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Original Research Article

Effect of Photosynthetic Cessation on the Traits Associated with Waterlogging Tolerance in Pigeonpea

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A B S T R A C T

Erratic monsoon causing flood in the river Ganga greatly affect the cultivation of pigeonpea in most of the regions of Bihar as they lie in and around the Ganga basin. Given the highly sensitive nature of the early stages of the crop there is dire need to identify and develop tolerant cultivars and parents for the stress breeding program. In this experiment, screening for waterlogging (WL) tolerance was done among twenty genotypes by sowing them into pots and providing controlled waterlogging treatment of 8 days. The genotypes were evaluated on the basis of three important characters viz. survival percent, dry weight per seedling and seedling vigour. Survival percent for the genotypes varied from 0.0% to 60.0%. Genotypes ICP-5028, ICPL-20126, LRG-30 and MAL-9 were genotypes having most plants survived out of WL stress on the contrary the genotypes ICP-7035, Manak, Pusa-991 and ICPL-20126 were with least survival percent. Similarly, to assess dry mass accumulation Dry Weight per Seedling (DWPS) was recorded for control and treated plants and reduction was measured. To quantify the overall tolerability seedling vigour was taken into accountwhich encompasses both survival percent and Dry Weight Per Seedling (DWPS). Tocher's method was used for clustering the genotypes using the data of survival percent. Plants were grouped in six clusters showing different waterlogging reaction viz. Highly tolerant, Tolerant, Moderately Susceptible, Moderately tolerant, susceptible and Highly Susceptible. Significant variability among the genotypes wasfound for waterlogging tolerance.

Introduction

Being a third world crop, pigeonpea has not got the kind of inquisitiveness other crops had. Changing climate and unstable natural ecosystem has led to drastic changes in the weather cycle especially in a sub-tropical country like India. Monsoon rain has become a matter of concern in the current scenario, and we have faced either very less downpour than required or heavy downpour causing flood like situations. Indian agriculture, which majorly depends upon the south-west monsoon, is adversely affected by this. It is well known that the pulse crop especially pigeonpea is much sensitive to waterlogging in its early to late vegetative stages. 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. Around 85% of total world's pigeonpea is being produced by India only, occupying area of around 46.5 lakh hectare giving 30.27 lakh tonnes of produce. In Bihar, it is cultivated on area of around 0.27 lakh hectare giving production of 0.39 lakh tonnes. The productivity of Bihar (1739 Kg/ha) is comparatively higher as compare to the national productivity (937 kg/ha).

Geographical presence of the river Ganga and its tributaries like kosi, gandak etc. cause this crop highly vulnerable to excessive hydrological stress every now and then in the state of Bihar. The sowing time of pigeonpea is around Mid-June to Mid-July in India, which makes the early stages of the crop to coincide with monsoonal downpours in the country. Being a kharif season crop, pigeonpea receives high rainfall. The presence of excessive moisture in the field or root zone enhances the incidence of *Phytophthora* and *Alternaria* blight.

Not only pods but also its other vegetative part is used as a feed, fodder, fuel and fertilizer by the rural section in Bihar. Survival percent is direct measure to assess the survival ability of plants under waterlogging stress. In addition, the reduction of dry matter is good indicator of plants internal well-being in terms of biosynthetic pathways and carbon assimilation under scarce conditions. Photosynthetic cessation is major setback, which plants start to experience during anoxic stress. Aquaporin is also an important membrane protein that helps in passage of water into the cell and is damaged due to reduced hydrolic conduction and water-uptake during waterlogging (Tournaire-Roux *et al.,* 2003). The accumulation of fresh and dry mass is reduced significantly during the waterlogging stress (Shabala *et al.,* 2014). There is dire need to be addressed to develop or identify waterlogging tolerant genotypes which can thrive in low-lying gangetic plains of Bihar. In the present study, we have evaluated pigeonpea genotypes for its waterlogging tolerance on the basis of different physiological parameters.

Materials and Methods

Freshly harvested seeds of 20pigeonpea genotypes were evaluated for waterlogging tolerance in a short period of time using a simple screening method using pots (Table 1). Pots were filled with 8 kg of soil and were mixed with soil: FYM in ratio 50:1(w/w) and Fertilizer (nitrogen, phosphorus and potassium, NPK) was also applied as basal doses. Ten seeds/pot were sown, for each genotype three pots were sown i.e. 2 treated and one as a control. To avoid the incidence of fungal infection, seed treatment was done with contact fungicide Thiram (dithiocarbamate) dust @ 2.5g per kg of the seeds before sowing. For each genotype, three pots were prepared (two pots for imposing stress treatment and one kept as a control, i.e. no treatment). Before application of waterlogging stress treatment, the number of plants in each pot was counted. The stress treatment imposed pots were kept in trench having depth slightly more than the height of pots to create anaerobic waterlogged conditions. Observations involving survival percent, dry weight per seedling and seedling vigour were recorded after 8 days of de-submergence.

Survival percentage

It was recorded by counting the number of plants before and after waterlogging treatment and then taking their ratio followed by multiplication of 100 to get it in percent.

Dry Weight per Seedling (DWPS)

Whole plants were first sundried in natural condition then inside incubator at 40°C till stable weight was attained. Then the plants were weighed and recorded in grams for treated and control plants.

Seedling vigour

It is a composite character, which depends upon the data of survival percent and Dry Weight per Seedling (DWPS). Seedling Vigour tells about the tolerability of plants towards waterlogging stress, which is the outcome of various adjustments in biosynthetic pathways to cope up with the adversity.

SEEDLING VIGOUR(SV) = SURVIVAL PERCENT X DRY WEIGHT PER SEEDLING (DWPS)

Analysis of generated data was done in Completely Randomized Design (CRD) with two replications over the control pots. Analysis of variance among genotypes for the character survival percent, dry weight per seedling and seedling vigour was done using Indostat software. Phenotypic Correlation was also determined among characters. Tocher's clustering was also done using the same software.

Results and Discussion

Survival percentage

Critical reduction was observed in the survival percentage of genotypes after 8th day of waterlogging stress. The genotypes ICP-5028 (60%) along with ICPL-20126 (55%) and MAL-9 (50%) were least affected or least mortile due the waterlogging stress while genotypesICP-7035 (0%), Manak (5%) and Pusa-991 (10%) were the most affected ones. Average performers include genotypes Maruti (19%), Asha (25%), Paras (27.5%), ICPL-20237 (30.5%), Mal-15 (40%) and ICPL-332 (40%) etc. At brief exposure of excessive moisture genotypes does show variation in their survival (Chauhan *et al.*, 1987).

Various studies on environment conditions of southeast Asian countries like India and Thailand unfolds that reduced diffusion of gases specially O_2 and along with this low irradiance sometimes are important towards plant mortality (Setter *et al.*, 1997). When a plant maintains constant level of growth as compared to normal conditions even in waterlogged ones then this is to be known as tolerance towards waterlogging (Setter and Waters, 2003). Kumutha *et al.*, (2009) suggested that waterlogging caused the loss as much as 96% in susceptible genotypes of Pigeonpea. Roots are most affected part due to shortage of oxygen which is further conducted to shoots (Yordanova *et al.*, 2001). This coupled with physiological absence of water to the plant tissues causes wilting and finally mortality of the plant. When tissues are hypoxic or anoxic, the oxygen-dependent pathways, especially the energy-generating systems, are suppressed, the functional relationship between roots and shoots are disturbed, and both carbon assimilation and photosynthetic utilisation are suppressed (Vartapetian and Jackson, 1997). Thus, leading to cessation of photosynthesis.

Dry Weight per Seedling (DWPS)

Genotypes like ICP-7035, Manak, ICPL-20125 and Paras exhibited drastic reductions in their dry weight per seedling i.e. 100%, 67%, 62.5% and 57% respectively as compared to genotypes ICP-5028, ICPL-20092, ICPL-20120 which showed almost same dry weight when compared with untreated control.

Waterlogging has shown significant reduction in photosynthetic efficiency and biological yield in maize (Zaidi *et al.*, 2003; Dhillon *et al.*, 1998; Ashraf and Rehman 1999; Scholowing and Teching, 1997), tomato (Else *et al.*, 2009), soybean (Cho *et al.*, 2006) and barley (Yordanova and Popova, 2001; Yan *et al.*, 1996). Short term exposure of 6 days waterlogging reduced dry weight of genotypes significantly in case of maize (Liu *et al.*, 2010). According to study on plants *Hyparrhenia rufa* and *Andropogon gayanus* by Filho and Lopes (2011) it has been found that the reduction in dry matter was more in waterlogged plants when compared with those grown at field capacity.

Singh *et al.*, (2017) suggested that waterlogging caused reduction in dry weight of seedlings with variations among genotypes. Dry weight enables to determine the biomass content of genotypes produced as result of photosynthetic assimilations after deducting the respiratory losses. Short duration exposure of waterlogging resulted in reduction of biomass by reducing the leaf area, which is mainly responsible for photosynthesis (Takele and Mcdavid, 1995). According to (Araki *et al.*, 2012), wheat roots and shoots were lighter when compared to control ones due to excessive moisture treatment in pots.

The response of photosynthesis to soil flooding resembled that produced by other stresses (osmotic shock and drought). The common events of this response are the slowed rate of carbon assimilation, inhibition of RuBPC activity, and changes in photorespiratory carbon metabolism (Kicheva *et al.*, 1994, Popova *et al.*, 1996, Tsonev *et al.*, 1998). All this contributes towards reduce carbon assimilation thus affecting the Dry weight per seedling.

Seedling Vigour (SV)

Seedling vigor was obtained by multiplying dry weight per seedling with Survival Percent. Results here correspond with that of DWPS and survival percent. Seedling vigour gives overall acceptability of plants for being used as waterlogging tolerant genotype.

Seedling vigour after treatment was found to be maximum with least reduction in genotypes ICP-5028(28%), LRG-30(48%), Mal-9(46%), Mal-15(58%) while at the lower end with maximum reduction compared to control in genotypes ICP-7035(100%), Manak (97%), ICPL-20238 (89%), Paras (89%), ICPL-20120 (83%), Pusa 991(89%) were observed. Seedling vigour is directly correlated to survival percent.

Significant positive correlation of Seedling Vigour with Survival percent and Dry Weight per Seedling was found i.e. r=0.85** and 0.62 ** respectively.

Shortage or insufficient oxygen supply attributes to reduced vigour of seedlings. 16.6 to 58.3 % reduction was found in pigeonpea genotypes after 8 days of waterlogging treatment (Lal M., 2014).

As oxygen is final electron acceptor in electron transport chain so the final sink for electron becomes unavailable thus affecting the whole respiration process thus hampering Adenosine Triphosphate (ATP) production, which results in decreased vigour and poor germination. This is also in lieu with reports of Johnson *et al.*, (1989). These results also had similarity with results of Wang *et al.*, (2012).

Clustering of genotypes using tocher's method

The character used for Tocher's Method clustering (using Indostat software) was Survival percent. As Survival percent is easily deducible and direct measure for waterlogging tolerance among pigeonpea genotypes. The results obtained were concurrent with classification on the basis of absolute values of Survival Percent. Details of various clusters include viz. Highly Susceptible (Cluster 6), Susceptible (Cluster 2), Moderately Tolerant (Cluster 4), Moderately Susceptible (Cluster 1), Tolerant (Cluster 3) and Highly Tolerant (Cluster 5).

Table.1 List of genotypes and mean performances for the traits recoded

S.NO.	GENOTYPE	SURVIVAL%	DWPS (WL)	DWPS (N)	SV (WL)	SV (N)
1	**ICP 5028**	60	0.07	0.06	3.8	5.27
2	**MAL 15**	40	0.06	0.07	2.57	6.14
3	**MAL 9**	50	0.06	0.08	2.83	5.22
4	**ICPL 332**	40	0.05	0.07	2.07	6.21
5	**ICPL20125**	40	0.03	0.06	1.09	4.91
6	**LRG 30**	50	0.06	0.07	2.84	5.49
7	**ASHA**	25	0.07	0.07	1.79	4.83
8	**ICPL20238**	12.5	0.05	0.06	0.59	5.26
9	**ICPL87051**	29.5	0.04	0.06	1.16	3.59
10	**ICPL20126**	55	0.03	0.08	1.64	5.38
11	**ICPL20237**	30.5	0.08	0.07	2.25	5.97
12	**ICPL20120**	11.3	0.06	0.05	0.59	3.51
13	**ICPL20092**	32	0.07	0.07	2.05	5.28
14	**ICPL99050**	41.5	0.05	0.07	2.17	6.61
15	**Paras**	27.5	0.03	0.07	0.62	5.62
16	**Pusa-991**	10	0.04	0.06	0.61	5.42
17	**Pusa-992**	12.1	0.07	0.07	0.85	4.4
18	**MARUTI**	19	0.07	0.08	1.24	7.57
19	**Manak**	5	0.02	0.06	0.13	4.48
20	**ICP-7035**	0	0	0.06	0	5.34
	Mean	**29.5**	**0.05**	**0.07**	**1.54**	**5.32**
	C.V.	**14.46**	**15.97**	**6.1**	**18.79**	**10.04**
	C.D. 5 %	**5.34**	**0.02**	**0.01**	**0.6**	**1.12**
	Ranges	**0.00-60.00**	**0.00-0.08**	**0.050-0.08**	**0.00-3.80**	**3.51-7.57**

Note: DWPS= Dry Weight per Seedling, SV= Seedling Vigor, WL- Waterlogging, N= Normal/Control

Fig.1 Comparison of survival percent and Reduction in Dry matter Per Seedling (Red DWPS) and Seedling Vigour (Red SV)

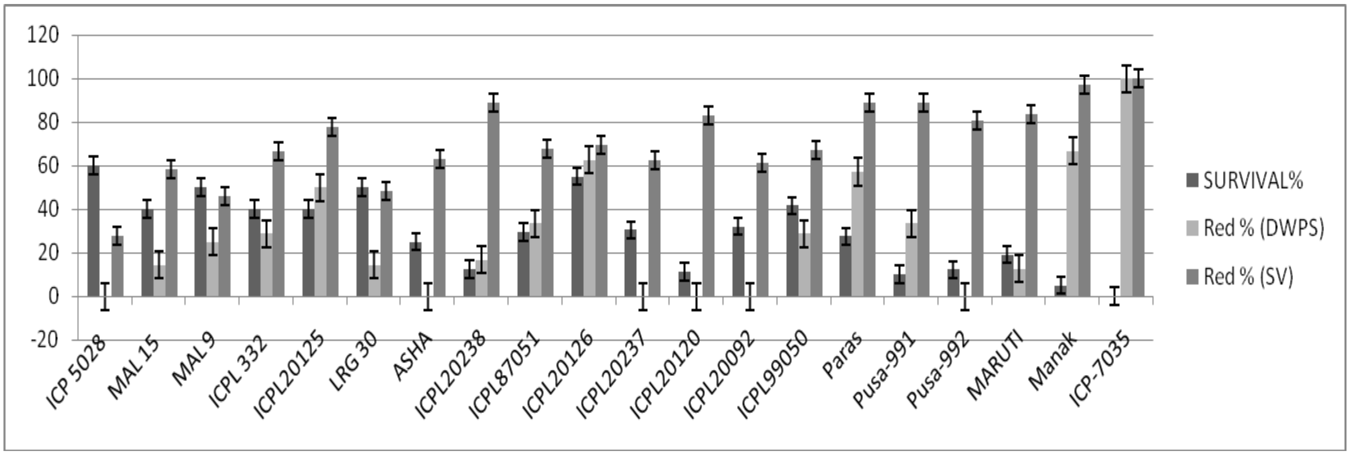
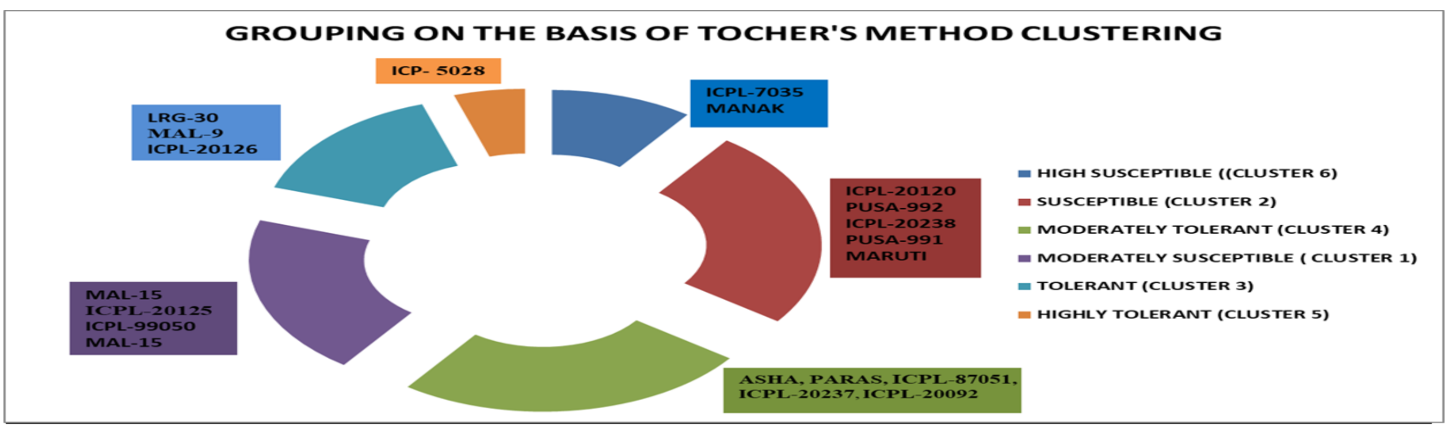


Fig.2 Classification of genotypes on the basis of Tocher's Method clustering by Survival % and cluster distances



	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6
Cluster 1	0.09	19.34	3.8	4.16	10.37	37.37
Cluster 2	19.34	0.47	39.66	6.01	57.77	3.33
Cluster 3	3.8	39.66	0.52	15.45	1.91	64.21
Cluster 4	4.16	6.01	15.45	0.38	27.28	17.16
Cluster 5	10.37	57.77	1.91	27.28	0	86.87
Cluster 6	37.37	3.33	64.21	17.16	86.87	0.72

When compared with cluster 6 the distances were in order: Cluster 2 (3.3) < Cluster 4 (17.16) < Cluster 1 (37.27) < Cluster 3 (64.21) <Cluster 5 (86.87). It is very clear from the distance table that cluster 6 (highly susceptible pool) is at maximum distance from cluster 5 (highly tolerant pool).

Cluster 5 which show highly tolerant group contains single genotypes ICP-5028, cluster 3 containing tolerant genotypes has genotypes LRG-30, Mal-9 and ICPL-20126. Cluster 1 is moderately susceptible group having genotypes Mal-15, ICPL-20125 and ICPL-99050 likewise other genotypes are grouped into remaining clusters shown in fig 2.

From above obtained reactions, it can be concluded that significant genotypic variability was observed among the pigeonpea genotypes screened for waterlogging tolerance; hence there is ample scope for selection of promising lines for waterlogging tolerance. The promising waterlogging tolerant lines may be utilized either directly or through introgression breeding to transfer waterlogging tolerance trait in high yielding cultivars of pigeonpea.

The photosynthetic capability and water uptake capability of plants are the two major factors which play key role in deciding the tolerability towards waterlogging. Therefore, the traits like survival percentage, DWPS and seedling vigour would help in evaluation, and thus genetic improvement through selection for these traits would be rewarding.

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