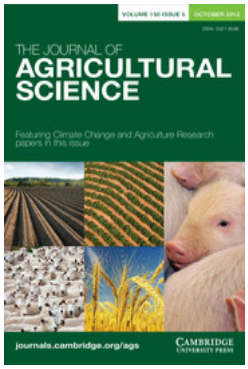

Waterlogging tolerance in pigeonpea (*Cajanus cajan* (L.) Millsp.): genotypic variability and identification of tolerant genotypes

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CROPS AND SOILS RESEARCH PAPER

Waterlogging tolerance in pigeonpea (*Cajanus cajan* (L.) Millsp.): genotypic variability and identification of tolerant genotypes

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SUMMARY

Pigeonpea is an important legume crop of the semi-arid tropics. In India, pigeonpea is mostly grown in areas prone to waterlogging, resulting in major production losses. It is imperative to identify genotypes that show tolerance at critical crop growth stages to prevent these losses. A selection of 272 diverse pigeonpea accessions was evaluated for seed submergence tolerance for different durations (0, 120, 144, 168 and 192 h) under *in vitro* conditions in the laboratory. All genotypes exhibited high (0·79–0·98) survival rates for up to 120 h of submergence. After 192 h of submergence, the hybrids as a group exhibited significantly higher survival rates (0·79) than the germplasm (0·71), elite breeding lines (0·68) and commercial varieties (0·58). Ninety-six genotypes representing the phenotypic variation observed during laboratory screening were further evaluated for waterlogging tolerance at the early seedling stage using pots, and survival rates were recorded for 8 days after completion of the stress treatment. Forty-nine of these 96 genotypes, representing the phenotypic variation for waterlogging tolerance, were chosen in order to evaluate their performance under natural field conditions. The following cultivated varieties and hybrids were identified as tolerant after three levels of testing (*in vitro,* in pots and in the field): ICPH 2431, ICPH 2740, ICPH 2671, ICPH 4187, MAL 9, LRG 30, Maruti, ICPL 20128, ICPL 332, ICPL 20237, ICPL 20238, Asha and MAL 15. These materials can be used as sources of waterlogging tolerance in breeding programmes.

INTRODUCTION

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is an important legume crop, grown mainly in the semi-arid tropical (SAT) regions of Asia, Africa, Latin America and the Caribbean (Saxena 2008). The total global area planted with pigeonpea was 4·5 million ha in 2009 (FAO 2009). India is the main producer (3·38 million ha) of pigeonpea and imports an additional 400 000 t (tonnes) from Myanmar and Africa to meet domestic needs. Although dozens of pigeonpea varieties have been released, productivity has remained stagnant at *c.* 700 kg/ha (FAO 2009) due to various genetic, management, biotic and abiotic constraints. Since the area of cultivation is not likely to increase, breeding efforts focusing on breaking the yield barrier through hybrid breeding (Saxena *et al.* 2010) and increasing sustainability of production through incorporating resistance to major biotic and abiotic stresses are needed to increase production and productivity.

In India, waterlogging during the monsoon season (June–September) is caused by erratic and prolonged rains and represents an important production constraint. Since pigeonpea is primarily grown in deep vertisols and in areas with mean annual rainfall of 600–1500 mm, waterlogging becomes a serious problem (Chaudhary *et al.* 2011). It occurs when the water table attains a level at which the soil pores in the root zone of the plants are fully saturated, and restricts normal air circulation. Consequently, oxygen levels in the soil decline and carbon dioxide concentration increases, which adversely affects the growth and development of plant roots (Vartapetian & Jackson 1997). The inability of dryland crop species, such as pigeonpea, to endure low oxygen conditions at the rhizosphere level, results in substantial yield losses.

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The roots of most plants are highly susceptible to anaerobic conditions, which support a unique microbial community; this severely affects the nutrient balance of the soil (Ponnamperuma 1972; Levitt 1980; Laanbroek 1990) and plant health. Soon after the onset of short periods of excessive moisture conditions, obligate aerobic bacteria become inactive, and facultative/obligate anaerobic bacteria become active and dominate the micro-flora in the inundated soils (Sachs *et al.* 1980; Jackson 1990). Another adverse effect of waterlogging is leaching of important minerals or essential intermediate metabolites from roots into water (Laanbroek 1990; Rathore *et al.* 1997). Waterlogging also induces certain changes in the physical and chemical properties of the rhizosphere. The gaseous diffusion rates in flooded soils are *c.* 100 times lower than normal (Kennedy *et al.* 1992) and respiration of plant roots, soil micro-flora and fauna leads to rapid exhaustion of soil oxygen, thereby causing anaerobiosis.

In India, *c.* 8·5 million ha of arable land is prone to waterlogging. A recent comparative analysis of pigeonpea growing regions revealed that *c.* 1·1 million ha of the total area (3·38 million ha) under pigeonpea is affected by excess soil moisture, causing an annual loss of 25–30% in production (Chaudhary *et al.* 2011).

Since waterlogging is an important yield constraint in pigeonpea, it is imperative to identify a viable economic solution for this problem. Although certain soil management options such as the use of raised sloping seed beds, ridge sowing and transplanting of seedlings help in reducing losses caused by waterlogging (Abebe *et al.* 1992), these options are not economically viable for the resource-poor farming community of the SAT. Hence, the use of tolerant genotypes is the most economical and simple way to minimize losses. According to Khare *et al.* (2002), the initial establishment of seedlings is the most critical factor for pigeonpea in waterlogging-prone areas. Therefore, the objective of the present study was to assess the genotypic variability for waterlogging tolerance in pigeonpea and to identify genotypes capable of withstanding waterlogging stress conditions at the sowing and early seedling stages.

MATERIALS AND METHODS

Critical evaluation of rainfall pattern during the monsoon season (June–September) at Patancheru, Andhra Pradesh, India (17°32′N, 78°16′E, 545 m asl) and its overlap with pigeonpea growing stages allowed identification of the most waterlogging-vulnerable stages as well as the time of occurrence. Pigeonpea receives maximum rain during the months of July and August (Fig. 1). Since the seed (just after sowing) and early seedling stage (15–35 days) in pigeonpea are very sensitive to waterlogging (Fig. 1), the screening methodology was optimized taking into account the crop growth stages that were most severely affected by waterlogging.

Laboratory screening (seed stage evaluation)

Seeds of 272 pigeonpea genotypes differing in maturity, seed colour and origin (Table 1) were evaluated for water submergence tolerance under laboratory conditions using a simple screening method that allowed evaluation of many genotypes in a short period of time. The genotypes used consisted of 114 elite breeding lines (ICPLs), 91 germplasm accessions (ICPs), 34 pure line varieties and 33 cytoplasmic male-sterility-based hybrids (ICPHs) (Table 1). Seeds of all genotypes were obtained from the global gene bank of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and from the ICRISAT pigeonpea breeding programme (Table 1). Seeds of all the genotypes were collected from the 2009 crop season and stored at 2–4 °C until used in the experiment. To avoid the incidence of fungal infection, the seeds were treated with *Thiram* (*dithiocarbamate*) dust (3 g/kg seeds) before imposing submergence treatments. The genotypes were classified into different groups based on maturity duration (short, medium or late) and seed coat colour (light or dark coloured). The materials included 196 medium-to-late (160–270 days) and 76 short (120–155 days to 75% maturity) maturing genotypes (Table 1). A total of 203 genotypes had dark coloured (black, purple, dark brown and brown) seeds, whereas 69 lines had light coloured (white, off-white and cream) seeds (Table 1). The experiment was conducted under laboratory conditions at ICRISAT, Patancheru, Andhra Pradesh, India in 2009.

The genotypes were subjected to water submergence treatments in 200 ml beakers (100 mm diameter) containing 100 ml of water at 23 ± 1 °C. The submergence treatments were established as a function of the submersion time (S120, S144, S168 and S192 for groups of seeds submerged for 120, 144, 168 and 192 h, respectively). A baseline (S0 = no submergence treatment) germination test was performed by placing 20 seeds of each genotype between two paper towels

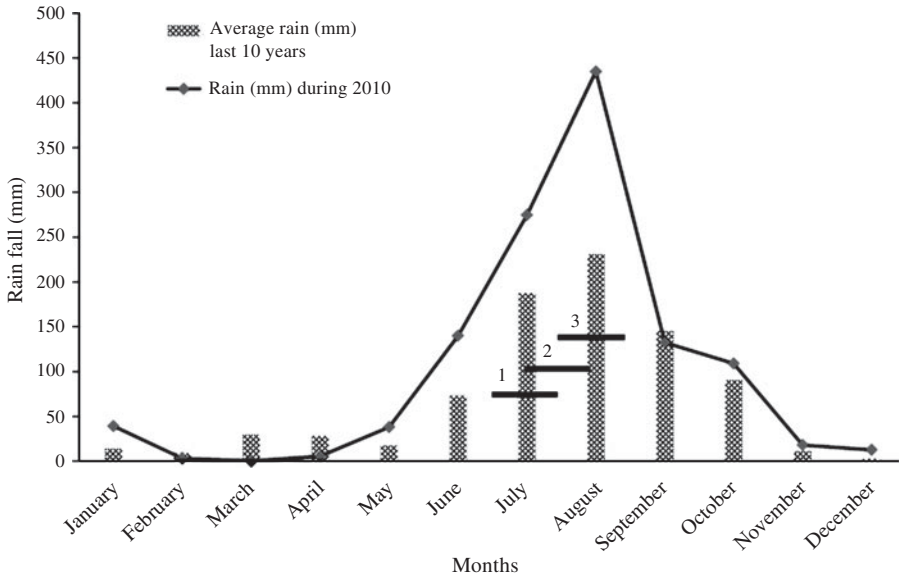

Fig. 1. Average rainfall distribution at Patancheru, India, for the last 10 years and during the 2010 pigeonpea growing season. The horizontal lines indicate the duration of the crop growth stages potentially affected by waterlogging. Line 1: planting time window (seed stage). Line 2: 15 days old seedling window (early seedling stage). Line 3: 35 days old seedling window (seedling stage).

in plastic Petri dishes and maintaining humidity as necessary. The durations of S120, S144 and S168 were comparable with field observations of soil waterlogging timing at the study site, especially during rainy years. The S192 duration was specifically selected for the present experiment in order to check seed viability under extended submergence (8 days). Each test sample consisted of 20 seeds and three replications. After completing each stress period, seeds were dried on a filter paper for 4–5 h to drain excess water and then placed on a paper towel in a Petri dish and kept for germination at a constant temperature (25 ± 2 °C) in a dark room. The seeds were considered to have germinated when radicle length reached a minimum of 2 mm. The germinated seeds were counted and percent survival was calculated 5–6 days after completing stress treatment.

Analysis of variance (ANOVA) was performed using SAS software (SAS 2008) to assess the variation among genotypes, submergence durations and their interactions. The germination data (per cent) were arcsine-transformed (Gomez & Gomez 1984) to induce normality of the data set. In addition, further analysis was also performed to compare relative survival rate of the four genotype groups within submergence durations using linear contrasts. The associations of survival rates under the different water submergence treatments with seed colour and maturity duration were assessed using a *t*-test.

Pot screening (early seedling stage evaluation)

Ninety-six out of 272 pigeonpea genotypes representing the four genotype groups (hybrids, lines, germplasm and varieties) that showed tolerance or moderate tolerance and susceptibility to water submergence at the seed stage during laboratory screening were further evaluated for waterlogging tolerance at the seedling stage (15 days). The evaluation was conducted using plastic pots of 102 mm diameter, with three 5·0 mm diameter perforations in the base. Pots were filled with a mixture of vertisols and farmyard manure (FYM); soil : FYM ratio was 50 : 1 (V/V). Fertilizer (nitrogen, phosphorus and potassium, NPK) was also applied as basal dose; the amount was calculated on a soil weight basis and thoroughly mixed into the soil. Each pot was weighed after filling in order to maintain the same quantity of soil and maintain constant moisture in each pot. For each genotype, five pots were prepared (four pots for imposing stress treatment and one kept as a control, i.e. no treatment). Filled pots were sown on 24 February 2010, with 5 seeds/pot at 20 mm depth using a completely randomized design. All pots were kept in a glasshouse at an average temperature of 32 ± 2 °C. Before application of water stress treatment, the number of plants in each pot was counted. The stress treatment was imposed by submerging four pots in a tray filled with water in such a way that the pots surface remained

Table 1. *Description of pigeonpea genotypes (n = 272) tested for waterlogging tolerance. The materials represent four genotypic groups: germplasm 'ICP'; elite inbred lines 'ICPL', 'ICPA', 'ICPB'; hybrids 'ICPH' and varieties (in italics) belonging to two maturity groups (late-to-medium and short) and of different seed coat colour intensities, bold font indicates dark (brown, black, purple) and non-bold font indicates light (cream or white) seed coat colour*

Genotype	Maturity duration	Genotype	Maturity duration	Genotype	Maturity duration	Genotype	Maturity duration
ICP 11149	LD	**ICPH 3371**	MD	**ICPL 99061**	MD	**ICPH 3313**	SD
ICP 12780	LD	**ICPH 3461**	MD	***Asha***	MD	**ICPH 3341**	SD
ICP 13581	LD	**ICPH 3467**	MD	***BDN 1***	MD	**ICPH 3362**	SD
ICP 8094	LD	**ICPH 3481**	MD	***GAUT 90-1***	MD	**ICPH 3629**	SD
ICP 7035	LD	**ICPH 3740**	MD	***JBP 110-B***	MD	**ICPH 4329**	SD
ICPH 2671	LD	**ICPH 3762**	MD	***JBP 36-B***	MD	**ICPA 2039**	SD
ICPL 20092	LD	**ICPH 3766**	MD	***LRG 30***	MD	**ICPB 2039**	SD
ICP 10948	MD	**ICPH 3964**	MD	***MAL 9***	MD	**ICPL 149**	SD
ICP 10960	MD	**ICPH 3992**	MD	***MAL 11***	MD	**ICPL 150**	SD
ICP 10987	MD	**ICPH 4031**	MD	***MAL 12***	MD	**ICPL 161**	SD
ICP 11059	MD	**ICPH 4104**	MD	***MAL 15***	MD	**ICPL 20**	SD
ICP 11130	MD	**ICPH 4183**	MD	***Maruti***	MD	**ICPL 20210**	SD
ICP 11145	MD	**ICPH 4187**	MD	ICP 11100	MD	**ICPL 20212**	SD
ICP 11150	MD	**ICPH 4275**	MD	ICP 11106	MD	**ICPL 20213**	SD
ICP 11378	MD	**ICPH 4301**	MD	ICP 11120	MD	**ICPL 20215**	SD
ICP 11447	MD	**ICPH 4304**	MD	ICP 11128	MD	**ICPL 20216**	SD
ICP 11681	MD	**ICPH 4305**	MD	ICP 11133	MD	**ICPL 20218**	SD
ICP 11811	MD	**ICPH 4322**	MD	ICP 11153	MD	**ICPL 20221**	SD
ICP 11813	MD	**ICPA 2043**	MD	ICP 11296	MD	**ICPL 20222**	SD
ICP 12024	MD	**ICPB 2043**	MD	ICP 1141	MD	**ICPL 20223**	SD
ICP 12057	MD	**ICPL 20058**	MD	ICP 11440	MD	**ICPL 20225**	SD
ICP 12176	MD	**ICPL 20093**	MD	ICP 11443	MD	**ICPL 20227**	SD
ICP 12714	MD	**ICPL 20094**	MD	ICP 12023	MD	**ICPL 20229**	SD
ICP 12739	MD	**ICPL 20095**	MD	ICP 12026	MD	**ICPL 20230**	SD
ICP 12747	MD	**ICPL 20096**	MD	ICP 12043	MD	**ICPL 20231**	SD
ICP 12750	MD	**ICPL 20097**	MD	ICP 12728	MD	**ICPL 20237**	SD
ICP 12751	MD	**ICPL 20099**	MD	ICP 12740	MD	**ICPL 20238**	SD
ICP 12761	MD	**ICPL 20100**	MD	ICP 12749	MD	**ICPL 20242**	SD
ICP 12792	MD	**ICPL 20101**	MD	ICP 1275	MD	**ICPL 81-9**	SD
ICP 12839	MD	**ICPL 20102**	MD	ICP 14085	MD	**ICPL 84031**	SD
ICP 13342	MD	**ICPL 20103**	MD	ICP 15200	MD	**ICPL 86005**	SD

ICP 13361	MD	**ICPL 20104**	MD	ICP 1575	MD	**ICPL 86022**	SD
ICP 13379	MD	**ICPL 20106**	MD	ICP 4928	MD	**ICPL 87**	SD
ICP 13384	MD	**ICPL 20108**	MD	ICP 7086	MD	**ICPL 87051**	SD
ICP 13389	MD	**ICPL 20110**	MD	ICP 7349	MD	**ICPL 87154**	SD
ICP 13391	MD	**ICPL 20113**	MD	ICP 7597	MD	**ICPL 88034**	SD
ICP 13392	MD	**ICPL 20114**	MD	ICP 7977	MD	**ICPL 90034**	SD
ICP 13395	MD	**ICPL 20116**	MD	ICP 8465	MD	**ICPL 91032**	SD
ICP 13402	MD	**ICPL 20117**	MD	ICPL20115	MD	**ICPL 92010**	SD
ICP 14092	MD	**ICPL 20119**	MD	ICPL 12761	MD	**ICPL 92041**	SD
ICP 14146	MD	**ICPL 20120**	MD	ICPL 20098	MD	**ICPL 92043**	SD
ICP 14282	MD	**ICPL 20122**	MD	ICPL 20105	MD	**ICPL 93101**	SD
ICP 14304	MD	**ICPL 20123**	MD	ICPL 20107	MD	**ICPL 93107**	SD
ICP 14318	MD	**ICPL 20125**	MD	ICPL 20109	MD	**ICPL 95040**	SD
ICP 14410	MD	**ICPL 20126**	MD	ICPL 20112	MD	**ICPL 98011**	SD
ICP 14712	MD	**ICPL 20127**	MD	ICPL 20118	MD	**ICPL 98013**	SD
ICP 14882	MD	**ICPL 20129**	MD	ICPL 20121	MD	***HPL 24***	SD
ICP 1571	MD	**ICPL 20130**	MD	ICPL 20124	MD	***UPAS 120***	SD
ICP 1941	MD	**ICPL 20132**	MD	ICPL 20128	MD	***VL-arhar 1***	SD
ICP 2376	MD	**ICPL 20133**	MD	ICPL 20131	MD	ICP 87051	SD
ICP 3782	MD	**ICPL 20219**	MD	ICPL 20135	MD	ICPL 87091	SD
ICP 4924	MD	**ICPL 20236**	MD	ICPL 20200	MD	ICPL 89	SD
ICP 5028	MD	**ICPL 20241**	MD	ICPL 96053	MD	ICPL 90030	SD
ICP 5429	MD	**ICPL 20243**	MD	ICPL 99044	MD	ICPL 90048	SD
ICP 5529	MD	**ICPL 20244**	MD	*BDN 2*	MD	ICPL 93017	SD
ICP 7193	MD	**ICPL 332**	MD	*BRG 2*	MD	*Kanchen*	SD
ICP 7201	MD	**ICPL 83057**	MD	*BRG 3*	MD	*SIPS 1*	SD
ICP 7741	MD	**ICPL 84060**	MD	*BRG1-(w)1*	MD	*SIPS 2*	SD
ICP 7815	MD	**ICPL 9048**	MD	*SGBS 3*	MD	*SIPS 4*	SD
ICP 8466	MD	**ICPL 92059**	MD	*SGBS 4*	MD	*SIPS 5*	SD
ICP 8920	MD	**ICPL 92067**	MD	*SGBS 6*	MD	*SIPS 6*	SD
ICP 8927	MD	**ICPL 96061**	MD	**ICPH 2363**	SD	*SIPS 7*	SD
ICP 8929	MD	**ICPL 990091**	MD	**ICPH 2364**	SD	*SIPS 8*	SD
ICP 9320	MD	**ICPL 99046**	MD	**ICPH 2431**	SD	*SIPS 9*	SD
ICP 9801	MD	**ICPL 99050**	MD	**ICPH 2433**	SD	*SIPS 10*	SD
ICP 9774	MD	**ICPL 99051**	MD	**ICPH 2438**	SD	*SIPS 15*	SD
ICPH 2740	MD	**ICPL 99054**	MD	**ICPH 2673**	SD	*SIPS 17*	SD
ICPH 2741	MD	**ICPL 99055**	MD	**ICPH 3310**	SD	*SIPS 18*	SD

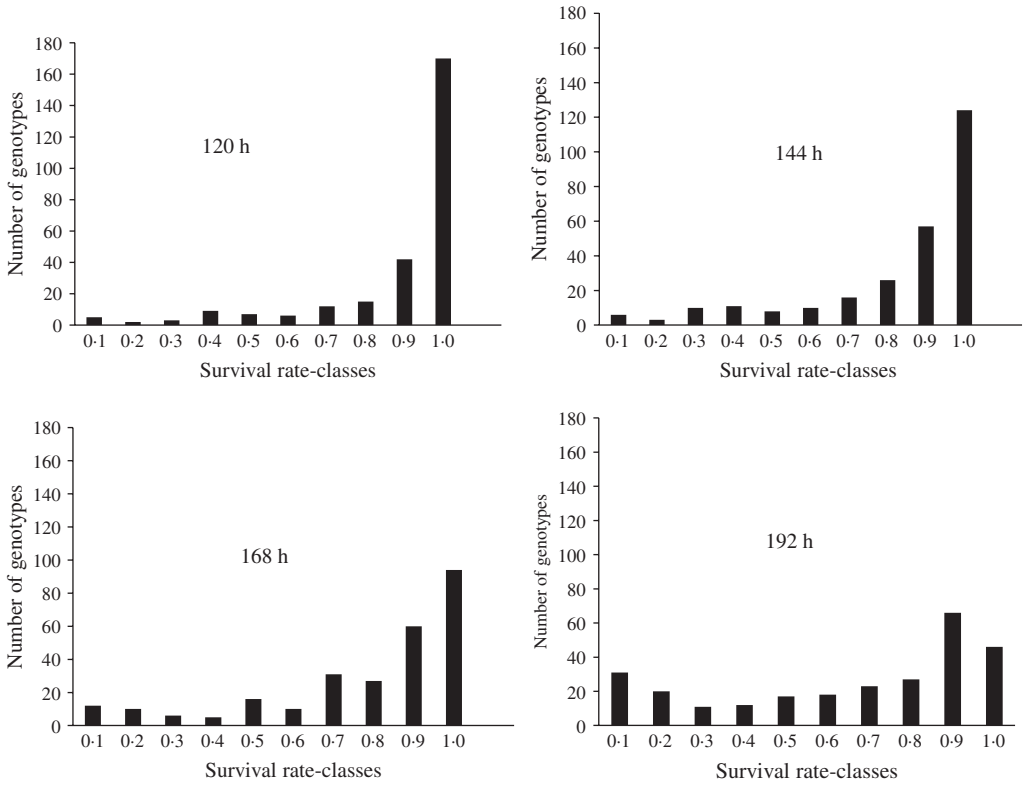

Fig. 2. Seed stage survival rate of 272 pigeonpea genotypes after 120, 144, 168 and 192 h of water submergence. 1 = 0–0·1, 2 = 0·1–0·2, 3 = 0·2–0·3, . . . ,10 = 1·0 survival rate.

at least 20 mm under water for 11 days, whereas the fifth pot was kept at normal moisture as a control. The water level in the tray was kept constant throughout the experiment and maintained for 11 days. Eight days after completion of the waterlogging stress treatment the number of plants that survived in each pot was counted and rate of survival was recorded with reference to the number of plants before treatment. ANOVA was performed using SAS software (SAS 2008) to assess the variation among genotypes for survival rates after stress imposition.

Field level evaluation (screening under natural conditions)

Forty-nine genotypes were further evaluated under natural field conditions to confirm the levels of tolerance observed under laboratory and pot screening. The field trial was conducted at ICRISAT, Patancheru, Andhra Pradesh, India (17°32′N, 78°16′E, 545 m a.s.l.) on 14 July 2010 with four replications using a 7 × 7 lattice design in deep vertisols on a flatbed rice field with no drainage. Seeds were planted in plots of four rows, 2·5 m long and 0·50 m apart, with spacing of 0·25 m within rows. Before planting, a basal dose of 46 kg N/ha in the form of diammonium phosphate was applied. A pendimethaline and atrazine mixture (both 0·75 kg/ha a.i.) was sprayed before emergence to keep the crop free from weeds. Soon after sowing, the rains commenced and continued for up to 60 days including 45 rainy days (minimum rainfall of 950 mm rain and 29 ± 1 °C average temperature). Thus, the crop was exposed to continuous natural water stress beginning 7 days after sowing with an average water depth of 20 ± 10 mm and continued for up to 53 days (Fig. 1). Plant survival counts were based on final plant stand at maturity (180 days from sowing). ANOVA was performed using SAS software (SAS 2008) to assess the variation among genotypes for survival rates before harvest.

RESULTS

Seed stage evaluation

Effect of submergence durations on seed survival

All genotypes exhibited ⩾0·90 survival irrespective of their origin when germinated under normal moisture conditions (S0, control = no submergence) (Fig. 2). The ANOVA showed highly significant differences

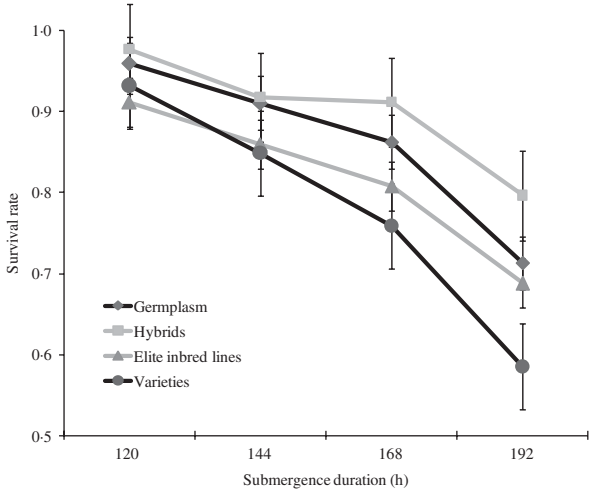

Fig. 3. Survival rates of the different groups of genotype submerged for 120, 144, 168 and 192 h under water, during seed stage screening, (G = germplasm; H = hybrids; L = lines and V = varieties); bars represent standard error (±).

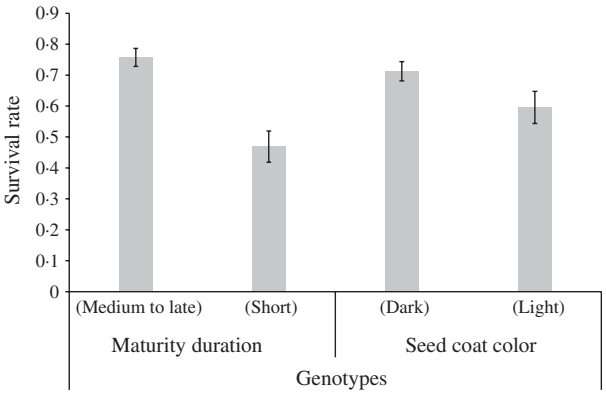

Fig. 4. Survival rate of pigeonpea genotypes (grouped based on maturity duration and seed coat colour) after 192 h of water submergence treatment under laboratory screening; bars represents standard error (±).

($P < 0{\cdot}01$) in seed survival rates among genotypes for all submergence durations. There were also significant survival rate differences among the various submergence durations (S120, S144, S168 and S192). The interactions between genotype and submergence duration were also significant; therefore, further analysis was carried out to understand genotypic performance after each submergence. This analysis revealed that the variation among genotypes for survival rate was highly significant at all the submergence durations. To explore further, the four distinct genotype groups (hybrids, germplasm, breeding lines and varieties) were compared using linear contrasts. Significant differences in survival rates between groups were recorded for the submergence durations. However, no significant differences were found between the individual groups at S144, and at S192 significant differences between groups were seen for all except lines and germplasm. The analysis further revealed that after 120 h submergence the genotypes, irrespective of origin, had high (>0·80) mean survival rates. Even after 168 h of submergence the mean survival rate was 0·73, which suggested that most of the genotypes had the potential to tolerate severe submergence stress. A sharp decline in seed survival was observed at the 192 h submergence period (Fig. 2). After 192 h of submergence the hybrids exhibited highest survival rate (>0·79) followed by germplasm accessions (0·71), advanced breeding lines (0·68) and released varieties (>0·58) (Fig. 3).

Relationship of maturity, seed colour and seed weight with survival rate

Medium-to-late maturing genotypes, irrespective of their origin, had significantly ($P < 0{\cdot}01$) higher mean survival rate (0·70) compared with short maturity types with mean survival rate of 0·42 (Fig. 4). Further group-wise analysis revealed that, in general, the medium-to-late maturing inbred lines had higher survival rates (0·78) than short (0·45) maturing types. Similar results were recorded among germplasm and varieties. However, hybrids exhibited consistently high survival rates irrespective of their maturity groups. It was also observed that the mean survival rate was significantly higher ($P < 0{\cdot}01$) in the genotypes with dark coloured seed coats (0·65) as compared with light coloured seed coats (0·54). In addition to maturity and seed coat colour, the seed size was found to be positively associated ($P < 0{\cdot}05$) with survival rate of the genotypes at all the levels of submergence treatment, S120 ($r = 0{\cdot}234$), S144 ($r = 0{\cdot}196$), S168 ($r = 0{\cdot}163$) and S192 ($r = 0{\cdot}152$).

Based on the results of laboratory survival rates, the genotypes were classified into four groups (Table 2); tolerant (>0·75), moderately tolerant (0·50–0·74), moderately susceptible (0·25–0·49) and susceptible (<0·25). Survival rate at the S192 duration varied from 0·20 to 1·00, 0·02 to 1·00, 0·02 to 1·00 and 0·0 to 0·93 in hybrids, germplasm, elite inbred lines and varieties, respectively.

Evaluation at early seedling stage

Ninety-six pigeonpea genotypes including tolerant ($n = 46$), moderately tolerant ($n = 10$) and susceptible

Table 2. *Pigeonpea genotypes representing tolerant (0·75–1·0), moderately tolerant (0·5–0·74), moderately susceptible (0·25–0·49) and susceptible (<0·25) on the basis of survival rate after the 192 h water submergence treatment at seed stage screening*

Survival rate (%)	Genotypic groups	Pigeonpea genotypes screened for waterlogging tolerance*				
Tolerant (1·0–0·75)	Elite inbred lines	ICPA 2039	ICPL 99051	ICPL 20100	ICPL 20118	ICPL 20129
		ICPL 150	ICPL 99054	ICPL 20103	ICPL 20119	ICPL 20130
		ICPL 332	ICPL 99055	ICPL 20107	ICPL 20120	ICPL 20131
		ICPL 83057	ICPL 99061	ICPL 20108	ICPL 20121	ICPL 20132
		ICPL 86005		ICPL 20109	ICPL 20122	ICPL 20133
		ICPL 87051	***ICPL 20092***	ICPL 20110	ICPL 20123	ICPL 20236
		ICPL 99048	ICPL 20093	ICPL 20112	ICPL 20124	***ICPL 20237***
		ICPL 92043	ICPL 20094	ICPL 20113	***ICPL 20125***	***ICPL 20238***
		ICPL 93101	ICPL 20095	ICPL 20114	ICPL 20126	ICPL 20241
		ICPL 99046	ICPL 20096	ICPL 20116	ICPL 20127	ICPL 20242
		ICPL 99050	ICPL 20099	***ICPL 20117***	***ICPL 20128***	ICPL 20243
	Hybrids and varieties	***Asha***	***ICPH 2740***	ICPH 3629	***ICPH 4104***	MAL 11
		BDN 1	ICPH 3341	ICPH 3740	***ICPH 4187***	***MAL 15***
		BRG1-(w)1	***ICPH 3362***	ICPH 3766	ICPH 4301	***MAL 9***
		ICPH 2431	ICPH 3371	ICPH 3964	ICPH 4322	SIPS 15
		ICPH 2671	ICPH 3461	ICPH 3992	***JBP 110-B***	SIPS 18
		ICPH 2673	ICPH 3481	ICPH 4031	***LRG 30***	SIPS 9
	Germplasm	ICP 10948	ICP 12176	ICP 13384	ICP 14318	ICP 7597
		ICP 11059	ICP 12739	ICP 13389	ICP 1571	ICP 7815
		ICP 11130	ICP 12740	ICP 13391	ICP 2376	ICP 7977
		ICP 11378	ICP 1275	ICP 13392	ICP 4924	ICP 8465
		ICP 11811	ICP 12750	ICP 13395	ICP 5028	ICP 8466
		ICP 11813	ICP 12751	***ICP 14085***	ICP 5429	ICP 8927
		ICP 12023	ICP 12839	ICP 14092	ICP 7086	ICP 8929
		ICP 12024	ICP 13361	ICP 14146	ICP 7193	
		ICP 12043	ICP 13379	ICP 14282	ICP 7201	

Moderately tolerant (0·50–0·74)	Elite inbred lines	***ICPB 2039***	ICPL 20101	ICPL 20106	ICPL 20244	ICPL 96061
		ICPL 161	ICPL 20102	ICPL 20135	ICPL 87154	
		ICPL 20097	ICPL 20104	ICPL 20219	ICPL 90030	
		ICPL 20098	ICPL 20105	ICPL 20229	ICPL 92059	
	Hybrids and varieties	BRG 2	ICPH 2741	ICPH 4329	SGBS 6	UPAS 120
		ICPH 2363	ICPH 3313	JBP 36-B	SIPS 10	
		ICPH 2364	ICPH 4183	***Maruti***	SIPS 17	
		ICPH 2438	ICPH 4275	SGBS 4	SIPS 5	
	Germplasm	ICP 10960	ICP 11296	ICP 14304	ICP 1575	ICP 8094
		ICP 10987	ICP 1141	ICP 14410	ICP 1941	ICP 8920
		ICP 11128	ICP 11440	ICP 14712	ICP 4928	ICP 87051
		ICP 11133	ICP 12057	ICP 14882	ICP 5529	
		ICP 11150	ICP 13342	ICP 15200	ICP 7741	
Moderately susceptible (0·25–0·49)	Elite inbred lines	ICPL 20200	ICPL 20222	ICPL 84060	ICPL 90034	ICPL 990091
		ICPL 20218	ICPL 84031	ICPL 87091	ICPL 95040	
	Hybrids and varieties	ICPH 2433	ICPH 3762	MAL 12	SIPS 1	
		ICPH 3467	ICPH 4304	SGBS 3	VL-arhar 1	
	Germplasm	ICP 11106	ICP 11443	ICP 12026	ICP 12792	ICP 7349
		ICP 11120	ICP 11447	ICP 12728	ICP 13402	
		ICP 11153	ICP 12747	ICP 12751	ICP 3782	
Susceptible (<0·25)	Elite inbred lines	ICPA 2043	ICPL 20212	ICPL 20227	ICPL 89	ICPL 93107
		ICPB 2043	ICPL 20213	ICPL 20230	ICPL 90048	ICPL 96053
		ICPL 20115	ICPL 20215	ICPL 20231	ICPL 91032	ICPL 98011
		ICPL 12761	ICPL 20216	ICPL 81-9	ICPL 92010	ICPL 98013
		ICPL 149	ICPL 20221	ICPL 86022	ICPL 92041	ICPL 99044
		ICPL 20	ICPL 20223	ICPL 87	ICPL 92067	
		ICPL 20210	ICPL 20225	ICPL 88034	ICPL 93017	
	Hybrids and varieties	BDN 2	ICPH 3310	Kanchen	SIPS 6	
		BRG 3	ICPH 4305	**SIPS 2**	SIPS 7	
		HPL 24	GAUT 90-1	SIPS 4	SIPS 8	
	Germplasm	ICP 11100	ICP 11681	ICP 12749	ICP 13581	ICP 9320
		ICP 11145	ICP 12714	ICP 12780	**ICP 7035**	ICP 9801
		ICP 11149	ICP 9774			

* Genotypes in italic and bold showed consistent higher survival rate after the *in vitro*, pot and field evaluations, while genotypes underlined and bold showed susceptible reaction for waterlogging tolerance across screenings.

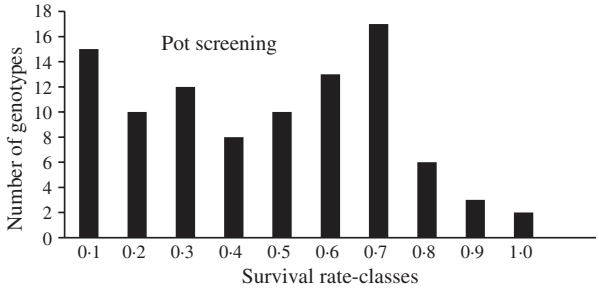

Fig. 5. Seedling stage (pot screening) survival rates of 96 pigeonpea genotypes after completion of submergence treatment. 1 = 0–0·1, 2 = 0·1–0·2, 3 = 0·2–0·3,. . .,10 = 1·0 survival rate.

($n = 40$) were further evaluated at the seedling stage for waterlogging tolerance. ANOVA revealed highly significant differences ($P < 0{\cdot}01$) among the genotypes for seedling survival, which ranged from 0 to 0·95 (Fig. 5). Most of the genotypes ($n = 54$) tested for survival rate at early seedling stage in pots were found to be sensitive to waterlogging and only a few genotypes exhibited higher (up to 1·0) survival. The dark-coloured, bold-seeded (100 seed weight $\geqslant$10 g), medium-maturing genotypes showed higher survival rate compared with light-coloured, small-seeded (100 seed weight <10 g) short-duration genotypes.

Field evaluation

The 49 genotypes screened under natural field conditions showed significant variation in the survival rate. A subset of genotypes that showed waterlogging tolerance at all the three level of screenings (laboratory, pot and field screening) during 2009 and 2010 were – short: ICPH 2431 and ICPB 2039; medium: ICPH 2740, ICPH 2671, ICPH 4187, Asha, ICPL 332, LRG 30, Maruti, ICPL 20117, ICPL 20125, ICPL 20128, ICPL 20237, ICPL 20238 and ICPL 99050; and late maturity: ICPL 20092, MAL 9 and MAL 15 (Table 2). All the tolerant genotypes had dark seed colour with 100 seed weight >10 g.

DISCUSSION

The erratic rainfall patterns in India render the country highly vulnerable to drought and floods. More than 90% of pigeonpea is grown under rainfed conditions (Saxena 2008). Like soybean (VanToai *et al.* 1994), chickpea (Cowie *et al.* 1996) and several other legumes (Whiteman *et al.* 1984), pigeonpea is highly sensitive to waterlogging (Chauhan *et al.* 1997; Perera *et al.* 2001; Khare *et al.* 2002). Despite recognizing that waterlogging is an important production constraint in pigeonpea, very few studies have been conducted to identify germplasm tolerant to this abiotic stress and few genotypes have been tested (Perera *et al.* 2001; Sarode *et al.* 2007) to assess the range of variation present in the overall pigeonpea gene pool.

For breeding purposes, a fast and reliable waterlogging screening method that allows evaluation of a large number of genotypes and does not require many seeds at early generation stages is necessary. The screening procedure used in the present paper is intended to be a systematic stepwise approach to filtering material through the breeding programme, starting with a large number of genotypes ($n = 272$) and reducing the number based on subsequent screening until the genotypes are validated and recommended to farmers. Past efforts to identify genotypic variability for waterlogging tolerance in pigeonpea were confined to *in vitro* and pot screenings using germplasm accessions and a few cultivated genotypes. The current study includes: (1) the most critical plant growth stages ($n = 3$) affected by waterlogging, (2) a large set of material that could be of direct interest or use for breeding purposes and (3) final selection of the promising genotypes based on the field evaluation. The results of screening a large set of materials ($n = 272$) with different genetic origins for waterlogging tolerance at seed level revealed that significant variability for waterlogging tolerance exists in cultivated pigeonpea genotypes. Chauhan *et al.* (1997) tested ten genotypes and Krishnamurthy *et al.* (2012) recently tested 160 accessions (146 mini core pigeonpea germplasm accessions, four control entries and ten previously tested genotypes). The present results re-confirmed the reactions of ICP 7035 previously reported by Chauhan *et al.* (1997) as sensitive and those of ICPH 2671, ICPH 2740, ICPH 3762 and ICPR 2671 (Asha) as tolerant (Krishnamurthy *et al.* 2012). To see the genotypic variability at seed level, the seeds of all genotypes ($n = 272$) were submerged for different durations (S120, S144, S168 and S192). The survival rates reduced drastically with increased duration of seed soaking and some of the susceptible materials started deteriorating within 120 h of soaking (skewed variation) while after 192 h of submergence, the range of variation for survival showed a normal distribution

(Fig. 2). Powell & Matthews (1978) noted that in legumes, injury to the seeds is caused by excessive water accumulation due to rapid water absorption. Waterlogging during seed germination, seedling establishment and early vegetative growth result in poor plant stand (Duke & Kakefuda 1981), which leads to significant yield losses and instability in production (Reddy & Virmani 1981). The genotypic differences for waterlogging tolerance at seedling level in pigeonpea have also been studied by Dubey & Asthana (1987), Takele & McDavid (1995), Chauhan *et al*. (1997), Perera *et al*. (2001), Sarode *et al*. (2007) and Krishnamurthy *et al*. (2012). Reductions in survival rate under prolonged submergence have been attributed to anoxia/hypoxia (Orchard & Jessop 1984). Respiration and electron transport under anoxic conditions are inhibited and adenosine tri-phosphate (ATP) formation is decreased (Johnson *et al*. 1989; Tsai *et al*. 1997), which results in decreased seed viability and poor germination. In the present paper, the hybrids exhibited greater survival rates (0·79) compared with germplasm accessions (0·71), elite inbred lines (0·68) or varieties (0·58). Differences in survival rates between four contrasting genotypic groups could be related to the origin of genotypes. It could also be related to the differences in the imbibition rates and the amounts of reserved materials present in the seeds and also to the fact that hybrid seeds may have experienced less oxygen deprivation during submergence, as compared with pure lines, due to greater biomass. Significant varietal differences in response to flooding tolerance have also been reported in maize (*Zea mays* L.) and it was found that hybrids performed better than inbred lines under excess soil moisture conditions (Sultana *et al*. 2009).

Evaluations of waterlogging tolerance in the laboratory, pot and field levels for medium-to-late maturing genotypes showed higher survival rates compared with short-duration types. Similar results were observed by Matsunaga *et al*. (1994). This indicates that medium-to-late maturing cultivars have enough time to recover from any sub-lethal waterlogging stress. Apart from maturity duration, Hou & Thseng (1991) also correlated flooding tolerance in soybean with seed coat colour: seeds with black coats germinated well even after 10 days of soaking. This was also observed for pigeonpea in the present work. Khare *et al*. (2002) found that the high levels of phenolic and tannin compounds found in dark seed coats slow down the rate of water uptake, which in turn increases the survival rate under extended periods of submergence. Besides origin, maturity and seed coat colour, seed size of each genotype played a significant role in survival after different water submergence treatments. However, in general a decrease in survival rate was recorded after S192 treatment in small-seeded elite inbred lines (< 10 g/100 seed weight). The marked differences in rates of survival may be related to different rates of imbibition in different seed sizes. The small seeds have large surface areas, which may facilitate fast water movement through micropyles as compared with larger seeds as suggested by de Jabrun *et al*. (1980).

The waterlogging-tolerant genotypes identified through natural field screening included hybrids (ICPH 2431, ICPH 2671, ICPH 2740 and ICPH 4187), varieties (Asha, LRG 30, Maruti, MAL 9, MAL 15 and ICPL 332) and advanced breeding lines (ICPL 20092, ICPL 20117, ICPL 20125, ICPL 20128, ICPL 20237, ICPL 20238 and ICPL 99050). It can be concluded that there is large extent of variation available in the cultivated groups of genotype for waterlogging tolerance, which contradicts the results obtained by Krishnamurthy *et al*. (2012). This may be due to the inclusion of only a few cultivated genotypes ($n = 21$) in Krishnamurthy *et al*. (2012). Very few waterlogging-tolerant varieties and hybrids (ICPH 2431 and ICPB 2039) are available for the short-duration group, whereas several are available for medium-to-late maturity group. Some of the accessions identified as tolerant to waterlogging could be promoted directly as cultivars after confirmation on farmer's field. Highly tolerant genotypes can also be used as donors of waterlogging-tolerant genes in breeding programmes; this is especially needed to incorporate waterlogging tolerance to the short-duration pigeonpea pool. This will eventually lead to reduction in overall losses caused by waterlogging in pigeonpea. However, more work is needed to understand the underlying mechanisms of tolerance to waterlogging and the post-waterlogging recovery.

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