

## Variability studies in soybean genotypes under different water regimes

### Abstract:

Soybean (*Glycine max* L.) productivity is frequently affected due to drought occurrence in India. Variability for root shoot traits under early seedling stage under drought stress may be important selection criteria for development of drought tolerant genotypes.

The present investigation was carried out at Net house of Experimental Farm, Mandsaur University, Mandsaur. Sixty genotypes, procured from ICAR-IISR, were used to know the extent of genetic variability under different water regimes [normal (100%), S<sub>1</sub>(50%), S<sub>2</sub> (25%) and S<sub>3</sub>(0%)] for root-shoot traits and relative leaf water content under early seedling stage. Significant genetic variability was recorded for all the traits among genotypes studied under normal and water stress conditions respectively which showed the presence of ample variability in studied material. High PCV than their corresponding GCV for the entire trait under different water regimes denoted influence of environment on these traits. The large genetic variation found in these genotypes may be used to develop varieties with better drought tolerance behavior and used as drought donor lines in drought breeding programs. We measured high heritability for selected characters under non water stress condition (75.05% to 100%) and water stress conditions S<sub>1</sub> (81.40% to 100%), S<sub>2</sub> (85.69% to 100%) and S<sub>3</sub> (92.60% to 99.26%) respectively. Additive gene action interacted to control characters under different water regimes because high broad sense heritability along with high genetic advance as percentage of mean were recorded for most of traits. It is indicating that simple phenotypic selection would be effective for these traits under water stress condition.

**Keyword:** Water regimes, Soybean, variability, water stress, heritability

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## Introduction

Soybean (*Glycine max* L. Merrill) is the world's most important oilseed crop (Narayanan S and Fallen B., 2019; Mishra and Patidar, 2023; Bairagi *et al.*, 2023). Soybean oil is secondly most widely consumed oil and rich source of protein in world (Sunaryo *et al.*, 2016). In International market soya oil trading is next only to palm oil (Bhuva *et al.* 2020). It contributes to 25 % of the global edible oil, about two-thirds of the world's protein concentrate for livestock feeding. The soybean is not only known for its high total protein content but the quality of soy protein which is higher than that of other plant proteins and similar to animal protein (Hughes *et al.* 2011). Soybean is known as the "Golden Bean" because of its health and nutritional benefits such as low Glycaemic index, low saturated fat, and cholesterol-free and is widely used as oilseed (Kumawat *et al.*, 2023). It is primarily produced by the United States, Brazil, Argentina, China, and India (Thu *et al.*, 2014). Soybean is also a very important *kharif* season crop of India. The top three soybean growing states in India are Madhya Pradesh, Maharashtra and Rajasthan. Madhya Pradesh state is leading state in both area and production point of view in all over India and emerged as India's Soy State (Kumawat *et al.*, 2023; Mishra and Patidar, 2023). Madhya Pradesh has 45% share in soybean production in the country. Soybean meal is a valuable ingredient in formulated feeds for poultry and fish.

Drought stress causes a decrease of 50% of the total yield soybean production (Sunaryo *et al.*, 2016). Water deficit affects physiological and agronomic traits of soybean plants, thus negatively influencing plant growth and development, resulting in grain yield reduction (Stolf-Moreira *et al.*, 2010; Giordani *et al.*, 2019, Nair *et al.* 2023; Kumawat *et al.*, 2023). Scientific forecasts draw a future with dark scenarios of water restrictions around the world (Dai, 2013; Mishra and Patidar, 2023).

Successful breeding programme depends on the extent of genetic variability present in the plant population of a crop for further improvement (Sileshi, 2019). Therefore the first step in any crop improvement programme is to assess the extent of variability in the base population under study. Information on the magnitude of variability and extent, to which desirable characters are heritable, is important for planning breeding programme and ascertaining the scope of its improvement. The success of phenotypic selection depends upon the range of genetic diversity available in the population, whereas estimate of heritability and genetic advance are useful in inferring the genetic factors. Root traits affect the amount of water and nutrient absorption, and are

important parameters for maintaining crop yields indirectly under water stress conditions (Fenta *et al.*, 2014; Mishra *et al.*, 2016; Mishra *et al.*, 2017; Vijay *et al.*, 2018; Aski *et al.*, 2022). Despite the importance of root traits in drought tolerance, few breeding programs take these traits into account when developing drought-tolerant soybean varieties. Information regarding the soybean genetic variability of the root traits is limited, and the exploitation of this variability can assist soybean breeding programs in the development of varieties with desired root traits for drought tolerance.

Keeping in view the above fact the aim of present study was to observe genetic variability among sixty genotypes of soybean in terms of root traits under seedling stage for the purpose of selection of parental genotypes for further crosses in order to develop progenies with drought tolerant types with increased early vigor of roots and stems.

#### Materials and methods

The experiment was carried out at Net house facility situated at Campus-I, Faculty of Agriculture Sciences, Mandsaur University, Mandsaur (M.P.). Net house and laboratory experiments were conducted to achieve the objective of present experiment. Seedlings were raised in 480 polythene bags during September- October 2022. Total sixty lines collected from ICAR-Indian Institute of Soybean Research, Indore were sown on dated 02/09/2023 in four different water regimes (nonstress-100 ml, 50 ml, 25 ml and 0 ml) using completely randomized block design with two replications for each set of experiment. The polythene bags were filled with standard soil having mixture of 1% sand, 1% FYM and 1% clay loamy soil. The soil was also treated with readymade 1/4<sup>th</sup> MS Media for obtaining healthy plants. The list of genotypes is presented in Table 1.

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**Table 1: Name of 60 genotypes of *Glycin max* L. used for study**

S.No	Name of genotypes	S.No	Name of genotypes	S.No	Name of genotypes	S.No	Name of genotypes
1	GW-34	16	GW-234	31	NRC-37(CHECK)	46	RSC-1107(CHECK)
2	GW-371(K-21C)	17	GW-196	32	GW-178	47	GW-212
3	GW-63(K-21)	18	GW-382	33	GW-87	48	NRC-138
4	GW-237(K-25)	19	GW-134	34	GW-45	49	GW-214
5	GW-155	20	AMS-2014-1(CHECK)	35	GW-89	50	NRC-142

6	GW-159	21	AGS-218	36	JS-2069	51	NRC-127
7	GW-99	22	GW-108	37	GW-207	52	JS-9560
8	GW-164	23	GW-132	38	GW-188	53	GW-203
9	GW-312	24	PK-472(CHECK)	39	GW-185	54	JS-20-116
10	GW-143	25	GW-100	40	GW-52	55	JS-2034
11	GW-152(K-21-C)	26	GW-10	41	GW-286	56	GW-253
12	GW-15 (CHECK)	27	IC-073710	42	GW-223	57	GW-225
13	GW-51(K-21)	28	GW-17	43	GW-251	58	TGX-9336E
14	GW-21	29	GW-13	44	GW-291	59	SQL-110
15	GW-161	30	GW-28	45	GW-221	60	AGS-25

### Water stress imposition:

Regular irrigation was continued till two leaf stage of plants under both normal and water stress conditions. After two leaf stage of plants (after 7 days), drought was imposed by applying water in proportion of 100% means 100 ml in non-water stress (control), 50% means 50 ml (stress S<sub>1</sub>), 25% means 25 ml (stress S<sub>2</sub>) and 0% (stress S<sub>3</sub>) conditions till 30 days after sowing. After seed sowing at 15-20 days, the extra plants were rouged out except one to two healthy seedlings. The whole plant from bags were picked up by making vertical cut on bags, washed with tap water and data were observed for root length, shoot length, root fresh weight, shoot fresh weight, root dry weight, shoot dry weight, root shoot ratio by length, root shoot ratio by weight and relative leaf water content respectively.

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### Statistical Analysis

The replicated values were subjected to statistical analysis of variance (ANOVA) as prescribed by Panse and Sukhatme (1978) for individual characters for each environment.

Estimation of variance components, coefficient of variation, broad sense heritability, and genetic advance were evaluated separately for each character both in water stress and non-stress conditions.

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PCV and GCV were calculated in range of low (if it is less than 10 percent), moderate (10-20 percent) and high (If more than 20 percent) as classified by Shivasubramanian and Menon (1973).

The range (low- < 30%, medium-30%-60%, high > 60%) of heritability and genetic advance as percentage of mean range (low- < 10%, medium-10%-20%, and high > 20%) was calculated as suggested by Johnson *et al.*, (1955).

### Results and discussion

The increasing population, biotic and abiotic factors are threatening the global food security (Saha and Choyal, 2022). The sudden change in environmental factors further reduces the water availability and causes drought stress in major agro-systems especially rain fed ecosystem worldwide. Drought is single abiotic factor which affects severely the crop production and productivity including soybean. However, the effect of drought stress varies with the crop stage of the crop as well as with the local environmental conditions (Okunlola *et al.*, 2017). Drought affects the seed germination, vegetative and reproductive growth and maturity stage of a crop especially seedling and germination stage by reducing seedling length and seedling biomass, seedling water content, biochemical and molecular attributes depending upon the frequency and duration of drought stress (Anjum *et al.*, 2017).

Results of genetic variability under non-water stress and water stress conditions have been presented in following heads:

#### Genetic variability under non-water stress (normal water) condition:

Many researchers have reported variability in root traits among soybean cultivars. This variability was observed during early growth (Manavalan *et al.*, 2009; Fenta *et al.*, 2014; Thu *et al.*, 2014; Fried *et al.*, 2018; Falk *et al.*, 2020; Dayoub *et al.*, 2021; Syiem *et al.*, 2022), at flowering stage (Zhao *et al.*, 2004; Mwamlima *et al.*, 2019) or at maturity (Ao *et al.*, 2010).

#### Analysis of variance

The results from analysis of variance under net house condition (**Table-2**) in non stress condition (100 ml water) indicated that high amount of variability was present among the genotypes for all the characters investigated *viz.*, root fresh weight, shoot fresh weight, root dry weight, shoot dry weight, root shoot ratio by weight, root length, shoot length and root shoot ratio by length and relative leaf water content because significant difference were observed among sixty

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genotypes. Similar results under non-water stress condition were reported by Meena *et al.* (2014) in chickpea for plant height and relative leaf water content, Prince *et al.* (2015), Falk *et al.* (2020), Yan *et al.* (2020), Mishra *et al.* (2021), Dayoub *et al.* (2021), Syiem *et al.* (2022) in soybean; Kumar *et al.* (2023) for root length, shoot length and shoot dry weight in wheat; for RWC, Bayoumi *et al.* (2008) in wheat and Kumar *et al.* (2021) in chickpea; Shankar *et al.*, (2019) for plant height, root length, root shoot ratio by length and RWC in groundnut, Priya *et al.* (2021) and Reddy *et al.* (2023) for root length in lentil, green gram and black gram respectively. Presence of sufficient amount of variability in the studied germplasm provides ample scope for selection superior and desired genotypes.

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**Table 2: Analysis of variance (mean sum of squares) for nine characters in soybean under non-stress (normal water) condition**

Source of variation	DF	Root length (cm)	Shoot length (cm)	Root fresh weight (g)	Shoot fresh weight (g)	RWC %	Root dry weight (g)	Shoot dry weight (g)	Root shoot ratio by length	Root shoot ratio by weight
Replication	1	0.09	0.00	0.000	0.047	0.74	0.000	0.001	0.001	0.007
Treatment	59	16.28**	394.02**	0.017**	0.269**	835.44**	0.0002**	0.008**	0.034**	0.046**
Error	59	1.62	2.86	0.000	0.014	1.78	0.000	0.000	0.001	0.001
Total	119	8.88	196.77	0.009	0.141	415.10	0.000	0.004	0.017	0.023

\*\* Significant at 1% (P= 0.01) level of significance

\* Significant at 5% (P=0.05) level of significance

### Genetic variability parameters ...

The estimates of variability parameters for all the characters are shown in **Table 3** under net house for non-water stress condition. Phenotypic coefficient of variation for all traits had generally greater, but closer values to their corresponding genotypic coefficient of variation value presented which indicated presence of lower environmental influence on the expression of these traits. Similar results were also reported by Kumar *et al.* (2023) in wheat.

The root length was ranged 5.05 cm to 18.20 cm with grand mean of 10.42 cm. The estimated values of PCV and GCV recorded were 24.48 and 21.21 per cent, respectively. High heritability (bs) 75.05 per cent coupled with medium genetic advance over percentage of mean 37.85 per cent were noticed for this trait. Similarly, high (More than 20%) PCV and GCV, (>60%) high heritability and high genetic advance (>30%) was recorded by Gobu *et al.* (2017) in eggplant, Kumar *et al.* (2023) in wheat; Shankar *et al.*, 2019 in groundnut and Hoque *et al.* (2021) in rice and Syiem *et al.* (2022) in soybean under normal water condition. High heritability for this trait was also reported by Reddy *et al.* (2023) in green gram and black gram.

A wide significant range of variation was found among all the germplasm accessions for shoot length. It varied from 12.20 cm to 79.45 cm with an overall mean of 46.94 cm. The recorded PCV and GCV for this character were 24.59 and 24.32 per cent, respectively. Similar results of high PCV and GCV were reported by Bayoumi *et al.* (2008) in wheat. This character revealed high  $h^2$  (97.85 per cent) along with high GA (49.57 per cent). Similar result of high heritability for this trait was also reported by Riaz *et al.* (2013) in cotton; Meena *et al.*, (2014) in chickpea. Similarly, high (10-30%) PCV and GCV, (>60%) high heritability and high genetic advance (>30%) was recorded by Gobu *et al.* (2017) in eggplant, Shankar *et al.*, 2019 in groundnut, Hoque *et al.* (2021) in rice and Syiem *et al.* (2022) in soybean under normal water condition.

The root fresh weight was ranged 0.042 gm to 0.389 gm with total mean of 0.199 gm. The phenotypic coefficient of variation (38.53) and genotypic coefficient of variation (36.95), heritability (91.93 per cent) and genetic advance over percentage of mean (72.98 per cent) respectively were found for this trait. Result of high GCV, PCV, heritability and high GA as percentage of mean was also noticed by Syiem *et al.* (2022) in soybean for root fresh weight under

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non-water stress condition. Similar result of high heritability for this trait was also recorded by Riaz *et al.* (2013) in cotton.

A wide range of variation was found among all the germplasm accessions for shoot fresh weight. This trait varied from 0.041 to 1.592 gm with an overall mean of 0.807 gm. The PCV (39.00), GCV (36.15), heritability ( $h^2$ ) (85.95%) and genetic advance over mean of (69.05%) were recorded respectively for shoot fresh weight. Similar findings for soybean for shoot fresh weight under seedling stage were also reported by Syiem *et al.* (2022) in soybean in normal water. Similar result of high heritability for this trait was also found by Riaz *et al.* (2013) in cotton.

The physiological character, relative leaf water content varied from 18.765 to 93.150% with an overall mean of 62.851%. The PCV (26.61), GCV (26.52), heritability ( $h^2$ ) (99.36%) and genetic advance over mean of percentage (54.46%) were observed respectively for this parameter. Similar high heritability results for relative leaf water content were also obtained by Bayoumi *et al.* (2008) in wheat, Garg *et al.* (2017) in rice and Kumar *et al.* (2021) in chickpea.

The root dry weight ranged from 0.009 to 0.079 with an overall mean of 0.025 gm. Recorded values of PCV and GCV were 39.41 and 39.41 per cent respectively. The values of high heritability (100.00%) together with high genetic advance as per cent mean (81.19) were recorded for this trait. Similar result of high heritability for this trait was also found by Riaz *et al.* (2013) in cotton; Lalitha *et al.* (2015) in chickpea.

The grand mean of shoot dry weight was 0.130 with a range of 0.0250 to 0.300. High phenotypic (40.10%) and genotypic coefficient of variability (39.72%) along with heritability (98.13%) and genetic advance as per cent mean (81.05) were recorded for this trait. Similar high (>30%) PCV and GCV, (>60%) high heritability and high genetic advance (>30%) was recorded by Kumar *et al.* (2023) in wheat. High heritability by Lalitha *et al.* (2015) in chickpea

Character, root shot ratio by length ranged from 0.101 to 1.023 with a grand mean value of 0.246. The PCV and GCV observed were 44.60 and 42.45 per cent, respectively. Heritability (bs) 90.59 per cent coupled with high genetic advance over percentage of mean 83.23 per cent were noticed for this trait. Similarly high PCV, GCV, heritability and genetic advance of root shot ratio by length were observed by Gobu *et al.* (2017) in eggplant, Shankar *et al.* (2019) in groundnut and Hoque *et al.* (2021) in rice under normal water condition.

A perusable mean values of different genotypes of trait root shoot ratio by weight revealed that overall mean value of it was 0.234. High PCV (53.60) and high GCV (52.20) were recorded for this parameter. The high heritability (94.85%) coupled with high genetic advance (104.73%) were noticed for it.

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**Table 3: Determination of mean, range and genetic variability parameters for nine traits in soybean under non-stress (normal water) condition**

Genotypes	Mean	Min	Max	var (g)	var (p)	Heritability (%)	GA	GA% mean	GCV (%)	PCV (%)
Root length (cm)	10.424	5.050	18.200	4.89	6.51	75.05	3.95	37.85	21.21	24.48
Shoot length (cm)	46.943	12.200	79.450	130.39	133.24	97.85	23.27	49.57	24.32	24.59
Root fresh weight (g)	0.199	0.042	0.389	0.005	0.006	91.93	0.15	72.98	36.95	38.53
Shoot fresh weight (g)	0.807	0.041	1.592	0.085	0.099	85.95	0.56	69.05	36.15	39.00
Rwc %	62.851	18.765	93.150	277.89	279.67	99.36	34.23	54.46	26.52	26.61
Root dry weight (g)	0.025	0.009	0.079	0.0001	0.0001	100.00	0.020	81.19	39.41	39.41
Shoot dry weight (g)	0.130	0.025	0.300	0.0027	0.0027	98.13	0.105	81.05	39.72	40.10
Root shoot ratio by length	0.246	0.101	1.023	0.0109	0.0121	90.59	0.205	83.23	42.45	44.60
Root shoot ratio by weight	0.234	0.043	0.756	0.0150	0.0158	94.85	0.245	104.73	52.20	53.60

\*\* Significant at 1% (P= 0.01) level of significance

\* Significant at 5% (P=0.05) level of significance

### Genetic variability under drought conditions

Genetic variability for drought tolerance has been reported by many researchers in different crops like (Thu *et al.*, 2014; Falk *et al.*, 2020; Yan *et al.*, 2020; Mishra *et al.*, 2021; Dayoub *et al.*, 2021; Bui *et al.*, 2022 in soybean), (Raina *et al.*, 2019; Amarapali 2022 in green gram), (Widuri *et al.*, 2018; Langat *et al.*, 2020 in common beans), (Upadhyay, 2005; Songsri *et al.* 2009 and Painwadee *et al.*, 2009 in pea), (Ali *et al.*, 2010 in chickpea) (Thakur *et al.*, 2013; Vaidya *et al.*, 2015; Shankar *et al.*, 2016; Barathi *et al.*, 2022 in groundnut), (Anita Kumari *et al.*, 2019; Aneja *et al.*, 2021 in mustard), (Praveen *et al.*, 2021 in sunflower) (Rajarajan *et al.* 2018 in sorghum), (Dutta and Borua, 2017; Hoque *et al.* 2020 in rice), (Li *et al.*, 2015 in maize), (Narayanan *et al.*, 2014; Fernandes *et al.*, 2020 in wheat) (Handi and Katageri, 2016 in cotton). Genetic variability is essential to know response to selection pressure. It has also been reported that the magnitude of genetic variability present in base population of any crop species is important in crop improvement and must be exploited by plant breeder for yield improvement (Akram *et al.*, 2011; Mehra *et al.*, 2020).

### Analysis of variance

Mean sum of squares of accessions in water stress condition S<sub>1</sub>(50% water imposition), S<sub>2</sub> (25% water imposition) and S<sub>3</sub> (0% water imposition) indicated significant difference among all the genotypes for all the nine traits showed good deal of variability in the material used (**Table 4,6 and 8**). Similarly significant differences for trait RWC was reported by Bayoumi *et al.* (2008) in wheat, Kanvi *et al.* (2020) in green gram and Ajayi (2022) in cow pea; Shankar *et al.*, (2019) for plant height, root length, root shoot ratio by length, root fresh weigh, shoot fresh weight, root dry weight, shoot dry weight and RWC in groundnut; Langat *et al.* (2019) for plant height in common beans (*Phaseolus vulgaris* L.); Priya *et al.* (2021) for root length in lentil and Wattoo *et al.* (2018) for relative leaf water content, root length, shoot length, root fresh weight, shoot fresh weight, root dry weight and shoot dry weight in maize.

**Table 4: Analysis of variance (mean sum of squares) for nine characters in soybean under S<sub>1</sub> (50% water) stress condition**

Source of variation	DF	Root length (cm)	Shoot length (cm)	Root fresh weight (g)	Shoot fresh weight (g)	RWC %	Root dry weight (g)	Shoot dry weight (g)	Root shoot ratio by length	Root shoot ratio by weight
Replication	1	0.20	0.71	0.001	0.002	7.87	0.000	0.001	0.000	0.000
Treatment	59	10.40**	279.82**	0.046**	0.196**	719.33**	0.0001**	0.005**	0.015**	0.047**
Error	59	0.74	4.11	0.001	0.013	8.36	0.000	0.000	0.001	0.001
Total	119	5.52	140.78	0.023	0.104	360.85	0.000	0.003	0.008	0.024

\*\* Significant at 1% (P= 0.01) level of significance

\* Significant at 5% (P=0.05) level of significance

**Table 5: Estimation of mean, range and genetic variability parameters for nine quantitative characters in Soybean under S<sub>1</sub> (50% water) stress condition**

Genotypes	Mean	Min	Max	var (g)	var (p)	Heritability (%)	GA	GA% mean	GCV (%)	PCV (%)
Root length (cm)	9.587	4.550	16.100	3.22	3.96	81.40	3.34	34.80	18.72	20.75
Shoot length (cm)	47.468	15.050	74.000	91.90	96.01	95.72	19.32	40.70	20.20	20.64
Root fresh weight (g)	0.198	0.044	0.995	0.015	0.016	95.15	0.25	123.88	61.65	63.20
Shoot fresh weight (g)	0.769	0.072	1.727	0.061	0.074	82.81	0.46	60.32	32.18	35.36
Rwc %	63.320	20.555	93.265	236.99	245.35	96.59	31.17	49.22	24.31	24.74
Root dry weight (g)	0.021	0.006	0.044	0.0001	0.0001	76.92	0.014	64.72	35.82	40.84
Shoot dry weight (g)	0.123	0.041	0.298	0.0018	0.0018	100.00	0.087	70.55	34.25	34.25
Root shoot ratio by length	0.217	0.101	0.641	0.0049	0.0055	90.08	0.137	63.20	32.32	34.06
Root shoot ratio by weight	0.205	0.032	0.942	0.0153	0.0166	92.63	0.246	119.97	60.51	62.87

**Table 6: Analysis of variance (mean sum of squares) for nine characters in Soybean under S<sub>2</sub> (25% water) stress condition**

Source of variation	DF	Root length (cm)	Shoot length (cm)	Root fresh weight (g)	Shoot fresh weight (g)	RWC %	Root dry weight (g)	Shoot dry weight (g)	Root shoot ratio by length	Root shoot ratio by weight
Replication	1	0.01	2.22	0.001	0.003	0.10	0.000	0.001	0.000	0.007
Treatment	59	13.03**	264.65**	0.030**	0.283**	657.89**	0.001**	0.008**	0.015**	0.118**
Error	59	0.69	3.61	0.001	0.014	1.86	0.000	0.000	0.000	0.001
Total	119	6.80	133.03	0.015	0.147	327.11	0.000	0.004	0.008	0.059

\*\* Significant at 1% (P= 0.01) level of significance

\* Significant at 5% (P=0.05) level of significance

**Table 7: Estimation of mean, range and genetic variability parameters for nine quantitative characters in Soybean under stress S<sub>2</sub> (25% watering) condition**

Genotypes	Mean	Min	Max	var (g)	var (p)	Heritability (%)	GA	GA% mean	GCV (%)	PCV (%)
Root length (cm)	9.546	3.750	16.450	4.12	4.80	85.69	3.87	40.52	21.25	22.96
Shoot length (cm)	46.749	17.900	73.250	87.01	90.63	96.01	18.83	40.28	19.95	20.36
Root fresh weight (g)	0.191	0.011	0.885	0.010	0.010	94.36	0.20	103.04	51.49	53.01
Shoot fresh weight (g)	0.757	0.104	1.739	0.090	0.104	86.22	0.57	75.66	39.55	42.60
Rwc %	64.274	26.435	92.115	218.68	220.54	99.16	30.33	47.19	23.01	23.11
Root dry weight (g)	0.025	0.006	0.084	0.0002	0.0002	100.00	0.026	107.55	52.21	52.21
Shoot dry weight (g)	0.123	0.032	0.319	0.0027	0.0027	100.00	0.107	87.33	42.39	42.39
Root shoot ratio by length	0.218	0.095	0.471	0.0048	0.0052	91.21	0.136	62.29	31.66	33.15
Root shoot ratio by weight	0.259	0.030	1.635	0.0389	0.0398	97.74	0.402	154.97	76.09	76.96

**Table 8: Analysis of variance (mean sum of squares) for nine characters in Soybean under S<sub>3</sub> (0% water) stress condition**

Source of variation	DF	Root length (cm)	Shoot length (cm)	Root fresh weight (g)	Shoot fresh weight (g)	RWC %	Root dry weight (g)	Shoot dry weight (g)	Root shoot ratio by length	Root shoot ratio by weight
Replication	1	0.03	4.73	0.000	0.004	5.40	0.000	0.002	0.000	0.007

Treatment	59	27.15**	411.19**	0.026**	0.497**	756.20**	0.001**	0.013**	0.028**	0.065**
Error	59	0.70	5.91	0.000	0.012	1.87	0.000	0.000	0.000	0.001
Total	119	13.81	206.84	0.013	0.252	375.90	0.000	0.007	0.014	0.033

\*\* Significant at 1% (P= 0.01) level of significance

\* Significant at 5% (P=0.05) level of significance

UNDER PEER REVIEW

## Genetic variability parameters

Genetic variability for drought tolerance has been reported by many researchers in different crops like in soybean (Dayob *et al.*, 2021), (Raina *et al.*, 2019; Amarapali 2022 in green gram), (Widuri *et al.*, 2018; Langat *et al.*, 2020 in common beans), (Upadhyay, 2005; Songsri *et al.* 2009 and Painwadee *et al.*, 2009 in pea), (Ali *et al.*, 2010 in chickpea) (Thakur *et al.*, 2013; Vaidya *et al.*, 2015; Shankar *et al.*, 2016; Barathi *et al.*, 2022 in groundnut), (Anita Kumari *et al.*, 2019; Aneja *et al.*, 2021 in mustard), (Praveen *et al.*, 2021 in sunflower) (Rajarajan *et al.* 2018 in sorghum), (Dutta and Borua, 2017; Hoque *et al.* 2020 in rice), (Li *et al.*, 2015 in maize), (Narayanan *et al.*, 2014; Fernandes *et al.*, 2020 in wheat) (Handi and Katageri, 2016 in cotton). Genetic variability is essential to know response to selection pressure. It has also been reported that the magnitude of genetic variability present in base population of any crop species is important in crop improvement and must be exploited by plant breeder for yield improvement (Akram *et al.*, 2011; Mehra *et al.*, 2020).

The use of morpho-physiological traits in relation to drought tolerance has been suggested by many researchers (Painwadee *et al.* 2009) because the inheritance of these characters is simpler than pod yield. The information related to heritability of characters is important for plant breeders to formulate appropriate breeding strategies to achieve breeding objectives.

The estimates of variability parameters of all genotypes for root shoot characters under water stress have been presented in **Table 5, 7 and 9**.

The root length showed significant good amount of genetic variation under stress conditions among the genotypes used for investigation. The biotic and abiotic factors affect plant growth. Among these, the scarcity of water great impact on root distribution system. A dry soil surface with high water stress often forces roots to increase their growth deep into the soil profile where water is more available (Adiku *et al.*, 1996, Amarapali, 2022). The deep and dense root system show better drought tolerance by extracting water from deeper soil layers (Parameshwarappa *et al.*, 2012). Under stress **S<sub>1</sub> (50% water)**, the range of mean was 4.55 cm to 16.10 cm with overall mean of 9.59 cm. **In stress condition S<sub>2</sub> (25% water)** the range of root length variability was observed from 3.75 cm to 16.45 cm with the grand mean of 9.55 cm. **Under stress condition S<sub>3</sub> (0% water)** highest root length was calculated by genotype NRC138 (20.50 cm) whereas lowest was recorded by GW312 (3.40 cm) with the overall mean of 0.41 cm. It indicates that germplasm lines are genetically variable. Similar increasing range of variation under water stress in comparison to normal condition was also noticed by Dayoub *et al.* (2021) in soybean; Raina *et al.* (2019) in mung bean. PCV and GCV recorded were (20.75% and 18.72%) under stress **S<sub>1</sub>**

(50% water); (22.96 and 21.25 per cent) under stress condition S<sub>2</sub> (25% water) and (22.96 and 21.25 per cent) under stress condition S<sub>2</sub> (25% water) respectively. Similar high variability was reported by Manickvelu *et al.* (2006), Dutta and Borua (2017), Sallleh *et al.* (2021) in rice; Rajkumar and Fakrudin (2018) in sorghum, Shankar *et al.*, (2019) in groundnut, Bayoumi *et al.* (2008) in wheat, Mishra and Sharma, (2015) in muskmelon, Wattoo *et al.* (2019) in maize. High heritability along with high genetic advance as percentage of mean was observed (81.40% and 34.80%) under stress S<sub>1</sub> (50% water); (85.69% and 40.52%) in stress condition S<sub>2</sub> (25% water) and (92.60% and 56.52%) in stress condition S<sub>3</sub> (0% water) respectively. High heritability coupled with high genetic advance represent that character root length is governed by additive gene action. It is **indicating that simple selection would be effective for this trait under stress condition**. Similar result of high heritability and high genetic advance for this trait was also observed by Irum *et al.*, (2011) and Riaz *et al.* (2013) in cotton, Dutta and Borua (2017) in rice, Gobu *et al.* (2017) in eggplant, Rajkumar and Fakrudin (2018) in sorghum, Shankar *et al.* (2019) in groundnut, Mishra and Sharma, (2015) in muskmelon, Gedam *et al.* (2021) in onion and **Kumar *et al.* (2023) in wheat under stress condition**. Bayoumi *et al.* (2008) in wheat, Wattoo *et al.* (2019) in maize and Pavitra *et al.* (2022) in rice for high heritability.

A wide variation was found among the germplasm accessions for shoot length. This character varied from 15.05 cm to 74.00 cm with an overall mean of 47.47 cm; 17.90 cm to 73.25 cm with the overall mean of 46.74 cm and 14.10 to 90.50 cm with a grand mean of 49.36 cm in stress conditions S<sub>1</sub> (50% water), S<sub>2</sub> (25% water) and S<sub>3</sub> (0% water) respectively. The PCV and GCV were 20.64 and 20.20 per cent; 20.36 and 19.95 per cent; 24.06 and 23.55 per cent respectively in stress conditions S<sub>1</sub> (50% water), S<sub>2</sub> (25% water) and S<sub>3</sub> (0% water). Similarly, high (More than 20%) PCV and GCV, was recorded by Mishra *et al.*, (2015) in muskmelon, Bayoumi *et al.* (2008), Ashfaq *et al.* (2022) and Kumar *et al.* (2023) in wheat. The estimates of h<sup>2</sup> (95.72%, 96.01% and 95.81 per cent) together with an expected GA over mean of (40.70%, 40.28% and 47.48 per cent) were recorded for this character in stress conditions S<sub>1</sub> (50% water), S<sub>2</sub> (25% water) and S<sub>3</sub> (0% water) respectively. Similar results of high heritability for this trait was also reported Meena *et al.* (2014) in chickpea, Mishra and Sharma, (2015) in muskmelon, Gobu *et al.* (2017) in eggplant, Shankar *et al.* (2019) in groundnut, Wattoo *et al.* (2019) in maize, Gedam *et al.* (2021) in onion; for high h<sup>2</sup> and high GA as percentage of mean by Riaz *et al.* (2013) in cotton and by Kumar *et al.* (2023) in wheat for this trait.

High PCV, GCV, heritability and genetic advance as percentage of mean was observed for root fresh weight under stress condition S<sub>1</sub>, S<sub>2</sub> and S<sub>3</sub> respectively. Root biomass and root length that aid in greater soil moisture extraction were identified as important root traits during terminal drought (Kashiwagi *et al.*, 2006; Varshney *et al.*, 2011). In stress conditions S<sub>1</sub> (50% water) the range of genetic variability for the trait root fresh

weight was found from 0.04 gm to 0.10 gm with the overall mean (0.20 gm), **in stress condition S<sub>2</sub> (25% water)** the grand mean of root fresh weight was 0.19 gm whereas highest root fresh weight was depicted by genotype GW312 (0.885 gm) and lowest by genotype GW212 (0.011 gm), **in stress condition S<sub>3</sub> (0% water)** the overall mean of it was 0.19 gm while highest root fresh weight was produced by genotype GW312 (0.79 gm) and lowest by GW212 (0.01 gm). The observed values of PCV, GCV, heritability and GA over percentage of mean were (63.20, 61.65 per cent, 95.15% and 123.28%); (53.01 and 51.49 per cent, 94.36% and 103.04%); (50.34, 49.06 per cent, 94.99% and 98.50%) under stress conditions **S<sub>1</sub>, S<sub>2</sub>** and **S<sub>3</sub>** respectively. The observed values of PCV, GCV, heritability and GA over percentage of mean were according to findings of by Riaz *et al.* (2013) in cotton and Rajkumar and Fakrudin (2018) in sorghum. Similar result of high PCV, GCV and heritability for this trait was also recorded by Wattoo *et al.* (2019) in maize. Similar result of high heritability for this trait was also recorded by Shankar *et al.* (2019) in groundnut.

A wide variation was observed among the germplasm accessions for shoot fresh weight. The character shoot fresh weight varied from (0.072 to 1.73 gm; 0.10 to 1.74 gm; 0.13 to 2.35 gm) with an overall mean of (0.77 gm; 0.76 gm; 0.85 gm) under **S<sub>1</sub>, S<sub>2</sub>** and **S<sub>3</sub>** respectively. The PCV, GCV,  $h^2$  together with an expected GA over mean under **S<sub>1</sub>, S<sub>2</sub>** and **S<sub>3</sub>** were (35.36%, 32.18%, 82.81% and 60.08%); (42.60%, 39.55%, 86.22% and 75.66 %); (48.82%, 47. %, 86.22 % and 75.66 %) respectively. Similar result of high PCV and GCV and heritability for this trait was also found by Wattoo *et al.* (2019) in maize; for high heritability by Irum *et al.* (2011), Riaz *et al.* (2013), Handi and Katageri (2016) in cotton; for high heritability and high genetic advance by Shankar *et al.* (2019) in groundnut.

Relative water content is the appropriate measure of plant water status in terms of the physiological consequence of cellular water deficit. In case of absorption of water by the plant by roots from soil, water potential is useful in dealing with water transport in the soil-plant-atmosphere continuum but does not account for osmotic adjustment (OA). Osmotic adjustment (OA) mechanism is responsible for conserving cellular hydration under drought stress and RWC expresses the effect of osmotic adjustment (OA) in this respect. Therefore, RWC analysis is an appropriate estimate of plant water status in terms of cellular hydration under the possible effect of both leaf water potential and OA. The method is simple and measure of water deficit in the leaf. RWC value generally range between 98% in turgid and transpiring leaves to about 40% in severely desiccated and dying leaves. In most crop species the typical RWC at about wilting is around 60% to 70%, with exceptions. A wide range of variation in **S<sub>1</sub>, S<sub>2</sub>** and **S<sub>3</sub>** stress (20.55 to 93.26%; 26.43 to 92.11%; 20.06% to 95.84%) was found among all the genotypes for relative leaf water content and the overall mean of physiological character was 63.32%, 64.27% and 63.18%. The PCV (24.74%, 23.11% and 25.19%), GCV (24.31, 23.01 and 25.10%), heritability ( $h^2$ ) (96.59%, 99.16% and

99.26%) and genetic advance over mean of percentage (49.22%, 47.19% and 51.51%) were observed respectively for this parameter under all the three water stress conditions (50%, 25% and 0%). Similar results were also reported by Manickvelu *et al.* (2006) in rice. High heritability for this parameter was also reported by Meena *et al.* (2014) in chickpea, Garg *et al.* (2017) in rice, Wattoo *et al.* (2019) in maize, Shankar *et al.* (2019) in groundnut and Gedam *et al.* (2021) in onion.

Data of variability for root dry weight under drought has been presented in Table 5, 7 and 9. Tolerance indices might be useful to screen the genotypes as dry weight of a plant at particular age is universally considered as more stable character than other morphological parameters (Dutta and Bera, 2008; Vijay *et al.* 2018). The PCV and GCV obtained for root dry weight were (40.84 and 35.82 per cent; 52.21% and 52.21%; 65.23% and 62.99%) respectively. The values of heritability (76.92%, 100.00% and 93.24%) along with high genetic advance as per cent mean (64.72%, 107.55% and 125.29%) were observed for this trait under water stress conditions S<sub>1</sub>, S<sub>2</sub> and S<sub>3</sub> respectively. High PCV, GCV, heritability and genetic advance were also reported for this trait by Riaz *et al.* (2013) in cotton and Rajkumar and Frakudin (2018) in sorghum; for high PCV, GCV and heritability by Wattoo *et al.* (2018) in maize. The result of high heritability for this trait was according to Irum *et al.* (2011), Handi and Katageri (2016) in cotton; Lalitha *et al.* (2015) in chickpea. Gurumurthy *et al.* (2019) also observed high heritability for this trait in black gram.

The results of shoot dry weight indicated that out of 60 genotypes, 30 genotypes showed high values in comparison to the grand mean (0.12gm). High phenotypic and genotypic coefficient of variability along with high heritability and genetic advance as per cent mean recorded were (34.25%, 42.39% and 46.87%), (34.25%, 42.39% and 46.52%), (100.00%, 100.00% and 98.50%) and (70.55%, 87.33% and 95.10%) respectively. Similar findings of high PCV, GCV, high  $h^2$  along with high genetic advance as percentage of mean was reported by Kumar *et al.* (2023) in wheat. Similar result of high heritability and high GA as percentage of mean for this trait was also found by Irum *et al.* (2011), Riaz *et al.* (2013), Handi and Katageri (2016) in cotton, Lalitha *et al.* (2015) in chickpea, Langat *et al.* (2019) in common beans (*Phaseolus vulgaris* L.), Gurumurthy *et al.* (2019) in black gram, Shankar *et al.* (2019) in groundnut. High PCV and GCV for this trait were also reported by Doumbia *et al.* (2022) in cowpea.

Perusal data of root shot ratio by length indicated the range of genetic variability (0.10-0.64), (0.10 to 0.47) and (0.01-0.91) with grand mean of (0.22, 0.22 and 0.23). The PCV and GCV observed were (34.06, 33.15, 43.88) and (32.32, 31.6642.44) per cent, respectively while high heritability (bs) of (90.08 per cent, 91.21 per cent, 96.14 per cent) coupled with high genetic advance over percentage of mean (63.20 per cent, 62.29 per cent,

85.71 per cent) were noticed under water stress conditions **S<sub>1</sub>**, **S<sub>2</sub>** and **S<sub>3</sub>** respectively. Similarly high PCV and GCV, heritability and genetic advance of root shoot ratio by length were recorded by Mishra and Sharma, (2015) in muskmelon, Gobu *et al.* (2017) in eggplant, Rajkumar and Frakudin (2018) in sorghum and Shankar *et al.* (2019) in groundnut.

The character root shoot ratio by weight expressed ranged from 0.03 to 0.94, 0.03 to 1.64 and 0.02 to 0.75 under all the three water stress conditions (**S<sub>1</sub>**, **S<sub>2</sub>** and **S<sub>3</sub>**). The grand mean of root shoot ratio in weight was 1.39, 0.26 and 0.23. The estimates of PCV (62.87%, 76.96 % and 65.60%), GCV (60.51%, 76.96% and 64.00%),  $h^2$  (92.63%, 97.74% and 95.17%) and G.A. (119.97%, 154.97% and 128.62%) respectively were recorded for this trait under all the three water regimes (**S<sub>1</sub>**, **S<sub>2</sub>** and **S<sub>3</sub>**) created for drought. Similar results of high GCV, heritability and high GA as percentage of mean was reported by Handi and Katageri (2016) in cotton.

High heritability along with high genetic advance as percentage of mean was recorded for most of the traits under stress condition. High heritability along with high genetic advance as percentage of mean indicating that simple selection would be effective for these traits under water stress conditions.

**Commented [SB10]:** Avoid repeating data points and phrases. For example, the phrase "high heritability" is mentioned multiple times in quick succession. Summarize where appropriate.

**Commented [SB11]:** Where's The conclusion with briefly summarized main findings and their implications for future research....also It should not merely reiterate the results. Suggest directions for future research or how these findings could be applied in practical breeding programs.

**Table 9: Estimation of mean, range and genetic variability parameters for nine quantitative characters in Soybean under stress S<sub>3</sub> (0% watering) condition**

Genotypes	Mean	Min	Max	var (g)	var (p)	Heritability (%)	GA	GA% mean	GCV (%)	PCV (%)
Root length (cm)	10.41	3.40	20.50	8.82	9.52	92.60	5.89	56.52	28.51	29.63
Shoot length (cm)	49.36	14.10	90.50	135.09	141.01	95.81	23.44	47.48	23.55	24.06
Root fresh weight (g)	0.186	0.013	0.789	0.008	0.009	94.99	0.18	98.50	49.06	50.34
Shoot fresh weight (g)	0.854	0.125	2.348	0.162	0.174	93.05	0.80	93.57	47.09	48.82
Rwc %	63.18	20.06	95.84	251.45	253.31	99.26	32.54	51.51	25.10	25.19
Root dry weight (g)	0.026	0.004	0.135	0.0003	0.0003	93.24	0.033	125.29	62.99	65.23
Shoot dry weight (g)	0.144	0.031	0.378	0.0045	0.0045	98.50	0.137	95.10	46.52	46.87
Root shoot ratio by length	0.227	0.093	0.911	0.0093	0.0097	96.14	0.195	85.71	42.44	43.28
Root shoot ratio by weight	0.229	0.023	0.750	0.0214	0.0225	95.17	0.294	128.62	64.00	65.60

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