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Independent and Combined Effects of Waterlogging and Salinity on Morpho-Physiological Parameters in Pigeonpea Genotypes at Early Growth Stages

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ABSTRACT: Occurrence of combined salinity and waterlogging stress is increasing throughout the world. According to the FAO, about 60-80 million hectares of land are affected to some extent by combined waterlogging and salinity stress. Waterlogging and salinity impair and effects the growth and development of pigoen pea, and reduces its productivity significantly. When water and salt exceed their optimum levels, it leads to waterlogging and salinity stress, respectively. The combination of waterlogging and salinity exacerbates the effects of salinity and vice versa. In this study, we examined the impact of waterlogging (W), salinity (S), and their combination (W+S) on plant survival percentage, shoot dry weight, total plant biomass, and total chlorophyll content in pigeonpea. The pot experiment was conducted during the rainy season of 2018-19, involving three genotypes (Bahar, UPAS-120 and Asha). Stress was imposed 40 days after sowing, and observations were taken at 6th and 10th day after the imposition of stress. Waterlogging + salinity was found to be more deleterious to plants, although the effect was less pronounced with salinity (30 mM NaCl) treatment alone. In terms of survival percentage, shoot dry weight, total plant biomass, and total chlorophyll content, the Bahar genotype demonstrated relatively higher tolerance compared to other genotypes in all treatments (waterlogging, salinity, and salinity + waterlogging). The order of performance among genotypes was found to be Bahar, UPAS-120, and Asha. These findings can contribute to the development of stress-tolerant pigeonpea genotypes or selection of a relatively tolerant genotype aimed at mitigating the negative effects of waterlogging and salinity stresses on crop productivity.

Keywords: Pigeonpea, *Salinity*, Waterlogging, Plant biomass, Chlorophyll content.

INTRODUCTION

Pigeonpea (*Cajanus cajan* (L.) Millspaugh) stands as a pivotal food legume crop, prominently cultivated in tropical and sub-tropical regions. In the Indian agricultural landscape, it holds the second position, following chickpea, as a vital pulse crop. Consumed widely in the form of de-hulled split peas, known as 'dal,' pigeonpea also finds culinary application as a fresh vegetable, particularly in regions such as Gujarat, Maharashtra, and Karnataka. Notably, tribal areas across various states integrate pigeonpea as a staple green vegetable in their diets (Saxena and Nadarajan 2010).

However, the production of pigeonpea faces significant challenges due to environmental stresses, with salinity and waterlogging emerging as major hindrances worldwide (Kumar *et al*., 2011). Salinity, a prevalent concern, affects over 800 million hectares globally, accounting for more than 6% of the total land area (Singh, 2022). Elevated levels of soil salinity, characterized by an excess of ions, particularly Na^+ and Cl^-, impair osmotic potentials and water availability to plant roots (Djanaguiraman and Prasad 2012). It induces ion toxicity, disrupting enzyme structures, damaging cell organelles, and interfering with overall cell metabolism (Maathuis and Amtmann 1999). Concurrently, waterlogging, affecting approximately 10% of the world's land area, possess a severe threat to crop yields, causing reductions of up to 80% (Shabala, 2011). In waterlogged conditions, soil gas exchange is impeded, leading to oxygen depletion and carbon dioxide accumulation, triggering hypoxia stress and a shift from aerobic to anaerobic metabolism in roots (Setter and Waters 2003). Notably, the combination of salinity and waterlogging stress, though less explored, are rise globally due to factors such as intensive irrigation, rising saline water tables, and seawater intrusion. This combined stress scenario exacerbates the challenges faced by plants, particularly in agricultural production systems (Smedema and Shiati 2002; Carter *et al*., 2006). Only a limited number of crop species exhibit tolerance to the dual stress of salinity and waterlogging, and the underlying physiological and molecular mechanisms of this tolerance remain elusive (Bennett *et al.*, 2014). The detrimental effects of waterlogging and salinization on plant growth are well-established. Waterlogging hampers growth by impeding soil aeration around the root zone, while salinization affects crops by increasing the osmotic potential of the soil solution. When occurring simultaneously, these processes lead to significant yield reductions, with the plant roots becoming shallow due to waterlogging and salts accumulating in the soil profile, rendering the land unsuitable for agriculture. In light of these challenges, understanding the interactive effects of salinity and waterlogging on pigeonpea becomes imperative for devising resilient agricultural strategies and enhancing global food security. This research endeavors to contribute to this understanding by investigating the combined impact of salinity and waterlogging on key physiological parameters, such as shoot dry weight and chlorophyll content, in pigeonpea genotypes.

MATERIALS AND METHODS

In the present study, crops were grown during the rainy seasons of 2018-19, and the results represent the average of this one-year data. Three genotypes, namely Bahar, UPAS-120, and Asha, were chosen for this experiment. All the genotypes were raised in plastic pots filled with 10 kg of soil and FYM manure mixture (3:1, v/v), and NPK was applied at a rate of 20:60:20 kg/ha. The treatments were as follows: T1 - Control, T2 - Waterlogging (6 days), T3 - NaCl 30 mM (6 days), T4 - Waterlogging (10 days), T5 - NaCl 30 mM (10 days), T6 - Waterlogging (6 days) + 30 mM NaCl (6 days), T7 - Waterlogging (10 days) + 30 mM NaCl (10 days). The treatments were imposed forty days after sowing. Plastic tanks were filled up to 4 cm above the height of pots with water, T2 and T4 were used for waterlogging treatment in which pots with plants were placed for 6 days and 10 days respectively, T6 and T7 were waterlogging (W) + Salinity (S) treatments with plants placed for 6 days and 10 days, respectively. Treatment T3 and T5 were treated with 30 mM NaCl solutions 40 days after sowing for 6 and 10 days, respectively. Tanks T2 and T6 were drained after 6 days of treatment, whereas treatments T4 and T7 were drained after 10 days. Pot T1 served as the control and was watered at appropriate intervals. The next day after completion of treatment duration observations were taken for survival percentage, shoot dry weight, plant total biomass, and total chlorophyll content as per procedure of Arnon (1949).

RESULT AND DISCUSSION

Morphological parameters: The genotypes tested were Bahar, UPAS-120, and Asha. Stress conditions included waterlogging (W), salinity (S), and their combination (W+S). The duration of stress imposition was upto 6 days and 10 days. After 6 days of stress imposition, all genotypes showed 100% survival under all the treatment conditions, However, at the 10 days under the waterlogging condition (W), the survival percentage was 67% for Bahar and UPAS-120, and 39 % for Asha. Under the combined stress of salinity and waterlogging (W+S), survival dropped to 33% for Bahar, 28% for UPAS-120, and 22% for Asha (Table 1). Overall, the results indicate that the genotypes exhibited varying degrees of tolerance to waterlogging, salinity, and their combination. Bahar showed better survival rates compared to UPAS-120 and Asha under most stress conditions and durations. Duhan *et al*, (2018) reported the similar result under similar stress conditions. Plant height data is presented in Table 2, the mean plant height for all genotypes was highest under control conditions (C). Under waterlogging (W), salinity (S), and combined stress (W+S), the plant height was lower compared to the control at 6 days of stress imposition, and after 10 days of stress imposition, also a same trend was observed. Plant height was highest under control conditions (C) and lowest under combined salinity and waterlogging stress (W+S) for all genotypes. The statistical analysis suggests that both genotype (G) and treatment (T) significantly influenced plant height. The interaction between genotype and treatment (G×T) also had a significant effect. The results indicate that the genotypes responded differently to waterlogging, salinity, and their combination, resulting in variations in plant height. The Table 3 presents the shoot dry weight data of pigeonpea genotypes under different stress conditions, the mean shoot dry weight was highest under control conditions (C) for all genotypes. Under waterlogging (W), salinity (S), and combined stress (W+S), the shoot dry weights were lower compared to the control. After 10 days of stress imposition, a similar trend was observed. Shoot dry weights were highest under control conditions (C) and lowest under combined salinity and waterlogging stress (W+S) for all genotypes. The statistical analysis suggests that both genotype (G) and treatment (T) significantly influenced shoot dry weight. The interaction between genotype and treatment (G×T) also had a significant effect. The results indicate that the genotypes responded differently to waterlogging, salinity, and their combination, resulting in variations in shoot dry weight. These results are consistent with previously published by Giaveno *et al*. (2007) in maize crop. Total plant biomass (g plant^{-1}) of pigeonpea genotypes under different stress conditions was highest under control conditions (C) for all genotypes compared to waterlogging (W), salinity (S), and combined stress (W+S), the total plant biomass decreased compared to the control (Table 4). after 10 days of stress imposition, a similar trend was observed. Total plant biomass was highest under control conditions (C) and lowest under combined salinity+ waterlogging (W+S) stress for all genotypes. The total plant biomass reduction was noticed under combined stress in Bahar genotypes it was 23.91%, for UPAS-120 29.79% and highest was in Asha genotypes 38.37% among all the genotypes studied. Bahar showed minimum Plant Biomass reduction, and highest reduction percent was found in Asha genotypes. The statistical analysis suggests that both genotype (G) and treatment (T) significantly influenced total plant biomass.The results indicate that the genotypes responded differently to waterlogging, salinity, and their combination, resulting in variations in total plant biomass.

Table 1: Effect of waterlogging, salinity and their combinations on survival percent (%) of pigeonpea genotypes after 6 and 10 days of stress imposition during 2018-2019.

Genotype	Duration of Stress							
	6 days*				10 days*			
	C	W	S	W+S	C	W	S	W+S
Bahar	100	100	100	100	100	67	100	33
UPAS-120	100	100	100	100	100	39	100	28
Asha	100	100	100	100	100	39	100	22

Stress was imposed after 40 days of sowing
*Stage of sampling, C= Control, W= waterlogging, S= salinity, S+W= salinity+ waterlogging

Table 2: Effect of waterlogging, salinity and their combinations on plant height (cm) of pigeonpea genotypes after 6 and 10 days of stress imposition during 2018-2019.

Genotype	Duration of Stress									Mean
	6 day*				Mean	10 day*				
	C	W	S	W+S		C	W	S	W+S	
Bahar	42.67	36.50	37.00	35.33	37.87	43.00	36.50	38.00	35.67	38.92
UPAS-120	45.00	36.67	42.23	34.83	39.76	45.67	36.67	43.00	35.00	40.34
Asha	42.00	28.67	41.67	28.00	38.08	42.67	30.00	42.24	29.67	36.16
Mean	43.22	34.05	40.30	32.72		43.78	34.72	41.11	33.45	
	SEm±		C.D (5%)			SEm±		C.D (5%)		
Genotype (G)	0.56		1.68			0.70		2.10		
Treatment (T)	0.65		1.95			0.81		2.44		
G×T	1.13		3.39			1.41		4.24		

Stress was imposed after 40 days of sowing
*Stage of sampling, C= Control, W= waterlogging, S= salinity, S+W= salinity + waterlogging

Table 3: Effect of waterlogging, salinity and their combinations on shoot dry weight (g plant^{-1}) of pigeonpea genotypes after 6 and 10 days of stress imposition during 2018-19.

Genotype	Duration of Stress									Mean
	6 days*				Mean	10 days*				
	C	W	S	W+S		C	W	S	W+S	
Bahar	0.983	0.752	0.879	0.629	**0.811**	1.282	0.765	0.893	0.633	**0.893**
UPAS-120	0.855	0.597	0.758	0.515	**0.681**	0.873	0.599	0.773	0.519	**0.691**
Asha	1.114	0.458	0.632	0.211	**0.619**	1.186	0.456	0.674	0.282	**0.649**
Mean	**0.984**	**0.602**	**0.756**	**0.472**		**1.114**	**0.607**	**0.780**	**0.478**	
	SEm±		**C.D at 5%**			**SEm±**		**C.D at 5%**		
Genotype (G)	**0.042**		**0.089**			**0.037**		**0.108**		
Treatment (T)	**0.050**		**0.103**			**0.042**		**0.125**		
G×T	**0.086**		**0.178**			**0.073**		**0.216**		

Stress was imposed after 40 days of sowing

*Stage of sampling, C= Control, W= waterlogging, S= salinity, S+W= salinity + waterlogging

Table 4: Effect of waterlogging, salinity and their combinations on Total Plant biomass (g plant^{-1}) of pigeonpea genotypes after 6 and 10 days of stress imposition during 2018-19.

Genotype	Duration of Stress									Mean
	6 days*				Mean	10 days*				
	C	W	S	W+S		C	W	S	W+S	
Bahar	1.28	0.78	0.91	0.69	**0.92**	1.46	0.78	0.92	0.70	**0.96**
UPAS-120	0.94	0.69	0.89	0.66	**0.79**	0.97	0.67	0.90	0.76	**0.81**
Asha	1.27	0.69	0.91	0.52	**0.85**	1.28	0.71	0.92	0.53	**0.86**
Mean	**1.16**	**0.72**	**0.90**	**0.63**		**1.24**	**0.73**	**0.91**	**0.63**	
	SEm±		**C.D at 5%**			**SEm±**		**C.D at 5%**		
Genotype (G)	**0.02**		**0.07**			**0.01**		**0.02**		
Treatment (T)	**0.03**		**0.09**			**0.01**		**0.03**		
G×T	**0.05**		**0.15**			**0.02**		**0.04**		

Stress was imposed after 40 days of sowing
*Stage of sampling, C= Control, W= waterlogging, S= salinity, S+W= salinity+ waterlogging

Table 5: Effect of waterlogging, salinity and their combinations on total chlorophyll content (mg g^{-1} fresh weight) of pigeonpea genotypes, 6 and 10 days after stress imposition during 2018-2019.

Genotype	Duration of Stress									
	6 days*				Mean	10 days*				Mean
	C	W	S	W+S		C	W	S	W+S	
Bahar	2.48	1.57	2.48	1.47	**2.00**	2.45	1.49	2.45	1.28	**1.93**
UPAS-120	2.43	1.56	2.45	1.25	**1.92**	2.42	1.32	2.93	1.08	**1.81**
ASHA	2.51	1.28	2.43	1.14	**1.84**	2.45	1.11	2.44	0.91	**1.73**
Mean	**2.46**	**1.47**	**2.45**	**1.24**		**2.44**	**1.31**	**2.44**	**1.09**	**1.73**
	SEm±		**C.D at 5%**			**SEm±**		**C.D at 5%**		
Genotype (G)	**0.02**		**0.06**			**0.02**		**0.05**		
Treatment (T)	**0.07**		**0.02**			**0.02**		**0.06**		
G×T	**0.04**		**0.12**			**0.03**		**0.10**		

Stress was imposed after 40 days of sowing*Stage of sampling, C= Control, W= waterlogging, S= salinity, S+W= Salinity + Waterlogging

Physiological parameter: Chlorophyll is an important component of photosynthesis and is imperative for plant physiological processes (Gu *et al*, 2016). Table 5 showed the total chlorophyll content (mg g^{-1} fresh weight) of pigeonpea genotypes under different stress conditions. After 6 days of stress imposition, the mean total chlorophyll content was highest under control conditions (C) for all genotypes. Under waterlogging (W), salinity (S), and combined stress (W+S), the total chlorophyll content decreased compared to the control. After 10 days of stress imposition, a similar trend was observed. These finding are consistent with the results of previous studies by Giaveno *et al*. (2007), Zeng *et al*. (2013). Total chlorophyll content was highest under control conditions (C) and lowest under combined salinity and waterlogging stress (W+S) for all genotypes. Bahar performed best in the tern of total chlorophyll content followed by UPAS-120 and Asha, the similar result reported by the wheat crop under the waterlogging condition by (Amri *et al*. 2014) and sugarcane (Bajpai and Chandra, 2015). Zeng *et al*. (2013) found that under the salinity stress after the two week of treatment no significant effect on leaf chlorophyll content in barley plants was observed. The combined stress found that more detrimental compared with waterlogging alone. The statistical analysis suggests that both genotype (G) and treatment (T) significantly influenced total chlorophyll content. The interaction between genotype and treatment (G×T) also had a significant effect. The results indicate that the genotypes responded differently to waterlogging, salinity, and their combination, resulting in variations in total chlorophyll content.

CONCLUSIONS

In conclusion, the study investigated the effects of waterlogging, salinity, and their combination on morpho-physiological parameter revealed that the genotypes exhibited varying degrees of tolerance to the imposed stresses. Survival percentages under combined waterlogging (W)+ Salinity(S) was more deleterious to pigeonpea compared to individual stresses. With respect to morphological parameters, plant height, shoot dry weight and total plant biomass were reduced under stress conditions compared to the control. The combined stress of salinity and waterlogging had the most detrimental effect on plant height, shoot dry weight and total plant biomass. Further, total chlorophyll content, was also significantly reduced under stress conditions compared to control. Among individual stress given alone, waterlogging stress was found to be more detrimental as compared to 30 mM NaCl salinity for recorded observations. Overall, Bahar genotype showed comparatively higher level of tolerance under the stress conditions, followed by UPAS-120 and Asha respectively. The findings can contribute to the development of stress-tolerant pigeonpea varieties and to select appropriate genotypes aimed at mitigating the negative effects of these stresses on crop productivity.

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