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Quantification of biochemical attributes of pigeonpea (*Cajanus cajan* L.) under waterlogged condition

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Abstract

The present study was conducted to assess the genotypic variability for waterlogging tolerance and related biochemical attributes which is vital in withstanding waterlogging stress in pigeonpea crop at the early seedling stages. Quantification of the amount of proline, sugar, starch and enzymes related to antioxidant defence mechanism in three pigeon pea genotypes under waterlogging condition was performed to satisfy objective of this research. Proline content increased significantly in leaves, and the increment was more in waterlogging resistant genotype. Soluble sugar content in leaves increased and it was attributed to increased hydrolysis of stored polysaccharides as the starch content concomitantly declined. Starch content in leaves of waterlogged plants undergoes significant reduction. Activities of enzymes super oxide dismutase and peroxidase increased significantly. Increments in super oxide dismutase and peroxidase were more in waterlogged plants of susceptible genotype MAL-18. Hydrogen peroxide content was almost unchanged in ICPL-84023, but increased in susceptible genotype MAL-18. Higher osmolytes accumulation, lesser H_2O_2 accumulation and antioxidant enzymes activities leads to lesser yield in tolerant genotype in comparison to susceptible genotype, leads to least yield decline in tolerant genotypes compared to susceptible.

Keywords: catalase, hydrogen peroxide, peroxidase, pigeonpea, proline, starch, sugar, superoxide dismutase and waterlogging

1. Introduction

India is the largest producer of pulses in the world, both in quantity and variety. Pigeonpea [*Cajanus cajan* (L.) Millsp.], commonly known as arhar, redgram, toovar, toor, or Gungopea member of the Fabaceae family. It is an important legume crop of rainfed agriculture and is known to be cultivated in more than 25 countries of the world such as Indian subcontinent, Africa and Central America in purview of the favourable climatic conditions. Pigeonpea are popular food in developing tropical countries. Nutritious and wholesome, the green seeds (pods) serve as vegetable. Dhal contains as much as 22% protein, depending on cultivar and location. Globally pigeonpea is cultivated on 4.92 million ha with an annual production of 3.65 metric tons and productivity of 898 kg ha^{-1}. According to FAO (2012) [12], India is a major pigeonpea producer in area 3.86 million ha having 687 kg ha^{-1} yield and 2.65 million tons production. Pigeonpea ranks second after chickpea among important pulse crops in India. Productivity of pigeonpea in India is low due to various biotic and abiotic stresses. In India, waterlogging is one of the most serious constraints for crop production and productivity, where about 8.5 mha of arable land is prone to this problem. Out of the total (3.9 mha) area under pigeonpea, about 1.1 mha is affected by excess soil moisture, causing an annual loss of 25-30% (Sultana 2010) [32]. The areas where rainfall is dependent on monsoon are more prone to waterlogging. Waterlogging occurs when rainfall or irrigation water is collected on the soil surface for prolonged periods without infiltrating into the soil (Choudhary *et al*. 2011) [8]. In India, pigeonpea is sown in June-July (rainy season). Annual and late cultivars flower in January and harvested in March-April. Being a summer rainy crop and erratic and prolonged rains during the monsoon, it is frequently exposed to the waterlogging conditions resulting in considerable loss in crop vigour and plant stand (Chauhan 1987; Choudhary *et al*. 2011) [9, 8]. Plants have evolved several mechanisms that allow perceiving the stresses and rapidly regulating their physiology and metabolism to cope them. The antioxidant defense mechanism provide an strategy to enhance drought tolerance by increase the rate of reactive oxygen species via enhanced electrolyte leakage in chloroplast and mitochondria. Plants with high levels of antioxidants either constitutive or induced have been reported to have greater resistance to the oxidative damage (Moussa and Aziz, 2008) [22].

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Under waterlogging stress condition, plant accumulates osmolytes such as proline and sugar, act as osmoprotectant. The present study was conducted to evaluate the effect waterlogging stress on biochemical and antioxidant enzymes activities in pigeonpea. This study would help to understand the responses under drought stress condition and its further improvement of present cultivar as well as to utter the genotypic variability for waterlogging tolerance in terms of vital biochemical and antioxidant enzymes activities in withstanding waterlogging stress in pigeonpea crop at the seedling stages.

2. Materials and Methods

Experiments were conducted during kharif in pots in the net house of the Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. Pigeonpea genotypes used were ICPL-84023, PTH-1, and MAL-18 procured from the Department of Genetics and Plant Breeding. Design applied was complete randomized design factorial with ten replicates. Excess soil moisture stress was imposed at 40 days after sowing by placing set of plastic pots of each genotype 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.

At the time of harvest, yield and yield attributes were analyzed at maturity. Proline, soluble sugar and starch contents was determined at 10 and 20 days in leaves tissues after imposing stress in normal and excess soil moisture as described by Bates *et al*. (1973) [6] and Dubois *et al*. (1956) [11] respectively. Starch content was estimated from the residue retained from the samples that was used for soluble sugar estimation.

All the enzymatic activity was assayed through first fully expanded leaf at 20 days after imposing stress in normal and excess soil moisture stressed plants. Peroxidase activity was assayed by the method Kar and Mishra, (1976) [14]. Superoxide dismutase activity was assayed by the method Dhindsa *et al*. (1981) [10]. Catalase activity was assayed spectrophotometrically in according to the protocol of Aebi *et al*. (1983) [1]. Hydrogen peroxide estimation was done as per the protocol of Mukherjee and Choudhary, (1983) [23].

3. Results

During the present investigation the total dry matter production per plant (Table-1) was found to be affected significantly by genotypes and stress conditions. Normal condition recorded more total dry matter production as compared to waterlogged condition. The maximum total dry matter production was recorded under waterlogged condition with genotype ICPL-84023 and it was at par to normal condition in same genotype. The minimum value of total dry matter production was recorded under waterlogged condition in MAL-18 followed by PTH-1 under same stress condition.

The data pertaining to 1000 grain weight was significantly influenced by genotypes and stress conditions (Table-1). The maximum 1000 grain weight was recorded under normal condition and it was significantly superior over waterlogged condition. The decline in 1000 grain weight under waterlogging compared to normal condition was highest in genotype MAL-18, followed by PTH-1 and minimum in genotype ICPL-84023.

The data related to seed yield per plant are presented in Table-1, was significantly influenced by genotypes and stress conditions. The maximum seed yield was recorded in genotype ICPL-84023 and PTH-1 under waterlogged conditions and it was found at par to same genotypes under normal conditions and significantly superior to MAL-18 under both normal and waterlogged conditions.

Data related to harvest index are also presented in Table-1, and are significantly influenced by genotypes and stress conditions. The maximum harvest index was recorded in genotype PTH-1 and it was superimposed over ICPL-84023 and MAL-18. The minimum harvest index value was recorded in MAL-18. In stress conditions, maximum harvest index was recorded under waterlogged condition and it was found at par to normal conditions.

Amount of proline was quantified in leaves of normal and waterlogged plants at ten and twenty days. Perusal of data indicate that, on an average, proline content in leaves (Table-2) under waterlogged condition increased with significant differences between treatments, genotypes and genotype × treatment at both stages of observations.

Soluble sugar content was estimated at two stages after imposing waterlogging stress in leaves (Table-3). Perusal of data revealed that on an average, soluble sugar content in waterlogged plants remained significantly higher though genotypic differences were not significant at any of the stage, but genotype × treatment interaction differed significantly.

In contrast to soluble sugar content, waterlogging resulted in significant reduction in leaf starch content (Table-4). The amount, on an average, under waterlogged condition gradually decreased with advancement in stage, but increased under normal condition, and the differences between normal and waterlogged treatment were significant at both stages. Genotypic as well as genotype × treatment interaction differences were also significant.

Changes in the content of hydrogen peroxide in leaves (Table-5) of pigeonpea genotypes were examined. Treatment and genotypic differences were significant. On an average, hydrogen peroxide content increased significantly due to waterlogging and genotype MAL-18 registered significantly higher amount of hydrogen peroxide than rest of the two genotypes. In all the genotypes, the differences between normal and waterlogged plants were significant only in MAL-18.

On an average peroxidase activity (Table-5) increased in waterlogged plants. Genotypic differences were also significant. In genotypes, where waterlogged plants had higher peroxidase activity than normal, the differences between normal and waterlogged plants were significant only in ICPL-84023.

On an average, Catalase activity (Table-6) increased significantly under waterlogged condition. Genotypic differences were also significant. Enzyme activity increased in all the studied genotypes in waterlogged plants, but percentage increase was highest in ICPL-84023.

On an average, waterlogging resulted in significant increase in SOD activity (Table-6). Genotypic differences were also significant, where MAL-18, on an average, registered significantly higher SOD activity than ICPL-84023.

Table 1: Total dry matter production (kg plant^{-1}), Seed yield (kg plant^{-1}), Harvest index (%) and 1000 grain weight (g) of plants of three genotypes of pigeonpea under normal and waterlogged conditions

Genotype	**Total dry matter production per plant**			**Seed yield**			**Harvest index**			**1000 grain weight**		
	Normal	**Waterlogged**	**Mean**	**Normal**	**Waterlogged**	**Mean**	**Normal**	**Waterlogged**	**Mean**	**Normal**	**Waterlogged**	**Mean**
ICPL-84023	0.90	0.99	0.95	0.13	0.14	0.14	14.91	14.98	14.94	111.4	110.6	111.0
PTH-1	0.66	0.52	0.59	0.13	0.14	0.14	19.72	27.74	23.73	129.6	126.6	128.1
MAL-18	0.54	0.33	0.44	0.06	0.02	0.04	10.76	6.21	8.48	130.0	121.3	125.6
Mean	0.70	0.62		0.11	0.10		15.13	16.31		12.37	119.5	
	SEm±		CD at 5%	SEm±		CD at 5%	SEm±	CD at 5%		SEm±	CD at 5%	
Treatment (T)	0.02		0.08	0.003		0.011	0.83	2.86		0.09	0.30	
Genotype (G)	0.03		0.08	0.002		0.006	0.75	2.18		0.10	0.29	
S AT SAME M	0.04		0.11	0.003		0.008	1.06	3.08		0.14	0.41	
M AT SAME/DIFF S	0.04		0.13	0.005		0.016	1.32	4.19		0.15	0.45	

*Plants were exposed to waterlogging stress after 40 days of sowing

Table 2: Proline content (mg g $^{-1}$ fresh weight) in the leaf tissue of three genotypes of pigeonpea under normal and waterlogged conditions

Genotype	**STAGE**							
	10 Days after imposing waterlogging stress*				**20 Days after imposing waterlogging stress***			
	Normal	**Waterlogged**		**Mean**	**Normal**	**Waterlogged**		**Mean**
ICPL-84023	0.110	1.910	(+1636.3)	1.01	0.150	2.400	(+1500.0)	1.28
PTH-1	0.130	1.840	(+1315.3)	0.99	0.210	2.490	(+1085.7)	1.35
MAL-18	0.370	1.920	(+1820.0)	1.15	0.180	2.240	(+1144.4)	1.21
Mean	0.20	1.89			1.05	0.18		2.38
	SEm±	*CD at 5%*			SEm±	*CD at 5%*		
Treatment (T)	0.07	0.20			0.03	0.08		
Genotype (G)	0.08	0.24			0.03	0.10		
TxG	0.12	0.34			0.05	0.14		

*Plants were exposed to waterlogging stress after 40 days of sowing
Values in parenthesis indicate % increase (+), or decrease (-) under waterlogged condition over normal

Table 3: Sugar content (μg g $^{-1}$ fresh weight) in the leaf of three genotypes of pigeonpea under normal and waterlogged conditions

Genotype	**Stage**							
	10 Days after imposing waterlogging stress*				**20 Days after imposing waterlogging stress***			
	Normal	**Waterlogged**		**Mean**	**Normal**	**Waterlogged**		**Mean**
ICPL-84023	30.600	50.170	(+63.9)	40.385	34.230	56.480	(+65.0)	45.355
PTH-1	20.480	55.210	(+169.5)	37.845	20.880	58.250	(+178.7)	39.565
MAL-18	20.520	54.390	(+165.0)	37.455	23.290	61.150	(+162.5)	42.220
Mean	23.87	53.26			38.56	26.13		58.63
	SEm±	CD at 5%			SEm±	CD at 5%		
Treatment (T)	0.53	1.52			0.97	2.76		
Genotype (G)	0.65	1.86			1.18	3.38		
TxG	0.92	2.63			1.67	4.78		

*Plants were exposed to waterlogging stress after 40 days of sowing.
Values in parenthesis indicate % increase (+), or decrease (-) under waterlogged condition over normal

Table 4: Starch content (μg g $^{-1}$ fresh weight) in the leaf of three genotypes of pigeonpea under normal and waterlogged conditions

Genotype	**Stage**							
	10 Days after imposing waterlogging stress*				**20 Days after imposing waterlogging stress***			
	Normal	**Waterlogged**		**Mean**	**Normal**	**Waterlogged**		**Mean**
ICPL-84023	20.220	1.550	(-63.4)	10.885	25.967	0.990	(-30.3)	13.479
PTH-1	7.280	0.870	(-71.1)	4.075	22.190	0.930	(-4.7)	11.560
MAL-18	6.970	0.620	(-38.0)	3.795	23.510	0.800	(-8.6)	12.155
Mean	11.49	1.01			6.25	23.89		0.91
	SEm±	CD at 5%			SEm±	CD at 5%		
Treatment (T)	1.34	3.83			0.33	0.94		
Genotype (G)	1.64	4.68			0.40	1.15		
TxG	2.32	6.63			0.57	1.62		

*Plants were exposed to waterlogging stress after 40 days of sowing.
Values in parenthesis indicate % increase (+), or decrease (-) under waterlogged condition over normal

Table 5: Enzyme activity (Units mg protein^{-1}) in the leaves of three genotypes of pigeonpea under normal and waterlogged conditions after 20 days of imposing waterlogging stress

Genotype	Hydrogen Peroxide			Peroxidase		
	Normal	Waterlogged	Mean	Normal	Waterlogged	Mean
ICPL-84023	202.00	210.77	206.39	20.97	31.33	26.15
PTH-1	208.80	243.29	226.05	20.48	21.75	21.12
MAL-18	214.11	455.97	335.04	27.80	27.50	27.65
Mean	208.30	303.34		23.08	26.86	
	SEm±	CD at 5%		SEm±	CD at 5%	
Treatment (T)	23.13	66.12		1.14	3.27	
Genotype (G)	28.33	80.98		1.40	4.00	
TxG	40.07	114.52		1.98	5.66	

*Plants were exposed to waterlogging stress after 40 days of sowing

Table 6: Enzyme activity (Units mg protein^{-1}) and Hydrogen peroxide (µM g $^{-1}$ fresh weight) content in the leaves of three genotypes of pigeonpea under normal and waterlogged conditions after 20 days of imposing waterlogging stress

Genotype	Catalase			Superoxide Dismutase		
	Normal	Waterlogged	Mean	Normal	Waterlogged	Mean
ICPL-84023	96.21	148.45	122.33	54.50	92.50	73.50
PTH-1	208.50	233.25	220.88	75.00	101.50	88.25
MAL-18	242.20	254.04	248.12	83.50	149.50	116.50
Mean	182.30	211.91		71.00	114.50	
	SEm±	CD at 5%		SEm±	CD at 5%	
Treatment (T)	0.726	2.261		7.29	20.82	
Genotype (G)	0.593	1.846		8.92	25.50	
TxG	1.026	3.198		12.62	36.07	

*Plants were exposed to waterlogging stress after 40 days of sowing

4. Discussion

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is a waterlogging-sensitive legume crop. Waterlogging caused reduction in dry weight of plant and plant parts (shoot and roots), seed yield, harvest index and 1000 grain weight in pigeonpea. Waterlogging induced reduction in plant height and delayed flowering in surviving plants, results into reduction in the number of pods, seeds/pod and seed yield in pigeonpea (Choudhary *et al*. 2011) [8]. The reduction in these parameters was found to be the minimum in genotype ICPL-84023 and characterized as waterlogging stress resistant genotype, generally, the maximum in MAL-18. Hence, MAL-18 is characterized as susceptible to waterlogging. Genotype PTH-1 behaved similar but little lesser in all parameters to ICPL-84023 under waterlogged condition, therefore, this genotype is classified as moderately resistant to waterlogging stress. Similar results were reported by Bansal and Srivastava, 2012 [5]. The loss in biomass and yield appeared to be related with slow metabolic activities of roots experiencing hypoxia (Mielke *et al*., 2003; Yiu *et al*., 2011) [20, 36] with impaired photosynthetic CO_2 assimilation regulated by source-sink phenomenon linked with xylem and phloem (Bai *et al*., 2010) [3].

Under waterlogging proline content was increased significantly in leaves of all pigeonpea genotypes and the increment was more in waterlogging resistant genotype. It has been suggested that accumulation of proline confers stress resistance in plants (Srivastava *et al*. 2007; Shah, 2007) [31, 28].

Under waterlogging stress condition, increase in soluble sugars content has been reported (Rai *et al*., 2004; Kumutha *et al*., 2008) [25, 17]. Increased soluble sugars content in leaves may be attributed due to increased hydrolysis of stored polysaccharides. It further proves that under waterlogging stress increased soluble sugars level is as a result of induced hydrolysis of stored insoluble sugars i.e., starch.

Enzymes superoxide dismutase, catalase and peroxidase tend to scavenge highly reactive oxygen species (Shah, 2007) [28]. SOD is a major scavenger of O_2 and its enzymatic action results in the formation of H_2O_2 and O_2. Catalases and peroxidases are major enzymatic cellular scavenger of CO_2. Removing the highly toxic H_2O_2 produced during dismutation is essential for the cell for the cell to avoid inhibition of the enzymes such as those controlling the calvin cycle in the chloroplast (Carvalho, 2008) [7]. Catalase, which is present in peroxisome, dismutates H_2O_2 into water and molecular O_2 whereas peroxidase decomposes H_2O_2 by oxidation of substrate such as phenolic compounds and/or antioxidants (Pan *et al*. 2006, Simova-Stoilova *et al*. 2007) [24, 29]. Antioxidant enzymes such as superoxide dismutase, catalase and peroxidase increased under waterlogging (Kumutha *et al*. 2009; Bansal and Srivastava 2012) [16, 5]. The increment in these parameters was found to be the maximum in genotype ICPL-84023 and minimum in MAL-18. Kumutha *et al*. (2009) [16] have described the comparatively greater antioxidant enzyme activities in tolerant genotype of pigeonpea (ICP 301) resulting in less oxidative stress could be one of the factor determining its higher tolerance to flooding as compared susceptible genotype (Pusa 207). If reactive oxygen species are not detoxified immediately then they cause severe damage to biomembranes and cellular structures (Monk *et al*., 1989., Ushimara *et al*., 1992; Ushimara *et al*, 1994; Yan *et al*., 1996; Ram *et al*., 2002) [21, 33, 34, 35, 26]. It appears that the concentration of reactive oxygen species (H_2O_2) is elevated to a very high level in susceptible genotype and detoxification mechanisms though activated, but are not able to detoxify them, and hence caused cellular damage to a greater extent (Sairam *et al*. 2009) [16], which leads to lesser yield in susceptible genotype MAL-18 in comparison to intermediate tolerant genotype PTH-1 and least yield decline in tolerant genotype ICPL-84023. The involvement of oxidative stress in soil flooding induces damage and antioxidant response as an indicator of flooding tolerance or sensitivity (Arbona *et al*., 2008) [2]. The enhanced stress induces activities of these enzymes in seedlings subjected to soil flooding to protect them from the stress (Liu *et al*., 2006; Arbona *et al*., 2008; Bailey-Serres and Voesenek, 2008) [18, 4, 2].

It is concluded that osmolytes accumulation and activation of antioxidant enzymes activities may plays important role in the maintenance of plant viability, conferring waterlogging resistance and stabilizing yield under waterlogging stress. Higher osmolytes accumulation, lesser H_2O_2 accumulation and antioxidant enzymes activities leads to lesser yield in tolerant genotype in comparison to susceptible genotype, leads to least yield decline in tolerant genotypes compared to susceptible. Higher antioxidant potential in ICPL-84023 as evidenced by enhanced peroxidase, catalase, and superoxide dismutase activities increased capacity for reactive oxygen species (ROS) scavenging and indicated relationship between waterlogging resistance and antioxidant defense system in pigeonpea.

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