INTERNATIONAL JOURNAL OF RESEARCHES IN BIOSCIENCES, AGRICULTURE & TECHNOLOGY
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www.vmsindia.org

IMAGE BASED PHENOTYPING OF SOYBEAN ROOTS FOR DROUGHT STRESS TOLERANCE

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ABSTRACT:

Drought stress is a major constraint to the production and yield stability of soybean [*Glycine max* (L.) Merr.]. Superior root phenotypes are currently considered to be key to improved drought tolerance characteristics that allow better plant performance through more efficient water uptake in crops such as soybean. A quantitative characterization of root parameters is currently being attempted for various reasons. Non-destructive, rapid analyses of root system are difficult to perform due to the hidden nature of the root. Hence, improved methods to measure root traits are necessary to support knowledge based plant physiology and to analyse root growth responses to drought stress. Here, we conducted a minirizotrone study to investigate drought stress tolerance soybean genotypes. The dynamic parameters of root traits associated with maintaining plant productivity under drought include root length, surface area, average diameter, root volume, number of tips, number of forks and number of crossings. A new technique has been established for non-destructive root growth studies and quantification of root traits. However, automation of the scanning process and appropriate software remains the bottleneck for high throughput analysis. The JS9752 (2.22) possessed the higher root volume though did not vary significantly with KDS344 (0.99). JS9752(455.03) and JS334 (450.31) recorded the significantly higher analysed region area. JS9752 (0.32) and CGSOYA (0.29) noted the significantly higher and MACS1188 (0.23) lower average diameter of root. These findings demonstrate that root phenotyping using minirhizotron that are easy-to-apply under drought conditions can be used to determine genotypic differences in drought tolerance in soybean.

Keywords: minirizotrone, high throughput, phenotyping, drought.

Introduction-

Soybean is the world's leading economic oilseed crop and the most widely grown oilseed worldwide, having reached a global 318.25 million metric ton in 2014/15 crop season (USDA, 2015). India cultivated in 10910.83 hectares area with the annual production of 10373.80 Million tonnes. Chhattisgarh contributes nearly 1.41 lakh ha areas and 0.728 mt in total production of Soybean in the country but the productivity of the crop is quite low as against the advanced countries (SOPA 2014-15). Current physiological strategies for crops such as soybean seek to identify new varieties with abiotic stress resistance and climate change. While considerable progress has been made recently in the development of imaging tools for the rapid phenotyping of root and shoots, the associated data processing and bio-informatic requirements remain challenging (Furbank et al. 2011). Moreover, despite intensive efforts, systems that are directly applicable in the field remain largely at the prototype stage (Araus and Cairns 2013). Therefore, classic manual plant phenotyping techniques remain valuable tools for plant physiology (Beebe et al. 2013). Such techniques are particularly important in many developing countries, particularly in India, which lack the essential underpinning infrastructure required to apply more sophisticated "phenomic" approaches (Sinclair et al. 2014).

Shoot morphology characteristics are commonly used for phenotyping (Manavalan et al. 2009). Shoot characteristics are generally easy to assess under field conditions, where they can often be determined simply by visual examination. Leaf movement and leaf area are commonly used in screening for drought tolerance. Decreases in leaf size and leaf expansion can also be used as measures of adaptation to drought. In general, plant breeders are often reluctant to apply physiological screening techniques extensively because they are regarded as expensive, time-consuming and more difficult to apply.

Roots are the first organs to perceive and respond to drought but below-ground phenotyping by screening of the root systems is rarely undertaken, particularly under field conditions. Root trait measurements are used in many kinds of physiological studies, particularly those involving water and mineral uptake by plants. One of the greatest constraints to measuring root traits has been the labour associated with the manual measurements made with rulers, curvimeters, calipers, and other devices. The distribution of roots, particularly those that can penetrate deeper in the soil, plays a crucial role in determining the ability of plants to capture key resources such as water and mobile nutrients. Root architecture therefore has a profound effect on the growth and yield of crop plants. Studies on the responses of root architecture to drought have been performed on soil or on solid support media, such as hydroponics or on agar plates (Prince et al. 2013).

Therefore, identifying crops with improved root architecture characteristics remains a major challenge to current plant biology, together with the development of appropriate technologies for the study of root growth in the soil, particularly under field conditions. Root architecture is greatly modified under drought stresses, which favour the production of greater numbers of longer lateral roots are root hairs to increase the total surface area for better water absorption (Osmont et al. 2007). Together with strategies that limit water loss, such as stomatal closure, leaf rolling and leaf abscission, the increase in root mass particularly deeper in the soil results in an improved plant water status that is required to support biomass production and yield.

Superior root phenotypes are currently considered to be key to improved drought tolerance characteristics that allow better plant performance through more efficient water uptake in crops such as soybean (Lopes et al. 2011). Genetic variability has been demonstrated in soybean root architecture and morphology, including traits such as root angle, root diameter, length, surface area and depth (Ao et al. 2010). To our knowledge, phenotyping of root using glass rhizotron system has not been widely used to select for superior root systems in soybean. The following studies were therefore undertaken to characterize drought-induced changes in soybean root under glass rhizotrons system in order to determine whether root traits can be linked with plant performance under drought, with a view to identifying drought-tolerant soybean cultivars. Root traits were characterized in six soybean cultivars that differ in drought tolerance, under drought conditions.

2. Materials and methods

2.1 Plant Material and Experimental Procedure

Glass rhizotron experiment was conducted at Research cum Instructional Farm, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.) during 2017-2018. Soybean (Glycine max L. Merr.) cultivars grown were KDS344, JS335, MACS1188, NRC37, JS9752 and CGSOYA. Three seeds sowing in rhizotron plates in eight replications. Watering is stopped and drought stress is created at vegetative stage.

Rhizotron made up of transparent glass plates filled with a mixture of cocopeat (60%), sand (20%) and field soil (20%). In order to increase the number of roots growing along the rhizotron surface, rhizotrons were stored at an angle of ~15°. After 45 days rhizotrons were open and plants were taken out. Root were separated from the shoot and cleaned from the substrate. Finer soil particles still attached to the root were removed using a small painting brush. Plant roots were stored in a 25% ethanol until the scanning procedure (fig.1).

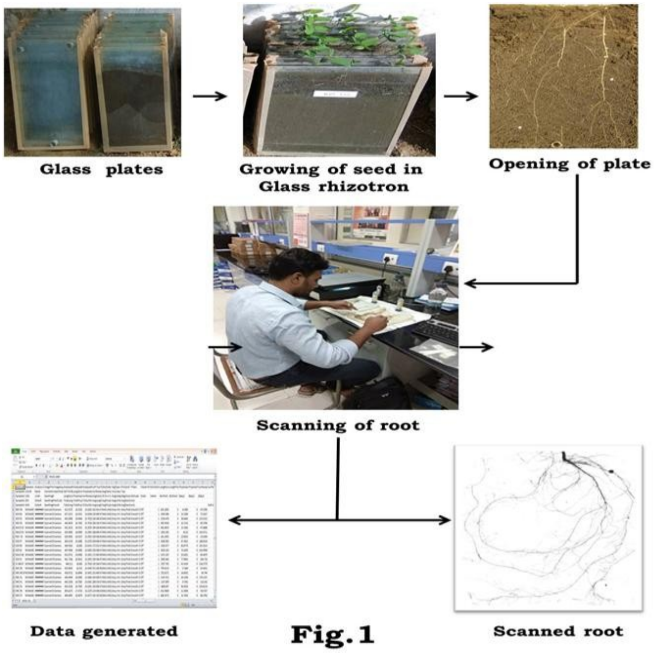

Fig.1

2.2 Root scanning

The root scanning was done by using root scanner machine Epson Perfection V700/ V750, 3.81 Version, WinRhizoReg 2009 (fig.1), which allows the roots to be light from above and below while the roots were being scanned. This is an important feature (called "Dual Scan" in Regent's documentation), which reduces shadows on the root image. The Regent Positioning System allows the trays to be consistently placed, thus obviating the need to preview each scan. Optimum scanning resolution depends on the type of samples. Generally roots scanned at 600 dpi in 10x15 cm trays. the data was recorded automatically in the computer for different root parameters including root length, average root diameter, root volume, number of tips, forks, surface area and number of crossings etc.

2.3 Analysing Scanned Images

The image was analysed by selecting the region of interest and it is analysed. When scanned images are analysed (fig.1), the software uses thresholding to determine what is root and what not root is. A few second later, the analysis was complete and roots found by WinRHIZO were identified by colored line in image. The colors used for drawing them are coded according to root diameter. Portions of the image can be excluded from analysis if necessary, and there are basic editing tools if minor image editing is required.

2.4 Save the measurement data

The last step of the analysis was data saving WinRHIZO knows when data was easily recordable by many programs including spreadsheet style like Excel (fig.1). Image and their analysis was also save to file for later validation, reanalysis, or for visualization in other software programs.

3. Statistical Analysis

The mean data of each replication was used for analysis of variance using CRD design. The replicated data were subjected to variance analysis and test of significance as per the method of Fisher (1935).

4. Result and Discussion

The present investigations have been carried out to reach deeper understanding of the variations in the root traits of soybean under drought condition. Treatments varied significantly (at 5%) among themselves for analysed region area, root length, surface area, average diameter, root volume, number of tips, number of forks and number of crossings (Table 1).

The results showed that the variety JS 9752(455.03), JS 334 (450.31), MACS 1188 (446.63) and CG SOYA (443.89) recorded the higher magnitudes for analysed region area though they did not differ significantly among them (Table.1, fig.2). The variety NRC 37 (431.92) recorded lowest analysed region area. Large increase in root growth was observed in non-irrigated plants, especially in the deeper soil layers. Root growth was less affected when drought was imposed at the R4 stage and ceased at the R5 stage (Hoogenboom et al. 1987)

Table 1. Root traits of soybean under drought condition

Veriety	Analysed Region Area(cm^2)	Length (cm)	Surface Area (cm^2)	Avg. Diam. (mm)	Root Volume(cm^3)	NO of Tips	No of Forks	No of Crossings
CGSOYA	443.89	1300.15	132.76	0.29	0.90	1227.50	1942.00	2436.38
JS334	450.31	1821.44	139.87	0.24	0.86	1767.88	2172.88	3859.13
KDS344	442.43	1888.39	153.15	0.26	0.99	1850.25	2596.88	3560.75
JS9752	455.03	1832.99	194.17	0.32	2.22	1824.38	3391.13	4122.63
MACS1188	446.63	1458.25	115.58	0.23	0.64	1461.00	1985.88	2964.63
NRC37	431.92	995.86	81.55	0.26	0.54	926.63	1289.88	1811.13
S.Em.+	**4.35**	**220.59**	**23.05**	**0.013**	**0.26**	**212.79**	**405.43**	**505.92**
CD at 5%	**12.50**	**633.32**	**66.19**	**0.038**	**0.73**	**611.63**	**1163.67**	**1452.51**

The results indicated that KDS344 (1888.39), JS9752 (1832.99) and JS334 (1821.44) higher values for length though did not differ significantly among them (Table.1, fig.2). NRC37 (995.86) recorded lower length. Root morph-architecture traits than those of XM6 as indicated by longer length. Under stress conditions, the number of lateral roots per unit of taproot length significantly increased (Read and Bartlett 1972).

The fibrous root trait of 'PI416937' also conferred increased water uptake through increased root surface area (Busscher et al. 2000). The JS9752 (194.17) noted the maximum (Table.1, fig.2) surface area though did not vary significantly with KDS344 (153.15), JS334 (139.87), CG SOYA (132.76) and MACS1188 (115.58). NRC37 (81.55) recorded lower surface area. The differences of root length, surface area and volume between two parental genotypes reached significant level from 2 weeks till 9 weeks after planting (Ao et al. 2010).

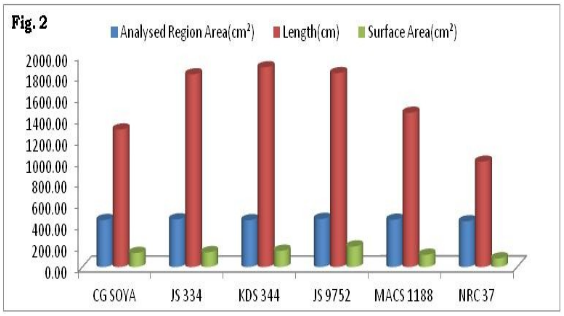
Fig. 2

The JS9752 (0.32), CG Soya (0.29) and NRC37 (0.26) recorded the higher (Table.1, fig.3) magnitudes for average diameter of root though they did not differ significantly among them. MACS1188 (0.23) noted the minimum average diameter of root. The roots diameter per unit of taproot length significantly increased under stress conditions (Read and Bartlett 1972). The JS9752 (2.22) possessed the higher (Table.1, fig.3) root volume though did not vary significantly with KDS344 (0.99), CG Soya (0.90) and JS334 (0.86). NRC37 noted the minimum root volume (0.54). Significant correlations exist in soybean between drought resistance and various root traits such as total length, and volume and number of lateral roots (Liu et al. 2005).

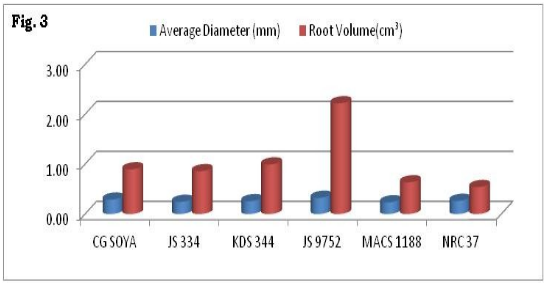
Fig. 3

The results indicated that treatment KDS344 (1850.25) noted the maximum (Table.1, fig.4) number of tips though did not vary significantly with JS9752 (1824.38). NRC37 noted minimum number of tips (926.63). Root surface area, longer root length, larger root volume, root tips, root forks as well as root crossings could facilitate root acquiring more water from soils to maintain water level in plants.

The JS9752 (3391.13) indicated the maximum (Table.1, fig.4) number of forks though did not vary significantly with KDS344 (2596.88) and JS334 (2172.88). NRC37 noted the minimum number of forks (1289.88). Dense fibrous roots when found in tandem with deep rooting genotypes were shown to be associated with improved growth of soybean in drought stress conditions (Fenta et al. 2011). The JS9752 (4122.63) possessed maximum (Table.1, fig.4) number of crossings though did not vary significantly with JS334 (3859.13) and KDS344 (3560.75). NRC37 (1811.13) recorded the significant lowest number of crossings. The quantitative nature of these root traits could be attributed to the complexity of root growth and development.

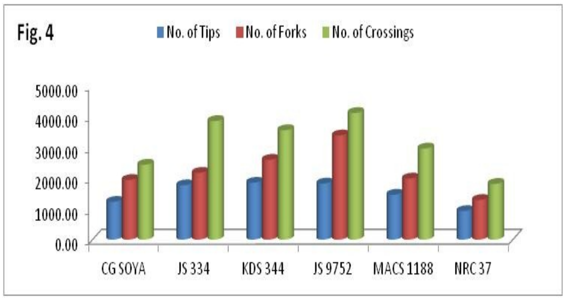
Fig. 4

5. Conclusions

In summary, the results presented here confirm the importance of root system architecture in drought tolerance to soybean. While manual phenotyping tools are labor-intensive and time consuming, they are still of great value particularly in developing countries, where high throughput phenotypic screening systems using imaging technologies are currently available. The six cultivars studied here in glass rhizotron experiments show similar relative drought tolerance characteristics and performance to those determined in experiment studies, with JS9752 being the most drought tolerant cultivar and JS 334 and KDS344 being the least drought tolerant cultivar. We conclude that this rizotron experiment root trait characteristics also have potential as targets for drought tolerant soybean cultivar development

6. References

1. Anonymous, 2014-15. Soybean Processors Association of India. www.sopa.org.
2. Ao, J., Fu J., Tian J., Yan X. and Liao H. (2010). Genetic variability for root morph-architecture traits and root growth dynamics as related to phosphorus efficiency in soybean. Funct. Plant Biol. 37:304–312.
3. Araus, J.L. and Cairns J.E. (2013). Field high-throughput phenotyping: The new crop breeding frontier. Trends Plant Sci. 19: 1–62.
4. Beebe, S.E., Rao I.M., Blair M.W. and Acosta-Gallegos J.A. (2013). Phenotyping common beans for adaptation to drought. Front Physiol.35.
5. Busscher, W.J., Lipiec J., Bauer P.J. and Carter T.E. (2000). Improved root penetration of soil hard layers by selected genotypes. Comm. Soil. Sci. Plant Anal. vol. 31 pg. 19-20.
6. Fenta, B.A, Martens C., Rombauts S., Holsters M., Kunert K. and Goormachtig S., (2011). Search for nodulation-related CLE genes in the genome of Glycine max. Journal of Experimental Botany. 426.
7. Fisher, R. A. 1935. The design of experiment. Oliver and Boyd Edinburgh.
8. Furbank, R.T. and Tester M. (2011). Phenomics—Technologies to relieve the phenotyping bottleneck. Trends Plant Sci.16: 635–644.
9. Hoogenboom, G., Peterson C.M. and Huck M.G. (1987). Shoot growth rate of soybeans as affected by drought stress. Agron. J. vol. 79: pg. 598-607.
10. Liu, F, Anderson M.N., Jacobson S.E. and Jensen C.R. (2005). Stomatal control and water use efficiency of soybean (Glycine max L.Merr.) during progressive soil drying. Environ. Exp. Bot.vol. 54:pg. 33-40.
11. Lopes, M.S., Araus J.L., Heerden P.D.R., Foyer C.H. (2011). Enhancing drought tolerance in C4 crops. J. Exp. Bot. 62:3135–3153.
12. Manavalan, L.P.; Guttikonda, S.K.; Phan Tran, L.S.; Nguyen, H.T.(2009). Physiological and molecular approaches to improve drought resistance in soybean. Plant Cell Physiol. 50:1260–1276.
13. Osmont, K.S., Sibout R. and Hardtke C.S. (2007). Hidden branches: Developments in root system architecture. Annu. Rev. Plant Biol. 58:93–113.
14. Prince, S.J., Mutava R.N., Pegoraro C., Oliveira A.C. (2013).Nguyen, H.T. Root characters. In Genomics and Breeding for Climate Resilient Crops; Kole, C. Ed.; Springer: Berlin, Germany. pp. 67–131.
15. Read, D.J. and Bartlett E.M. (1972).The physiology of drought resistance in soybean plant (Glycine max). J. Appl. Ecol. vol. 9:pg. 487-489.
16. Sinclair, T.R., Marrou H., Soltani A. and Valdez V. (2014). Soybean production in Africa. Glob. Food Biol.31.
17. USDA – United States Department of Agriculture, 2017. World agricultural supply and demand estimates, 542. http://www.usda.gov/oce/commodity/wasde/latest.pdf.

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