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A Preliminary Study of Soybean Genotype Responses to Glyphosate

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SUMMARY

The effect of four application rates of glyphosate (Roundup, 360 g a.i./l) – 180; 360; 720 and 1440 g a.i./ha on the survival, dynamics of growth, and accumulation of fresh biomass in g per plant was studied on eight newly-developed Bulgarian lines, varieties and candidate varieties of soybean bred by different methods under greenhouse conditions. The objective of this study was to determine and compare the sensitivities of different soybean genotypes to glyphosate. The studied soybean genotypes showed different levels of glyphosate sensitivity due to their genetic differences. Glyphosate rates of 180, 360, 720 and 1440 g a.i./ha, applied at the stage of three trifoliate leaves (V_4) of soybean had effect on the survival of the studied genotypes and can be presented conditionally in the following order: *H* (40.6%) < *G* (40.7%) < *D* (51.3%) < *C* (52.6%) < *F* (58.9%) < *E* (60.5%) < *B* (62.0%) < *A* (65.3%). The depression coefficients (B) of the studied characteristics depended mainly on soybean genotypes and the applied herbicide rates. The tested glyphosate rates showed a high (GI 26.5-51.6%) to relatively low degree of toxicity (GI 16.0-18.7%) in the studied soybean genotypes.

Keywords: Genotype; Soybean; Glyphosate; Response

INTRODUCTION

Weed infestation is one of significant factors influencing soybean grain yields.

In the past decade, research work has focused on the study of sensitivity to glyphosate in non-genetically (non-GM) modified soybean genotypes. Glyphosate is the active ingredient of the systemic total herbicide Roundup. Studies conducted by Duke (1998) and King et al. (2001) showed that the herbicide suppressed the EPSPS enzyme responsible for amino acid synthesis in plants. Padgette et al. (1995), Davis (2006), Zambrano et al. (2003), Main et al. (2004) reported naturally-arising genetically controlled herbicide resistance to glyphosate that was not due to gene engineering in soybean and sugarcane. According to these authors, the soybean genotype showing herbicide tolerance of glyphosate can be transferred to other soybean plants by conventional breeding methods. Similar results were also reported by Nelson and Renner (2001), according to whom the application of 840 g a.i./ha glyphosate at the three trifoliate leaf stage (V_4) of GM and non-GM

soybean plants had no significant effect on leaf area of the crop. The studies of Al-Khatib and Peterson (1999) and Cedergreen (2008) showed that glyphosate application at reduced rates at the initial developmental stages of soybean plants (phenological stages V_3-V_4) did not affect soybean grain yields significantly.

The objective of this study was to determine and compare the sensitivities of different soybean genotypes to glyphosate under greenhouse conditions.

MATERIAL AND METHODS

The study was conducted under greenhouse conditions at the Institute of Forage Crops, Pleven, in 2008. Two factors were studied: factor A – the applied rate of glyphosate (Roundup, 360 g a.i./l): control, 180 g a.i./ha, 360 g a.i./ha, 720 g a.i./ha, 1440 g a.i./ha; factor B – new Bulgarian genotypes (lines, varieties and candidate varieties) of soybean developed by different breeding methods (experimental mutagenesis and/or hybridization): *A*, *B*, *C*, *D*, *E*, *F*, *G*, *H* (Table 1).

In order to determine and compare the sensitivities of different soybean genotypes to glyphosate in the laboratory, 27 plants were sown in individual plastic containers of 0.5 l with five replications for factor A. Untreated plants sown in the same pot volume were used as a control. At the phenological stage of three trifoliate leaves (V_4) the tested soybean genotypes were sprayed with different rates of glyphosate and with distilled water in the control to test factor B.

The following characteristics were studied based on factors A and B: the percentage of surviving plants, %; herbicide phytotoxicity, depending on application rates, in the tested soybean genotypes according to an EWRS scale: score 1 – no damage; score 9 – completely killed plants, the recording being performed on the 7th, 15th and 30th day after treatment (DAT) using the accepted methodology for herbicides intended for application during vegetation; plant length (root + stem), cm; fresh and dry biomass, g per plant, on the 30th DAT.

Table 1. Studied soybean genotypes

Genotype	Method of development
A	Hybridization
B	Hybridization
C	Hybridization
D	Experimental mutagenesis
E	Hybridization
F	Experimental mutagenesis
G	Hybridization
H	Interspecies hybridization

The formula of Shabanov et al. (1982) was used to determine the depression coefficients (B) depending on the studied factors.

$$\begin{cases} n \lg Y_0 + B\sum_{i=1}^{n} \lg x_i = \sum_{i=1}^{n} \lg Y_i \\ \lg Y_0 \sum_{i=1}^{n} \lg x_i + B\sum_{i=1}^{n} (\lg x_i)^2 = \sum_{i=1}^{n} (\lg x_i - \lg Y_i) \end{cases}$$

Where: n = volume of pair samplings depending on the studied factors – A and B for the used rates of glyphosate; Y_0 = tested characteristics of all soybean genotypes ($Y_0 = 1$, surviving plants, %; $Y_0 = 2$, plant length; $Y_0 = 3$, fresh biomass produced, and $Y_0 = 4$, dry biomass in g per plant in the control variants taken for 100%; x_i = the used g a.i./ha glyphosate for each of the studied soybean genotypes were transformed on a 5-score scale; Y_i = the studied characteristics of all soybean genotypes: (Y_i =1, surviving plants, %; Y_i = 2, plant length; $Y_i = 3$, fresh biomass formed, and $Y_i = 4$, dry biomass in g per plant); B = depression coefficient. Plant development index (*GI*) was determined by the formula of Gariglio et al. (2002): $GI = G/G_0 \cdot L/L_0 \cdot 100$; where: G = percentage of surviving plants in the studied variant; G_0 = percentage of surviving plants in the control variant; L = length (cm) of plants in the studied variant; L_0 = length (cm) of plants in the control variant. The percentage of surviving plants was preliminarily transformed by the formula $Y = \arcsin\sqrt{(x_{\%}/100)}$ (Hinkelmann and Kempthorne, 1994).

The rate of growth (K) of the studied soybean genotypes was determined with regard to height of growth (K/cm), as well as fresh biomass ($K/g_{/fb}$) and dry biomass ($K/g_{/db}$) produced, in all variants of the trial depending on the studied factors – glyphosate rate (A) and kind of genotype (B) by the formula of Mamonov and Kim (1978):

$$K = (W_2 - W_1)/(t_2 - t_1)$$

where: W_1 = initial stage of recording of the parameter; W_2 = final stage of recording of the parameter; $t_2 - t_1$ = time interval between W_1 and W_2.

Mathematical statistical processing of the experimental data was performed using the software product STATGRAPHYCS plus for Windows.

RESULTS AND DISCUSSION

The rates of 180, 360, 720 and 1440 g a.i./ha glyphosate 360 g/l, applied at the soybean phenological stage of three trifoliates (V_4) had an effect on the survival of the studied genotypes and can be presented conditionally in the following order: *H* (40.6%) < *G* (40.7%) < *D* (51.3%) < *C* (52.6%) < *F* (58.9%) < *E* (60.5%) < *B* (62.0%) < *A* (65.3%).

With increasing herbicide concentrations there was a general tendency of its decrease in all soybean genotypes of 12.1-100%, as against the control variants (Figure 1). An exception was observed only in genotypes *A*, *B*, *E* and *F*, where no statistically significant differences were found at the lower glyphosate rates of 180 and 360 g a.i./ha. The genotypes *C*, *D*, *G* and *H* can be considered sensitive because their survival under all rates varied from 87.9 to 0.0% as against the control variant, the differences being statistically significant at P=0.05, and *r* ranging from -0.859 to -0.972.

A specific genotype response was also observed with regard to phytotoxicity of the used glyphosate tothe studied soybean genotypes. The herbicide applied at the rate of 180 g a.i./ha did not induce phytotoxicity until the 7th, 15th and 30th DAT, except in genotypes *G* and *H,* where chlorotic spots were observed among leaf veins (score 3) on the 30th DAT. Under the higher rates of 360 and 720 g a.i./ha, phytotoxicity to the studied soybean genotypes was low-to-moderate (score 2-3) and manifested as chlorotic spots among leaf veins. The rate of 1440 g a.i./ha had stronger phytotoxicity (score 5-6), but only in genotypes *G* and *H,* manifesting as epinastic bending of leaf petioles, while the score was intermediate (2-4) in the other soybean genotypes. On the 15th DAT, the soybean genotypes showed low to moderate phytotoxicity. The newly appearing leaves

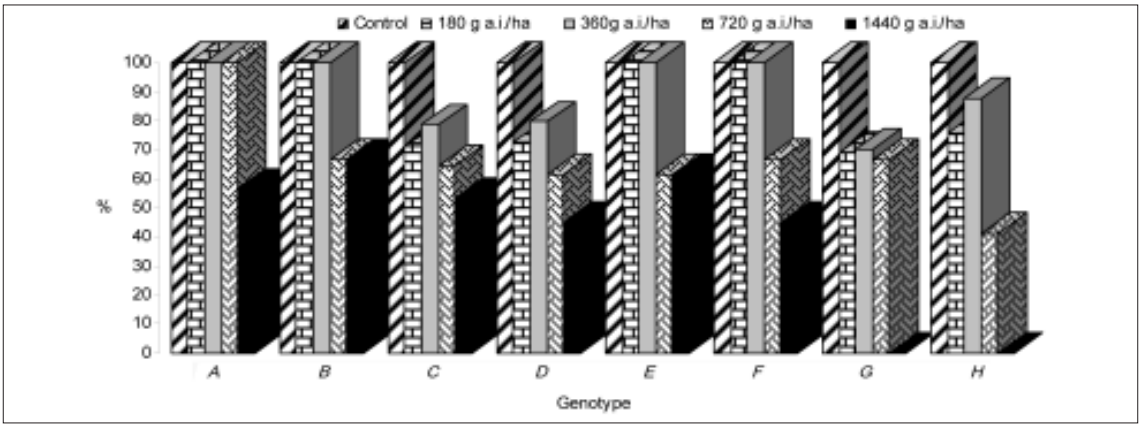

Figure 1. Survival of the studied soybean genotypes, depending on glyphosate application rate as percentage of the control

Table 2. Phytotoxicity of glyphosate to the studied soybean genotypes in greenhouse conditions according to EWRS*

	DAT	Rate g a.i./ha	Genotype							
			A	*B*	*C*	*D*	*E*	*F*	*G*	*H*
Phytotoxicity	7	180	0	0	0	0	0	0	0	0
		360	2	2	1	1	3	1	1	0
		720	3	2	1	3	3	2	2	3
		1440	5	2	4	4	4	4	6	6
	15	180	0	0	0	0	0	0	0	0
		360	2	2	1	2	3	4	4	3
		720	3	2	1	3	3	4	4	3
		1440	5	3	4	4	4	4	7	7
	30	180	0	0	0	0	0	0	3	3
		360	2	2	2	2	3	4	5	4
		720	3	3	5	5	5	4	7	8
		1440	5	3	7	8	6	8	9	9

*EWRS: (score 1 – no damages; score 9 – completely killed plants)

and stems had no visible changes due to herbicide effect at the lower rates of 180, 360 and 720 g a.i./ha, but the genotypes responding with higher sensitivity were completely killed – score 9 (Table 2).

Biometric measurement of plant length allows objective estimation of herbicide phytotoxicity at the initial developmental stages of soybean genotypes (Table 3).

Table 3. Effects of the herbicide glyphosate on sensitivity of different soybean genotypes under greenhouse conditions

Genotype	Application rate, g a.i./ha	Parameters		
		length of growth, cm	biomass produced, g/plant	
			fresh	dry
A	0	55.9 bc	5.2 a	1.1 a
	180	57.0 bc	8.2 b	1.7 b
	360	43.1 a	3.4 a	0.7 a
	720	49.2 ab	3.5 a	0.7 a
	1440	47.3 c	5.7 a	1.2 ab
B	0	56.2 b	4.9 b	1.0 a
	180	57.3 b	4.1 ab	0.9 a
	360	52.8 ab	3.7 ab	0.8 a
	720	59.8 b	3.3 a	0.7 a
	1440	44.9 a	3.5 ab	0.8 a
C	0	55.1 a	5.0 a	1.1 a
	180	57.3 a	6.2 a	1.3 a
	360	52.8 a	5.8 a	1.2 a
	720	59.8 a	4.0 a	0.9 a
	1440	44.9 a	3.1 a	0.6 a
D	0	54.0 a	4.4 a	0.8 ab
	180	59.4 a	5.2 a	1.1 b
	360	54.6 a	4.2 a	0.9 ab
	720	54.6 a	3.4 a	0.7 a
	1440	54.0 a	4.6 a	0.9 ab
E	0	54.8 b	5.1 a	1.0 b
	180	54.2 b	4.7 a	1.0 b
	360	51.6 ab	3.9 a	0.8 ab
	720	57.3 b	4.2 a	0.9 ab
	1440	44.1 a	3.7 a	0.6 a
F	0	56.1 b	5.5 b	1.0 b
	180	52.2 b	4.6 b	0.9 b
	360	50.3 b	3.4 ab	0.7 ab
	720	55.1 b	5.9 b	1.3 b
	1440	21.0 a	1.2 a	0.2 a
G	0	51.3 c	7.7 c	1.6 c
	180	41.2 b	4.3 b	0.9 b
	360	49.5 bc	4.3 bc	0.8 b
	720	43.5 bc	5.7 bc	1.3 bc
	1440	0.0 a	0.0 a	0.0 a
H	0	54.1 c	6.1 b	1.3 b
	180	38.0 b	5.1 b	1.2 b
	360	45.7 bc	3.8 ab	0.8 ab
	720	43 bc	1.9 b	0.5 ab
	1440	0.0 a	0.0 a	0.0 a

The numbers in columns followed by diferrent letter differ significantly, P=0.05

The studied glyphosate concentrations had no statistically significant inhibitory effect on plant growth of the soybean genotypes tested (Table 3). An inhibitory effect (61.7 to 100%) was observed only at the highest concentration in genotypes *A, B, F, G* and *H*, being statistically significant at P=0.05, while an exception was observed only in genotypes *C* and *D*, where the differences were statistically nonsignificant. A similar specific genotypic response of soybean to glyphosate was found by King et al. (2001).

The production of fresh biomass in g per plant varied in the studied variants depending on the genotype and application rate of glyphosate. At the lowest glyphosate rate (180 g a.i./ha), the soybean genotypes produced more fresh biomass, measured in g per plant on the average. With increasing glyphosate rates, the produced fresh biomass decreased disproportionately to the increase in the rate of herbicide (Table 3). There was a negative correlation between the quantity of the applied glyphosate and accumulated fresh biomass in the studied genotypes, as follows: *A* -0.173; *B* -0.725; *C* -0.848; *D* -0.169; *E* -0.731; *F* -0.718; *G* -0.847 and *H* -0.978. An exception to the relationship described was observed only in genotype *A*, where the lowest glyphosate rate had a stimulatory effect, being statistically significant at P=0.05. The obtained experimental data were consistent with the results published by Cedergreen (2008) and Velini et al. (2008), according to which the low application rates of glyphosate had stimulatory effect on the dynamics of growth and accumulation of fresh biomass in different crops. Therefore, the observed differences between soybean genotypes with regard to their sensitivity to glyphosate can be attributed to their genetic differences because comparisons between them were made under the same conditions and herbicide application rates.

The obtained results were analogous in terms of the dynamics of production of dry biomass in g per plant (Table 3).

Growth rate (K/cm) and accumulation of fresh (K/g_{fb}) and dry biomass (K/g_{db}) in the studied variants depended on the applied rate of glyphosate, and soybean

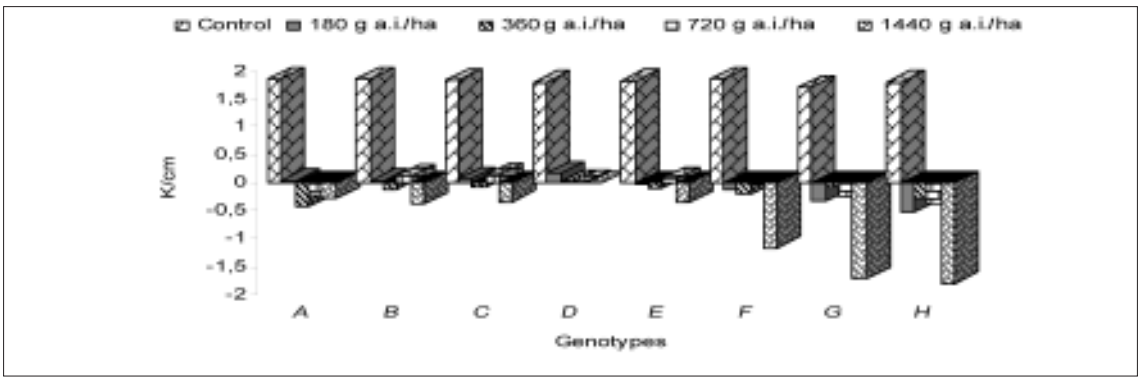

Figure 2a. Rate of growth (K/cm) of the soybean genotypes depending on the rate of applied glyphosate

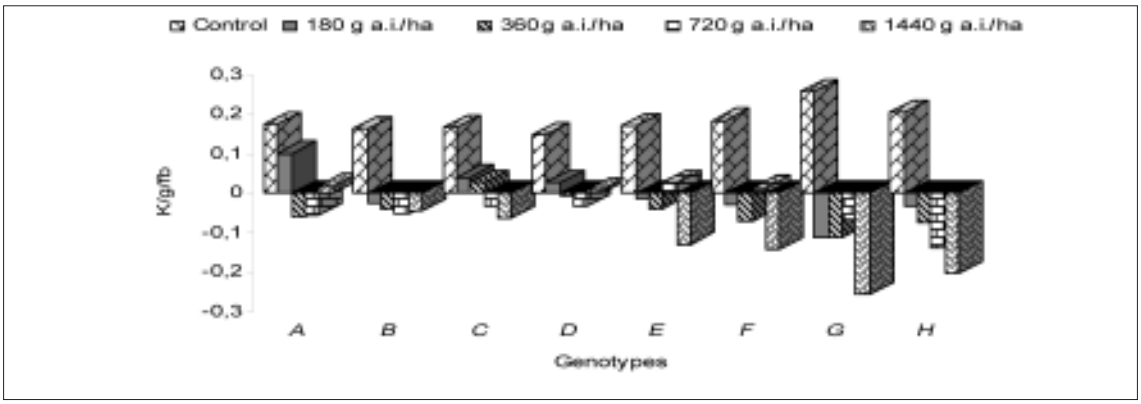

Figure 2b. Rate of accumulation of fresh biomass ($K/g/_{fb}$) in g per plant of the studied soybean genotypes depending on the rate of applied glyphosate

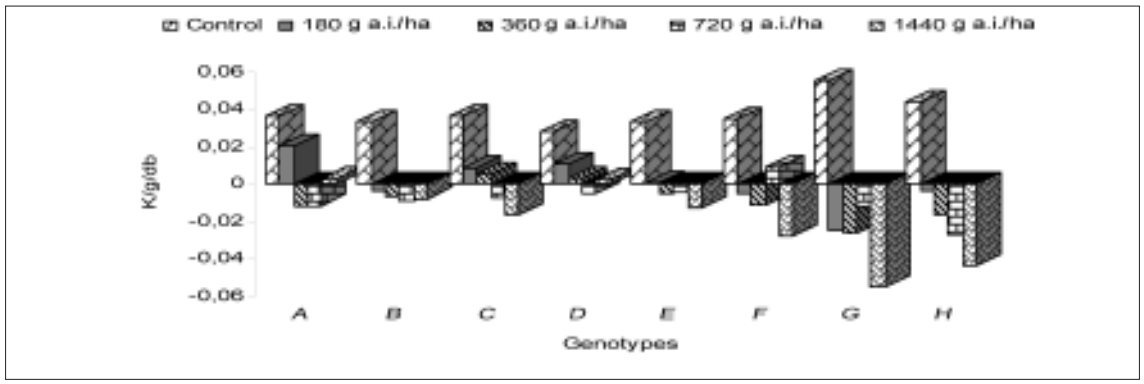

Figure 2c. Rate of accumulation of dry biomass ($K/g_{/db}$) in g per plant of the studied soybean genotypes depending on the rate of applied glyphosate

genotype (Figure 2). The lowest application rate studied (180 g a.i./ha) had a stimulatory effect, but only to genotypes *A*, *B*, C and *D,* while growth rate (K/cm) decreased with the increasing rates of 360, 720 and 1440 g a.i./ha.

A strong suppression of growth rate was found in genotypes *F*, *G* and *H*, and relatively slighter one in genotypes A, *B*, *C* and *E*. An exception to the described relationship was found only in genotype *D* (Figure 2a).

The same relative tendency was observed regarding the rate of accumulation of fresh ($K/g_{/db}$) and dry biomass ($K/g_{/fb}$) in g per plant (Figures 2b and 2c).

An exception to the described relationship was observed in genotypes *C* and *D* under treatment with 360 g a.i./ha. Similar results were reported by Cedergreen (2008), who found that plant responses to glyphosate can have variable degrees and directions of manifestation, expressed as a change in the dynamics of growth and rate of accumulation of fresh and dry biomass, but only in the first six weeks after treatment.

The depression coefficients (B) of the studied characteristics depended mainly on the soybean genotype and the applied glyphosate concentration. It is evident from Table 4 that the depression coefficients (B) were relatively lowest at the lowest application rate of 180 g a.i./ha, while they increased with application rates increasing to 360, 720 and 1440 g a.i./ha.

Our data analysis showed that the depression coefficients (B) were in a negative correlation (*r*) with plant survival in genotypes *A*, *B*, *C*, *D*, *E* and *F* (*r* varied from -0.878 to -0.913), and in a positive correlation in genotypes *G* and *H* (*r* was 0.192 and 0.194, respectively). The depression coefficients relating to growth and production of fresh and dry biomass in g per plant were in a positive correlation with the applied herbicide rate – *r* varied from 0.958 to 0.999 (Table 5).

Estimating the complex effect of the studied characteristics by the averaged development index GI in the tested soybean genotypes, they can be presented conditionally in the following descending order: *B* -84.0%

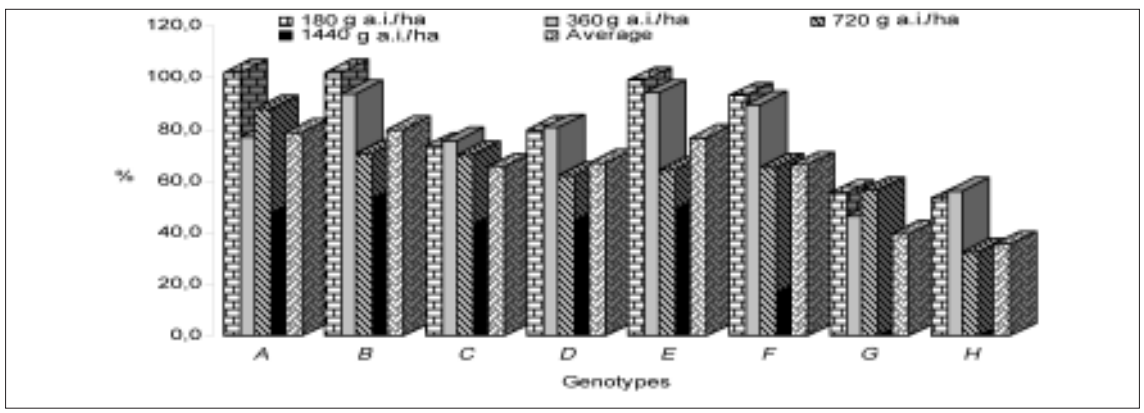

Figure 3. Development index (GI) of the soybean genotypes depending on the application rate of glyphosate

Table 4. Depression coefficients (B) of the studied parameters depending on soybean genotype and the applied rate of glyphosate

Genotype	Application rate, a.i./ha	Depression coefficients (B):				
		percentage of surviving plants	length of growth, cm	biomass produced g/plant		GI
				fresh	dry	
A	180	-2.7	-2.9	-3.1	-3.1	-2.9
	360	-3.2	-3.1	-3.0	-3.0	-3.1
	720	-4.0	-4.1	-3.8	-3.8	-4.1
	1440	-5.5	-6.4	-6.8	-6.7	-5.6
Average		-9.0	-9.4	-9.5	-9.5	-9.1
B	180	-2.7	-2.9	-2.8	-2.8	-2.9
	360	-3.2	-3.3	-3.1	-3.2	-3.3
	720	-3.6	-4.2	-3.8	-3.9	-3.9
	1440	-5.7	-6.3	-6.2	-6.2	-5.7
Average		-9.0	-9.5	-9.2	-9.3	-9.1
C	180	-2.5	-2.9	-3.0	-3.0	-2.7
	360	-3.0	-3.3	-3.4	-3.4	-3.1
	720	-3.6	-4.3	-4.0	-4.0	-3.9
	1440	-5.4	-6.3	-6.0	-5.7	-5.4
Average		-8.8	-9.5	-9.3	-9.2	-9.0
D	180	-2.5	-2.9	-3.0	-3.1	-2.7
	360	-3.0	-3.3	-3.3	-3.4	-3.2
	720	-3.5	-4.2	-4.0	-4.0	-3.8
	1440	-5.2	-6.6	-6.7	-6.7	-5.5
Average		-8.7	-9.6	-9.6	-9.6	-9.0
E	180	-2.7	-2.9	-2.8	-2.9	-2.9
	360	-3.2	-3.3	-3.1	-3.2	-3.3
	720	-3.5	-4.2	-4.3	-4.1	-3.8
	1440	-5.6	-6.3	-4.6	-6.0	-5.6
Average		-8.9	-9.5	-8.8	-9.3	-9.1
F	180	-2.7	-2.8	-2.8	-2.8	-2.8
	360	-3.2	-3.2	-3.0	-3.0	-3.2
	720	-3.6	-4.2	-4.3	-4.4	-3.8
	1440	-5.2	-5.2	-4.4	-4.3	-4.1
Average		-8.8	-9.0	-8.7	-8.7	-8.4
G	180	-2.5	-2.7	-2.5	-2.5	-2.5
	360	-2.6	-3.3	-2.9	-2.8	-2.8
	720	-3.6	-4.0	-3.9	-4.0	-3.7
	1440	0.0	0.0	0.0	0.0	0.0
Average		-6.6	-6.9	-6.7	-6.7	-6.6
H	180	-2.5	-2.6	-2.8	-2.8	-2.5
	360	-2.9	-3.2	-3.0	-3.0	-2.9
	720	-3.2	-4.0	-3.1	-3.3	-3.2
	1440	0.0	0.0	0.0	0.0	0.0
Average		-6.7	-7.3	-6.9	-7.0	-6.7

Table 5. Correlation (*r*) of the studied parameters with rates of the applied glyphosate and soybean genotypes

Genotype	Parameters				
	procentage of survival plants	length of growth, cm	biomass produced, g/plant		GI
			fresh	dry	
	r				
A	-0.895	0.992	0.995	0.999	0.981
B	-0.907	0.997	0.998	0.999	0.996
C	-0.913	0.999	0.997	0.999	0.999
D	-0.902	0.996	0.999	0.999	0.993
E	-0.901	0.996	0.958	0.965	0.997
F	-0.878	0.991	0.986	0.999	0.988
G	0.192	0.995	0.998	0.999	0.998
H	0.234	0.993	0.978	0.999	0.998

> *A* -83.0% > *E* -81.3%> *D* -73.5% > *F* -73.0% > *C* -72.5%>*G* -51.7% >*H* -48.4% (Figure 2).

The analyses showed that the studied glyphosate rates had a relatively high degree of toxicity – GI in soybean genotypes *C*, *D*, *F*, *G* and *H* (26.5% to 51.6% on the average), whereas in genotypes *A*, *B* and *E*, GI varied from 16.0 to 18.7% (GI > 80% – Tiquia et al., 1996) (Figure 3).

CONCLUSIONS

The studied soybean genotypes showed different levels of sensitivity to glyphosate (Roundup, 360 g a.i./l) resulting from their genetic differences.

The rates of 180, 360, 720 and 1440 g a.i./ha of glyphosate applied at the three trifoliate leaf stage (V_4) of soybean, affected the survival of the studied genotypes and can be presented conditionally in the following order: *H* (40.6%) < *G* (40.7%) < *D* (51.3%) < *C* (52.6%) < *F* (58.9%) < *E* (60.5%) < *B* (62.0%) < *A* (65.3%).

The depression coefficients (B) of the studied characteristics depended mainly on soybean genotype and the rate of the applied glyphosate.

The studied application rates of glyphosate showed a relatively high degree of toxicity (GI) in soybean genotypes *C*, *D*, *F*, *G* and *H* (26.5% to 51.6%), while GI varied from 16.0 to 18.7% in genotypes *A*, *B* and *E*.

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Preliminarno proučavanje odgovora različitih genotipova soje na glifosat

REZIME

Proučavano je delovanje četiri doze primene glifosata (Roundup, 360 g a.m./l) – 180, 360, 720 i 1440 g a.m./ha na preživljavanje, dinamiku rasta i akumulaciju sveže biomase (g/biljci) osam novih bugarskih linija, varijeteta i predloženih varijeteta soje oplemenjenih različitim metodama u uslovima staklenika. Cilj proučavanja je bio da se utvrdi i uporedi osetljivost različitih genotipova soje na glifosat. Proučavani genotipovi soje pokazali su različite nivoe osetljivosti na glifosat kao posledicu genetskih razlika. Doze primene glifosata od 180, 360, 720 i 1440 g a.m./ha u fazi tri troliska (V_4) soje su uticale na preživljavanje proučavanih genotipova i uslovno se mogu predstaviti sledećim redosledom: *H* (40.6%) < *G* (40.7%) < *D* (51.3%) < *C* (52.6%) < *F* (58.9%) < *E* (60.5%) < *B* (62.0%) < *A* (65.3%). Koeficijent opadanja (B) proučavanih karakteristika zavisi prevashodno od genotipa soje i doze primene herbicida. Ispitivane doze glifosata pokazale su stepen toksičnosti u rasponu od visokog (*GI* 26.5-51.6%) do relativno niskog (GI 16.0-18.7%) za proučavane genotipove soje.

Ključne reči: Genotip; soja; glifosat; odgovor