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Tolerance Mechanism of Some Sugarcane Genotypes under Flood Stress

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ABSTRACT

A pot study was conducted in an artificially created flood in a concrete water tank at Bangladesh Sugarcane Research Institute during the cropping seasons of 2013-2014 to screen flood tolerant genotypes. Biochemical analysis of juice for determination of brix, pol, purity and reducing sugar were done. Morphological observations on adventitious roots, growth rate and senesced leaves, green leaves of seven sugarcane genotypes viz., Isd 20, Isd 34, Isd 37, Isd 38, Isd 39, Isd 40 and I 25-04 were done under flood stress conditions. The genotype Isd 34 showed the highest tolerance to flooding for 30, 60 and 90 days under 30 cm depth above pot soil floods. The variety had >40% green leaf after 120 days stress periods. Genotypes Isd 34 and Isd 39 showed tolerant reactions following 30, 60 and 90 days flood stress periods with > 33% green leaf after 120 days. Genotype Isd 34 produced the highest adventitious root (AR) (842.0 g/plant) followed by Isd 38 (836.0 g/plant). The genotypes Isd 38 and Isd 39 showed highest growth rate. Isd 38 and Isd 34 showed highest brix percent (20.55, 20.35) and pol percent (14.48, 14.34) followed by Isd 20 (brix 19.05% and pol 13.78%). Results of this experiment indicated that genotypes clone Isd 34 and Isd 38 performed better under flood stress conditions than other genotypes for their better survival mechanism under stress condition.

Key words: Sugarcane, morphological observations, biochemical analysis, anatomical view of adventitious root

INTRODUCTION

Among abiotic stresses, flood is an important stress for sugarcane cultivation in Bangladesh. It is because of increased cultivation of sugarcane in low lying char areas prone to periodic inundation by flood water (Begum *et al.*, 2013). The effect of excess water stress from temporary or continuous flooding has been studied extensively (Scott *et al.*, 1989; Jackson *et al.*, 1978). Sugarcane root density is greatest near the soil surface with 60% in the 0 to 30 cm depth, but roots may penetrate to 180 cm in well- drained soils (Gascho and Shih, 1983; Paz-Vergara *et al.*, 1980). Flooding tolerance depends on physiological and anatomical changes of the genotypes. Plants develop a suite of anatomical, morphological and physiological responses in order to deal with partial submergence imposed by flooding (Colmer and Voesenek, 2009; Striker *et al.*, 2005). The most common anatomical response is the generation of aerenchyma in tissues

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(Seago *et al.*, 2005), which facilitates the transport of oxygen from shoots to roots (Colmer, 2003). At morphological level, usual responses to flooding include adventitious rooting and increases in plant height and consequently, in the proportion of biomass above water level (Naidoo and Mundree, 1993; Grimoldi *et al.*, 1999). This also helps to facilitate the oxygenation of submerged tissues through the aerenchyma tissue (Colmer, 2003) and at physiological level, flooding modifies water relations and plants carbon fixation. Closing of stomata, with or without leaf dehydration, reduction of transpiration and inhibition of photosynthesis, are responses that can occur in hours or days, depending on the tolerance to flooding of each plant species (Striker *et al.*, 2005; Insausti *et al.*, 2001; Mollard *et al.*, 2008; Mollard *et al.*, 2010). The following sections show the main plant responses at those levels associated with tolerance to flooding.

One morphological change in sugarcane roots growing under high water tables is a greater proportion of fibrous to thick roots in the soil layer above the water table (Eavis, 1972; Webster and Eavis, 1972). The reason is probably an adaptation to lower O_2 levels. A thin root has a smaller path-length for O_2 diffusion to respiring tissue than a thicker root (Eavis, 1972). Presence of root aerenchyma is a key requisite for sustained root activity in flooded soil. The roots of all of the 40 sugarcane genotypes examined contained aerenchyma (Ray *et al.*, 1996; Heyden *et al.*, 1998). In species that are flood tolerant, aerenchyma formation is usually constitutive, meaning that it requires no external stimulus, such as flood (Drew, 1997). Glaze *et al.* (2002) grew nine sugarcane cultivars under 15 and 38 cm water table depths. They reported a mean yield reduction of 8.3% at the 15 cm water table, but two cultivars had similar yields at both water tables, and the yield of one cultivar was reduced by 25% at the 15 cm water table. Generally, sugarcane is not considered a flood tolerant species, but when it exposed to flood sugarcane produces adventitious roots which contains aerenchyma which helps sugarcane genotypes to survive under flood stress. In previous studies, germination and early seedling growth stages were found most susceptible to flood (Miah and Rahman, 2002). In Bangladesh floods occur at millable cane stage when they grown up much to fight against stress. Mainly this experiment was conducted to observe the mechanism of tolerance of some genotypes under flood stress condition.

MATERIALS AND METHODS

An experiment was carried out to observe mechanism of tolerance under flood stress during the cropping seasons of 2013-2014. Sugarcane genotypes Isd 20, Isd 34, Isd 37, Isd 38, Isd 39, Isd 40 and I 25-04 were grown in plastic pots (2 pots per clone). One pre-germinated sett cutting was transplanted in each pot. Irrigations and other cultural practices were done as and when required to all plant in pot for natural growth. Six months after transplanting two pots of each genotype were placed in a concrete tank and inundated in running water (30 cm deep above pot soil), while the remaining three pots per clone were kept as non-flooded controls. Green and dry leaves counts were taken after 60, 90 and 120 days of inundation. Data on fresh and dry weight of adventitious roots as well as volume of adventitious roots (ARs) were taken at harvest. ARs were collected and taken in paper bags of known weight and oven dried at 85°C until constant weight. Sections of ARs were made with a sharpe blade and viewed under microscope and photographed. Tolerance rating scale was recorded on greenness of leaves and other factors recorded. Data were recorded on growth rate at 60, 90, 120 days floods. Laboratory analysis of cane juice was done after 11 months of growth. The cane samples were crushed in a three-roller power crusher. Soluble solids (Brix %) was determined by brix hydrometer standardized at 20°C and Horne's dry lead method was used for sucrose determination using an automatic polarimeter (Bellingham and Stanley ADP-220®). Juice purity was calculated as the ratio of the sucrose content and corrected brix reading. Reducing sugars were determined by the method described in Queensland Laboratory Manual [Bureau Sugar Experiment Stations (BSES, 1970).

The collected data were compiled and analyzed statistically using the analysis of variance (ANOVA) technique with the help of a computer package program Statistix 10 and the mean differences were compared by least significance difference test at 5% level of probability.

RESULTS AND DISCUSSION

Morphological observations in different parameter

Green and dry leaves

Significant differences were observed on dry leaf, green leaf and growth rate by different genotypes Table 1. Isd 34 produced the highest number of green leaves (44.07%) followed by Isd 38 (40.0%). Highest growth rate was recorded in Isd 38 (1.290 cm day^{-1}) followed by Isd 39 (1.2750 cm day^{-1}). Dry leaf, green leaf and growth rate were also affected significantly due to flood and control conditions. Dry and green leaves were affected significantly and growth rate were unaffected due to different days after initiation on stress condition. Interaction of factor A (variety) and factor B (Flood, Control), factor A and factor C (Different days after initiation of flood), factor B and factor C, factor A, factor B and factor C has significant effect on dry leaf, green leaf and growth rate. All the genotypes under flood condition at different stress period produced higher no. of green leaf, and showed higher growth rate than in control condition except in I 25-04 which are in agreement with Tetsushi and Karim (2007) who found that plant height of the flooded plants was noticeably higher than that of the control plants. It is possible because sugarcane has constitutive aerenchyma. For this reason when it falls under stress it can easily survive by using oxyzen which is preserved by aerenchyma cell (Begum *et al.*, 2013). Aerenchyma formation in the root cortex is the most studied plastic response to flooding (Seago *et al.*, 2005; Visser *et al.*, 2000; McDonald *et al.*, 2002; Evans, 2003; Grimoldi *et al.*, 2005; Striker *et al.*, 2007). This aerenchyma tissue provides a continuous system of interconnected aerial spaces (aerenchyma lacunae) of lower resistance for oxygen transport from aerial shoots to submerged roots, allowing root growth and soil exploration under anaerobic conditions (Colmer and Greenway, 2005). It is predictable that stress from soil flooding on roots also alters shoot morphology because of the close functional interdependence between both of them. In this way, flooded plants of tolerant species are often taller than their non-flooded counterparts as a result of increases in the insertion angles and length of their aerial organs. These responses were well characterized in the dicotyledonous Rumex palustris by Cox *et al.* (2003 and 2004) and Heydarian *et al.* (2010) among others.

Interaction effects of genotypes to situation and days after plantation on dry leaf, green leaf and growth rate differed significantly (Table 7). Genotype Isd 34 produced lowest dry leaves after 60 days under flood stress condition while the highest were observed on Isd 39 after 120 days in control condition. Highest green leaves were produced by Isd 34 after 60 days under flood stress condition followed by Isd 38 after 60 days under flood condition and Isd 39 after 60 days under flood condition while the lowest were produced by Isd 39 after 120 days in control condition. Highest growth rate was observed in Isd 39 after 60 days under flood condition followed by Isd 20 after 60 days flood condition and Isd 37 after 60 days flood condition while the lowest were observed in Isd 38 after 120 days in control condition.

Adventitious root

Amount of adventitious roots of various genotypes differed significantly due to flood stress (Table 8). Among all genotype Isd 34 produced highest adventitious roots (842.0 g $plant^{-1}$) followed by Isd 38 (836.0 g $plant^{-1}$). Flooding induces morphological changes in roots and shoots. In the sugarcane, the formation of adventitious roots is highlighted as a common response of flood-tolerant species. These adventitious roots, which have high porosity, help plants to continue with water and nutrient uptake under flooding conditions, replacing in some way the functions of older root system (Kozlowski *et al.*, 1984). It is frequent that these adventitious roots are positioned near the better-aerated soil surface. Following the review by Jackson (2004), there are three mechanisms for generating these 'replacement' root systems: (i) stimulation of the outgrowth of pre-existing root primordia in the shoot base (Jackson *et al.*, 1981), (ii) induction of a new root system that involves initiation of root primordia and their subsequent outgrowth (Jackson and Armstrong, 1999; Shimamura *et al.*, 2007) and (iii) placing roots at the soil surface involving the re- orientation of the root extension as seen for woody species by Pereira and Kozlowski (1977) and for herbaceous species by Gibberd *et al.* (2001). The two first mechanisms appear to be triggered by ethylene, which is thought to increase the sensitivity of plant tissues to auxin (Bertell *et al.*, 1990; Liu and Reid, 1992) (Figures 2 and 3). Cross section of the adventitious roots showed a lot of aerenchyma cells (Figure 3). These aerenchyma cell preserved a lot of air which helps the plants to survive under stress condition. Aerenchyma formation in the root cortex is the most studied plastic response to flooding (Seago *et al.*, 2005; Visser *et al.*, 2000; McDonald *et al.*, 2002; Evans, 2003; Grimoldi *et al.*, 2005; Striker *et al.*, 2007). This aerenchyma tissue provides a continuous system of interconnected aerial spaces (aerenchyma lacunae) of lower resistance for oxygen transport from aerial shoots to submerged roots, allowing root growth and soil exploration under anaerobic conditions (Colmer and Greenway, 2005).

Figure 1. Pictorial view of the experiment conducted under induced flood stress in a concrete water tank

Figure 2. Formation of adventitious root due to flood stress

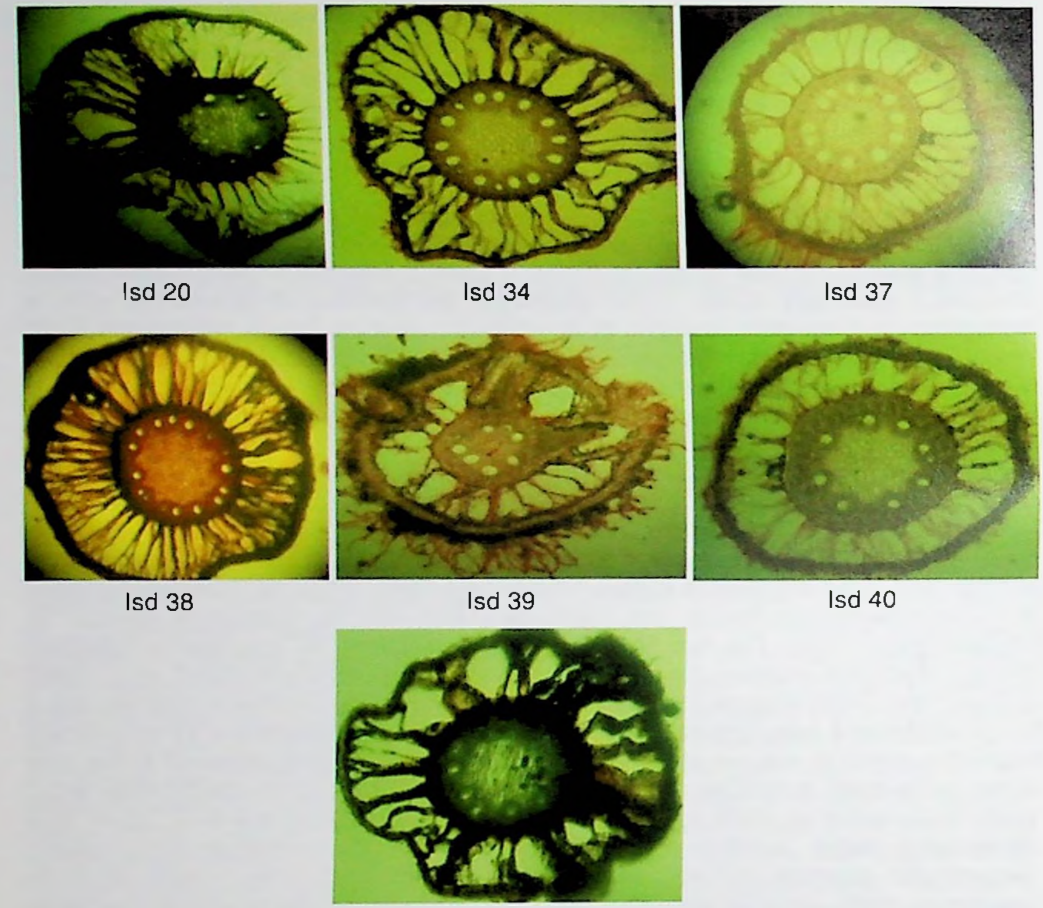

Figure 3. Cross section of adventitious root of some sugarcane genotypes showing aerenchyma cell.

Table 1. Effects of genotypes on morphological parameter

Name of Genotypes	Dry leaf percentage	Green leaf percentage	Growth rate (cm day^{-1})
Isd 20 (V_1)	61.700	38.300	1.0244
Isd 34 (V_2)	55.933	44.067	1.0689
Isd 37 (V_3)	61.333	38.000	1.2000
Isd 38 (V_4)	58.967	40.200	1.2900
Isd 39 (V_5)	60.367	38.967	1.2750
Isd 40 (V_6)	60.550	38.783	0.8989
I 25-04 (V_7)	63.117	36.217	0.8900
LSD (0.05)	0.3950	0.4055	0.0557

Table 2. Effects of situation on morphological parameter

Name of situation	Dry leaf percentage	Green leaf percentage	Growth rate (cm day^{-1})
Flood (F)	59.800	39.200	1.2019
Control (C)	60.762	39.238	0.9830
LSD (0.05)	0.2111	NS	0.029

Table 3. Effects of days after plantation on morphological parameter

Name of situation	Dry leaf percentage	Green leaf percentage	Growth rate (cm day^{-1})
60 days	52.655	46.869	1.1424
90 days	61.705	37.748	1.1014
120 days	66.483	33.040	1.0336
LSD (0.05)	0.2586	0.2655	0.0365

Table 4. Interaction Effects of Genotypes x situation on morphological parameter

Genotypes x situation	Dry leaf percentage	Green leaf percentage	Growth rate (cm day^{-1})
V_1F	60.567	39.433	0.8656
V_1C	62.833	37.167	1.1833
V_2F	59.167	40.833	1.2233
V_2C	52.700	47.300	0.9144
V_3F	60.133	38.533	1.4733
V_3C	62.533	37.467	0.9267
V_4F	58.133	40.200	1.4667
V_4C	59.800	40.200	1.1133
V_5F	59.133	39.533	1.4067
V_5C	61.600	38.400	1.1433
V_6F	59.433	39.233	0.9644
V_6C	61.667	38.333	0.8333
V_7F	62.033	36.633	1.0133
V_7C	64.200	35.800	0.7667
LSD (0.05)	0.5586	0.5735	0.0788

Table 5. Interaction Effects of Genotypes x days after plantation on morphological parameter

Genotypes x days after plantation	Dry leaf percentage	Green leaf percentage	Growth rate (cm day^{-1})
V_1D_1	53.717	45.617	1.1950
V_1D_2	62.967	36.367	1.3900
V_1D_3	67.317	32.017	1.2400
V_2D_1	50.267	49.067	1.1750
V_2D_2	62.917	36.417	1.2950
V_2D_3	67.917	31.417	1.1300
V_3D_1	53.100	46.900	1.0900
V_3D_2	62.850	37.150	1.0750
V_3D_3	69.150	30.850	1.0417
V_4D_1	51.567	47.767	0.9850
V_4D_2	65.867	33.467	0.8500
V_4D_3	71.917	27.417	0.8350

(Contd.)

Genotypes x days after plantation	Dry leaf percentage	Green leaf percentage	Growth rate (cm day^{-1})
V_5D_1	51.917	47.417	1.4700
V_5D_2	59.067	39.767	1.2200
V_5D_3	65.917	33.417	1.1800
V_6D_1	52.917	46.417	0.9667
V_6D_2	61.767	37.567	0.8550
V_6D_3	66.967	32.367	0.8750
V_7D_1	55.100	44.900	1.1150
V_7D_2	56.200	43.800	0.9333
V_7D_3	56.500	43.500	1.0250
LSD (0.05)	0.6841	0.7024	0.0965

Table 6. Interaction Effects of situation x days after plantation on morphological parameter

situation x days after plantation	Dry leaf percentage	Green leaf percentage	Growth rate (cm day^{-1})
FxD_1	51.881	47.167	1.2333
FxD_2	61.424	37.481	1.2300
FxD_3	66.095	32.952	1.1424
CxD_1	53.429	46.571	1.0514
CxD_2	61.986	38.014	0.9729
CxD_3	66.871	33.129	0.9248
LSD (0.05)	0.3657	0.3754	0.0516

Table 7. Interaction Effects of Genotypes x situation x days after plantation on morphological parameter

Genotypes x situation x days after plantation	Dry leaf percentage	Green leaf percentage	Growth rate (cm day^{-1})
V_1FD_1	52.033	46.633	1.6300
V_1FD_2	60.633	38.033	1.2400
V_1FD_3	65.633	33.033	1.3500
V_1CD_1	53.800	46.200	1.1500
V_1CD_2	62.900	37.100	1.1500
V_1CD_3	68.300	31.700	1.1300
V_2FD_1	48.433	50.233	1.0533
V_2FD_2	59.400	40.600	0.9200
V_2FD_3	60.700	39.300	0.9200
V_2CD_1	52.100	47.900	0.8800
V_2CD_2	63.700	36.300	0.7900
V_2CD_3	69.000	31.000	0.8300
V_3FD_1	52.433	46.233	1.4200
V_3FD_2	61.133	37.533	1.6300
V_3FD_3	66.833	31.833	1.3700
V_3CD_1	55.000	45.000	0.9300
V_3CD_2	64.800	35.200	0.9600
V_3CD_3	67.800	32.200	0.8900
V_4FD_1	51.100	48.900	1.2200
V_4FD_2	62.500	37.500	1.2800

(Contd.)

Genotypes x situation x days after plantation	Dry leaf percentage	Green leaf percentage	Growth rate (cm day^{-1})
V_4FD_3	68.100	31.900	1.1700
V_4CD_1	55.100	44.900	0.9600
V_4CD_2	63.200	36.800	0.8700
V_4CD_3	70 200	29.800	0.9133
V_5FD_1	50.533	48.133	1.1200
V_5FD_2	64.833	33.833	0.9300
V_5FD_3	70.733	27.933	0.9900
V_5CD_1	52.600	47.400	0.8500
V_5CD_2	66.900	33.100	0.7700
V_5CD_3	73.100	26.900	0.6800
V_6FD_1	51.233	47.433	1.8200
V_6FD_2	58.033	39.633	1.2900
V_6FD_3	65.133	33.533	1.2900
V_6CD_1	52.600	47.400	1.1200
V_6CD_2	60.100	39.900	1.1500
V_6CD_3	66.700	33.300	1.0700
V_7FD_1	57.400	42.600	0.7600
V_7FD_2	62.133	36.533	0.9300
V_7FD_3	66.833	31.833	0.9067
V_7CD_1	52.800	47.200	1.4700
V_7CD_2	52.300	47.700	1.1200
V_7CD_3	53.000	47.000	0.9600
LSD (0.05)	0.9675	0.9933	0.1365

Table 8. Adventitious roots (AR) of BSRI bred sugarcane clones under induced flood stress condition (pot experiment).

Name of Genotypes	Fresh wt. of adventitious root plant^{-1} (g)
Isd 20	62.0
Isd 34	842.0
Isd 37	54.8
Isd 38	836.0
Isd 39	380.0
Isd 40	315.7
I 25-04	24.5
LSD (0.05)	1.7512

Biochemical Analysis of Juice

All the parameters viz., brix percentage, purity percentage, pol percentage and reducing sugar (RS) etc. indicating juice quality Table 9 showed significant difference among different genotypes. Among them Isd 38 produced the highest brix % (20.55), highest pol % (14.48) and the highest purity (89.54%) followed by Isd 34 (brix 20.25% , pol 14.34%, purity 89.39%). Flood and control condition showed significant difference on brix and RS and insignificant effect on pol and purity percentage Table 10. All the genotypes produced higher brix, pol, purity percentage and lower RS in flood condition than in the control condition except I 25-04 which are in agreement with Hasan *et al.* (2003) who grew some sugarcane genotypes under waterloged condition and found that all the genotypes had higher brix, pol, purity percentage and lower RS in stress than in control condition.

Interaction effects of genotypes to situation on juice quality differed significantly Table 11. Genotype Isd 34 produced the highest brix % and pol % followed by genoypes Isd 38 and Isd 20 under flood stress condition. Isd 38 produced comparatively higher brix and pol percentage both in stress and control condition. Highest purity percentage were obtained in genotypes Isd 34 and Isd 38 both stress and control conditions. Isd 20 also showed better purity percentage in control condition. All the genotypes produced comparatively lower reducing. Isd 40 produced the highest reducing sugar in control condition.

Table 9. Effects of genotypes on juice quality

Name of Genotypes	Brix (%)	Pol (%)	Purity (%)	Reducing sugar (RS)
Isd 20 (V_1)	19.450	13.780	88.460	0.4500
Isd 34 (V_2)	20.250	14.340	89.390	0.3400
Isd 37 (V_3)	17.500	12.50	87.515	0.4300
Isd 38 (V_4)	20.550	14.480	89.540	0.4050
Isd 39 (V_5)	19.600	13.590	87.020	0.7100
Isd 40 (V_6)	18.800	12.825	83.730	0.7100
I 25-04 (V_7)	18.850	12.530	85.910	0.2800
LSD (0.05)	0.6344	0.4395	0.9991	0.008619

Table 10. Effects of situation on juice quality

Name of Genotypes	Brix (%)	Pol (%)	Purity (%)	Reducing sugar (RS)
Flood (F)	19.443	13.536	87.881	0.5657
Control (C)	19.129	13.334	86.851	0.3843
LSD (0.05)	0.3391	0.2349	0.5341	0.004607

Table 11. Interaction effects of genotypes x situation on juice quality

Genotypes x situation	Brix (%)	Pol (%)	Purity (%)	Reducing sugar (RS)
V_1F	20.000	13.020	87.170	0.2500
V_1C	18.900	14.540	89.750	0.6500
V_2F	20.800	14.680	89.900	0.3300
V_2C	19.700	14.000	88.880	0.3500
V_3F	18.000	13.000	87.670	0.4100
V_3C	17.000	12.000	87.360	0.4500
V_4F	20.600	14.600	89.800	0.4000
V_4C	20.500	14.360	89.280	0.4100
V_5F	19.800	13.840	89.040	0.6700
V_5C	19.400	13.340	85.000	0.7500
V_6F	19.000	13.000	85.650	0.3800
V_6C	18.600	12.650	81.810	1.0400
V_7F	18.700	12.450	85.880	0.3100
V_7C	19.000	12.610	85.940	0.2500
LSD (0.05)	0.8971	0.6215	1.4130	0.0122

Observing the tolerance mechanism of the genotypes which were used in this study we can concluded that all the genotypes showed better performance under stress than control condition which produced more adventitious roots with spread aerenchyma cell are suitable for cultivation under flood stress considering their morphological characters and biochemical analysis except the genotype I 25-04.

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