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Screening of Pigeonpea [*Cajanus cajan* (L.) Millsp.] Genotypes for Waterlogging, Salinity and Combined Waterlogging & Salinity Tolerance

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ABSTRACT

Pigeonpea is very sensitive to waterlogging and salinity. These two stresses adversely affect the germination and early vegetative stages of pigeonpea as compared to mature plants. Being a rainy season crop, pigeonpea is invariably exposed to intermittent waterlogging conditions for different durations from germination to early vegetative growth stages. The objective of this study was to screen thirty pigeonpea [Cajanus cajan (L.) Millsp.] genotypes HO6-1, HO6-12, HO3-41, HO9-27, HO9-33, HO9-34, HO9-36, HO9-38, MANAK, PARAS, ICPH 2431, ICPH 2671, ASHA, MARUTI, ICPL 87051, ICP 5028, ICPL 20096, ICPL 87091, ICPL 20241, LRG 30, ICPL 20120, MAL 9, ICPL 20238, ICPL 20237, MAL 12, SIPS 2, SGBS 6, ICP 8857, UPAS 120 and ICP 7035 for waterlogging, salinity (60mM NaCl) and combined waterlogging plus salinity (30mM NaCl) tolerance. These treatments were resulted in visible yellowing and senescence of leaves, decreased plant survival, chlorophyll content and chlorophyll fluorescence. The genotypes ICPH 2431, PARAS, HO9-33, HO6-1, HO6-12, HO9-36 were found relatively tolerant while UPAS 120, SGBS 6, MAL-12, ICPL 20237, HO9-34, LRG 30 were relatively sensitive to waterlogging and salinity treatments.

Key words: *Pigeonpea, Waterlogging, Salinity, Saline Waterlogging, Plant Survival, Chlorophyll Fluorescence.*

INTRODUCTION

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is an important legume crop widely grown in many parts of Indian sub-continent. It is mainly used as human food, animal feed and an effective green manure crop[17]. It is adapted to a wide range of environments and cropping systems. Major abiotic stresses encountered by pigeonpea are waterlogging, salinity and drought. Pigeonpea is reported to be highly sensitive to waterlogging[13] and salinity[20]. Being a rainy season crop, pigeonpea is invariably exposed to intermittent waterlogging conditions for different durations from germination to early vegetative growth stages. According to Singh *et al.*[16] germination and early vegetative stages of pigeonpea are more sensitive to waterlogging stress as compared to mature plants.

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Soil salinity is another major abiotic stress that affects plant growth, development and yield by causing physiological and biochemical changes in plants[12]. Germination of seeds, one of the most critical phases of plant life, is greatly influenced by salinity[11]. Salinity together with waterlogging can cause deleterious effects in plants posing major threat to crop productivity[2]. These two abiotic stresses are related with each other as water logging results in rise of water table causing development of salinity in many parts of India[5] .Waterlogging and salinity stresses are important yield constraints in pigeonpea as water logging blocks oxygen supply to roots which hampers root permeability and salinity impairs seed germination, reduces nodule formation, retards plant development and finally reduces crop yield[17]. Therefore, the present study was carried out to screen the pigeonpea genotypes for waterlogging, salinity and combine waterlogging plus salinity tolerance.

MATERIAL AND METHODS

Thirty genotypes of pigeonpea selected for their comparative analysis were HO6-12, HO6-1, HO3-41, HO9-27, HO9-33, HO9-34, HO9-36, HO9-38, MANAK, PARAS, ICPH 2671, ICPH 2431, ASHA, MARUTI, ICPL 87051, ICP 5028, ICPL 20096, ICPL 87091, ICPL 20241, LRG 30, ICPL 20120, MAL 9, ICPL 20238, ICPL 20237, MAL 12, SIPS 2, SGBS 6, ICP 8857, UPAS 120 and ICP 7035. Fresh seeds were obtained from ICRISAT, Andhra Pradesh (India) and Pulses Section, Department of Genetics and Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar (India). The screening was done in the screen house conditions.

Surface sterilized seeds 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 three treatments were given to the plants, T1- waterlogging, T2-waterlogging plus salinity (30 mM NaCl) and T3- salinity (60 mM NaCl) and one set is taken as control. For treatments T1 and T2, the polythene bags were placed in cemented tanks (length 160 cm, breadth 125 cm and depth 65 cm) filled with water and NaCl solution (30 mM), 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 T3 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:

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

Leaf senescence: Scoring for leaf senescence was observed among the plants at eight days after drainage. The number of yellow leaves was counted in three plants of each genotype eight days after the removal of treatments.

Chlorophyll Content: Chlorophyll content was measured using SPAD chlorophyll meter from third fully expanded leaf of three healthy plants of each genotype just before the treatment and eight days after removal of treatment. To measure the chlorophyll content, the leaf was cleaned with tissue paper to remove the dust. The leaf was then inserted in the sensor of the SPAD chlorophyll meter and the reading, shown on the display was recorded. The data was expressed as SPAD units.

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 expended 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.

Figure: Visual effects of various treatments on pigeonpea plants

RESULTS AND DISCUSSION

Waterlogging occurs over a vast region of the world, adversely affecting about 10% of the global land area[14] and reducing crop yields by as much as 80% [15]. Under waterlogging condition, soil gas exchange is severely impeded, that results in a significant depletion of free oxygen (O_2)[1]. Excessive soil salinization is also a major ecological and agronomic problem throughout the world. When combined with waterlogging, salinity can cause even greater damage to plants, so having a major impact on agricultural production[2]. Only a very few crop species can tolerate the combination of salinity and waterlogging[3] and the physiological and molecular mechanisms conferring this tolerance remain elusive.

Survival percentage: Plants survival was adversely affected by waterlogging and salinity treatments (Table 1). Waterlogging treatment resulted in a 0 to 75% decline in percent survival with no mortality in genotype ICPH 2431 and 12.5% in PARAS, HO6-12, H09-33 while 75% in UPAS 120 and SGBS 6. Kumatha *et al.*[8] observed 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 in green grams. At ICRISAT, scientists reported that till 120 hours of submergence, high survival rates (78.7 to 98.6%) were observed in pigeonpea, which declined rapidly as the duration increased. After 192 hours of submergence, survival reduced to less than 40%.The effect of combined waterlogging & salinity (30mM NaCl) was more deleterious to plants and resulted in 50 to 100% mortality. The most sensitive genotype was SGBS 6 (100%). PARAS and ICPH 2431 showed 50% mortality. 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[21]. The salinity treatment of 60mM NaCl showed no deleterious effects and 100% survival was observed in all the genotypes.

Leaf senescence score: Leaf senescence score is an important visible symptom associated with waterlogging and salinity stress and is further exacerbated by the combined waterlogging and NaCl treatment. The increased in leaf senescence score was 14% to 400% after the various treatments (Table 2). After combine waterlogging & salinity treatments the plants were most affected (129-400%), followed by waterlogging (86-233%) and salinity (14-133%). The least.

Table 1: Effect of different treatments on percent survival of pigeonpea genotypes

Genotypes	Survival Percentage				
	Control	**WL**	**WL+30mMn NaCl**	**60mM NaCl**	**Mean**
HO6-12	100	88	13	100	75
HO6-1	100	63	13	100	69
HO3-41	100	75	25	100	75
HO9-27	100	63	25	100	72
HO9-33	100	88	38	100	81
HO9-34	100	38	25	100	66
HO9-36	100	75	38	100	78
HO9-38	100	63	25	100	72
MANAK	100	63	25	100	72
PARAS	100	88	50	100	84
ICPH 2671	100	63	25	100	72
ICPH 2431	100	100	50	100	88
ASHA	100	50	25	100	69
MARUTI	100	63	38	100	75
ICPL 87051	100	38	38	100	69
ICP 5028	100	50	25	100	69
ICPL 20096	100	50	38	100	72
ICPL 87091	100	63	25	100	72
ICPL 20241	100	63	38	100	75
LRG 30	100	50	13	100	66
ICPL 20120	100	63	50	100	78
MAL 9	100	63	25	100	72
ICPL 20238	100	63	38	100	75
ICPL 20237	100	38	25	100	66
MAL 12	100	38	13	100	63
SIPS 2	100	38	25	100	66
SGBS 6	100	25	---	100	56
ICP 8857	100	38	38	100	69
UPAS 120	100	25	13	100	59
ICP 7035	100	63	38	100	75
Mean	100	58	28	100	
C.D. at 5% level of significance	Genotypes = NS Treatments = 7.03 Genotypes x Treatments = NS				

Table 2: Effect of different treatments on leaf senescence score of pigeonpea genotypes

Genotypes	Leaf senescence score				
	Control	**WL**	**WL+30mM NaCl**	**60mM NaCl**	**Mean**
HO6-12	1.8	3.5	5.0	2.5	3.2
HO6-1	1.5	3.3	6.0	2.5	3.3
HO3-41	1.5	3.3	5.5	2.5	3.2
HO9-27	1.8	3.0	5.5	2.8	3.3
HO9-33	1.5	2.8	5.5	2.3	3.0
HO9-34	1.5	4.0	6.0	2.8	3.6
HO9-36	1.8	3.5	5.5	2.0	3.2
HO9-38	1.8	3.8	6.0	2.5	3.5
MANAK	1.5	3.5	5.8	2.5	3.3
PARAS	1.8	3.3	4.0	2.3	2.8
ICPH 2671	1.8	3.8	6.0	2.5	3.5
ICPH 2431	1.8	3.0	4.5	2.3	2.9
ASHA	1.8	4.0	5.8	2.5	3.5
MARUTI	1.8	4.0	5.5	2.3	3.4
ICPL 87051	1.8	4.0	5.3	2.5	3.4
ICP 5028	1.5	4.5	5.8	2.5	3.6
ICPL 20096	1.8	3.3	5.5	2.8	3.3
ICPL 87091	1.5	3.3	5.5	2.5	3.2
ICPL 20241	1.8	3.8	5.5	2.8	3.4
LRG 30	1.5	4.0	6.0	2.5	3.5
ICPL 20120	1.5	3.8	5.0	2.5	3.2
MAL 9	1.5	3.5	5.5	2.8	3.3
ICPL 20238	1.8	3.8	5.0	2.8	3.3
ICPL 20237	1.5	4.5	6.0	2.8	3.7
MAL 12	1.8	4.3	6.0	2.5	3.6
SIPS 2	1.8	3.8	6.0	2.5	3.5
SGBS 6	1.5	5.0	---	3.3	4.4
ICP 8857	1.8	4.0	6.3	2.5	3.6
UPAS 120	1.5	4.5	7.5	3.5	4.3
ICP 7035	1.5	3.8	5.5	2.8	3.4
Mean	1.6	3.7	5.7	2.6	
C.D. at 5% level of significance	*Genotypes* = *0.36* *Treatments* = *0.13* *Genotypes* x *Treatments* = *0.72*				

Table 3: Effect of different treatments on total chlorophyll content (SPAD value) of pigeonpea genotypes

Genotypes	Total chlorophyll content (SPAD value)				
	Control	**WL**	**WL+30mM NaCl**	**60mM NaCl**	**Mean**
HO6-12	37.23	28.38	22.50	32.38	30.12
HO6-1	35.85	27.13	21.20	31.88	29.01
HO3-41	35.73	27.05	22.88	31.60	29.31
HO9-27	36.95	28.28	24.40	32.78	30.60
HO9-33	38.28	29.60	26.98	33.80	32.16
HO9-34	36.10	26.65	23.28	31.13	29.29
HO9-36	36.50	27.83	23.00	31.83	29.79
HO9-38	37.40	28.63	23.78	33.03	30.71
MANAK	36.45	28.58	23.78	31.20	30.00
PARAS	37.28	29.63	24.98	33.13	31.25
ICPH 2671	37.88	28.53	24.80	32.63	30.96
ICPH 2431	37.68	29.55	25.50	33.15	31.47
ASHA	35.65	25.60	21.08	30.95	28.32
MARUTI	36.65	27.70	21.60	32.00	29.49
ICPL 87051	37.43	27.20	24.68	32.60	30.48
ICP 5028	35.80	27.63	23.20	30.63	29.31
ICPL 20096	37.93	29.30	23.90	32.90	31.01
ICPL 87091	35.20	26.33	22.58	30.15	28.56
ICPL 20241	35.93	27.30	21.58	30.73	28.88
LRG 30	33.73	25.60	22.30	28.95	27.64
ICPL 20120	33.38	25.40	19.18	28.35	26.58
MAL 9	33.73	24.98	19.88	29.20	26.94
ICPL 20238	34.88	26.00	21.20	30.10	28.04
ICPL 20237	33.78	25.70	19.50	28.78	26.94
MAL 12	34.55	24.68	22.20	30.10	27.88
SIPS 2	34.68	25.43	22.40	30.35	28.21
SGBS 6	32.15	21.40	---	26.90	20.11
ICP 8857	34.50	25.70	21.90	29.68	27.94
UPAS 120	34.95	24.50	18.60	29.20	26.81
ICP 7035	35.63	25.48	24.20	31.25	29.14
Mean	35.79	26.86	21.90	31.04	
C.D. at 5% level of significance	*Genotypes* = *1.04* *Treatments* = *0.38* *Genotypes* x *Treatments* = *2.08*				

Table 4: Effect of different treatments on chlorophyll fluorescence score (Fv/Fm) of pigeonpea genotypes

Genotypes	Chlorophyll fluorescence score (Fv/Fm)				
	Control	**WL**	**WL+30mM NaCl**	**60mM NaCl**	**Mean**
HO6-12	0.675	0.599	0.508	0.656	0.609
HO6-1	0.680	0.595	0.504	0.659	0.610
HO3-41	0.675	0.587	0.535	0.656	0.613
HO9-27	0.668	0.580	0.513	0.648	0.602
HO9-33	0.676	0.595	0.557	0.654	0.620
HO9-34	0.674	0.582	0.533	0.654	0.611
HO9-36	0.677	0.591	0.543	0.656	0.617
HO9-38	0.682	0.599	0.521	0.662	0.616
MANAK	0.678	0.593	0.534	0.658	0.616
PARAS	0.680	0.607	0.556	0.662	0.626
ICPH 2671	0.668	0.594	0.508	0.647	0.604
ICPH 2431	0.674	0.600	0.547	0.656	0.619
ASHA	0.658	0.567	0.491	0.635	0.588
MARUTI	0.659	0.579	0.528	0.639	0.601
ICPL 87051	0.668	0.572	0.512	0.645	0.599
ICP 5028	0.668	0.588	0.496	0.646	0.599
ICPL 20096	0.659	0.576	0.502	0.636	0.593
ICPL 87091	0.665	0.589	0.495	0.642	0.598
ICPL 20241	0.664	0.571	0.497	0.641	0.593
LRG 30	0.669	0.587	0.484	0.646	0.597
ICPL 20120	0.662	0.580	0.498	0.637	0.594
MAL 9	0.665	0.557	0.495	0.645	0.590
ICPL 20238	0.663	0.582	0.499	0.641	0.596
ICPL 20237	0.662	0.556	0.534	0.636	0.597
MAL 12	0.665	0.565	0.496	0.642	0.592
SIPS 2	0.665	0.579	0.521	0.642	0.601
SGBS 6	0.655	0.548	---	0.628	0.458
ICP 8857	0.663	0.578	0.501	0.638	0.595
UPAS 120	0.658	0.548	0.465	0.632	0.576
ICP 7035	0.663	0.576	0.492	0.641	0.593
Mean	0.668	0.581	0.495	0.646	
C.D. at 5% level of significance	*Genotypes* = *0.012* *Treatments* = *0.004* *Genotypes* x *Treatments* = *0.024*				

Affected genotypes were PARAS, ICPH 2431, HO6-12, HO9- 27 and most affected was UPAS 120 (400%). However, no plant was survived in SGBS 6 genotype with combined treatment of waterlogging and salinity. Kumutha *et al.*[9] also reported increased leaf senescence with waterlogging in pigeonpea. They found that waterlogging resulted in yellowing and ultimately drying of leaves and death of shoots/branches, more in Pusa 207 (sensitive) than ICP 301 (tolerant). One of the major factors inducing leaf senescence is the decrease of chlorophyll content under saline conditions[4]. Leaf senescence is also correlated with increased membrane permeability at high salt concentration[7]. Zeng *et al.*[22] reported drastic increased in clorotic and necrotic leaves in barley genotypes. The effect was more on the sensitive genotypes Naso Nijo leaves. The earlier studies refer specifically to increased leaf senescence under conditions of waterlogging (hypoxia) and salinity[2].

Chlorophyll content and Chlorophyll fluorescence: The waterlogging and salinity induced decrease in plant survival and leaf senescence was accompanied by a

considerable decrease in chlorophyll content (SPAD value) and chlorophyll fluorescence (Table 3 & 4). The treatments of waterlogging and combine waterlogging & salinity resulted in a significant decrease in SPAD value and chlorophyll fluorescence. The effects was less on salinity treated plants. The genotypes PARAS, ICPH 2431, MANAK, HO6-1, HO9-27, HO9-33, ICPH 2671were performed better while SGBS 6, UPAS 120, MAL 12. ICPL 20237 have very poor performance. Kumutha *et al.*[9] 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. Collaku and Harrison[6] also reported a decrease in chlorophyll content in waterlogged wheat plants. Zeng *et al.*[22] 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). However, WL and NaCl/WL treatments caused a massive reduction in the chlorophyll content in both varieties.

The maximum photochemical efficiency of PSII (chlorophyll fluorescence Fv/Fm value) was also significantly affected by WL and NaCl/WL treatments[22]. A reduction in the maximum quantum yield of photosystem II (Fv/Fm) after the onset of waterlogging has been reported in some plant species[18,19]. Cork oak (*Quercus variabilis*) and China wingnut (*Pterocarya stenoptera*) show a prominent decrease in maximum quantum efficiency (Fv/Fm) when subjected to waterlogging[8]. The maximum photochemical efficiency of PSII (chlorophyll fluorescence *F*v/*F*m value) was also significantly affected by WL and NaCl/WL treatments[22].

CONCLUSION

Among the thirty genotypes ICPH 2431, PARAS, HO9-33, HO6-1, HO6-12, HO9-36 were found relatively tolerant while UPAS 120, SGBS 6, MAL-12, ICPL 20237, HO9-34, LRG 30 were relatively sensitive to waterlogging and salinity stress. The tolerant genotypes can be further used by plant breeders to generate higher yielding varieties under waterlogging and salinity stresses.

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