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Seedling Shoot and Root Growth Responses among Soybean (*Glycine max*) Genotypes to Drought Stress

Obed J. Mwenye, Leon Van Rensburg, Angeline Van Biljon and Rouxlene Van der Merwe

Abstract

Drought stress is a major limiting factor in soybean production in South Africa. The development of soybean varieties with enhanced tolerance to soil waterlimited induced stress (WLIS) is one sustainable way to deal with drought. Root traits have shown strong potential for improvement of drought tolerance through breeding. The objectives of this study were to evaluate seedling shoot- and root growth responses under WLIS in order to study root morphology as a mechanism to cope with drought stress and to determine if there were genotypic differences in shoot- and root morphology between drought tolerant and -sensitive soybean genotypes. Seedlings of three drought tolerant and one sensitive genotype were subjected to soil WLIS in deep-root-pots for 21 days. Results suggested significant genotypic differences for shoot length, number of leaves, tap root length and root-to-shoot length ratio. Soybean tolerant genotypes were associated with moderate shoot biomass, deep rooting abilities and maintained a large root-to-shoot ratio under WLIS conditions. In contrast, the sensitive genotype was associated with a reduced root-to-shoot ratio and shallower root system. Soybean genotypes showed varying seedling root growth responses to soil WLIS, while shoot biomass characteristics were similar.

Keywords: root, drought, tolerance, selection, soybean

1. Introduction

Soybean (*Glycine max* L. Merrill) is the world's leading economic oilseed crop [1]. It is the largest source of vegetable oil and protein in the world [2] and a primary source of protein in the livestock feed industry in South Africa [3]. Soybean production in South Africa is limited by inadequate rainfall and soil water-limited-induced-stress (WLIS) which characterises most production regions [3, 4], consequently affecting the livestock industry and food security of the country [5].

Direct selection for morphological and physiological parameters related to soil WLIS tolerance has been used in developing drought tolerant soybean cultivars [6]. Drought tolerant mechanisms in soybean have been closely associated with the

rooting system and/or rooting pattern [7, 8]. The response of root traits to the immediate effects of soil WLIS determines the soybean plants' defence to drought [7, 9]. This is because the root system is responsible for exploring and acquisition of all the water the plant requires from the soil [10]. Plants sense water shortage around their roots and respond instantaneously by sending chemical signals to shoots to initiate various adaptive responses to soil WLIS [11].

Genetic variation for consultative ability and phenotypic plasticity in root growth patterns have been reported in soybean [12, 13]. Although root traits have shown strong potential in breeding for drought tolerance in soybean [6], the laborious and difficult procedure involved to phenotype roots without breaking the tap root is a big setback [14]. However, a "deep-pot" root screening method, which facilitates removal of the root system with minimal damage and evaluation of the soybean root architecture, has been developed [9]. This screening method facilitates root evaluation efficiently at seedling stage. The present study was conducted with the aim of discriminating drought tolerant and –sensitive genotypes through root- and shoot morphology. Specific objectives were to determine the responses of seedling shoot- and root length, dry matter and root length density among soybean genotypes grown under soil WLIS using the "deep-pot" method. Study using the same four PANNAR SEED® (Pty) LTD soybean cultivars, under similar experimental conditions as at the University of Free State, South Africa has not been done before.

2. Materials and methods

2.1 Plant material and trial site

Glasshouse trials were conducted in duplicate (trial 1 from 06/09/2014 to 07/10/2014 and trial 2 from 06/11/2014 to 08/12/2014) at the Department of Soil, Crop and Climate Sciences of the University of the Free State, South Africa in the summer season. The plant material used included three drought tolerant genotypes (BL1, BL4 and CV2) and one susceptible genotype (CV3) obtained from the PANNAR SEED® (PTY) LTD breeding programme (**Table 1**). The plant materials in the study are grouped in the medium quick (5.7), medium (6.7) and medium late (7.0–7.4) maturity groups (**Table 1**) [15].

Glasshouse trials were conducted in deep transparent polythene pots (10 cm diameter and 110 cm length) according to [9] with modifications. Polythene pots were placed in polyvinyl chloride (PVC) tubes (10 cm diameter and 10 cm length) for support and the pots were filled with soil, leaving a 5 cm space at the top (**Figure 1**). Soil used was an aridic ustothent soil (Bainsvlei 2300) with a reddish brown colour

Genotype	Growth habit	Maturity group	Drought sensitivity
BL1	Determinate	6.7	Tolerant
BL4	Indeterminate	7.4	Tolerant
CV2	Indeterminate	7.0	Tolerant
CV3	Determinate	5.7	Sensitive

Maturity group (length of the growing season of a cultivar, mostly determined by day length and temperatures): 5.7 = medium quick, 6.7 = medium, 7.0 &7.4 = medium late.

Table 1.
Soybean genotypes used in the study and their levels of drought sensitivity.

Figure 1.
"Deep-pot" system used for screening shoot- and root morphology of four soybean seedling genotypes grown under water-limited-induced-stress conditions for 21 days from sowing.

and a fine sandy texture, and contained 8–14% clay and 2–4% silt [16]. Pots were fully saturated with water and left to drain. After seven days, three seeds from each genotype were sown in the pots.

2.2 Experimental layout and data collection

The pots were laid out in a randomised complete block design with six replications. After germination, seedlings were thinned to one per pot. For the first 12 days after sowing, plants were watered daily with 100 ml of water to ensure strong seedlings, thereafter soil WLIS was induced by irrigating 100 ml every third day. Twenty-one days after sowing (**Figure 1**), shoot length (SL) (cm), was measured from the soil contact point to the tip of the plant and number of trifoliate leaves was counted. Shoot dry weight (biomass) (g) was recorded after drying the shoots in the glasshouse at room temperature for 72 hours.

For root measurements, the polythene pots were carefully taken from the PVC tubes and cut longitudinally in order to safely isolate the whole root system from the soil. Tap root length (TRL) was measured on intact roots from the soil contact point to the tip. Total root dry weight (root biomass) (g) was recorded after separating the roots from the soil by washing the samples on a 0.5 mm sieve. The root samples were then dried at room temperature, weighed and root biomass determined. After recording tap root length, shoot and root biomass, all roots including tap root and primary roots were cut into 10 cm sections, corresponding to the actual depths from the tubes. For root length density, root samples in the 10 cm sections were counted using a modified infra-red root counter [17] and were converted into root length per soil layer using a standard curve generated from standard samples and actual root length. Mass of the total root per layer was used to calculate the root weight density per layer.

2.3 Data analysis

Data was statistically analysed using GenStat Release 18 statistical package [18]. A combined analysis of variance (ANOVA) (**Table 2**) was conducted for the duplicated trials on the data collected to partition the different sources of variation. Means were separated using least significant difference (LSD) test (**Table 2**).

Source of variation	Shoot length (cm)	No. of trifoliate leaves	Shoot dry weight (g)	Tap root length (cm)	Root dry weight (g)	TRL/ SL	RDW/ SDW
Replication	2.38	1.02	0.07	70.70	0.03	0.39	0.01
Trial (T)	1.02	1.02	1.56**	78.70	0.51**	0.22	0.01
Genotype (G)	40.34**	7.74**	0.11	1552.60**	0.02	4.76*	0.02
G×T	12.56	1.74	0.05	53.50	0.04	1.53	0.02
Residual	10.80	1.07	0.13	170.50	0.02	1.28	0.01
CV%	18.80	26.80	23.70	16.30	23.60	24.00	24.70

*, **, significant at $P < 0.05$, $P < 0.01$, respectively. CV = coefficient of variation, TRL/SL = tap root length to shoot length ratio, RDW/SDW = root dry weight to shoot dry weight ratio, G×T = genotype by trial interaction.

Table 2.
Combined analysis of variance showing mean square values for shoot- and root traits of soybean seedlings under water-limited-induced stress at 21 days after sowing.

3. Results

3.1 Response of shoot and root traits

Significant genotype differences were observed for shoot length ($p < 0.01$), number of leaves ($p < 0.01$), tap root length ($p < 0.01$) and root-to-shoot length ratio (TRL/SL) ($p < 0.05$) (**Table 2**). Trial effects were significant for shoot and root dry weight ($p < 0.01$). No significant genotype by treatment (G×T) interaction effects were observed for any of the traits measured (**Table 2**).

This suggested that genotypes responded the same between the two trials for all traits studied. Tolerant genotypes BL1 and BL4 grouped together for shoot length (both large), while the other tolerant genotype CV2 grouped with the sensitive genotype CV3 (**Table 3**). This, together with shoot dry weight (non-significant genotype effects), indicated that the sensitive genotype CV3 generally showed a good shoot biomass and was not different from the tolerant genotypes, especially BL4 and CV2 (**Table 3**).

Source of variation	Shoot length (cm)	No. of trifoliate leaves	Shoot dry weight (g)	Tap root length (cm)	Root dry weight (g)	TRL/ SL	RDW/SDW
BL1	19.58[a]	4.42[a]	1.55	80.10[b]	0.60	4.22b[c]	0.40
BL4	18.12[ab]	3.08[b]	1.59	95.50[a]	0.69	5.37[a]	0.45
CV2	15.25[c]	3.25[b]	1.37	75.30[bc]	0.67	5.13[ab]	0.49
CV3[*]	16.96[bc]	4.67[a]	1.50	68.80[c]	0.67	4.13[c]	0.45
Mean	*17.33*	*3.85*	*1.50*	*79.90*	*0.66*	*4.71*	*0.45*
LSD$_{0.05}$	2.72	0.86	0.29	10.82	0.13	0.94	0.09

[*]Drought sensitive genotype. TRL/SL = tap root length to shoot length ratio, RDW/SDW = root dry weight to shoot dry weight ratio, LSD = least significant difference. Means followed by the same letter of the alphabet in the column are not significantly different.

Table 3.
Combined mean values for shoot- and root characteristics of soybean seedlings under water-limited-induced stress conditions at 21 days after sowing.
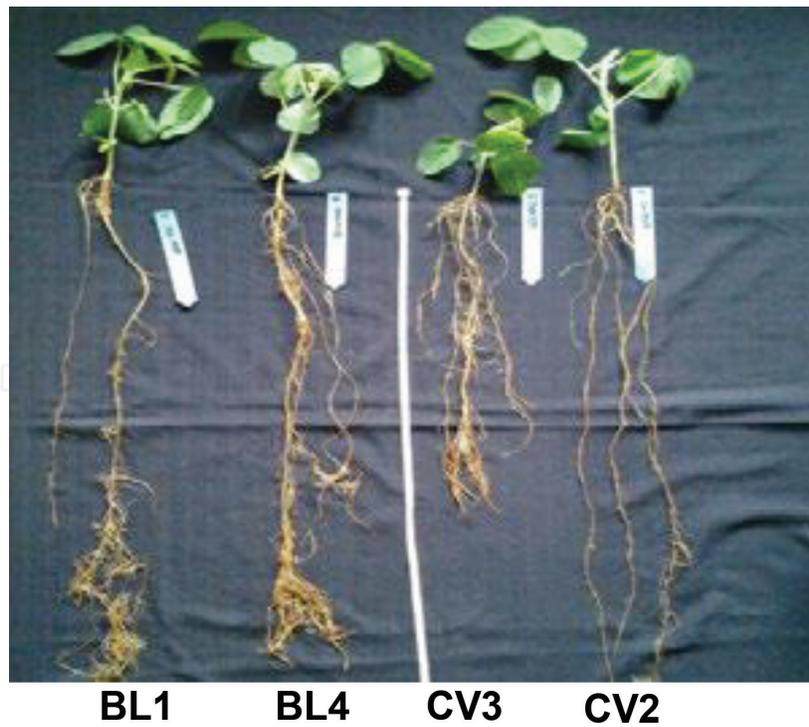
Figure 2.
Root lengths of four soybean seedling genotypes with CV3 as the sensitive genotype under soil water-limited-induced-stress conditions at 21 days after sowing.
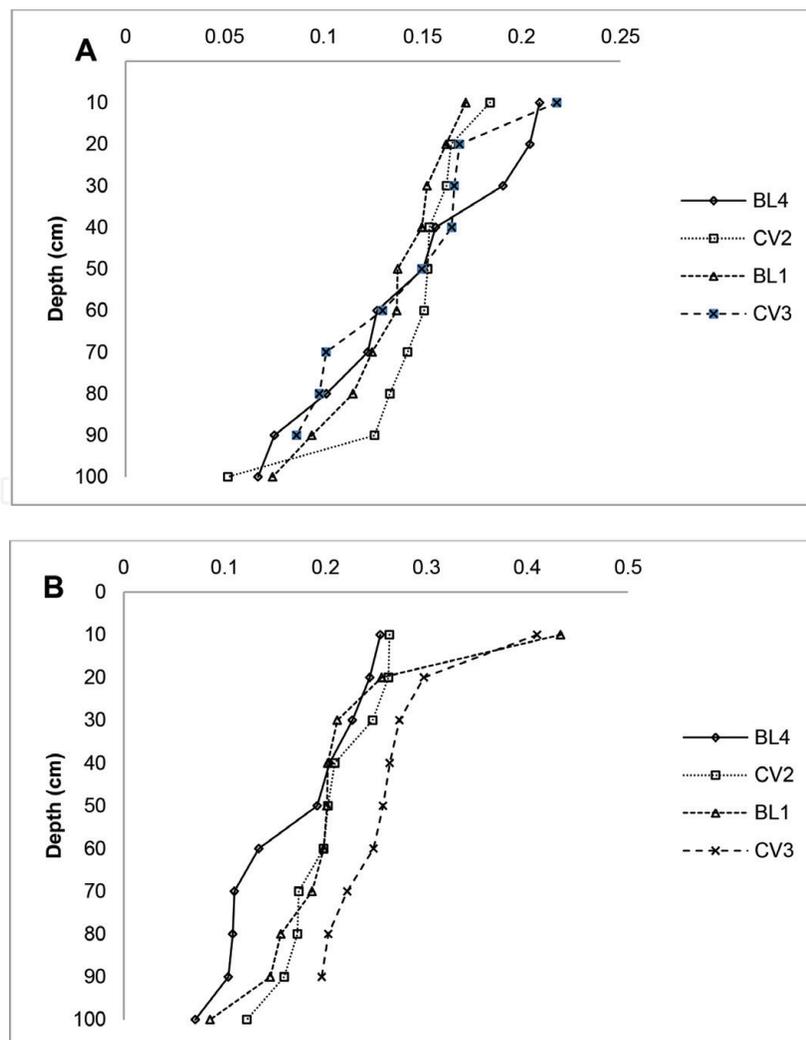

Figure 3.
Root length density (cm cm^{-3}) distribution with depth for four soybean seedlings grown under soil water-limited-induced stress conditions for duplicate trials: (A) trial 1 and (B) trial 2.
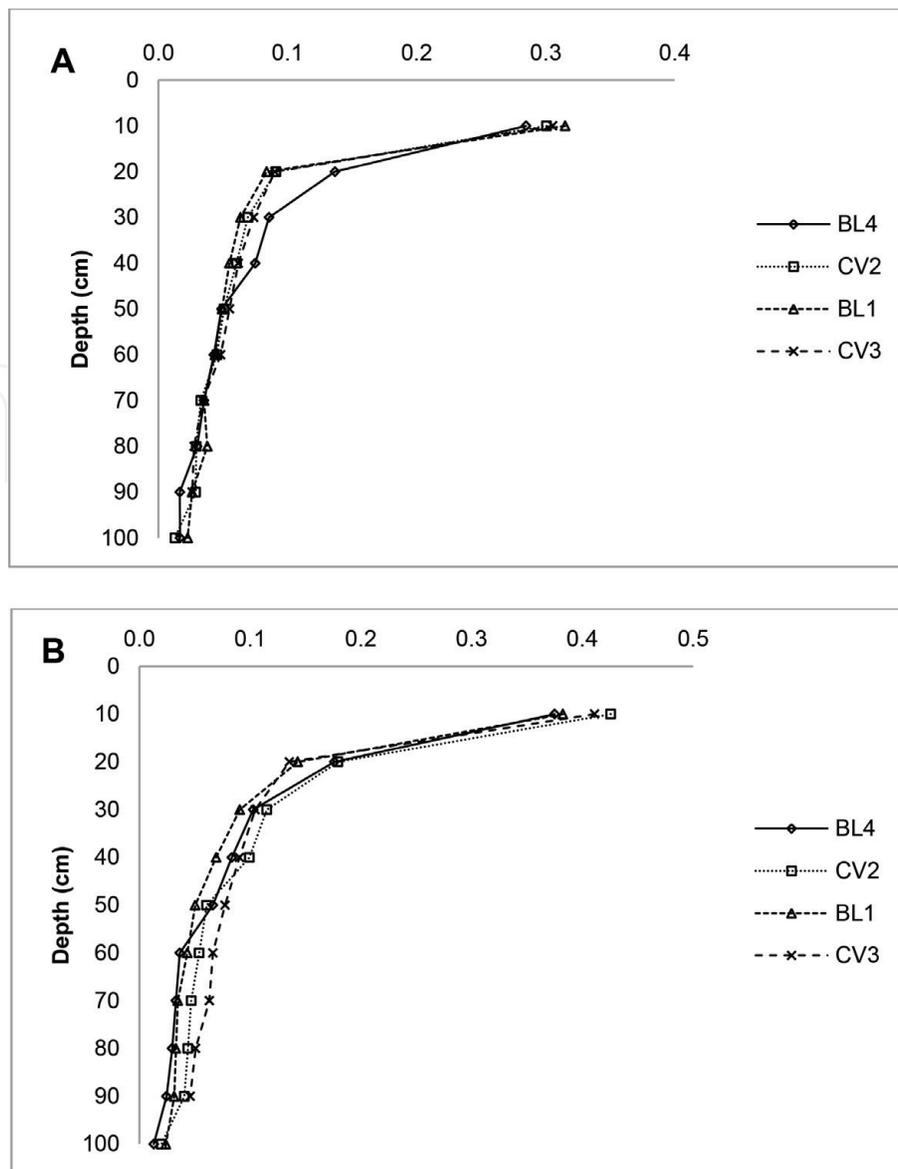
Figure 4.
Root weight density (mg cm⁻³) distribution with depth for four soybean seedlings grown under soil water-limited-induced stress conditions for duplicate trials: (A) trial 1 and (B) trial 2.

The three tolerant genotypes (BL1, BL4 and CV2) ranked in the top three positions for both tap root length and TRL/SL ratio, while the drought sensitive genotype (CV3) ranked last (**Table 3**). This suggested that the drought sensitive genotype developed a shorter tap root (as indicated in **Figure 2**) or had a reduced root elongation rate compared to the tolerant genotypes due to the soil WLIS. In **Figure 2** it is evident that the three tolerant genotypes had longer root systems than the sensitive genotype (CV3).

The root architecture system under soil WLIS was investigated by determining the distribution of the root length density (cm cm⁻³) and dry matter (root weight density) (mg cm⁻³) through the soil profile (**Figures 3** and **4**). Results showed that the root systems of all four cultivars were mostly contained in the top 0–40 cm soil profile (**Figures 3** and **4**). There were no marked differences in root distribution among the tolerant genotypes (BL1, BL4 and CV2) (**Figures 3** and **4**). The tolerant genotypes' roots explored the whole soil profile to the deepest part (**Figures 3** and **4**).
On the other hand, it was evident that the sensitive genotype (CV3) had the same root distribution (root length density and dry matter) like the tolerant genotypes in the top 0–90 cm soil profile (**Figures 3** and **4**). However, the sensitive genotype's roots could not explore the deeper profile beyond the 90 cm depth mark (**Figures 3** and **4**).

This suggested that the tolerant genotypes, unlike the sensitive one, could endure the soil WLIS by exploring moisture trapped in the deep soil profiles.

4. Discussion

Drought stress tended to increase biomass partitioning to the roots. This was demonstrated by the increase in the tap root length to shoot length ratio (TRL/SL) especially among the tolerant soybean genotypes (**Figures 3** and **4**). Similar trends were reported by [19] using a deep-rooted pot system with 24 soybean cultivars including exotic plant introductions (PI) lines in where it was suggested that TRL/SL determines the effective proportion of the roots supporting the above ground shoots. This is significant for survival under soil WLIS conditions. The large tap root length to shoot length ratio observed for the drought tolerant genotypes might have translated to the ability of the tolerant cultivars to access and get more edaphic resources per unit of the above ground shoot [20, 21]. A large TRL/SL suggests a large root surface area per unit of shoot length and this significantly increases the capacity of the tolerant genotypes to efficiently absorb and utilise soil water per unit dry matter [10]. The drought sensitive soybean genotype indicated to have a lower ability to penetrate the deeper soil (**Table 3**).

The seedling genotypic variation observed between drought tolerant and –sensitive genotypes for root and shoot traits, in response to soil WLIS (**Table 3**) agrees with results of [7, 11, 22, 23]. Drought tolerant soybean genotypes were associated with a deep-rooting phenotype and a large root-to-shoot ratio (length and/or mass), unlike the drought sensitive soybean genotypes (**Table 3**). Thus, drought tolerant genotypes tend to demonstrate a phenomenon called "balanced growth", whereby plants respond to drought by stimulating or maintaining root growth while effectively reducing shoot growth [24]. Increases in root versus shoot growth under drought conditions enhance the genotype's drought coping ability due to increased root-to-leaf surface ratio, continued production of new root tips, and enhancement of plant capacity for acquiring water to support existing shoots [14]. The observed shift in allometry under drought stress and the ability of the tolerant genotypes to grow roots according to the distribution of available soil water thus increase the productivity of tolerant genotypes under soil WLIS conditions. This has been attributed to the action of ethylene and abscisic acid in the tolerant soybean genotypes [25, 26] and is triggered by soil WLIS conditions.

5. Conclusions and recommendations

Soybean genotypes showed varying seedling shoot- and root morphology in response to soil WLIS conditions. The tolerant and sensitive genotypes did not differ much for shoot- and root biomass but significant differences were observed for shoot length, tap root length and tap root length to shoot length ratio. Drought tolerant genotypes showed deep rooting ability and larger root-to-shoot ratios compared to the drought sensitive genotype. Consequently, the drought tolerant genotypes increased biomass partitioning to the roots as a coping mechanism to soil WLIS. The "deep-pot" system was effectively used to phenotype the roots of soybean seedlings in a non-destructive manner. The system showed potential to differentiate between drought tolerant and -sensitive genotypes at seedling growth stage. However, in future studies more genotypes and more root traits need to be analysed in order to efficiently classify genotypes as drought tolerant or drought sensitive.

Acknowledgements

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