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Screening of pigeonpea genotypes for resistance to short term water logging stress

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Abstract
Ten genotypes of pigeonpea were investigated for their response to short term root zone waterlogging stress imposed at an early stage of growth. Waterlogging caused significant reduction in plant dry weight. Number of leaf-lets/plant, leaf area/plant, photosynthetic rate and chlorophyll content decreased under the influence of waterlogging. Genotype ICPL-84023 and PTH-1 were recorded lesser reduction in plant dry weight, maintained higher leaf area and photosynthetic rate among the studied genotypes. The reduction in all the observed parameters was higher in genotype MAL-18. It is observed that the product of leaf area/plant × photosynthetic rate is a better parameter to identify waterlogging resistant genotypes in pigeonpea instead of reduction in leaflet number/plant, leaf area/plant or photosynthetic rate individually.

Keywords: pigeonpea genotypes, resistance, short term water logging stress

Introduction
Pigeonpea [*Cajanus cajan* (L.) Millsp.], commonly known as *arhar*, *redgram*, *toovar*, *toor*, or *Gungopea* is member of the Fabaceae family. It is an important legume crop of rainfed agriculture, primarily cultivated in Asia, Africa, Latin America and the Caribbean region. It is of high nutritive value and being a legume, it forms root nodules in association with *Rhiozobium* and is capable of fixing atmospheric nitrogen. According to FAO (2007) [8], India is a major pigeonpea producer, having productivity of 711 kg/ha with 2.51 million tons production. Productivity of pigeonpea in India is low due to various biotic and abiotic stresses. Waterlogging is the main problem in pigeonpea production. In the North-East-Plain-Zone (NEPZ) of India, pigeonpea is sown in the beginning of rainy season (*kharif*) and heavy or continuous rains result in considerable loss in crop vigour and plant stand (Chauhan *et al.* 1997) [2]. Major factor for reduced plant growth and poor crop productivity under waterlogging condition is reduced oxygen concentration in root zone resulting in derangement in morphological, physiological and biochemical parameters of the plant (Kozlowski 1984, Srivastava *et al.* 2007) [19, 18]. Though parameters/traits associated with waterlogging resistance in crop plants *viz*., maize (Zaidi *et al.* 2003, Singh 2008) [23, 14], wheat (Malik *et al.* 2002) [12] and barley (Yordanova and Popova, 2001) [22] etc., are widely studied, but such studies are rather limited in pigeonpea (Chauhan *et al.* 1997) [2]. In the present study, an attempt has been made to investigate the genotypicvariability in waterlogging resistance in pigeonpea and to identify the morphophysiological parameters associated with waterlogging resistance in this crop for rapid screening at early growth stage.

Materials and Methods
Seeds of pigeonpea genotypes *viz*., ICPL-84023, MAL-26, MAL-6, MAL-23, ICPL-7035, PTH-1, MA-3, MAL-13, MAL-24 and MAL-18 were procured from the Department of Genetics and Plant Breeding, Institute of Agricultural Science, B. H. U., Varanasi. Experiment was conducted in pots in the net house of the Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India during rainy season (*kharif*) 2007-08 and 2008-09. Seeds were sown in plastic pots (diameter 15 cm) filled with well pulverized 1.5 kg soil collected from field in which pigeonpea was not sown for many years. Five healthy and uniform seedlings were maintained in each pot. Plants ware maintained at optimum soil moisture and fertilizers. Waterlogging stress was imposed at 40 days after sowing by placing pots in water filled cemented container (55 cm x 55 cm x 55 cm) in such a way that the pots were completely submerged and the water level in the container was maintained 5 cm above the soil surface in the pots. The water level was maintained daily.

This treatment was referred to as 'waterlogging treatment'. One set of pots of each genotype was maintained at optimal supply of soil moisture, and termed as 'normal'.
The normal and waterlogged plants were removed from pots carefully after 20 days of imposing stress. Plants were cleaned with tape water, followed by distilled water, and blotted carefully, oven dried at 105°C for 1 hours followed by at 65°C till constant weight and weighed for total dry matter. Leaf area of green leaves per plant (cm^2 per plant) was measured by leaf area meter (Systronics Leaf Area Meter 211) at 4 to 20 days after imposing stress. Green leaflets plant-1 were counted separately at 20 days after imposing stress. Carbon exchange rate in fully expanded leaf from top was measured between 9.00 to 10.30 hr after 20 days of imposing waterlogging stress in waterlogged and normal plants with the help of Infrared Gas Analyzer (IRGA, LCi Portable Photosynthesis System, ADC). Chlorophyll content was measured during 11 to 20 days after imposing stress with the help of SPAD meter (Minolta). For chlorophyll content, first fully expanded leaf from top was tagged in normal and waterlogged plants on very first day of observation and latter on all the observations were recorded on this leaf. Data were analyzed by CRD factorial with two factor analysis of variance and depicted as mean value of three replicates. Critical difference (CD) for mean values was calculated at 5% (Gomez and Gomez, 1984) [9].

Result

On the basis of reduction in plant dry weight after 20 days of waterlogging genotype ICPL-84023 and PTH-1 were identified as relatively resistant, as these genotypes tend to maintain plant dry weight under the influence of waterlogging stress, whereas MAL-18 as most susceptible, and rest of the genotypes performed in between (Table 1). Genotype PTH-1 registered marginal improvement in plant dry weight under waterlogging but it did not differ significantly with respect to normal. Per cent reduction in plant dry weight, under the influence of waterlogging was the minimum in genotype ICPL-84023 (9.6%) and the maximum in MAL-23 (46.6%) followed by genotype MAL-18 (41.7%). Leaflet number plant-1 decreased significantly due to waterlogging treatment during both the years of experimentation (Table 1). On an average, leaflet/plant under normal as well as waterlogged conditions was more during 2008-09 than during 2007-08. Genotypes ICPL-84023 and PTH-1 maintained the maximum number of leaflets/plant under waterlogged condition while genotype MAL-18 the minimum. Rest of the genotypes performed in between.
Waterlogging reduced leaf area/plant. Differences were significant with respect to genotype, treatment and their interactions (Table 1). On an average, leaf area/plant was more during 2008-09 than during 2007-08. During 2007-08, per cent reduction in leaf area in waterlogged plants as compares to normal was the minimum in genotype PTH-1 (16.1 %), while during 2008-09, the reduction was the minimum in ICPL-84023 (28.7%) followed by PTH-1 (31.0 %). During both the years' there was the maximum reduction in leaf area of waterlogged MAL-18 (66.6 %-75.3 %). Rate of photosynthesis (μ mol CO_2 m^{-2} s^{-1}) was recorded 20 days after imposing waterlogging stress (Table 2). Differences were significant with respect to genotype, treatment and their interactions. Though as compared to normal plants, photosynthetic rate in waterlogged plants was much lower in all the genotypes but the differences were not significant in genotypes ICPL-84023, MAL-23 and ICPL- 7035. Chlorophyll content reduced significantly as a consequence of waterlogging (Fig.1).
During 2007-08, reduction in chlorophyll content was observed in waterlogged plants of all the genotypes at different stages of observation. In genotype MAL-23, per cent reduction in chlorophyll content was maximum after 11 days of imposing stress and in genotype MAL-18, after 16 and 20 days of imposing stress. Among the studied genotypes MAL-24 maintained maximum chlorophyll content under waterlogging. During 2008-09 at all the stages, treatment and genotypic differences were significant and in all the genotypes, where chlorophyll content in leaves of waterlogged plants of all the genotypes decreased, except in PTH-1, where it increased marginally at 11 days after imposing stress. When per cent reduction was calculated at 20 days after imposing stress, the reduction was the maximum in genotype MAL-18 and the minimum in ICPL-84023.

Discussion

In the present investigation, waterlogging caused significant reduction in plant dry weight, number of leaflets/plant, leaf area/plant and chlorophyll content, which are in agreements with the earlier observations on mungbean (Laosuwan *et al.* 1994) [11] and cotton (Conaty *et al.* 2008) [5]. Waterlogging resistant genotypes ICPL-84023 and PTH-1 maintained significantly higher number of leaflets/plant as compared to susceptible genotype MAL-18 under stress condition. Reduced leaflet number $plant^{-1}$ in waterlogged plants was mainly due to enhanced senescence of leaves. Under the influence of waterlogging there was significant reduction in leaf area/plant in susceptible genotype MAL-18. Induction in leaf senescence and reduction in leaf area development has been reported to be the most sensitive features in pigeonpea (Kumutha *et al.* 2009) [10].
Waterlogging resulted in significant reduction in chlorophyll content in barley (Yordanova and Popova 2001) [22], soybean (Cho *et al.* 2006) [3], tomato (Else *et al.* 2009) [7] and pigeonpea (Kumutha *et al.* 2008) [20]. In the present investigation chlorophyll content in leaves of waterlogged pigeonpea genotypes generally decreased at all the stages of observation during both the years. In genotype PTH-1, reduction in leaf chlorophyll content was less as compared to that in genotype MAL-18, which had least chlorophyll content under waterlogged condition at all the stages. It is apparent that in waterlogging resistant genotypes of pigeonpea certain mechanism (s) operate (s) either to protect chlorophyll loss or to maintain chlorophyll biosynthesis under waterlogged condition. Reports indicate increased chlorophyllase activity under abiotic stress (Pereira and Kozlowski 1977, Pezashki 2001, Shah 2007) [15, 16, 13] and, therefore, this aspect requires further investigation in pigeonpea.
Waterlogging caused significant reduction in photosynthetic rate. Reduced photosynthetic rate along with reduced stomatal conductance under waterlogged condition are documented in maize (Yan *et al*. 1996, Dhillon *et al*. 1998, Zaidi *et al*. 2003, Singh 2008, Srivastava *et al.* 2010) [21, 6, 23, 17, 14], barley (Yordanova and Popova 2001) [22], soybean (Cho *et al*. 2006) [3], mung bean (Ahmed *et al.* 2002) [1], tomato (Else *et al*. 2009) [7], lucerne (Christiane and Sergey 2005) [4], cotton (Conaty *et al*. 2008) [5] and pigeonpea (Srivastava *et al*. 2010) [17]. Waterlogging resistant genotype ICPL-84023 registered lower photosynthetic rate under normal condition, while PTH-

1 and MAL-18 exhibited significantly much higher photosynthetic rates. Though waterlogging caused significant reduction in photosynthetic rate in the studied genotypes, but the reduction was not significant in genotypes ICPL-84023, MAL-23 and ICPL-7035. The reduction in photosynthetic rate, chlorophyll content, leaf area/plant or the number of leaflets/plant seems to be inadequate to relate with the relative performance of the genotypes (in terms of dry matter production/plant) under waterlogged condition. Nevertheless, if the product of photosynthetic rate and leaf area/ plant is taken into account, it relates well with the relative performance of the genotypes under waterlogged condition (Table 2). Therefore, it is suggested that this parameter be taken for screening waterlogging resistant pigeonpea genotypes.

Table 1: Total dry weight (g/plant), Number of leaflets/plant and leaf area/plant (cm^{-2}) in pigeonpea genotypes after 20 days of imposing waterlogging stress

Treatment	Total dry weight		No of leaflet per plant				Leaf area /plant			
Genotype			2007-08		2008-09		2007-08		2008-09	
	Normal	Waterlogged	Normal	Waterlogged	Normal	Waterlogged	Normal	Waterlogged	Normal	Waterlogged
ICPL-84023	0.62	0.56	23.67	12.00	44.67	42.00	104.13	58.47	325.70	231.93
MAL-26	0.89	0.69	25.00	10.67	44.67	34.67	141.93	65.10	303.47	175.10
MA 6	1.20	0.70	32.00	10.67	39.00	36.00	176.63	88.13	278.70	156.60
MAL-23	1.78	0.95	28.00	14.33	43.33	27.00	131.77	98.63	296.23	131.60
ICPL-7035	0.83	0.62	26.00	10.67	42.67	22.67	141.60	92.93	311.87	123.37
PTH-1	0.55	0.69	21.00	12.33	43.00	35.33	97.00	81.37	313.67	216.13
MA-3	0.68	0.55	27.33	12.00	42.33	24.33	116.27	62.47	296.93	124.60
MAL-13	0.79	0.54	29.33	8.33	41.00	22.67	105.33	52.80	312.93	127.33
MAL-24	1.02	0.88	30.33	12.00	44.00	34.67	166.93	64.47	307.83	167.00
MAL-18	0.91	0.53	24.67	7.00	37.00	16.33	134.53	33.17	297.63	99.33
CD (P=0.05)										
Genotype (G)	0.37		6.49		8.77		33.00		38.48	
Stress (S)	0.17		2.90		3.92		14.76		17.21	
G x S	0.52		9.17		12.41		46.67		54.42	

Mean (n=3) with in a column are not significantly different at $P<0.05$

Table 2: Photosynthetic rate (μmol m^{-2} s^{-1}) and Leaf area × Photosynthetic rate (μmol s^{-1}) in pigeonpea genotypes during 2008-09 after 40 Days of imposing stress

Treatment	Photosynthetic rate		Leaf area x photosynthetic rate	
Genotype	Normal	Waterlogged	Normal	Waterlogged
ICPL-84023	10.55	9.18	0.34	0.21
MAL-26	14.09	6.50	0.42	0.11
MA 6	18.50	5.06	0.51	0.07
MAL-23	10.08	8.12	0.29	0.10
ICPL-7035	10.26	8.08	0.31	0.09
PTH-1	24.33	16.50	0.76	0.35
MA-3	14.31	7.50	0.42	0.09
MAL-13	28.50	17.90	0.89	0.22
MAL-24	23.20	19.80	0.71	0.33
MAL-18	22.49	6.40	0.66	0.06

Mean (n=3) with in a column are not significantly different at $P<0.05$

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