academicJournals

Vol. 12(19), pp. 1661-1668, 11 May, 2017
DOI: 10.5897/AJAR2017.12251
Article Number: E12136C64153
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Characterization of soybean population with sulfonylurea herbicides tolerant alleles

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Received 22 February, 2017; Accepted 11 April, 2017

With the introduction of commercial soybean genotypes with *Als1* and *Als2* alleles that confer tolerance to different active ingredients of sulfonylurea group, this work aims to test soybean populations for the presence/absence of *Als1* and *Als2* alleles and evaluate the agronomic impact of these alleles addition. These trials were conducted in experimental stations of DuPont Pioneer at Sorriso, Mato Grosso state and Planaltina, Federal District. Four populations were evaluated with 40 genotypes each; 10 genotypes without *Als1* and *Als2* (null), 10 genotypes containing *Als1*, 10 containing *Als2* and 10 genotypes containing both alleles. These populations were tested for different traits. The grain yield average at Planaltina and Sorriso were 2888 and 2456 kg ha^{-1}, respectively. Yield for the genotypic classes null, Als1, Als2 and Als1+Als2 were 2672, 2671, 2631 and 2657 kg ha^{-1}, respectively, and they were not statistically different from each other. Also, the other traits indicated similar behavior among classes. As the studied populations were developed for this study, they were inferior than the checks. This work demonstrated that in the four studied populations, the addition of *Als1* and/or *Als2* alleles did not cause significant differences in the evaluated traits.

Key words: *Glycine max* L., *Als1*, *Als2,* grain yield.

INTRODUCTION

During the development of agriculture in Brazil, several species of weeds were selected due to a continuous exposure to herbicides with a similar mode of action. This occurred in conventional soybeans and corn crops, and thereafter due to overuse of glyphosate in genetically modified soybeans. The selection of resistant species is associated with genetic changes in the population under a selection pressure for such products. Therefore, the rotation of herbicide with different action modes is of fundamental importance in production areas (Powles, 2008).

Sulfonylureas are herbicides that block the synthesis of essential amino acids by inhibiting the acetolactate synthase (ALS) enzyme. ALS is the first enzyme to act on

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the biosynthesis of the amino acids valine, leucine and isoleucine. It catalyzes two parallel reactions: condensation of 2 moles of pyruvate forming acetolactate, and condensation of 1 mol of pyruvate with 1 mol of 2-oxybutyrate forming aceto-hydroxybutyrate (Eberlein et al., 1997). The inhibition of this enzyme disrupts the production of proteins, interfering with cell growth and consequently resulting in the death of the plant. Sulfonylureas have been widely used in more than 80 countries and approximately 25 crops. There is a wide variety of sulfonylureas. Some are not selective or effective in the control of all plants, while other products are selective, acting in some species and being tolerated by other species that metabolize the product and detoxify before undergoing a significant damage due to inhibition of ALS activity (Green, 2007).

Having proof the soybean capacity to tolerate some active ingredients of sulfonylureas, such as ethyl chlorimuron, through its fast metabolic inactivation, this active ingredient has become widely used in soybean crops (Zawoznik and Tomaro, 2005). Currently, in Brazil, chlorimuron ethyl is used during pre and post-emergence for the control mainly of weeds resistant to glyphosate. However, higher resistance to this component and to other sulfonylureas was given to soybean through specific mutations in the ALS genes, causing this enzyme to be less susceptible to inhibition by sulfonylureas and maintaining its active vital capacity (Walter et al., 2014).

By using mutagenic techniques and conventional breeding, the cultivar W20 was developed in the 1980s. It derived from Williams and presented a resistance to sulfonylurea herbicides (Sebastian et al., 1989). This was the first cultivar of the group commercially known as STS® (sulfonylurea tolerant soybean). This technology provided a greater flexibility in the use of different sulfonylureas with a wider weed control action spectrum, and was widely used by different companies in North and South America. After a period without use due to the introduction of genetically modified cultivars with resistance to glyphosate, the emergence of weeds resistant to this active ingredient reactivated the use of the STS® technology, reappearing in the market combined with the glyphosate resistance gene (Green and Owen, 2011). Later, the mutant allele used in the STS® technology became known as *Als1*. Its wild version is the *als1* (Walter et al., 2014).

After the incorporation of the allele *Als1* into modern cultivars, a new cycle of changes began aiming to develop mutants even more tolerant to sulfonylurea. The line W4-4 was then created. It underwent a second mutagenic event, giving rise to a second independent allele called *Als2* (Walter et al., 2014).

The type of gene mutation that occurred with the *Als1* and *Als2* alleles was base substitution. For the allele *Als1*, located on the chromosome 4, there was the substitution of proline for serine at the position 178 of the soybean protein. For the allele *Als2*, located on the chromosome 6, tryptophan was replaced for leucine at the position 560 of the soybean protein (Walter et al., 2014).

Soybean containing *Als1* and/or *Als2* alleles were developed as an alternating tool to control weeds showing herbicide resistance especially to glyphosate. Herbicides from sulfonylurea group are intended to auxiliary in the dicotyledonous weed control such as *Conyza* spp. which is glyphosate resistant. It is mentioned in the literature (Vargas et al., 2007; Moreira et al., 2007; Lamego and Vidal, 2008) that *C. bonariensis* and *C. canadensis* are glyphosate resistant.

Given the possibility of using soybean lines containing *Als1* and *Als2* alleles, tolerant to different active ingredients of sulfonylureas and presenting a higher tolerance to the current used sulfonylureas, it is necessary to conduct tests to prove that the addition of such mutant alleles into new soybean genotypes do not cause agronomic losses to the crop. Therefore, this study aims to test different soybean populations regarding the presence/absence of *Als1* and *Als2* and evaluate the agronomic impact of these alleles addition.

MATERIALS AND METHODS

Obtaining the families

This study was conducted using recombinant inbred lines from four populations. The development of these genotypes using modified bulk method started during the 2011/2012 season, when crosses involving the donor of *Als1* and *Als2* alleles (CD250RRSTS) with genotypes adapted to central Brazil (BG4277, 98Y30, YB84C12 and XB85C12) were made. In the winter of 2012, F_1 seeds were sown and the confirmation of the crosses was made by molecular analysis using markers according to Walter et al. (2014). During the next season (2012/2013), seeds were sown as F_2, and in the 2013 winter as F_3. When advanced to F_4, during the 2013/2014 season, another molecular analysis (Walter et al., 2014) was made to classify and select homozygous plants considering the presence/absence of *Als1* and *Als2* alleles. In the 2014 winter, $F_{4:5}$ recombinant lines from the four populations were sown (Table 1). The development of the populations was made at the DuPont Pioneer research center of Planaltina, Federal District (DF), except for F_3 and $F_{4:5}$ generations, whose developments were conducted at DuPont Pioneer research center of Palmas, Tocantins (TO) state.

For each population, four classes of genotypes (genotypic classes) were obtained: Null genotypes (without *Als1* and *Als2* alleles), genotypes containing *Als1* allele, genotypes containing *Als2* allele, and genotypes containing both alleles (Table 1). All evaluated ALS alleles were homozygous. 10 $F_{4:6}$ recombinant lines from each class were selected among the four populations based on agronomic characteristics, uniformity, maturity and germination. They were tested during the 2014/2015 season, into which four variety checks without *Als1* and *Als2* alleles were included. Variety checks; 97R21, 97R73, 98Y12 and 98Y30; were included in the statistical analysis as the fifth genotypic class.

Field evaluation

Forty genotypes of each population were sown during the 2014/2015 season, being 10 genotypes of each class plus the four

Table 1. Number of genotypes obtained from each class and population after marker assisted selection.

Population		Number of genotypes				
Parental	**Code**	**Null**	**Als1**	**Als2**	**Als1+Als2**	**Total**
BG4277/CD250RRSTS	Pop001	69	73	162	183	487
98Y30/CD250RRSTS	Pop002	14	19	20	31	84
YB84C12/CD250RRSTS	Pop003	78	164	32	129	403
XB85C12/CD250RRSTS	Pop004	164	131	142	123	560
Total		325	387	356	466	1534

checks. The experiments were conducted at DuPont Pioneer research centers of Sorriso, Mato Grosso (MT) state, and Planaltina (DF). Although both sites have soils classified as Latosol, both locations represent two distinct environments. The Sorriso site is located at the center-northern part of MT at 12°44´39.63´´S and 55°49´54.23´´W with an altitude of 398 m. The Planaltina site is located at the northeastern part of the DF at 15°43´18.12´´S and 47°36´10.21´´W with an altitude of 1,163 m.

The experimental design was randomized blocks with three replications. Each population was planted and randomized separately. Each plot consisted of four rows of five meters long with 0.50 m between rows. The two center rows were harvested. Plant populations were 240,000 and 280,000 plants ha^{-1} at Planaltina and Sorriso, respectively. Experiments were sowed in Sorriso on November 6th and in Planaltina on November 21th, 2014. Field management was done following EMBRAPA soja (2001) recommendation. Crop field was desiccated at the R7.3 stage with Gramoxone, which contain 200 g L^{-1} of active ingredient Paraquat, using product dosage of 2 L ha^{-1}. Subsequently each population was harvested with mechanical combine according to maturity level.

The evaluated agronomic traits were: Seedling emergence (EMG), which consists in a visual percentage of emerged seedlings at V2 stage; plant height (PH), measured the distance in cm from the soil surface until the apex of a representative plant and evaluated during the maturity stage of the treatment in R8; maturity (MAT), it is the number of days from planting to the date when 95% of the treatment reached the R8 maturity stage; plot evaluation (PLEV), a percentage score for the experimental unit aiming to measure the plot quality based on the number of plants and their distribution during the maturity stage in R8; visual treatment evaluation (VTE), percentage score for visual appearance of the treatment at R8 stage based on desired agronomic characteristics; non-lodging (NLOD), which consists in the percentage of plants that did not incline more than 45% during R8 stage; and grain yield (GY) that is the seed weight of a plot converted to kg ha^{-1} and corrected to 13% moisture. The soybean stages were classified according to Fehr et al. (1971).

The collected data were analyzed using the statistical program R (R Core Team, 2016). The adopted statistical model was mixed with locations and blocks as random effects, and populations and gene genotypic classes as fixed effects. An analysis of variance was performed for each location, followed by a Bartlett's test (homogeneity of variances) at 0.005 probability in order to validate a combined analysis of variance (Ramalho et al., 2012). The means were compared by Tukey test at 5% probability.

RESULTS AND DISCUSSION

As the errors of the variances were homogeneous in both locations for all evaluated traits in the experiments, a combined variance analysis was performed (Table 2). The means of the experiments, populations, gene genotypic classes, all interactions and Tukey test performed for traits with significant difference are shown in Table 3.

During the crop cycle of the 2014/2015 season, the climatic conditions of Planaltina were favorable to the development of the crop, except during the final phase of the vegetative stage, when rainfalls decreased. At the Sorriso station, the weather conditions were inadequate at the beginning of the vegetative stage due to low rainfalls, complicating early crop growth and significantly compromising final grain production (Figure 1). Therefore, the yield average of the experiment in Planaltina was 2,888 kg ha^{-1}, and in Sorriso was 2,456 kg ha^{-1}. The average of the two experiments was 2,674 kg ha^{-1} (Table 3).

The difference in yield between the two locations can be explained partly by a decrease in seedling emergence and plot evaluation. The average emergence score of Planaltina was 91%; in Sorriso, such score was 76% (Table 3). For the trait evaluation of the plot, the average in Planaltina was 96%, and in Sorriso 61% (Table 3).

According to grain yield data, the only significantly different sources of variation were population and location*class (Table 2). Pop002 population was significantly better than Pop003 and Pop004 populations, but it did not differ statistically from Pop001. In the interaction, checks were superior than genotypic classes in Sorriso (Table 3). Checks had better capability to overcome the adversity conditions in Sorriso due to the whole breeding and selection process they went through, different of the populations that were developed for this type of study. The means of the genotypic classes null, Als1, Als2 and Als1+Als2 had similar values and they were not statistically different. The other sources of variation for grain yield did not show significant differences, indicating that the addition of *Als1* and/or *Als2* alleles did not change the most important characteristic in soybeans (Table 3).

For seedling emergence, the significantly different sources of variation were class and location *population* class (Table 2). Checks were better than null and Als1 classes, and did not vary from Als2 and Als1+Als2. The values in Sorriso were inferior than in Planaltina (Table 3). The adverse conditions during emergence and early

Table 2. Summary of the combined analysis of variance with the evaluated traits in the experiments conducted at Planaltina, DF and Sorriso, MT during 2014/2015 season.

Source of variation	DF	GY	EMG	PH	PLEV	NLOD	VTE	MAT
Local	1	-	-	-	-	-	-	-
Rep(Local)	4	-	-	-	-	-	-	-
Pop	3	6060313*	859^{ns}	5652^{ns}	409^{ns}	9238^{ns}	1140^{ns}	2262^{ns}
Class	4	727597^{ns}	568*	10179*	464^{ns}	5005**	3258^{ns}	2151*
Pop*Class	12	304056^{ns}	177^{ns}	650**	160^{ns}	1229**	156^{ns}	142^{ns}
Local*Pop	3	240059^{ns}	135^{ns}	1227**	422**	1127**	1501**	410**
Local*Class	4	463476**	70^{ns}	972**	704**	64^{ns}	836**	156**
Local*Pop*Class	12	186123^{ns}	338*	110^{ns}	179**	229^{ns}	186*	73**
Error	999	113723	155	107	59	192	100	17
CV (%)		12.6	14.9	11.4	9.8	16.1	14.2	3.3

^{ns}Non significant; * and ** significant at the 0.05 and 0.01 probability levels, respectively. DF = degrees of freedom; GY = grain yield; EMG = seedling emergence; PH = plant height; PLEV = plot evaluation; NLOD = non-lodging; VTE = visual treatment evaluation e MAT = maturity.

Table 3. Means of evaluated traits for locations, populations, genotypic classes, and all interactions in the experiments conducted at Planaltina (Plan), DF and Sorriso (Sorr), MT, in 2014/2015 season (Tukey test was applied for traits with significant difference).

Description	Variable	GY (kg ha^{-1})	EMG (%)	PH (cm)	PLEV (%)	NLOD (%)	VTE (%)	MAT (days)
Mean	Both locations	2674	84	91	79	86	71	124
Loc. mean	Planaltina	2888	91	91	96	87	69	140
Loc. mean	Sorriso	2456	76	91	61	85	72	107
Four populations means	Pop001	2755^{bc}	82	91	77	91	71	126
	Pop002	2835^{c}	86	84	79	90	72	120
	Pop003	2492^{a}	84	94	80	78	68	126
	Pop004	2615^{ab}	82	94	79	85	71	123
Five genotypic classes means	Null	2672	82^{a}	94^{b}	78	83^{a}	68	125^{b}
	Als1	2671	82^{a}	91^{b}	79	87^{b}	70	125^{b}
	Als2	2631	85^{ab}	95^{b}	78	82^{a}	70	125^{b}
	Als1+Als2	2657	84^{ab}	91^{b}	79	87^{b}	70	124^{b}
	Checks	2832	87^{b}	72^{a}	83	98^{c}	81	115^{a}
Populations and classes interaction means	Pop001xNull	2765	79	94^{bcde}	75	91^{cde}	68	126
	Pop001xAls1	2844	82	92^{bcd}	75	90^{cde}	70	127
	Pop001xAls2	2635	85	93^{bcde}	77	87^{bcde}	72	130
	Pop001xAls1+Als2	2723	82	92^{bcd}	78	95de	71	125
	Pop001xChecks	2889	86	76^{a}	84	98^{e}	82	115
	Pop002xNull	2878	88	86^{b}	81	92de	72	121
	Pop002xAls1	2761	84	86^{b}	78	88^{bcde}	70	121
	Pop002xAls2	2836	85	86^{b}	77	88^{bcde}	42	121
	Pop002xAls1+Als2	2826	86	86^{b}	78	89^{bcde}	70	120
	Pop002xChecks	2934	88	70^{a}	85	98^{e}	82	115
	Pop003xNull	2474	81	99de	78	66^{a}	64	129
	Pop003xAls1	2395	83	91^{bcd}	81	83^{bcd}	68	126
	Pop003xAls2	2527	85	99de	79	78^{abc}	66	127
	Pop003xAls1+Als2	2449	87	96^{cde}	79	78^{abc}	67	128
	Pop003xChecks	2797	87	74^{a}	82	97^{e}	81	116
	Pop004xNull	2582	80	97^{cde}	78	83^{bcd}	69	125
	Pop004xAls1	2686	80	97^{cde}	80	88^{bcde}	72	124

Table 3. Contd.

	Pop004xAls2	2527	84	102^{e}	78	76^{ab}	68	123
	Pop004xAls1+Als2	2631	82	88^{bc}	78	88^{bcde}	72	123
	Pop004xChecks	2707	87	70^{a}	81	99^{e}	81	114
	PlanaltinaxPop001	2933	90	93^{cd}	93^{b}	92de	71^{bc}	140^{f}
	PlanaltinaxPop002	3063	93	85^{a}	96^{bc}	93^{e}	73^{c}	137^{e}
Locations and populations interaction means	PlanaltinaxPop003	2689	91	95de	99^{c}	77^{a}	63^{a}	144^{g}
	PlanaltinaxPop004	2870	90	91^{bc}	96^{bc}	88^{cde}	69^{b}	139^{f}
	SorrisoxPop001	2572	74	89^{b}	60^{a}	91de	71^{bc}	111^{d}
	SorrisoxPop002	2606	79	83^{a}	62^{a}	87^{cd}	72^{bc}	103^{a}
	SorrisoxPop003	2294	78	94^{cde}	61^{a}	80^{ab}	72^{bc}	109^{c}
	SorrisoxPop004	2357	74	97^{e}	61^{a}	83^{bc}	73^{c}	106^{b}
	PlanaltinaxNull	2888^{bc}	90	95^{d}	96^{c}	84	65^{a}	142^{e}
	PlanaltinaxAls1	2901^{c}	90	93^{cd}	96^{c}	89	69^{abc}	141^{e}
	PlanaltinaxAls2	2854^{bc}	92	95^{d}	97^{c}	84	67^{ab}	141^{e}
Locations and classes interaction means	PlanaltinaxAls1+Als2	2899^{c}	92	91^{cd}	97^{c}	89	69^{abc}	141^{e}
	PlanaltinaxChecks	2921^{c}	93	66^{a}	95^{c}	97	85^{e}	129^{d}
	SorrisoxNull	2454^{a}	74	93^{cd}	60^{a}	82	71^{bc}	108^{c}
	SorrisoxAls1	2440^{a}	75	90^{c}	61^{a}	86	71^{bc}	108^{c}
	SorrisoxAls2	2408^{a}	77	95^{d}	59^{a}	81	72^{c}	109^{c}
	SorrisoxAls1+Als2	2407^{a}	76	90^{c}	60^{a}	86	71^{bc}	106^{b}
	SorrisoxChecks	2743^{b}	81	78^{b}	70^{b}	98	78^{d}	100^{a}
	PlanxPop001xNull	2893	86^{bcdefgh}	98	89^{e}	93	68^{bcdef}	140^{jk}
	PlanxPop001xAls1	2958	90^{defgh}	94	93^{ef}	93	70^{bcdef}	142^{jkl}
	PlanxPop001xAls2	2922	91^{efgh}	95	94^{ef}	86	69^{bcdef}	141^{jkl}
	PlanxPop001xAls1+Als2	2936	93^{gh}	96	94^{ef}	95	70^{bcdef}	140^{jk}
	PlanxPop001xChecks	2984	94^{h}	69	97^{ef}	97	86^{h}	129^{i}
	PlanxPop002xNull	3122	92^{fgh}	87	96^{ef}	95	72^{cdef}	138^{j}
	PlanxPop002xAls1	3085	91^{efgh}	88	96^{ef}	89	69^{bcdef}	138^{j}
	PlanxPop002xAls2	2970	95^{h}	88	96^{ef}	93	73^{defg}	138^{j}
	PlanxPop002xAls1+Als2	3073	93^{gh}	86	97^{ef}	93	73^{defg}	138^{j}
	PlanxPop002xChecks	3067	95^{h}	67	97^{ef}	98	86^{h}	129^{i}
	PlanxPop003xNull	2676	92^{fgh}	102	99^{f}	61	55^{a}	145^{l}
	PlanxPop003xAls1	2590	90^{defgh}	93	99^{f}	82	64^{abcd}	144^{kl}
	PlanxPop003xAls2	2740	92^{fgh}	100	99^{f}	77	61^{ab}	144^{kl}
Locations, populations and classes interaction means	PlanxPop003xAls1+Als2	2690	92^{fgh}	96	99^{f}	77	62^{abc}	145^{l}
	PlanxPop003xChecks	2843	90^{defgh}	65	95^{ef}	98	84^{h}	129^{i}
	PlanxPop004xNull	2860	89^{cdefgh}	94	98^{f}	88	65^{abcd}	141^{jkl}
	PlanxPop004xAls1	2970	90^{defgh}	96	96^{ef}	90	70^{bcdef}	141^{jkl}
	PlanxPop004xAls2	2784	91^{efgh}	98	97^{ef}	78	66^{bcde}	140^{jk}
	PlanxPop004xAls1+Als2	2895	90^{defgh}	86	96^{ef}	90	70^{bcdef}	140^{jk}
	PlanxPop004xChecks	2791	93^{gh}	64	93^{ef}	97	83^{gh}	129^{i}
	SorrxPop001xNull	2628	72^{a}	91	59^{a}	90	68^{bcdef}	109^{defg}
	SorrxPop001xAls1	2730	75^{ab}	89	57^{a}	87	68^{bcdef}	112^{g}
	SorrxPop001xAls2	2349	79^{abcdef}	90	60^{a}	87	76^{efgh}	118^{h}
	SorrxPop001xAls1+Als2	2494	70^{a}	88	60^{a}	95	71^{bcdef}	108^{defg}
	SorrxPop001xChecks	2795	78^{abcde}	82	70^{cd}	99	78^{fgh}	100^{a}
	SorrxPop002xNull	2634	83^{abcdefgh}	84	65^{abcd}	90	72^{cdef}	103^{abc}
	SorrxPop002xAls1	2437	77^{abcd}	84	61^{ab}	87	72^{cdef}	103^{abc}
	SorrxPop002xAls2	2699	74^{ab}	84	57^{a}	83	71^{bcdef}	103^{abc}
	SorrxPop002xAls1+Als2	2578	79^{abcdef}	85	60^{a}	84	68^{bcdef}	103^{abc}

Table 3. Contd.

SorrxPop002xChecks	2801	80^{abcdefg}	73	72^{d}	98	78^{fgh}	99^{a}
SorrxPop003xNull	2272	71^{a}	96	57^{a}	71	72^{cdef}	111^{fg}
SorrxPop003xAls1	2199	76^{abc}	89	63^{abc}	85	71^{bcdef}	108^{defg}
SorrxPop003xAls2	2313	79^{abcdef}	100	59^{a}	79	71^{bcdef}	110^{efg}
SorrxPop003xAls1+Als2	2209	82^{abcdefgh}	97	62^{abc}	79	71^{bcdef}	110^{efg}
SorrxPop003xChecks	2752	83^{abcdefgh}	82	69^{bcd}	96	78^{fgh}	102^{ab}
SorrxPop004xNull	2303	70^{a}	101	57^{a}	78	72^{cdef}	108^{defg}
SorrxPop004xAls1	2392	70^{a}	98	64^{abcd}	86	73^{defg}	107^{cdef}
SorrxPop004xAls2	2280	77^{abcd}	107	59^{a}	75	70^{bcdef}	106^{bcde}
SorrxPop004xAls1+Als2	2347	74^{ab}	91	59^{a}	87	73^{defg}	105^{bcd}
SorrxPop004xChecks	2624	82^{abcdefgh}	75	69^{bcd}	100	78^{fgh}	99^{a}

Means with the different letter in the column within a source of variation are different according to Tukey test (P<00.5). GY = grain yield; EMG = seedling emergence; PH = plant height; PLEV = plot evaluation; NLOD = non-lodging; VTE = visual treatment evaluation; MAT = maturity.

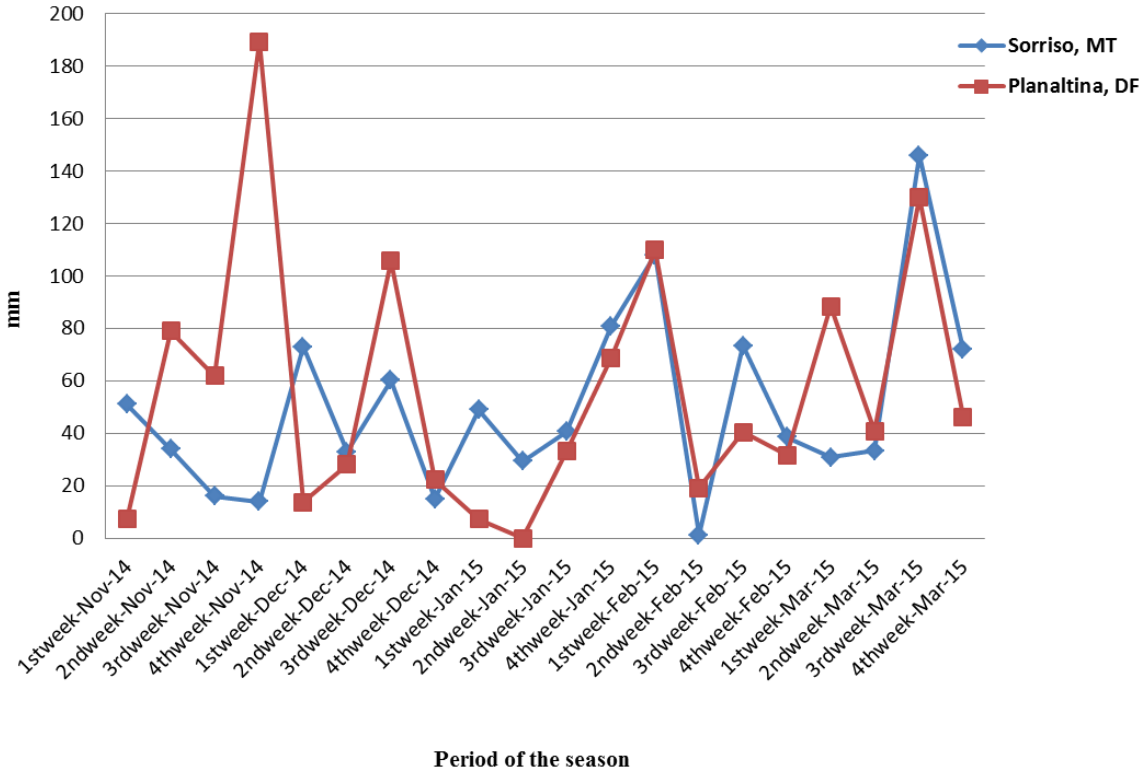

Figure 1. Rainfall at DuPont Pioneer research centers of Sorriso, MT and Planaltina, DF during 2014/2015 season. Data collected by DuPont Pioneer weather station located at the research sites.

growth of the crop in Sorriso are because of the lack of rain in the beginning of the crop cycle.

Plant height was statistically different regarding the sources of variation class, population*class, location*population and location*class (Table 2). Checks were lower than the genotypic classes of *Als1* and/or *Als2* alleles (Table 3). This difference is highly associated with the cycle of the evaluated genotypes. The checks reached maturity significantly earlier than the other genotypic classes. Comparing populations, Pop002 was significantly lower than other populations at Sorriso and Planaltina. In Planaltina, the highest population was Pop003. In Sorriso, the highest populations were Pop004 and Pop003 (Table 3). These higher populations were therefore more subject to lodging.

Another trait that reflected the environmental conditions

was plot evaluation, which was significantly different for location*population, location*class and location*population*class (Table 2). The populations and classes in Sorriso were statistically inferior than the populations and classes in Planaltina. In the Sorriso experiment, the checks were more tolerant to adverse conditions and had higher scores when compared with genotypic classes (Table 3). Checks, once again, showed superiority when in unfavorable conditions due to all testing and selection process they passed by during years before become commercial varieties different of the genotypic classes.

Three sources of variation were statistically different for non-lodging: Class, population*class and location*population (Table 2). Due to the low plant height of the checks, they lodged less than the genotypic classes. Among populations, those that stood out were Pop001 and Pop002 (Table 3).

Regarding the visual evaluation of the treatment, there were differences for location*population, location*class and location*population*class (Table 2). In Sorriso, populations had similar appearance and behavior, and were statistically equal. However, in Planaltina, Pop002 was significantly superior to Pop003 and Pop004 populations (Table 3). In Planaltina, Pop001 and especially Pop002 stood out visually if compared to the other populations. Pop002 was highly homogeneous and its genotypic classes were very similar. Thus, it was difficult to mark any visual difference between them. Despite the lower homogeneity of the other populations, they also showed a high visual similarity between the four gene genotypic classes (Table 3).

For the maturity trait, the genotypes in Sorriso completed the cycle faster than in Planaltina (Table 3). This is due to the geographical position of the evaluated locations. Latitude and altitude affect day length and temperature during the day and the night, causing soybean genotypes in Sorriso to accelerate the cycle if compared to Planaltina. The sources of variation class, location*population, location*class and location*population*class were significantly different regarding this trait (Table 2). The checks completed the cycle faster than genotypic classes both in Sorriso and Planaltina. Genotypic classes did not differ among themselves in each location, except for Als1+Als2 in Sorriso, which had a slightly earlier cycle than the others. Among populations, the Pop002 cycle was significantly earlier than the other populations. In Sorriso, the Pop001 cycle was delayed, while in Planaltina the Pop003 cycle required the longest time to reach maturity (Table 3). Once more, the genotypic classes of the alleles were very similar, however with a longer cycle than the average of the four checks.

The breeding process, to which the checks were submitted, played an important role. Checks presented better ability to excel under unfavorable conditions compared to the populations that were developed aiming to evaluate *Als1* and *Als2* alleles. The genotypic classes of the alleles were very similar overall, however it is necessary that the best lines containing one or both alleles go through a whole breeding process before become a commercial variety or entry in a breeding cross.

There are no reports in the literature comparing the effects of adding *Als1* and *Als2* alleles on the agronomic characteristics of soybean genotypes. In transgenic, which involves the inclusion of a generally exogenous gene into a given genotype, there is a great concern about whether their inclusion could cause agronomic concerns, such as a decrease in yield, and change crop cycle, plant height, germination, flowering and other traits. This is due to the inclusion of a gene which may be added to an undesired region of a chromosome, disrupting endogenous genes, preventing the formation of essential proteins or causing fusion of undesirable proteins. These changes may result in phenotypes with undesirable agronomic characteristics (Que et al., 2010).

Some studies (Minor, 1998; Elmore et al., 2001) reported that the addition of a transgenic gene resistant to the first-generation of glyphosate in soybeans decreased grain yield. However, another line (Carpenter, 2001) stated that this happened because the transgenic gene was added to genotypes that were not superior, and the decrease in yield was due to the genotype and not to the transgenic gene; thus, with the introduction of new superior cultivars containing the transgenic gene, that difference in productivity would decrease until inexistent. In addition, Hungria et al. (2014) reported no yield drag on genetic modified soybean and EFSA (2010) concluded that the transgenic version is agronomically equivalent to its conventional counterpart.

Mutagenic induction aiming to generate variability and hence the appearance of new forms of a gene may also raise suspicion that such mutant allele may cause changes in the formation of essential proteins, development of improper proteins and emergence of undesirable phenotypes.

In soybeans, the mutant gene *FAD2-1A*, found in the cultivar M23, which provides a high-quality oil to soybeans seeds by increasing the oleic acid, is often associated with decrease in grain yield (Scherder and Fehr, 2008; Clemente and Cahoon, 2009).

In tomato crops, the impact of a mutation known as *ovate*, which promotes a drastic change in the tomato fruit shape, also caused negative changes in the phenotype. This mutation resulted in a decrease in soluble solids, average fruit and seed weight, fruit fixation and productivity (Faria, 2014).

On the other hand, other mutations did not significantly change agronomic characteristics. Spano et al. (2003) worked with four mutants in durum wheat with the capability of delay leaf senescence. They concluded that the extended period of flag leaf photosynthetic competence in the mutant lines generated higher seed

weights and grain yield per plants in the mutant lines compared to their parental lines.

In fig trees, the mutation process, induced by irradiation with gamma rays aiming to increase genetic variability, generated five mutant lines, which were then evaluated in performance tests and compared to other commercial cultivars. The results showed that the mutants had a performance similar to the commercial cultivars, and that the mutant PI-189 was superior to the commercial cultivars regarding important characteristics such as number of fruits per plant, average weight per fruit and yield (Rodrigues et al., 2009).

The type of gene mutation that generated *Als1* and *Als2* alleles was base substitution. Only a single amino acid was changed in each gene (Walter et al., 2014). The results of this study show that such a minimal change in amino acids, in general, did not generate statistically significant differences between the four classes of *Als1* and *Als2* alleles.

Further studies evaluating the addition of *Als1* and/or *Als2* alleles, involving populations or commercial varieties with a similar germplasm and in different environments, become crucial to confirm that the addition of such alleles does not change the agronomic characteristics in soybeans.

Conclusions

For the four studied populations, Pop001 (BG4277/ CD250RRSTS), Pop002 (98Y30/CD250RRSTS), Pop003 (YB84C12/CD250RRSTS) and Pop004 (XB85C12/ CD250RRSTS), the incorporation of *Als1* and/or *Als2* alleles aiming a greater resistance to herbicides from the sulfonylurea group did not cause significant changes in the evaluated agronomic traits.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Carpenter JE (2001). Comparing Roundup Ready and conventional soybean yields: 1999. National Center for Food and Agricultural Policy, Washington, DC.

Clemente TE, Cahoon EB (2009). Soybean oil: genetic approaches and modification of functionality and total content. Plant Physiol. 151(3):1030-1040.

Eberlein CV, Guttieri MJ, Thill DC, Baerg RJ (1997). Altered acetolactate synthase activity in ALS - inhibitor resistant prickly lettuce (*Lactuca serriola*). Weed Sci. 45(2):212-217.

EFSA – European Food Safety Autority (2010). Scientific opinion of the panel on Genetically Modified Organisms on applications (EFSA-GMO-RX-40-3-2) for the renewal of authorization for the continued marketing of (1) food containing, consisting of, or produced from genetically modified soybean 40-3-2; (2) feed containing, consisting of, or produced from soybean 40-3-2; (3) other products containing or consisting of soybean 40-3-2 with the exception of cultivation, all under Regulation (EC) No 1829/2003 from Monsanto. EFSA J. 8(12):1-38.

Elmore RW, Roeth FW, Nelson LA, Shapiro CA, Klein RN, Knezevic SZ, Martin A (2001). Glyphosate-resistant soybean cultivar yields compared with sister lines. Agron. J. 93(2):408-412.

EMBRAPA Soja (2001). Tecnologias de produção de soja – Região Central do Brasil – 2001/2002. Londrina: Documentos 167:267.

Faria JPB (2014). Alterações hormonais no mutante ovate de tomateiro (*Solanum lycopersicum* L. cv Micro-Tom) e seu impacto na morfologia, qualidade do fruto, produtividade e partenocarpia. 68 f. Masters dissertation (Master in Plant Physiology and Biochemistry). Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba.

Fehr WR, Caviness CE, Burmood DT, Pennington JS (1971). Stage of development descriptions for soybeans, *Glycine max* (L.) Merrill. Crop Sci. 11(6): 929-931.

Green JM (2007). Review of glyphosate and ALS-inhibiting herbicide crop resistance and resistant weed management. Weed Technol. 21(2):547-558.

Green JM, Owen MDK (2011). Herbicide-resistant crops: utilities and limitations for herbicide-resistant weed management. J. Agric. Food Chem. 59(11):5819-5829.

Hungria M, Mendes IC, Nakatani AS, Reis-Junior FB, Morais JZ, Oliveira MCN, Fernandes MF (2014). Effects of glyphosate-resistant gene and herbicides on soybean crop: Field trials monitoring biological nitrogen fixation and yield. Field Crop. Res. 158(1):43-54

Lamego FP, Vidal RA (2008). Resistance to glyphosate in *Conyza bonariensis* and *Conyza canadensis* biotypes in Rio Grande do Sul, Brazil. Planta Daninha 26(2):467-471.

Minor H (1998). Performance of GMOs vs. traditional varieties: a southern perspective. In: Proceeding of the 28th Soybean Seed Research Conference, 1998, Chicago, IL. American Seed Trade Association, Washington, DC. pp. 1–9.

Moreira M, Nicolai M, Carvalho SJP, Christoffoleti PJ (2007). Resistência de *Conyza canadensis* e *C. bonariensis* ao herbicida glyphosate. Planta Daninha 25(1):323-326.

Powles SB (2008). Evolution in action: glyphosate-resistant weeds threaten world crops. Outlooks on Pest Management 19(6):256-259.

Que Q, Chilton MDM, Fontes CM, He C, Nuccio M, Zhu T, Wu Y, Chen JS, Shi L (2010). Trait stacking in transgenic crops: challenges and opportunities. GM Crops Food 1(4):220-229.

R Core Team (2016). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria. https://www.R-project.org/

Ramalho MAP, Ferreira DF, Oliveira AC (2012). Experimentação Em Genética E Melhoramento De Plantas. 3.Ed. Lavras: UFLA 305 p.

Rodrigues MGF, Correa LS, Boliani AC (2009). Avaliação de seleções mutantes de figueira cv. Roxo-de-Valinhos. Rev. Bras. Frutic. 31(3):771-777.

Sebastian SA, Fader GM, Ulrich JF, Forney DR, Chaleff RS (1989). Semidominant soybean mutation for resistance to sulfonylurea herbicides. Crop Sci. 29(6):1403-1408.

Scherder CW, Fehr WR (2008). Agronomic and seed characteristics of soybean lines with increased oleate content. Crop Sci. 48(5):1755-1758.

Spano G, Di Fonzo N, Perrotta C, Platani C, Ronga G, Lawlor DW, Napier JA, Shewry PR (2003). Physiological characterization of 'stay green' mutants in durum wheat. J. Exp. Bot. 54(386):1415-1520.

Vargas L, Bianchi MA, Rizzardi MA, Agostinetto D, Dal Magro T (2007). Buva (*Conyza bonariensis*) resistente ao glyphosate na região sul do Brasil. Planta Daninha 25(3):573-578.

Walter KL, Strachan SD, Ferry NM, Albert HH, Castle LA, Sebastian SA (2014). Molecular and phenotypic characterization of *Als1* and *Als2* mutations conferring tolerance to acetolactate synthase herbicides in soybean. Pest Manage. Sci. 70(12):1831-1839.

Zawoznik MS, Tomaro ML (2005). Effect of chlorimuron-ethyl on *Bradyrhizobium japonicum* and its symbiosis with soybean. Pest Manage. Sci. 61(10):1003-1008.