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EFFECTS OF WATERLOGGING, SALINITY AND THEIR COMBINATION ON PERCENT SURVIVAL, CHLOROPHYLL CONTENT AND CHLOROPHYLL FLUORESCENCE IN PIGEON PEA (*CAJANUS CAJAN* L. MILLSP.) GENOTYPES

SAVITA DUHAN*[1], NIDHI SHARMA[2], SUMAN BALA[1], MANOHAR LAL[1] AND SUNITA SHEOKAND[1]
[1]Department of Botany & Plant Physiology, CCS HAU, Hisar - 125 004, INDIA
[2]Department of Horticulture, CCS HAU, Hisar - 125 004, INDIA
e-mail: savitaduhan53@gmail.com

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***Corresponding author**

ABSTRACT

The objective of this study was to examine the effects of waterlogging (W), salt (S) and their combination (W+S) on percent survival, chlorophyll content, chlorophyll fluorescence in pigeonpea (*Cajanus cajan* L. Millsp.) genotypes (ICPH-2431, PARAS, UPAS-120, H09-33). Waterlogging salinity and waterlogging + salinity treatments were given for 8 and 12 days and observations were taken 1 and 8 days after removal from treatments. A 40 to 50 % decline was observed in percent survival under waterlogging treatment for 8 days was observed which further increased to 50 to 60 % with 12 days waterlogging treatment. No decline in percent survival was observed under salinity treatment. Waterlogging and salinity treatment in combination were more deleterious resulting in 70 to 80 % decrease in percent survival after 8 days and 96 to 100 % after 12 days. A decline of 20 to 30 % was observed in chlorophyll content and chlorophyll fluorescence in waterlogging and waterlogging+salinity treatment for 8 days which further increased to 30 to 40 % when waterlogging was given for 12 days. However, no significant change was observed with salinity treatment. In all treatments waterlogging, salinity and waterlogging +salinity ICPH-2431 was found relatively more tolerant as compared to other genotypes.

Abbreviations: W- Waterlogging, S- Salinity, W+S- Waterlogging+salinity, DAR- Day after removal

INTRODUCTION

Pigeonpea is an important pulse crop of South Asia. Being a summer-rainy season crop, pigeonpea is exposed to waterlogging condition during germination and early vegetative growth phases. This is the crucial period, which determines the crop stand and ultimately crop growth and productivity (Kumutha *et al.,* 2008). About 0.28 million tons loss in pigeonpea has been reported annually due to waterlogging (ICRISAT Report, 2011). In India, where most of world's pigeonpea is produced, around 2.95 million hectares land is also affected by salinity and in Haryana 49.2 thousand hectares is affected by salinity (NRSA, 1996).

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. Decrease in germination ability has been attributed to a shortage of oxygen due to waterlogging (Orchard and Jessop, 1984). Decrease in Chl content under waterlogging has been reported in wheat (Huang *et al.*, 1994; Collaku and Harrison, 2002), maize (Younis *et al.*, 2003; Prasad *et al.*, 2004) and *V. sinensis* (Younis *et al.*, 2003). Significantly greater damage to Chl b than Chl a have been reported under flooding (Ashraf and Arfan, 2005) and water stresses (Wentworth *et al.*, 2006). Under waterlogged condition the productivity of sugarcane also found lower than normal condition in various studies (Kumar *et al.*, 2015).

Salinity is a particular problem in irrigated land: more than 25% of irrigated land is saline in Egypt, Iran, Iraq, India, Pakistan and Syria. 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 (Marked *et al.*, 2014). The chlorophyll content of tissues decreased with an increase in salinity. Zhao *et al.* (2007) reported that salinity reduced leaf chlorophyll in the oat plant. The decrease in Chl content under salt stress is a commonly reported phenomenon and in various studies the Chl concentration were used as a sensitive indicator of the cellular metabolic state (Chutipaijit *et al.* 2011). Chlorophyll fluorescence has been used to evaluate the integrity of photosystem II upon exposure to stress (Shabala, 2002). The in vivo effects of salinity on chlorophyll fluorescence have been described for several crop species (Smillie and Nott, 1982; Sayed, 2003).

In recent years, an impressive amount of knowledge has accumulated on plant physiological and molecular responses to salinity or waterlogging stresses. However, studies dealing with the combined effects of these two stresses are much rarer and often controversial (Barrett-Lennard, 2003). Nonetheless, the occurrence of combined salinity and waterlogging stress is increasing throughout the world This is due to intensive irrigation in agricultural production systems (Smedema and Shiati, 2002), rise of saline water tables (Hatton *et al*., 2003), and seawater intrusion in coastal environments (Carter *et al*., 2006). When combined with waterlogging, salinity can cause even greater damage to plants, so having a major impact on agricultural production (Barrett-Lennard, 2003). Only a very few crop species can tolerate the combination of salinity and waterlogging (Bennett *et al*., 2009) and the physiological and molecular mechanisms conferring this tolerance remain elusive. So, the present investigation was conducted to study the effects of waterlogging, salinity and their combination on percent survival, chlorophyll content, chlorophyll fluorescence in pigeonpea (*Cajanus cajan* L. Millsp.) genotypes.

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 per ha). Twenty and forty days after sowing the pots were placed in cemented tanks (length 160 cm, breadth 125 cm and depth 65 cm). Waterlogging, salinity (30 mM NaCl) and waterlogging + salinity (30 mM NaCl) treatments were given for 8 and 12 days.

Statistical design

Factorial Randomized Design (4 Replications)

Sampling stages

One and 8 days after draining out the wáter and NaCl solutions.

Survival percentage

Eight and twelve days after removal from the treatments the living plants were counted and expressed in the term of percent survival.

Chlorophyll content

Leaf discs (0.03 g) were washed, blotted dry and dipped in test tubes containing 3mL of dimethyl sulfoxide (DMSO) for overnight as described by Sawhney and Singh, (2002). The extracted chlorophyll in DMSO was estimated by recording its absorbance at 663 and 645 nm, respectively and its amount was calculated from the formula:

$$\text{Chl 'a'} = \frac{12.3\ A_{663} - 0.86\ A_{645}}{a \times 1000 \times W} \times V$$

$$\text{Chl 'b'} = \frac{19.3\ A_{645} - 3.6\ A_{663}}{a \times 1000 \times W} \times V$$

Where,

V = Volume of DMSO

a = Path length

W = Weight of tissue taken

Chlorophyll fluorescence

Chlorophyll fluorescence was recorded using CIP chlorophyll fluorescence Os-30P meter at midday (between 10.00 AM to 12:00 AM). The fully expanded leaf was first acclimated to dark for minimum two minutes by fixing clip on it. The dark adapted leaf was then continuously irradiated for one second (1500 μmol $m^{-2}s^{-1}$) provided by an array of three light emitted diodes in the sensor. The Fv/Fm ratio was recorded.

RESULTS AND DISCUSSION

Percent survival

A 40 to 50 % decline was observed in percent survival 1 DAR which further increased to 65 to 90 % 8 DAR (day after removal) from waterlogging (8 days) treatment in 20 days old plants. Twelve days waterlogging treatment was more deleterious and resulted in 50 to 75% decrease in percent survival 1 DAR which further increased to 100% 8 DAR. 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 70 to 80 % decrease in percent survival 1 DAR and 100 % 8 DAR from 8 days treatment. No survival was observed with 12 days waterlogging + salinity treatment 1 and 8 DAR from treatment (Table 1 A). Forty day old plants were more sensitive to waterlogging and waterlogging + salinity treatment resulting in 40 to 80% decrease 1 DAR was which further increased to 100% 8 DAR from 8 days waterlogging. No survival was observed with 12 days waterlogging and waterlogging +

Table 1: Effect of waterlogging, salinity and their combination (8 and 12 days) on survival percent (%) of pigeonpea
A) 20 DAS

	8day* (1 day)**			12day* (1 day)**				8day* (8 day)**			12day* (1 day)**			
Genotype	C	W	S	W+S	W	S	W+S#	C	W	S	W+S#	W#	S	W+S#
ICPH 2431	100	58	100	35	50	100	0	100	38	100	0	0	100	0
UPAS 120	100	46	100	19	24	100	0	100	15	100	0	0	100	0
HO9 33	100	47	100	26	41	100	0	100	21	100	0	0	100	0
PARAS	100	47	100	33	43	100	0	100	26	100	0	0	100	0

B) 40 DAS

	8day *(1 day)**				12day* (1 day)**			8day* (8 day)**				12day* (1 day)**		
Genotype	C	W	S	W+S	W#	S	W+S#	C	W#	S	W+S#	W#	S	W+S#
ICPH 2431	100	40	100	20	0	100	0	100	0	100	0	0	100	0
UPAS 120	100	20	100	10	0	100	0	100	0	100	0	0	100	0
HO9 33	100	20	100	10	0	100	0	100	0	100	0	0	100	0
PARAS	100	30	100	20	0	100	0	100	0	100	0	0	100	0

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

Table 2: Effect of waterlogging, salinity and their combination (8 and 12 days) on chlorophyll content (mg/g dry weight) in pigeonpea leaves
A) 20 DAS

			8day* (1 day)**			12day *(1 day)**			8day *(8 day)**				12day* (8 day)**	
Genotype	C	W	S	W+S	Mean	W	S	Mean#	C	W	S	Mean	S	Mean#
ICPH 2431	2.95	2.37	3.00	1.84	2.54	2.18	2.89	2.67	2.96	2.50	2.94	2.80	2.92	2.94
UPAS 120	2.86	1.89	2.88	1.50	2.28	1.61	2.66	2.37	2.87	2.02	2.83	2.57	2.72	2.79
HO9 33	2.92	1.96	2.96	1.63	2.37	1.90	2.75	2.52	2.95	2.18	2.92	2.68	2.86	2.91
PARAS	2.94	2.24	2.99	1.77	2.49	2.15	2.83	2.64	2.95	2.35	2.92	2.74	2.89	2.92
Mean	2.92	2.11	2.96	1.69		1.96	2.78		2.93	2.26	2.90		2.85	
C.D. at 5%			T - 0.03, G- 0.03, TXG- 0.07				T- 0.04, G- 0.05, TXG- 0.08				T- 0.04, G- 0.05, TXG- 0.09		T- 0.04, G- 0.05, TXG- N.S.	

B) 40 DAS

	8day* (1 day)**					12day* (1day)**		8day *(8 day)**			12day *(8 day)**	
Genotype	C	W	S	W+S	Mean	S	Mean#	C	S	Mean	S	Mean#
ICPH 2431	3.01	1.87	3.02	1.82	2.43	2.85	2.93	3.02	3.00	3.01	2.95	2.99
UPAS 120	2.93	1.54	2.92	1.47	2.22	2.63	2.78	2.93	2.90	2.91	2.71	2.82
HO9 33	2.98	1.65	2.98	1.58	2.30	2.71	2.84	2.98	2.95	2.97	2.82	2.90
PARAS	3.00	1.79	3.00	1.74	2.38	2.80	2.90	2.99	2.97	2.98	2.90	2.95
Mean	2.98	1.71	2.98	1.66		2.75		2.98	2.95		2.85	
C.D. at 5%	T - 0.03, G- 0.03, TXG- 0.05					T- 0.03, G-0.04, TXG- 0.05		T- N.S., G- 0.07, TXG- N.S.			T- 0.05, G- 0.07, TXG-N.S.	

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

salinity treatment 1 and 8 DAR from treatment. No decline in percent survival was observed under salinity treatment also in 40 days old plants (Table 1 B). ICPH-2431 performed best under waterlogging and waterlogging + salinity treatments (8 days and 12 days) followed by PARAS, HO9-33 and UPAS-120 in 20 day as well as 40 day old plants . Kumutha *et al.,* (2008) reported in green gram that tolerant genotype MH96-1 did not show any mortality even after 8 days of waterlogging and recovery while susceptible genotype MH 1K- 24 showed more than 60% mortality during recovery after 8 days of waterlogging. The negative effects of salinity have been attributed to increase in Na^+ and Cl^- ions in different plants hence these ions produce the critical conditions that affect plant survival by intercepting different plant mechanisms. Although both Na^+ and Cl^- are the major ions which produce many physiological disorders in plants, Cl^- is the most dangerous (Tavakkoli *et al.*, 2010). Not surprisingly, for most genotypes tested, there were adverse interactions between waterlogging and salinity on plant survival. Carter *et al.,* (2006) reported that the regulation of ions uptake and production of organic solutes (i.e. methyl proline) is related to combined salt and flood stress tolerance in some wetland plants.

Chlorophyll content

Chlorophyll content decreased under waterlogging and salnity stress and maximum decline was observed with combined waterlogging and salinity treatment (Table 2 A and B). Waterlogging (8 days) treatment resulted in a decline of 20 to 34 % 1 DAR which further decreased to 16 to 30% 8 DAR in 20 day old plants. Twelve days waterlogging treatment resulted in 26 to 44 % decline 1 DAR however no survival was observed 8 DAR. Salinity showed positive results initially with 0.8 to 1.6 % increase in chlorophyll content 1 DAR from treatment but 0.7 to 1.4 % decrease was noticed 8 DAR from treatment. Twelve days salinity treatment resulted in 2 to 7% decline 1 DAR and 1 to 5 % 8 DAR. Waterlogging + salinity (8 days) showed maximum adverse effects with 38 to 47 % decrease 1 DAR. No survival was observed 8 DAR from 8 days and 1 and 8 DAR 12 days waterlogging + salinity treatment in 20 day old plants. Decline in chlorophyll content was more in 40 day old plants resulting in 38 to 48 % and 40 to 50 % decrease 1 DAR from waterlogging (8 day) and waterlogging + salinity (8 days) treatment respectively. Salinity did not show significant effects initially also in case of 40 day plants but 0.8 to 1.3 % decline was noticed 8 DAR from 8 days salinity treatments which further increased to 6 to 10 % 1 DAR and 2.4 to 7.5 % 8 DAR from 12 days salinity treatment. No survival was observed 8 DAR from waterlogging (8 days) and 1 and 8 DAR from 8 and 12 days waterlogging and waterlogging + salinity treatment in 40 day old plants. ICPH 2431 performed best in terms of chlorophyll content followed by PARAS and HO9 33. Kumutha *et al.,* (2009) reported that total chlorophyll content drastically decreased in pigeonpea genotypes. The decrease in chlorophyll content was 56% in Pusa 207 and 49% in ICP 301 after 6 days of waterlogging. Decline in chlorophyll content under waterlogging was also observed in wheat (Amri *et al.*, 2014) and sugarcane (Bajpai and Chandra, 2015). Zeng *et al.* (2013) reported that after 2 weeks of treatment, NaCl alone had no significant effect on the leaf chlorophyll content (SPAD value) for either of the barley variety (CM72 and Naso Nijo) grown in either (sandy loam-grown and vermiculite grown) growth media. However, waterlogging and combined waterlogging and salinity treatments caused a massive reduction in the chlorophyll content in both varieties. The combined waterlogging and salinity treatment was more detrimental compared with waerlogging alone in both varieties. The observed decline in chlorophyll content was genotype-specific, with chlorophyll content in tolerant CM72 plants being significantly higher than that in sensitive Naso Nijo plants for each treatment, except

Table 3: Effect of waterlogging, salinity and their combination (8 & 12 days) on chlorophyll fluorescence in pigeonpea
A) 20 DAS

	8day* (1 day)**					12day* (1 day)**			8day* (8 day)**				12day* (8 day)**	
Genotype	C	W	S	W+S	Mean	W	S	Mean#	C	W	S	Mean	S	Mean#
ICPH 2431	0.67	0.53	0.65	0.47	0.60	0.49	0.64	0.60	0.67	0.62	0.66	0.65	0.66	0.66
UPAS 120	0.65	0.44	0.63	0.38	0.51	0.42	0.61	0.56	0.65	0.52	0.64	0.60	0.62	0.64
HO9 33	0.65	0.46	0.63	0.39	0.50	0.43	0.61	0.56	0.65	0.54	0.64	0.61	0.63	0.64
PARAS	0.66	0.50	0.65	0.46	0.49	0.48	0.63	0.59	0.66	0.59	0.65	0.63	0.64	0.65
Mean	0.65	0.48	0.64	0.43		0.45	0.62		0.66	0.57	0.65		0.64	
C.D. at 5%	T- 0.01, G- 0.01, TXG- 0.02					T- 0.01, G- 0.01, TXG- 0.02				T- 0.01, G- 0.02, TXG- 0.03			T- 0.01, G- 0.02, TXG- N.S.	

B) 40 DAS

	8day* (1 day)**					12day *(1day)**		8day* (8 day)**			12day* (8 day)**	
Genotype	C	W	S	W+S	Mean	S	Mean#	C	S	Mean	S	Mean#
ICPH 2431	0.67	0.52	0.66	0.46	0.58	0.63	0.65	0.68	0.67	0.67	0.64	0.66
UPAS 120	0.65	0.39	0.63	0.34	0.50	0.59	0.62	0.66	0.64	0.65	0.60	0.63
HO9 33	0.66	0.46	0.64	0.40	0.54	0.60	0.63	0.66	0.64	0.65	0.61	0.64
PARAS	0.67	0.51	0.65	0.43	0.56	0.63	0.65	0.67	0.66	0.66	0.63	0.65
Mean	0.66	0.47	0.65	0.41		0.61		0.67	0.65		0.62	
C.D. at 5%	T- 0.01, G- 0.01, TXG- 0.03					T- 0.01, G- 0.01, TXG- N.S.		T- 0.02, G-N.S., TXG- N.S.			T- 0.01, G- 0.02, TXG- N.S.	

* duration of treatment, ** stage of sampling, # mean value were calculated with the respective control values

for the control.

Chlorophyll fluorescence

A 21 to 31 % decline was observed in chlorophyll fluorescence 1 DAR and 7 to 19 % 8 DAR from waterlogging (8 days) treatment which further increased to 25 to 36 % 1 DAR from 12 days waterlogging treatment in 20 day old plants however no plant survived 8 DAR. Salinity showed less adverse effects with a 1.7 to 2.9 % decline 1 DAR and 0.9 to 2.3 % decline 8 DAR from salinity (8 days) treatment. Under 12 days salinity treatment decline was 3.9 to 6.2 1 DAR and 1.5 to 4.9 % 8 DAR from treatment. Waterlogging + salinity treatment for 8 days resulted in 29 to 41 % decline 1 DAR from treatment. No survival was observed 8 DAR from 8 days and 1 and 8 DAR from 12 days waterlogging + salinity treatment in 20 day old plants. Forty day old plants showed more sensitivity to waterlogging and waterlogging + salinity stress resulting in 23 to 41 % and 32 to 48 % decline 1 DAR from waterlogging (8 day) and waterlogging + salinity (8 days) treatment. A 2.1 to 3.3 % decline was observed 1 DAR and 1.5 to 3 % decline was observed 8 DAR from salinity (8 days) treatment which further increased to 5.9 to 9.5% 1 DAR and 4.9 to 9.1% 8 DAR from salinity (12 days) treatment. No survival was observed 8 DAR from waterlogging (8 days) and 1 and 8 DAR from 8 and 12 days waterlogging and waterlogging + salinity treatment in 40 day old plants. Again ICPH 2431 performed best in terms of chlorophyll fluorescence followed by PARAS, HO9-33 and UPAS-120 in both the treatments waterlogging and waterlogging + salinity. A reduction in the maximum quantum yield of photosystem II (Fv/Fm) after the onset of waterlogging has been reported in some plant species (Smethurst and Shabala, 2003; Smethurst *et al.*, 2005). Cork oak (*Quercus variabilis*) and China wingnut (*Pterocarya stenoptera*) show a prominent decrease in maximum quantum efficiency (Fv/Fm) when subjected to waterlogging (Hua *et al.*, 2006). Zeng *et al.* (2013) reported the combined effect of waterlogging and salinity in barley genotypes (CM 72 and Naso Nijo). The maximum photochemical efficiency of PSII (chlorophyll fluorescence *F*v/*F*m value) was also significantly affected by waterlogging and combined waterlogging and salinity treatments. A substantial decline in *F*v/*F*m value was reported for both treatments. The chlorophyll fluorescence (*F*v/*F*m value) was not affected by NaCl alone. On averaging the two varieties, the effect of the combined waterlogging and salinity stress was much more severe than waterlogging alone.

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