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Influence of Waterlogging on Certain Biochemical and Yield Parameters of Pigeonpea (*Cajanus cajan* (L.) *Millsp*)

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ABSTRACT

A pot culture experiment was conducted at Department of Crop physiology, S.V. Agricultural College,Tirupati during kharif 2013 to know the effect of waterlogging on certain biochemical and yield parameters of Redgram (Cajanus cajan (L.) Millsp).The experiment was conducted in a spilt pot design with different time periods of waterlogging as main treatments and genotypes as subplots. LRG 30, Maruti and Asha were the genotypes tested. Waterlogging affected all the biochemical and yield parameters viz., reducing and total reducing sugars, superoxide dismutase, membrane stability index, number of pods per plant, pod dry weight and seed yield. The three different periods of stress imposition were 40 DAS (vegetative stage), 80 DAS (reproductive stage) and 120 DAS (pod formation stage). Sensitive stage of crop to watelogging was recorded. Water logging at 40DAS affected super oxide dismutase, reducing sugars and total sugars. When stress was imposed at 80DAS only pod dry weight affected. A greater decrease in number of pods per plant and seed yield was observed when stress was imposed at 120 DAS. The present study forms a biochemical basis to understand the sensitive stage of redgram to waterlogging stress.

Key words: *Redgram, Waterlogging, Biochemical, Yeld parameters.*

INTRODUCTION

Waterlogging is a serious problem, which effects the crop growth and yield. Waterlogging may occur as a result of unpredictable high rainfall when evaporative demand is low and unpredictable rainfall[2]. It can also occur when the amount of water added through rainfall or irrigation is more than what can percolate into the soil within one or two days. The situation is further aggravated in clay soils which have characteristically poor internal drainage.

During recent times a recurrent event of untimely rains and an associated excessive soil moisture situation is causing an impediment to crop production. This is truer in case of khariff pulse crops like red gram.

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Pigeon pea is highly sensitive to water logging. It is generally grown under rainfed conditions in the rainy season and often exposed to drought as well as extended episodes of transient water logging during the peak rainy days, leading to a heavy loss of individuals in the plant stand. In Andhra Pradesh pigeon pea is mainly cultivated in heavy soils of Krishna and Guntur districts. Thus the problem of transient waterlogging here has caused for a decrease in yield of pulse crops in general and redgram in particular.

Waterlogging is known to inhibit vegetative and flowering stages and yield of several plant species. This is accompanied by poor uptake of water and minerals from the soil[9], epinasty, senescence and abscission of leaves and derangement in the hormonal metabolism of the plant. Further, waterlogging also predisposes pigeon pea plants to Fusarium wilt and Phytopthera blight infections which are common fungal diseases of rainy season resulting in upto a 100% yield losses.

MATERIALS AND METHODS

The experiment was laid out in a Split Plot Design with three stress imposing stages *viz*., 40 DAS, 80 DAS and 120 DAS as main plots and three as sub plots replicated four times. LRG 30 (Palanadu), ICPL 8863(Maruti) and ICPL 87119 (Asha) were the genotypes selected based on the literature as waterlogging resistant, moderately resistant and susceptible cultivars. Thus, three sets of pots (4 pots in each set) were maintained at each stage of stress imposition for each variety. Further each set of pots were used to record the data 1) before imposition of stress 2) after imposition of stress and 3) to record the final yield data with respect to the stage of stress imposition. Eight days of waterlogging followed by six days of drainage was uniformly at each stage of stress imposition (viz, 40, 80 and 120 DAS) Biochemical parameters viz, Superoxide dismutase, Reducing sugars. Total sugars and Membrane stability was measured as par the procedures mentioned below.

Superoxide dismutase

Superoxide dismutase was estimated using the method given by Dhindsa *et al*[3]. 1 gm of fresh root sample was taken and homogenized with liquid nitrogen then ground with 10 ml of pre cold Potassium phosphate buffer (250 mM of potassium mono hydrogen orthophosphate (K_2HPO_4) and 250 mM of potassium dihydrogen orthophosphate (KH_2PO4) for 500 ml adjusting pH 7.8). The grounded root sample was centrifuged at 10,000 rpm for 10 minutes at 4°C. After centrifugation the supernatant was collected and refrigerated. 50 µl of enzyme extract was added to test tubes containing 600 µl of potassium phosphate buffer, 60 µl of ethylene diamine tetra acetic acid, 390 µl of methionine, 0.6 µl of riboflavin and 300 µl of nitro blue tetrazolium. Along with the sample test tubes, blank (without nitro blue tetrazolium and enzyme extract) and reference (without enzyme extract) were also maintained. The sample, reference and blank tubes were kept under fluorescent light for 15 minutes and absorbance was recorded at 560 nm and expressed as g (fr.wt) min^{-1}. One unit is defined as change in absorbance per gram fresh weight per minute.

Reducing sugars

Procedure as suggested by Somogyi[11] was employed for estimation of reducing sugars. Reagent A was prepared by mixing 4 ml of copper sulphate solution (15 g of $CuSO_4$ dissolved in a small volume of distilled water with a drop of H_2SO_4 added to it, the final volume was made up to 100ml) and 96 ml of alkaline copper tartarate reagent (2.5 g anhydrous Na_2CO_3, 2 g of Na_2HCO_3, 2.5 g of potassium sodium tartarate and 20 g of anhydrous sodium sulphate were dissolved in 80 ml water and made upto 100 ml).Reagent B was prepared by dissolving 2.5 g of ammonium molybdate in 45 ml of distilled water adding 2.5 ml H_2SO_4 0.3 g of disodium hydrogen arsenate (Na_2HSO_4. $7H_2O$) was separately dissolved in 25 ml distilled water. Both the solutions were mixed and placed in an incubator at 37°C for 24 to 48 hours.100 mg of glucose was dissolved in 100 ml of distilled water in a volumetric flask to prepare

standard glucose stock. 10ml of stock was diluted to 100 ml in a volumetric flask to prepare working standard.100 mg of sample was weighed and grinded with mortar and pestle. Sugars were extracted with 5 ml of hot 80 per cent ethanol twice. The extract was centrifuged at 3500rpm for 10 minutes. Supernatant was collected and the ethanol was evaporated by keeping the test tubes in a water bath at 80°C for 3 to 4 hrs. Sugars collected at the base of the test tube were dissolved with 5 ml distilled water and thoroughly mixed. Aliquots of 0.5 ml of sample were pippeted out in separate test tubes and the volume was made up to 1 ml with distilled water. One ml of reagent A was added to the sample and placed in boiling water bath for 10 minutes. After cooling the test tubes, 1 ml of reagent B was added and the volume was made up to 8 ml with distilled water. The absorbance values were recorded at 620 nm.

Total sugars

Total sugars were estimated by the method suggested Somogyi[11]. 1 ml of alcohol free extract of plant sample was placed in a test tube and evaporated to dryness. Add 1 ml of distilled water and 1 ml of 1N H_2SO_4 to the residue and hydrolyze by heating at 49^0C for 30 minutes. Cool the test tubes, add 1-2 drops of methyl red indicator and neutralize the contents by adding 1N NaOH. Maintain appropriate reagent blank (1ml water+1 ml H_2SO_4 + two drops of methyl red indicator+ NaOH). The solution was made up to 10 ml of final volume with distilled water.The amount of total sugars was estimated using a standard graph prepared with glucose and expressed in percentage.

Membrane stability index

Membrane stability index was estimated as per the protocol mentioned by Sairam *et al*[8]. 100 mg root material, in two sets, was taken in test tubes containing 10 ml of double distilled water. One set was heated at 40^0C for 30 min in a water bath, and the electrical conductivity of the solution was recorded on a conductivity bridge (C_1). Second set was boiled at 100 °C on a boiling water bath for 10 min, and its conductivity was measured on a conductivity bridge (C_2). Membrane stability index (MSI) is calculated as:

$$MSI = \left\{1 - \frac{C1}{C2}\right\} \times 100$$

Yield parameters

At the time of harvesting yield parameters were recorded from the pots left for this purpose. The data on number of pods per plant, pod dry weight (g) and seed yield was collected from three plants of each pot and averaged.

RESULTS AND DISCUSSION

A significant difference was observed in reducing sugars content among treatments, genotypes and their interaction after imposition of stress. Among treatments and genotypes a higher reducing sugars content was recorded at 120 DAS (2.41 %) and LRG 30 (2.20 %) respectively (Table: 1A). The highest percentage increase of reducing sugars was recorded at 80 DAS (61.80) among treatments and LRG 30 (74.28) among the genotypes (Table: 1B). Similar results were observed by kumutha *et al*[5]., in Mungbean.A significant difference was observed in total sugars (%) at both before and after stress impositions among treatments, genotypes and in their interactions. The mean values pertaining to total sugars decreased at all the stress imposing stages. A significant difference in total sugars among different stress imposing treatments was observed. Percent total sugars recorded was higher at 120 DAS (2.43 %). Among genotypes LRG 30 was found significantly higher total sugars % (1.80) (Table: 2A) (fig 1). The percentage decrease in total sugars differed significantly among different stress imposing treatments and a lower decrease in percentage of sugars was

observed at 120 DAS (20.44). Among different genotypes the percentage decrease of total sugars was more in Maruti (40.68) (Table: 2B). A similar decrease in total sugars was reported by Kumutha *et al*[5]., in green gram due to water logging. SOD content after imposition of stress differed significantly at different treatment and among genotypes (Table: 3A). The percentage increase in SOD at different treatments and genotypes was found significant. Higher percentage increase in SOD was observed at 80 DAS (45.35%) Among genotypes LRG 30 recorded high increase in SOD (44.86%) (Table: 3B). Ruchi and Srivastava[7] reported a similar increase in antioxidant enzymes like SOD, APX, Glutathione reductase and Catalase due water logging in Pigeonpea.Cell membrane stability has been widely used to express stress tolerance. Higher membrane stability could be correlated with abiotic stress tolerance.A significant difference in percentage decrease in membrane stability index due to stress imposition was observed among genotypes only. A lower reduction in membrane stability index was observed in LRG 30 (18.65%) (Table: 4A & 4B). The results obtained were in conformity with Premchandra *et al*[6].A significant difference was observed among different stages of water logging among genotypes and their interaction with respect to number of pods per plant. Water logging stress at 120 DAS recorded least number of pods per plant (44.39) (Table: 5). However, water logging stress at 40 DAS recorded highest number of pods per plant (92.19). Among varieties highest number of pods per plant were observed in LRG 30 (93.91) (Fig. 1). Similar results were obtained by Jafar Ullah[4] in green gram. Significant difference was observed among treatments with respect to pod dry weight. While among genotypes and their interaction were found to be non significant. Watelogging at 120 DAS recode more pod dry weight (48.71 g plant^{-1}) among the treatments (Fig. 1). Such reduction in pod dry weight was also reported by Bishnoi and Krishnamoorthy[1] in Chick pea when stress was imposed at flowering stage (i.e 80 DAS). Significant differences with respect to seed yield were observed among different treatments and genotypes. However, their interaction was found non-significant. The reduction in seed yield was less when stress was imposed at 40 DAS (31.31 g plant^{-1}) compared to other stage of stress imposition (viz 80 and 120 DAS) Among varieties a higher seed yield was observed in LRG 30 (27.51 g plant^{-1}) at all the stages of waterlogging stress compared to other varieties (Fig. 1). Such effect of water logging on seed yield was also explained by Singh and Srivastava[10] in Pigeonpea.

Table: 1A. Effect of waterlogging on reducing sugars (%) at different crop growth stages of Pigeonpea

Treatments	BEFORE IMPOSING STRESS				AFTER IMPOSING STRESS			
	LRG 30	MARUTI	ASHA	Mean	LRG 30	MARUTI	ASHA	Mean
40 DAS	0.58	0.46	0.49	**0.51**	0.97	0.54	0.78	**0.76**
80 DAS	1.25	1.37	1.25	**1.29**	2.74	1.49	1.83	**2.02**
120 DAS	1.62	1.79	1.75	**1.72**	2.91	1.93	2.39	**2.41**
Mean	**1.15**	**1.21**	**1.16**		**2.20**	**1.32**	**1.67**	
	T	**V**	**T × V**		**T**	**V**	**T × V**	
SEm±	**0.041**	**0.041**	**0.071**		**0.03**	**0.04**	**0.05**	
CD (P=0.05)	**0.14**	**N.S.**	**N.S.**		**0.10**	**0.11**	**0.19**	

Table: 1B. Effect of waterlogging on percentage increase in reducing sugars

Treatments	LRG 30 (V_1)	MARUTI (V_2)	ASHA(V_3)	Mean
40 DAS (T_1)	48.15	36.23	51.28	**45.22**
80 DAS (T_2)	108.23	30.98	46.20	**61.80**
120 DAS (T_3)	66.48	25.73	24.73	**38.98**
Mean	**74.28**	**30.98**	**40.73**	
	T	**V**	**TXV**	
SEm±	**3.65**	**11.41**	**6.32**	**3.65**
CD (P=0.05)	**12.60**	**33.92**	**N.S.**	**12.60**

Table: 2A. Effect of waterlogging on total sugars (%) at different crop growth stages of Pigeonpea

Treatments	BEFORE IMPOSING STRESS				AFTER IMPOSING STRESS			
	LRG 30	MARUTI	ASHA	Mean	LRG 30	MARUTI	ASHA	Mean
40 DAS	0.82	1.53	1.14	**1.16**	0.65	0.79	0.66	**0.70**
80 DAS	1.84	3.54	2.64	**2.67**	1.64	2.06	1.84	**1.85**
120 DAS	2.28	3.74	3.03	**3.02**	2.14	2.55	2.33	**2.34**
Mean	**1.65**	**2.94**	**2.27**		**1.47**	**1.80**	**1.61**	
	T	**V**	**T × V**		**T**	**V**	**T × V**	
SEm±	**0.010**	**0.008**	**0.017**		**0.005**	**0.007**	**0.009**	
CD (P=0.05)	**0.035**	**0.025**	**0.045**		**0.017**	**0.022**	**0.039**	

Table: 2B. Effect of waterlogging on percentage decrease in total sugars

Treatments	LRG 30 (V_1)	MARUTI (V_2)	ASHA(V_3)	Mean
40 DAS (T_1)	21.59	48.20	42.09	**37.29**
80 DAS (T_2)	10.89	41.85	30.30	**27.68**
120 DAS (T_3)	6.36	32.00	22.98	**20.44**
Mean	**12.95**	**40.68**	**31.79**	
	T	**V**	**TXV**	
SEm±	**0.29**	**0.40**	**0.50**	
CD (P=0.05)	**1.00**	**1.20**	**2.14**	

Table: 3A. Effect of waterlogging on Superoxide Dismutase (units g^{-1} (fr.wt) min^{-1}) at different crop growth stages of Pigeonpea

Treatments	LRG 30 (V_1)	MARUTI (V_2)	ASHA(V_3)	Mean
40 DAS (T_1)	39.34	25.70	19.55	**28.19**
80 DAS (T_2)	59.37	31.38	45.32	**45.35**
120 DAS (T_3)	35.89	30.83	40.25	**35.66**
Mean	**44.86**	**29.30**	**35.04**	
	T	**V**	**TXV**	
SEm±	**3.59**	**3.64**	**6.23**	
CD (P=0.05)	**12.41**	**10.82**	**N.S.**	

Table: 4A. Effect of waterlogging on membrane stability index (%) at different crop growth stages of Pigeonpea

Treatments	BEFORE IMPOSING STRESS				AFTER IMPOSING STRESS			
	LRG 30	MARUTI	ASHA	Mean	LRG 30	MARUTI	ASHA	Mean
40 DAS	62.00	54.13	59.63	**58.58**	40.25	26.68	39.93	**35.62**
80 DAS	77.50	56.60	66.25	**66.78**	60.25	39.25	47.13	**48.88**
120 DAS	79.75	69.00	72.75	**73.83**	60.13	48.25	57.00	**55.13**
Mean	**73.08**	**59.91**	**66.21**		**53.54**	**38.06**	**48.02**	
	T	**V**	**T × V**		**T**	**V**	**T × V**	
SEm±	**1.10**	**0.99**	**1.91**		**1.90**	**5.11**	**3.30**	
CD (P=0.05)	**3.81**	**2.95**	**5.43**		**6.57**	**N.S.**	**N.S.**	

Table: 4B. Effect of waterlogging on percentage decrease in membrane stability index

Treatments	LRG 30 (V_1)	MARUTI (V_2)	ASHA(V_3)	Mean
40 DAS (T_1)	14.75	65.91	21.97	**34.21**
80 DAS (T_2)	14.75	48.84	18.82	**27.47**
120 DAS (T_3)	26.47	62.65	16.96	**35.36**
Mean	**18.65**	**59.13**	**19.25**	
	T	**V**	**TXV**	
SEm±	**1.90**	**1.39**	**3.30**	
CD (P=0.05)	**N.S.**	**4.14**	**7.73**	

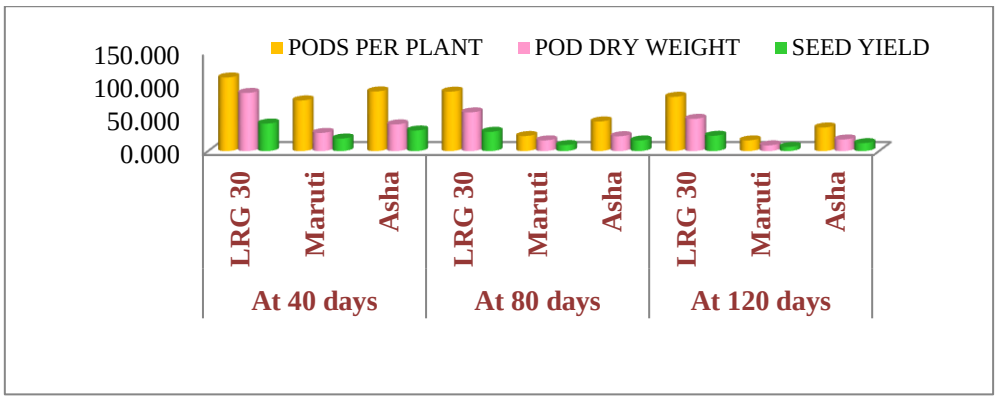

Fig. 1: Effect of waterlogging on yield components at different crop growth stages in redgram genotypes

CONCLUSION

The superiority of LRG-30 in most of the biochemical parameters and yield parameters forms the basis for its tolerance to waterlogging stress. Among yield parameters number of pods per plant was affected mostly by water logging stress. At all the three crop growth stages pod development stage (120 DAS) was found to be the most sensitive stage for water logging. A higher variability among genotypes was observed with respect to water logging stress tolerance for further crop improvement.

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