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RESEARCH ARTICLE

SELECTION OF SOYBEAN GENOTYPES USING MORPHOLOGICAL MARKERS

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ABSTRACT

This paper was aimed at evaluating the morphological differences in six soybean genotypes and also correlates some morphological features with yield-indicating parameters, which will enhance selection and improvement programmes of the crop especially, using molecular tools. Seeds from six genotypes of soybeans: TGX923-2E, TGX1844-18E, TGX1448-2E, TGX1740-1E, TGX1448-1E and TGX1440-E were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Three seeds were sown per hole per genotype at a depth of 3cm and 30cm between genotype and were randomized 6 times per block using randomized complete block design (RCBD). Each genotype was later thinned down to two stands. Cultural practices were observed when appropriate. Data on the number of leaves per plant, plant height, root length per plant, number of nodules per plant and dry matter yield (g) per plant were obtained at intervals of 2, 3, 4, and 5 weeks after planting. The results showed significant differences ($P < 0.05$) in all the morphological parameters studied, which expectedly increased with weeks of data collection. Our results are explicit indication that TGX1740-1E, TGX923-2E and TGX1448-1E soybean genotypes could be selected based on the number of leaves and number of nodules produced per plant for further improvement. This is with the understanding that increase nodulation will lead to increase yield. Our results also revealed that increasing nodulation in TGX1740-1E will result to increase foliage production, which will invariably affect photosynthetic activity and productivity. Though the correlation analysis of these morphological traits, in other selected genotypes was not consistent with TGX1740-1E, our results threw light to other relationships that might directly and indirectly influence yield.

INTRODUCTION

Selection of superior genotypes using morphological, biochemical or molecular markers should be the aspiration of any crop breeder with the view of having optimal yield. However, using molecular (DNA) marker assisted selection through Quantitative Trait Loci (QTLs) mapping will require sophisticated instrumentations, which more often than not are found in research institutes depriving local breeders of high yielding genotypes. Conventional methods of improving crops especially, through morphological markers plays vital role breeding programme and only complement molecular approach (DNA marker-assisted selection) of crop breeding and improvement. A crop of interest is soybean – a crop laden with protein.

Soybean (*Glycine max*) is a member of the family legumineceae and subfamily papilionadeae. It is essentially a subtropical crop but its cultivation now extends to the tropics and temperate regions, up to latitude 52^0N (Onochie, 1998). Soybean is a good source of protein as its seed contain approximately 49% protein and 20-30% fat and oil (Ezechoma, 2004).

According to Dutta (2005), soybean has a lot of industrial benefits such as production of soap, textile, plastics, adhesive, cosmetics, etc. However, the importance attached to soybean nutritionally and industrially notwithstanding, its production in Nigeria has been erratic mainly because much of the production is left to the initiatives of the local farmers; the poor genetic makeup of available genotypes; unfavourable climatic conditions, and prevailing government policies (Onochie, 1998).

It has been reported that *Rhizobia,* which are gram-negative bacilli belonging to the genera *Rhizobium, Bradyrhizobium* and *Azarhizobium* (Schutze,1994) elicit nitrogen-fixing nodules on the roots of leguminous host plants indicted for improve yield in soybean (Egamberdiyeva *et al*., 2004). Usually, a particular *Rhizobium* species elicits nodules on a limited number of host legumes. For instance, *Rhizobium meliloti* nodulates Medicago, Melilotus, and Trignonella species, *R. trifolii* nodulates Trifollium species; while *R. leguminosarum* nodulates Pisum and Vicia species. However, nodulation in soybean is said to be caused by *Rhizobium fedii, Bradyrhizobium japonicum*, and/or *Bradyrhizobium elkanii*. In all of these symbioses, nodulation is a complex process involving the following steps: recognition

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between the symbionts, attachment of *Rhizobium* to root hairs, root hair curling, infection thread formation within root hairs, initiation of a nodule meristem, and nodule organogenesis (Dudley *et al*., 1987; Ausubel *et al*., 1988). Raverkar and Konde (1988) reported increase in nodulation and growth of *Arachis hypogeal* under field condition after *Rhizobium japonicum* and *Azospirillum lipoferum* inoculation. Dashti *et al*. (1997) in their report observed that inoculation of soybean plant with plant growth-promoting *rhizobacteria* in the presence of *Bradyrhizobium japonicum* increased soybean grain yield, grain protein yield and total plant protein production.

The interaction between particular strains of *Rhizobium* and the appropriate legume is mediated by nodulation genes (nod genes): nodA. nodB, and nodC genes, referred to as common nodulation genes, which are conserved both structurally and functionally between *Rhizobium* and *Bradyrhizobium* species (Djordjevic *et al*., 1987). It has been reported that the common nodulation genes code for essential, evolutionarily conserved steps in the nodulation process that are common to all *Rhizobium*-legume symbioses (Ausubel *et al*., 1988). Though they are common nodulation genes, different species have extra nodulation genes for special functions. For instance, *Rhizobium meliloti* has a set of genes: nodFE, nodG and nodH. Although nodF and nodE are conserved at the nucleotide sequence level among several *Rhizobium* species (Fisher *et al*., 1987), mutation in these genes cannot be complemented by the homologous genes from other *Rhizobium* species (Ausubel *et al*., 1988). The nodulation genes more often than not activate the expression of the other nod genes in the presence of flavones root exudates (Fisher and Long, 1992). Recent evidence from *Rhizobium* species indicates that the nod genes play a role in host range determination by modulating gene expression as a function of the composition of the legume host root exudates. *Rhizobium* species that acquires nodulation genes from another species still nodulates its original host although it may acquire the ability to nodulate a heterologous host as well. Several experiments involving the transfer of symbiotic plasmids (pSym) or cloned nodulation genes between various rhizobium species have been reported (Ausubel *et al*., 1988). It is possible that the nodulation genes may code for enzymes that can alter a specific substrate required for a particular cultivar nodulation into a product required for another plant nodulation (Ausubel *et al*., 1988). This paper was aimed at evaluating the morphological differences in soybean genotypes and also correlates some morphological features with yield-indicating parameters. This will enhance selection and improvement programmes of the crop, especially using molecular/biotechnological tools.

MATERIALS AND METHODS

Seeds from six genotypes of soybeans: TGX923-2E, TGX1844-18E, TGX1448-2E, TGX1740-1F, TGX1448-1E and TGX1440-E were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. These seeds were checked for viability through floatation test. 108 polythene bags were filled with about 10.5kg of soil per bag where the seeds of the different soybean genotypes were sown. The 108 polythene bags were divided into 3 blocks containing 36 bags per block. The six genotypes were randomized 6 times per block using randomized complete block design (RCBD) in a 6 x 4 factorial experiment. Four seeds were sown in each polythene bag at a depth of 3cm. Picking of weeds was done routinely to avoid unpleasant competition with the plants for nutrient, space, sunlight. Data on the number of leaves per plant, plant height per plant, root length per plant, number of nodules per plant and dry matter yield (g) per plant were obtained at intervals of 2, 3, 4, and 5 weeks after planting. These data were subjected to the analysis of variance (ANOVA) (Obi, 2002) while correlation analysis was done for selected genotypes based on morphological performances using SPSS (8.0 version).

RESULTS

Table 1 presents the results of the analysis of the morphological performances of six genotypes of soybean screened. The results showed significant differences ($P < 0.05$) in all the morphological parameters studied, which expectedly increased with weeks of data collection. Our results revealed that TGX1448-1E, TGX 1740-1E and TGX 923-2E genotypes had the same average height and average root length per plant when compared with other genotypes. TGX 1740-1E soybean genotype produced the highest number of nodules followed by TGX 923-2E. However, there was no significant diference ($P > 0.05$) in the average number of nodules produced by TGX 1448-1E, TGX 1844-1E and TGX 1440-e, respectively (Table 1). TGX 1740-1E produced significantly ($P < 0.05$) the highest number of leaves per plant, which differed slightly from that produced by TGX 1448-11E and TGX 923-2E. TGX1448-1E genotype produced plants with the longest root length, which differed slightly from those of TGX 1740-1E and TGX 923-2E genotypes. The same trend occurred for the average dry weight per plant.

Correlation analyses were carried out for TGX 1740-1E, TGX 1448-1E and TGX 923-2E. Results for TGX 1740-1E genotype showed that there was significant positive correlation ($P < 0.5$) between the number of leaves and the number of root nodules. Though there were no significant relationships between the root length and number of leaves, root length and number of nodules, our results revealed that there were negatively correlated. For TGX 1448-1E variety of soybean, there was significant positive correlation between plant height and number of nodules and a significant negative relationship ($P < 0.5$) between the number of leaves and dry weight. There were weak associations (negative and positive) among other morphological traits though not significant. TGX 923-2E genotype showed a significant positive correlation ($P < 0.01$) between the root length and the number of leaves per plant. There were negative correlation between the number of leaves and plant height; number of leaves and dry weight and root length and dry weight (Table 2).

DISCUSSION

It is obvious that for better morphological and yield performances of legumes especially, soybean, many factors are indispensible, which include the amount of nutrients in the soil, the rate of photosynthetic activities in the leaves and the nodulation rate, which is a function of the amount and species of *Rhizobia* invading into the roots nodules. These factors however, seem to be functioning in tandem with one another. Nodulation being a complex

Table 1: Morphological performances of six genotypes of soybean screened

Parameter	Weeks	Soybean genotypes					
		TGX923-2E	TGX1844-18E	TGX1448-2E	TGX1740-1E	TGX1448-1E	TGX1440-E
Av.Plant height per plant	2	$9.78^{d}\pm0.33$	$7.80^{b}\pm0.61$	$6.32^{a}\pm0.30$	$8.24^{c}\pm0.46$	$9.82^{d}\pm0.36$	$7.82^{b}\pm0.34$
	3	$16.21^{gh}\pm0.43$	$13.23^{f}\pm0.40$	$11.45^{e}\pm0.40$	$15.35^{fg}\pm0.70$	$17.43^{h}\pm0.20$	$14.20^{f}\pm0.42$
	4	$19.29^{ij}\pm0.68$	$15.54^{g}\pm0.20$	$14.40^{f}\pm0.70$	$19.40^{j}\pm0.50$	$20.59^{j}\pm0.59$	$17.84^{hi}\pm0.18$
	5	$22.05^{k}\pm0.37$	$18.40^{i}\pm0.09$	$17.46^{h}\pm0.77$	$23.13^{k}\pm0.7$	$24.11^{kl}\pm0.32$	$21.06^{jk}\pm0.08$
Av. Root length per plant	2	$13.97^{c}\pm0.50$	$9.27^{a}\pm0.38$	$7.61^{a}\pm0.41$	$13.71^{c}\pm0.66$	$13.28^{c}\pm0.38$	$9.99^{b}\pm0.37$
	3	$21.75^{f}\pm0.25$	$16.41^{d}\pm0.4$	$14.29^{c}\pm0.83$	$21.38^{f}\pm0.38$	$22.30^{f}\pm0.61$	$18.90^{e}\pm1.21$
	4	$30.64^{i}\pm0.12$	$25.47^{g}\pm0.38$	$22.20^{f}\pm1.09$	$29.83^{i}\pm0.37$	$31.24^{i}\pm0.86$	$27.70^{h}\pm0.83$
	5	$41.02^{k}\pm0.31$	$36.32^{j}\pm0.74$	$30.33^{i}\pm0.81$	$40.37^{k}\pm0.51$	$42.29^{kl}\pm0.75$	$37.21^{j}\pm0.98$
Av. No. of nodules	2	$32.11^{cd}\pm0.49$	$20.55^{b}\pm0.40$	$14.67^{a}\pm0.38$	$35.66^{de}\pm0.67$	$30.11^{c}\pm1.13$	$22.00^{b}\pm0.88$
	3	$39.22^{f}\pm0.78$	$29.56^{c}\pm0.48$	$22.78^{b}\pm0.29$	$44.44^{h}\pm0.59$	$34.22^{d}\pm0.62$	$32.11^{cd}\pm0.29$
	4	$50.44^{i}\pm0.48$	$41.66^{g}\pm0.67$	$32.67^{d}\pm0.38$	$59.22^{j}\pm0.95$	$51.18^{i}\pm1.44$	$44.34^{h}\pm0.33$
	5	$60.89^{j}\pm0.95$	$52.00^{i}\pm0.97$	$38.22^{f}\pm1.24$	$66.70^{k}\pm0.77$	$52.78^{i}\pm1.93$	$52.78^{i}\pm0.29$
Av. No of leaves	2	$9.22^{bc}\pm0.29$	$7.22^{a}\pm0.11$	$6.33^{a}\pm0.19$	$8.00^{ab}\pm0.19$	$8.44^{b}\pm0.49$	$7.22^{a}\pm0.44$
	3	$12.45^{e}\pm0.40$	$10.33^{cd}\pm0.19$	$9.22^{bc}\pm0.11$	$11.44^{e}\pm0.29$	$12.44^{e}\pm0.68$	$10.22^{c}\pm0.45$
	4	$15.89^{g}\pm0.11$	$14.66^{f}\pm0.33$	$12.45^{e}\pm0.22$	$16.00^{g}\pm0.39$	$15.78^{g}\pm0.59$	$14.00^{f}\pm0.33$
	5	$20.00^{i}\pm0.19$	$18.11^{h}\pm0.40$	$15.45^{fg}\pm0.22$	$20.78^{ij}\pm0.40$	$19\ .44^{i}\pm0.59$	$17.89^{h}\pm0.29$
Av. Dry weight (g)	2	$0.62^{c}\pm0.04$	$0.17^{a}\pm0.02$	$0.14^{a}\pm0.03$	$0.38^{b}\pm0.01$	$0.46^{b}\pm0.03$	$0.21^{a}\pm0.01$
	3	$1.11^{e}\pm0.02$	$0.87^{d}\pm0.08$	$0.58^{c}\pm0.01$	$0.92^{d}\pm0.02$	$1.11^{e}\pm0.03$	$0.79^{d}\pm0.03$
	4	$2.11^{i}\pm0.13$	$1.49^{fg}\pm0.03$	$1.31^{f}\pm0.04$	$2.16^{i}\pm0.03$	$1.81^{h}\pm0.05$	$1.46^{f}\pm0.03$
	5	$2.98^{k}\pm0.11$	$2.89^{k}\pm0.07$	$2.37^{j}\pm0.05$	$3.17^{l}\pm0.08$	$3.09^{l}\pm0.09$	$2.46^{j}\pm0.09$

* Means followed by the same case letter along each horizontal array indicate not significant ($P > 0.05$).

Table 2: Correlation matrices for the morphological traits evaluated in TGX1740-1E, TGX 1448-1E and TGX 923-2E

Morphological parameter	Number of leaves per plant	Number of nodules per plant	Plant height per plant	Root length per plant	Dry weight
Number of leaves per plant	1.00	$0.833^{a}*$ 0.600^{b} -0.222^{c}	0.027^{a} 0.458^{b} -0.869^{c}	-0.080^{a} -0.105^{b} 0.982^{**c}	0.429^{a} -0.879^{*b} -0.854^{c}
Number of nodules per plant		1.00	0.460+ 0.884^{*b} 0.085^{c}	-0.868^{a} 0.741^{b} -0.340^{c}	0.410^{a} -0.503^{b} 0.061^{c}
Plant height per plant			1.00	0.590^{a} -0.545^{b} -0.776^{c}	0.230^{a} -0.340^{b} 0.618^{c}
Root length per plant				1.00	0.194^{a} 0.284^{b} -0.846^{c}
Dry weight per plant					1.00

a = TGX 1740-1E; b = TGX 1448-1E; c =TGX 923

process is chiefly influenced by the nodule-eliciting *Rhizobia* whose function is to fix nitrogen in the roots of legumes in the form of nitrates, which enhances vegetative (foliage) growth. Expectedly, increase in the foliage of the crop, increases photosynthetic activity leading to increase in yield. If this assumption is correct, it then implies that the trend in yield is TGX1740-1E genotype >TGX923-2E=TGX1448-1E>TGX184418E = TGX1440-E>TGX1448-2E. It thus follows that the number of nodules per plant produced by each genotype equals the number of leaves per plant of the said genotype (Table 1).

It should be a common sense to assume that the number of nodules produced per plant per genotype will be proportional to the length of the roots produced. Based on the morphological markers evaluated, TGX1448-1E had the lowest number of leaves and nodules per plant when compared with TGX1740-1E and TGX923-2E but however, produced plant with the longest root length. It should be understood that the production of nodules by legumes is never infinite as it is probable that the nodulation genes could have regulatory mechanisms in controlling nodule production in legumes, the length of roots notwithstanding. This was also the trend in the plant height. The different morphological performances observed in the different genotypes (Table 1) could be traceable to the different genetic compositions of the genotypes screened, recognition between symbionts and the attachment of *Rhizobium* to the root hairs of soybean plant (Ausubel *et al*., 1988) without interruption and the nutrients available to the crop during the infection phase of nodulation. This is an indication that even if superior soybean genotypes are cultivated on a soil devoid of the necessary species of *Rhizobium* and adequate nutritional base, yield improvement might be impaired. It is also probable that the *Rhizobial* signal from other *Rhizobia* species in the form of a surface component or a diffusible molecule may have been synthesized and modified by the soybean range gene products during the process of nodulation thus eliciting the infectious process (Ausubel *et al*., 1988), which may have resulted in the enhancement of morphological traits in some genotypes. It might make sense to suggest that the poor morphological performances of some soybean genotypes could be linked to the failure of some genotypes to secrete flavones for *Rhizobia* recognition the genotypic differences notwithstanding.

Correlation analysis of TGX1749-1E showed that there was a significant positive relationship ($P < 0.05$) between the number of nodules per plant produced by the genotype and the number of leaves per plant. This implies that as nodule number increases, the number of leaves also increases proportionally, which might probably lead to increase yield. Thus selecting genotype with increased nodule number will inevitably mean selecting genotype that will yield maximally. It implies that manipulating soybean genetically towards increasing the number of leaves, will inevitably lead to increase in nodule production with the resultant increase in yield, which was the position of Egamberdiyeva *et al*. (2004). For TGX1448-1E increase in dry weight per plant significantly ($P < 0.05$) led to the decrease in the number of leaves per plant and verse visa. This is sequel to the fact that the leaves contribute integrally to the dry weight of the plant. There was also significant positive correlation ($P < 0.05$) observed between plant height per plant and the number of nodules (Table 2). It is suggestive of the fact that the amount of nitrogen fixed into the plant, led to the increase in leaf number, which in turn influenced the photosynthetic activity, leading to high growth rate. For TGX923-2E soybean genotype, there was significant positive relationship ($P < 0.01$) between the root length and the number of leaves. Unfortunately, the length of the root does not necessarily mean increase nodule number because of the internal gene regulatory mechanism. Thus basing selection of superior genotype on this relationship might be misleading and unrealistic.

It is disheartening that most genetic manipulations of crop plants in the recent past have been on cassava and cereals with little or no attention on legumes, especially soybean. Our results are explicit indication that TGX1740-1E, TGX923-2E and TGX1448-1E soybean genotypes could be selected based on the number of leaves and number of nodules produced per plant for further improvement. This is with the understanding that increase nodulation will lead to increase yield. Our results also revealed that increasing nodulation in TGX1740-1E will result to increase foliage production, which invariably affect photosynthetic activity and high productivity. Though the correlation analysis of these morphological traits, in other selected genotypes was not consistent with TGX1740-1E, our results threw light to other relationships that might directly and indirectly influence yield.

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