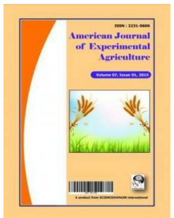

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Genetic Variability of Pigeonpea (*Cajanus cajan* (L.) Millsp.) for Water Logging and Salinity Tolerance under *in vitro* and *in vivo* Conditions

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Authors' contributions

This work was carried out in collaboration between all authors. Authors PG, IS and OPC conducted field experiments. Authors Jagmeet Kaur and Jasdeep Kaur conducted laboratory experiments. Authors PG and AH prepared the first draft. Authors SS, OPC and KBS designed the experiments. Author PS performed the statistical analysis. Authors SS, CVSK and KBS prepared the final draft. All authors read and approved the final manuscript.

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ABSTRACT

Pigeonpea (*Cajanus cajan* (L.) Millsp.) genotypes acquired from diverse sources, were screened for water logging and salinity tolerance under laboratory and field conditions. Analysis of variance revealed significant differences among the genotypes for various traits. Based on seedling vigor index, six genotypes (AL 1756, AL 1849, AH-06-7, H-2000-14, H-2003-14, ICP 5028) were found highly tolerant, 10 tolerant, 24 moderately tolerant, 12 sensitive and eight highly sensitive to water logging. In case of salinity treatment, seven genotypes (AL 1849, AH-06-7, H-2000-14, H-2001-25,

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H-2003-14, ICP 5028, JBP 110B) were found tolerant, 14 moderately tolerant, 17 sensitive and 22 highly sensitive. Mean values for germination percentage, seedling length, seedling dry weight and seedling vigor index were generally lower in salinity treatment than water logging treatment. Based on results of laboratory studies, 28 genotypes were screened in pot/field for tolerance to water logging and salinity and were categorized on the basis of plant survival percentage after treatments. Based on plant survival, four genotypes (AL 15, AL 1849, H-2000-14, H-02-28) were found highly tolerant, five tolerant, nine moderately tolerant, four sensitive and six were found highly sensitive in water logging treatment. In case of salinity treatment, six genotypes were found tolerant, five moderately tolerant, three sensitive and 14 were found highly sensitive. Based on higher plant survival (%) under both water logging and saline conditions, common genotypes were identified for their further use in breeding programme.

Keywords: Germination; abiotic stresses; phenotypic variability; stress tolerance.

1. INTRODUCTION

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is an important legume crop widely grown in many parts of Indian sub-continent. It is mainly used as human food, animal feed and an effective green manure crop. It is adapted to a wide range of environments and cropping systems. Major abiotic stresses encountered by pigeonpea are water logging, salinity and drought. These abiotic stresses adversely affect its productivity causing severe yield losses [1,2]. Pigeonpea is reported to be highly sensitive to water logging [3,4] and salinity [5,6]. Water logging and salinity stresses are important yield constraints in pigeonpea as water logging blocks oxygen supply to roots which hampers root permeability [7] and salinity impairs seed germination, reduces nodule formation, retards plant development and finally reduces crop yield [8]. It is reported that germination and early vegetative growth stages in pigeonpea are more affected by water logging than the flowering stage [9]. Water logging is reported to delay flowering, reduce vegetative growth, photosynthetic rate, biomass and grain yield [10,11]. It has also been reported that short duration varieties of pigeonpea are more prone to the risk of yield reduction due to water logging as compared to medium or long duration varieties [12]. Anoxic soil conditions are produced by excess water and consequently, plant roots suffer hypoxia or anoxia. Min and Bothalomew [13] reported gradual decrease in relative water content under water logging and plants wilt visually within a few hours of imposing water logging stress. It is observed that water logging induced decrease in leaf water potential [14] and membrane damage [15]. Water logging also caused reduction in stomata conductance and plants exposed to water logging exhibit increased stomata resistance and limited water uptake leading to internal water deficit [16].

Soil salinity is another major abiotic stress that affects plant growth, development and yield by causing physiological and biochemical changes in plants [17]. It leads to osmotic stress and interferes with mineral nutrients uptake [18]. Seedling and reproductive stages in plants are mainly affected by salinity stress [19]. Salinity together with water logging can cause deleterious effects in plants posing major threat to crop productivity [20]. These two abiotic stresses are related with each other as water logging results in rise of water table causing development of salinity in many parts of India [21]. In India, saline water are defined as water having EC > 2 dS m^{-1} [22,23]. A large genetic variation has been found in different cultivated and wild species of pigeonpea for salinity tolerance [5,24]. In spite of the fact that both these stresses pose serious threat to pigeonpea production, very few efforts have been made to identify tolerant sources [4,11]. Thus, it becomes important to identify the diverse sources of pigeonpea tolerant to water logging and salinity stresses to develop high yielding and tolerant cultivars keeping in view the present climatic conditions [25]. Therefore, the present study was undertaken to screen and identify pigeonpea genotypes tolerant to water logging and salinity stresses under both *in vitro* and *in vivo* conditions.

2. MATERIALS AND METHODS

2.1 Experimental Materials

The experimental material comprised a set of 60 pigeonpea genotypes, including advance breeding lines and some commercially released cultivars, acquired from different sources namely, International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru;

CCS Haryana Agricultural University, Hisar; Indian Agricultural Research Institute, New Delhi and Punjab Agricultural University, Ludhiana.

2.2 *In vitro* Screening

The 60 genotypes were evaluated under *in vitro* conditions in the laboratory of Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India, using two treatments viz., water logging and salinity stresses during rainy seasons of 2012-13 and 2013-14. The water logging treatment comprised distilled water while the salinity treatment was having 30mM NaCl solution. Prior to the treatment, seeds of different genotypes were surface sterilized with 0.1% mercuric chloride for 1-2 minutes and residual chlorine was eliminated by rinsing the seeds with sterile distilled water several times. Seeds were submerged in beaker containing 200 ml normal distilled water in case of water logging treatment and in 200 ml 30 mM NaCl solution in case of salinity treatment, respectively, for 7 days (168 hrs) at 25±1℃. After the submergence treatment, seeds were then placed in sterilized petri-plates containing germination paper moistened with distilled water. For each genotype, 3 replications each were kept for both the treatments using 15 seeds per replication. Petri-plates were then kept in BOD incubator maintained at 25±1℃ for 7 days.

2.3 Observations

Data were recorded after 7 days of incubation for both the treatments for germination percentage, seedling length (plumule and radicle), seedling dry weight (plumule and radicle) and seedling vigor index. Germination count was recorded by counting the number of germinated seeds out of the total seeds. Seed was considered to be germinated when emerging radicle elongated upto 1 mm length and then average germination percentage was computed. Seedling length was recorded by measuring the length of plumule and radicle and seedling dry weight was recorded after drying them in oven at 60℃ for 3 days. Seedling vigor index was calculated using the formula: Seedling vigor index (VI) = mean germination% X mean seedling dry weight (mg). Based on seedling vigor index, the genotypes were categorized as highly tolerant (VI=>2000), tolerant (VI=1501-2000), moderately tolerant (VI=1001-1500), sensitive (VI=501-1000) and highly sensitive (VI=≤500).

2.4 *In vivo* Screening

Based on results of laboratory screening, a set of 28 genotypes, including highly tolerant to highly sensitive, was chosen for *in vivo* screening in the pots/field. Plants of 28 genotypes were raised in 27 cm diameter plastic pots containing 10 kg of soil fertilized with recommended fertilizers as per package of practices for pigeonpea. Seeds of each genotype were sown in pots and replicated thrice for both water logging and salinity treatments. After germination, six plants were maintained finally for recording observations. After 30 days of sowing, the pots were kept for 5 days in ponds of 20 ft x 10 ft size containing normal water (water logging) and 30 mM NaCl solution (salinity). The water level was maintained at top surface level of pots. Five days after the water logging and salinity treatments, pots were removed from the ponds and placed on ground for the recovery of plants. During recovery of plants, normal water was applied as and when required.

2.5 Observations

Survival of plants was recorded after 5 days and then the final survival was recorded one month after water logging and salinity treatments. Chlorophyll content of plants on the third leaf from the top of plant was measured with SPAD (502-plus) before and 5 days after the water logging and salinity treatments. In each replication three plants were taken randomly for recording chlorophyll content. Percent reduction in chlorophyll content was calculated using the formula: Reduction in chlorophyll content (%) = (chlorophyll content before treatment – chlorophyll content after treatment)/chlorophyll content before treatment X 100. As suggested by Dua [26], the genotypes were categorized based on final plant survival percentage (PS), as highly tolerant (>90% PS), tolerant (75-90% PS), moderately tolerant (50-74% PS), sensitive (25-49% PS) and highly sensitive (<25% PS). Data on initiation and completion of leaf senescence were also recorded visually after water logging and salinity treatments.

2.6 Experimental Design and Statistical Analysis

Both the experiments (*in vitro* and *in vivo*) were conducted following Completely Randomized Design with three replications. The analysis of variance (ANOVA) was performed on single year data as well as on pooled data of two years using statistical analysis as per the procedure given by Snedecor and Cochran [27] and adapted by Cheema and Singh [28] using the following equation: $Y_{ije} = \mu + \tau_i + \beta j + ke + \varepsilon ije$; where the parameter μ is an overall mean, τi are the treatment effects, βj are the block effects, ke are the years' effect and εije is the random error. All comparisons were made at 5% level of significance. Tukey test was performed to group the genotypes based on germination percentage, seedling vigor index for *in vitro* and on plant survival for *in vivo* studies. The correlation coefficients were also worked out among the various traits for *in vitro* and *in vivo* experiments following Al-Jibouri et al. [29].

3. RESULTS

3.1 *In vitro* Screening

Analysis of variance, performed on single year data as well on pooled data of two years, revealed significant differences among the genotypes for all the traits studied viz., germination percentage, seedling length, seedling dry weight and seedling vigor index under both water logging and saline conditions (data not shown). In case of water logging conditions, six genotypes viz., AL 1756, AL 1849, AH-06-7, H-2000-14, H-2003-14 and ICP 5028 were found as highly tolerant, 10 genotypes (AL 15, AL 1758, AH-06-9, AH-06-12, H-2001-25, H-02-28, ICPL 332, ICPA 2039, ICPL 20128, JBP 110B) as tolerant, 24 as moderately tolerant, 12 as sensitive and eight as highly sensitive (Table 1). Under saline conditions, none of the genotypes was found to be highly tolerant, however, seven genotypes viz., AL 1849, AH-06-7, H-2000-14, H-2001-25, H-2003-14, ICP5028 and JBP 110B were found as tolerant, 14 as moderately tolerant, 17 as sensitive and 22 as highly sensitive (Table 1). Data on germination percentage, seedling length, seedling dry weight and seedling vigor index of 28 genotypes including some highly tolerant to highly sensitive genotypes are presented in Tables 2 and 3, respectively for water logging and saline conditions. It can be seen that genotype AH-06-7 had the maximum seedling vigor index (2655.0) followed by H-2000-14 (2636.3), AL 1756 (2099.8) and AL 1849 (2091.0) under water logging condition. The germination percentage of highly tolerant and tolerant genotypes was more than 75%. Out of the 60 genotypes, 3 highly sensitive genotypes recorded zero values for germination percentage, seedling length, seedling dry weight and seedling vigor index (data not shown). Under saline conditions, the genotype H-2000-14 had the maximum seedling vigor index (1841.9) followed by ICP 5028 (1706.3), AH-06-7 (1668.5) and H-2001-25 (1656.4). Germination of tolerant genotypes was more than 75%. Of the total 22 highly sensitive genotypes, five genotypes, having 0-15% germination, showed zero values for seedling length, seedling dry weight and seedling vigor index. However, some genotypes like AL 1760, ICPL 99051 and UPAS 120 recorded comparatively higher germination percentage (30-38.5%), but their seedling vigor index values (180.0-231.0) were very low due to low seedling dry weight as a result of less seedling growth after germination due to sensitivity to saline conditions.

Overall under *in vitro* condition, it was observed that mean values of all the genotypes for all the traits studied like germination percentage, seedling length, seedling dry weight and seedling vigor index were lower under saline conditions (63.1%, 6.4 cm, 15.4 mg, 1090.9, respectively) as compared to water logging conditions (72.8%, 7.7 cm, 19.1 mg, 1477.3, respectively). Overall there were 23 genotypes which showed similar reactions, i.e. tolerant (2 genotypes), moderately tolerant (5 genotypes), sensitive (8 genotypes) and highly sensitive (8 genotypes), under both water logging and salinity conditions. Of the six highly tolerant genotypes under water logging conditions, five genotypes namely AL 1849, AH-06-7, H-2000-14, H-2003-14 and ICP 5028 showed tolerant reaction, whereas one genotype, AL 1756 showed moderately tolerant reaction to saline conditions. Of the 10 tolerant genotypes under water logging conditions, two genotypes (H-2001-25, JBP 110B) showed the same reaction, i.e. tolerant, while seven genotypes namely AL 15, AL 1758, AH-06-9, AH-06-12, H-02-28, ICPA 2039 and ICPL 20128 changed their reaction to moderately tolerant, and one genotype, ICPL 332 changed its reaction to highly sensitive under saline conditions. Of the 24 genotypes that showed moderately tolerant reaction under water logging conditions, five genotypes namely AL 1779, AL 1839, AH-07-3, H-02-59 and SGBS 6 showed similar reaction (moderately tolerant), while the remaining 19 genotypes changed their reaction to either sensitive or highly sensitive under saline conditions. On the other hand, interestingly the genotype AH-09-9, which was found sensitive under water logging conditions, showed moderately tolerant reaction under saline conditions.

Table 1. Grouping of pigeonpea genotypes based on seedling Vigor Index (VI) evaluated under water logging and saline conditions *in vitro*

Categories based on seedling Vigor Index (VI)	Name of genotypes (Number of genotypes)	
	Water logging	**Salinity**
Highly tolerant (VI=>2000)	AL 1756, AL 1849,AH-06-7, H-2000-14, H-2003-14, ICP 5028 (Total=6)	-
Tolerant (VI=1501-2000)	**Common genotypes:** H-2001-25, JBP 110B (Total=2)	
	AL 15, AL 1758, AH-06-9, AH-06-12, H-02-28, ICPL 332, ICPA 2039, ICPL 20128(Total:2+8=10)	AL 1849, AH-06-7, H-2000-14, H-2003-14, ICP 5028 (Total:2+5=7)
Moderately Tolerant (VI=1001-1500)	**Common genotypes:** AL 1779, AL 1839, AH-07-3, H-02-59, SGBS 6 (Total=5)	
	AL 1812, AL 1847, AL 1873, AH-06-5, H-03-29, H-03-30, H-2005-5, Pusa 992, Pusa 2002, Pusa 2001-10, Pusa 2010-5-2, Pusa 2012-2, ICP 8857, ICP 14085, ICPL 2376, LRG 30, RG-06-1, ASJ 123, Sel. 107-1 (Total:5+19=24)	AL 15, AL 1756, AL 1758, AH-06-9, AH-09-9, AH-06-12, H-02-28, ICPA 2039, ICPL 20128 (Total:5+9=14)
Sensitive (VI=501-1000)	**Common genotypes:** AL 201, AL 1747, AL 1881, PAU 881, AH-06-1, Pusa 2011-1, ICP 4924,ICPL 99050 (Total=8)	
	AL 1760, AH-09-9, ICPL 96061, ICPL 99051 (Total:8+4=12)	AL 1812, AH-06-5, H-03-29, H-2005-5, Pusa 992, Pusa 2001-10, ICP 8857, ICP 14085, LRG 30 (Total: 8+9=17)
Highly Sensitive (VI=<500)	**Common genotypes:** AL 1593, H-61-21, Pusa 33, MAL 9, MAL 12, MAL 15,ICP 11811,UPAS 120 (Total=8)	
	-	AL 1760, AL 1847, AL 1873, H-03-30, Pusa 2002, Pusa 2010-5-2, Pusa 2012-2, ICPL 332, ICPL 2376, ICPL 99051, ICPL 96061, RG-06-1, ASJ 123, Sel. 107-1 (Total: 8+14=22)

Correlation coefficients between various traits, worked out for the 28 genotypes studied under *in vitro* conditions, are presented in Table 4. The results showed highly significant positive correlation among germination percentage, seedling length, seedling dry weight and seedling vigor index under both water logging and saline conditions.

3.2 *In vivo* Screening

A set of 28 genotypes was also evaluated under field (*in vivo*) conditions for chlorophyll content and plant survival (%) under water logging and salinity conditions. Analysis of variance, performed on single year data as well on pooled data of two years, revealed significant differences for chlorophyll content before as well as after treatments, reduction in chlorophyll content after treatment and for plant survival percentage under both water logging and saline conditions (data not shown). It was observed that four genotypes namely AL 1849, H-02-28, AL 15 and H-2000-14 showed 100% plant survival and very less reduction (<10.0%) in chlorophyll content, and thus, categorized as highly tolerant, whereas five genotypes namely AH-06-12, H-2003-14, AL 1756, AH-06-9 and AL 1758 showed comparatively less plant survival (75.0 to 83.0%) and higher reduction (11.76 to 20.24%) in chlorophyll content, and thus, categorized as tolerant under water logging conditions (Table 5). Of the remaining 19 genotypes, nine genotypes were categorized as moderately tolerant, four as sensitive and six genotypes as highly sensitive based on final plant survival percentage. The reduction in chlorophyll content was found to be <10% in highly tolerant genotypes, while it was >31% in sensitive or highly sensitive genotypes.

Table 2. Germination percentage, seedling length, dry seedling weight, seedling vigor index and tolerance category of pigeonpea genotypes under water logging conditions (mean of two years' data)

Sr. no.	Genotypes	Germination (%)	Seedling length (mm)	Dry seedling weight (mg)	Seedling Vigor Index (VI)	Tolerance category*
1	AH-06-7	90.0[a#]	10.6	29.5	2655.0[a]	HT
2	H-2000-14	92.5[a]	10.5	28.5	2636.3[a]	HT
3	AL 1756	92.5[a]	7.7	22.7	2099.8[ab]	HT
4	AL 1849	82.0[a]	11.9	25.5	2091.0[abc]	HT
5	H-2003-14	90.0[a]	8.5	22.5	2025.0[abc]	HT
6	ICP 5028	87.5[a]	9.4	23.0	2012.5[abc]	HT
7	H-2001-25	81.5[a]	8.9	23.5	1915.3[bc]	T
8	AL 1758	79.5[a]	6.5	24.0	1908.0[bc]	T
9	ICPA 2039	84.5[a]	8.7	22.3	1884.3[bc]	T
10	H-02-28	85.0[a]	8.8	22.1	1878.5[bc]	T
11	AH-06-9	75.0[a]	9.0	24.7	1852.5[bc]	T
12	ICPL 332	84.0[a]	10.6	19.2	1612.8[bcd]	T
13	ICPL 20128	83.0[a]	8.0	18.8	1560.4[bcde]	T
14	JBP 110B	85.0[a]	10.4	18.3	1555.5[bcde]	T
15	AH-06-12	75.0[a]	7.3	20.3	1512.4[bcdef]	T
16	AL 15	75.0[a]	7.0	20.0	1500.0[bcdef]	T
17	SGBS 6	72.5[a]	7.4	20.0	1450.0[cdef]	MT
18	ICP 14085	70.0[a]	7.6	17.1	1197.0[defg]	MT
19	H-02-59	60.0[a]	9.2	19.5	1170.0[defg]	MT
20	Pusa 992	65.0[a]	7.8	17.6	1144.0[defg]	MT
21	AH-06-5	67.5[a]	8.1	16.6	1120.5[defg]	MT
22	PAU 881	59.5[a]	8.2	16.1	958.0[defgh]	S
23	AH-09-9	57.5[ab]	6.4	16.2	931.5[efgh]	S
24	AL 1760	61.0[a]	5.6	14.2	866.2[fgh]	S
25	ICPL 99050	67.0[a]	3.7	11.7	783.9[gh]	S
26	ICPL 99051	60.0[a]	2.9	11.9	714.0[gh]	S
27	UPAS 120	45.0[ab]	4.1	6.8	306.0[hi]	HS
28	MAL 15	10.0[b]	0.5	2.3	23.0[i]	HS
	Mean	**72.8**	**7.7**	**19.1**	**1477.3**	
	CD (0.05)	24.47	3.63	5.67	345.34	
	MSD	48.19			654.92	

**HT: Highly tolerant (VI: >2000); T: Tolerant (VI: 1500-2000); MT: Moderately Tolerant (VI: 1000-1499.99); S: Sensitive (VI: 500-999.99; HS: Highly Sensitive (VI: <500); MSD: Minimum significant difference; #Same letters indicate non-significant while different letters indicate significant differences among the genotypes*

In case of saline conditions, none of the genotypes showed more than 90% plant survival, however six genotypes namely AL 1756, AL 1849, H-2-28, H-2001-25, AL 1758 and AL 15 showed higher plant survival (75-83%) and less reduction (<21.0%) in chlorophyll content, and thus, categorized as tolerant, whereas five genotypes namely PAU 881, H-2000-14, H-02-259, SGBS 6 and H-2003-14 showed moderate plant survival ranging from 56.0 to 66.0% and higher reduction (23.77 to 35.88%) in chlorophyll content, and thus, categorized as moderately tolerant under saline conditions (Table 6). Of the remaining 17 genotypes, three genotypes were categorized as sensitive and 14 as highly sensitive based on final plant survival. The reduction in chlorophyll content was also found to be <20% in tolerant genotypes, while it was >37% in sensitive or highly sensitive genotypes.

Of the four genotypes which were found highly tolerant under water logging condition, three genotypes, AL 1849, H-02-28 and AL 15, changed their category to tolerant, while one genotype, H-2000-14 changed its category to moderately tolerant under saline conditions. Of the five tolerant genotypes under water logging condition, two genotypes (AL 1756, AL 1758) gave similar reaction, while one genotype (H-2003-14) changed its reaction to moderately tolerant, and two genotypes (H-06-9, H-06-12) changed their reaction to highly sensitive under saline conditions. Of the nine moderately tolerant genotypes under water logging conditions, three genotypes (H-02-59, PAU 881, SGBS 6) gave similar reaction, while one genotype (AL 1760) changed its reaction to sensitive, four genotypes (ICPL 332, AH-09-9, AH-06-5, AH-06-7) changed their reaction to highly sensitive, and interestingly one genotype, H-2001-25, changed its reaction to tolerant under saline conditions.

Table 3. Germination percentage, seedling length, dry seedling weight, seedling vigor index and tolerance category of pigeonpea genotypes under saline (30 mM NaCl) conditions (mean of two years' data)

Sr. no.	Genotypes	Germination (%)	Seedling length (mm)	Dry seedling weight (mg)	Seedling Vigor Index (VI)	Tolerance category*
1	H-2000-14	81.5 [a#]	10.8	22.6	1841.9 [a]	T
2	ICP 5028	75.5 [ab]	7.0	22.6	1706.3 [ab]	T
3	AH-06-7	75.5 [ab]	9.6	22.1	1668.5 [ab]	T
4	H-2001-25	82.0 [a]	9.2	20.2	1656.4 [abc]	T
5	H-2003-14	83.0 [a]	9.2	19.9	1651.7 [abc]	T
6	AL 1849	85.0 [a]	8.1	19.4	1649.0 [abc]	T
7	JBP 110B	82.5 [a]	7.4	18.3	1509.8 [abcd]	T
8	AL 1756	78.5 [ab]	5.7	18.4	1444.4 [abcd]	MT
9	ICPL 20128	78.5 [ab]	6.6	17.9	1405.1 [abcde]	MT
10	SGBS 6	66.5 [abc]	8.4	21.9	1456.4 [abcd]	MT
11	H-02-28	73.5 [ab]	7.1	18.4	1352.4 [abcde]	MT
12	AL 1758	76.0 [ab]	4.4	17.6	1337.6 [abcde]	MT
13	AL 15	72.5 [ab]	8.7	18.3	1326.8 [abcde]	MT
14	ICPA 2039	75.0 [ab]	6.7	16.2	1215.0 [bcdef]	MT
15	AH-06-9	69.0 [abc]	6.2	16.6	1145.4 [bcdef]	MT
16	AH-06-12	58.5 [abc]	7.2	19.5	1140.8 [bcdef]	MT
17	H-02-59	69.0 [abc]	8.0	16.4	1131.6 [bcdef]	MT
18	AH-09-9	60.0 [abc]	7.0	17.3	1038.0 [cdef]	MT
19	AH-06-5	68.5 [abc]	6.7	14.5	993.3 [def]	S
20	Pusa 992	60.0 [abc]	6.7	15.1	906.0 [def]	S
21	PAU 881	66.5 [abc]	7.3	13.6	904.4 [def]	S
22	ICP 14085	53.0 [abc]	6.0	15.2	805.6 [efg]	S
23	ICPL 99050	60.0 [abc]	3.9	11.0	660.0 [fg]	S
24	AL 1760	38.5 [bcd]	3.8	6.0	231.0 [gh]	HS
25	ICPL 99051	38.5 [bcd]	2.8	4.9	188.7 [gh]	HS
26	UPAS 120	30.0 [cd]	5.0	6.0	180.0 [gh]	HS
27	ICPL 332	5.0 [d]	0.0	0.0	0.0 [h]	HS
28	MAL 15	5.0 [d]	0.0	0.0	0.0 [h]	HS
	Mean	**63.1**	**6.4**	**15.4**	**1090.9**	
	CD (0.05)	21.72	3.46	5.17	315.42	
	MSD	40.57			626.72	

** T: Tolerant (VI: 1500-2000); MT: Moderately Tolerant (VI: 1000-1499.99); S: Sensitive (VI: 500-999.99; HS: Highly Sensitive (VI: <500); MSD: Minimum significant difference; #Same letters indicate non-significant while different letters indicate significant differences among the genotypes*

Table 4. Correlation between various traits in pigeonpea genotypes under *in vitro* conditions

Traits	Seedling length (cm)		Seedling dry weight (mg)		Seedling vigor index	
	WL#	**SAL#**	**WL**	**SAL**	**WL**	**SAL**
Germination%	0.782**	0.841**	0.872**	0.917**	0.899**	0.930**
Seedling length (cm)			0.839**	0.893**	0.801**	0.858**
Seedling dry weight (mg)					0.967**	0.964**

*# WL: Water logging conditions; SAL: Saline conditions; **Significant at 1% level*

Table 5. Chlorophyll content and plant survival of some tolerant and sensitive pigeonpea genotypes under water logging conditions (mean of two years' data)

Sr. no.	Genotype	Chlorophyll content			Plant survival (%)	Tolerance category*
		Before treatment	After treatment	Reduction after treatment (%)		
1	AL 1849	43.6	41.3	5.28	100[a#]	HT
2	H-02-28	43.1	39.3	8.82	100[a]	HT
3	AL 15	39.2	35.6	9.18	100[a]	HT
4	H-2000-14	38.7	34.9	9.82	100[a]	HT
5	AH-06-12	42.5	37.5	11.76	83[ab]	T
6	H-2003-14	35.0	29.4	16.00	83[ab]	T
7	AL 1756	41.6	36.1	13.22	80[ab]	T
8	AH-06-9	47.2	40.5	14.19	80[ab]	T
9	AL 1758	42.0	33.8	19.52	75[abc]	T
10	ICPL 332	32.5	25.5	21.53	67[abcd]	MT
11	H-2001-25	47.3	36.4	23.04	67[abcd]	MT
12	AH-09-9	47.6	35.4	25.63	67[abcd]	MT
13	AL 1760	42.1	30.7	27.08	67[abc]	MT
14	H-02-59	37.9	26.7	29.55	66[abcde]	MT
15	PAU 881	43.4	32.4	25.35	60[abcde]	MT
16	AH-06-5	41.4	30.8	25.60	60[abcde]	MT
17	SGBS 6	34.4	24.8	27.90	55[bcdef]	MT
18	AH-06-7	41.1	32.2	21.65	50[bcdef]	MT
19	ICPA 2039	35.7	20.6	42.30	34[cdefg]	S
20	Pusa 992	38.2	21.4	43.98	33[cdefg]	S
21	ICP 5028	35.6	24.5	31.18	25[defg]	S
22	UPAS 120	46.0	30.2	34.35	25[defg]	S
23	JBP 110B	32.5	19.5	40.00	23[efg]	HS
24	ICPL 20128	36.7	21.4	41.69	15[fg]	HS
25	MAL 15	33.3	19.3	42.04	0[g]	HS
26	ICP 14085	30.2	16.5	45.36	0[g]	HS
27	ICPL 99051	38.4	20.5	46.61	0[g]	HS
28	ICPL 99050	36.2	18.6	48.62	0[g]	HS
	Mean	**39.41**	**29.15**	**26.78**	**54.11**	
	CD (0.05)	12.43	11.26	17.67	21.46	
	MSD				42.36	

**HT: Highly tolerant (>90% PS); T: Tolerant (75-90%PS); MT: Moderately Tolerant (50-75%PS); S: Sensitive (25-49%PS); HS: Highly Sensitive (<25%PS); MSD: Minimum significant difference; #Same letters indicate non-significant while different letters indicate significant differences among the genotypes*

In water logging treatment, the leaf senescence started on 4th day from the lower part of plants and moved upwards and was nearly 100% after 10th day of treatment in highly sensitive genotypes such as MAL 15, ICP 14085, ICPL99051 and ICPL 99050. In case of salinity treatment, the leaf senescence started on 2nd day of the treatment in similar fashion as in case of water logging treatment and was nearly 100% after 6th day of treatment in highly sensitive genotypes such as ICPL 14085, ICPL 99051, MAL 15, ICP 5028, ICPL 332, AH-06-9, AH-06-9 and ICPL 99050. In rest of the highly sensitive genotypes, complete leaf senescence was observed after 15-20 days of treatment. Comparatively lesser leaf senescence was observed in highly tolerant genotypes, while low to moderate leaf senescence was recorded in tolerant or moderately tolerant genotypes under both water logging and saline conditions.

Chlorophyll content measured before the water logging and salinity treatments revealed that the genotype AH-09-9 had the maximum value (47.6) followed by H-2001-25 (47.3) and AH-06-9 (47.2). After water logging treatment, the maximum chlorophyll content was observed in AL 1849 (41.3) followed by AH-06-9 (40.5) and H-02-28 (39.3), whereas after salinity treatment, the maximum chlorophyll content was recorded by H-2001-25 (38.5) followed by AL 1849 (37.6) and AL 1756 (37.3). Overall, there were nine genotypes (highly tolerant or tolerant) under water logging conditions and six genotypes (tolerant) under saline conditions that showed <20% reduction in chlorophyll content (Tables 5, 6). On the average of all genotypes under *in vivo* conditions, it was observed that reduction in chlorophyll content was higher (36.19%) in saline conditions compared to water logging conditions (26.78%).

Results of the present study revealed that five genotypes namely AL 1756, AL 1849, H-2-28, AL 1758 and AL 15 recorded higher plant survival (>75%) under water logging as well as saline conditions, whereas six genotypes namely JBP 110B, ICPL 20128, MAL 15, ICP 14085, ICPL 99051 and ICPL 99050 were found highly sensitive under both conditions. The correlation coefficients between different traits, worked out for the 28 genotypes studied under *in vivo* conditions, are presented in Table 7. Plant survival percentage showed highly significant positive correlation with chlorophyll content both before and after water logging treatment. The plant survival also showed highly significant positive correlation with chlorophyll content after saline treatment, while before saline treatment the correlation between these two traits was non-significant. The reduction in chlorophyll content showed highly significant negative correlation with plant survival percentage and chlorophyll content both after the water logging and saline treatments. The reduction in chlorophyll content also showed significant negative correlation with chlorophyll content before water logging treatment, while after salinity treatment

Table 6. Chlorophyll content and plant survival of some tolerant and sensitive pigeonpea genotypes under saline conditions (mean of two years' data)

Sr. no.	Genotype	Chlorophyll content			Plant survival (%)	Tolerance category*
		Before treatment	After treatment	Reduction after treatment (%)		
1	AL 1756	41.6	37.3	10.34	83[a#]	T
2	AL 1849	43.6	37.6	13.76	83[a]	T
3	H-02-28	43.1	36.1	16.24	78[ab]	T
4	H-2001-25	47.3	38.5	18.60	75[ab]	T
5	AL 1758	42.0	36.1	14.05	75[ab]	T
6	AL 15	39.2	31.6	19.38	75[ab]	T
7	PAU 881	43.4	30.5	29.72	66[abc]	MT
8	H-2000-14	38.7	29.5	23.77	62[abc]	MT
9	H-02-59	37.9	24.3	35.88	62[abc]	MT
10	SGBS 6	34.4	22.7	34.01	58[abcd]	MT
11	H-2003-14	35.0	24.3	30.57	56[abcde]	MT
12	AL 1760	42.1	26.4	37.29	44[bcdef]	S
13	Pusa 992	38.2	20.2	47.12	37[cdefg]	S
14	ICPA 2039	35.7	18.6	47.90	37[cdefg]	S
15	UPAS 120	46.0	27.6	40.00	23[defg]	HS
16	AH-06-5	41.4	25.4	38.65	20[efg]	HS
17	ICPL 20128	36.7	22.3	39.24	18[fg]	HS
18	JBP 110B	32.5	17.8	45.23	18[fg]	HS
19	AH-06-12	42.5	24.3	42.82	11[fg]	HS
20	AH-06-7	41.1	24.5	40.39	6[g]	HS
21	ICP 14085	30.2	17.6	41.72	0[g]	HS
22	ICPL 99051	38.4	21.3	44.53	0[g]	HS
23	MAL 15	33.3	17.8	46.55	0[g]	HS
24	ICP 5028	35.6	18.7	47.47	0[g]	HS
25	ICPL 332	32.5	16.7	48.62	0[g]	HS
26	AH-06-9	47.2	23.4	50.42	0[g]	HS
27	AH-09-9	47.6	22.3	53.15	0[g]	HS
28	ICPL 99050	36.2	16.3	54.97	0[g]	HS
	Mean	**39.40**	**25.33**	**36.19**	**35.25**	
	CD (0.05)	12.43	10.32	19.17	18.76	
	MSD				37.59	

** T: Tolerant (75-90%PS); MT: Moderately Tolerant (50-75%PS); S: Sensitive (25-49%PS); HS: Highly Sensitive (<25%PS); MSD: Minimum significant difference; #Same letters indicate non-significant while different letters indicate significant differences among the genotypes*

Table 7. Correlation between various traits in pigeonpea genotypes under *in vivo* conditions

Traits	Chlorophyll content before treatment		Chlorophyll content after treatment		Reduction in chlorophyll content (%)	
	WL#	SAL#	WL	SAL	WL	SAL
Plant survival (%)	0.498**	0.286	0.878**	0.830**	-0.950**	-0.902**
Chlorophyll content before treatment			0.799**	0.652**	-0.481**	-0.309
Chlorophyll content after treatment					-0.908**	-0.919**

*# WL: Water logging conditions; SAL: Saline conditions; **Significant at 1% level*

the correlation was non-significant between these two traits.

Considering results of both *in vitro* and *in vivo* conditions together, one genotype, AL 1849, was found highly tolerant or tolerant under both water logging and saline conditions, while six genotypes namely H-2000-14,H-2003-14, H-02-28, AL 15, AL 1756 and AL 1758 were found highly tolerant or tolerant under water logging and tolerant or moderately tolerant under saline conditions. Four genotypes namely MAL 15, UPAS 120, ICPL 99050 and ICPL 99051 were found sensitive or highly sensitive to both water logging and saline treatments under *in vitro* and *in vivo* conditions.

4. DISCUSSION

The results of the present study indicated existence of large phenotypic variability among the genotypes screened *in vitro* under water logging and saline conditions. These results were in accordance with the results of previous studies [30-32]. None of the genotypes was found to be highly tolerant under saline conditions, while six genotypes were found highly tolerant under water logging conditions. However, some of the genotypes showed similar reactions under water logging and saline conditions, but majority of the genotypes changed their reaction and showed lower level of tolerance to saline conditions as compared to their reaction under water logging conditions. Also the overall lower mean values for all the traits like germination percentage, seedling length, seedling dry weight and seedling vigor index under saline conditions as compared to water logging conditions. These results indicated that most of the pigeonpea genotypes used in the present study were more sensitive to saline conditions as compared to water logging conditions. Water logging results in anoxic conditions due to which respiration and electron transport are inhibited and ATP formation is decreased resulting in low seed viability and poor germination [33,34]. Seed germination rate has been used as phenotypic marker for selection of salinity tolerant pigeonpea cultivars [35] where they observed genotypic variability to a greater extent under salt stress conditions. Earlier in chickpea, the parameters like germination percentage, radicle and plumule length and plant survival at maturity have been found effective in differentiating tolerant and sensitive genotypes under saline conditions [36].

The correlation coefficients between various traits indicated that genotypes having higher germination percentage will generally have higher seedling length, seedling dry weight and seedling vigor index. For instance the genotypes like AH-06-7 and H-2000-14 recorded higher germination percentage, also had higher seedling length, seedling dry weight and seedling vigor index, whereas genotypes like UPAS 120 and MAL 15 recorded lower germination percentage, also had lower seedling length, seedling dry weight and seedling vigor index. Seedling vigor index of seedlings was also found to be positively correlated with germination percentage, root length and seedling dry weight in previous studies [37,38].

In case of *in vivo* studies, significant differences were observed for chlorophyll content before as well as after treatments, reduction in chlorophyll content after treatment and for plant survival under both water logging and saline conditions indicated the existence of large genetic variability for both water logging and salinity tolerance in pigeonpea. Reduction in survival rate might be due to anoxia caused by water logging and salinity stresses. Overall, the lower mean values for chlorophyll content after salinity treatment and plant survival and high values for reduction in chlorophyll content indicated that saline conditions adversely affected the metabolism of plants which led to plant mortality.

The results revealed large extent of phenotypic variability in pigeonpea germplasm for tolerance to water logging and salinity stresses based on plant survival and reduction in chlorophyll content under field conditions. This variability can be exploited in breeding programme for developing high yielding cultivars having tolerance to water logging and salinity stress conditions [39]. In chickpea also, plant survival was taken as an important parameter for identifying tolerant genotypes under salinity stress [26]. Generally, it has been observed that water logging leads to low levels of oxygen in plants leading to internal water deficit. Oxygen deficiency leads to decline in net photosynthetic rate [40] which is due to reduced chlorophyll content and leaf area [41] and also due to stomata closure under stress [42]. Water logging results in wilting, chlorosis, senescence and abscission of leaves which may be due to reduced uptake and mobilization of nutrients. Reduction in chlorophyll content was also reported in pigeonpea [43,44], wheat [45], maize [46] and *Vigna sinensis* [47]. The highly significant negative correlation between reduction in chlorophyll content and plant survival indicated that high reduction in chlorophyll after water logging and saline treatments disrupted plant metabolism which led to plant mortality.

The genotype AL 1849 which was found highly tolerant or tolerant in both *in vitro* and *in vivo* condition under both water logging and saline conditions and the six genotypes namely H-2000-14, H-2003-14, H-02-28, AL 15, AL 1756 and AL 1758 which were found highly tolerant or tolerant in water logging and tolerant or moderately tolerant under saline conditions can be further evaluated under field conditions for their utility as cultivar as such or as donor for breeding for water logging and salinity tolerance. The genotypes which were found highly tolerant or tolerant and highly sensitive in both *in vitro* and *in vivo* conditions under water logging and saline conditions can be used for developing appropriate populations for genetic studies. Subarao et al. [6] studied comparative salinity tolerance among pigeonpea genotypes and their wild relatives. A large genetic variation was also observed earlier in cultivated and wild species of pigeonpea, especially *C. scarabaeoides*, for salinity tolerance [5]. As the wild relatives of pigeonpea are rich reservoir of genes for various abiotic stresses, including water logging and salinity tolerance, they can be exploited for introgressing desirable genes into cultivated background to minimize the adverse effects caused by such stresses [48]. It will help to broaden the narrow genetic base of pigeonpea which has always been a limiting factor in its breeding [37,49].

5. CONCLUSIONS

Results of the present study indicated that both water logging and saline conditions affect the germination under *in vitro* conditions and plant survival under *in vivo* conditions to varying extent suggesting that both stresses alone or together pose a serious threat to pigeonpea cultivation. Thus, a holistic approach towards the management of these stresses is needed by developing water logging and salinity tolerant cultivars. Some of the genotypes were found promising under both *in vitro* and *in vivo* conditions there by indicating correlation for seedling and adult plant tolerance which can be exploited in breeding programmes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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