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INFLUENCE OF WATERLOGGING, SALINITY AND THEIR COMBINATION ON MEMBRANE INJURY, LIPID PEROXIDATION, PLANT BIOMASS AND YIELD IN PIGEON PEA (*CAJANUS CAJAN* L. MILLSP.) GENOTYPES

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ABSTRACT

An experiment was conducted with four pigeonpea [*Cajanus cajan* (L.) Millsp.] genotypes ICPH 2431, PARAS (relatively tolerant) and SGBS 6, UPAS 120 (relatively sensitive) for physiological and biochemical changes under waterlogged and saline conditions. Waterlogging, salinity and the combination of waterlogging and salinity had adverse effect on membranes as was evident from increased electrolyte leakage, lipid peroxidation levels and decrease in plant biomass and yield and it's component. The adverse effects were more on the sensitive genotypes SGBS 6 and UPAS 120 as compared to the relatively tolerant genotypes ICPH 2431 and PARAS. The effect of waterlogging + salinity was significantly greater than that of waterlogging and salinity alone. It was concluded that waterlogging and waterlogging + salinity treatments resulted in increase in membrane injury, lipid peroxidation and decrease in plant biomass and yield.. However, salinity treatment had less adverse effects. The order of performance of genotypes was ICPH 2431>PARAS>UPAS120> SGBS 6.

INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is the sixth most important grain legume of tropics and subtropics. Because of its multiple uses, it plays an important role in subsistence agriculture. It is an important pulse crop that performs well in poor soils and regions where moisture availability is unreliable or inadequate (Kimani, 2001). In India, pigeonpea is mainly grown in the regions lying between 14^{0}N and 29^{0}N latitudes with mean annual rainfall ranging between 600 and 1500 mm. Waterlogging is a perennial production problem in these areas which are characterized with alluvial or deep vertisols. Soil waterlogging has long been identified as a major abiotic stress and the constraints it imposes on roots have marked effect on plant growth and development. Under waterlogged condition the productivity of sugarcane also found lower than normal condition in various studies (Kumar *et al.*, 2015).Waterlogged plants are affected by various stresses, such as limitations to gas, mineral nutrient deficiencies and microelement toxicities (Setter and Waters, 2003; Setter *et al.*, 2009).Excess water in the root environment may be injurious or even lethal because it blocks the transfer of oxygen and other gases between the soil, the roots of the plant and the atmosphere. Due to waterlogging, the solute leakage increased (Johnson *et al.*, 1989), so electrical conductivity is also increased (Givelberg *et al.*, 1984; Lott *et al.*, 1991). Membrane disintegration is one of the consequences of oxygen deprivation (Rawyler *et al.*, 2002), resulting in more than 40 times increase in solute leakage from 4d waterlogged pea plants (Jackson *et al.*, 1982).

Salinity is one of the most significant abiotic factors limiting crop productivity (Munns, 1993; Gama *et al.*, 2007). At present about 20% of the world's cultivated land and approximately half of all irrigated land is affected by salinity (Zhu, 2001). Abiotic stresses such as drought and high salinity adversely affect the growth and productivity of crop plants. High concentrations of salt resulting from natural processes or disarrangement in irrigated agriculture result in inhibition of plant growth and yield (Demiral and Turkan, 2006). Under saline environments, the plant lipid metabolism is interrupted as a result of oxidative damage to membrane lipids by reactive oxygen species and lipid peroxidation (Misra and Gupta, 2006). Salinity proved to have a significant impact on dry and fresh weight, root volume and stem diameter. Pak *et al.* (2009) found that salt stress caused a decrease in growth and a dry matter accumulation in *Brassica napus* leaves.

Much of the world's saline land is also subjected to waterlogging (saturation of the soil) because of the presence of shallow water-tables or decreased infiltration of surface water due to sodicity (Ghassemi *et al.,* 1995; Qureshi and Barrett-Lennard, 1998). It is widely recognized that waterlogging and salinity interact to increase Na^+ and/or Cl^- concentrations in shoots, which can have adverse effects on plant growth and survival (Barrett-Lennard and Shabala, 2013, Zeng *et al.,* 2013). The combination of waterlogging, and salinity is much more deleterious than the individual stress and cause greater damage to plants, so having a major impact on agricultural production (Barrett-Lennard, 2003). So present study was conducted to study the effects of waterlogging, salinity and their combination on membrane injury, lipid peroxidation and yield of pigeonpea genotypes.

MATERIALS AND METHODS

The present study was carried out to understand the tolerance mechanism of waterlogging and salinity stress in pigeonpea [*Cajanus cajan* (L.) Millsp.] genotypes. Four genotypes PARAS, ICPH 2431 (Relatively tolerant) and SGBS 6, UPAS 120 (Relatively sensitive) were raised in polythene bags filled with half kg soil + FYM manure mixture (3 soil: 1manure w/w), NPK (@20:40:20 kg per ha). Twenty one days after sowing the polythene bags were placed in cemented tanks (length 160 cm, breadth 125 cm and depth 65 cm). T2 and T3 tanks were filled with water and NaCl solution, respectively. The water and solution levels were maintained for eight days. After eight days the water and solution was drained out of the tanks. In T4 treatment, the plants were treated with 60mM NaCl solutions twenty one days after sowing. Eight days after the removal of treatment, following physiological observations were recorded:

Electrolyte leakage or Membrane injury (MI)

Membrane injury was analysed according to the method of Zhang *et al.* (2006).

Procedure

After waterlogging and salinity treatment, leaf samples were collected from control as well as treated plants. From these plants, 100 mg of leaf tissue was taken separately in 20 ml test tubes containing 10 ml of de-ionized water. These samples were incubated for 24 hrs at 4ºC. The conductance of decanted liquid containing refluxed electrolytes was determined at 25ºC with a conductivity meter and designated as ECa (Before boiling). Then the samples were subjected to heating at 100ºC in a water bath for 10 minutes. After cooling, the electrical conductivity of the solutions was measured and designated as ECb (After boiling). The electrolyte leakage was expressed by the following formula.

$$\text{Electrolyte leakage (\%)} = \frac{ECa}{ECb} \times 100$$

Lipid peroxidation

The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) present in leaf tissues. MDA is a product of lipid peroxidation and was measured by thiobarbituric acid (TBA) reaction with minor modifications of the method of Heath and Packer (1968).

Reagents

0.1% Trichloroacetic acid (TCA)

20 % TCA containing 0.5 % thiobarbituric acid (TBA)

Extraction

Three hundred mg of fresh third leaves were homogenized separately with 5 mL of 0.1 % TCA (w/v) solution. The homogenate was centrifuged at 8000 rpm for 15 min. The supernatant was then directly used for the assay.

Procedure

One ml of the supernatant was taken in a test tube and precipitated by 4 ml of 20 % TCA containing TBA. The mixture was heated in a water bath shaker at 95ºC for 30 min and quickly cooled in an ice-bath. After centrifugation at 8000 rpm for 10 min the absorbance of the reaction mixture was read at 532 nm and the value for non- specific absorption at 600 nm was subtracted. The concentration of MDA was calculated using its extinction coefficient of 155 $mM^{-1}cm^{-1}$.

Total plant biomass

Three random plants from each genotype were collected from the pots, washed and its root, stem, leaves were separated and wrapped in a paper bag and was kept at room temperature for one day. Then these samples were dried in an oven at 70ºC till a constant weight was obtained.

Yield and yield components

Seed yield per plant (g)

At the time of harvesting the seeds of each plant were collected separately and weighed. The seeds weight was expressed in grams per plant.

Test weight (g)

Hundred seeds were counted from each genotype and the weight was recorded in grams. This weight was recorded as test weight.

RESULTS AND DISCUSSION

Electrolyte leakage

Waterlogging and salinity treatments were reported to have adverse effect on membrane integrity which resulted in electrolyte leakage. With comparison to control plants the increase in electrolyte leakage was 22 to 32% during waterlogging. Kumutha *et al.* (2009) reported in pigeonpea genotypes that membrane stability index (MSI) in the roots and leaves decreased under waterlogging with greater decline in Pusa 207 (sensitive), while ICP 301 (tolerant) managed to maintain higher MSI even after 6 days of waterlogging. Similar results are obtained in the present investigation as the tolerant genotypes ICPH 2431 and PARAS maintained lower membrane injury as compared to sensitive genotypes. Similar results were obtained in green gram by Kumutha *et al.* (2008a).

In salinity treated plants maximum increase in electrolyte leakage was observed in UPAS 120 (23%) genotype and minimum increase was observed in ICPH 2431 (15%). Bayat *et al.* (2012) reported that in *Calendula* plants electrolyte leakage was intensively increased by salt treatment. Supporting evidence was shown when SA reduced electrolyte leakage in salt stressed tomato (Stevens *et al.,* 2006) and maize (Turan and Aydin, 2005) leaves.

The combined waterlogging and salinity (30mM NaCl) treatments had a more adverse effect on membrane stability. A 30 to 43% increase in electrolyte leakage was observed with combined waterlogging + salinity (30mM NaCl) treatments. Maximum increase in electrolyte leakage was observed in UPAS 120 (43%) genotype and minimum increase

Table 1: Effect of different treatments on electrolyte leakage (%) of pigeonpea genotypes.

Genotypes	Electrolyte leakage (%)				
	Control	WL	WL+30mM NaCl	60mM NaCl	Mean
ICPH 2431	16.1	38.0	46.2	31.4	32.9
PARAS	17.4	40.2	50.2	32.2	35.0
UPAS 120	18.6	52.0	61.6	41.2	43.3
SGBS 6	18.2	50.1	59.9	39.7	42.0
Mean	17.6	45.1	54.5	36.1	
C.D. at 5% level of significance	*Genotypes*		=	*2.25*	
	Treatments		=	*2.25*	
	Genotypes x *Treatments*		=	*4.50*	

Table 2: Effect of different treatments on MDA content (μ mol gm^{-1}) of pigeonpea genotypes.

Genotypes	MDA Content (μ mol gm^{-1})				
	Control	WL	WL+30mM NaCl	60mM NaCl	Mean
ICPH 2431	6.0	7.6	8.1	6.8	7.1
PARAS	6.1	7.9	8.6	7.1	7.4
UPAS 120	5.8	8.7	9.5	8.1	8.0
SGBS 6	5.6	8.5	9.3	7.9	7.8
Mean	5.9	8.2	8.9	7.5	
C.D. at 5% level of significance	*Genotypes*		=	*0.28*	
	Treatments		=	*0.28*	
	Genotypes x *Treatments*		=	*0.56*	

Table 3: Effect of different treatments on biomass per plant (gm) of pigeonpea genotypes

Genotypes	Biomass per plant (gm)				
	Control	WL	WL+30mM NaCl	60mM NaCl	Mean
ICPH 2431	12.1	9.4	6.7	11.9	10.0
PARAS	10.7	8.6	5.9	10.5	8.9
UPAS 120	10.0	7.2	5.4	8.9	7.9
SGBS 6	9.6	6.9	-	8.8	6.3
Mean	10.6	8.0	4.5	10.0	
C.D. at 5% level of significance	*Genotypes*	=	*0.19*		
	Treatments	=	*0.19*		
	Genotypes x *Treatments*	=	*0.38*		

was observed in ICPH 2431 [30% (Table 1)]. Glynn *et al.* (1998) also reported the increase in electrolyte leakage in trees grown in waterlogged and salt amended solutions.

Lipid peroxidation level

Lipid peroxidation was measured in term of malondialdehyde (MDA) content. The MDA content increased with waterlogging and salinity treatments (Table 2). Higher the MDA content was, higher the oxidation degree of plant cell membranes resulting in greater damage to membranes (Cheruth *et al.*, 2009). In the present investigation, ICPH 2431(28.3%) showed minimum increase among the four genotypes and SGBS 6(51.5%) showed maximum increase in MDA content. Increase in MDA content with flooding treatment has been reported by Yan *et al.* (1996) in *Zea mays*. Arbona *et al.* (2008) reported that waterlogging also leads to an oxidative stress through an increase in ROS, such as $^1O_2$, H_2O_2, $OH^\bullet$, which adversely affect the membrane by increasing the lipid peroxidation levels. Higher accumulation of H_2O_2 (Jaiswal *et al.*, 2014) and lipid peroxidation under anaerobic condition has been reported by several researchers (Hossain *et al.*, 2009; Kumutha *et al.*, 2009; Sairam *et al.*, 2011). Salinity treatments aggravate the membrane lipid peroxidation in plants. In the present experiment, a 14.5 to 41.6% increase in MDA content was observed with 60mM NaCl treatments. The maximum damage was observed in the relatively sensitive genotype SGBS 6. Weisany *et al.* (2012) reported that in leaf and root (soybean), lipid peroxidation was significantly influenced by salt stress. In pumpkin, MDA accumulation in salt sensitive genotypes was higher than tolerant genotypes (Sevengor *et al.,* 2011).

The combined waterlogging and salinity treatment more severely affected lipid peroxidation levels. A 36.0 to 67.0% increase in MDA content was observed where, relatively tolerant genotype ICPH 2431 showed minimum increase among the four genotypes and relatively sensitive genotype SGBS 6 showed maximum increase in MDA content. Turkan *et al.* (2013) measured lipid peroxidation level by thiobarbutiric acid reactive substances (TBARS). The salt sensitive *P.media* showed increased TBARS content with all stress treatments and salt tolerant genotype *P. maritima* showed a less adverse effect on salinity, waterlogging and combined waterlogging and salinity treatments.

Total plant biomass

Total plant biomass decreased with various treatments (Table 3). The decline in total plant biomass was 19.3 to 28.7% with waterlogging, 1.8 to 10.7% with salinity (60mM NaCl) and 44.8 to 45.8% with waterlogging plus salinity (30mM NaCl)

Table 4: Changes in seed yield per plant (g) in pigeonpea genotypes affected by various treatments.

Genotypes	Seed yield per plant (gm) Control	WL	WL+30mM NaCl	60mM NaCl	Mean
ICPH 2431	5.88	4.24	2.87	4.77	4.44
PARAS	5.55	3.43	2.35	4.74	4.02
UPAS 120	5.42	2.20	1.80	3.78	3.30
SGBS 6	5.28	1.98	-	3.50	2.69
Mean	5.53	2.96	1.76	4.20	
C.D. at 5% level of significance	*Genotypes*		=	*0.26*	
	Treatments		=	*0.26*	
	Genotypes x *Treatments*		=	*0.52*	

Table 5: Changes in test seeds weight (g) in pigeonpea genotypes affected by various treatments.

Genotypes	100 seeds test weight (gm) Control	WL	WL+30mM NaCl	60mM NaCl	Mean
ICPH 2431	8.3	7.3	6.8	7.7	7.5
PARAS	8.2	6.3	6.2	7.4	7.0
UPAS 120	7.9	6.2	5.1	7.0	6.5
SGBS 6	7.5	5.8	-	6.6	5.0
Mean	8.0	6.4	4.5	7.2	
C.D. at 5% level of significance	*Genotypes*	=	*0.19*		
	Treatments	=	*0.19*		
	Genotypes x *Treatments*	=	*0.38*		

treatments. Maximum decline in plant biomass was observed in SGBS 6 (28.7%), minimum decline in PARAS (19.3%) with waterlogging treatments. Root O_2 deficiency under waterlogged conditions restricts aerobic respiration and reduced the plant biomass (Bailey-Serres and Voesenek, 2008). Kumutha *et al.* (2009) also reported in pigeonpea genotypes that total dry matter decreased with different durations of waterlogging, and greater reduction over control was recorded in Pusa 207 (sensitive) than ICP 301 (tolerant). Smethurst and Shabala (2003) reported significant reduction in fresh and dry weight for both shoots and roots after 16 days of waterlogging stress in lucerne (*Medicago sativa*) cultivars.

Salinity leads to a decrease in growth and many plants can't tolerate high levels of salt. It also limits plant growth by increasing osmotic pressure, disrupting the balance between nutrients and causing toxicity of some elements (Xiao-fang *et al.*, 2000; Yong *et al.*, 2005). In the present observation salinity (60mM NaCl) treatments decreased the plant biomass, maximum decline was observed in UPAS 120 (10.7%) and minimum decline was observed in ICPH 2431 (1.8%). Munns (2002) also observed decreased in plant biomass with salt stress.

The combined treatment of waterlogging and salinity was found more deleterious with a maximum decline in UPAS 120 (45.4%) and minimum decline in ICPH 2431 (44.8%) & PARAS (44.8%). No plant was survived in SGBS 6 with combined treatment. These results are in confirmation with the results of Hollington (1998) which reported that saline hypoxia reduces growth, grain and straw yields in wheat, but NaCl or hypoxia alone had smaller effects. Zeng *et al.* (2013) reported that two weeks of combined salinity and waterlogging treatment significantly decreased plant biomass in two barley genotypes, CM72 and NasoNijo.

Yield and yield attributes

The present investigation showed that the seed yield per plant and seed test weight decreased with waterlogging and salinity treatments (Table 4 and 5). The decline in seed yield per plant was 27.9 to 62.5% and seed test weight was 12.2 to 23.3% with waterlogging treatment. Maximum decline was observed in SGBS 6 and minimum decline was observed in ICPH 2431. Shabala (2011) reported that waterlogging adversely affects about 10% of the global area and reduces crop yields by as much as 80%. Waterlogging reduces plant height and delays flowering in surviving plants, resulting in reduction in the number of pods, seeds/pod and seed yield in pigeonpea (Choudhary *et al.*, 2011).

Salinity (60mM NaCl) treatments had a less adverse effect on seed yield. A 14.6 to 33.7% decline in seed yield per plant and 7.2 to 12.4% decline in seed test weight was observed with salinity treatment. Maximum decline in yield per plant was observed in SGBS 6 (33.7%) and minimum decline was observed in PARAS (14.6%) while the decline in test weight was maximum in SGBS 6 (12.4%) and minimum in ICPH 2431 (7.2%). Ghassemi *et al.* (2009) observed that salinity stress reduced number of pods and grains per plant, grain weight and grain yield in soybean. Katerji *et al.* (1992) indicated that salinity stress decreased grain yield of mung-bean by about 28% and the main factor in yield reduction was difference in grain weight. Reduction in crop yield as a result of salt stress has also been reported for sunflower (Khatoon *et al.*, 2000), cotton and wheat (Cullu, 2003), canola (Bybordi, 2010), broad bean (Katerji *et al.*, 1992), chickpea (Sohrabi, 2008), rice (Mahmood *et al.*, 2009) and soybean (Ghassemi *et al.*, 2009).

The combined effect of waterlogging and salinity treatment on seed yield was more severe. Hollington (1998) has concluded that saline hypoxia reduces growth, grain and straw yields in wheat, but NaCl or hypoxia alone had smaller effects. Similar results were obtained in the present investigation. A 51.2 to

66.8% decline in yield per plant and 18.1 to 34.8% decline in seed test weight were observed with waterlogging + salinity (30mM NaCl) treatments. Maximum decline was observed in UPAS 120 and minimum decline was observed in ICPH 2431.

A significant effect on seed test weight was observed under different treatments. The seed test weight decreased from 12.2 to 23.3% with waterlogging, 7.2 to 12.4% with salinity (60mM NaCl) and 18.1 to 34.8% with waterlogging + salinity (30mM NaCl) treatments as compared to their respective control. Maximum decline with waterlogging was reported in SGBS 6 (23.3%) and minimum decline was reported in ICPH 2431 (12.2%). Salinity treatments resulted in a comparatively lower decline in seed test weight as compared to other treatments. Maximum decline in SGBS 6 (12.4%) and minimum decline in ICPH 2431 (7.2%) was observed. Waterlogging and salinity treatments in combination were more deleterious. No plant was survived in SGBS 6 in combined treatment. Maximum decline was observed in UPAS 120 (34.8%) and minimum decline was observed in ICPH 2431 (18.1%).

The tolerance of the genotypes ICPH 2431& PARAS could be due to well developed aerenchyma, higher total soluble sugar and reducing sugar content, lesser membrane injury, higher chlorophyll content as compared to sensitive genotypes. These genotypes can be further used by plant breeders to generate higher yielding varieties under waterlogging and salinity stresses.

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