Current Journal of Applied Science and Technology

39(12): 21-33, 2020; Article no.CJAST.56510
ISSN: 2457-1024
(Past name: British Journal of Applied Science & Technology, Past ISSN: 2231-0843, NLM ID: 101664541)

Characterization of Pigeonpea Genotypes for Waterlogging Tolerance Based on Morpho-physiological and Molecular Traits

Deepak Kumar[1], Rafat Sultana[1*], Ravi Ranjan Kumar[2] and Megha Kirti[1]

[1]Department of Plant Breeding and Genetics, Bihar Agricultural University, Sabour, Bihar, India.
[2]Department of Molecular Biology and Genetic Engineering, Bihar Agricultural University, Sabour, Bihar, India.

Authors' contributions

This work was carried out in collaboration among all authors. Authors DK and RS designed the study, performed the statistical analysis. Author DK wrote the protocol and wrote the first draft of the manuscript. Author RRK helped in molecular section. Author MK helped in data collection and literature search. All authors read, scrutinized and approved the final manuscript.

Article Information

DOI: 10.9734/CJAST/2020/v39i1230660
Editor(s):
(1) Dr. Belisario Dominguez-Mancera, University Veracruz, Mexico.
Reviewers:
(1) Necat Togay, Fethiye ASMK Vocational High School, Mugla S.K. University, Turkey.
(2) Justice Okona Frimpong, Ghana.
Complete Peer review History: http://www.sdiarticle4.com/review-history/56510

Original Research Article

Received 07 January 2020
Accepted 12 March 2020
Published 31 May 2020

ABSTRACT

Aim: To screen and characterize pigeonpea genotypes using morpho-physiological, biochemical and molecular traits.
Study Design: The field trial was conducted using the Randomized Block Design (RBD) while Completely Randomized Design was used for laboratory and pot screening experiments.
Place and Duration of Study: The research trial was conducted at Laboratory and Pulse Research Farm, Model Bhitti, BAU, Sabour, which lies between 25°15'40" N latitude to 87°2'42" E longitude and 46 meters above sea level. Study was undertaken between July, 2018 to March, 2019.
Methodology: Sixty pigeonpea genotypes were screened for submergence tolerance at seed stage in the laboratory. Based on results of laboratory screening, 40 genotypes with sufficient genotypic variability for waterlogging tolerance were further taken for seedling stage screening at field, finally 20 genotypes were taken to pots for waterlogging tolerance evaluation and characterization on the basis of morpho-physiological, biochemical and molecular traits. According to pot results, six contrasting genotypes were considered for RAPD primers amplification.
Results: The three levels of sieving of genotypes fetched results directing the opportunity of particular genotypes to be sown inlow land areas. Character like seed colour varied from brown,

**Corresponding author: E-mail: rafat.hayat@gmail.com;*

dark brown to black, which showed significant relationship with level of tolerance. Significant (p=0.01) higher germination with less reduction due to waterlogging stress was observed in genotypes such as ICP-11809, ICPL-20098, NDA-1 and ICP-5028. Maximum survival percent was found in ICP-5028 (62.28%) while least survival percent was observed in ICP-7035 (10.98%). At the field stage, genotypes such as ICP-5028, ICPL-990985, ICPL-20238 were best performing genotypes. SPAD Chlorophyll Content results exhibited significant reduction among the susceptible genotypes. However, there was least reduction among tolerant genotypes such as LRG-30, Mal-9, Pusa-992 and ICP-5028.Genotypes namely: Manak, Pusa-991 and Pusa-992 faced hastened senescence under waterlogging condition as compared to ICP-5028, Mal-15, Mal-9, LRG-30. Molecular evaluation results of six genotypes chosen across screening showed that ICP-7035 and Manak were clubed together in one cluster. Nevertheless, ICP-5028 and Mal-9 were grouped in another cluster of Dendrogram, constructed using Jaccard similarity coefficient. In the present investigation two unique amplicons were amplified by primers OPA-13 and OPC-01. OPA-13 amplified unique band was linked with susceptible genotypes of size ~1240 bp while the unique amplicon given by OPC-01 was of ~980 bp size linked with tolerant genotypes.

Conclusion: Available waterlogging tolerance in pigeonpea gene pool to some extent can provide source for breeding waterlogging tolerant cultivars. Physiological and genetic approach involving efficient screening techniques and evaluation of breeding material/lines under targeted environment for the traits linked to tolerance is likely to lead to the identification of specific component traits and high yielding varieties with improved stress tolerance.

Keywords: Waterlogging; seed colour; senescence; rapd; dendrogram.

1. INTRODUCTION

Waterlogging is a phenomenon manifesting the detrimental hydrological capabilities of nature. In nature due to undesired hydrological levels from excessive downpour situations like soil saturation, submergence, flooding etc., often arises. In terms of agriculture, there is fine difference between waterlogging and submergence. According to Sasidharan et al. when roots of a plantis dipped into water the situation is called waterlogging while in case of submergence the whole aerial parts of plant is submerged into water [1].

The harmful effects due to the condition of waterlogging arises as result of deprivation of proper aeration of crop root zone and the obvious reason for this is the presence of excessive moisture or water content in all the air pores in soil. Consequently, creating hypoxic and finally anoxic conditions, which has been the main problem of waterlogging (WL) soils. During waterlogging stress the activity of nitrifying soil microbe is inhibited causing depletion of soil nitrogen (N) levels thus reducing its availability to the plants [2,3]. The paucity of oxygen hinders respiration in plants by causing cessation of Krebs cycle as oxygen is final electron acceptor in the electron transport chain.

Waterlogging has proved devastating for agriculture in last decade. According to FAO, flood accounted for around one-third of the total losses and damage caused to crop plants between 2006 to 2016 [4]. In India itself, 8.5 million ha of arable land is prone to waterlogging. According to the previous available data one-third (1.1 mha) of the total cultivated area (3.38 mha) of pigeonpea is affected by waterlogging causing annual loss of 25-30% [5,6].

Despite being the largest producer, India is also the largest importer and consumer (23-24 million tonnes) of pulses in the world. Among pulses, pigeonpea hold second position after the chickpea in terms of area and production. Pigeonpea is one of important component of diet used mainly as 'Dal', a recipe cooked with spilt beans of the pulse. Around 85% of total pigeonpea of the world is produced by India only, which occupies an area of around 46.5 lakh hectare with 30.27 lakh tonnes of produce. In Bihar, it is cultivated on area of around 0.27 lakh hectare with a production of 0.39 lakh tonnes. The productivity of the Bihar state (1739 kg/ha) is surprisingly highest among the states.

In pigeonpea waterlogging stress induces various morphological, physiological and biochemical adaptations leading to alterations in underlying pathways ofthe cells. Agronomic practices like growing pigeonpea on raised sloping seed beds, ridge sowing and transplanting of seedlings helped in reducing losses caused by waterlogging [7], but are not

economically viable for the resource-poor farming community. There is therefore an immense need to breed superior cultivars that can serve as "genetic defense" to ensure a superior yield in the face of a flooding event or even in multi-stress conditions [8]. In the present research, we have screened 60 diverse pigeonpea genotypes for the waterlogging tolerance on the basis of morpho-physiological parameters. For responsive remedy towards waterlogging stress, plants show changes in their expression profile of genes at epigenetic level [9], transcriptional [10,11] and translational level [12]. These changes are different among genotypes due to differences existing in their genetic make-up. To identify such differences contributing towards waterlogging tolerance further biochemical and molecular evaluation was also carried out among the identified tolerant and susceptible genotypes.

2. MATERIALS AND METHODS

Stepwise screening and characterization were carried out at various crop growth stages, for accessing effect of waterlogging at the most vulnerable periods i.e. seed stage at laboratory level and seedling stage in field and pots. Seeds were accessed at laboratory in a controlled environment where the temperature and relative humidity were 25°C and >70% respectively.

2.1 Laboratory Screening

For laboratory screening 60 genotypes were taken for study (Table 1). 20 seeds of each genotype were submerged in 50 ml water in test tube to create anaerobic waterlogged condition *in vitro*. They were kept in water for three different periods i.e. 120 hrs, 144 hrs and 168 hrs, after which they were de-submerged and taken for germination onto petri-plates with germination paper. Data was recorded on 9th day of de-submergence. Germination percentage, radicle-plumule length were recorded over the control plates of same genotypes plated on same day. Then the selected genotypes were taken to field conditions for further evaluation. Out of total 60 genotypes, 40 were taken to the field stage for evaluation and screening at the seedling stageof the crop.

2.2 Field Screening

The field experiment was undertaken at Pulse Research Station Model Bhitti (BAC, Sabour, Bhagalpur), located on the coordinates 25°15'40" N latitude to 87°2'42" E longitude and 46 meters above sea level. 40 pigeonpea genotypes were sown in field arranged in a Randomized Block Design (RBD) with two replications. Row to row distance was kept 70 cm and plants were spaced at distance of 25 min a row. Fertilizer application to soil was done as basal application at 25 kg ha^{-1} Nitrogen, 50 kg ha^{-1} P_2O_5 (Phosphorus) and 30 kg ha^{-1} K_2O_5 (Potassium). Waterlogging treatment was given at knee high stage (70 days after germination) by flooding the field in a way so that water level of 5±1 cm was maintained throughout the treatment period (5 days). Later on, water was drained from the treatment plot and various observations were recorded, thus survival percent of genotypes was accessed by recording plant stand before and after submergence. Analysis of data collected from field was done using the ANOVA test for Randomized Block Design (RBD).

2.3 Morpho-physiological, Biochemical and Molecular Characterization

From the results obtained through the field data, 20 plants showing variability for waterlogging tolerance was further taken to the next stage of evaluation into pots for morpho-physiological, biochemical and molecular characterization at seedling stage. The evaluation was conducted using plastic pots of 90 mm diameter, with 3.0 mm diameter perforations in the base. Each pot was filled with a mixture of soil and farmyard manure (FYM). Soil: FYM ratio was 50:1(V/V). Fertilizer (nitrogen, phosphorus and potassium) was applied as basal dose and was thoroughly mixed into the soil. Each pot was weighed after filling in order to maintain the same quantity of soil and constant moisture in each pot. For each genotype three pots (10 seeds /pot) were sown i.e. two treated and one as a control. Pots were kept in large trench and water was maintained up to the level of surface of soil in pot (3±1 cm). All the observations were recorded after 8 days of draining of the water except the leaf samples for Alcohol Dehydrogenase Assay, which was taken in between treatment of waterlogging. Analysis of generated data was done by subjecting data to the ANOVA test in a CRD style over the control pots data. Observations recorded at this stage include senescence and chlorophyll content measured by SPAD meter along with Alcohol Dehydrogenase Assay. For the results obtained from pot level characterization, six genotypes were chosen with contrasting nature towards waterlogging stress. Three genotypes

Table 1. Mean performances of characters recorded at laboratory stage

S. No.	Genotypes	Seeds Index (G)	Seed Colour	Germ %	Length (mm)168 HRS	
				168 Hrs	Radical	Plumule
1	ICP-13359	5.44	B	8.67	47.67	42.33
2	ICP-6739	6.22	B	4.67	23.33	26.67
3	BRA-303	6.22	B	7.33	44.67	31.33
4	ICP-11627	6.29	B	6.59	51.67	42.33
5	Manak	6.50	B	5.70	57.33	35.33
6	ICP-11477 (DT)-1	6.58	B	6.00	40.33	40.33
7	ICPL-20098	6.72	B	6.15	45.00	37.00
8	ICP-11477 (NDT)	6.89	B	6.68	31.33	31.67
9	LRG 30	6.90	D B	8.00	44.33	32.67
10	ICPL-99095	6.99	B	6.83	27.67	27.33
11	BRA-304	7.01	BL	6.67	32.67	25.67
12	PAU-881	7.10	D B	5.71	32.00	28.33
13	ICPL-20238	7.20	B	6.22	35.33	26.33
14	PATHAM	7.24	D B	8.33	47.00	38.33
15	BAHAR	7.39	D B	5.33	32.00	36.00
16	ICPL-2011246	7.46	D B	6.00	38.33	37.33
17	ICP-11089	7.56	B	7.06	44.67	40.00
18	ICPL-2011242	7.67	B	5.67	43.33	41.67
19	ICP-15185	7.75	B	6.67	45.00	46.00
20	ICP-11910	7.76	B	5.56	47.00	49.67
21	Pusa-991	7.90	B	3.81	42.00	35.33
22	ICPL-99098	7.93	B	6.33	43.67	32.00
23	ICP-772	7.98	B	7.93	40.67	43.00
24	ICPL-2011247	8.02	D B	7.67	40.67	43.00
25	ICP-144903	8.04	B	2.78	24.67	28.33
26	ICP-9414	8.05	B	8.00	43.33	38.00
27	ICPL-99090	8.09	B	7.67	56.67	43.67
28	ASHA	8.23	D B	7.30	49.67	52.67
29	ICPL-20093	8.27	B	8.63	49.00	36.67
30	ICPL-99088	8.30	B	7.93	43.33	52.67
31	ICPL-99091	8.34	B	5.67	36.00	37.33
32	Paras	8.40	B	6.00	35.67	36.33
33	Maruti	8.50	B	7.33	54.00	39.67
34	Pusa-992	8.50	B	8.59	35.33	50.00
35	ICPL-20177	8.64	B	6.33	26.67	33.00

S. No.	Genotypes	Seeds Index (G)	Seed Colour	Germ %	Length (mm)168 HRS	
				168 Hrs	Radical	Plumule
36	ICPL-20116	8.78	B	7.17	30.33	40.33
37	ICPL-20123	8.81	B	8.33	44.33	54.00
38	PA-291	8.82	B	2.11	22.00	20.67
39	ICPL-990102	9.00	B	5.85	27.67	21.67
40	BRA 306	9.00	B	2.11	3.33	10.00
41	ICP-5028	9.49	BL	1.04	5.33	0.00
42	ICPL-20125	9.50	B	5.33	18.33	28.33
43	ICPL-20338	9.50	B	2.85	16.00	25.67
44	NDA-1	9.50	D B	3.33	21.33	28.33
45	ICPL-20178	9.50	D B	4.85	30.00	22.00
46	ICPL-20126	9.60	B	4.15	24.00	29.33
47	ICPL-20103	9.68	B	5.56	25.00	35.00
48	PUSA-9	9.77	D B	8.00	43.67	32.33
49	ICPL-87051	9.80	B	4.33	27.67	24.33
50	MAL-9	10.23	B	4.19	27.33	34.00
51	ICPL-332	10.30	D B	5.19	41.67	26.67
52	MAL-13	10.38	B	4.33	65.33	34.00
53	ICPL-99050	10.50	B	8.00	30.00	16.67
54	ICPL-20237	10.50	B	3.41	27.67	26.00
55	ICPL-20092	10.80	D B	2.48	7.67	9.67
56	ICPL-20090	11.10	B	2.96	37.33	33.00
57	ICPL-99087	11.10	D B	7.67	53.67	39.67
58	ICPL-20120	11.50	B	2.00	22.33	34.00
59	MAL 15	12.30	D B	1.83	17.00	16.33
60	ICP-7035	19.80	B	9.00	36.67	16.33
	Mean			**5.80**	**35.63**	**32.94**
	C.V.			**19.79**	**20.68**	**22.81**
	C.D. 5%			**1.85**	**11.91**	**12.15**
	S. E.			**0.66**	**4.25**	**4.34**
	Range			**1.04-9.00**	**3.33-65.33**	**0.00-54.00**

GERM%= Germination Percent, B= Brown, DB= Dark Brown, BL= Black

each of tolerant and susceptible nature were selected. These genotypes were accessed for genetic variability with Random Amplification of Polymorphic DNA (RAPD) primers. All the statistical analysis performed on measured responses was done using Indostat Software Services.

3. RESULTS AND DISCUSSION

Pigeonpea has almost 90% of area of cultivation under rainfed condition [13,14]. As a wild plant, pigeonpea is perennial in nature but in cultivation its crop duration varies from 90 days to 280 days, this makes it one of the hardy legume crops. It thrives in wide range of environmental conditions of Asian, African, Latin American and Caribbean Islands. However, like most of the legumes, waterlogging is highly detrimental to pigeonpea [15,16,17]. In spite, being a crucial constraint in pigeonpea production, the studies in this direction for developing tolerant and susceptible genotypes have been insufficient [16]. Agronomic practices to mitigate the problem have now become insufficient and economically unviable, so to meet the needs of resource poor farmers of Afro-Asia, especially Indian farmers of north eastern parts, where there is urge to develop tolerant varieties with minimum effect to desired yield related characters. This research carried out at Bihar Agricultural University was done with main target of identifying tolerant genotypes for under-privileged farmers of Gangetic flood plains of Bihar. The sections below discuss the results obtained for various character recorded in the present experiment.

3.1 Seeds Characteristics

The seed morphology has many crucial impacts on the ability of a particular genotype to tolerate the waterlogging stress. Therefore, in this study seed colour was observed and this varied from brown, dark brown to black. This intensification of the seed colour towards darker shade of brown and sometimes to black has to do with mainly tannin content. Tannins are type of poly-phenolic compounds, which have antibacterial and antifungal activities by means of which their presence in plant tissues prevent their petrification. They also have inhibitory activity towards hydrolytic enzymes like pectinases, cellulases, xylanases, cutinases etc. In the present investigation most of the tolerant genotypes genotypes exhibited dark colour as compared to susceptible ones viz. Paras, Maruti, Manak, ICP-7035, Pusa-991 and Pusa-992 were brown in colour and they behaved as susceptible genotypes. Contrary to genotypes namely ICPL-332, Mal-15, and Mal-9, which were dark brown and ICP-5028 was black in colour [Table 1]. These genotypes were found to have tolerance towards waterlogging stress. Tannin content in seeds of most legumes has imparted tolerance to water logging [18,19,20]. Seed index varied from 5.435 g (ICPL-13359) to 19.801 g (ICP-7035) [Table 1]. Seeds index directly tells about the size of the seeds. As per reports lager seeds have less tolerability as compared to smaller ones. The larger surface area of the smaller seeds has advantage over the counterparts due to enhanced water conductivity [21]. Thus, the bigger seeds, the more it suffers to flooding stress as compared to smaller ones. Similarly, Sayama et al., found that pigmented seed coat and small seed weight tended to have a positive effect on seed-flooding tolerance [22]. Similar results in pigeonpea were reported by Khare et al. [17], Sultana et al.[14] and Hingane et al. [23].

3.2 Germination Percentage

Germination percent is a measure to assess the pre-germination response of the seed biomass towards waterlogging stress. Generally, reduction in germination percent was foundas the treatment duration increases. Significant variation among genotypes was found in the present investigation for germination percent. The reduction in germination percentage was quite significant (p=0.01) with increased duration of seed submergence, most of the genotypes exhibited more than 75% germination till 120 hours of submergence treatment, which went on to reduce drastically with 168 hours of submergence, the range of germination gave a normal distribution curve. Higher germination with less reduction after imposing waterlogging treatment was observed among the pigeonpea genotypes such as ICP-11809, ICPL-20098, NDA-1 and ICP-5028 compared to control. In case of 120 hours of submergence, maximum germination percent was achieved by genotype ICP-99098 (100%) and minimum by MAL-13 (31.7%), similarly in case of 144 hours treatment maximum germination was shown by NDA-1 (100%) and Maruti (20.38%) while at third treatment of 168 hours submergence highest germination was shown by ICP-5028 (90%) and lowest by Maruti (10.38%) exhibited minimum [Fig. 1 and Table 1]. This also relates to differential amount of seed reserve present and imbibition rates of the water into seeds through the layers of germinating seeds [14,24]. During

anoxia condition, the rate of alcoholic fermentation and carbohydrates combustion for energy generation can be correlated with coleoptile elongation and varies among genotypes. In older seedlings, survival during submergence is highly correlated with carbohydrate supply and its utilization via respiration. The anoxic conditions created hampered the respiration. Similar results were observed by Matsunaga et al. Coutts and Philipson, Khare et al. and Sultana et al. [14,17,25,26].

3.3 Radical and Plumule Length

The presence of hypocotyl and epicotyl in the seeds are pre-factors for plumule and radical emergence. Any damage to these tissues will lead to retarded or obsolete growth of plumule and radical. Waterlogging stress leads to reduction in radical and plumule length of most of the genotypes. After the three submergence treatments (120 hours, 144 hours, 168 hours), the genotypes (Maruti, Manak, Pusa-991, Pusa-992 and ICP-7035), which exhibited susceptible behavior in most of the observations were found to have less radical and plumule length as compared to genotypes Mal-15, Mal-9, ICPL-332, ICP-5028, which exhibited tolerance towards waterlogging in most of the other observations. For 168 hours submergence of seeds, the data is depicted in Table 1. These results were in lieu with the reports of Hsu et al. who reported that progressive waterlogging treatment had detrimental effect on radicle and plumule length due to serious damage to hypocotyl and radicle by waterlogging stress [27]. In addition, during waterlogging, allocation of biomass or dry matter to radicle, seminal roots and aerenchyma decreases if treatment persisted for more than 4 days [28]. Inhibition of root elongation due to low metabolic activity and slow growth during flooding in trees is very common response [29].

3.4 Survival Percentage

Encompassing all the internal and external adaptations attributes, survival percent enables in evaluating the suitability of particular genotype towards waterlogging stress in nutshell. The average survival percentage in the current investigation varied from 10.99% to 62.28% with standard error of 7.4. Maximum survival percent was found in ICP-5028 (62.28%) while least was in ICP-7035 (10.98%).At field stage genotypes ICP-5028, ICPL-990985, and ICPL-20238 were best performing genotypes [Table 2]. Survival percentage after 8 days of treatment was found to have significant interaction with genotypes. Critical reduction was seen in survival percent of genotypes when observation was recorded on the 8^{th} day. During the course of waterlogging two vital plant processes, respiration and photosynthesis are affected adversely and also the anaerobiasis created due to waterlogging causes hampering to the aerobic metabolism leading to energy crisis resulting an imbalance between consumption and production, finally causing plant mortality [30]. The roots cannot transport water and nutrients efficiently under hypoxic or anoxic conditions; thus, the shoot functions are affected and visible symptoms such as wilting, senescence, and death may be observed.

3.5 Chlorophyll Content

Photosynthetic ability is directly attributed to chlorophyll content of the leaves as they are vital component from plant side in the light harnessing machinery of plants. It has been reported that waterlogging stress causes reduction in soluble protein content, thus influencing carbon assimilation, and it degrades chlorophyll, resulting in the decline of photo-assimilation. SPAD results exhibited reduction in chlorophyll, which was least in genotypes LRG-30, Mal-9, Pusa-992, and ICP-5028 while it was found to be maximum in ICP-7035, Manak, ICPL-20092 and Pusa -991 [Table 3]. Waterlogging treatment for 5 days had reducing effect on chlorophyll content. Imposition of any kind of stress to the green parts of the plant causes significant reduction in photosynthetic capacity of the leaves by damaging the chloroplast morphology and ultrastructure of functional leaves [31,32]. Ample amount of variability was found in 20 genotypes for which chlorophyll was taken. Here, it is clearly exhibited that chlorophyll reduction is one of the criteria in characterizing better genotypes for waterlogging tolerance. Similar results were also reported by Yordanova and Popova, in Barley and Kumutha et al. in Pigeonpea [33,34].

3.6 Senescence

Senescence in general terms is ageing of plants, it can be either natural due to intrinsic factors (hormonal) or another type, which is of interest in this very investigation is due to external environmental cues or stresses like drought, flood, heat etc. Waterlogging like situation

hastens the process of senescence and its effects is distinctly visible. The rapid de-colourization of leaves and retarded shoot growth in flooded situation is due to inhibition of nitrogen (N) and other nutrients' uptake and its remobilization and redistribution within the shoots [35]. In this investigation visual scoring of 1 to 5 for accessing degree of senescence was done for the genotypes at pot stage. Senescence is also explained in terms of loss in turgidity from plant organs and withdrawal of mobile elements from leaves and other plant parts. Genotypes namely Manak, Pusa-991 and Pusa-992 faced hastened senescence as compared to ICP-5028, Mal-15, Mal-9, and LRG-30, which were capable of withstanding waterlogging stress as compared to former lot [Table 3]. In some cases, there is early remobilization of protein nitrogen due to advent of senescence even when leaves had not lost turgidity [36]. Other stress like drought, heat, nutrient deprivation also quickens senescence progression [37,38].

3.7 Alcohol Dehydrogenase Activity

Alcohol dehydrogenase (ADH) converts acetaldehyde to ethanol under oxygen deprivation. Its activity was found to be increased under hypoxia/ anoxia conditions. Among the genotypes of which ADH activity was assayed, variation from 3-4 fold increase to almost same levels in some genotypes was observed. Genotypes showing maximum increase were; ICP-5028 (4 folds), Mal-9 (3 folds), and ICPL-332 (3 folds) while almost no increase was found in Manak, Paras, Pusa-991, Pusa 992 and ICP-7035 (Fig. 2). ADH drives the recycling of NADH to NAD^+, which is foremost requirement of glycolytic pathway's continuation [39,40]. Studies conducted using ADH null mutants exhibited the importance of ethanolic fermentation in several plant species [41,42,43]. Thus, it can be concluded that triggering of ethanolic fermentation is one of the pathways by which plants thrive in anaerobic environment [44] .

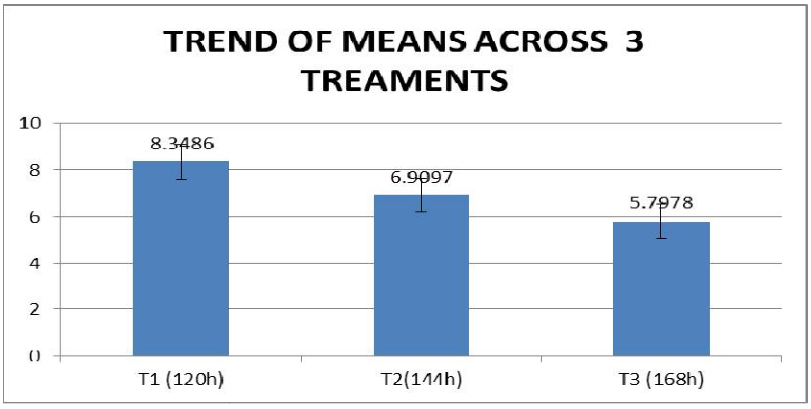
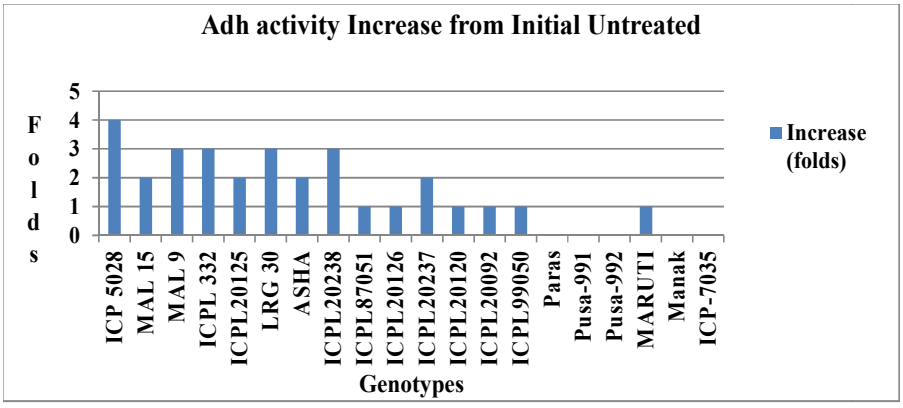


Fig. 1. Trend of germination percent means of all three waterlogging treatment



1 fold increase= 2 folds of initial

Fig. 2. Enzymatic activity (alcohol dehydrogenase) increase in waterlogging stress condition

Table 2. Mean performances for characters recorded at field stage

S.No.	Genotypes	Survival%
1	ICP-5028	62.28
2	MAL 15	58.34
3	ICPL-99088	55.00
4	ICPL-20128	47.22
5	MAL-9	42.49
6	BAHAR	39.34
7	Maruti	38.98
8	ICPL-20238	38.34
9	ICP-9414	37.99
10	Paras	37.96
11	ICPL-990102	35.42
12	MAL 13	35.23
13	ICP 6739	34.31
14	Pusa-992	34.05
15	ICPL-20237	33.39
16	BRA-303	33.33
17	ICPL-99050	32.21
18	ASHA	31.37
19	PUSA-9	30.81
20	ICPL-99087	30.68
21	PAU 881	30.55
22	ICPL-20125	28.99
23	ICPL-20092	28.67
24	ICPL-99098	28.43
25	NDA-1	26.79
26	Pusa-991	26.68
27	ICPL-99090	26.34
28	PATHAM	25.37
29	ICP-13359	23.67
30	ICP-772	23.38
31	Manak	23.28
32	PA-291	23.22
33	ICPL-2011247	22.87
34	LRG-30	20.00
35	ICPL-99091	19.21
36	ICP-11477 (DT)-1	16.82
37	ICPL-332	14.67
38	BRA-304	14.55
39	ICPL-99095	13.66
40	ICP-7035	10.99
	Mean	**30.92**
	C.V.	**33.89**
	C.D. 5%	**21.20**
	S. E.	**7.41**
	Range	**10.99-62.28**

3.8 Assessment of Genetic Diversity Using RAPD Marker

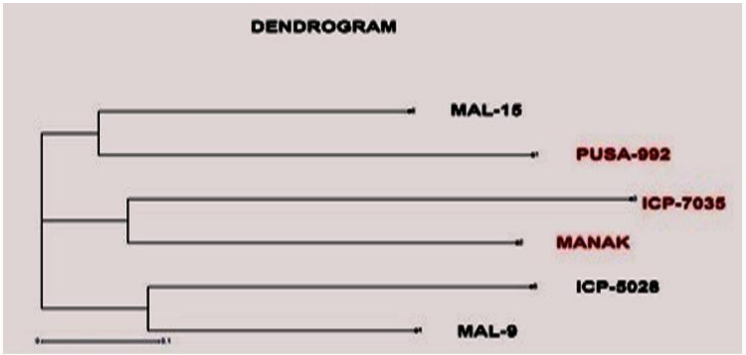
Random Amplification of Polymorphic DNA (RAPD) is one of the pioneer DNA based markers with the advantage of having less pre-requisite knowledge requirements, as it does not require any sequence-based information and specific primers. Any RAPD primers (10 mers generally) can be used for any organism whether plants or animals. In current study, genetic diversity of contrasting pigeonpea genotypes for waterlogging tolerance was assessed using 24 RAPD primers. Out of 24 primers, 9 primers were found to be polymorphic. The polymorphism percentage exhibited by these 9 polymorphic primers was 96.08% with an average of 5.67 amplicons per primer, which was in lieu with the results obtained by Choudhury et al. and Rathnaparkhe et al. [45,46]. In the present investigation two unique amplicons were amplified by primers OPA-13 and OPC-01. OPA-13 exhibited unique amplicon linked with susceptible genotypes of size ~1240 bp while the

unique amplicon given by OPC-01 was of ~980 bp linked with tolerant genotypes. These can be further taken for development of linked SCAR (Sequence Characterised Amplified Regions) markers by validating the present result and increasing the number of RAPD primers. Prasanthi et al. used 200 RAPD primers to develop SCAR in Pigeonpea using contrasting genotypes for sterility mosaic disease [47]. Dendrogram was constructed using amplicon scoring of 9 primers with the help of DARwin 6.0 based on Jaccard's similarity constant grouped genotypes namely; ICP-7035 and Manak together in one cluster while ICP-5028 and Mal-9 were grouped [Fig. 3] in another cluster. Khoiriyah et al., also, used 14 RAPD primers to assess genetic diversity of 30 Pigeonpea genotypes and constructed dendrogram [48].

Table 3. Mean performances for the traits recoded at pot stage

S. No.	Genotype	CHL BF	CHL AF	SENE(1-5)	CHL RED (%)
1	ICP 5028	37.68	32.65	2.00	13.42
2	MAL 15	38.18	32.50	2.50	14.67
3	MAL 9	36.98	32.70	2.00	11.55
4	ICPL 332	36.68	30.45	3.00	16.95
5	ICPL20125	39.00	28.85	4.00	26.06
6	LRG 30	37.63	34.70	2.00	7.73
7	ASHA	34.63	26.20	3.50	24.33
8	ICPL20238	36.18	26.55	2.50	26.64
9	ICPL87051	39.85	30.65	3.50	23.07
10	ICPL20126	35.50	26.45	3.00	25.48
11	ICPL20237	37.23	32.25	2.50	13.36
12	ICPL20120	39.53	29.65	2.50	24.99
13	ICPL20092	37.83	21.00	3.00	44.48
14	ICPL99050	35.35	25.05	4.00	29.41
15	Paras	36.83	29.85	3.50	18.94
16	Pusa-991	34.65	24.00	4.50	30.68
17	Pusa-992	37.53	32.60	3.50	13.14
18	MARUTI	38.13	28.40	2.50	25.51
19	Manak	38.88	21.00	4.00	45.95
20	ICP-7035	18.40	0.00	2.50	50.00
	Mean	**36.33**	**27.28**	**3.03**	**24.32**
	C.V.	**16.24**	**7.72**	**34.28**	**68.79**
	C.D. 5%	**NS**	**4.39**	**NS**	**NS**
	S. E.	**4.17**	**1.49**	**0.73**	**11.83**
	Ranges	**18.40-39.85**	**0.00-34.70**	**2.00-4.50**	**7.73-50.00**

CHL BF= Chlorophyll before WL, CHL AF= Chlorophyll After WL, SENE= Senescence, CHL RED= Chlorophyll Reduction, NS= Non Significant



	A	B	C	D	E
B	0.822				
C	0.920	0.760			
D	0.737	0.723	0.821		
E	0.835	0.822	0.919	0.556	
F	0.633	0.717	0.815	0.631	0.730

Fig. 3. Dendrogram of genotypes on which RAPD marker was run and their Jaccard's constant

4. CONCLUSION

Genotypic/ Genetic approach involving efficient screening techniques and evaluation of breeding lines under waterlogging environment for the traits linked to tolerance is likely lead to identification of high yielding varieties under waterlogging stress. Available waterlogging tolerance in pigeonpea gene pool to some extent can provide source for breeding waterlogging tolerant cultivars as well as for resource poor farming community of the north-eastern region of India, where pigeonpea is extensively grown.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sasidharan R, Bailey-Serres J, Ashikari M, Atwell BJ, Colmer TD, Fagerstedt K, Fukao T, Geigenberger P, Hebelstrup KH, Hill RD, Holdsworth MJ. Community recommendations on terminology and procedures used in flooding and low oxygen stress research. New Phytologist. 2017;214(4):1403-7.
2. Jaiswal A, Srivastava J. Changes in reactive oxygen scavenging systems and protein profiles in maize roots in response to nitric oxide under waterlogging stress. Indian journal of biochemistry & biophysics. 2018; 55.
3. Nguyen LT, Osanai Y, Anderson IC, Bange MP, Tissue DT, Singh BK. Flooding and prolonged drought have differential legacy impacts on soil nitrogen cycling, microbial communities and plant productivity. Plant and Soil. 2018;431(1-2):371-87.
4. FAO (Food and Agriculture Organization of the United Nations), Faostat; 2017.
5. Sultana R. Can a drowning pigeonpeaperform.SA Trends. 2010;102.
6. Choudhary AK, Sultana R, Pratap A, Nadarajan N, Jha UC. Breeding for abiotic stresses in pigeonpea. Journal of Food Legumes. 2011;24(3):165-74.
7. Abebe M, Mamo T, Duffera M, Kidanu S. Durum wheat response to improved drainage of Vertisols in the central highlands of Ethiopia. In seventh regional wheat workshop for Eastern, Central and Southern Africa. Nakuru, Kenya: CIMMYT 1992;407-414.
8. Mickelbart MV, Hasegawa PM, Bailey-Serres J. Genetic mechanisms of abiotic stress tolerance that translate to crop yield stability. Nature Reviews Genetics. 2015; 16(4):237-51.
9. Tsuji H, Saika H, Tsutsumi N, Hirai A, Nakazono M. Dynamic and reversible changes in histone H3-Lys4 methylation and H3 acetylation occurring at submergence-inducible genes in rice. Plant and cell physiology. 2006;47(7):995-1003.
10. Mustroph A, Lee SC, Oosumi T, Zanetti ME, Yang H, Ma K, Yaghoubi-Masihi A, Fukao T, Bailey-Serres J. Cross-kingdom comparison of transcriptomic adjustments to low-oxygen stress highlights conserved and plant-specific responses. Plant Physiology. 2010 ;152(3):1484-500.
11. Lee SC, Mustroph A, Sasidharan R, Vashisht D, Pedersen O, Oosumi T, Voesenek LA, Bailey-Serres J. Molecular characterization of the submergence response of the Arabidopsis thaliana ecotype Columbia. New Phytologist. 2011; 190(2):457-71.
12. Juntawong P, Sirikhachornkit A, Pimjan R, Sonthirod C, Sangsrakru D, Yoocha T, Tangphatsornruang S, Srinives P. Elucidation of the molecular responses to waterlogging in Jatropha roots by transcriptome profiling. Frontiers in Plant Science. 2014;5:658.
13. Saxena KB. Genetic improvement of pigeon pea—a review. Tropical plant biology. 2008;1(2):159-78.
14. Sultana R, Vales MI, Saxena KB, Rathore A, Rao S, Rao SK, Mula MG, Kumar RV. Waterlogging tolerance in pigeonpea (*Cajanus cajan* (L.) Millsp.) genotypic variability and identification of tolerant genotypes. The Journal of Agricultural Science. 2012;151(5):659-71.
15. Chauhan YS, Silim SN, Rao JK, Johansen C.A pot technique to screen pigeonpea cultivars for resistance to waterlogging. Journal of Agronomy and Crop Science. 1997;178(3):179-83.
16. Perera AM, Pooni HS, Saxena KB. Components of genetic variation in short-duration pigeonpea crosses under waterlogged conditions. Journal of Genetics & Breeding. 2001;55(1):21-38.
17. Khare D, Rao S, Lakhani JP, Satpute RG. Tolerance for flooding during germination in pigeonpea. Seed Research. 2002;30:82-7.

18. Hou FF, Thseng FS. Studies on the flooding tolerance of soybean seed: varietal differences. Euphytica. 1991; 57(2):169-73.
19. Ueno K, Takahashi H. Varietal variation and physiological basis for inhibition of wheat seed germination after excessive water treatment. Euphytica. 1997;94(2): 169-73
20. Zhang Z, Wei L, Zou X, Tao Y, Liu Z, Zheng Y. Submergence-responsive micro RNAs are potentially involved in the regulation of morphological and metabolic adaptations in maize root cells. Annals of botany. 2008;102(4):509-19.
21. Hunter MN, Jabrun P, Byth DE. Response of nine soybean lines to soil moisture conditions close to saturation. Australian Journal of Experimental Agriculture. 1980;20(104):339-45.
22. Sayama T, Nakazaki T, Ishikawa G, Yagasaki K, Yamada N, Hirota N, Hirata K, Yoshikawa T, Saito H, Teraishi M, Okumoto Y. QTL analysis of seed-flooding tolerance in soybean (Glycine max [L.] Merr.). Plant Science. 2009;176(4):514-21.
23. Hingane AJ, Saxena KB, Patil SB, Sultana R, Srikanth S, Mallikarjuna N, Vijaykumar R, Sameer Kumar CV. Mechanism of water-logging tolerance in pigeonpea. Indian J Genet Pl Br (The). 2015; 75(2):208.
24. Setter TL, Ellis M, Laureles EV, Ella ES, Senadhira D, Mishra SB, Sarkarung S, Datta S. Physiology and genetics of submergence tolerance in rice," Annals of Botany; 1997.
25. Matsunaga R, Ito O, Tobita S, Rao TP, Johansen C. Response of short-duration pigeonpea to nitrogen application after short-term waterlogging on a Vertisol. Field Crops Research. 1994;38(3):167-74.
26. Coutts MP, Philipson JJ. Tolerance of tree roots to waterlogging: ii. Adaptation of sitka spruce and lodgepole pine to waterlogged soil. New Phytologist. 1978;80(1):71-7.
27. Hsu FH, Lin JB, Chang SR. Effects of waterlogging on seed germination, electric conductivity of seed leakage and developments of hypocotyl and radicle in sudangrass. Botanical Bulletin of Academia Sinica. 2000;41.
28. Ghobadi ME, Ghobadi M, Zebarjadi A. Effect of waterlogging at different growth stages on some morphological traits of wheat varieties. International journal of biometeorology. 2017;61(4):635-45.
29. Mielke MS, de Almeida AA, Gomes FP, Aguilar MA, Mangabeira PA. Leaf gas exchange, chlorophyll fluorescence and growth responses of Genipaamericana seedlings to soil flooding. Environmental and experimental botany. 2003;50(3):221-31.
30. Sasidharan R, Voesenek LA. Ethylene-mediated acclimations to flooding stress. Plant Physiology. 2015;169(1):3-12.
31. Niki T, Yoshida S, Sakai A. Studies on chilling injury in plant cells I. Ultra structural changes associated with chilling injury in callus tissues of *Cornus stolonifera*. Plant and Cell Physiology. 1978;19(1):139-48.
32. Giles KL, Cohen D, Beardsell MF. Effects of water stress on the ultrastructure of leaf cells of Sorghum bicolor. Plant physiology. 1976;57(1):11-4.
33. Yordanova RY, Popova LP. Photosynthetic response of barley plants to soil flooding. Photosynthetica.2001;39(4):515-20.
34. Kumutha D, Sairam RK, Ezhilmathi K, Chinnusamy V, Meena RC. Effect of waterlogging on carbohydrate metabolism in pigeon pea (*Cajanus cajan* L.): upregulation of sucrose synthase and alcohol dehydrogenase. Plant Science. 2008;175(5):706-16.
35. Drew MC, Sisworo EJ. Early effects of flooding on nitrogen deficiency and leaf chlorosis in barley. New Phytologist. 1977; 79(3):567-71.
36. Feller U, Keist M. Senescence and nitrogen metabolism in annual plants. In Fundamental, ecological and agricultural aspects of nitrogen metabolism in higher plants Springer, Dordrecht. 1986;219-234.
37. Dungey NO, Davies DD. Protein turnover in the attached leaves of non-stressed and stressed barley seedlings. Planta. 1982; 154(5):435-40.
38. Roy-Macauley H, Zuily-Fodil Y, Kidric M, Thi AP, de Silva JV. Effect of drought stress on proteolytic activities in Phaseolus and Vigna leaves from sensitive and resistant plants. Physiologia Plantarum. 1992;85(1):90-6.
39. Ismond KP, Dolferus R, De Pauw M, Dennis ES, Good AG. Enhanced low oxygen survival in Arabidopsis through increased metabolic flux in the fermentative pathway. Plant physiology. 2003;132(3):1292-302.
40. Keyhani E, Keyhani J. Hypoxia/anoxia as signaling for increased alcohol

dehydrogenase activity in saffron (*Crocus sativus* L.) corm. Annals of the New York Academy of Sciences. 2004;1030(1):449-57.

41. Matsumura H, Takano T, Yoshida KT, Takeda G. A rice mutant lacking alcohol dehydrogenase. Japanese Journal of Breeding. 1995;45(3):365-7.
42. Ellis MH, Dennis ES, Peacock WJ. Arabidopsis roots and shoots have different mechanisms for hypoxic stress tolerance. Plant Physiology. 1999; 119(1):57-64.
43. Saika H, Matsumura H, Takano T, Tsutsumi N, Nakazono M. A point mutation of Adh1 gene is involved in the repression of coleoptile elongation under submergence in rice. Breeding Science. 2006;56(1):69-74.
44. Magneschi L, Perata P. Rice germination and seedling growth in the absence of oxygen. Annals of Botany. 2009; 103(2):181-96.
45. Choudhury PR, Singh IP, George B, Verma AK, Singh NP. Assessment of genetic diversity of pigeonpea cultivars using RAPD analysis. Biologia Plantarum. 2008;52(4):648-53.
46. Ratnaparkhe MB, Gupta VS, Murthy MV, Ranjekar PK. Genetic fingerprinting of pigeonpea [*Cajanus cajan* (L.) Millsp.] and its wild relatives using RAPD markers. Theoretical and applied genetics. 1995; 91(6-7):893-8.
47. Prasanthi LR, Reddy BV, Rani KR, Sivaprasad Y, Rajeswari T, Reddy KR. RAPD and SCAR marker linked to the sterility mosaic disease resistance gene in PigeonPea (*Cajanus cajan* L. Millsp.). Asian and Australasian Journal of Plant Science and Biotechnology. 1996;3(1):16-20.
48. Khoiriyah N, Yuniastuti E, Purnomo D. Genetic diversity of pigeon pea (*Cajanus cajan* (l.) Millsp.) based on molecular characterization using randomly amplified polymorphic DNA (RAPD) markers. In IOP Conference Series: Earth and Environmental Science. IOP Publishing. 2018;129(1):012016.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/56510