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Symbiotic Nitrogen Fixation, Phosphorus and Potassium Uptake Capacity of a Number of Soybean Mutant Lines in a Calcareous Soil

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ABSTRACT

The objectives of the present study was to evaluate biological nitrogen fixation, P and K uptake ability of 31 soybean mutant lines (induced by Gamma irradiation) and their parent cultivar at greenhouse level. Initially, 10 Rhizobial isolates and strains were tested for infectiveness and symbiotic effectiveness using Leonard jars, and the strain *Bradyrhizobium japonicum* strain RS 152 was selected as the most efficient strain. The experiment was conducted under proper greenhouse condition with a randomized complete block design on a soil with no indigenous rhizobia and low nitrogen and phosphorus content. Thirty-one soybean mutant lines and two inoculated and un-inoculated wild cultivar blanks were replicated three times. Each pot contained 3.5 kg air-dried homogenized soil, and at the time of planting each seedling was inoculated with l mL of inoculum RS 152 containing approximately 9×10^8 cells per mL. During about 4 months of growth, the plants were irrigated to maintain the soil moisture approximately 0.8 field capacity. The plants were harvested at the plant developmental stage of pod filling (R6), and several parameters, such as dry matter of shoot, nodule and roots, number of nodules, and proportion and amount of Nitrogen derived from air (Ndfa% and N-fixed) were measured. According to the results, in most parameters, the mutant lines were significantly different with each other and also with the L17 parent soybean cultivar. The mutant line 5 showed the maximum value of %Ndfa (73.068) compared to the cultivar L17 (48.762), indicating significant increase of biological nitrogen fixation, by about fifty percent, through physical mutagenesis. In addition, the mutant lines 21 and 31 had higher phosphorus and potassium uptake than the others, however, significant differences were observed for phosphorus uptake capacity compared to the cultivar L17. Although more detailed studies are needed to evaluate effects of these mutations on nitrogen fixation, macro- and microelements absorption capacity, and also on yield and quality parameters, these findings show that gamma irradiation could be helpful to induce new nutritional properties in soybean and release new mutant cultivars.

Keywords: Biological nitrogen fixation; Ndfa, Nutrient efficient genotypes, Physical mutagenesis.

INTRODUCTION

Because of problems related to the agronomic, economic, and environmental impacts of chemical fertilizers, other complementary solutions to overcome the shortage of the essential nutritional elements should be considered. In recent decades, development of nutrient efficient crop genotypes and to improve nutrient efficiency, especially uptake of phosphorus, potassium, and micronutrients, has been widely studied.

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Therefore, an appropriate strategy to deal with the constraints of nutrient deficiency and to prevent the reduction of production is development and application of plant genotypes with efficient absorbance of nutrients. This could lead to increasing the plant resistance to pathogens, yield, and nutritional quality of grain (Khoshgoftarmanesh, 2010).

In this regard, application of mutation techniques to generate plant variation is known as one of the main ways for improvement of plant quality. Mutagenesis is usually made by using physical, chemical, and biological agents (Bradshaw, 2016). Physical mutagens include X-rays, gamma rays, alpha particles, fast neutrons, UV and cosmic rays; while chemical mutagens are sodium azide, ethyl methanesulphonate, methyl methanesulphonate, hydroxylamine and N-methyl-N-nitrosourea. The spontaneous mutation rate is too low (10^{-5}–10^{-8}) and, therefore, is inadequate to be utilized for enhancing genetic variability in crop breeding. Induced mutations with mutagen agents enhances mutation rate, enabling mutant lines to be used in plant breeding programs, especially in the crops with limited genetic variability such as legumes (Jain, 2005; Jain and Suprasanna, 2011).

In the past 6-7 decades, application of induced mutations has been on the forefront of activities in developing and developed countries to produce several superior crop varieties, and that has made great economic impact on food production and feeding people around the world (Kharkwal and Shu, 2009; Shu, 2009). Several mutant varieties have been released in China, India, the former USSR, Netherlands, Japan, Sweden, and USA as the leading countries. In view of the applied mutagen types, more than 50% of the mutants have been developed by using gamma rays (Mba, 2013).

The FAO/IAEA Mutant Variety Database show 3,249 entries, out of which 2,456 are seed generated and 367 are vegetatively grown plants. This grouping is based on the common name of the entry. The top six are rice, barley, chrysanthemum, wheat, soybean, and maize. In total, improved characters are described 5,569 times for 3,222 varieties. These are classified in five general categories: agronomic and botanic traits (48%), quality and nutrition traits (20%), yield and contributors (18%), resistance to biotic stresses (9%), and tolerance to abiotic stresses (4%). Agronomic and botanic traits include maturity, flowering time, and plant structure (Jankowicz-Cieslak *et al*., 2017). Among cereals and all crops, rice tops the list with 700 mutants/mutant varieties followed by barley, wheat, maize, durum wheat, oat, millet, sorghum and rye (Suprasanna *et al*., 2015). These mutant varieties have made great economic impact, contributing millions of dollars annually to farmers' local economies (Ahloowalia *et al*., 2004; Jain, 2005).

Soybean [*Glycine max* (L.) Merrill] has become the miracle crop of the 21 century (Ferri, *et al*., 2017). It has high amount of oil and protein in seed and is being used a lot in the industry, food and pharmaceuticals (Argaw, 2012). Furthermore, it has one of the largest cultivation areas among the oil seeds in Iran and the world (FAO, 1984; Keyser and Li, 1992). Besides, soybean as oil seed legume is a strategic crop to meet the protein and oil needs of the growing population in the world. It has been cultivated about five decades in Iran. Thus, the present study was undertaken for comparison and determination of the symbiotic nitrogen fixation and K and P uptake ability of 31 soybean mutant lines 8th generation (which has been induced by gamma irradiation) and their parent cultivar.

MATERIALS AND METHODS

In the preliminary studies, eight native rhizobia isolates and 2 commercial strain of *B. japonicum*, namely, Biosoy (which was isolated from commercial inoculum Biosoy) and RS 152 were received from Soil Biology Section, Soil and Water Research Institute, Iran. Purity of the strains was tested using culture on YMA (Yeast extract Mannitol Agar)+Congo red and other indicators, such as Bromthymol Blue, Bromcresol Purple and Bromcresol Green (Beck, *et al*., 1993). Furthermore, infectiveness of the strains was evaluated by using Most Probable Number Plant Infection Test (MPN-PIT) in fivefold dilution series (Vincent, 1982; Beck *et al*., 1993). The Symbiotic Effectiveness (SE) was measured by 35, 70, and zero mg/l N using Leonard jar test in a proper glass house condition (Somasegaran and Hoben, 1994). According to the results of these experiments, one strain was selected for t evaluation of the soybean mutant lines at greenhouse level. The growth conditions were as follows; 28/18^{o}C light/dark temperatures with 12-14 hours daily photoperiod. The light intensity and humidity was about 30,000 Lux and 60%, respectively. To evaluate the nitrogen fixation and P uptake of the soybean mutant lines, several soils were collected from around Karaj, Iran, and were tested for N, P, K content and native *B. japonicum* using Most Probable Number Plant Infection Test (MPN-PIT) in fivefold dilution series (Vincent, 1982; Beck *et al*., 1993). A soil with no native *B. japonicum* and low N and P content was selected (Table 1).

Ninety-nine plastic pots (18 cm deep, 17 cm top diameter) were filled with 3.5 kg sieved homogenized soil (< 4 mm) with low N and P content (Table 1). The experiment involved 33 treatments, 31 selected M8 generation soybean mutant lines, which were evolved by gamma ray (cobalt-60) derived from L17 cultivar seeds irradiated with absorbed doses 150, 200, and 250 Gray (Younessi Hamzekhanlu *et al*., 2011), an inoculated and a non-inoculated L17 parent cultivar were used as the control.

Experiment was conducted in a Completely Randomized Block Design (CRBD), with three replications, in glasshouse of Nuclear Agriculture Research School, Karaj, Iran. The soybean seeds were germinated on water agar. Seven pre-germinated seeds were planted per pot; and after one week, thinned out to three. The inoculation was performed at sowing by pipetting 1 mL (containing about 9×10^{8} cells) of a suspension (Yeast extract Manitol Broth) of the selected strain RS 152 onto the seed with regard to treatment. To estimate biological nitrogen fixation, the nitrogen difference approach was used as the following equation (Beck, *et al*., 1993; Unkovich *et al*., 2008). The amount of N_2 fixed was calculated as the difference in uptake of N of the N2-fixing and reference plants. Here, the non N2-fixing (control or reference) plant is an un-inoculated L17 soybean.

N2 fixed= N yield N2-fixing plant–N yield reference plant

In this method of calculation of biological nitrogen fixation, percentage of Nitrogen derived from air (%Ndfa) could be calculated as follows;

$$\%\text{Ndfa} = \frac{\text{N2 fixed}}{\text{N yield N2}-\text{fixing plant}}$$

Then, percentage of Nitrogen derived from soil (%Ndfs)= 100-%Ndfa

Based on the soil analysis, all pots were given 12 mg P kg^{-1} in the form of Triple SuperPhosphate (TSP). Throughout the experimental period, the pots were kept weed-free and watered up to 80% field capacity. At pod filling stage (R6) (Fehr *et al*., 1971), above ground parts were harvested, dried at 70^{o}C for 72 hours, weighed and ground to pass a 40 mesh sieve.

Table 1. Some physicochemical and bacteriological characteristics of the soil used in the study.

Depth	Texture	pH (H_2o)	Organic matter	Total N	TNV	P (Available)	K (Available)	*B. japonicum* in soil
m			%			mg kg^{-1}		number g^{-1}
0-0.2	Sandy Clay loam	8.18	0.37	0.035	9	3.4	261	0

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The ground samples were then used to determine the N content by the Kjeldhal method (Hardarson and Danso, 1990), P contents was measured according to the method of Vanadate/molybdate method (yellow method), and K contents was evaluated using Flame emission spectrometry (Amami, 1996).

Also, roots were washed carefully and a number of parameters, such as dry matters of shoots, roots, and nodules as well as number of nodule were determined. Analysis of variance was done by SPSS16 package and the means were compared and ranked by Duncan Multiple Range Test.

RESULTS AND DISCUSSION

All the preliminary tests with indicators and microscopic observations showed that all the (ten) studied isolates and strains of *B. japonicum* were probably pure and free of any contamination. Results from MPN-PIT showed that four strains were able to make symbiotic association with soybean i.e., form nodule on the root of soybean. Results obtained from Symbiotic Effectiveness (SE) test using Leonard jar showed that, although all the tested isolates and strains were completely efficient (SE>100%) (Table 2), the strains RS 152, Biosoy, RS 117 and Br-41 were the most effective, respectively. Besides, results for two nodule parameters i.e. nodule number and nodule dry matter were not the same as symbiotic effectiveness. According to these results, the strain RS 152 was selected for the next experiments for detailed evaluation of soybean mutant lines and blanks in the pot experimental assays.

An important characteristic of legumes is their ability to fix atmospheric nitrogen in symbiosis with nodule-forming rhizobia. In this way, it can be avoid use of much nitrogen fertilizers, which, in addition to higher production costs, may cause environmental pollutions. Furthermore, this ability of legumes is particularly important in countries where the cost of nitrogen fertilizer is high or availability is limited. Adopting a suitable method for accurate evaluation of the amount of nitrogen fixation is an important requirement in any program aiming at maximizing symbiotic nitrogen fixation. In the present study, several simple methods, such as nitrogen difference method, nodule observation, dry matter yield, and total nitrogen uptake were used to accurately evaluate nitrogen fixed (Hardarson and Danso, 1993). According to the Tables 3 and 4, soybean mutant lines were significantly different in most of the studied parameters. For example, most of the mutant lines showed significantly different shoot dry matter yield parameter, however, the lines 21, 27, and 31 did not

Table 2. Effect of nitrogen and inoculation with different strains of *Bradyrhizobium japonicum*on on parameters in L17 soybean cultivar.[a]

Treatment[B]	Nodule dry matter (g plant^{-1})	Nodule number per plant	Shoot dry matter yield (g plant^{-1})	N-Uptake (mg plant^{-1})	Total dry matter (g plant^{-1})	SE (%)
N0	-	-	0.66 d	4.48 e	0.9 d	
N1	-	-	1.20 c	11.19 e	1.53 c	
N2	-	-	2.25 a	26.96 d	2.81 a	
Br-41	0.168 a	52.33 a	1.80 b	40.46 c	2.30 b	1.69 b
Biosoy	0.145 a	39.25 b	2.25 a	50.29 b	2.79 a	2.17 ab
RS 117	0.145 a	44.00 ab	2.06 ab	50.44 b	2.53 ab	2.10 ab
RS 152	0.170 a	42.34 ab	2.39 a	63.72 a	2.93 a	2.68 a

[a] Means followed by similar letters are not significantly different at the 95% confidence level. [b] N0, N1 and N2 are 0, 35, and 70 mg kg^{-1} N with no rhizobia inoculation, respectively.

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Table 3. Comparison of nitrogen fixation efficiency and yield parameters in some soybean mutant lines using Duncan's Multiple Range Test.[a]

Mutant lines	Shoot dry matter yield (g plant^{-1})		Nodule number (Number plant^{-1})		Nodule dry matter (g plant^{-1})		Nitrogen percent (% plant^{-1})		N yield (g plant^{-1})		N fixed (g plant^{-1})		%Ndfa		%Ndfs	
1	4.261	a-e	25.555	e-g	0.171	bc	3.659	c-h	0.158	a-d	0.097	a-d	58.196	a-e	41.804	c-g
2	4.494	a-d	16.000	g-i	0.187	b	4.412	a	0.198	a-c	0.137	a-c	69.054	a-c	30.946	e-g
3	4.293	a-e	17.000	g-i	0.146	bc	3.892	a-f	0.168	a-d	0.107	a-d	60.678	a-e	39.322	c-g
4	3.776	b-e	17.667	f-i	0.125	b-d	4.036	a-e	0.152	a-e	0.091	a-e	59.738	a-e	40.262	c-g
5	5.187	a-c	32.056	a-e	0.194	b	4.399	a	0.228	a	0.167	a	73.068	a	26.932	g
6	5.140	a-c	38.778	a-d	0.175	bc	4.189	a-c	0.214	a	0.153	a	71.287	ab	28.713	fg
7	4.580	a-d	24.111	e-h	0.183	b	4.433	a	0.205	ab	0.143	ab	68.166	a-c	31.834	e-g
8	4.500	a-d	27.222	d-g	0.171	bc	3.918	a-f	0.177	a-d	0.116	a-d	62.973	a-d	37.027	d-g
9	5.211	a-c	32.000	a-e	0.200	b	4.310	ab	0.225	a	0.164	a	71.297	ab	28.703	fg
10	4.688	a-d	42.111	ab	0.156	bc	3.979	a-e	0.187	a-c	0.126	a-c	64.207	a-d	35.793	d-g
11	4.270	a-e	21.111	e-i	0.133	b-d	4.060	a-d	0.175	a-d	0.114	a-d	61.928	a-d	38.072	d-g
12	4.386	a-e	23.333	e-h	0.138	b-d	4.272	a-c	0.187	a-c	0.125	a-c	66.913	a-d	33.087	d-g
13	3.099	de	25.000	e-g	0.303	a	3.461	d-h	0.107	d-f	0.046	d-f	42.752	e	57.248	c
14	5.330	a-c	40.333	a-c	0.206	b	4.040	a-e	0.215	a	0.154	a	71.450	ab	28.550	fg
15	4.736	a-d	43.667	a	0.202	b	4.268	a-c	0.202	a-c	0.141	a-c	69.624	a-c	30.376	e-g
16	4.180	a-e	28.444	c-f	0.165	bc	3.871	a-g	0.162	a-d	0.101	a-d	61.825	a-d	38.175	d-g
17	5.295	a-c	28.222	c-f	0.193	b	4.010	a-e	0.212	a	0.151	a	70.830	a-c	29.170	e-g
18	4.101	a-e	31.667	a-e	0.159	bc	3.752	b-h	0.158	a-d	0.097	a-d	53.224	b-e	46.776	c-f
19	4.332	a-e	27.222	d-g	0.167	bc	3.744	b-h	0.161	a-d	0.100	a-d	60.179	a-e	39.821	c-g
20	4.665	a-d	22.667	e-h	0.178	bc	4.283	a-c	0.199	a-c	0.138	a-c	68.958	a-c	31.042	e-g
21	5.688	a	22.500	e-h	0.170	bc	3.469	d-h	0.196	a-c	0.135	a-c	68.740	a-c	31.260	e-g
22	4.949	a-c	30.889	b-f	0.174	bc	4.013	a-e	0.199	a-c	0.138	a-c	68.252	a-c	31.748	e-g
23	4.902	a-d	22.917	e-h	0.180	bc	3.501	d-h	0.172	a-d	0.110	a-d	64.176	a-d	35.824	d-g
24	5.215	a-c	19.500	e-i	0.183	b	3.491	d-h	0.182	a-d	0.121	a-d	66.264	a-d	33.736	d-g
25	4.999	a-c	18.222	f-i	0.129	b-d	3.804	a-h	0.189	a-c	0.128	a-c	67.361	a-d	32.639	d-g
26	3.700	c-e	9.500	ij	0.042	de	3.539	d-h	0.131	b-f	0.069	b-f	51.914	c-e	48.086	c-e
27	5.553	ab	27.056	d-g	0.177	bc	4.021	a-e	0.223	a	0.162	a	72.427	a	27.573	g
28	4.489	a-d	11.500	h-j	0.127	b-d	3.339	f-h	0.150	a-e	0.089	a-e	58.811	a-e	41.189	c-g
29	4.179	a-e	18.722	e-i	0.145	bc	3.686	b-h	0.154	a-e	0.092	a-e	58.194	a-e	41.806	c-g
30	2.606	e	8.500	ij	0.076	c-e	3.197	h	0.083	ef	0.022	ef	26.040	f	73.960	b
31	5.656	a	17.167	g-i	0.170	bc	3.413	e-h	0.194	a-c	0.133	a-c	61.524	a-d	38.476	d-g
L17-fix	3.842	a-e	23.444	e-h	0.160	bc	3.265	gh	0.124	c-f	0.062	c-f	48.762	de	51.238	cd
L17- nonfix	3.657	c-e	0.000	j	0.000	e	1.685	i	0.062	f	0.000	f	0.000	g	100.000	a

[a] Means followed by similar letters are not significantly different at the 95%confidence level. %Ndfa: Percentage of Nitrogen derived from atmosphere; %Ndfs: Percentage of Nitrogen derived from soil; L17-fix and L17- nonfix are parent soybean cultivar which are inoculated as mutant lines and not inoculated, respectively.

Table 4. Comparison of some yield and P and K uptake parameters in some soybean mutant lines using Duncan's Multiple Range Test.[a]

Mutant lines	Root dry matter ($g\ plant^{-1}$)		Root+Nodule dry matter ($g\ plant^{-1}$)		Total plant dry matter ($g\ plant^{-1}$)		Potassium percent ($\%\ plant^{-1}$)		Potassium uptake ($g\ plant^{-1}$)		Phosphorous percent ($\%\ plant^{-1}$)		Phosphorous uptake ($g\ plant^{-1}$)	
1	0.887	a-d	1.058	ab	5.318	a-d	1.375	a-c	0.057	a-f	0.262	a-e	0.011	c-e
2	0.464	d	0.651	bc	5.145	a-e	1.280	a-f	0.058	a-f	0.260	a-e	0.012	b-e
3	0.851	a-d	0.997	a-c	5.290	a-e	1.146	f	0.048	c-g	0.243	a-e	0.010	de
4	0.685	b-d	0.810	a-c	4.586	b-e	1.437	a	0.054	a-g	0.242	a-e	0.009	de
5	1.023	ab	1.216	a	6.403	a-c	1.173	d-f	0.061	a-f	0.247	a-e	0.013	b-e
6	0.944	a-c	1.119	ab	6.259	a-c	1.294	a-f	0.066	a-d	0.247	a-e	0.012	b-e
7	0.759	a-d	0.942	a-c	5.522	a-d	1.276	a-f	0.058	a-f	0.269	a-e	0.012	b-e
8	0.782	a-d	0.952	a-c	5.452	a-d	1.152	ef	0.051	b-g	0.205	de	0.009	de
9	0.936	a-c	1.136	ab	6.347	a-c	1.217	c-f	0.063	a-e	0.250	a-e	0.013	b-e
10	0.824	a-d	0.980	a-c	5.669	a-d	1.224	c-f	0.057	a-f	0.193	e	0.009	de
11	0.715	a-d	0.849	a-c	5.119	a-e	1.141	f	0.048	d-g	0.229	a-e	0.009	de
12	0.752	a-d	0.890	a-c	5.276	a-e	1.156	ef	0.051	b-g	0.220	c-e	0.010	de
13	0.565	cd	0.868	a-c	3.967	de	1.296	a-f	0.040	fg	0.301	a-c	0.009	de
14	0.838	a-d	1.044	ab	6.374	a-c	1.228	c-f	0.065	a-d	0.217	c-e	0.012	c-e
15	0.798	a-d	1.000	a-c	5.736	a-d	1.379	a-c	0.065	a-d	0.278	a-e	0.013	b-e
16	0.734	a-d	0.899	a-c	5.078	a-e	1.302	a-f	0.054	a-g	0.269	a-e	0.011	c-e
17	0.926	a-c	1.119	ab	6.414	a-c	1.338	a-d	0.071	ab	0.220	c-e	0.011	c-e
18	0.754	a-d	0.912	a-c	5.014	a-e	1.354	a-c	0.056	a-g	0.263	a-e	0.010	de
19	0.842	a-d	1.009	a-c	5.341	a-d	1.402	ab	0.059	a-f	0.248	a-e	0.010	de
20	0.703	a-d	0.881	a-c	5.545	a-d	1.288	a-f	0.060	a-f	0.269	a-e	0.012	b-e
21	1.112	ab	1.282	a	6.969	a	1.292	a-f	0.074	a	0.293	a-c	0.017	ab
22	0.862	a-d	1.036	ab	5.985	a-d	1.247	b-f	0.062	a-e	0.269	a-e	0.013	b-d
23	0.844	a-d	1.023	a-c	5.925	a-d	1.344	a-c	0.066	a-d	0.258	a-e	0.013	b-e
24	0.958	a-c	1.141	ab	6.357	a-c	1.345	a-c	0.070	a-c	0.303	a-c	0.016	a-c
25	0.890	a-d	1.019	a-c	6.018	a-d	1.210	c-f	0.061	a-f	0.227	b-e	0.011	c-e
26	0.863	a-d	0.905	a-c	4.605	b-e	1.289	a-f	0.048	d-g	0.254	a-e	0.010	de
27	1.001	a-c	1.178	a	6.731	a-c	1.212	c-f	0.067	a-d	0.238	a-e	0.013	b-e
28	0.748	a-d	0.875	a-c	5.364	a-d	1.349	a-c	0.060	a-f	0.261	a-e	0.012	c-e
29	0.682	b-d	0.827	a-c	5.005	a-e	1.320	a-e	0.055	a-g	0.290	a-d	0.012	c-e
30	0.448	d	0.524	c	3.130	e	1.350	a-c	0.035	g	0.298	a-c	0.008	e
31	0.974	a-c	1.144	ab	6.801	ab	1.273	a-f	0.072	ab	0.312	ab	0.018	a
L17-fix	1.148	a	1.308	a	5.150	a-e	1.405	ab	0.054	a-g	0.297	a-c	0.011	c-e
L17- nonfix	0.847	a-d	0.847	a-c	4.505	c-e	1.162	ef	0.043	e-g	0.316	a	0.012	c-e

[a] Means followed by similar letters are not significantly different at the 95%confidence level. L17-fix and L17-nonfix are parent soybean cultivar which are inoculated as mutant lines and not inoculated, respectively.

show significant difference with the L17 parent cultivar.

In addition, a number of the mutant lines showed significantly higher root and nodules dry matter, as well as number of nodules and nitrogen contents, than L17 soybean parent cultivar. This indicates that mutation could cause enough variability to increase symbiotic nitrogen fixation properties of L17 soybean cultivar. This is in agreement with report of Hafiz Khan and Dutt Tyagi (2013) as well as FAO/IAEA mutant database which has listed more than 170 new mutant soybean cultivars or lines by using different mutagens especially gamma irradiation in countries like China, Australia, Japan, India and some others (IAEA, 2017).

In addition, Table 3 shows comparison between nitrogen difference method parameters, such as parameters N-fixed and percentage of Nitrogen derived from air (%Ndfa), as the best method in this assay, and parameters of other methods such as nodule observation, dry matter yield, and total nitrogen show that nodule observation and shoot dry matter methods are not proper enough to be used in such studies. Lines 5, 9, 14, and 27 were significantly higher than L17 soybean parent cultivar, while these are not the same for shoot dry matter, nodule number, and nodule dry matter indexes. As it is shown in the Table 3, mutation in the L17 parent cultivar could increase value of %Ndfa from 48.762 to 73.068 in mutant line 5, indicating about fifty percent increase in biological nitrogen fixation through physical mutagenesis by Gamma irradiation.

Phosphorus as a major component in plant DNA and RNA is critical in root development, crop maturity, and seed production. Although, potassium role in plants is indirect, in other words, it does not make up any plant part. However, potassium is required for the activation of more than 80 enzymes throughout the plant. It's important for a plant's ability to withstand harsh condition, such as extreme cold and hot temperatures, drought, and pests. Potassium increases water use efficiency and transforms sugars to starch in the grain-filling process (Halvin *et al*., 1999). Considering the effect of mutation on P and K uptake capacity from the soil, as Table 4 shows, mutation could have increased the uptake of these nutrients in mutant lines 21 and 31, whose differences are only significant for phosphorus uptake in comparison with L17 wild cultivar. However, root system indexes, which are usually related to uptake of P and K from the soil, in this trait as Table 4 shows, do not show such variability in relation with. This is indicating that, probably, some genetic changes occurred in mutant lines via physical mutation that caused differences in P and K uptake from the soil between mutant lines and the wild cultivar.

Many studies have demonstrated that there is substantial genetic variation for plant P efficiency from the soil (Furlani *et al*., 2002; Tsvetkova and Georgiev, 2003; Vance, 2001; Vinod and Heuer, 2012). Wang *et al.* (2010) has identified P-efficient varieties in soybean that grow much better than standard varieties under P-deficient conditions in acid soils, where the concentration of available soil P was often lower than 20 mg kg^{-1}, and show yields similar to standard varieties in P-fertilized soils. There are some potential adaptive mechanisms that P-efficient plants can use for better growth on low-P soils, including changes in root morphology and architecture, root symbiosis, activation of phosphate transporters, enhancement of internal phosphatase activity, and, finally, secretion of organic acids and phosphatases into the rhizosphere (Raghothama, 1999; Vance *et al*., 2003; Gahoonia and Nielsen, 2004). Accordingly, reasons for our data could be one or all of the mentioned changes in the root secretions and exudates. In other words, increased P uptake from the soil in mutant lines 31 and 21 compared to the wild cultivar L17 needs to be studied in details with more different treatments, probably is caused because of changes in their root secretion compounds by gamma irradiation.

Moreira *et al*. (2015) described that using cultivars with nutrient-use efficiency is an important strategy in the management of

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plant nutritional status, particularly for potassium (K), because it is highly required, and the progressive depletion in soil, caused by the use of inadequate amounts of fertilizers, initiate frequent deficiency symptoms observed in soybean crops. These researchers assessed the effect of applying two rates of K (50 and 200 mg kg^{-1}) on growth, shoot dry weight, yield, and seed yield, nutritional status, yield components, and efficiency of K use in eleven cultivars in greenhouse conditions. Their results revealed differences between cultivars in view of efficiency and response to the K in soil. Our findings (Table 4) are related to K uptake of different mutant lines and wild soybean cultivar from the soil and are in agreement with the latter study.

CONCLUSIONS

The results demonstrated that mutation could make noticeable variation in soybean cultivar L17 in terms of symbiotic nitrogen fixation as well as phosphorus and potassium uptake capacity from soil. Although it is necessary to test the best lines in more detailed and more comprehensive experiments, especially in different fields and locations, these results could increase hope for development of improved cultivars via mutation and release of nutrient-efficient plant cultivars to enhance farmers' income in regards to sustainable agricultural production.

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تثبیت زیستی نیتروژن و ظرفیت جذب فسفر و پتاسیم لاین های موتانت سویا در یک خاک آهکی

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چکیده

هدف این مطالعه گلخانه ای تعیین تثبیت زیستی نیتروژن و جذب عناصر غذایی فسفر و پتاسیم ۳۱ لاین موتانت سویا با اشعه گاما در مقایسه با رقم مادری L17 بود. در ابتدا از میان ۱۰ جدایه باکتری *ریزوبیومی* با استفاده از آزمایشات شاخص های خلوص، توانایی ایجاد غده و آزمون کارایی همزیستی بهترین سویه، یعنی RS 152 انتخاب شد. سبس در گلدان های پلاستیکی در قالب بلوک های کامل تصادفی، یک خاک فاقد باکتری بومی همزیست و دارای نیتروژن و فسفر کم با سه تکرار قرارگرفت. در هر گلدان به میزان ۳/۵ کیلوگرم خاک یکنواخت شده توزین و هر بذر با یک میلی‌لیتر از مایه تلقیح سویه کاملاً مؤثر RS 152 با غلظت $۱۰^{۸}$ باکتری درمیلی لیتر تلقیح شد. در طول ۴ ماه دوره رشد گیاهان، رطوبت گلدان‌ها در حدود ۸۰ درصد ظرفیت رطوبی مزرعه نگه داشته شد. عملیات برداشت گیاهان به صورت سبز، در مرحله دانه‌بندی کامل سویا صورت گرفت. پانزده شاخص کیفی و کمی رشد گیاهان در هر گلدان جداگانه اندازه‌گیری و مورد تجزیه و تحلیل آماری قرار گرفت. بر اساس نتایج حاصل، لاین های موتانت در غالب شاخص ها نسبت به هم و رقم مادری (L17) دارای اختلاف معنی دار هستند. لاین موتانت ۵ نسبت به رقم مادری (L17) افزایش معنی دار تثبیت زیستی نیتروژن مولکولی هوا از ۴۸/۷۶۲ به ۷۳/۰۶۸ یعنی در حدود ۵۰ درصد نشان داد. لاین های موتانت ۲۱ و ۳۱ از لحاظ جذب فسفر و پتاسیم در گیاه نسبت به دیگر لاین ها برتری نشان دادند که این اختلاف تنها در میزان جذب فسفر از خاک نسبت به رقم مادری (L17) معنی داری بود. این نتایج بیانگر آن است که با اشعه گاما می توان احتمالاً لاین های گیاهی و به تبع آن ارقام جدید موتانت در سویا ایجاد کرد که دارای توانایی بالاتری در جذب عناصر غذایی نسبت به رقم مادری هستند.

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