Soybean cultivar BR-16-AHAS tolerance to the herbicide imazapyr

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Abstract – The objective of this work was to evaluate the effect of the transgenic soybean BR-16-AHAS genetic constitution on the tolerance to the herbicide imazapyr. BR-16-AHAS was crossed with ten other genotypes. The experimental design was a complete randomized block, in a 2x12x3 factorial arrangement, with two sowing periods (winter and summer), twelve crossing groups and three plant positions (upper, mid and lower), with three replicates. The plants were treated with 100 g ha^{-1} a.i. of imazapyr at the V3/V4 stage. For each position of the plant (upper, mid and lower), the following variables were assessed: number of pods, number of seeds, seed weight, number of seeds per pod and the 100 seeds weight. The effect of the herbicide varied according to the more affected plant position (upper, mid and lower) of each genotype. The use of the same gene *ahas* of BR-16-AHAS, in various genotypes, results in materials with good tolerance to imazapyr; tolerance levels depend not only on the *ahas* gene, but also on the presence of other genes.

Index terms: *Arabidopsis thaliana*, *Glycine max*, epistasis, genetic transformation.

Tolerância da soja BR-16-AHAS ao herbicida imazapyr

Resumo – O objetivo deste trabalho foi avaliar o efeito da constituição genética da soja transgênica BR-16-AHAS sobre a tolerância ao herbicida imazapyr. Dez genótipos foram cruzados com BR-16-AHAS. O delineamento experimental foi o de blocos completos ao acaso, em esquema fatorial 2x12x3, com dois períodos de semeadura (inverno e verão), doze grupos de cruzamentos de plantas e três posições (superior, médio e inferior), com três repetições. As plantas foram submetidas ao tratamento com imazapyr, 100 g ha^{-1} do i.a., nos estádios V3 e V4. Para cada posição da planta (superior, médio e inferior), foram avaliados: número de vagens, número de sementes, peso de sementes, número de sementes por vagem e peso de 100 sementes. Os efeitos do herbicida variaram quanto à posição da planta (alta, média e baixa) mais afetada em cada genótipo. O uso do mesmo gene *ahas* da BR-16-AHAS, em diferentes genótipos, resulta em materiais com boa tolerância ao imazapyr. O nível de tolerância depende não só do gene *ahas*, mas também da presença de outros genes.

Termos para indexação: *Arabidopsis thaliana*, *Glycine max*, epistasia, transformação genética.

Introduction

Agricultural research and development are basic requirements to increase agricultural yield and improve food quality. There are several ways to sustainable increase of yield, such as using of chemical products (fertilizers and pesticides), organic manure, integrated pest control, natural resource conservation and improved varieties which can be obtained by traditional methods or by biotechnology (Herrera-Estrella, 1999). Among the biotechnological techniques available, the most promising is the development of genetically modified organisms (GMO). The creation of genetic combination that does not exist in nature, and specially gene transference between reproductive distinct species, can result in better quality plants, resistant to diseases or herbicides.

Herbicide tolerance is normally a characteristic governed by few genes and this simplifies the development of resistant transgenic varieties. The increase in the number of efficient alternatives for weed control, affecting only the infesting species without harming the development of the commercial variety, is very advantageous for the producers (Hain & Schreier, 1995).

The first plant into which a bacterial gene was introduced was obtained in 1983, coding for resistance to the antibiotic kanamycin. The gene was introduced by *Agrobacterium tumefaciens*, in tobacco plants (Frizzas et al., 2004). In soybean, one of the first applications of

genetic engineering was the development of tolerance to glyphosate (Padgette et al., 1995). Later, other transgenic plants as soybean, rice, cotton, potato, rape seed, sugarcane, corn, eucalyptus and pine were obtained (Vargas et al., 1999; Monquero, 2005).

Aragão et al. (2000) obtained soybean plants tolerant to imazapyr herbicide, where a mutant *ahas* gene, isolated from *Arabidopsis thaliana*, was inserted by biobalistics, conferring tolerance to this product. Imazapyr is a wide spectrum herbicide, which controls most of the annual and perennial grasses and also broadleaf species (Beardmore et al., 1991). This herbicide belongs to the chemical group of imidazolinones, which inhibit the AHAS enzyme (acetohydroxyacid synthase EC 4.1.3.18) that acts in the biosynthesis of the branched chain amino acids (valine, leucine and isoleucine), and can lead to plant death. AHAS-inhibiting herbicides are largely used, because of their low toxicity to animals and high efficiency at low dosage (Vargas et al., 1999).

Kiihl & Arias (2004) compared the performance of conventional genotypes nontreated with imazapyr herbicide to AHAS-treated genotypes and evaluated the effect of imazapyr treatment on BR-16-AHAS and Doko-AHAS events. They observed that BR-16-AHAS genotype was much more affected by imazapyr than Doko-AHAS, when compared to the respective conventional cultivars, besides, the perception of the herbicide effect differed, mainly for most affected plant position (upper, mid and lower), in each genotype. The question that arose after these observations was whether this different response was due to problems of gene expression or to the genetic background of these materials.

The objective of this work was to assess the effect of the genetic constitution of BR-16-AHAS on the tolerance to the herbicide imazapyr.

Materials and Methods

Ten soybean cultivars including BRS 133, Conquista, Celeste, Jataí, Chapadão, EMG 308, FT 106, E96 125, Uirapuru and Pintado were crossed with BR-16-AHAS (each cross represented one group), and some F_5 descendent lines were selected. Three lines were selected within each group, except for two groups where four lines were selected. The treatments consisted of groups of crosses between commercial cultivars and BR-16-AHAS, treated with the herbicide. BR-16-AHAS and Doko-AHAS were used as control group.

The selected lines were sown and assessed in two sowing periods, June 2001 (winter) and December 2001 (summer). The experiments were carried out in greenhouse and the plants were cultivated in 23 tall plastic pots containing 8 L of substrate. The substrate consisted of a mixture of 20% sand and 20% organic compost. Fertilizer was applied according to the substrate analysis. Nitrogen was not used and inoculation with *Bradyrhizobium japonicum* was performed. Seeds were placed in a germinator and transplanted at the fourth day.

Fifteen days after transplant, the plants were treated with the herbicide imazapyr (100 g ha^{-1} a.i.) at the V3/V4 stages. The herbicide was applied with hand-held sprayer. For the application, the pots were separated and later reassembled to the original positions. Extra light was supplied in the winter experiment up to July.

The experimental design was a complete randomized block, distributed in a 2x12x3 factorial arrangement, with two sowing periods (winter and summer), twelve crossing groups and three plant positions (upper, mid and lower), with three replicates. The plot consisted of one pot, with two plants per pot. After maturation, the plants were divided into thirds and, for each position of the plant (upper, mid and lower), the following variables were assessed: number of pods, number of seeds, seed weight, number of seeds per pod, and 100 seeds weight. Individual analyses of variance were performed for each sowing period x group combination. In order to verify the effect of plant position, a joint analysis of variance was performed for sowing periods, ascertaining group effects. The means were compared by Tukey's test, at 5% of probability.

Results and Discussion

There was sowing period effect for most of the traits, except for 100 seeds weight (Table 1). Almost all groups presented superior means, in the summer, for most of the traits, except for the number of pods in the 'Conquista' group and the 100 seeds weight in 'Chapadão', 'Emgopa 308' and 'Uirapuru' groups (Table 2). The following factors were significant ($p<0.01$) for all traits: group, sowing period x group interaction, the line within group and the sowing

period x line interaction (Table 1). In the first part of the analysis, for which the effect of plant position was not considered, it was observed that the groups and the lines within each group differed regarding trait expression, and that there was differential genotype response to variations in the sowing period (Table 2).

There was significance ($p<0.01$) for the effect of plant position for all traits, for the effect of double interactions (sowing period x plant position, group x plant position and line x plant position) for the traits number of pods, number of seeds, and seed weight and the sowing period x plant position interaction for number of seeds per pod. There was no significance regarding traits for the triple interactions (sowing period x group x plant position, and sowing period x line x plant position).

In order to simplify the comparison among the genetic groups, the traits number of pods, number of seeds and weight were represented in percent and compared to the plant total (Table 3). Differences were observed among the groups and within plant positions, mainly in the winter. For example, 'BR-16-AHAS' concentrated 12.5% of the pods in the upper part, and 48.2% in the lower part, in the winter experiment, when the herbicide was applied; 'Conquista' concentrated 31% in the upper part, and 30% in the lower part. In the summer, the line group performance for the number of pods, number of seeds and weight differed from the performance observed in the winter, showing once again that there was sowing period difference, therefore, the genotype assessment period should be taken into account, in order to obtain good results when distinguishing genotypes with good acceptability for

Table 1. Mean squares obtained in the analyses of variance for number of pods (Np), number of seeds (Ns), seed weight (W, g), number of seeds per pod (Nsp), and of 100 seeds weight (W100, g) assessed for each group derived from conventional soybean cultivars, evaluated after sowing in winter and summer, in greenhouse.

Source of variation	df	Np	Ns	W	Nsp	W100
Sowing period (SP)	1	10,380.2**	54,082.5**	1,844.8**	2.19**	0.02^{ns}
Block/SP	4	165.9	405.1	2.9	0.14	23.83
Group (G)	11	545.5**	2,845.8**	57.2**	0.57**	125.54**
SPxG	11	381.4**	1,409.3**	36.1**	0.47**	15.34**
Lines/group (L/G)	22	208.2**	1,237.2**	18.8**	0.29**	79.97**
SPxL/G	22	110.4**	512.6**	11.3**	0.13**	36.00**
Position (P)	2	2,138.2**	9,066.1**	297.2**	1.23**	191.93**
SPxP	2	2,159.9**	11,180.4**	392.4**	0.53**	2.39^{ns}
GxP	22	263.1**	1,018.7**	33.2**	0.05^{ns}	2.56^{ns}
LxP/G	44	103.1**	417.4**	13.7**	0.05^{ns}	1.53^{ns}
SPxGxP	22	69.4^{ns}	287.9^{ns}	8.8^{ns}	0.03^{ns}	1.71^{ns}
SPxLxP/G	44	57.2^{ns}	263.7^{ns}	6.9^{ns}	0.03^{ns}	1.86^{ns}
Error	401	56.6	239.0	7.3	0.05	4.76
Mean		23.7	47.1	8.3	1.9	18.19
CV (%)		31.7	32.8	32.7	10.9	11.99

^{ns}Nonsignificant. * and **Significant at 5 and 1% probability, respectively.

Table 2. Effect of sowing period (winter and summer) on the number of pods, number of seeds, seed weight, number of seeds per pod and 100 seeds weight, evaluated in F_5 lines of group of crosses of BR-16-AHAS x commercial soybean cultivars.

Group	No. of pods		No. of seeds		Seed weight (g)		No. of seeds per pod		100 seeds weight (g)	
	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
BR-16-AHAS	18.7ABa	17.4Ea	30.2C a	37.9Ga	5.7CDb	7.9Da	1.6Eb	2.2ABa	20.2Aa	21.2Aa
Doko-AHAS	19.4ABb	36.8Aa	40.3ABCb	73.6ABa	7.5ABb	13.7Aa	2.1ABa	2.0BCDa	19.2ABCa	18.6BCDa
BRS 133	18.6ABa	20.3DEa	32.6BCb	41.7FGa	6.0BCDb	8.4CDa	1.7Eb	2.0ABCa	19.6ABa	20.2ABa
Conquista	17.0Bb	25.3BCDEa	36.5ABCb	51.2CDEFGa	7.1ABCDb	10.9ABCDa	2.1Aa	2.0BCDb	20.1Aa	21.4Aa
Celeste	19.2ABb	38.4Aa	35.8ABCb	79.6Aa	5.5Db	12.6ABa	1.8CDEb	2.1ABCa	17.4CDEb	16.6DEa
Jataí	20.9ABb	29.6ABCDa	38.3ABCb	59.4BCDEFa	6.1ABCDb	10.1BCDa	1.7DEb	2.0ABCDa	16.7DEa	17.1CDEa
Chapadão	21.5ABa	23.6CDEa	35.7ABCb	44.1EFGa	6.2ABCDb	7.5Da	1.6Eb	1.8Da	18.6ABCDa	17.4CDEb
EMG 308	20.2ABb	29.4ABa	41.6ABb	65.6ABCDa	7.3ABCb	10.9ABCDa	2.0ABCb	2.2Aa	17.5BCDEa	16.6DEb
FT 106	19.9ABb	33.6ABa	42.0ABb	68.6ABCa	6.4ABCDb	10.8ABCDa	2.1ABa	2.0ABCa	15.4Ea	16.2Ea
E96 125	19.5ABb	26.4BCDEa	39.1ABCb	52.4CDEFGa	6.7ABCDb	8.8CDa	2.0ABCa	2.0BCDa	17.6BCDEa	16.8CDEa
Uirapuru	18.2ABb	24.1BCDEa	35.0ABCb	48.7DEFGa	6.5ABCDb	8.2Da	1.9BCDa	2.0ABCDa	19.4ABCa	17.7CDb
Pintado	22.8Ab	32.0ABCa	45.0Ab	61.9ABCDEa	7.8Ab	11.8ABCa	2.0ABCa	1.9CDa	17.6BCDb	19.1ABCa

(1)Means followed by the same capital letters in a column and by the same small letters in a line do not differ by Tukey's test, at 5% probability.

the *ahas* gene. The herbicide effects varied according to the most affected plant positions in each genotype; some genotypes showed pod distribution on the plant similar to distribution in Doko-AHAS (e.g. Conquista, Celeste, EMG 308, FT106, E96 125, Uirapuru, and Pintado), and others were more similar to BR-16-AHAS (e.g. BRS 133, Jataí, and Chapadão).

Kiihl & Arias (2004) found significant differences between Doko-AHAS and BR-16-AHAS for the capacity to produce pods on plants upper part, both in the winter and in the summer. A possible cause for this effect could be related to the insertion point of the *ahas* gene in BR-16, which would be unsuitable for allowing good levels of expression and for conferring tolerance to the plants. This hypothesis can be now discarded, because using the same gene as BR-16-AHAS in various genotypes, the materials obtained presented tolerance comparable or even superior to Doko-AHAS. Thus, the tolerance levels shown by the various genotypes tested depended not only on the *ahas* gene, but also on the presence of other genes, characterizing a type of epistasis. Lines within the groups, considered promising, may or may not inherit these additional genes. Nevertheless, lines within the groups, considered nonpromising, may arise gene combinations favorable to increase imazapyr tolerance level, indicating that it may be a polygenic heredity, which has been called genetic constitution or genetic background in this work.

Table 3. Means of each group of crosses of BR-16-AHAS x commercial soybean cultivars, for each plant position, for number of pods, number of seeds, seed weight, number of seeds per pod, and 100 seeds weight, evaluated after sowing in winter (w) and summer (s) in a greenhouse(1).

Group	Plant position	No. of pods (%)		No. of seeds (%)		Seed weight (%)		No. of seeds per pod		100 seeds weight (g)	
		w	s	w	s	w	s	w	s	w	s
BR-16-AHAS	Upper	12.5b	26.80a	11.77b	27.38a	11.85b	30.08a	1.63a	2.22a	19.69a	22.96a
	Mid	39.3ab	37.40a	40.44ab	38.06a	40.28a	37.94a	1.66a	2.24a	20.96a	21.06a
	Lower	48.2a	35.70a	47.78a	34.55a	47.87a	31.99a	1.61a	2.10a	19.85a	19.23a
Doko-AHAS	Upper	26.57a	41.02a	25.66a	42.04a	27.65a	42.04a	2.00a	2.05a	20.34a	18.79a
	Mid	41.43a	42.53a	43.31a	42.42a	43.19a	43.14a	2.19a	2.00a	18.61a	18.94a
	Lower	32.00a	16.44b	31.03a	15.55b	29.16a	14.82b	1.97a	1.90a	18.59a	17.94a
BRS 133	Upper	17.22b	31.62a	16.93b	32.18a	18.45c	33.45a	1.66a	2.05a	21.21a	21.51a
	Mid	45.15a	36.21a	45.71a	36.61a	45.88a	36.20a	1.68a	2.11a	19.53ab	19.88ab
	Lower	37.63a	32.17a	37.36a	31.21a	35.67b	30.15a	1.73a	1.96a	18.06b	19.16b
Conquista	Upper	31.21a	42.08a	32.39ab	44.05a	34.69a	45.60a	2.24a	2.14a	21.04a	22.04a
	Mid	38.06a	33.61ab	38.71a	33.12ab	37.99a	32.45ab	2.16a	1.98ab	19.47a	20.79a
	Lower	30.73a	24.31b	28.89b	22.83b	27.32b	21.94b	1.98b	1.81b	19.75a	21.23a
Celeste	Upper	22.84b	33.96a	23.61b	34.42a	23.97b	35.16a	1.85a	2.07a	17.77a	17.73a
	Mid	42.67a	34.88a	44.80a	35.12a	42.43a	34.16a	1.88a	2.11a	17.76a	16.28a
	Lower	34.49a	31.16a	31.60b	30.46a	33.60ab	30.68a	1.74a	2.03a	16.70a	15.79a
Jataí	Upper	19.11b	31.31a	19.06b	32.84a	20.12c	35.53a	1.66a	2.11a	16.61ab	18.43a
	Mid	44.60a	34.63a	45.14a	35.48a	46.40a	35.17a	1.76a	2.07a	17.36a	16.99ab
	Lower	36.29a	34.06a	35.80a	31.68a	33.48b	29.30a	1.80a	1.87a	16.09b	15.92b
Chapadão	Upper	13.45b	28.76a	13.24b	30.27a	15.05b	31.61a	1.63a	1.97a	20.22a	18.70a
	Mid	44.56a	32.84a	45.33a	32.87a	45.78a	33.30a	1.65a	1.85a	18.40a	17.02a
	Lower	41.99a	38.40a	41.43a	36.86a	39.17a	35.09a	1.60a	1.60a	17.12a	16.27a
EMG 308	Upper	26.53b	43.50a	24.55b	45.27a	25.49b	46.21a	1.86a	2.35a	18.38a	17.45a
	Mid	50.51a	36.44a	51.47a	36.40a	50.85a	35.43a	2.10a	2.24a	17.31ab	16.19a
	Lower	22.96b	20.05b	23.98b	18.33b	23.66b	18.36b	1.93a	2.04b	16.86b	16.19a
FT 106	Upper	27.32b	31.74a	27.65b	34.09a	28.92b	36.06a	2.11a	2.19a	16.13a	17.62a
	Mid	40.99a	34.16a	41.33a	34.41a	41.49a	33.52a	2.15a	2.06a	15.41ab	15.61a
	Lower	31.69b	34.11a	31.02b	31.50a	29.60b	30.42a	2.03a	1.86b	15.45b	14.69a
E96 125	Upper	26.59b	36.64a	26.14b	38.76a	28.24b	40.88a	2.00a	2.12a	18.77a	18.12a
	Mid	45.58a	36.49a	45.65a	35.53a	46.67a	35.34a	2.01a	1.88a	17.50a	16.65a
	Lower	27.82b	26.87a	28.22b	25.71a	25.09b	23.78a	1.90a	1.90a	16.43a	15.75a
Uirapuru	Upper	25.17b	36.00a	25.28b	38.08a	26.27b	40.17a	1.90a	2.14a	20.47a	19.01a
	Mid	40.48a	37.23a	41.82a	36.29a	42.14a	36.00ab	1.97a	1.95a	19.29a	17.74a
	Lower	34.35a	26.77a	32.90b	25.64a	31.59ab	23.83b	1.86a	1.88a	18.37a	16.21a
Pintado	Upper	24.61b	32.91a	25.34b	36.21a	27.30b	38.15a	2.03a	2.14a	18.73a	20.07a
	Mid	43.46a	31.58a	44.30a	30.70a	43.69a	30.26a	2.02a	1.87b	17.25ab	18.88ab
	Lower	31.93b	35.51a	30.36b	33.09a	29.01b	31.59a	1.88a	1.78b	16.86b	18.40b

(1)Means followed by the same letters in a column do not differ by Tukey's test, at 5% probability.

Conclusion

Using the same gene *ahas* of BR-16-AHAS in various genotypes, the materials obtained present good tolerance to imazapyr; the tolerance level depends not only on the *ahas* gene but also on the presence of other genes.

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References

ARAGÃO F.J.L.; SAROKIN, L.; VIANNA, G.R.; RECH, E.L. Selection of transgenic meristematic cells utilizing a herbicidal molecule results in the recovery of fertile transgenic soybean [*Glycine max* (L.) Merril] plants at a high frequency. **Theoretical and Applied Genetics**, v.101, p.1-6, 2000.

BEARDMORE R.A.; HART, R.; IVERSON, R.; RISLEY, M.A.; TRIMMER, M. Imazapyr herbicide. In: SHANER, D.L.; O'CONNOR, S.L. (Ed.). **The imidazolinone herbicides**. Boca Raton: CRS Press, 1991. p.211-227.

FRIZZAS, M.R.; CUNHA, U.S. da; MACEDO, L.P.M. Plantas transgênicas resistentes a insetos. **Revista Brasileira de Agrociência**, v.10, p.13-18, 2004.

HAIN, R.; SCHREIER, P.H. Genetic engineering in crop protection: opportunities, risks, and controversies. **Pflanzenschutz Nachrichten Bayer**, v.48, p.25-120, 1995.

HERRERA-ESTRELLA, L. Transgenic plants for tropical regions: some considerations about their development and their transfer to the small farmer. **Proceedings of the National Academy of Sciences of the United States of America**, v.96, p.5978-5981, 1999.

KIIHL, T.A.M.; ARIAS, C.A.A. Behavior of soybean genotypes transformed with the *ahas* gene which confers tolerance to herbicide imazapyr. **Crop Breeding and Applied Biotechnology**, v.4, p.267-272, 2004.

MONQUERO, P.A. Plantas transgênicas resistentes aos herbicidas: situação e perspectivas. **Bragantia**, v.64, p.517-531, 2005.

PADGETTE, S.R.; KOLACZ, K.H.; DELANNAY, X.; RE, D.B.; LAVALLÉE, B.J.; TINIUS, C.N.; RHODES, W.K.; OTERO, Y.I.; BARRY, G.F.; EICHHOLTZ, D.A.; PESCHKE, V.M.; NIDA, D.L.; TAYLOR, N.B.; KISHORE, G.M. Development, identification and characterization of a glyphosate-tolerant soybean line. **Crop Science**, v.35, p.1451-1461, 1995.

VARGAS, L.; SILVA, A.A.; BORÉM, A.; REZENDE, S.T.; FERREIRA, F.A.; SEDIYAMA, T. **Resistência de plantas daninhas a herbicidas**. Viçosa: UFV, 1999. 131p.

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