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Glyphosate resistance trait into soybean Cuban varieties: agronomical assessment of transgenic lines until F6 generation

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Abstract

Glyphosate-resistant (GR) soybean was one of the first major applications of genetic engineering in field crops and offered farmers a vital tool in fighting weeds. Weeds are a problem for soybean production in Cuba, so our work aim was the GTS 40-3-2 event introgression into Cuban varieties. Two local cultivars were crossed with transgenic genotypes that carry the event. From F1 to F3 generations, individual plants that produced more than 60 g of seeds per plant were chosen to obtain next generation. Individual lines were selected from F4 generation. F5 and F6 generations of five selected transgenic lines and their relatives were chosen to evaluate seven agronomic traits throughout the summers of 2012 and 2013. A Random Block experimental design was done. First flowering (R1) and maturity (R8) stages of all genotypes were affected by planting date. Plant height of I1B2-3, I1B4, I36B4 and RP5 lines ranged from 80 to 111 cm. I1B2-2 and I1B2-3 lines would be suitable for mechanized harvesting because they had the insertion of the first pod at 14.63 cm and 13.93 cm respectively. I36B4 line produced the greatest number of pods per plant (127). Transgenic lines produced more than 180 seeds per plant and 100-seed weight ranged from 13.75 g to 17.46 g. Seed yield per plant of transgenic lines and their parents IncaSoy36, CEB2 and CEB4 weren't statistically different. These results could be a start point for other studies involving larger areas, different planting dates and localities.

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Introduction

Improvement of all major crops including cereals, legumes, and oilseeds has been a constant activity for agricultural development. Soybean (*Glycine max* L. Merrill) is, no doubt, one of the most important crops at global level for the widespread applicability (food, biodiesel, secondary metabolites, among others) and economic value of its products in the global market (Sharma *et al.*, 2011).

Modern methods of plant breeding, whether classical or through genetic engineering, have been used to enhance soybean attributes. Yield (Wilcox, 2001; Toshiyuki *et al.*, 2002; De Bruin and Pederson, 2008), quality and seed composition (Krishnan, 2005; Cicek *et al.*, 2006; Haun *et al.*, 2014), disease and pest resistance (Calvo *et al.*, 2008; Cunha *et al.*, 2010; Yu *et al.*, 2013), drought and salinity tolerance (Chen *et al.*, 2007; Wang *et al.*, 2011; Zhang *et al.*, 2013), herbicide resistance (Padgette *et al.*, 1995; Walter *et al.*, 2014) among others have been some of the principal goals for soybean improvement.

Glyphosate-resistant (GR) soybean was one of the first major applications of genetic engineering in field crops (Elmore *et al.*, 2001). The soybean genetic modification involved the insertion of a gene (cp4 EPSPS:5-enolpyruvylshikimate-3 phosphate synthase), which is responsible to the production of cp4-EPSPS enzyme from *Agrobacterium* sp. This enzyme confers tolerance to glyphosate (N-fosfometil glycine). Glyphosate-tolerant crops offered farmers a vital tool in fighting weeds and were compatible with no-till methods, which help preserve topsoil.

Weeds are considered the number one problem in all major soybean producing countries. According to estimates, weeds, alone, cause an average reduction of 37% on soybean yield (Oerke and Dehne, 2004). The introduction of GR soybean contributed to standardization of weed management (Vivian *et al.*, 2013). Plants genetically modified can normally develop in the presence of this herbicide, providing farmers with a strong management tool for soybean crop production. In the US, more than 93% of soybean has the GR technology. In Brazil and Argentina, these values represent 80% and 99%, respectively (Vivian *et al.*, 2013). In 2014, the US planted a record area of 34.3 million hectares of soybean (James, 2014).

In Cuba, soybeans were cultivated for the first time in 1904, and both climate and soil are suitable for planting, with the potential to grow up to three times a year. Research institutions belonging to the ministries of Agriculture and Higher Education have developed a series of varieties and introductions of foreign genotypes (Ponce *et al.*, 2002) in order to provide soybean germplasms that could be planted at different times (Iznaga *et al.*, 2009; Romero *et al.*, 2013); appropriate for grain and fodder production (Díaz *et al.*, 2003) with adequate characteristics for mechanical harvesting and good tolerance to major diseases and pests that attack this crop. Agronomic evaluations of this soybean germplasm were performed (Fundora *et al.*, 2003), in different localities (Ortiz *et al.*, 2004) and under stressful abiotic conditions (Ortiz *et al.*, 2000).

Since 2008, a soybean genetic breeding program have been developed by our group that include the introgression of GTS 40-3-2 event into Cuban varieties mediated by artificial hybridization. In this paper, we describe a crossing procedure and selection strategy for introgression of GTS 40-3-2 event into two Cuban varieties. In addition, we show the results of preliminary evaluation of five soybean Glyphosate-resistant lines obtained in this program. The trials were carried out in summer over two years and we evaluated seven agronomic traits of the transgenic lines and its relatives. Based on our results, we recommend these transgenic lines for further extension in larger areas, different planting dates and localities. This is the first report of agronomic evaluation of transgenic lines obtained by GTS 40-3-2 event introgression into Cuban varieties.

Materials and methods

GTS 40-3-2 event introgression

IncaSoy1 and IncaSoy36 cultivars, obtained from the

National Institute of Agriculture Sciences (INCA), Cuba were selected for GTS 40-3-2 event introgression. As male parent, the transgenic genotypes CEB2 and CEB4 obtained in the soybean breeding program of the Centre for Genetic Engineering and Biotechnology (CIGB), Cuba were used.

Three seeds from each individual parent were sown in pots with organic material and zeolite (1:1) and were cultivated in greenhouses under natural conditions of light and dripping irrigation. Weekly sowing of parents was done for synchronizing reproductive stages R1 and R2 of female and male parents respectively. When female floral buds were swollen and the corolla was visible through the calyx or just emerging from it, the calyx and the corolla were carefully removed. After the female flowers were prepared, they were pollinated with recently opened male parent flowers. Each pollinated flower was identified with a tag showing the date and the corresponding parents of crosses.

F1 seeds were sown in pots, when F1 plants achieved V3 stage Glyphosate was applied. F1 glyphosate resistant plants were self-pollinated to obtain F2 generation. Some F2 plants were chosen to backcross with IncaSoy36. From F1 to F3 generations, individual plants that produced more than 60 g of seeds per plant were chosen to obtain next generation. Individual lines were selected from F4 generation. Finally, five transgenic lines, representing all genotypes combination except IncaSoy36 x CEB2, were chosen for obtaining F5 and F6 generations. Fig. 1 shows the procedure followed for the selection of soybean Glyphosate resistant lines.

Growth Conditions

All trials were carried out at the CIGB experimental farm (23^0 04'25''N, 82^0 27'08''W). F1 and F2 plants were grown in pots of 20 cm of diameter with organic material and zeolite (1:1) and cultivated in greenhouses. F3 and F4 plants were cultivated in the field in rows with 20 m long, 0.5 m of inter row and 0.1m inter plant distances. Planting dates were June 3th (F3) and November 19th (F4) of 2011. Ten plants of each line were randomly selected to quantify agronomic traits in F4 generation.

I1B2-2 (Incasoy1 × CEB2), I1B2-3 (Incasoy1 × CEB2), I1B4 (Incasoy1 × CEB4), I36B4 (Incasoy36 × CEB4), RP5 (backcrossing with Incasoy36) transgenic lines were selected to obtain F5 and F6 generations. Each study included the line parents IncaSoy1, IncaSoy36, CEB2 and CEB4. Planting dates were June 29th of 2012 (F5) and August 20th of 2013 (F6). The experimental design in both years was Split-plot replicated three times. A subplot consisted of 5 rows of 6 m long and 2.5 m of width. The inter subplots, inter rows and inter plants distances were 1.5 m, 0.5 m and 0.1 m respectively. All seeds were inoculated with Bradyr*hizobium japonicum* strain (liquid inoculum containing 1×10^9 viable cells) before sown in the field. The experiments were maintained weed-free by Glyphosate (3.5 L/ha) application when was needed except in the non-transgenic genotypes subplot where weed control was by hand. Ten random plants from each replication were used as source of data.

Trait Measurements

Several agronomical traits were measured in all generations of transgenic lines such as plant height, height of first pod insertion, number of branches per plant, number of pods per plant, seeds per plant, and seed yield per plant. In F5 and F6 generations the number of days to first flowering (R1), days to maturity (R8) and hundred seed weight were also evaluated.

Statistical analysis

Mean and standard deviation were calculated for each trait. Collected data from F5 and F6 generation assays was subjected to two-way ANOVA analysis. Mean performance of genotypes were compared using Tukey's multiple comparison test. Pearson correlation coefficients were used to measure the relationships among variables of interest. All data was collected with the statistical software PRISMA version 6.1.

Results and discussion

Introgression of GTS 40-3-2 event into IncaSoy1 and IncaSoy36 cultivars was done by crossing with transgenic genotypes CEB2 and CEB4, followed by a backcross with IncaSoy36. Different individuals from each parental combination were obtained, which were self-pollinated until F6 generation. Glyphosate was used to select plants that carry the event in each generation.

Table 1. Means, standard deviations and ranges of yields per plant of soybean Glyphosate resistant lines selected over each generation.

	IncaSoy1 × CEB2			IncaSoy1 × CEB4		
	Mean	SD	Range	Mean	SD	Range
F1	19.19	5.28	15.5 - 25.24	7.82	2.87	2.96-10.88
F2	36.7	31.97	2.56 - 85.35	42.52	25.48	3.45-89.06
F3	86.13	25.65	37.84-35.00	80.78	27.55	34.32-128.4
F4	14.75	4.127	10.1 - 20.00	14.71	4.70	10.2 - 24.20

	IncaSoy36 × CEB2			IncaSoy36 × CEB4			IncaSoy36 backcrossing		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
F1	15.4	3.34	11.99-18.66	14.75	7.645	6.44-29.39	2.68	0.57	2.28-3.08
F2	34.33	22.3	7.02-65.89	51.35	26.99	10.86-95.56	40.91	18.89	15.87-69.02
F3	66.93	20.09	33.89-94.78	66.29	21.82	25.46-108.3	83.86	34.21	33.17- 142.6
F4	10.80	2.52	7.1-12.5	12.68	2.89	8.8-16.5	15.3	2.44	12.1-17.90

Three F1 plants were obtained from each combination IncaSoy1 × CEB2 and IncaSoy36 × CEB2; all of them were resistant to Glyphosate. Combinations IncaSoy1 × CEB4 and IncaSoy36 × CEB4 produced six and seven F1 Glyphosate resistant plants respectively. From F2 generation 61 plants were selected based on their seed yield per plant, all of them had higher yields than 60 g of seed per plant. In the F3 generation a total of 88 plants selected for their superior agronomic characteristics were evaluated. Plant height ranged from 57 to 134 cm, the first pod height was from 4 to 19 cm, the number of branches was from 4 to 13 and the number of seeds per plant was from 248 to 1124. To obtain F4 generation, 25 plants that produced more than 500 seeds per plant and representing all cross combinations were chosen. All plants in this generation were resistant to Glyphosate. In addition, phenotypic homogeneity was observed in plants of each line. Plant height ranged from 27.7 to 57.1 cm, the lines obtained by crossing between Incasoy36 and CEB2 had the lower plant height (27.7 to 30.5 cm). In general, the first pod height ranged from 4.1 to 11.4 cm, the number of branches from 2 to 5, the number of pods from 28 to 64 and the number of seeds per plant from 50 to 115. Table 1 shows the mean, standard deviation and ranges of yield per plant assessed in each generation. Comparing plant performances of lines from different crossing combinations in F4 generation, we observed that the lines belonging to the combination IncaSoy36 × CEB2 had the lowest yield averaging 10.8 g per plant. Also, the lines obtained by crossing between transgenic cultivar and IncaSoy1 had higher performance than those obtained by crossing with IncaSoy36; as well as backcross lines had the highest yield (average 15.3 g per plant) of all combinations tested (Table 1). Although Romero *et al.* (2013) reported that the cultivar IncaSoy1 presented lower yield potential among a group of evaluated varieties, our results of crossbreeding suggest that the combination of IncaSoy1 with transgenic parental, besides providing a new basis for resistance to glyphosate, increased yield potential of IncaSoy1 offspring. On the other hand, the offspring of IncaSoy36 crossing had lower yield per plant of backcross progeny, indicating that the backcrossing strategy was needed to introgressed transgenic event and maintain the yield characteristics of parental IncaSoy36.

Five transgenic lines representing each cross combinations except IncaSoy36 × CEB2 were selected to evaluate the F5 and F6 generations. The trials were done in the summer of 2012 and 2013 respectively. Table 2 shows vegetative cycles and reproductive stages (R1 and R8) of transgenic lines and their relatives.

Table 2. Reproductive stages (R1 and R8) and vegetative cycles of five soybean transgenic lines and their relatives evaluated over two years.

Planting date	June 29th, 2012			August 20th, 2013		
Phenological stages and vegetative cycle	R1	R8	Harvest	R1	R8	Harvest
I1B2-2	42	117	122	36	106	111
I1B2-3	40	117	122	36	106	111
I36B4	40	115	120	34	104	108
RP5	40	115	120	34	104	108
I1B4	36	109	112	34	104	108
CEB2	42	120	125	38	109	115
CEB4	42	120	125	38	109	115
IncaSoy36	42	119	122	36	105	108
IncaSoy1	30	88	91	28	86	90
Mean	39	113	118	35	104	108
Difference of R1-R8	74			69		

The vegetative cycles, in general, were longer in 2012 than in 2013, with averages of 118 and 108 days respectively; this result is consistent considering that the culture in late August takes place mainly in shorter days which leads to decrease juvenile period and crop cycle. According to the characteristics of the genotypes used as parents, IncaSoy1 had the shortest cycle while CEB2 and CEB4 had the longest in both years. Similarly there were differences between the onset of flowering (R1) and the end of the reproductive cycle (R8) in both years.

Table 3. Mean square values of combined analysis of variance for seven agronomic traits of nine soybean genotypes over two years.

Source of variation	DF	Plant height (cm)	First pod height (cm)	Number of branches per plant (no.)	Number of pods per plant (no)	Number of seeds per plant (no)	Seed weight per plant (g)	Weight of 100 seeds (g)
Replication	2	84.27	0.4999	0.6936	47.84	1165	71.64	11.36
Genotype (G)	8	3787****	93.66****	15.09****	8607****	37781****	927.2****	26.81*
Year (Y)	1	24317***	443.8***	87.25****	101493**	416992*	15277**	237.7**
G x Y	8	307.4*	2.163 ns	8.233****	5102****	23540***	654.4****	7.527 ns
Error	16	107.6	11.12	0.4552	500.5	2889	62.21	5.793

****Significant differences at $P < 0.0001$ level; ***significant at $P < 0.001$ probability level; **significant at $P < 0.01$ probability level; *significant at $P < 0.05$ probability level and *ns* no significant.

The genotypes studied reached the R1 stage on an average of 39 days and the R8 in 113 days with a difference between these phases of 74 days (2012). Meanwhile, in 2013 the average was 35 days for R1 and 104 days for R8; the difference between R1 and R8 was 69 days (Table2). As the trials were performed under the same experimental conditions in both years, the difference could be explained by planting date. A study by Ibrahim (2012), where six sowing dates were evaluated from mid-June to late August, showed that the number of days to flowering and maturing of six soybean cultivars decreased as delaying planting date. In our assay, experiments were sown on June 29, 2012 and August 20, 2013 and in both days to R1 and R8 stages were reduced in all genotypes in the August sowing date, similar to what happened in the mentioned trial.

The days to flowering of all genotypes ranged from 34 to 42 days considering both planting dates, except for IncaSoy1 female parent which was less than 31 days. This result is similar to that obtained by Ibrahim (2012) and Peluzio *et al.* (2012) who had ranges of 36-47 days (sown in late June and August) and 36-42 days (June planting date) respectively. Meanwhile, Malik *et al.* (2006) and Neves *et al.* (2013) had mixed results because they had days to flowering ranges between 45 and 58 days. The difference in results may be due to genetic variability of the genotypes in each of these studies and the influence of environmental conditions.

Table 4. Means of seven agronomic traits assessed over two years from nine soybean genotypes.

Genotypes	Plant height (cm)	First pod height (cm)	Number of branches per plant (no.)	Number of pods per plant (no)	Number of seeds per plant (no)	Seed weight per plant (g)	Weight of 100 seeds (g)
I1B2-3	111.1 a	13.93 b	5 b	113 abc	255 a	39.26 a	15.06 ab
I36B4	102.1 ab	7.19 c	4 bc	127 ab	266 a	37.95 a	13.75 ab
RP5	80.74 cd	10.30 bc	5 bc	104 abc	206 a	36.99 a	17.13 a
I1B4	91.78 bc	10.71 bc	5 bc	89 bc	185 a	33.16 a	17.46 a
I1B2-2	65.94 de	14.63 b	5 bc	103 abc	214 a	31.64 a	14.59 ab
CEB2	68.05 de	19.44 a	4 bc	82 c	186 a	30.46 a	15.60 a
CEB4	106.3 ab	12.66 b	6 a	135 a	286 a	40.56 a	13.45 ab
IncaSoy36	60.08 e	6.45 c	4 c	128 ab	264 a	46.24 a	13.74 ab
IncaSoy1	35.00 f	11.60 bc	1 d	10 d	22 b	2.73 b	10.46 b
Mean	80.12	11.88	4.333	99.00	209.3	33.22	14.58
SD	25.12	3.952	1.414	37.86	79.39	12.43	2.113
SEM	8.374	1.317	0.4714	12.62	26.46	4.144	0.7043

Means followed by the same letters do not differ significantly.

The lines had days to maturity ranges from 104 to 117 (Table 2). I1B4 line had the shortest cycle, probably because the genetic contribution of the female parent IncaSoy1. I1B2-2 and I1B2-3 had the longest cycle, only overcome for CEB2 parent (120 days). Similar results was obtained by Ortiz *et al.* (2004) in the evaluation of Cuban soybean cultivars, whose growing cycles ranged from 87 to 130 days in spring season.

Considering the days to flowering and days to maturity, we can classify the transgenic lines evaluated in our work, as early and medium cycle cultivars according to the scale used by Ortiz *et al.* (2000). The soybean genotypes classified as early and medium cycle cultivars are more likely to planting in the Cuba´s climate conditions (Ortiz *et al.*, 2000).

Plant height, first pod height, number of branches, number of pods and number of seeds, seed yield per plant and 100- seeds weight are agronomic traits that have been evaluated in different types of studies in soybean (Oz *et al.*, 2009; Iqbal *et al.*, 2010; Rahman *et al.*, 2011; Ibrahim, 2012; Ngalamu *et al.*, 2013). We evaluated these agronomic traits in the transgenic lines and their relatives. Table 3 shows the results of ANOVA performed for each trait. Genotype, year and genotype × year interaction were affected significantly ($P \leq 0.001$) almost in all traits, except the first pod height and 100-seed weight. There were no significant differences in the genotype × year interaction. Similar results were obtained by Ngalamu *et al.* (2013) for these characters in this type of interaction.

In the soybean commercial production, plant height and first pod height are important for mechanical harvesting (Ibrahim, 2012). If the cultivar is very tall, plants stuck in the machine and if it is too low may leave residues in the soil and damage the pods (Mebrahtu and Devine, 2008). Plant height of I1B2-3, I1B4, I36B4 and RP5 lines ranged from 80-111 cm. They were taller than their relatives except CEB4 (Table 4). Similar results were obtained by Ponce *et al.* (2002) in the evaluation of eight soybean cultivars in Cuba over three years in spring season, whose plants reached from 79.62 to 119.47 cm of height.

First pod height ranged from 6.4 cm (IncaSoy36) to 19.44 cm (CEB2). The high operational production of combines, associated with minimal loss at harvest, demand a height of first pod of at least 12 cm (Ramteke *et al.*, 2012). I1B2-2 and I1B2-3 lines would be suitable for mechanized harvesting, as they had the insertion of the first pod to 14,63cm and 13.93 cm respectively. I36B4 line had the lowest first pod insertion (7.19 cm), however, it produced the greatest number of pods per plant (Table 4). The lines produced 89 to 127 of pods per plant. It was superior than the number of pods obtained by Fundora *et al.* (2003) in evaluating the Cuban soybean germplasm, whose genotypes produced pods in a range of 18 to 55.

Table 5. Correlation coefficients for agronomic traits in nine soybean genotypes during two years.

Trait	Plant height (cm)	First pod height (cm)	Branches per plant (no)	Pods per plant (no)	Seeds per plant (no)	Seed yield per plant (g)	Weight of 100 seeds (g)
Plant height (cm)	1						
First pod height (cm)	-0.053	1					
Branches per plant (no)	0.877**	0.084	1				
Pods per plant (no)	0.719*	-0.270	0.867**	1			
Seeds per plant (no)	0.749*	-0.194	0.882**	0.993***	1		
Seed yield per plant (g)	0.663	-0.248	0.826**	0.965***	0.960***	1	
Weight of 100 seeds (g)	0.453	0.150	0.571	0.396	0.382	0.533	1

* Significant at P <0.05 probability level. ** Significant at P < 0.01 probability level. *** Significant at P < 0.001 probability level.

Seed number per plant is the most important contributor to yield gain genetically (Jin *et al.*, 2010). Transgenic lines produced more than 180 seeds per plant, superior to that obtained by other authors (Morrison *et al.*, 2000; Ibrahim, 2012). Morrison *et al.* (2000) concluded that plant breeders in short regions have to increase performance by selecting cultivars that produce greater numbers of seeds per plant. From this statement, we recommend the use of transgenic lines evaluated in our work for soybean production in Cuba.

The transgenic line 100-seed weight ranged from 13.75 g to 17.46 g, superior to its parents (10.46 to 15.60 g). Peluzio *et al.* (2012) and Romero *et al.*, (2013) obtained similar results to those with ranges from 11.3 to 20.4 g and 12.85 to 21.10 g respectively, while Malik *et al.* (2006) had mixed results with a range from 3.87 to 13.5 g.

Seed yield per plant can be used to estimate the cultivars yield potential in a given area. We compared this trait in the transgenic lines and their parents. There were no significant differences between the transgenic lines and the parents CEB2, CEB4 and IncaSoy36 (Table 4). However, this difference was significantly higher when they were compared to the parent IncaSoy1 (P<0.05). The seed yield per plant superior to 30 g was our goal when we selected the transgenic lines because we expect to reach high yield (more than 5 ton/ha) using genotypes with this yield potential. All lines had a seed yield per plant from 31.64 g to 39.26 g. Also, some of them (I36B4 and RP5 lines) had other characters desirable of their parents e. g. size large seeds with a light hilum well accepted for soymilk and flour production (Seibel *et* *al.,* 2013).

Additionally, other benefits of using Glyphosate resistant genotypes should be considered such as easier weed management because only one herbicide is needed, easy crop rotation due to its non-residual effects, fewer herbicide treatments and reduction of toxicological and ecotoxicological risks because of the replacement of more toxic weed killers by Glyphosate (Bonny, 2009).

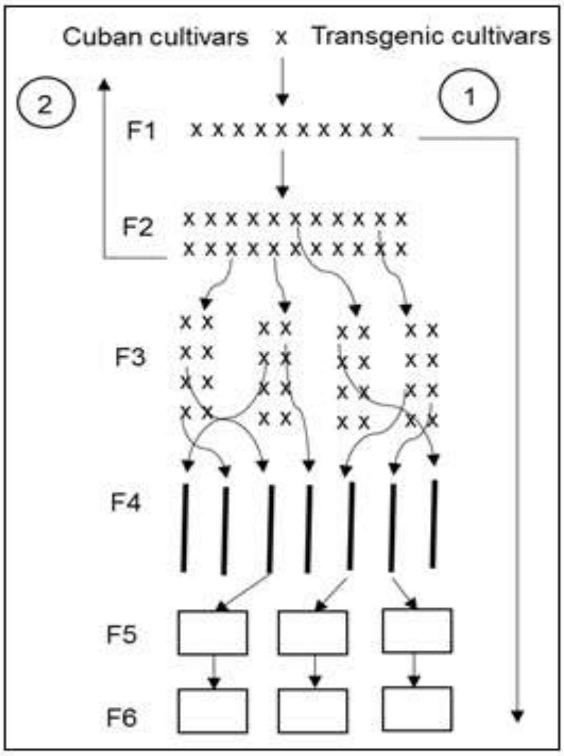

Fig. 1. Procedure for selection of soybean Glyphosate resistant lines obtained by crossing Cuban soybean varieties with transgenic genotypes (event GTS 40-3-2). From F1 to F3 generations, individual plants that produced more than 60 g of seeds per plant were chosen to obtain next generation. Individual lines were selected from F4 generation. Agronomical traits of selected lines were evaluated in the F5 and F6 generations. Legend: 1 self-pollination; 2 backcrossing; x individual plant; I groove; □ plot.

Analysis of correlation

Correlation coefficient quantifies the direction and magnitude varying two variables together. Table 5 shows the correlation coefficient between agronomic traits evaluated in this study.

Plant height correlated positively with all evaluated characters, except with the first pod height. This result disagrees with Malik *et al.*, (2006) who obtained a positive correlation between these two traits. Significant correlation between plant height and number of branches, number of pods and number of seed was observed, while Ngalamu *et al.,* (2013) correlated significantly only with the number of seeds per pod and not significantly with other characters. Negative correlation was observed between first pod height and number of pods, number of seeds and seed yield per plant, similar to that obtained by Oz *et al*, (2009).The number of branches per plant correlated positively and significantly with the number of pods, number of seeds and seed yield per plant. Iqbal *et al.*, (2010) are in line with this, reporting a correlation coefficient between the number of branches per plant and grain yield per plant positively and significantly (r=0.19*). Number of pods per plant correlated strongly with the number of seeds per plant (r = 0.99***) and seed yield per plant (r = 0.96***), similar to that obtained by Mahmoodi *et al.* (2013), who found a significant and positive correlation between these characters (r = 0.92 **). On the other hand, Oz *et al.*, (2009) established that associations between these traits were the highest and the most stable in their study. The number of seeds per plant and seed yield per plant correlated positively and significantly, similar reveled by Mahbub *et al.* (2015). Positive correlation between 100-seed weight and all traits was observed in our study. This result disagrees with other researchers. It was negatively associated with number of pods per plant, number of branches per plant (Malik *et al.*, 2006) and plant height (Iqbal *et al.,* 2010). Based on our correlation analyses, number of branches, number of pod and number of seed per plant should be considered to improve performance in soybean.

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