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Genetics and Plant Breeding

Morpho-physiological Basis of Waterlogging Tolerance in Pigeonpea [*Cajanus cajan* (L.) Millsp.]

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Abstract

Global climate change predictions suggest new scenarios with large arid areas and extreme climatologic events. Thus it is essential to understand how plants respond to different abiotic stresses in order to improve crop performance. A pot experiment was carried in a net house in four replicates of normal (no waterlogging) and waterlogging stress. Excess soil moisture stress was imposed at 21 days after sowing by placing the pots of each genotype in water filled troughs for 7 and 14 days. The data revealed that the genotypes ICPB 2039 and KPBR 80-2-1 were showed significantly superiority during 7 and 14 days waterlogging stress and during recovery for survival percentage. However, during 7 days waterlogging stress genotype KPBR 80-2-1 was superior for plant height and chlorophyll content, ICPH 2431 for root length, ICPL 20128 for leaf area, JBP 110-B for relative water content and JKM 7 for total dry matter production. When the waterlogging duration exceeded 14 days the genotypes showed the highest plant height and relative water content by ICPL 87051, root length and total dry matter production by JKM 7, leaf area by ICPH 2431 and chlorophyll content by C 11 under waterlogged conditions.

Highlights

- ICPB 2039 and KPBR 80-2-1 were significantly superior for 7 and 14 days waterlogging.
- Seedling mortality increased with increasing waterlogging period.
- Decline in the oxygen level of the soil adversely affecting root growth.

Keywords: Pigeonpea, Submergence, Leaf Area, Chlorophyll Content, Relative Water Content, Dry Matter Production

Among the abiotic stresses waterlogging during June to September is one of the basic constraints in pigeonpea production. Waterlogging is caused due to erratic and intense rainfall for a prolonged period and occurs when the soil water table attains a level during which the soil pores in the root zone get saturated and thus restricts normal air circulation. There is a decline in the oxygen level of the soil with an increase in the carbon dioxide concentration, thus adversely affecting root growth. Drastic reduction in oxygen level is the primary stress to which the plants are exposed to under waterlogging conditions. Pigeonpea cannot withstand low oxygen conditions at the rhizosphere level, caused by waterlogging, resulting in substantial yield losses. During 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.

Considering such huge losses caused by waterlogging, there is a need to develop waterlogging tolerant pigeonpea genotypes. Although some agronomic management options as the use of raised beds, planting on ridges, transplanting of seedlings, could be a partial solution to the waterlogging problem. These options are not economically viable for the resource poor farming community. Thus the use of waterlogging tolerant genotypes would be the most economical, easiest and a time effective way to minimize losses. The investigations would be highly meaningful through screening suitable genotype which can be grown successfully under such conditions. The traits identified in the study may later be utilized for further breeding programme in developing tolerant lines.

Materials and Methods

The experiment was conducted in the net house of the Department of Plant Physiology, College of Agriculture, Jawaharlal Nehru Agricultural University, Jabalpur, Madhya Pradesh, India during 2011-12 and 2012-13. Seeds were sown in pots in 4 replications with control and data were statistically analyzed through Complete Randomized Design.

Seed of 31 pigeonpea genotypes viz UPAS 120, ICPL 20123, ICPB 2039, ICPL 87051, Maruti, ICPL 20125, ICPH 2740, ICPL 20241, ICPH 2431, JBP 110-B, LRG 30, ICPL 20128, KPBR 80-2-1, RG 135, RG 137, RG 188, RG 190, RG 198, RG 199, RG 211, C 11, JA 4, ICP 8863, JKM 7, BRG 1, GT 100, H 04-26, JP 10, JP 11, JP 12 and JP 14 were sown. The evaluation was conducted by using plastic pots of 30 cm x 30 cm (diameter x height). Filled with soil and farmyard manure (FYM) in a ratio of 3:1 v/v. Inorganic fertilizers were added @ 20:60:20 kg NPK ha-1 as basal dose. Eight pots were prepared for each genotype (four pots for imposing stress treatment and four pots were kept as a control, i.e. no waterlogged) and the seeds were sown at a depth of 5 cm. Seedlings were maintained with sufficient moisture. Later, five healthy and uniform seedlings were maintained in each pot.

Waterlogging stress: Excess soil moisture stress was imposed after 21 days of sowing by placing one set of plastic pots (four pots) of each genotype in water filled plastic troughs in such a way that the pots were completely submerged and the water level in the troughs was maintained 3-5 cm above the soil surface of the pots and kept constant throughout the experimentation. The remaining sets of pots (four pots) were maintained at optimum soil moisture. Treatments consisted of control, 7 and 14 days of waterlogging, and recovery 6 and 8 days after drainout.

The observations were recorded by sampling of plants under normal and stressed conditions. Survival percent of the plants were recorded on seven and fourteen days after waterlogging and six and eight day after drainout. The morphological attributes viz; plant height, leaf area (Laser area meter Model LI-300), chlorophyll content (SPAD-502 plus), relative water content, root length and total dry matter production were recorded at eight days after termination of treatment.

Results and Discussion

The results showed that, during seven days waterlogging only fifteen lines were survived when the waterlogging period was increased with fourteen days only twelve lines were survived out of 31 lines.

Survival (%): The waterlogging stress cause a significant reduction in survival percentage was recorded on seven and fourteen days after waterlogging, six and eight days after drain out among the genotypes. The highest survival percentage was noted on seven day of waterlogging stress. However, it reduced constantly on the six and eight days after drain out.

The present study demonstrated that during seven days waterlogging stress the highest survival percentage was attained by ICPB 2039 (64.38) while it was minimum in BGR 1 (20.00) under seven days after waterlogging (Fig 1 A). Six days after drainout, genotypes ICPB 2039 (53.75) and H-04-26 (16.88) were exhibited maximum and minimum survival percentage (Fig 1B). However, eight days after drain out the genotype ICPB 2039 (48.75) was showed maximum and JP 10 (16.25) minimum survival percentage (Fig 1C). Moreover, during fourteen days waterlogging stress the highest survival percentage (48.75, 35.00 and 31.88) was showed by genotype KPBR 80-2-1, ICPB 2039 and ICPL 87051 whereas the lowest (26.88, 16.25 and 20.00) by ICPL 20128, RG 188 and JP 10 under fourteen days after waterlogging, six and eight

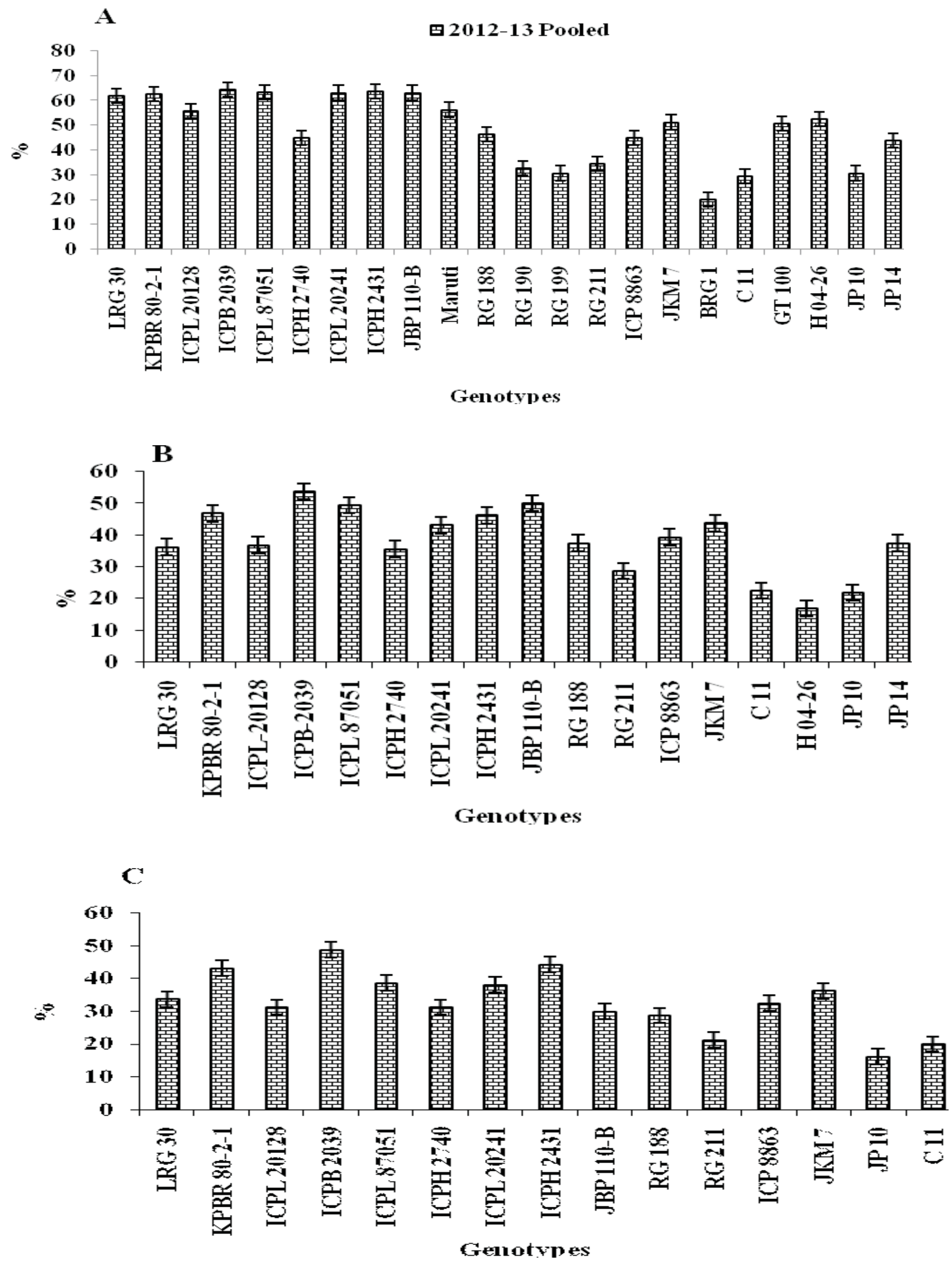

Fig 1. Effect of 7 days waterlogging on survival percentage of pigeonpea genotypes at 7 days after waterlogging (A), 6 days after drainout (B) and 8 days after drainout (C)

days after drainout respectively (Fig 2A, B and C). However, closely related work by Bansal and Srivastava (2012) emphasized that pigeonpea is a waterlogging sensitive legume crop. During waterlogging stress the loss was more in pigeonpea susceptible genotype, which also suffered 96% mortality during recovery (Kumutha *et al.,* 2009). Due to shifting of energy metabolism from aerobic to anaerobic mode under hypoxia or anoxia the energy requirements of the tissue is greatly restricted as very few ATPs are generated per molecule of glucose. A high level of anaerobic metabolism in hypoxic or anoxic roots is therefore very important to supply the energy charge high enough which can sustain metabolism in roots for the survival of plants. Thus, maintaining adequate levels of readily metabolizable (fermentable) sugars in hypoxic or anoxic roots is one of the adaptive mechanisms to waterlogging or oxygen deficient environment (Sairam *et al.,* 2009b; Irfan *et al.,* 2010; Singla and Inubushi 2013).

Plant height (cm plant^{-1}): The present findings revealed that genotype ICPL 20128 (63.58), KPBR 80-2-1 (52.98) and RG 211 (39.41 and 31.36) possessed the highest and the lowest plant height under normal and waterlogged conditions respectively (Table 1) during seven days waterlogging stress. Fourteen days waterlogging stress (Table 3) revealed that maximum plant height was attained by ICPL 87051 (61.23 and 42.62) and it was minimum in ICPH 2431(34.45 and 43.58) under normal and waterlogged conditions respectively. The waterlogging stress caused a significant decreased plant height compared with the non-waterlogged control and it was significantly reduced (23 to 30%) due to waterlogging treatment according to (Habibzadeh *et al.,* 2012; Shimono *et al.,* 2012).

Table 1. Effect of 7 days waterlogging (WL) on plant height, leaf area and chlorophyll content of pigeonpea genotypes

Genotypes	Plant height		Leaf area		Chlorophyll content	
	Normal	WL	Normal	WL	Normal	WL
LRG 30	51.45	41.35	289.30	179.02	46.87	40.62
KBPR 80-2-1	59.62	52.93	294.80	229.12	53.23	45.74
ICPL 20128	63.58	46.31	316.87	275.25	41.14	36.70
JBP 110-B	55.60	39.60	278.69	222.42	61.91	38.63
ICPB 2039	51.52	40.62	223.69	206.59	36.99	34.25
ICPL 87051	54.98	40.62	225.50	177.98	40.45	30.21
ICPH 2740	48.97	34.97	197.76	125.84	43.17	30.76
ICPL 20241	43.04	32.53	311.63	209.04	36.66	28.77
ICPH 2431	47.19	35.11	228.22	186.44	52.90	43.26
RG 188	44.32	34.04	298.49	203.84	54.21	44.07
RG 211	39.41	31.96	254.86	175.48	45.87	26.28
ICP 8863	42.64	36.67	247.35	179.94	43.96	40.27
JKM 7	45.22	38.81	309.63	226.56	55.22	42.80
C 11	44.64	35.49	227.93	179.20	50.19	40.38
JP 10	44.64	34.41	204.81	173.24	54.88	43.66
Mean	49.12	38.36	260.64	196.66	47.84	37.76
SEm ±	4.26	3.12	23.05	19.12	4.18	3.49
CD 5%	12.11	8.88	65.54	54.37	11.89	9.92

Table 2. Effect of 7 days waterlogging (WL) on relative water content, root length and total dry matter production of pigeonpea genotypes

Genotypes	RWC		Root length		TDM	
	Normal	WL	Normal	WL	Normal	WL
LRG 30	69.92	60.80	24.77	19.84	2.97	2.42
KBPR 80-2-1	71.82	67.02	31.15	20.52	3.44	2.94
ICPL 20128	77.18	63.96	28.39	21.00	3.04	2.46
JBP 110-B	76.74	72.35	30.09	22.08	3.23	2.06
ICPB 2039	80.63	70.04	27.94	21.58	3.14	2.24
ICPL 87051	80.48	70.75	25.48	21.40	2.50	1.90
ICPH 2740	67.52	54.81	28.73	16.33	3.49	2.07
ICPL 20241	59.46	47.44	26.71	16.04	2.55	1.65
ICPH 2431	54.18	43.03	29.45	22.49	3.76	2.62
RG 188	66.69	57.63	26.08	9.96	2.26	1.42
RG 211	70.66	60.51	26.53	15.06	2.85	1.59
ICP 8863	47.29	34.57	24.68	12.87	3.19	2.24
JKM 7	67.16	55.83	31.20	20.50	3.62	2.99
C 11	53.92	41.61	19.85	12.64	2.74	1.93
JP 10	67.65	43.58	27.04	11.81	3.12	2.20
Mean	67.42	56.26	27.21	17.61	3.06	2.18
SEm ±	5.56	6.63	1.64	2.39	0.24	0.26
CD 5%	15.81	18.84	4.66	6.80	0.68	0.75

Table 3. Effect of 14 days waterlogging (WL) on plant height, leaf area and chlorophyll content of pigeonpea genotypes

Genotypes	Plant height		Leaf area		Chlorophyll content	
	Normal	WL	Normal	WL	Normal	WL
ICPB 2039	61.10	40.02	275.46	229.46	42.72	39.02
ICPL 87051	61.23	42.62	221.37	176.57	38.05	36.45
ICPL 20241	49.31	37.41	202.48	130.37	42.53	31.57
ICPH 2431	43.58	34.45	307.99	230.58	36.81	27.70
ICP 8863	45.21	37.94	261.49	202.91	49.21	44.01
JKM 7	45.78	38.81	311.02	222.02	55.16	40.94
JP 10	47.53	39.01	223.33	185.65	51.02	39.56
C 11	45.15	36.05	206.60	152.10	54.11	45.64
Mean	49.86	38.29	251.22	191.21	46.20	38.11
SEm ±	1.78	1.56	11.62	7.20	1.97	1.67
CD 5%	5.18	4.56	33.92	21.02	5.75	4.86

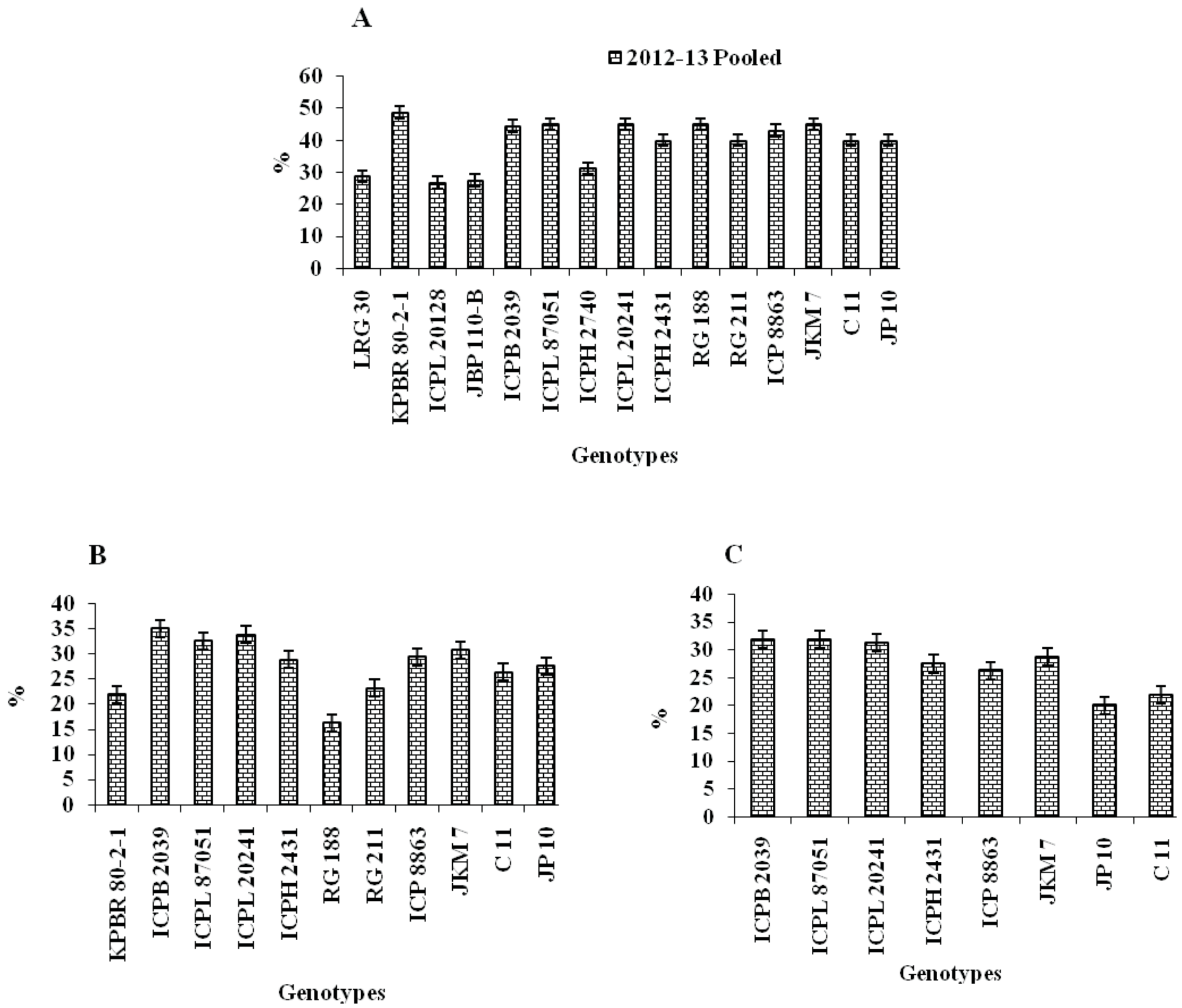

Fig 2. Effect of 14 days waterlogging on survival percentage of pigeonpea genotypes at 14 days after waterlogging (A), 6 days after drainout (B) and 8 days after drainout (C)

Leaf area (cm^2 plant-1): The data in Table 1 and 3 revealed that during seven days waterlogging treatments under normal conditions the genotype ICPL 20128 (316.87) exhibited maximum leaf area while minimum in ICPH 2740 (197.76) however, under waterlogged conditions genotype ICPL 20128 (275.25) was achieved maximum leaf area and ICPH 2740 (125.24) recorded minimum value for this trait (Table 1). When the waterlogging stress duration exceeded fourteen days, under normal conditions JKM 7 (311.02) was achieved maximum and ICPL 20241 (202.48) minimum leaf area however, under waterlogged conditions ICPH 2431 (230.58) accumulated the highest and ICPL 20241 (130.37) the lowest leaf area (Table 3). Leaf area was used to assess the adverse effect of flooding on plant growth. It was determined that flooding treatment decreased the leaf area (21% and

18%) in common bean genotypes (Celik and Turhan 2011). Under waterlogged condition, the minimum leaflet number per plant was mainly due to enhanced senescence of lower leaves. According to (Zhou and Lin 1995) the most significant effect of waterlogging was on the lowest leaves and gave less than the control. Waterlogging stress could promote the degradation of chlorophyll therefore accelerate leaf senescence. (Kumutha *et al.,* 2009) expressed that six days of waterlogged pigeonpea genotypes suffered a severe loss in leaf area and leaf senescence is induced. Leaf area development is the most sensitive feature in pigeonpea under waterlogged condition. However, closely related work by Kumutha *et al.,* (2008) and Shimono *et al.,* (2012) emphasizes that waterlogging treatment significantly reduced leaf number on the main stem.

Chlorophyll content (SPAD): In the present study during 7 days waterlogging stress exhibited that genotype JBP 110-B (61.91) was accumulated maximum chlorophyll content while minimum chlorophyll content was noted in JP 10 (36.66) under normal condition. Genotype KPBR 80-2-1 (45.74) and RG 211 (26.28) were attained maximum and minimum chlorophyll content under waterlogged conditions data presented in Table 1. Moreover, 14 days waterlogging stress showed that JKM 7 (55.16) exhibited maximum chlorophyll content but it was minimum in ICPH 2431(36.81) under normal conditions. Under waterlogged genotype C 11 (45.64) was recorded the highest and ICPH 2431 (27.70) the lowest values for this trait (Table 3). The present study demonstrated that, reduction in chlorophyll content from seven days of waterlogging treatment. Photosynthetic pigments were significantly lowered under the waterlogging stress as compared to normal conditions. Many researchers found that waterlogging induced destruction of chlorophyll. This decrease in chlorophyll directly or indirectly affects the photosynthesis capacity of plant under waterlogged conditions (Ashraf *et al.,* 2011). Similar results were observed by (Kumutha *et al.,* 2008). With, advancement of waterlogging among the genotypes the chlorophyll concentration decreased (Wang and Jiang, 2007). (Shimono *et al.,* 2012) chlorophyll content by the SPAD reading at the end of the treatments was significantly reduced due to waterlogging.

Table 4. Effect of 14 days waterlogging on chlorophyll content and relative water content of pigeonpea genotypes

Genotypes	RWC		Root length		TDM	
	Normal	WL	Normal	WL	Normal	WL
ICPB 2039	80.24	71.69	30.08	18.74	2.89	2.12
ICPL 87051	81.66	71.71	28.00	23.56	3.02	2.16
ICPL 20241	67.35	52.13	27.42	12.77	3.37	2.45
ICPH 2431	65.83	50.52	33.03	17.33	2.68	1.89
ICP 8863	50.28	35.13	27.22	17.87	3.22	2.20
JKM 7	69.68	53.50	35.75	23.61	3.92	2.92
JP 10	55.91	40.99	22.79	14.13	2.65	2.04
C 11	68.96	48.31	30.09	15.80	3.24	2.23
Mean	67.49	53.00	29.30	17.97	3.12	2.25
SEm ±	1.86	2.02	1.25	0.86	0.12	0.07
CD 5%	5.44	5.91	3.65	2.51	0.34	0.20

Relative water content (% plant-1): As a result of waterlogging pigeonpea genotypes suffered a severe loss in RWC in leaves eight days after drainout. Seven days waterlogging treatment showed that ICPB 2039 (80.63) and JBP 110-B (72.35) were maintained the highest and ICP 8863(47.29 and 37.57) the lowest values for RWC under normal and waterlogged conditions respectively (Table 2). Perusal (14 days waterlogging) of pooled results genotype ICPL 87051 (81.66 and 71.17) and ICP 8863 (50.28 and 35.13) existed maximum and minimum values for RWC under normal and waterlogged conditions (Table 4) respectively. Environment stresses such as waterlogging is major constants of yield potential. It is the ratio of water content of a tissue to that of the same when the tissue is fully turgid expressed as percentage and indicates the water status of tissues. Significant reduction was observed in RLWC during waterlogging, which further declined with the duration of waterlogging. Similar findings have been reported by Min and Bartholomew (2005). (Kumutha *et al.,* 2008) observed that the waterlogging decreased leaf relative water content in pigeonpea genotypes. Sairam *et al.,* (2009a) concluded that waterlogging resulted in greater decline in relative water content. (Kumutha *et al.,* 2009) recorded that at 6 days of waterlogging caused a more severe decrease in RWC both in roots and leaves compared to non waterlogged plants.

Root length: (cm plant-1): The present investigations pertaining to performance revealed that, during seven days waterlogging stress under normal conditions genotype JKM 7 (31.20) and C 11 (19.85) were showed the highest and the lowest root length. However, under waterlogged conditions genotype ICPH 2431 (22.49) possessed maximum and RG 188 (9.96) minimum for root length (Table 2). The investigations of pooled (14 days waterlogging) analysis of variance exhibited that JKM 7 (35.75 and 23.61) recorded maximum root length and it was minimum in JP 10 (22.79) and ICPL 20241 (12.77) under normal and waterlogged conditions respectively (Table 4). The root growth of pigeonpea genotypes is significantly suppressed by waterlogging stress. However, the tolerant genotypes have the ability to continue their root growth under the stress in some extent. (Huang *et al.,* 1994) reported a drastic decrease in length of the longest seminal root and also in total length of seminal roots by 14 days hypoxia for waterlogging intolerant genotypes. The growth of many species that are tolerant to hypoxic conditions can also be reduced when the roots are waterlogged, but unlike sensitive ones, tolerant species rapidly resume their growth a short period after the resumption of aeration in roots (Crawford, 1982). Decrease in root length caused by low metabolic activity and slow growth is also a common response to flooding in trees (Mielke *et al.,* 2003).

Total dry matter production (TDM g plant-1): The present findings revealed that genotype ICPH 2431 (3.76) and RG 188 (2.26) were attained maximum and minimum TDM under normal conditions. However, under waterlogged condition ICP 8863 (2.99) and RG 188 (1.42) accumulated maximum and minimum TDM respectively (Table 2) during seven days waterlogging stress. Pooled data revealed that JKM 7 (3.92 and 2.92) maintained the highest value but JP 10 (2.65) and ICPH 2431 (1.89) were showed the lowest value for TDM under normal and waterlogged conditions respectively (Table 4) during fourteen days waterlogging stress. During early stage of growth dry matter production mainly depends on the development of the leaf area while the later growing period is strongly influenced by the respiratory consumption. (Kumutha *et al.,* 2008, 2009) observed that the waterlogging decreased dry matter in pigeonpea. (Liu *et al.,* 2010) in maize observed that dry weight of shoot and root of all lines significantly reduced at 6 days of waterlogging compared to control. Similar kind of results have been reported by (Talbot *et al.,* 1987) dry weight of root, stem and leaf which significantly reduced due to waterlogging in Salix caprea L. and Salix cinerea species. (Filho and Lopes 2011) reported that under flooding, Hyparrhenia rufa and Andropogon gayanus reduced dry matter production as compared to plants grown under field capacity. According to Araki *et al.,* (2012) waterlogging reduced roots and shoot weights. Shimono *et al.,* (2012) total dry weight at the end of the treatment was significantly reduced by waterlogging.

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