

Original Research Article

Genetic variability for water logging and salinity tolerance in pigeonpea (*Cajanus cajan* (L.) Millsp.) under *in vitro* and *in vivo* conditions

Pigeonpea (*Cajanus cajan* (L.) Millsp.) genotypes acquired from diverse sources, were screened for water logging and salinity tolerance under laboratory (60 genotypes) and field conditions (28 genotypes). Analysis of variance revealed significant differences among the genotypes for various traits. Based on vigour 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, none of the genotypes was found highly tolerant, however, seven genotypes (AL 1849, AH-06-7, H-2000-14, H-2001-25, H-2003-14, ICP 5028, JBP 110B) were found tolerant, 14 moderately tolerant, 17 sensitive and 22 highly sensitive. Mean values for germination%, seedling length, seedling dry weight and 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, none of the genotypes was found highly tolerant, however, 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.

Key words: Pigeonpea, water logging, salinity, tolerance, abiotic stresses

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 (Abdul et al. 2006, Rodriguez et al. 2005). Pigeonpea is reported to be highly sensitive to water logging (Chauhan et al. 1997; Perera et al. 2001) and salinity (Rao et al. 1981, Subbarao et al. 1991). Water logging and salinity stresses are important yield constraints in pigeonpea as water logging blocks oxygen supply to roots which hampers root permeability (Else et al. 2001) and salinity impairs seed germination, reduces nodule formation, retards plant development and finally reduces crop yield (Greenway &



Munns 1980). It is reported that germination and early vegetative growth stages in pigeonpea are more affected by water logging than the flowering stage (Singh et al. 1986). Water logging is reported to delay flowering, reduce vegetative growth, photosynthetic rate, biomass and grain yield (Chauhan et al. 1997, Matsunaga et al. 1996, Takele and McDavid 1995, Sarode et al. 2007). 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 (Matsunaga et al. 1991). Soil salinity is another major abiotic stress that affects plant growth, development and yield by causing physiological and biochemical changes in plants (Munns 2002). It leads to osmotic stress and interferes with mineral nutrients uptake (Chikelu et al. 2007). Seedling and reproductive stages in plants are mainly affected by salinity stress (Munns and James 2003). Salinity together with water logging can cause deleterious effects in plants posing major threat to crop productivity (Barrett-Lennard 2003). 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 (Choudhary et al. 2011). A large genetic variation has been found in different cultivated and wild species of pigeonpea for salinity tolerance (Rao et al. 1981; Karajol and Naik 2011). 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 (Pereraa et al. 2001; Sarode et al. 2007). 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 (Ceccarelli et al. 2010). 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.

MATERIALS AND METHODS

Experimental material

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.

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 (0.1g HgCl₂ dissolved in 100 ml water) 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 30mM NaCl solution in case of salinity treatment, respectively, for 7 days (168 hrs). 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°C for 7 days.

Observations

Data were recorded after 7 days of incubation for both the treatments for germination%, seedling length (plumule and radicle), seedling dry weight (plumule and radicle) and 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 1mm 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°C for 3 days. Vigor index was calculated using the formula: Vigor Index (VI) = mean germination% X mean seedling dry weight (mg). Based on vigour 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).

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.

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 (1992), the genotypes were categorized based on final plant survival% (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.

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 (1989) and adapted by Cheema and Singh (1991) using the following equation: $Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$, where: Y_{ij} is the j^{th} observation of the i^{th} treatment, μ is the population mean, τ_i is the treatment effect of the i^{th} treatment, and ε_{ij} is the random error. All comparisons were made at 5% level of significance. The correlation coefficients were also worked out among the various traits following Al-Jibouri et al. (1958).

RESULTS

***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%, seedling length, seedling dry weight and 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%, seedling length, seedling dry weight and 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 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% of highly tolerant and tolerant genotypes was more than 75%. Out of the 60 genotypes, 3 highly sensitive genotypes recorded zero values for germination%, seedling length, seedling dry weight and vigor index (Data not shown). Under saline conditions, the genotype H-2000-14 had the maximum 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 vigor index (data not shown). However, some genotypes like AL 1760, ICPL 99051 and UPAS 120 recorded comparatively higher germination% (30-38.5%), but their 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%, seedling length, seedling dry weight and vigor index were lower under saline conditions (63.1%, 6.4cm, 15.4mg, 1090.9; in that order) as compared to water logging conditions (72.8%, 7.7cm, 19.1mg, 1477.3; in that order). 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.

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%, seedling length, seedling dry weight and vigor index under both water logging and saline conditions.

***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% 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%. The reduction in chlorophyll content was found to be <10% in highly tolerant genotypes, while it was >31% in sensitive or highly sensitive 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.

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 (Table 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% 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% 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 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.

DISCUSSION

254 In the present study a diverse set of 60 genotypes was screened *in vitro* and significant
 255 differences among the genotypes for all the traits studied viz., germination%, seedling length,
 256 seedling dry weight and vigor index for individual years as well as pooled data clearly indicated
 257 that large genetic variability exists among pigeonpea genotypes included in the study for both
 258 water logging and saline conditions. These results were in accordance with the results of
 259 previous studies (Dubey and Asthana 1987, Krishnamurthy et al. 2012, Sultana et al. 2012).
 260 Interestingly, none of the genotypes was found to be highly tolerant under saline conditions,
 261 while six genotypes were found highly tolerant under water logging conditions. However, some
 262 of the genotypes showed similar reactions under water logging and saline conditions, but
 263 majority of the genotypes changed their reaction and showed lower level of tolerance to saline
 264 conditions as compared to their reaction under water logging conditions. Also the overall lower
 265 mean values for all the traits like germination%, seedling length, seedling dry weight and vigor
 266 index under saline conditions as compared to water logging conditions. These results indicated
 267 that most of the pigeonpea genotypes used in the present study were more sensitive to saline
 268 conditions as compared to water logging conditions. Water logging results in anoxic conditions
 269 due to which respiration and electron transport are inhibited and ATP formation is decreased
 270 resulting in low seed viability and poor germination (Johnson et al. 1989; Tsai et al. 1997). Seed
 271 germination rate has been used as phenotypic marker for selection of salinity tolerant pigeonpea
 272 cultivars (Krishna and Naik 2011) where they observed genotypic variability to a greater extent
 273 under salt stress conditions. Earlier in chickpea, the parameters like germination%, radicle and
 274 plumule length and plant survival at maturity have been found effective in differentiating tolerant
 275 and sensitive genotypes under saline conditions (Chauhan et al. 2012).

276 The correlation coefficients between various traits indicated that genotypes having higher
 277 germination% will generally have higher seedling length, seedling dry weight and vigor index.
 278 For instance the genotypes like AH-06-7 and H-2000-14 recorded higher germination%, also had
 279 higher seedling length, seedling dry weight and vigor index, whereas genotypes like UPAS 120
 280 and MAL 15 recorded lower germination%, also had lower seedling length, seedling dry weight
 281 and vigor index. Vigor index of seedlings was also found to be positively correlated with
 282 germination%, root length and seedling dry weight in previous studies (Kumar et al. 2011;
 283 Bharathi and Saxena 2012).

284 Severe yield losses are caused by prolonged rainfall during rainy season in tropical and
 285 sub-tropical regions. Anoxic soil conditions are produced by excess water and consequently,
 286 plant roots suffer hypoxia or anoxia. Min and Bothalomew (2005) reported gradual decrease in
 287 relative water content under water logging and plants wilt visually within a few hours of
 288 imposing water logging stress. It is observed that water logging induced decrease in leaf water
 289 potential (Else et al. 1995) and membrane damage (Rawyler et al. 2002). Water logging also
 290 caused reduction in stomata conductance and plants exposed to water logging exhibit increased
 291 stomata resistance and limited water uptake leading to internal water deficit (Parent et al. 2008).
 292 In case of *in vivo* studies, significant differences were observed for chlorophyll content before as
 293 well as after treatments, reduction in chlorophyll content after treatment and for plant survival%
 294 under both water logging and saline conditions indicated the existence of large genetic variability
 295 for both water logging and salinity tolerance in pigeonpea. Reduction in survival rate might be
 296 due to anoxia caused by water logging and salinity stresses. Interestingly, none of the genotypes
 297 was found highly tolerant under saline conditions and the change in reaction of highly tolerant
 298 genotypes to tolerant and from tolerant reaction under water logging to moderately tolerant
 299 reaction under saline conditions indicated more sensitivity of genotypes to saline conditions as
 300 also observed in case of *in vitro* studies. Overall, the lower mean values for chlorophyll content
 301 after salinity treatment and plant survival% and high values for reduction in chlorophyll content
 302 indicated that saline conditions adversely affected the photosynthesis of plants which led to plant
 303 mortality.

304 The results revealed large extent of genetic variability in pigeonpea germplasm for
 305 tolerance to water logging and salinity stresses based on plant survival (%) and reduction in
 306 chlorophyll content (%) under field conditions. This variability can be exploited in breeding
 307 programme for developing high yielding cultivars having tolerance to water logging and salinity
 308 stress conditions (Dua and Sharma 1996). In chickpea also, plant survival% was taken as an
 309 important parameter for identifying tolerant genotypes under salinity stress (Dua 1992).
 310 Generally, it has been observed that water logging leads to low levels of oxygen in plants leading
 311 to internal water deficit. Oxygen deficiency leads to decline in net photosynthetic rate (Ashraf et
 312 al. 2011) which is due to reduced chlorophyll content and leaf area (Malik et al. 2001) and also
 313 due to stomata closure under stress (Ashraf and Arfan 2005). Water logging results in wilting,
 314 chlorosis, senescence and abscission of leaves which may be due to reduced uptake and

mobilization of nutrients. Kumutha et al. (2008, 2009) also reported reduction in chlorophyll content in pigeonpea genotypes due to water logging. Decrease in chlorophyll content under water logging condition was also observed in wheat (Collaku and Harrison 2002), maize (Prasad et al. 2004) and *Vigna sinensis* (Younis et al. 2003). 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 photosynthesis 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 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. (1991) studied comparative salinity tolerance among pigeonpea genotypes and their wild relatives. Techniques like embryo rescue and doubled haploid breeding were also used to identify stress tolerant recombinants in pigeonpea (Pratap et al. 2010). A large genetic variation was also observed earlier in cultivated and wild species of pigeonpea, especially *C. scarabaeoides*, for salinity tolerance (Rao et al. 1981). 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 (Sharma et al. 2003). It will help to broaden the narrow genetic base of pigeonpea which has always been a limiting factor in its breeding (Yang et al. 2006; Kumar et al. 2011).

CONCLUSIONS

It is evident from the results obtained from the present and previous studies 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 water logging and salinity 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. Further, there is a need to study the genetics of water logging and salinity tolerance taking lead from the present study and to design appropriate breeding strategies for developing water logging and salinity tolerant cultivars by using tolerant genotypes as donors in breeding programmes to minimize the yield losses caused by these abiotic stresses in pigeonpea. 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.

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490

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

Categories based on 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)

496 **Table 2. Germination%, seedling length, dry seedling weight, vigor index and tolerance category of**
 497 **pigeonpea genotypes under water logging conditions (mean of two years' data)**
 498

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

499 *HT: Highly tolerant (VI: >2000); T: Tolerant (VI: 1500-2000); MT: Moderately Tolerant (VI: 1000-
 500 1499.99); S: Sensitive (VI: 500-999.99); HS: Highly Sensitive (VI: <500)

Table 3. Germination%, seedling length, dry seedling weight, 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)	Vigor Index (VI)	Tolerance Category*
1	H-2000-14	81.5	10.8	22.6	1841.9	T
2	ICP 5028	75.5	7.0	22.6	1706.3	T
3	AH-06-7	75.5	9.6	22.1	1668.5	T
4	H-2001-25	82.0	9.2	20.2	1656.4	T
5	H-2003-14	83.0	9.2	19.9	1651.7	T
6	AL 1849	85.0	8.1	19.4	1649.0	T
7	JBP 110B	82.5	7.4	18.3	1509.8	T
8	AL 1756	78.5	5.7	18.4	1444.4	MT
9	ICPL 20128	78.5	6.6	17.9	1405.1	MT
10	SGBS 6	66.5	8.4	21.9	1456.4	MT
11	H-02-28	73.5	7.1	18.4	1352.4	MT
12	AL 1758	76.0	4.4	17.6	1337.6	MT
13	AL 15	72.5	8.7	18.3	1326.8	MT
14	ICPA 2039	75.0	6.7	16.2	1215.0	MT
15	AH-06-9	69.0	6.2	16.6	1145.4	MT
16	AH-06-12	58.5	7.2	19.5	1140.8	MT
17	H-02-59	69.0	8.0	16.4	1131.6	MT
18	AH-09-9	60.0	7.0	17.3	1038.0	MT
19	AH-06-5	68.5	6.7	14.5	993.3	S
20	Pusa 992	60.0	6.7	15.1	906.0	S
21	PAU 881	66.5	7.3	13.6	904.4	S
22	ICP 14085	53.0	6.0	15.2	805.6	S
23	ICPL 99050	60.0	3.9	11.0	660.0	S
24	AL 1760	38.5	3.8	6.0	231.0	HS
25	ICPL 99051	38.5	2.8	4.9	188.7	HS
26	UPAS 120	30.0	5.0	6.0	180.0	HS
27	ICPL 332	5.0	0.0	0.0	0.0	HS
28	MAL 15	5.0	0.0	0.0	0.0	HS
	Mean	63.1	6.4	15.4	1090.9	
	CD (0.05)	21.72	3.46	5.17	315.42	

* T: Tolerant (VI: 1500-2000); MT: Moderately Tolerant (VI: 1000-1499.99); S: Sensitive (VI: 500-999.99; HS: Highly Sensitive (VI: <500)

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

Traits	Seedling length (cm)		Seedling dry weight (mg)		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**

508 # WL: Water logging conditions; SAL: Saline conditions

509 **P = 0.01

510

511 **Table 5. Chlorophyll content and plant survival of some tolerant and sensitive pigeonpea genotypes**
512 **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	HT
2	H-02-28	43.1	39.3	8.82	100	HT
3	AL 15	39.2	35.6	9.18	100	HT
4	H-2000-14	38.7	34.9	9.82	100	HT
5	AH-06-12	42.5	37.5	11.76	83	T
6	H-2003-14	35.0	29.4	16.00	83	T
7	AL 1756	41.6	36.1	13.22	80	T
8	AH-06-9	47.2	40.5	14.19	80	T
9	AL 1758	42.0	33.8	19.52	75	T
10	ICPL 332	32.5	25.5	21.53	67	MT
11	H-2001-25	47.3	36.4	23.04	67	MT
12	AH-09-9	47.6	35.4	25.63	67	MT
13	AL 1760	42.1	30.7	27.08	67	MT
14	H-02-59	37.9	26.7	29.55	66	MT
15	PAU 881	43.4	32.4	25.35	60	MT
16	AH-06-5	41.4	30.8	25.60	60	MT
17	SGBS 6	34.4	24.8	27.90	55	MT
18	AH-06-7	41.1	32.2	21.65	50	MT
19	ICPA 2039	35.7	20.6	42.30	34	S
20	Pusa 992	38.2	21.4	43.98	33	S
21	ICP 5028	35.6	24.5	31.18	25	S
22	UPAS 120	46.0	30.2	34.35	25	S
23	JBP 110B	32.5	19.5	40.00	23	HS
24	ICPL 20128	36.7	21.4	41.69	15	HS
25	MAL 15	33.3	19.3	42.04	0	HS
26	ICP 14085	30.2	16.5	45.36	0	HS
27	ICPL 99051	38.4	20.5	46.61	0	HS
28	ICPL 99050	36.2	18.6	48.62	0	HS
	Mean	39.41	29.15	26.78	54.11	
	CD (0.05)	12.43	11.26	17.67	21.46	

513 *HT: Highly tolerant (>90% PS); T: Tolerant (75-90%PS); MT: Moderately Tolerant (50-75%PS);
514 S: Sensitive (25-49%PS); HS: Highly Sensitive (<25%PS)

515
516

517 **Table 6. Chlorophyll content and plant survival of some tolerant and sensitive pigeonpea genotypes**
518 **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	T
2	AL 1849	43.6	37.6	13.76	83	T
3	H-02-28	43.1	36.1	16.24	78	T
4	H-2001-25	47.3	38.5	18.60	75	T
5	AL 1758	42.0	36.1	14.05	75	T
6	AL 15	39.2	31.6	19.38	75	T
7	PAU 881	43.4	30.5	29.72	66	MT
8	H-2000-14	38.7	29.5	23.77	62	MT
9	H-02-59	37.9	24.3	35.88	62	MT
10	SGBS 6	34.4	22.7	34.01	58	MT
11	H-2003-14	35.0	24.3	30.57	56	MT
12	AL 1760	42.1	26.4	37.29	44	S
13	Pusa 992	38.2	20.2	47.12	37	S
14	ICPA 2039	35.7	18.6	47.90	37	S
15	UPAS 120	46.0	27.6	40.00	23	HS
16	AH-06-5	41.4	25.4	38.65	20	HS
17	ICPL 20128	36.7	22.3	39.24	18	HS
18	JBP 110B	32.5	17.8	45.23	18	HS
19	AH-06-12	42.5	24.3	42.82	11	HS
20	AH-06-7	41.1	24.5	40.39	6	HS
21	ICP 14085	30.2	17.6	41.72	0	HS
22	ICPL 99051	38.4	21.3	44.53	0	HS
23	MAL 15	33.3	17.8	46.55	0	HS
24	ICP 5028	35.6	18.7	47.47	0	HS
25	ICPL 332	32.5	16.7	48.62	0	HS
26	AH-06-9	47.2	23.4	50.42	0	HS
27	AH-09-9	47.6	22.3	53.15	0	HS
28	ICPL 99050	36.2	16.3	54.97	0	HS
	Mean	39.40	25.33	36.19	35.25	
	CD (0.05)	12.43	10.32	19.17	18.76	

519 * T: Tolerant (75-90%PS); MT: Moderately Tolerant (50-75%PS); S: Sensitive (25-49%PS);
520 HS: Highly Sensitive (<25%PS)

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522

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524 **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**

525 # WL: Water logging conditions; SAL: Saline conditions

526 **P = 0.01

527