Indian J. Genet. Plant Breed., (2023); 83(3): 316-325
https://doi.org/10.31742/ISGPB.83.3.3

ISSN: 0975-6906

www.isgpb.org

RESEARCH ARTICLE

Genetic analysis of tolerance to transient waterlogging stress in pigeonpea (*Cajanus cajan* L. Millspaugh)

P. S. Basavaraj*, Jagadish Rane, K. M. Boraiah, Prakash Gangashetty[1] and C. B. Harisha

Abstract

Pigeonpea (*Cajanus cajan* L. Millspaugh) is mainly grown in the rainfed ecosystem during the monsoon season on deep vertisols, and it often faces transient waterlogging problems due to continuous and heavy downpours during early monsoon seasons. Thus, waterlogging has become a significant issue in most of the pigeonpea growing areas of the semi-arid tropics due to heavy rain during the initial crop growth stage, which causes 30 to 40% economic yield loss in India and around the world. Only a few sources of waterlogging tolerant genotypes are available in pigeonpea but are not in the required duration. Hence, the present investigation was carried out to identify waterlogging tolerant pigeonpea genotypes in different durations suitable for different agroecologies of India and to find ideal selection indices as appropriate strategies to identify waterlogging tolerant genotypes. In this study, 162 genotypes were screened under *in vitro* conditions for 2, 4, 6, and 8 days by submerging seeds. Of these, 33 genotypes could survive for eight days. Further, following a standard screening protocol; these 33 pigeonpea genotypes were screened under pot culture for two years (2020 and 2021). Based on different morpho-physiological parameters and waterlogging tolerant coefficient, genotypes ICP-10397, ICP-7507, ICP-7869, ICP-7148, ICP-4903 ICP-16309, ICP-7375, ICP-6815, ICP-7507, and ICP-6128 were identified as tolerant to transient waterlogging. In addition, this study reports the existence of substantial genetic variation for waterlogging tolerant traits in pigeonpea. Stress indices, such as stress tolerance index) (STI), mean relative performance (MRP) and relative efficiency index (REI) were identified as the ideal selection index. Therefore, identified genotypes are further validated and can be deployed in waterlogging tolerance pigeonpea breeding programs.

Keywords: Waterlogging, pulse, flood, pigeonpea, abiotic stresses

Introduction

Pigeonpea (*Cajanus cajan* L. Millspaugh) is a vital pulse crop native to India (Van der Maesen 1980). Due to its considerable variation in maturity group, it is adapted to diverse agroecologies and cropping systems. It is extensively cultivated in the arid and semi-arid tropics for food, fodder, and other uses. India is the world's largest pigeonpea producer, producing 5.02 million tons from 6.09 million ha (India-stat 2020-21). Though this crop can be grown in diverse soil types; however, it grows best in well-drained fertile soils. Although several improved varieties were released for commercial cultivation across the country, their productivity remains low, ~852 kg/ha, which is mainly due to various constraints such as genetic, agronomic, biotic, and abiotic factors (Sultana et al. 2013).

In India, pigeonpea is mainly grown in the rainfed ecosystem during the monsoon season (June to December) on deep vertisols, and thus often, an early stage of the crop is exposed to transient waterlogging due to continuous and heavy downpours during early monsoon seasons (Hingane et al. 2015; Kumar et al. 2020). Therefore, the initial establishment is the most crucial phase in flood-prone areas (Khare et al. 2002; Sultana et al. 2013; Kumar et al. 2020). Waterlogging stress occurs when soil pores around a plant's root zone are filled with water. This prevents the normal movement of air in the root zone, resulting in a decline in oxygen levels in the soil and, conversely, increases the

ICAR-National Institute of Abiotic Stress Management, Baramati 413 115, India

[1]International Crops Research Institute for the Semi-Arid Tropics-Hyderabad 502 324, India

***Corresponding Author:** P. S. Basavaraj, ICAR-National Institute of Abiotic Stress Management, Baramati 413 115, India, E-Mail: bassuptl@gmail.com

How to cite this article: Basavaraj P.S., Rane J., Boraiah K.M., Gangashetty P. and Harisha C.B. 2023. Genetic analysis of the tolerance to transient waterlogging stress in pigeonpea (*Cajanus cajan* L. Millspaugh) . Indian J. Genet. Plant Breed., **83**(3): 316-325.

Source of support: ICAR-NICRA

Conflict of interest: None.

Received: April 2023 **Revised:** Aug. 2023 **Accepted:** Aug. 2023

carbon dioxide concentration. This state of minimal aeration necessitates the plant to shift its metabolism from aerobic to anaerobic dependency rapidly.

Consequently, the ATP and NADP production is limited, eventually altering the reserve energy (Kumutha et al. 2008). Thus, the fermentation process deprives the plant's stored energy and releases by-products such as alcohols, aldehydes, and reactive oxygen species (ROS), which are detrimental to plant growth (Sairam et al. 2011). Further, these anaerobic conditions support a unique microbial community leading to a severe nutrient imbalance in the soil (Laanbroek 1990). Moreover, it causes deficiency of N, Mg, and Mn under waterlogged conditions due to increased denitrification and nitrate leaching from the top soils has been reported (Srivastava et al. 2009). This shift in nutrient balance results in visible yellowing, a decrease in leaf area, dry matter, relative water content, and degradation of chlorophyll pigments, directly affecting the plant's photosynthetic ability (Kumutha et al. 2008). Reports suggest that waterlogging stress causes a reduction in soluble protein content, which further influences the carbon assimilation of the pigeonpea (Kumar, 2020).

Another notable impact observed is the reduction of seminal root growth and root dry mass (Dubey and Asthana 1987; Chauhan et al. 1997). The amalgam of these effects in the plant would profoundly impact the quality and yield of the plant (Kumutha et al. 2008). Further, these episodes of stagnant water film over the upper soil would also promote the infestation and spread of *Phytophthora* species (Duncan and Kennedy 2007; Saxena et al. 2018).

The impact of waterlogging stress on pigeonpea can be managed through agronomic interventions such as raised sloping seed beds, sowing on ridges, transplanting of seedlings, and use of growth regulators (Abebe et al. 1992). However, they add extra cost to the production and are not an economically viable solution. Therefore, developing and deploying potential waterlogging tolerant cultivars is an economical and feasible alternative approach.

Earlier experiments on waterlogging tolerance in pigeonpea were focused only on the survival ability of the crop alone. However, based on past efforts in the identification and development of stress-tolerant crop varieties in many crops (Basavaraj et al. 2021; Wasae 2021; Nagaraju et al. 2022) by involving grain yield as a main selection criterion (Kumar et al. 2021) under stress condition. Although the yield under stress (S) and non-stress (NS) had a weak correlation, but positive wave emerged (Kumar et al. 2008), suggesting that the yield could be used as a primary selection criterion.

In the earlier studies, different selection criteria have been proposed for identifying the best genotypes for stress and normal conditions in other crops for drought and high temperature. However, there is no information on using indices to identify tolerant genotypes for waterlogging stress in pigeonpea. These quantitative indices enable breeders to identify a superior performance of genotypes under all circumstances. Hence, the study was indented to identify early seedling stage waterlogging tolerant pigeonpea genotypes based on appropriate selection indices.

Materials and methods

A total of 162 genotypes which includes notified varieties, checks, and diverse germplasm of pigeonpea collected from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India, was used in the present study (Supplementary Tables S1 and S2) during the wet season of 2020 and 2021 for two successive years at ICAR-National Institute of Abiotic Stress Management-Baramati, India (18° 09′ 30.62′′N and 74° 30′ 03.08′′E and 570 m above mean sea level).

In vitro seed submergence experiment

The *Invitro* experiment was carried out at the seed stage under controlled conditions following the methods of Sultana et al. (2013) and Hingane et al. (2015). In brief, each test genotype had 20 healthy seeds of uniform size in four replicates. The test genotypes were subjected to different durations of water submergence treatments in 250 mL beakers containing 150 mL of water at 25 ± 1°C. The submergence duration (treatment) was 2, 4, 6, and 8 days. For control, i.e. (no submergence treatment), the germination test was carried out by placing 20 seeds of each genotype in a paper towel method. Upon completion of each of the stress treatment duration, seeds were taken out and dried on a filter paper for 3 to 4 hours to remove excess moisture and then placed on a paper towel in a petri dish and kept at a steady temperature for germination (25 ± 2°C) in a dark room. When the radicle length reached a minimum of 2 mm, the seeds were considered to have germinated. After 5 to 6 days of stress treatment, the germinated seeds were counted, and the percent survival was estimated. The seedling length (cm) was measured for each genotype under the control and submergence conditions after respective days of treatment (2, 4, 6, and 8 days). In addition, the waterlogging tolerance coefficient (WTC) was estimated for each genotype using the following formula (Habibullah et al. 2021).

$$\text{WTC} = \frac{\text{Seedling length under waterlogged condition}}{\text{Seedling length under control condition}}$$

Screening of pigeonpea genotypes in the pot culture

Out of 162, thirty-three genotypes showed tolerance to water submergence (8 days) in the *in vitro* seed submergence experiment. They were further used to screen for transient waterlogging tolerance at the early stages of crop establishment (20 days after emergence). The screening was carried out using plastic pots of 25 cm diameter, with four 6 mm diameter holes in the base. Pots were filled with a mixture of black soil and farmyard manure (FYM) in a ratio of 50:1 (V/V). Fertilizer (nitrogen, phosphorus, potassium, NPK) was also applied as a basal dose; the amount was calculated on a soil weight basis and adequately blended into the soil. After filling, each pot was weighed to 12 kg to ensure the same amount of soil mixture and constant moisture in each pot. Eight pots were used for each genotype (four pots for imposing waterlogging stress and four kept as a control). Following a completely randomized design, filled pots were sown, taking three seeds/pot at 18 to 20 mm depth. Before the stress imposition, the number of plants in each pot was counted. Waterlogging stress was imposed by immersing pots in a cement tank filled with water for ten days and maintaining a water level of 20 mm above the pot surface during the entire duration of the whole experiment (10 days), while the control pots were kept at normal moisture. Throughout the stress treatment, the water level in the pot was maintained at the same level for ten days. After ten days of stress treatment, excess water in the pots was drained out and allowed to recover. The number of plants that survived in each pot was counted five days after the waterlogging stress treatment, and the rate of survival was calculated based on the number of plants in each pot before and after the treatment (Sultana et al. 2013; Hingane et al. 2015).

Measurement of yield and its attributing and physiological traits

The yield and its attributing traits, such as days to 50% flowering (DFF), plant height in centimetres (PH), number of primary branches (NPB), number of secondary branches (NSB), number of pods per plant (PP), number of seeds per pod (SPP), 100 seed weight in gram (TW) and grain yield per plant in gram (GY) were recorded in each treatment and each replication. The amount of chlorophyll content (µg/g of Fresh weight) present in the pigeonpea leaves was estimated by following the method of Lichtenthaler and Wellburn (1983).

The maximum quantum yield of PS-II (Qmax) (Qmax)- (Fv/Fm)

which indicates the efficiency of PS-II of genotype, was assessed by using dark-adapted leaves and was measured with a portable open gas exchange fluorescence system GFS-3000-C equipped with a high-precision CO_2/H_2O infrared gas analyzer and a standard measuring head 3010-S (Heinz Walz GmbH, Effeltrich, Germany).

Stress indices

The following stress indices were calculated based on Yield under stress and control.

Where Ysi and Yci, are the yield of ith variety under stress and control, respectively. Ys and Yc are the average yields of all varieties under stress and normal condition.

Table 1. Caption missing

Stress tolerance indices	Formula	Reference
Stress Tolerance Index (STI)	$\text{STI}=\frac{Ysi*(Ysi-Yci)}{Ys}$	Lambers et al., 2008
Percent Yield Reduction (PYR)	$\text{PYR}=\frac{(Yci-Ysi)}{Yci}*100$	Simane and Struik1993
Stress susceptibility percentage index (SSPI)	$\text{SSPI}=\frac{(Yci-Ysi)}{2Yc}$	Darkwa et al. 2016
Relative waterlogging index (RWI)	$\text{RWI}=\frac{(Ysi/Yci)}{Ys/Yc}$	*Wortmann et al. 1995*
Mean relative performance (MRP)	$\text{MRP}=\frac{(Ysi)}{Ys}+\frac{(Yci)}{Yc}$	*Benjamin et al. 2003*
Relative efficiency index (REI)	$\text{REI}=\frac{(Ysi)}{Ys}*\frac{(Yci)}{Yc}$	*Manjeru et al. 1995*
Yield Stability Index (YSI)	$\text{YSI}=\frac{Ysi}{Yci}$	*Porch et al. 2009*

Statistical analysis

The data recorded for all the parameters were used for statistical analysis. Analysis of variance using Factorial RBD was carried out. Correlation and PCA were also calculated. All the analyses were performed using R statistical software version 4.4.1.

Results

In-vitro experiment

The 162 diverse pigeonpea genotypes' seeds were subjected to different transient water submergence durations (2, 4, 6, and 8 days) to identify tolerant and intolerant genotypes. A total of 102 out of 162 genotypes survived only for two days of submergence treatment; however, 13, 14, and 33 genotypes survived 4, 6, and 8 days of submergence, respectively.

The survival percentages were calculated for thirty-three genotypes that survived up to 8 days of submergence and are presented in (Table 3). The survival percent varied from 20% (MAL-15) to 100% (GRG-811, ICP-6815, ICP-6845, ICP-7223, ICP-10228, ICP-10397, ICP-4903, ICP-7869) under eight days of submergence (Fig. 1).

Seedling measurement and submergence tolerance

After eight days of submergence treatment, seedling lengths of 33 pigeonpea genotypes were measured. The genotypes *viz.*, ICP-4903, ICP-7148, ICP-7869, 7507, and ICP-10397 recorded significantly highest seedling lengths under both control as well eight days of submergence treatment

Table 2. Analysis of variance (ANOVA) for the yield and its attributing traits of pigeonpea genotypes as influenced by waterlogging stress

Source of Variation	DF	DFF	PH	NPB	NSB	PP	SPP	TW	GY
Factors A (Stress and Control)	1	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Factor B (Genotypes)	32	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Interaction	32	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Years	1	NS	NS	NS	NS	NS	NS	NS	NS

*NS-Non-significant

Note: DF: Degrees of Freedom, DFF: Days to 50% flowering, PH: Plant Height, NPB: Number of Primary branches, NSB: Number of Secondary Branches, PP: Pods per plant, SPP: Seeds per pod, TW: Test Weight, GY: Grain yield per plant

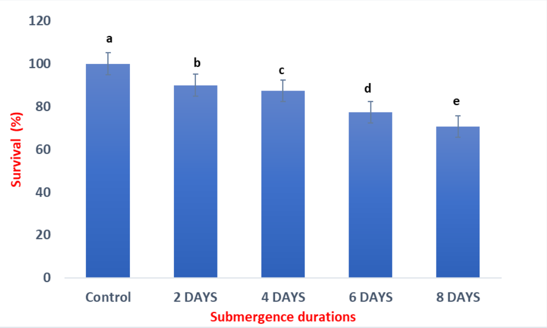

Fig. 1. Survival percent of diverse genotypes of pigeonpea under different submergence durations. The bars on the columns represent the SE, and different letters differ significantly by LSD (p < 0.01)

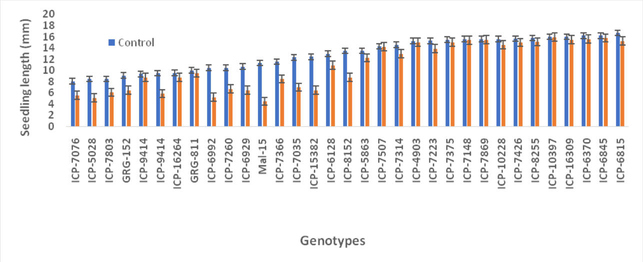

Fig. 2. Seedling length (mm) of thirty-three pigeonpea genotypes after 8 days of submergence

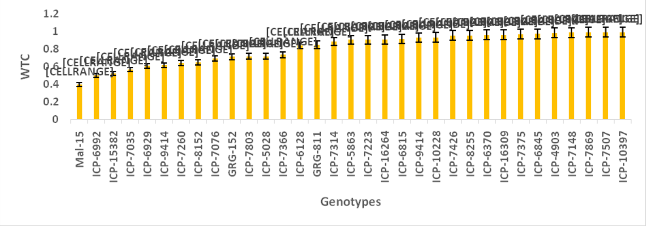

Fig. 3. Waterlogging tolerant coefficient of diverse pigeonpea genotypes

(Fig. 2). Conversely, genotypes such as ICP-7076, ICP-7803, and GRG-152 exhibited the lowest seedling length under eight days of submergence. Further, waterlogging tolerant coefficient (WTC) was also estimated. Genotypes ICP-10397, ICP-7507, ICP-7869, ICP-7148, ICP-4903 ICP-16309, ICP-7375, ICP-6815, ICP-7507, and ICP-6128 exhibited significant highest waterlogging tolerant coefficients, whereas genotypes such as ICP-7426, ICP-8255, ICP-6370, ICP-16309, ICP-7375 are at far and MAL-15 recorded lowest WTC (Fig. 3).

Screening of pigeonpea genotypes in the early seedling stage

Thirty-three out of 162 pigeonpea genotypes that survived up to 8 days of submergence at the seed stage were further screened for waterlogging tolerance at the early seedling stage in the pot. Combined analysis of variance revealed a significant difference among normal and waterlogging stress conditions for all the traits studied at a 0.5% probability level. The genotypes also showed significant differences for all the traits at a 1% probability level, and their interaction was also significant (Table 1).

Physiological response of pigeonpea genotypes to the transient waterlogging

Waterlogging at the early seedling stage of pigeonpea significantly reduced the chlorophyll content compared to the control (p<0.05). Genotypes ICP-10397, ICP-7375, ICP-10228, ICP-7869, ICP-4903, ICP-6370, ICP-7148, ICP-8255, ICP-6845, and ICP-7426 recorded highest total chlorophyll content under control and waterlogging condition, whereas ICP-8602 showed lowest total chlorophyll content among all the genotypes under both waterlogging and control conditions (Fig. 4).

The quantum efficiency of PS-II decreased under waterlogging conditions compared to the control condition in all the genotypes. Photosynthetic health of (Quantum efficiency of PS-II) of pigeonpea genotypes ICP-10397, ICP-7375, ICP-10228, ICP-7869, ICP-4903, ICP-6370, ICP-7148, ICP-8255, ICP-6845, and ICP-7426 was significantly high under both control and waterlogging condition, whereas MAL-15 and ICP-7803 had lower PS-II efficiency under waterlogging stress (Fig. 5).

Morphological and yield traits

The pigeonpea genotypes exhibited significant differences for the traits such as days to 50% flowering, plant height, number of primary branches, number of secondary branches, pods $plant^{-1}$, seeds pod^{-1}, test weight and grain yield $plant^{-1}$ under both control and waterlogging conditions and their interactions were also significant (Table 2). Among the diverse pigeonpea genotypes, ICP-5863 was the earliest

Table 3. The pattern of survivability percentages of 33 pigeonpea genotypes in 2, 4, 6, and 8 days of seed submergence

S. No.	Genotypes	Survival %			
		2 Days	4 Days	6 days	8 Days
1	GRG-152	100	90	40	30
2	GRG-811	100	100	100	100
3	ICP-5028	100	100	90	83
4	Mal-15	60	60	40	20
5	ICP-7035	100	100	40	40
6	ICP-6929	100	80	40	40
7	ICP-6992	80	50	40	40
8	ICP-7076	100	100	70	50
9	ICP-6815	100	100	100	100
10	ICP-6845	100	100	100	100
11	ICP-7375	100	100	100	100
12	ICP-7426	100	100	100	100
13	ICP-7507	100	100	100	100
14	ICP-7260	100	60	60	50
15	ICP-9414	100	100	80	70
16	ICP-7148	100	100	100	100
17	ICP-7314	100	100	100	100
18	ICP-15382	100	80	50	30
19	ICP-16309	100	100	100	100
20	ICP-16264	100	100	100	50
21	ICP-7803	100	100	80	80
22	ICP-7869	100	100	100	100
23	ICP-8152	100	100	90	60
24	ICP-8602	100	100	90	85
25	ICP-8255	100	100	100	100
26	ICP-5863	100	100	100	100
27	ICP-6128	100	100	100	100
28	ICP-6370	100	100	100	100
29	ICP-7366	100	80	80	80
30	ICP-7223	100	100	100	100
31	ICP-10228	100	100	100	100
32	ICP-10397	100	100	100	100
33	ICP-4903	100	100	100	100

to flower under both control and waterlogging treatment (81 and 83 days, respectively). Plant height was highest in genotype ICP-8152 and ICP-7035 under control and waterlogging conditions, respectively, whereas the lowest plant height was observed in ICP-7148. Pods plant^{-1} is notably highest under control conditions compared to waterlogging conditions. In control and stress conditions, a higher number of pods plant^{-1} was recorded in ICP-7314 and ICP-7507. Test weight was significantly reduced under waterlogging stress in pigeonpea genotypes compared to non-stress. The test was highest among ICP-7035 under normal conditions and ICP-7869 under waterlogged conditions. Grain yield plant^{-1} is one of the essential traits for increased yield production. In the present study, ICP-7314 and ICP-7507 produced the highest grain yield plant^{-1} under normal and waterlogging stress conditions, respectively.

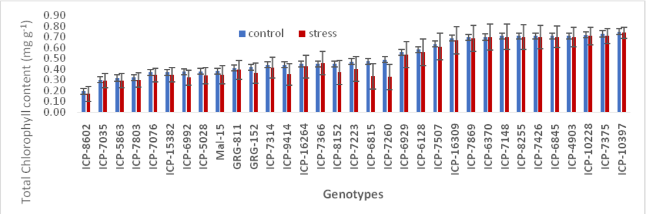

Fig. 4. Total chlorophyll content of pigeonpea genotypes under both waterlogging and controlled conditions

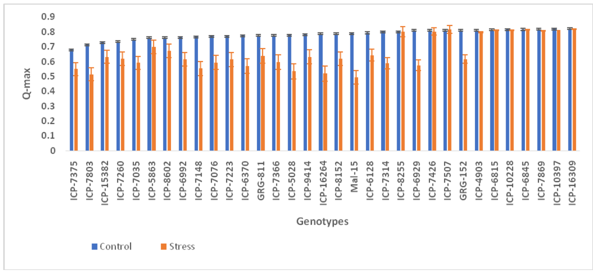

Fig. 5. Quantum efficiency of PS-II of diverse pigeon pea genotypes under control and transient waterlogging condition

Stress tolerance indices

Different selection indices were calculated and presented in Table 4 based on the yield under stress and control conditions. The highest value for STI was recorded by genotypes ICP-16309, ICP-7148, ICP-8255, ICP-6845, ICP-6815, ICP-10228, ICP-6370, ICP-10397, ICP-4903, ICP-7869, ICP-7507, whereas the least STI was observed for ICP-7366. However, tolerant checks ICP-5028 (0.19) and MAL-15 (1.73) recorded low and high STI values. Genotypes ICP-7869 (0.73), ICP-6370 (1.4), ICP-4903 (1.98), ICP-10228 (1.99), ICP-7507 (2.52), ICP-6845 (3.55), ICP-7148 (3.79), ICP-10397 (3.97), ICP-6815 (5.34), ICP-8255 (6.5), ICP-7426 (6.74), ICP-16309 (6.77) recorded notably very low per cent yield reduction (PYR) compared to tolerant check, MAL-15 (8.19) and ICP-5028 (58.75). SSPI was highest in genotype ICP-7314, whereas it was very low in genotypes viz., ICP-7869, ICP-6370, ICP-10228, and ICP-4903. A higher value of RWI was noted in ICP-7148, ICP-10397, ICP-6845, ICP-7507, ICP-10228, ICP-4903, ICP-6370, and ICP-7869. Higher yield stability was observed in genotypes *viz.*, ICP-7375, ICP-7426, ICP-16309, ICP-8255, ICP-6815, ICP-6845, ICP-7148, ICP-10397, ICP-7507, ICP-10228, ICP-4903, ICP-7869 and ICP-6370.

Table 4. Estimates of Stress Tolerance indices for different pigeonpea genotypes

Genotype	STI	PYR	SSPI	RWI	MRP	REI	YSI
GRG-152	0.15	77.8	0.78	0.35	1.70	1.39	0.22
GRG-811	0.11	76.1	0.49	0.37	1.75	0.60	0.24
ICP-5028	0.19	58.8	0.21	0.64	1.15	0.32	0.41
Mal-15	1.73	8.19	0.05	1.43	3.21	2.50	0.92
ICP-7035	0.35	35.6	0.10	1.01	1.10	0.30	0.64
ICP-6929	0.40	27.1	0.07	1.14	1.04	0.27	0.73
ICP-6992	0.32	62.5	0.47	0.59	2.35	1.28	0.37
ICP-7076	0.14	61.9	0.19	0.60	0.98	0.22	0.38
ICP-6815	1.45	5.34	0.03	1.48	2.57	1.60	0.95
ICP-6845	1.32	3.55	0.02	1.51	2.29	1.26	0.96
ICP-7375	0.93	8.2	0.03	1.43	1.73	0.73	0.92
ICP-7426	0.77	6.74	0.02	1.46	1.39	0.47	0.93
ICP-7507	3.07	2.52	0.03	1.52	5.25	6.60	0.97
ICP-7260	0.63	13.8	0.04	1.35	1.29	0.41	0.86
ICP-9414	0.15	52.7	0.12	0.74	0.76	0.14	0.47
ICP-7148	1.15	3.79	0.02	1.50	2.00	0.97	0.96
ICP-7314	0.70	68	1.50	0.50	0.59	9.63	0.32
ICP-15382	0.25	35.3	0.07	1.01	0.78	0.15	0.65
ICP-16309	1.12	6.77	0.03	1.46	2.04	1.01	0.93
ICP-16264	0.44	11.3	0.02	1.39	0.86	0.18	0.89
ICP-7803	0.21	42.6	0.09	0.90	0.78	0.15	0.57
ICP-7869	1.83	0.73	0.00	1.55	3.04	2.21	0.99
ICP-8152	0.27	20.8	0.03	1.24	0.63	0.10	0.79
ICP-8602	0.09	67.5	0.20	0.51	0.87	0.17	0.32
ICP-8255	1.21	6.5	0.03	1.46	2.20	1.17	0.93
ICP-5863	0.11	63.8	0.18	0.57	0.86	0.17	0.36
ICP-6128	0.54	36.5	0.16	0.99	1.70	0.73	0.63
ICP-6370	1.57	1.4	0.01	1.54	2.65	1.67	0.99
ICP-7366	0.05	83.9	0.54	0.25	1.62	0.42	0.16
ICP-7223	0.19	62.6	0.28	0.58	1.40	0.45	0.37
ICP-10228	1.55	1.99	0.01	1.53	2.63	1.65	0.98
ICP-10397	1.60	3.97	0.02	1.50	2.79	1.88	0.96
ICP-4903	1.76	1.98	0.01	1.53	2.99	2.14	0.98

Note: STI: Stress tolerance index, PYR: Percent Yield reduction SSPI: Stress susceptibility percentage index, RWI: Relative waterlogging index, MRP: Mean relative performance, REI: Relative efficiency index, YSI: Yield Stability Index

Interrelationships among the stress indices and genotypes

Principal component analysis was conducted to study the interrelationship between stress indices and genotypes. A total of 7 components were formed; among them, PC1 and PC2 together explained 91.12 % of the total variability (Fig. 6). Among the various indices, YSI, REI, STI, and RWI contributed the highest to PC1, whereas PYR and SSPI contributed the highest to PC2. PC1 mainly had genotypes viz., ICP-10397, ICP-7375, ICP-10228, ICP-7869, ICP-4903, ICP-7869, ICP-6370, ICP-7148, ICP-8255, ICP-6845, and ICP-7426 MAL-15, and are situated on the positive side of the axis of

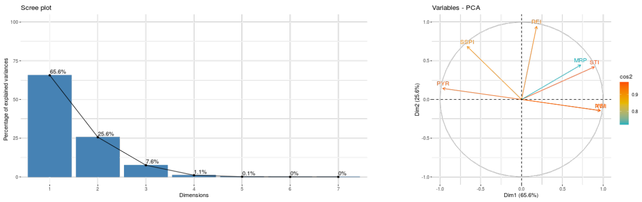

Fig. 6. Scree plot depicting the percent of variance explained by each principal component and Contribution of each variable to total variability in PC1 and PC2

PC1, and the sensitive ones ICP-7366, ICP-8602 are located on the negative side of the PC2 (Fig. 7).

Discussion

Climate change-induced erratic precipitation patterns render agriculture highly vulnerable to drought and flood events. The inability of dryland legumes such as pigeonpea (Krishnamurthy et al. 2011; Sultana et al. 2013; Kumar et al. 2020), soybean (Chandra et al. 2022), chickpea (Palta et al. 2010), etc. to endure hypoxia or anoxia conditions at the root zone, results in significant yield losses which in turn affects pigeonpea productivity and food security. Anoxia or hypoxia conditions caused by waterlogging instigate a series of modifications in plants' morpho-physiological and biochemical traits. That negatively impacts growth, development, and yield (Langan et al. 2022). Therefore, developing and deploying waterlogging tolerant varieties is urgently needed to cope with production demand. Hence, it is imperative to screen and identify waterlogging-tolerant pigeonpea genetic resources. However, efforts have been made to identify sources of waterlogging tolerance in pigeonpea, but they have been limited to very few studies (Chauhan et al. 1997; Krishnamurthy et al. 2012; Sultana et al. 2013).

Most economically essential traits otherwise absent in the modern-day varieties persist in the landraces and wild species. Therefore, the present experimental material was designed in such a way as to include as much genetic variation as possible for the traits being studied. It consists of a diverse collection of genotypes, including high-yielding notified cultivars (9), breeding lines (68), and worldwide germplasm collections of landraces (85). Among the 162 genotypes studied, 124 are native to India, 38 are exotic collections from Bangladesh, Myanmar, China, Italy, Kenya, Zambia, Uganda, Thailand, USA, Australia, Trinidad and Tobago, Nepal, Malawi, Myanmar, and Sri Lanka, origins representing widespread geographical diversity. As a result of the abundance of genetic variability, this genetic material is a good source for selecting superior genotypes for the traits under consideration. Sultana et al. (2013) used 272 diverse pigeonpea genotypes originating from different countries with different maturity duration and seed colours to screen for waterlogging tolerance at seed, pot, and field conditions. Based on *in-vitro* screening, 162 genotypes were grouped into tolerant (33), moderately tolerant (13), susceptible (14), and highly sensitive (102). The mortality rate increased with the increased duration of seed soaking, and many of the highly susceptible genotypes started putrefying immediately after two days of submergence. However, some of the tolerant genotypes survived even after eight days of seed soaking. *In-vitro* studies demonstrated the existence of substantial genetic variation for waterlogging tolerance in pigeonpea, even at the seed stage. If the seeds of grain legumes are soaked in water for a longer time, over accumulated water leads to rapid damage to the seeds, resulting in decay and improper germination (Powell and Matthews 1978). The differences between mortality rates observed in the present study might be attributed to the diverse origin of these genotypes and their genetic nature. Additionally, it might be linked to variations in imbibition rates and the levels of reserved elements in the seeds. (Sultana et al. 2009, 2013).

Screening of genotypes in the pot culture revealed that most survived genotypes are medium to late in maturity. The present results were in agreement with the results of Matsunaga et al. 1994; Sultana et al. 2013 and Kumar et al.

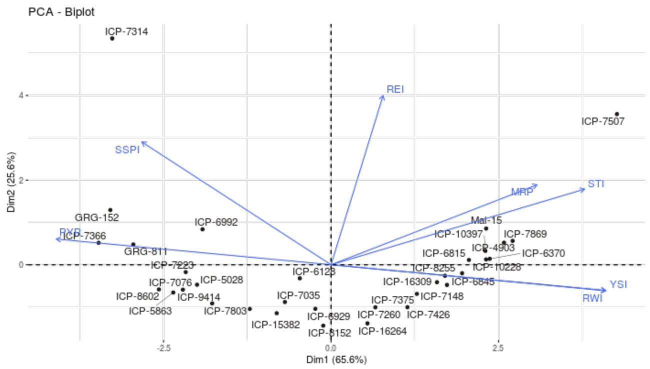

Fig. 7. Biplot depicting the interrelationship between stress indices and pigeonpea genotypes

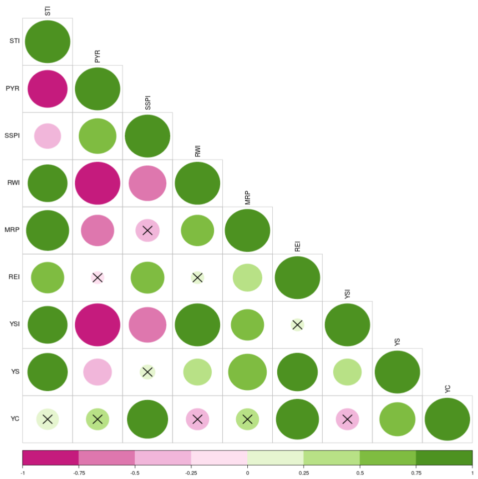

Fig. 8. Correlation among different stress indices with grain yield under stress (Ys) and control (Yc)

2020. This implies that medium to late-maturing genotypes will receive ample time for recovery compared to short-duration genotypes. Besides these, waterlogging tolerance is also associated with seed coat color. The dark seed coat is associated with high tannin and phenolic compounds, which help slow water uptake and enhance survival. Seeds with dark colors would survive and germinate even after more than ten days of submergence in soybean (Hou and Thseng 1991) and pigeonpea (Sultana et al. 2013). In the present study, interestingly, along with dark-coated genotypes, some of the white (cream color) genotypes (ICP-6815, ICP-7507, ICP-7869, and ICP-8152) also germinated well despite eight days of submergence.

The effect of waterlogging stress on physiological and yield-related traits in pigeonpea genotypes was also assessed. Waterlogging stress negatively influenced the growth, development, and yield of pigeonpea genotypes by reducing plant height, number of branches per plant, pods per plant, number of seeds per pod, test weight, and final economic grain yield. Reduction in these traits is associated with decreased photosynthetic efficiency under stress and plant height (Figs. 2 and 4). It may be due to a decline in cell growth and expansion that limits the overall plant architecture under waterlogging stress (Kumar et al. 2020).

The stress indices were calculated to ascertain the most effective and desirable index for identifying waterlogging tolerant pigeonpea genotypes based on correlations between the grain yield of genotypes under stress and non-stress conditions. In the recent past, researchers exploited these indices for drought tolerance in rice (Hussain et al. 2021), low phosphorous tolerance rice (Kale et al. 2021; Basavaraj et al. 2021), salinity (Singh et al. 2015), and low nitrogen (Khan and Mohammad 2016). Based on these indices, genotypes were identified as tolerant and intolerant to different stresses in crops like rice (Hussain et al. 2021; Basavaraj et al. 2021), wheat (Farshadfar et al. 2012), and maize (Naghavi et al. 2013). Based on the previous investigations, the present study was carried out to group genotypes tolerant and intolerant to waterlogging stress. An ideal selection index or indices could correlate with grain yield under contrasting conditions (Mitra 2001). According to Farshadfar et al. (2001), a perfect selection index is effectively and highly associated with grain yield under stress and control environments.

Stress indices such as STI, MRP and REI were ideal selection indices due to significant positive association with grain yield under stress (Ys) (Fig. 8). These findings revealed that higher tolerance index and low susceptible index values could identify tolerant genotypes. The genotypes such as ICP-16309, ICP-7148, ICP-8255, ICP-6845, ICP-6815, ICP-10228, ICP-6370, ICP-10397, Mal-15, ICP-4903, ICP-7869, and ICP-7507 were tolerant to waterlogging, and all other genotypes were moderate to susceptible to waterlogging conditions. On the other hand, indices SSPI was a better indicator for grain yield under non-stress conditions based on significant positive association.

In summary, based on *In-vitro* and pot experiment genotypes ICP-10397, ICP-7507, ICP-7869, ICP-7148, ICP-4903 ICP-16309, ICP-7375, ICP-6815, ICP-7507, and ICP-6128 identified as tolerant to transient waterlogging at an early stage of the pigeonpea as these possess high WTC, survival rate, seedling length, etc. A considerable genetic variation exists for waterlogging tolerance among pigeonpea genotypes. Therefore, there is an opportunity for genetic improvement of the trait through a breeding program. Hence, identified genotypes can be further validated, and then they can be deployed in waterlogging tolerance pigeonpea breeding programs.

Acknowledgment

The authors thank ICRISAT, Hyderabad, for sharing seeds and ICAR-NIASM for supporting this study. Authors acknowledge the ICAR-NICRA for funding this research.

Authors' contribution

Conceptualisation of research (BPS, JR), Designing of experiment (BPS, JR, BKM), Contribution of Experimental materials (PG, BPS), Execution of field/lab experiment and data collection (BPS, BKM), Analysis of Data and interpretation (BPS, JR), Preparation of the Manuscript (BPS, JR, BKM, PG, HCB).

References

Abebe M., Mamo T., Duffera M. and Kidam S. 1992. Durum wheat response to improved drainage of Vertisols in the central highlands of Ethiopia. In Seventh Regional Wheat Workshop for Eastern Central and Southern Africa, held in Nakuru, Kenya, 16–19 September 1991 (Eds. D. G. Tanner & W. Mwangi), pp. 407–414. Mexico: CIMMYT.

Basavaraj P.S., Gireesh C., Murali B., Manoj C.A., Ishwarya Lakshmi V.G., Honnappa., Ajitha V., Senguttuvel P., Sundaram R.M. and Anantha M.S. 2021. Stress tolerance indices for the identification of low phosphorus tolerant introgression lines derived from *Oryza rufipogon* Griff. Plant Genetic Resources: Characterization and Utilization, **19**(4): 328-338. doi:10.1017/S1479262121000381.

Benjamin J.G., Nielsen D.C., Vigil M.F. 2003. Quantifying effects of soil conditions on plant growth and crop production. Geoderma, **116**(1-2): 137–148.

Chandra S., Reecha T., Das., Nagar S., Satpute G.K., Kumawat G., Ratnaparkhe M.B., Gupta S., Rajesh V., Nataraj V., Shivakumar M., Srivastva M. and Meena S. 2022. Soybean improvement for waterlogging tolerance. S. H. Wani et al. (eds.) Soybean Improvement: Physiological, Molecular and Genetic Perspectives. Switzerland: Springer International Publishing https://doi.org/10.1007/978-3-031-12232-3_3.

Chauhan Y.S., Silim S.N., Kumar Rao, J.V.D.K. and Johansen C. 1997. A pot technique to screen pigeonpea cultivars for resistance to waterlogging. J. Agron. Crop Sci., **178**: 179–183.

Darkwa K., Ambachew D., MohammedH., AsfawA. and Blair M.W.

2016. Evaluation of common bean (*Phaseolus vulgaris* L.) genotypes for drought stress adaptation in Ethiopia. Crop J., **4**(5): 367-376.

Dubey S.D. and Asthana A.N. 1987. Selection of plant type for resistance to water logging in pigeonpea. In ACIAR proceedings series.

Duncan J.M. and Kennedy D.M. 1989. The effect of waterlogging on Phytophthora root rot of red raspberry. Plant pathol., **38**(2): 161-168.

Farshadfar E., Farshadfar M.and Dabiri S. 2012. Comparison between effective selection criteria of drought tolerance in bread wheat landraces. Iran Ann. Biol. Res., **3**(7): 3381–3389.

Farshadfar E., Farshadfar, M. and Sutka J. 2001. Combining ability analysis of drought tolerance in wheat over different water regimes. Acta. Agron. Hung., **48**(4): 353–361.

Habibullah M., Sarkar S., Islam M.M., Ahmed K.U., Rahman M.Z., Awad M.F., ElSayed, A.I., Mansour E.and Hossain M.S. 2021. Assessing the response of diverse sesame genotypes to waterlogging durations at different plant growth stages. Plants, **10:** 2294. https://doi.org/10.3390/plants10112294

Hingane A., Rafat S., Patil S., Vijaykumar R., Saxena, K.B., Mallikarjuna N., Malayee S. and Sameer Kumar C.V. 2015. Mechanism of water-logging tolerance in pigeonpea. Indian J. Genet. Plant Breed., **75**(2): 208-214.

Hou F.F. and Thseng F.S. 1991. Studies on the flooding tolerance of soybean seed: varietal differences. Euphytica, **57**: 169–173.

Hussain T., Hussain N., Ahmed M., Nualsri C. and Duangpan S., 2021. Responses of lowland rice genotypes under terminal water stress and identification of drought tolerance to stabilize rice productivity in Southern Thailand. Plants, **10**: 2565. https:// doi. org/ 10. 3390/plant s1012 2565.

India Stat. 2021. https://www.indiastat.com/data/agriculture/land-use-classification/data-year/all-years.

Kale R.R., Anila M., Mahadeva Swamy H.K., Bhadana V.P., Durga Rani C.V., Senguttuvel P. and Sundaram R.M. 2021.Morphological and molecular screening of rice germplasm lines for low soil P tolerance. J. Plant. Biochem. Biotechnol., **30**(2): 275–286.

Khan F.U. and Mohammad F. 2016. Application of stress selection indices for assessment of nitrogen tolerance in wheat (*Triticum aestivum* L.). J. Anim. Plant. Sci., **26**(1): 201–210.

Khare D., Rao S., Lakhani J.P., Satpute R.G. 2002. Tolerance for flooding during germination in pigeonpea. Seed Res., **30**: 82-87.

Krishnamurthy L., Upadhyaya H.D., Saxena, K.B. and Vadez V. 2012. Variation for temporary waterlogging response within the mini core pigeonpea germplasm. J. Agric. Sci., **150**: 357–364.

Kumar A., Bernier J., Verulkar S., Lafitte H.R. and Atlin G.N. 2008. Breeding for drought tolerance: direct selection for yield, response to selection and use of drought-tolerant donors in upland and lowland adapted populations. Field. Crop. Res., **107**:221–231.

Kumar A., Raman A., Yadav S., Verulkar S.B., Mandal N.P., Singh O.N., Swain, P., Ram, T., Badri J., Dwivedi J.L. and Das S.P. 2021. Genetic gain for rice yield in rainfed environments in India. Field. Crop. Res., **260**:107977.DOI: 10.9734/cjast/2020/v39i1230660

Kumar D., Sultana R., Kumar R.R.and Kirti M. 2020. Characterization of pigeonpea genotypes for waterlogging tolerance based on morpho-physiological and molecular traits. *Current J. Appl. Sci. Technol.,* **38** *(12):* 21-33.

Kumutha D., Sairam R.K., Ezhilmathi K., Chinnusamy V. and Meena R.C. 2008. Effect of waterlogging on carbohydrate metabolism in pigeonpea (*Cajanus cajan* L.): upregulation of sucrose synthase and alcohol dehydrogenase. Plant Sci., **175**(5): 706–716. doi:10.1016/j.

Laanbroek H.J. 1990. Bacterial cycling of minerals that affect plant growth in waterlogged soils: a review. Aquatic Bot., **38**(1): 109-125.

Lambers H., Chapin F.S. and Pons T.L. 2008. Plant Physiological Ecology, Springer Science & Business Media, New York, NY, USA.

Langan P., Bernád V., Walsh J., Henchy, J., Khodaeiaminjan M., Mangina, E.and Negrão E. 2022. Phenotyping for waterlogging tolerance in crops: current trends and future prospects. J. Expt Bot., **73**(15): 5149–5169, https://doi.org/10.1093/jxb/erac243.

Lichtenthaler H.K. and Wellburn A.R 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochemical Society Transactions, **11**: 591-592.

Manjeru P.T., Madonzi B., Makeredza A. and Nciizah Sithole M. 1995. Effects of water stress at different growth stages on yield and yield components of common bean (*Phaseolus vulgaris*). African Crop Sci., **8**: 299–303.

Matsunaga R., Ito O., Tobita S., Rao T.P. and Johansen C. 1994. Response of short-duration pigeonpea to nitrogen application after short-term waterlogging on a vertisol. Field Crop Res., **38**: 167–174.

Mitra J. 2001. Genetics and genetic improvement of drought resistance in crop plants. Curr Sci., **80**: 758–763.

Nagaraju P., Beulah P., Jaldhani V., Manasa, V., Madhusudan R., Sundaram, R.M., Hariprasad A.S., Sruti K., Sheshumadhav M., Kota S., Kalyani M.B., Mahender Kumar R., Sreedevi B., Surekha Rani H., Pawar S.C., Roja Rani A. and Senguttuvel P. 2022. Assessment of reproductive stage drought tolerance using stress indices in improved restorer lines of KMR-3R in rice. Cereal Res Commun., https://doi.org/10.1007/s42976-022-00334-6.

Naghavi M.R., Pourabоughadareh A. and Khalili M. 2013. Evaluation of drought tolerance indices for screening some of corn (*Zea mays* L.) cultivars under environmental conditions. Not. Sci. Biol., **5**(3): 388–393.

Palta J.A., Ganjeali A., Turner N.C.and Siddique K.H.M.2010. Effects of transient subsurface waterlogging on root growth, plant biomass and yield of chickpea. Agric. Water Manage., **97**(10): 1469–1476.doi: 10.1016/j.agwat.2010.05.001.

Porch T.G., Ramirez V.H., Santana D. and Harmsen E.W. 2009. Evaluation of common bean for drought tolerance in Juana Diaz, Puerto Rico. J. Agron. Crop Sci., **195**(5): 328–334.

Powell A.A. and Matthews, S.1978. The damaging effect of water on dry pea embryos during imbibition. J. Expt Bot., **29**: 1215–1229.

Sairam R.K., Dharmar K., Lekshmy S. and Chinnusamy V. 2011. Expression of antioxidant defense genes in mung bean (*Vigna radiata* L.) roots under water-logging is associated with hypoxia tolerance. Acta Physiol. Plant., **33**(3): 735-744.

Saxena K.B., Choudhary A.K., Saxena R.K. and Varshney R.K. 2018. Breeding pigeonpea cultivars for intercropping: synthesis and strategies. Breed. Sci., **68**(2): 159-167.

Simane B. and Struik P.C. 1993. Agroclimatic analysis: a tool for planning sustainable durum wheat (*Triticum turgidum* var. durum) production in Ethiopia. Agric. Ecosyst. Environ., **47**(1): 31-46.

Singh S., Sengar R.S., Kulshreshtha N., Datta D., Tomar R.S. and Rao V.P. 2015. Assessment of multiple tolerance indices for salinity stress in bread wheat (*Triticum aestivum* L.). J. Agri. Sci., **7**(3): 49.

Srivastava J.P., Singh P., Singh V.P. and Bansal R. 2009. Effect of waterlogging on carbon exchange rate, stomatal conductance and mineral nutrient status in maize and pigeonpea. Plant stress, **4**(1): 94-99.

Sultana R., Singh P.P., Singh R.P., Singh D. K., Jat M.L., Dass S., Zaidi P.H. and Singh I. 2009. Studies on gene effects of traits associated with excessive soil moisture tolerance in tropical maize (*Zea mays* L.). In Abstracts of Invited Papers: 4th World Congress on Conservation Agriculture, 4–7 February 2009, New Delhi, India, p. 81.

Sultana R., Vales M., Saxena K., Rathore A., Rao S. and Kumar R. 2013. Waterlogging tolerance in pigeonpea (*Cajanus cajan* (L.) Millsp.): Genotypic variability and identification of tolerant genotypes. The J. Agric. Sci., **151**(5): 659-671.

Van der Maesen L.J.G. 1980. India is the native home of the pigeonpea. In: Arends J.C., Boelema G., Groot C.T. and Leeuwenberg A.J.M. Eds., Liber Gratulatorius in nonerem H.C.D. de Wit, Agricultural University, Wa-geningen, 257-262.

Wasae A. 2021. Evaluation of drought stress tolerance based on selection indices in haricot bean varieties exposed to stress at different growth stages. International J. Agron., Article ID 6617874, 9.https://doi.org/10.1155/2021/6617874.

Wortmann C.S.L., Lunze V.A. and Ochwoh L. J. 1995. Bean improvement for low fertility soils in Africa. African. Crop. Sci. J., **3**(5): 469-477.