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Waterlogging, Salinity and Combined Stress a Major Problem in Pigeonpea (*Cajanus cajans* L. Mill sp.)

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Abstract

The present investigation was conducted in Department of Botany and Plant Physiology, CCS Haryana Agricultural University, Hisar during 2014–15 and 2015–16. Waterlogging and salinity are most severe stress all around the world. When water and salt is present in excess amount than its optimum requirement it refers to waterlogging and salinity respectively. Waterlogging when combined with salinity exaberate the effect of salinity. Four pigeonpea genotypes (ICPH-2431, PARAS, UPAS-120, H09-33) were raised in polythene bags filled with half kg soil+FYM manure mixture and Carbohydrate metabolism and aerenchyma formation under twelve days waterlogging, salinity and combined stress was studied in roots of 20 and 40 day old pigeonpea plants 1, 4 and 8 days after removal from treatment under pot house conditions. Decline in total and nonreducing sugar content and a significant increase in reducing sugar content, sucrose synthase activity and alcohol dehydrogenase activity was observed with waterlogging and salinity stress. No survival was observed under combined waterlogging and salinity treatment in any genotypes. Waterlogging was found more deleterious compared to alone salinity treatment. Aerenchyma formation was observed only under waterlogging treatment. Both the stresses are found more deleterious when given at later stages of growth. ICPH performed best among all the genotypes followed by PARAS, HO9-33 and UPAS-120 in terms of total sugar, nonreducing sugar, reducing sugar, sucrose synthase activity, alcohol dehydrogenase activity and aerenchyma formation.

Keywords: Aerenchyma, carbohydrate metabolism

1. Introduction

Abiotic stresses like waterlogging and salinity affects large area of world (Zeng et al., 2013; CSSRI, 2016). Both of these stresses affect growth of plant by affecting a number of physiological processes like photosynthesis, water uptake and respiration etc (Bajpai and Chandra, 2015). Increased level of water decreases the availability of oxygen to plant by creating hypoxic condition and finally leads to death of plant due to anoxia (complete absence of oxygen) (Ashraf, 2012; Akhtar and Nazir, 2013). Since oxygen is a terminal acceptor in aerobic respiration its absence blocks the kreb cycle and electron transport chain. However plant uses several strategies to cope up with stresses like anerobic respiration to produce adenosine triphosphate (ATP) and aerenchyma formation (Hossain and Uddin, 2011; Kulkarni and Chavan, 2013). Similarly salinity stress also affects the water relation of the plant. Initially plant faces water scarcity which in turn reduces leaf expansion but later when this water scarcity is prolonged it results into decreased photosynthesis and sucrose phosphate synthase activity which converts hexose phosphates into sucrose (Amirjani, 2010). Plants also have some mechanism like accumulation of proline, soluble sugar, glycine betain and sugar alcohols to cope up with salinity stress (Celik and Atak, 2012). 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. In India, pigeonpea is mainly grown in the regions lying between 14 °N and 29 °N latitudes with mean annual rainfall ranging between 600 and 1500 mm. Majority of these areas are endowed with a dependable and high rainfall pattern. Waterlogging is a perennial production problem in these areas which are characterized with alluvial or deep vertisols (Bansal and Srivastava, 2012). Salinity is an ever-increasing abiotic stress affecting about 95 mha or around 20% of land worldwide (Aksoy and Dinler, 2014). These environmental stress significantly contribute in reduction of crop yield (Basiri et al., 2013; Javed et al., 2014). So, soil salinity can be a major constraint to pigeonpea in regions where it is predominantly grown (Choudhary et al., 2011).

The combined effects of salinity and waterlogging are common in saline areas, particularly where shallow saline-water tables exist or where soils are also sodic, reducing

water infiltration and causing water to pond on the soil surface (Barrett-Lennard, 2003). For many plant species, when salinity and waterlogging occur together, a large increase in Na^+ and Cl^- concentrations in shoots occurs due to increased entry of these ions into oxygen deficient roots (Barrett-Lennard, 2003). Thus, waterlogging can exacerbate the effects of salinity (e.g. aize, (Drew et al., 1988). Therefore, the present work was conducted to study the independent and interactive effects of twelve days waterlogging and salinity on carbohydrate metabolism and aerenchyma formation in pigeonpea genotypes.

2. Materials and Methods

Four genotypes were raised in polythene bags filled with half kg soil+FYM manure mixture (3 soil: 1manure v/v), NPK (@ 20:60:20 kg ha^{-1}). Twenty and forty days after sowing the pots were placed in cemented tanks (length 160 cm, breadth 125 cm and depth 65 cm). Three treatments i.e. alone waterlogging, alone salinity (30 mM NaCl) and waterlogging+salinity (30 mM NaCl) in combination were given for 12 days. Under waterlogging and combined treatment of waterlogging and salinity, plants with polythene bags were put in cemented tanks filled with simple water and 30 mM NaCl solution respectively. Solutions were drained out 12 days after treatment and observations were recorded 1, 4 and 8 days after removal from treatment in the roots of plants. Alone salinity (30 mM NaCl) treatment was given by only irrigating plants with 30 mM NaCl solution instead of water time to time. Under waterlogging treatment, plants were waterlogged with pure water and under waterlogging+salinity treatment plants were waterlogged in saline solution. In case of alone salinity treatment only time to time irrigation was given saline solution and no waterlogged condition was created in alone salinity treatment Statistical design was two factorial randomized design (4 Replications)

2*.1. Survival percentage was calculated after the living plants*

were counted and expressed in the term of percent survival. Total soluble sugar content was estimated using method prescribed by Yemm and Willis (1954), reducing sugar content by DNS method (Miller, 1959). Non-reducing sugar content was measured by deducing reducing sugar content from the total sugar content.

2.2. Sucrose synthase

Extraction buffer consisted of 200 mM HEPES containing+1 Mm DTT+5 mM magnesium chloride+1 mM EDTA+20 mM sodium ascorbate+1 mM PMSF+10% (w/v) polyvinyl polypyrrolidone, and pH was adjusted to 7.5. One gram of plant material was ground followed by extraction with chilled extraction buffer (10 ml). Extract was centrifuged at 14,000 rpm at 4 °C for 10 min. Supernatant was dialyzed at 4 °C for 24 h against extraction buffer diluted 1:40, which was changed at least three times during dialysis. The reaction mixture contained 50 mM HEPES–NaOH buffer (pH 7.5), 15 mM magnesium chloride, 10 mM fructose, 5 mM UDP-glucose, 50 ml enzyme extract and water to make final volume 3.0 ml. Assay was conducted at 30 °C for 30 min in a shaking water bath incubator, and reaction was terminated by addition of 1 ml of 30% KOH. Controls were terminated at 0 min. The unreacted fructose was removed by subsequent heating at 100 °C for 10 min. After cooling, each assay mixture was incubated with 1 ml of 0.14% anthrone in H_2SO_4 at 40 °C for 20 min and absorbance recorded at 620 nm.

2.3. Alcohol dehydrogenase

The extraction buffer consisted of 50 mM Tris–HCl+15 mM DTT, pH 8.0. Plant tissue of 0.5 g was homogenized with 5.0 ml of extraction buffer. The extract was centrifuged at 12,000 rpm for 15 min at 4 °C. The 3 ml reaction mixture contained 50 mM Tris buffer, 0.867 mM NAD, 20% ethanol, 50 ml enzyme extract and double distilled water. Reaction mixtures except NAD were prepared in test tubes, and each sample used as blank to adjust zero. NAD was added to initiate the reaction and increase in absorbance due to NADH at 340 nm recorded for 1 min. Amount of NADH formed is computed by drawing a standard curve of NADH at 340 nm and activity is expressed as nmol NADH formed per mg protein per minute.

2.4. Aerenchyma formation in roots

Root was sampled below the root-shoot transition region. The materials were fixed in formalin-acetic acid-alcohol solution FAA (Sass, 1964) at vegetative stage one day after removal of eight treatments. After 24–48 hrs, the materials were washed and preserved in 70% alcohol till further use. The preserved materials when used were dehydrated through ethanol xylene series and then infiltrated and embedded in paraffin wax (congealing point 58–60 °C). Serial transverse sections of roots were cut at 8–10 µm on rotary microtome Johansen, 1940 (Spencer 820 microtome, USA). Affixing of the paraffin ribbons to the slides was made by using synthetic gum. Conventional combination like safranin and light green stain (Johansen, 1940) were used for studying the aerenchyma formation in roots.

3. Results and Discussion

3.1. Percent survival

Twelve days W treatment resulted in 50–75% decrease in percent survival 1 DAR which further increased to 65–90% and 100% 4 and 8 DAR respectively in 20 day old plants (Table 1). Salinity treatment alone had no deleterious effect and no decline in percent survival was observed. Waterlogging and salinity treatment in combination was more deleterious resulting in 100% 1, 4 and 8 DAR from 12 days treatment. Forty day old plants recorded no survival with 12 days W and W+S treatment 1, 4 and 8 DAR. No decline in percent survival was observed under S treatment also in 40 day old plants. ICPH 2431 performed best under W and W+S treatments (8 days and 12 days) followed by PARAS, HO9-33 and UPAS-120 in 20 day as well as 40 day old plants.

Table 1: Effect of waterlogging, salinity and their combination (12 days) on survival percent (%) of pigeonpea genotypes

Genotype	20 DAS											40 DAS										
	12 day* (1 day)**				12 day* (4 day)**				12 day* (8 day)**			12 day* (1 day)**				12 day* (4 day)**				12 day* (8 day)**		
	C	W	S	W+S#	C	W	S	W+S#	W#	S	W+S#	C	W#	S	W+S#	C	W#	S	W+S#	W#	S	W+S#
ICPH 2431	100	50	100	0	100	38	100	0	0	100	0	100	0	100	0	100	0	100	0	0	100	0
UPAS 120	100	24	100	0	100	15	100	0	0	100	0	100	0	100	0	100	0	100	0	0	100	0
HO9 33	100	41	100	0	100	21	100	0	0	100	0	100	0	100	0	100	0	100	0	0	100	0
PARAS	100	43	100	0	100	26	100	0	0	100	0	100	0	100	0	100	0	100	0	0	100	0

*Duration of treatment; ** stage of sampling; # no survival was observed

3.2. Carbohydrate metabolism

3.2.1. Total soluble sugar content

A decline of 14–26% and 7–14% was observed in total sugar content of 20 day old plant roots 1 DAR from 12 days W and S treatment which further decreased to 5–7% and 3–7% 4 DAR in case of 12 days W and S treatment respectively (Table 2). Salinity treatment also resulted 3 to 7% increase 8 DAR from 12 days treatment. Waterlogging+Salinity was found more deleterious to all genotypes and resulted in no survival. Forty day old plant roots showed more decline in comparison to 20 days plant roots. A decline of 11 to 22% 1 DAR from 12 days S treatment was observed. Salinity (12 days) treatment resulted in 2 to 5% decline 4 DAR from treatment. The decline was 6 to 11% 8 DAR from 12 days S treatment. No plant survived 4 DAR from 12 days combined treatment, 8 DAR from 12 days waterlogging and 1 and 8 DAR from 12 days combined treatment in 20 day old pigeonpea plants and 4 DAR from 12 days and 1 and 8 DAR from 12 days waterlogging and waterlogging+salinity treatment in 40 day old pigeonpea plants. ICPH 2431 performed best among the genotypes under all treatments. Sairam et al. (2009b) also noticed that during waterlogging roots of comparatively tolerant genotypes showed greater sugar content than susceptible one in pigeonpea. Horchani et al. (2010) also investigated the interactive effects of salinity and hypoxia on the physiological responses of tomato (*Solanum lycopersicum* L.) plants and

Table 2: Effect of waterlogging, salinity and their combination (12 days) on total sugar content (mg g^{-1} dry weight) in pigeonpea roots

Genotype	20 DAS										40 DAS		
	12 day* (1 day)**				12 day* (4 day)**				12 day* (8 day)**		12 day* (1 day)**		
	C	W	S	Mean#	C	W	S	Mean	S	Mean#	C	S	Mean#
ICPH 2431	24.3	20.9	22.6	22.6	24.8	23.5	27	24.9	24.1	24.5	25.1	22.3	23.7
UPAS 120	23.6	17.4	20.4	20.5	23.8	22.1	24	23.4	22.2	23	24.2	18.9	21.6
HO9 33	23.9	18.4	20.9	21.1	24.1	22.7	25	24	22.6	23.4	24.4	19.6	22
PARAS	24.1	19.9	22.1	22	24.4	23.2	26	24.5	23.5	24	24.7	21.8	23.3
Mean	24	19.2	21.5		24.3	22.9	26		23.1		24.6	20.7	
CD (p=0.05)	T-0.41, G-0.48, T×G-0.82				T-0.24, G-0.27, T×G-0.47				T-0.42, G-0.59, T×G-N.S.		T-0.54, G-0.76, T×G-1.08		

Table 2: Continue...

Genotype	40 DAS				
	12 day* (4 day)**			12 day* (8 day)**	
	C	S	Mean	S	Mean#
ICPH 2431	25.3	24.8	25.1	23.9	24.6
UPAS 120	24.3	23.2	23.8	21.7	23
HO9 33	24.5	23.5	24	22.1	23.3
PARAS	24.9	24.5	24.7	23.1	24
Mean	24.8	24		22.7	
CD (p=0.05)	T-0.60, G-0.85, T×G N.S.			T-0.48, G-0.68, T×G- N.S.	

reported an increase in root carbohydrate content and a decrease in leaf carbohydrate content. Though, an increase in TSC in roots was observed under waterlogging treatments.

3.2.3. Non-reducing sugars

Twelve days W and S treatment resulted in 30–40% and 13–19% decline in non-reducing sugar content 1 DAR from treatment. Decline was 9–10% 4 DAR from 12 days W treatment and 6–10% 8 DAR from 12 days S treatment. An increase of 2–7% was also observed 4 DAR from 12 days S treatment in 20 day old plant roots (Table 3). Forty day old plants recorded a 17–28% decline 1 DAR from 12 days S treatment. Twelve days S treatment resulted in 4–7% and 8–13% decline in non-reducing sugar content 4 and 8 DAR from treatment. No plant survived 4 DAR from 12 days combined treatment, 8 DAR from 12 days waterlogging and 1 and 8 DAR from 12 days combined treatment in 20 day old pigeonpea plants and 4 DAR from 12 days and 1 and 8 DAR from 12 days waterlogging and waterlogging+salinity treatment in 40 day old pigeonpea plants. ICPH 2431 performed best among all genotypes with minimum decline in non reducing sugar content. Sairam et al. (2009a) reported that level of nonreducing sugars declined under waterlogging and reached a lowest value on the 8th days of treatment in

Table 3: Effect of waterlogging, salinity and their combination (12 days) on non reducing sugar content (mg g^{-1} dry weight) in pigeonpea roots

Genotype	20 DAS										40 DAS		
	12 day* (1 day)**				12 day* (4 day)**				12 day* (8 day)**		12 day* (1 day)**		
	C	W	S	Mean#	C	W	S	Mean	S	Mean#	C	S	Mean#
ICPH 2431	20	14	17.5	17.2	20.4	18.6	21.8	20.3	19.2	19.8	20.6	17	18.9
UPAS 120	19.8	11.8	16.1	15.9	19.9	17.9	20.3	19.4	18	19	20.2	15	17.4
HO9 33	20	12.5	16.5	16.3	20.1	18.4	20.9	19.8	18.3	19.2	20.2	15	2.4
PARAS	20.1	13.6	17.4	17	20.2	18.6	21.4	20.1	18.9	19.6	20.4	17	3.1
Mean	20	13	16.9		20.1	18.4	21.1		18.6		20.4	16	
CD (p=0.05)	T-0.42, G-0.49, T×G-0.85				T-0.46, G-0.53, T×G-N.S.				T-0.47, G- N.S., TXG- N.S.		T-0.47, G-0.67, T×G-0.95		

Table 3: Continue...

Genotype	40 DAS				
	12 day* (4 day)**			12 day* (8 day)**	
	C	S	Mean	S	Mean#
ICPH 2431	20.8	20	20.4	19.1	20
UPAS 120	20.1	18.8	19.5	17.4	18.8
HO9 33	20.3	19.1	19.7	17.7	19
PARAS	20.5	19.8	20.2	18.4	19.5
Mean	20.4	19.4		18.2	
CD (p=0.05)	T-0.21, G-0.29, T×G- N.S.			T-0.26, G-0.37, T×G- 0.52	

*: Duration of treatment; **: stage of sampling; #: mean value were calculated with the respective control values.

mung bean. However, V. luteola and T44 (relatively tolerant) retained higher content of nonreducing sugar content than PB (relatively susceptible). However Naureen and Naqvi (2010) reported that salinity stress of 200 mM significantly increased the amount of nonreducing sugar in wheat.

3.2.3. Reducing sugars

Under 12 days W treatment an increase of 47–60% was observed 1 DAR from treatment in 20 day old plant roots. Twelve days S treatment resulted in 13–19% and 8–11% increase in reducing sugar 1 and 8 DAR from treatment in 20 day old plant roots. An increase of 8 to 11% under W (12 days) and 5–7% under S (12 days) 4 DAR from treatment was recorded (Table 4). Maximum increase in ICPH 2431 and minimum in UPAS 120 in 20 day old plant roots was recorded. Forty day old plant roots recorded 8–16%, 5–7% and 2–7% increase 1, 4 and 8 DAR from 12 days S treatments. No plant survived 4 DAR from 12 days combined treatment, 8 DAR from 12 days waterlogging and 1 and 8 DAR from 12 days combined treatment in 20 day old pigeonpea plants and 4 DAR from 12 days and 1 and 8 DAR from 12 days waterlogging and waterlogging+salinity treatment in 40 days old pigeonpea plants. ICPH 2431 was found best among the genotypes under all treatments in terms of reducing sugar content. Increased reducing sugar content in *Vigna luteola* was reported by Sairam et al. (2009a). Javed et al. (2014) investigated the effect of salinity (8 dSm^{-1}) on reducing sugar content in six varieties of safflower and reported increased content of reducing sugar under salt stress. Increase in reducing sugar content was more in ICPH 2431 among all the genotypes which reflects the mechanism behind the tolerance of that genotype to waterlogging and salinity stress as maintaining adequate levels of readily metabolizable sugars in hypoxic roots is one of the adaptive mechanisms to waterlogging.

3.2.4. Sucrose synthase

Under 12 days W treatment an increase of 49–72% was observed 1 DAR from treatment in 20 days old plant roots

Table 4: Effect of waterlogging, salinity and their combination (12 days) on reducing sugar content (mg g^{-1} dry weight) in pigeonpea roots

Genotype	20 DAS										40 DAS		
	12 day* (1 day)**				12 day* (4 day)**				12 day* (8 day)**		12 day* (1 day)**		
	C	W	S	Mean#	C	W	S	Mean	S	Mean#	C	S	Mean#
ICPH 2431	4.3	6.9	5.1	5.4	4.4	4.9	4.7	4.7	4.9	4.7	4.5	5.2	4.9
UPAS 120	3.8	5.6	4.3	4.6	3.9	4.2	4.1	4.1	4.2	4.1	4	4.3	4.2
HO9 33	3.9	5.9	4.4	4.7	4	4.3	4.2	4.2	4.3	4.2	4.2	4.6	4.4
PARAS	4.0	6.3	4.7	5.0	4.2	4.6	4.5	4.4	4.6	4.4	4.3	4.9	4.6
Mean	4.0	6.2	4.6		4.1	4.5	4.4		4.5		4.3	4.8	
CD (p=0.05)	T-0.46, G-0.53, T×G-N.S.				T-N.S., G-N.S., T×G-N.S.				T-N.S., G-N.S., T×G-N.S.		T-N.S., G-N.S., T×G-N.S.		

Table 4: Continue...

Genotype	40 DAS				
	12 day* (4 day)**			12 day* (8 day)**	
	C	S	Mean	S	Mean#
ICPH 2431	4.5	4.8	4.7	4.8	4.7
UPAS 120	4.2	4.4	4.3	4.3	4.3
HO9 33	4.2	4.4	4.3	4.4	4.3
PARAS	4.4	4.7	4.6	4.7	4.6
Mean	4.3	4.6		4.6	
CD (p=0.05)	T-N.S., G-N.S., T×G- N.S.			T-N.S., G-N.S., T×G- N.S.	

which further reduced to 11–16% 4 DAR from 12 days W treatment (Table 5). Twelve days S treatment resulted in 13–25%, 1–5% and 5–9% increase in sucrose synthase activity 1, 4 and 8 DAR from treatment in 20 days old plant roots. Forty day old plant roots showed lower increase in sucrose synthase activity. Twelve days S treatment resulted in 9–21%, 2–4% and 3–6% increase 1, 4 and 8 DAR from treatment. No plant survived 4 DAR from 12 days combined treatment, 8 DAR from 12 days waterlogging and 1 and 8 DAR from 12 days combined treatment in 20 days old pigeonpea plants and 4 DAR from 12 days and 1 and 8 DAR from 12 days waterlogging and waterlogging+salinity treatment in 40 days old pigeonpea plants. ICPH 2431 was found best among the genotypes under all treatment in terms of sucrose synthase activity**.** Stress induces to increase the content of reducing sugar through increased activity of SS in tolerant genotype to cope up with stress (Hossain and Uddin, 2011). The results suggest that waterlogging tolerance of pigeonpea genotypes ICPH 2431 and PARAS depends on the availability of sufficient sugar reserve in the roots, activity of sucrose synthase to provide reducing sugars for glycolytic activity.

3.2.5. Alcohol dehydrogenase

Twelve days W and S treatments resulted in 49–61% and 16–31% increase in enzyme activity 1 DAR from treatments in 20 days old plant roots. A partial recovery was observed 4 DAR from 12 days W and S treatments with 19–34% and 9–14% increase in enzyme activity. A higher increase of 11 to 18% 8 DAR from 12 days S treatment was observed (Table 6). In 40 days old plant roots, an increase of 15–30%, 9–11% and 8–16% in ADH activity was observed 1, 4 and 8 DAR from 12 days S treatment. No plant survived 4 DAR from 12 days combined treatment, 8 DAR from 12 days waterlogging and 1 and 8 DAR from 12 days combined treatment in 20 day old pigeonpea plants and 4 DAR from 12 days and 1 and 8

Table 5: Effect of waterlogging, salinity and their combination (12 days) on sucrose synthase (µl mg^{-1} protein min^{-1}) in pigeonpea roots

Genotype	20 DAS										40 DAS		
	12 day* (1 day)**				12 day* (4 day)**				12 day* (8 day)**		12 day* (1 day)**		
	C	W	S	Mean#	C	W	S	Mean	S	Mean#	C	S	Mean#
ICPH 2431	1.05	1.81	1.31	1.39	1.06	1.23	1.11	1.13	1.16	1.11	1.07	1.3	1.18
UPAS 120	1.01	1.5	1.14	1.22	1.02	1.13	1.03	1.06	1.07	1.05	1.03	1.1	1.08
HO9 33	1.02	1.62	1.16	1.27	1.03	1.15	1.05	1.08	1.09	1.06	1.04	1.2	1.09
PARAS	1.04	1.78	1.28	1.37	1.04	1.2	1.08	1.11	1.12	1.08	1.05	1.3	1.15
Mean	1.03	1.68	1.22		1.04	1.18	1.07		1.11		1.05	1.2	
CD (p=0.05)	T-0.05, G-0.05, T×G- 0.09				T-0.06, G-N.S., T×G- N.S.				T-0.06, G-N.S., T×G- N.S.		T-0.07, G-N.S., T×G- N.S.		

Continue...

Table 5: Continue...

Genotype	40 DAS				
	12 day* (4 day)**			12 day* (8 day)**	
	C	S	Mean	S	Mean#
ICPH 2431	1.08	1.12	1.11	1.15	1.12
UPAS 120	1.03	1.05	1.04	1.06	1.04
HO9 33	1.05	1.07	1.05	1.08	1.07
PARAS	1.06	1.09	1.08	1.12	1.09
Mean	1.06	1.08		1.1	
CD (p=0.05)	T- N.S., G- N.S., T×G- N.S.			T-0.04, G-N.S., T×G- N.S.	

DAR from 12 days waterlogging and waterlogging+salinity treatment in 40 days old pigeonpea plants. Maximum increase was observed in ICPH 2431 and minimum in UPAS 120. Wang et al. (2009) reported increase in the activity of ADH in the roots of Kentucky bluegrass under three days of waterlogging conditions. Similar results were noticed by Chen and Quallis (2003) in roots of perennial pepper weed. Akhtar et al. (1998) measured ADH activity in wheat roots and reported comparatively higher activity under saline-hypoxic compared to salinity and hypoxia alone. These results suggest that waterlogging tolerance of pigeonpea genotypes ICPH 2431 and PARAS depends on the availability of sufficient ADH for the recycling of NADH, essential for the continuation of glycolysis, the major source of energy under hypoxia.

Table 6: Effect of waterlogging, salinity and their combination on ADH activity (µ mol NADH mg^{-1} protein min^{-1}) in pigeonpea roots

Genotype	20 DAS										40 DAS		
	12 day* (1 day)**				12 day* (4 day)**				12 day* (8 day)**		12 day* (1 day)**		
	C	W	S	Mean#	C	W	S	Mean	S	Mean#	C	S	Mean#
ICPH 2431	0.55	0.91	0.72	0.73	0.55	0.74	0.63	0.64	0.65	0.6	0.55	0.7	0.64
UPAS 120	0.54	0.8	0.63	0.65	0.54	0.64	0.59	0.59	0.6	0.57	0.54	0.6	0.59
HO9 33	0.54	0.81	0.65	0.67	0.54	0.67	0.6	0.6	0.62	0.58	0.55	0.6	0.6
PARAS	0.54	0.86	0.69	0.7	0.55	0.72	0.61	0.63	0.64	0.59	0.55	0.7	0.62
Mean	0.541	0.85	0.67		0.55	0.69	0.61		0.63		0.55	0.7	
CD (p=0.05)	T-0.02, G- 0.03, TXG-0.05				T-0.02, G-0.02, TXG-0.03				T-0.02, G-0.02, TXG- N.S.		T-0.03, G-0.04, TXG- N.S.		

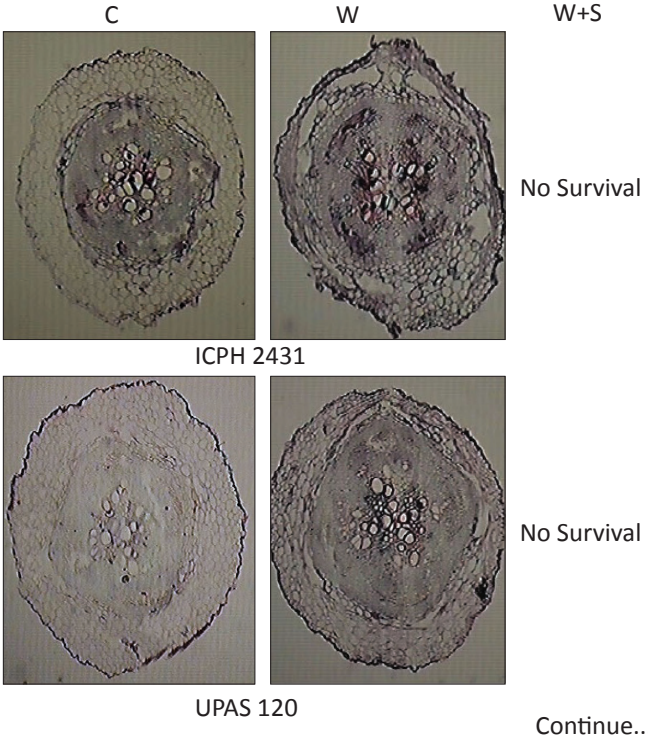
Table 6: Continue...

Genotype	40 DAS				
	12 day* (4 day)**			12 day* (8 day)**	
	C	S	Mean	S	Mean#
ICPH 2431	0.56	0.62	0.59	0.65	0.61
UPAS 120	0.55	0.6	0.58	0.59	0.57
HO9 33	0.55	0.61	0.58	0.61	0.58
PARAS	0.56	0.62	0.59	0.63	0.6
Mean	0.55	0.62		0.62	
CD (p=0.05)	T-0.02, G-N.S., TXG- N.S.			T-0.02, G-N.S., TXG- N.S.	

*: Duration of treatment; **: stage of sampling; #: Mean value were calculated with the respective control values

3.2.6. Aerenchyma formation

Aerenchyma is a special tissue which consists of continuous gas filled channels or much enlarged gas spaces. Aerenchyma formation was observed under W and W+S treatments (Figure 1, 2). The aerenchyma formation was less in sensitive genotypes as compared to tolerant genotypes. Hossain and Uddin (2011) in wheat and De-Souza et al. (2009) in maize also noticed formation of aerenchyma in root tissue of tolerant variety of wheat in response to anoxia. In control plant there was no aerenchyma formation. Alone salinity treatment also



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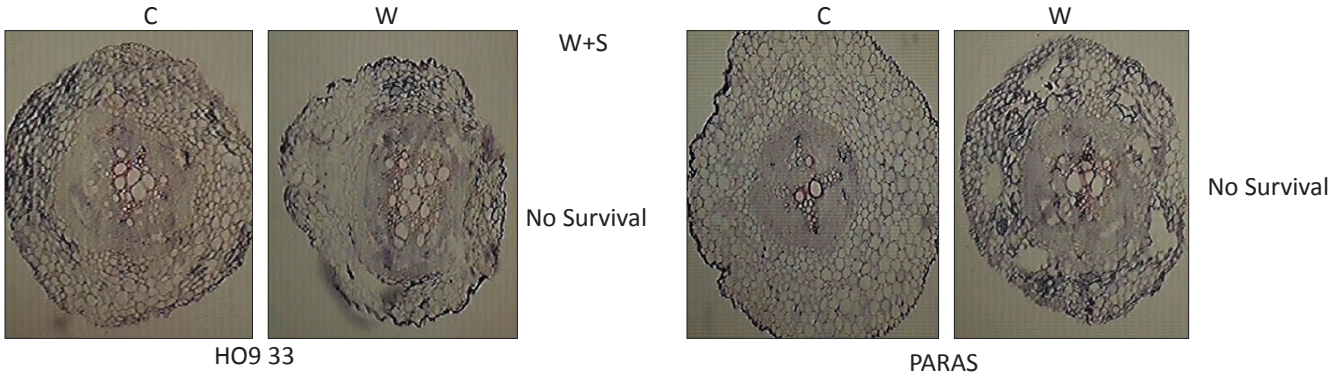

Figure 1: Aerenchyma formation in roots of 20 days old pigeonpea genotypes 1 DAR from 12 days W and W+S treatments

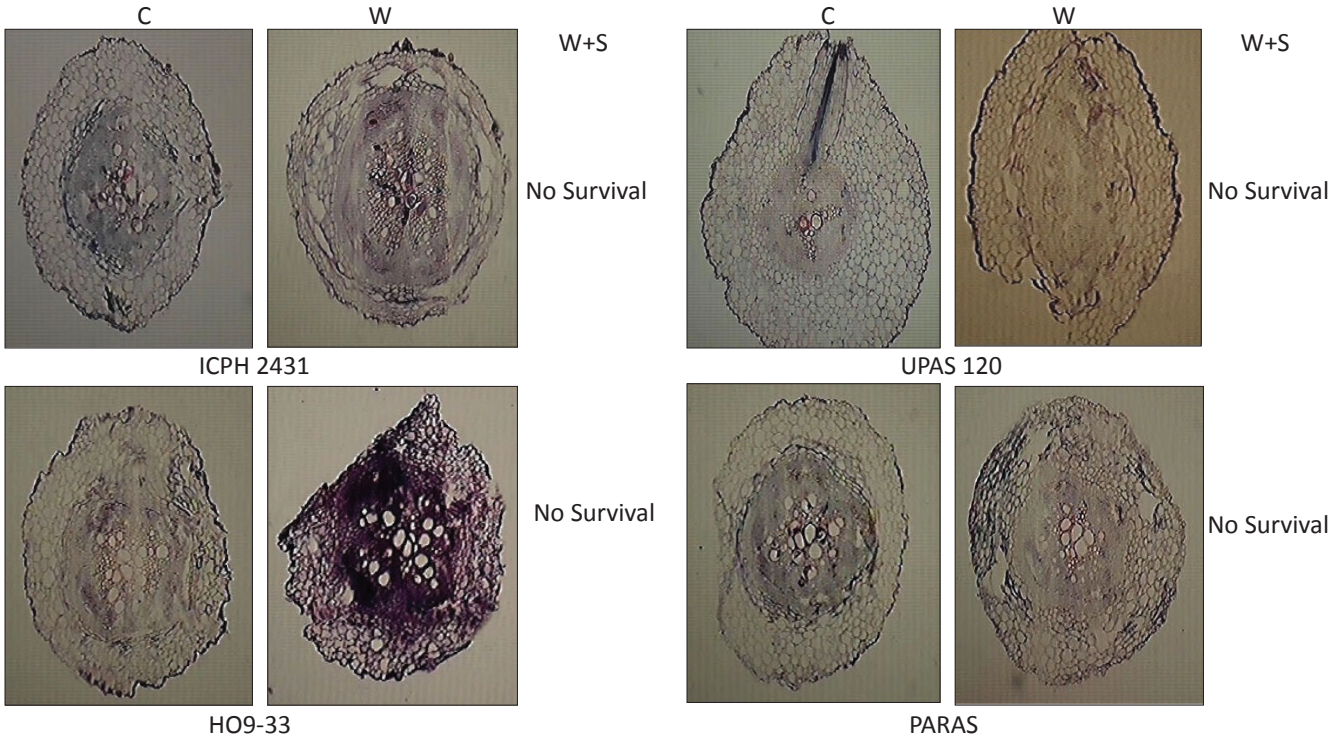

Figure 2: Aerenchyma formation in roots of 20 days old pigeonpea genotypes 4 DAR from 12 days W and W+S treatments

resulted in no aerenchyma formation. It may concluded from this study that combined stress of waterlogging and salinity is more deleterious compared to these stresses alone and aerenchyma formation is one of the strategy which plants opt to cope up with waterlogging and combined stress. More aerenchyma formation in ICPH 2431 and PARAS represent tolerant behavior of these genotypes towards waterlogging and combined stress. It is also concluded from the study that stress become more deleterious when given for long time and at later vegetative stages.

4. Conclusion

Waterlogging and salinity in combination are more deleterious to plants of pigeonpea as compared to these stresses alone. All the three stresses are more deleterious when given at later stages of growth. Tolerant pigeonpea genotypes had a better carbohydrate metabolism and more aerenchyma formation to cope with waterlogging and salinity and this represent the tolerant behavior of these genotypes towards waterlogging and salinity stress.

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