige and Skoog, 1962) to which three concentrations of metsulfuron-methyl were added. The first concentration (I) was the standard field-dose. The other two concentrations were calculated as double (II) and triple (III) field-doses. Metsulfuron-methyl is usually used to control broadleaf weeds and some annual grasses in wheat, barley, and oats, as a pre- or post-emergence application in a field-dose of 4-7.5 g per hectare. The active ingredient of the herbicide used in this study had a technical purity of 95%.

The applied concentrations of metsulfuron-methyl were: I – 30 g l^{-1}, II – 60 g l^{-1}, and III – 90 gl^{-1}. The control groups of embryos were cultivated on herbicide-free medium.

Data collection and *analysis*

For each treatment, 20 embryos were isolated. The experiment had a completely randomized block design, with two replicates. Each replication consisted of five culture tubes (100 x 25 mm) with two embryos in each tube.

The embryos were cultivated for 30 days at 25-27°C and light intensity of 1,500 lx, with a photoperiod of 16 h of light. After the period of cultivation, fresh callus weight (FCW) was measured. Reduction of fresh callus weight (RFCW) on media with different metsulfuron-methyl concentrations was calculated in relation to the control and expressed in percents.

All results were processed in the computer program Statistika 7 (StatSoft, Inc. Corporation, Tulsa, OK, USA). Before applying the analysis of variance, homogeneity was verified by the Hartley, Cochran, and Bartlett tests. If the tests indicated it as necessary, appropriate data transformations and adequate variance analysis models were applied.

RESULTS

Table 1 shows the results of measuring fresh callus weight (FCW) of the six wheat genotypes from the *in vitro* test and calculated values for reduction of fresh callus weight (RFCW). As no homogeneity of the variances was established for FCW, logarithmic transformation of the data was performed before the statistical analysis. Highly significant effects of the genotype and the herbicide concentration on callus tissue growth were found (Table 2).

The presence of the herbicide in the nutrient medium inhibited the growth of wheat calli. When average values for the six genotypes are considered (Table 1), a significant decrease of FCW in relation to the control group can be noted already in the first treatment, i.e., when metsulfuron-methyl was added in a concentration of 30 g l^{-1}. The average FCW of 23.0 mg on this medium was a reduction by 71.3% in relation to the control. Further increase of herbicide concentration to 60 g l^{-1} caused a similar reaction of callus tissue (RFCW of 73.7%), while at the highest herbicide concentration (90 g l^{-1}) RFCW was 77.0%.

Table 1. Fresh callus weight (FCW) and reduction of fresh callus weight (RFCW) of six wheat genotypes in response to different herbicide treatments (Control-herbicide free, I – 30 g l^{-1}, II – 60 g l^{-1}, and III – 90 g l^{-1} metsulfuron-methyl).

		FCW (mg)				RFCW (%)		
	Genotype	Control	I	II	III	I	II	III
1	Lr-12	51.49	10.38	9.5	9.07	80.5	81.5	82.6
2	Durin	54.22	16.1	12.63	9.3	71.1	74.4	78.8
3	NS 55-25	103.77	27.69	21.36	22.79	72.3	76.0	75.5
4	Pobeda	68.63	24.69	23.37	18.93	64.1	66.6	72.4
5	Florida	73.17	25.42	17.05	19.2	65.1	76.6	73.7
6	Vel	113.07	41.96	24.13	23.32	63.3	78.4	79.1
	Mean	77.4	23.0	19.4	17.1	71.3	73.7	77.0

Table 2. Test of significance for effects of genotype ("Var. 1"), metsulfuron-methyl concentration ("Var. 2"), and their interaction ("Var. 1" * "Var. 2") on fresh callus weight of six wheat genotypes. **Significance at P < 0.01.

Effect	SS	Df	MS	F	P
Intercept	94.13704	1	94.13704	4301.273	0.000**
"Var. 1"	1.22792	5	0.24558	11.221	0.000**
"Var. 2"	3.37811	3	1.12604	51.450	0.000**
"Var. 1" * "Var. 2"	0.16138	15	0.01076	0.492	0.922
Error	0.52526	24	0.02189		

Table 3. Dunnett's test for the effect of different herbicide treatments (I – 30 g l-1, II – 60 g l-1, and III – 90 g l-1 metsulfuron-methyl) on fresh callus weight of six wheat genotypes. *Significance at P < 0.05; **Significance at P < 0.01.

		Probabilities for Post Hoc Tests (M < Control) Error: Between MS = 0.02189, Df = 24		
No	Genotype	I	II	III
1	Lr-12	0.0004**	0.0004**	0.0002**
2	Durin	0.0093**	0.0035**	0.0005**
3	NS 55-25	0.0068**	0.0013**	0.0021**
4	Pobeda	0.0381*	0.0234*	0.0068**
5	Florida	0.0258*	0.0016**	0.0046**
6	Vel	0.0392*	0.0011**	0.0008**

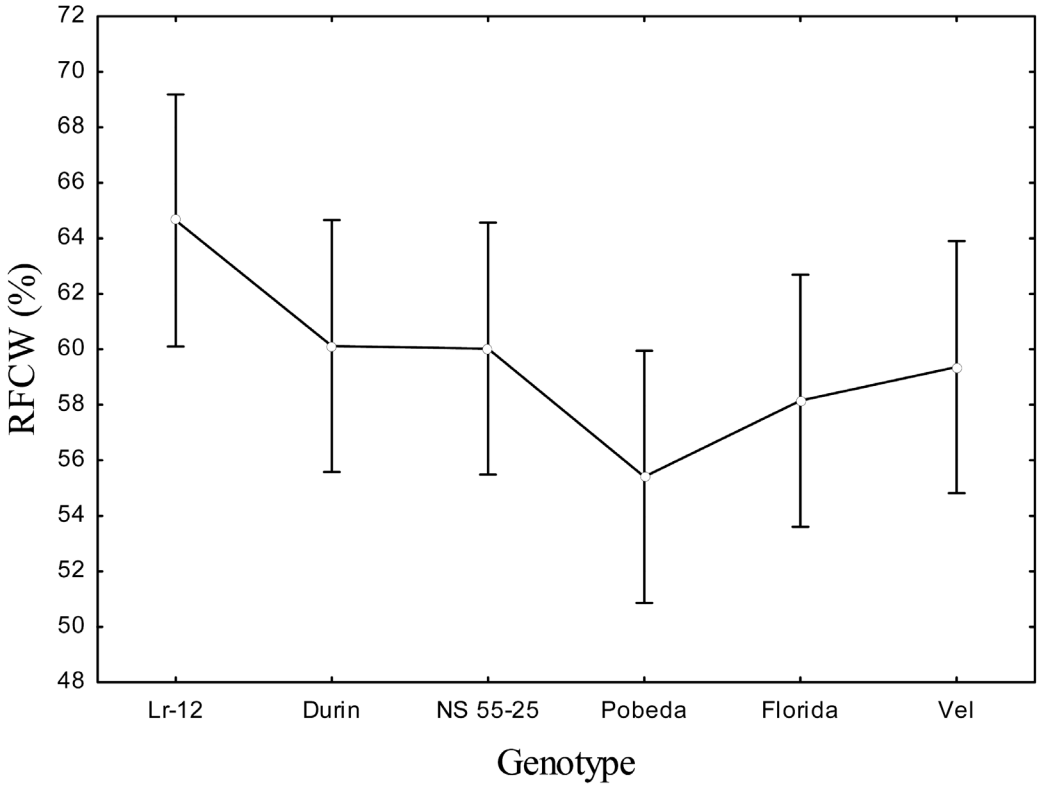

Fig. 1. Average reductions of fresh callus weight (RFCW-transformed data) for three metsulfuron-methyl concentrations in six wheat genotypes. Vertical bars denote 0.95 confidence intervals.

The genotypes mostly reacted in accordance with the average reaction. In the one-sided Dunnett's test, significantly lower values for FCW in response to the herbicide treatments were found in all of the genotypes when compared to the control. However, the level of significance was different among the genotypes (Table 3). Thus, at the lowest herbicide concentration (30 g l^{-1} metsulfuron-methyl), the values for FCW in cvs. Pobeda, Florida, and Vel were significantly lower in relation to the control at the level of $P < 0.05$, while in other genotypes the level of significance was $P < 0.01$. In cv. Pobeda decrease of callus growth in response to herbicide treatment II was significant at the level of $P < 0.05$, while in all other genotypes the decrease was significant at the level of $P < 0.01$.

Since the data on RFCW were expressed in percents, transformation was performed prior to statistical analysis. Factorial ANOVA indicated that all of the genotypes had similar RFCW in response to the applied herbicide treatments. However, the average reactions of the genotypes to all herbicide treatments, expressed by RFCWs (Fig. 1), showed a significant difference between the Lr-12 and Pobeda genotypes. The Lr-12 genotype had the highest RFCW, while cv. Pobeda had the lowest RFCW.

DISCUSSION

The results of callus fresh weight determinations indicated that the presence of metsulfuron-methyl in the medium had an inhibitory effect on callus growth in all genotypes (Table 1). However, the *in vitro* culture test showed significant differences among the genotypes regarding their response to herbicide treatments (Fig. 1). This confirmed that differences in genotype reactions to herbicide toxicity are visible at the cellular level and that they can serve as a selection criterion. These results are in agreement with our earlier investigations (Kondić-Špika and Jevtić, 2002), as well as with the results of other authors (Bozorgipour and Snape, 1997; Kintzios et al., 1999; Singh and Wright, 2002).

Fresh callus weight was chosen for evaluation of herbicide tolerance based on the results of our previous comparative *in vitro* and *in situ* experiments (Kondić-Špika et al., 2007). The study showed that of three *in vitro* parameters, fresh callus weight was the best one for separating tolerant from sensitive genotypes. Validity of the tolerance level expressed by FCW was confirmed at the level of whole plants in an *in situ* experiment. This could be due to the unique mode of action of sulfonylurea herbicides, which inhibit acetolactate synthase (ALS), a key enzyme required for plant cell growth (Pornprom et al., 2005).

Callus growth of all genotypes was inhibited by 71.3% at a concentration of 30 g l^{-1} metsulfuron-methyl (Table 1), which was considered as the standard field-dose. It is a very sensitive reaction, considering that metsulfuron-methyl is a selective herbicide. The results of some investigations have shown that callus tissues are much more susceptible to the herbicide than are whole *in situ* plants (Kintzios et al., 1999; Taregyan et al., 2001; Pornprom et al., 2005). They suggested that the less sensitive reaction of sprayed plants could be due to partial degradation of the herbicide during its uptake and translocation to the target tissues. On the other hand, callus tissues cultured on a selective medium are directly exposed to the herbicide and should be more susceptible to lower herbicide concentrations. Because of that, lower concentrations than 30 g l^{-1} of metsulfuron-methyl should be investigated in future studies in order to identify the best selective concentration for detecting different levels of herbicide tolerance in wheat genotypes.

Besides identification of naturally tolerant genotypes, *in vitro* cell cultures could be used for the development of crop herbicide tolerance by selection at physiologically inhibitory concentrations of herbicides (also referred to as brute force selection). Using cell culture procedures, BASF Inc. produced a corn hybrid (DK404SR) that is resistant to the sulfonylurea herbicide sethoxidim (Mulwa and Mwanza, 2006).

Cell culture at lethal concentrations of certain herbicides also results in gene amplification in surviving cell, which leads to resistance through the overproduction of enzymes targeted by herbicides. Caretto et al. (1994) selected carrot cells and subse-

quently regenerated plants that were resistant to the sulfonylurea herbicide chlorsulfuron. Resistance in these plants was due to amplification of the acetolactate synthase gene. With this in mind, the concentration of 30 g l^{-1} metsulfuron-methyl should be tested in future studies for this kind of selection pressure and for the development of new herbicide-tolerant wheat genotypes.

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ТОЛЕРАНТНОСТ ГЕНОТИПОВА ПШЕНИЦЕ ПРЕМА СУЛФОНИЛУРЕИ У КУЛТУРИ "ЗИГОТНОГ" ЕМБРИОНА

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Толерантност генотипова пшенице према метсулфурон-метилу, хербициду из групе сулфонилуреа препарата, испитана је у *in vitro* култури зиготног ембриона. За изолацију коришћено је шест генотипова пшенице. Ембриони су инокулисани на модификовану MS подлогу, којој је метсулфу-

рон-метил додат у три концентрације: I – 30 g l^{-1}, II – 60 g l^{-1} и III – 90 g l^{-1}. Контролна група ембриона гајена је на подлози без хербицида. После месец дана гајења измерена је свежа маса калуса. Резултати су показали да је присуство метсулфурон-метила у подлози изазвало инхибицију раста калуса код свих генотипова. Међутим, генотипови су се значајно разликовали међу собом у погледу реакције на овај хербицид. На најнижој концентрацији метсулфурон-метила (I – 30 g l^{-1}), генотип Lr-12 имао је највишу редукцију свеже масе калуса, у односу на контролу (80.5 %), док је најнижу редукцију имао генотип Вел (63.3 %). Више концентрације хербицида имале су још јаче инхибиторно дејство код свих генотипова. Резултати су показали да је сорта Победа била најтолерантнија, док је генотип Lr-12 био најосетљивији на присуство метсулфурон-метила у подлози.