

## Drought Susceptibility Index; a Preferred Criterion in Screening for Tolerance in Soybean

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## Cover Page Footnote

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## DROUGHT SUSCEPTIBILITY INDEX; A PREFERRED CRITERION IN SCREENING FOR TOLERANCE IN SOYBEAN

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### ABSTRACT

Soybean (*Glycine max* L.) yield and yield related traits are constrained by drought. Adaptation of soybean to changing environment could be improved by exploitation and introgression of diverse germplasm in breeding program. In present study, the response to drought conditions, especially at flowering stage, was evaluated to determine the potential soybean germplasm for future soybean breeding programs in Pakistan. Field experiment was conducted under two water regimes i.e. well-water and water-limited, to assess the effect of drought in seed yield and yield related traits. Although, drought led to overall reduction of ~15 % in thousand seed weight but still some soybean genotypes performed relatively better under water-limited conditions. These genotypes were also tolerant to drought, with a drought susceptibility index of < 0.5. PCA also explained the pattern of variation existing in soybean germplasm grown under given water regimes i.e. well-water and water-limited conditions. The identified soybean genotypes could be a favorable resource to introduce high yielding soybean in local environment.

**Keywords:** Drought, screening, soybean, principal component analysis.

### INTRODUCTION

Soybean (*Glycine max* L.), is becoming an important oilseed crop for Asia Pacific region because of its nutritional and health benefits (Foyer et al., 2016). Advantages derived from soybean cultivation is not just restricted as a food commodity; it is also a vital industrial crop used in the production of fibre, wax, dyes, paints, edible oil and also as a meat substitutes (Rezaei et al., 2002; Tang, 2017). Soybean yield and yield contributing traits are constrained by drought (Manavalan et al., 2009). Drought can have drastic effects on soybean growth and development, particularly in semi- and arid zones of the world (Sinclair et al., 2014, 2020). Several reports documented significant reduction in soybean yield

under drought conditions (Specht et al., 1999; Yan et al., 2020; Arya et al., 2021;). Effects of drought on soybean depend upon several factors such as timing and duration of stress. Flowering time and the period subsequent to flowering are the most crucial stages for drought stress in soybean (Yan et al., 2020). Desclaux et al., (2000) studied the drought induced phenomics of early maturity soybean cultivars and reported that drought stress even at the vegetative stage led to reduced plant height, seed weight (late reproductive stages) and seed numbers (early reproductive stages). Significant reduction (73-82 %) in soybean yield (under drought stress conditions) has been reported in semi humid and semi-arid zones of Huaibei, China (Wei et al., 2018). Long

term drought during flowering stages reduces biomass allocation to reproductive tissues, hence reducing seed weight (Du et al., 2020).

Breeding for yield and yield contributing traits is the utmost objective of any crop improvement program. Hence, yield and its components have extensively been practiced as drought tolerance indicators (Pinto et al., 2010). Since trait evaluation is expensive in terms of resource and time, therefore heat and/or drought susceptible indices have been used to discriminate between tolerant and intolerant genotypes. Efforts have been made in breeding soybean with primary objective of improving the performance of yield contributing traits under drought (Gao et al., 2020). Although, conventional breeding efforts have resulted in the development of improved soybean cultivars but still there is a need to add additive genetic variance in soybean germplasm to ensure fast forward genetic gain.

In the current climate-change scenario, water scarcity and heat stress episodes are casting a considerable threat for human food. Exploitation and usage of diverse crop genetic resources is suggested as vital soybean breeding approach to cope with climate-change scenario. The present work was performed to compare field based performance of introduced and indigenous soybean germplasm under drought conditions.

## MATERIALS AND METHODS

Soybean germplasm was collected from national and international germplasm resource centers with the aim of strengthening indigenous soybean germplasm for future breeding purposes. The collected soybean genotypes were planted at the research farm of MNS University of Agriculture, Multan in spring 2021 under two water regimes i.e. “well-water” and “water-limited” conditions using augmented design

(UAM-SB-200 check-accession). Soybean field was prepared by laser leveling followed by standard agronomic practices. Briefly, one bag of urea  $\text{acer}^{-1}$  and one bag of Di-ammonium Phosphate (DAP)  $\text{acer}^{-1}$  were applied before sowing. Sowing (2-3 seeds per planting site) was done beds having  $15 \times 2.5$  ft (Length  $\times$  Width) dimensions. Plant to plant distance was maintained at 1 ft. The “well-water” experimental units were irrigated after an interval of  $\sim 14$  days while “water-limited” experimental units were subjected to drought condition especially at flowering stage. After thirty days of sowing, plant thinning was performed to eradicate unhealthy plants. Weeds were controlled manually. Phenotypic trait data was obtained for number of pods per plant, pod length, number of seeds per pod, plant height, seed weight per plant, thousand seed weight. Seed width and seed thickness were measured through Document Scanner S500A3B (Shenzhen Eloam Technology, China.) from both water regimes. Plant height (PH), was recorded at plant’s physiological maturity as the distance from soil level to topmost part of each plant. Thousand seed weight (TSW) was measured by weighing duplicate samples of  $\sim 500$  seeds from each soybean genotype.

The drought susceptibility index for TSW ( $DSI_{TSW}$ ) was computed using the formula:  $DSI_{TSW} = (1 - Y/Y_p)/D$  where  $Y$  is the TSW of a soybean genotype grown under water-limited condition,  $Y_p$  is the mean of TSW of soybean genotypes grown under well-water conditions, and  $D$  (stress intensity)  $= 1 - X/X_p$ ,  $X$  is the average  $Y$  of all soybean genotypes and  $X_p$  is the mean  $Y_p$  of all soybean genotypes. The soybean genotypes were categorized as intolerant ( $DSI_{TSW} > 1$ ) and tolerant ( $DSI_{TSW} \leq 0.5$ ) to drought (Fischer & Maurer, 1978; Khanna-Chopra & Viswanathan, 1999).

## **Statistical Analyses**

Analysis of variance (ANOVA) for augmented block design was applied using R Studio 1.4.1717. ANOVA with least significant difference (post hoc) approach was applied to validate differences between the means. The relationship among studied parameters were computed using Pearson's correlation tests using XLSTAT 2014 (Singh et al., 2020). On the basis of correlation matrix approach, principal component analyses (PCA) was computed using XLSTAT 2014 (Vidal et al., 2020). In PCA, all soybean genotypes were regarded as cases against phenotypic attributes/traits averaged throughout the field trials as variables.

## **RESULTS**

### ***Analyses of Variance and Mean Performance of Soybean Genotypes***

ANOVA was computed for all studied traits. Significant variations were observed for the yield contributing traits under both water regimes (Table 1). Thousand seed weight, seed length, seed width and seed thickness were reduced by 15, 16.1, 17.3 and 2 % in water-limited conditions compared to well-water conditions, respectively. Thousand seed weight was reduced with an average of 127.54 (g) in well-water and 108.36 (g) in water-limited. Seed length was reduced with an average of 0.631(mm) in well-water and 0.529 in water-limited. Seed width was also reduced with an average of 0.640 (mm) in the well-water and 0.528 in water-limited conditions. In case of seed thickness, the averaged thickness was recorded 0.888 (mm) under well-water and 0.871 under water-limited conditions (Figure 1). Number of seeds per pod and pod length showed non-significant difference for soybean genotype and water treatment (Figure 1).

Yield stability was assessed according to thousand seed weight ranking under both water regimes. The agronomic

performances of the highest thousand seed weight (g) are presented in Table 1. All these soybean accessions showed tolerance ( $DSI_{Tsw} > 0.5$ ) to drought on the basis of thousand seed weight in both environments.

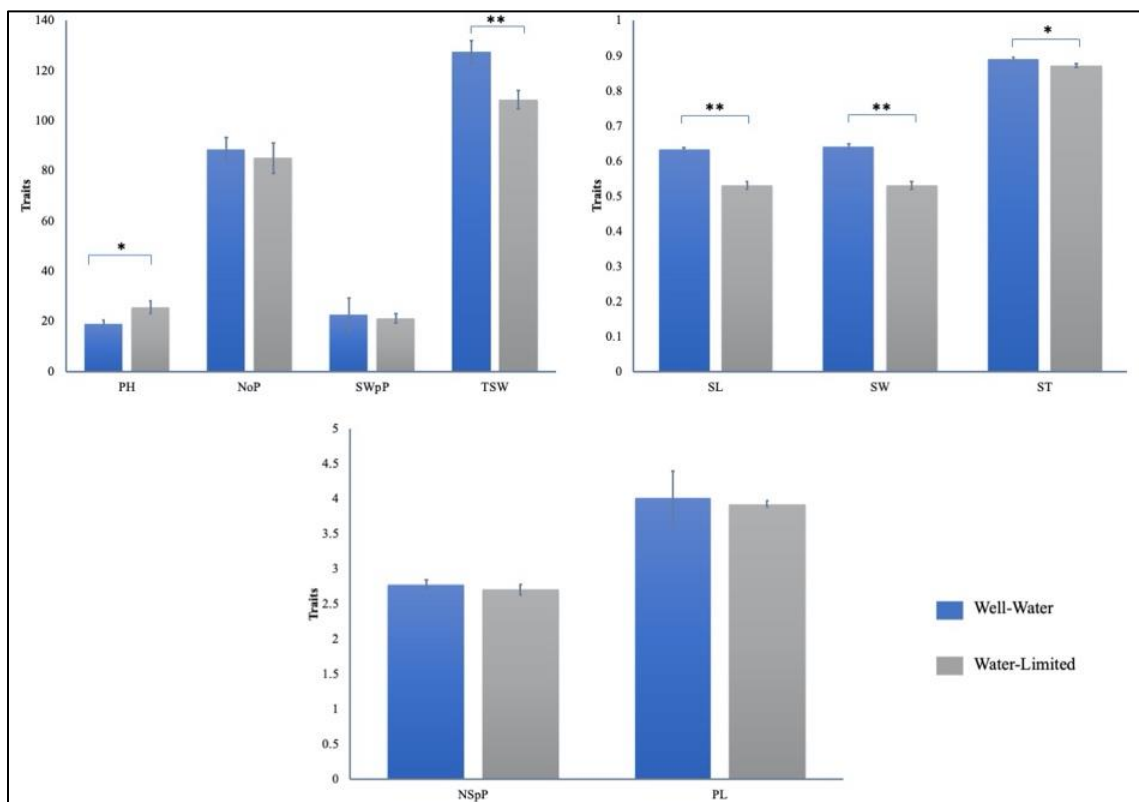
### ***Principal Component and Biplot Analysis***

Field data from both water regimes was averaged and the non-hierarchical multivariate analysis (PCA) was performed to investigate relationship among variables. The data was averaged just to minimize the error. Recently, this approach has been used to interpret the PCA results under abiotic stress conditions (Aziz et al., 2018). Out of nine principal components (PCs), first four PCs showed Eigen values more than 1. Among these four PCs, first two PCs showed a cumulative 44.68 % variability among soybean genotypes for the parameters under study. Hence these two PCs were given due importance for further explanation. The vector length presented the degree of variations explained by each variable in PCA (Figure 2). The vector angle between traits is predicting a correlation between them. The vector angle  $< 90$  (acute angle) between the two traits shows positive correlation whereas the traits having obtuse angle between the traits shows negative correlation. In scatterplots, thousand seed weight, seed length and seed width showed positive correlation (Figure 2). On the basis of thousand seed weight, the scatterplot showed that soybean genotypes presented in Table 1 and yellow highlighted in Figure 2 were tolerant to water-limited conditions. Intolerant soybean genotypes are highlighted in blue color. Briefly, CH-1, CH-3, Ch-96, Kuell and 205/1 showed good performance and these genotypes were also positioned closed to thousand seed weight in the biplot.

**Table 1: Analyses of Variance based on mean square values of traits under well water (WW) and water limited (WL) conditions.**

Sources of Variations	Df	PH		NSpP		NoP		PL		SWpP		TSW		SL		SW		ST	
		WW	WL	WW	WL	WW	WL	WW	WL	WW	WL	WW	WL	WW	WL	WW	WL	WW	WL
Block	10	47.1	669	0.135	0.094	1041	1957	0.143	0.1022	81	154.5	374	337	0.00129	0.00529	0.001953	0.00528	0.000706	0.00226
Treatment	45	69.5	175	0.183	0.2300*	812*	1268*	0.292	0.1892**	46.7*	126.6*	1001	611*	0.002152*	0.00477	0.002065*	0.00458	0.00129*	0.00152*
Check	1	174.3	30	0.142	0.28	2127	1375	0.9	0.0071**	75.2	73.6	4139	2182	0.000881	0.00785	0.000582	0.00158	0.001925	0.01282
Check + check vs. augmented	44	67.2	178	0.184	0.229	782	1265	0.279	0.1933**	46	127.9	930	575	0.002181	0.0047	0.002098	0.00465	0.001276	0.00127
Residuals	1	1	3	0.02	8E-04	0	2	0.005	0	0	0.4	5	2	0.000002	0.00224	0	0.00008	0	0

*Plant height PH (inches), number of seeds per pod – NSpP, number of pods per plant – NoP, pod length – PL (cm), seed weight per plant – SWpP (g), thousand seed weight – TSW (g), seed length – SL (mm), seed width – SW (mm), seed thickness – ST (mm).*

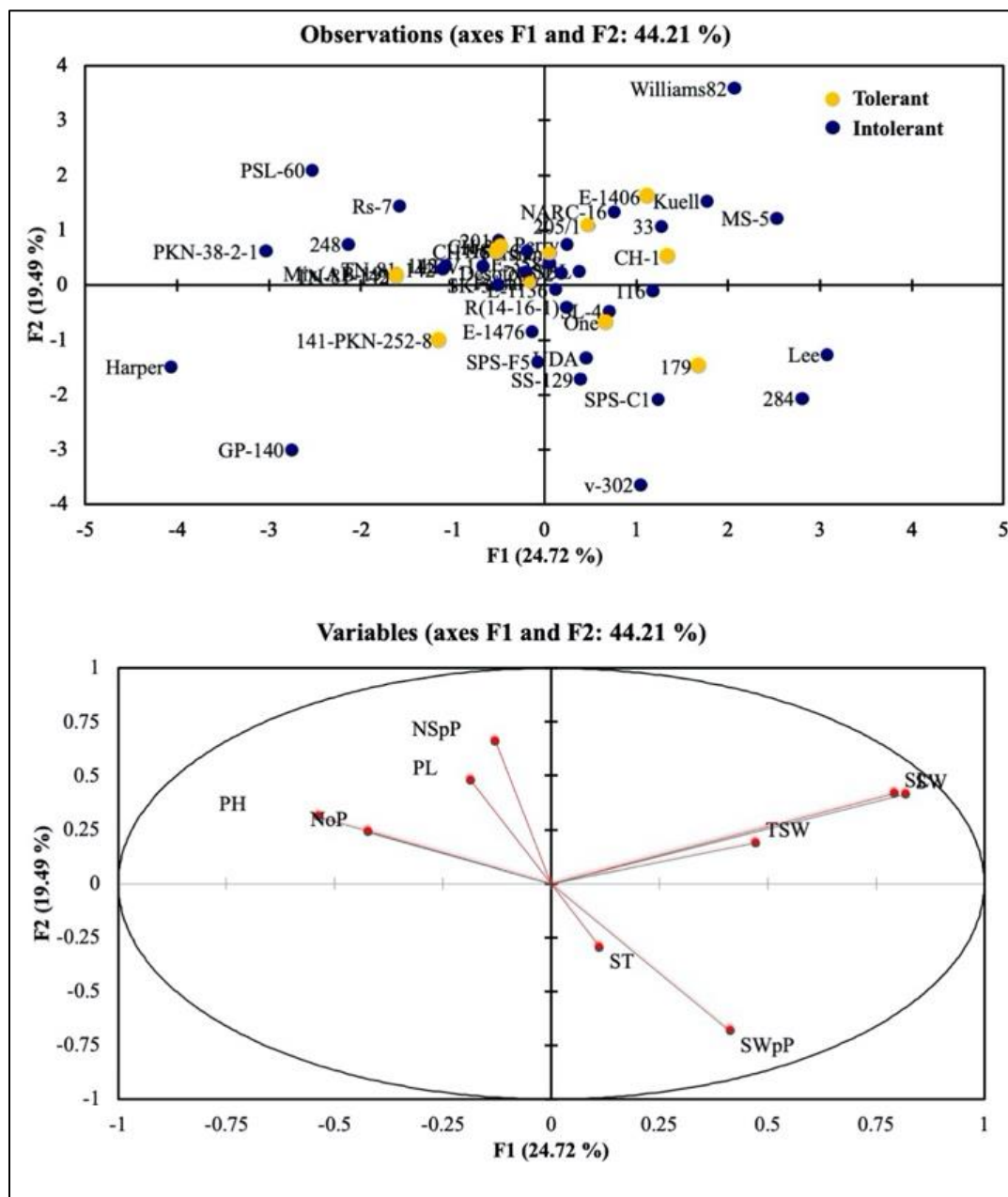


**Figure-1: Phenotypic comparison of soybean accession under well-water and water limited conditions.**

Traits are plant height – PH (inches), number of pods per plant – NoP, seed weight per plant – SWpP (g), thousand seed weight – TSW (g), seed length – SL (mm), seed width – SW (mm), seed thickness – ST (mm), number of seeds per pod – NSpP, pod length – PL (cm). \* $P < 0.05$ , \*\* $P < 0.01$ . Error bar shows standard error.

**Table 2: Thousand seed weight comparison of promising soybean genotypes under well-water and water-limited conditions along with drought susceptibility index for thousand seed weight ( $DSI_{TSW}$ )**

Genotype	TSW in Water-limited	TSW in Well-Water	$DSI_{TSW}$
205/1	115	118	0.33170874
One	102	104	0.25090789
E-1136	104	106	0.24617378
141-PKN-252-8	110	112	0.2329859
CH-3	120	122	0.21388869
CH-96	140	142	0.18376353
179	87	90	0.43490701
SPS-F5	96	97	0.13450732



**Figure-2: Scatterplot and principal component analyses.**

Scatterplot showing soybean accessions sorted into tolerant and intolerant calculated on the phenotypic traits to the first two principal components. Principal component analyses based on all studied phenotypic traits averaged over both water regimes.

## DISCUSSION

The performance of forty-six soybean genotypes was evaluated to classify the genetic variation for important agronomic traits under well-water and water-limited conditions. ANOVA exhibited considerable effects due to soybean genotypes and environment. The

results were consistent with those previously published literature for soybean in terms of significant variation in soybean seed vigor (Wijewardana et al., 2019).

PCA also confirmed the presence of significant variation under water-limited conditions for the studied parameters in given soybean genotypes. Seed weight has been the direct selection benchmark for



breeding in soybean; however, further progress through direct selection of the seed weight might be difficult due to low heritability which has been reported previously (Hayati et al., 2022). Hence, selection for stable yield in soybean based upon drought susceptibility index (DSI) is desired selection criteria in selection for drought tolerance to drought (Aziz et al., 2018; Hayati et al., 2022).

Considerable decline in yield and yield related traits under water-limited conditions agrees with the previous reports on drought response in soybean. Drought resulted in the reduction of seed number and size (Wijewardana et al., 2019), seed weight (Hayati et al., 2022), total above ground biomass and number of pods (Jumrani & Bhatia, 2018). In current study, seed weight (TSW) was reduced to 15 % which is in accordance to Jumrani & Bhatia (2018), who reported that soybean hundred seed weight decreased by 9 to 36 %.

It is very challenging to apply an environmental variable like drought as a treatment under field conditions without keeping in-view several additional factors i.e. photoperiod, soil conditions and precipitation. These factors might be reduced in a controlled condition, but the shortcomings might be a restraint in mimicking different external factors achievable under field conditions (Aziz et al., 2018). We suggest, experiments at different field locations and the over years can be helpful to reduce the effects of environmental factors and will give understanding into actual envirotyping.

The  $DSI_{TSW}$  is regarded as a dependable trait to pick drought tolerant germplasm and has been reported in several studies for measuring the tolerance to abiotic stress factors in different crop plant (Aziz et al., 2018; Mirzaghaderi & Mason, 2019). The soybean genotypes were categorized into tolerant and intolerant to drought on the basis of  $DSI_{TSW}$ . The positive association of seed length and seed width with thousand seed

weight in PCA also suggested that seed length and seed width are also essential traits that are contributing to higher thousand seed weight under both water regimes.

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## CONFLICT OF INTEREST

All authors declare not conflict of interest.

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## AUTHOR'S CONTRIBUTION

Muhammad Zeshan Zafer, Chahat Fatima, Ayesha Aftab – executed the experiments. Muhammad Hammad Nadeem Tahir, – provided financial and technical support. Muhammad Amir Bakhtavar, Mahmood Alam Khan, Zulqurnain Khan, Shoaib Ur Rehman – provided technical support. Muhammad Zeshan Zafer and Chahat Fatima – written initial draft. Essam Darwish and Zulqurnain Khan – review and editing. Shoaib Ur Rehman – supervised the research.

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