Forage Res., **46** (4) : pp. 368-373 (2021) **http://forageresearch.in**

EFFECT OF SALINITY STRESS ON DIFFERENT SEED VIABILITY INDICES IN SINGLE AND MULTI CUT BERSEEM (*TRIFOLIUM ALEXANDRINUM*) VARIETIES

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(Received : 11 March 2021; Accepted : 31 March 2021)

SUMMARY

In the present investigation, four berseem varieties *viz*., Wardan, BB2, BB3 and JBSC-1 were tested for their relative salt tolerance to increasing levels of salinity in the combinations of salts which nearly exist in the natural salt affected soils in India. Seeds were germinated in petri dishes and were exposed to four salinity [(4,8,12 and 16EC (dS m^{-1})] levels. The germination percentage, shoots and root length, fresh and dry weight of seedling were recorded. The results revealed that the effect of salt levels on these parameters was significant ($p < .05$). Higher loss of germination percentage was found in BB2 at different levels of salinity than in JBSC-1. It was inferred that salinity stress significantly decreased germination and growth parameters of seedlings of four berseem varieties; however, varieties JBSC-1 and Wardan were found relatively tolerant to salinity than BB2.

Key words : Salinity, berseem, germination percentage, shoot length and seed viability indices

Salinity affects about six per cent of the world's total land area, including 20% of arable land and 33% of irrigated land (Kuang *et al.*, 2019; Safdar *et al.,* 2019), causing estimated yield losses of 20% (Ashraf and Harris2005; Pirasteh-Anosheh *et al*., 2016). Salinity is one of the rising problems causing tremendous yield losses in many regions of the world especially in arid and semiarid regions (Hasanuzzaman *et al*., 2014). In India, about 5.95 Mha areas have been affected by salinity. The loss of productivity due to salinity has been reported 6.2 Mt. Five states *viz*. Haryana, Punjab, Rajasthan, Gujarat and Andhra Pradesh accounts for 48% of the total salt affected soils of the country (Singh *et al.,* 2020, Mandal *et al*., 2010). Legumes/pulses are very important food and feed crops, known for their health benefits (Arnoldi *et al*., 2014), vital ingredient of Indian and Mediterranean diets and considered staple food in other regions (Patto *et al*., 2014), have high demand as forage for producing high-quality meat and milk (Boelt *et al*., 2015). Salinity diminishes seedling growth and development by causing ion toxicity, physiological drought, mineral imbalance and osmotic stress which negatively affect plant productivity (Shrivastava *et al*., 2015). To fulfill the food, feed, fodder and industrial raw material demands of growing population, development of salt tolerant and high yielding crop genotypes is necessary. Establishment of seedlings at early growth stages of crop plants is one of the most important determinants of high yield in severely affected by soil salinity (Akhtar *et al*., 2008). Increasing salinity levels are reported to reduce germination significantly and other parameters like shoot and root length, shoot and root fresh and dry weights of some of forage legumes (Dheeravathu *et al*., 2017). Berseem or Egyptian clover (*Trifolium alexandrinum* L.), is commonly cultivated as winter annuals in the tropical and subtropical regions and it has been established as one of the best winter season fodder crop in entire North West Zone, Hill Zone and part of Central and Eastern Zone of the country (Mehta and Swaminathan, 1965), occupy around two million hectare area (Singh *et al*., 2019). Berseem is popular due to its multi cut (4-8 cuts) nature, providing fodder for a long duration (November to May), very high quantum of green fodder (85 t/ha) and better quality of fodder (20% crude protein), high digestibility (up to 65%) and palatability (Singh *et al*., 2019 and 2020). Legumes are considered a relatively salt sensitive family (Mass and Hoffman, 1977) within which limited variability for salinity tolerance has been detected (Johansen *et al*., 1990). Dheeravathu *et al.,* (2017) reported that increasing salinity levels affected germination percentage, germination rate, seedling shoot and root length, seed viability index-I and II, seedling fresh and dry weight of three range legumes *viz.* centro, clitoria and siratro. Berseem clover like other plants in the legume family, is placed among the most sensitive plants, because its tolerance threshold was estimated to be close to 1.5 dS m^{-1} (Maas and Hoffman, 1977). Therefore, in the present investigation three multi cut and one single cut berseem varieties were tested for their relative salt tolerance to increasing levels of salinity in those combinations of salts which nearly exist in the natural salt affected soils.

MATERIALS AND METHODS

This study was conducted at Crop Improvement Division of ICAR- Indian Grassland and Fodder Research Institute, Jhansi (25°45'N, 78°58'E, 243m above MSL), during 2020-2021. Experiment consisted of three multi cut *viz.,* Wardan, BB-2, BB-3 and one single cut JBSC-1 varieties, with different salinity levels *i.e.* 0 as control (distilled water), 4, 8, 12 and 16EC (dSm^{-1}) as prepared by mixing the different salts according to Dheeravathu *et al.,* (2017), which were evaluated in a Completely Randomized Block Design in petri plates and replicated thrice. The seeds were surface sterilized with 0.01% mercuric chloride (w/v) for 10 min to avoid fungal invasion, followed by washing with distilled water. These treated seeds were used for the experiment. For each variety 150 seeds for each of the four salt treatments were used. Seeds were allowed to germinate in laboratory condition on filter paper (Whatman No. 2) in sterilized 15 cm (diameter) petri dishes soaked in a solution of the respective salt concentration. Small amount (10g) of artificial sand was added to each petri dish, after the germination for support and early growth of seedling in petri plates, respective salt concentration was maintained up to 10 days. The number of germinated seeds was counted every day up to 10 days and the seeds were considered germinated when the radical emerged. At the 10^{th} day (starting of germination), 5 seedlings were randomly selected and seedling and root length with their fresh weight and dry weight were measured, the fresh weight and dry weight of seedling were obtained after the length assessment. For dry weight recording, the seedlings were wrapped in brown paper bags and placed in oven at 80 °C for 24 h. The dry weight of the seedlings was recorded. Seed salinity viability indices-I, II, III were computed as suggested by Azizi *et al.* (2011) and Dheeravathu *et al.*, (2017) by the following equations:

Seed salinity viability index-I =Final germination percentage × average seedling length [(shoot + root length (cm)] /100,

Seed salinity viability index-II =Final germination percentage × fresh weight of seedling [(shoot + root (mg)]/ 100,

Seed salinity viability index-III =Final germination percentage × dry weight of seedling [(shoot + root (mg)] / 100.

Seedling total water content (STWC) was calculated by following the method described by Dheeravathu *et al.*, (2018) using the formula: seedling fresh weight-seedling dry weight/ seedling fresh weight.

Statistical design and analysis :

Analysis of variance of data was performed with Microsoft Excel and SAS 9.3 statistical program using completely randomized design and mean values were compared using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The main effects of salinity on varieties and the interaction effects among varieties were significant in all the measured parameters (Table 1). Salinity reduced germination percentage, seedling shoot length, seedling root length, seedling vigor indices (SVI-I, II and III), seedling fresh weight but seedling dry weight increased at increasing levels of salinity in different berseem varieties compared to control.

Effect of salt stress on seed germination percentage, seedling shoot length, root length and root to shoot ratio

The results of seed germination showed that, germination percentage reduced with increasing salt concentrations. Lowest mean germination percentage was observed in BB2 and BB3 (91%), while the highest mean value was observed in JBSC-1 (97%) (Table 2). The highest percentage of reduction over the control

TABLE 1

ANOVA results of the effect of salt stress on germination percentage, seedling shoot length, seedling root length, seedling fresh weight and dry weight

Source	DF	Mean Square				
		GP	SSL	SRL	SFWT	SDWT
Replication	2	32.55	0.04	0.03*	0.002**	0.00**
Variety	3	1506.86**	0.42**	2.13**	0.05**	0.00**
Salinity level	4	265.29**	15.59**	2.96**	0.37**	0.00**
Variety *Salinity level	12	148.51**	0.26**	0.03**	0.02**	0.00**
Error	38	4.27	0.02	0.01	0.00	0.00

**, significance level, $p < .01$. GP-Germination percentage, SSL-Seedling shoot length, SRL-Seedling root length, SFWT -Seedling fresh weight, SDWT- Seedling dry weight

rate was found in BB2 (7.65) followed by BB3 whereas the least reduction was noticed in JBSC-1 (2.01).The highest reduction of germination was evident in all four varieties at highest salt concentration of 16 EC (dS m^{-1}). Under stress condition, seedling shoot length and root length, was reduced in all the varieties but highest reducing seedling shoot length and root length was observed at 16EC (dS m^{-1}) compared to 4, 8 and 12EC (dS m^{-1}), (Table 2) but increasing root to shoot ratio was observed at increasing salinity levels compared to control (Table 2).

Effect of salt stress on berseem seedling fresh, dry weight and seedling total water content

The fresh and dry weight of seedling of all four berseem varieties was strongly affected by all salinity levels. Seedling fresh and dry weight were reduced at high salinity levels [(8 and 16EC (dS m^{-1})], whereas fresh weight was reduced more as compared to dry weight of seedling at increasing level of salinity, relatively (Fig 1). The reducing total seedling water content was observed in all the four varieties and highest reduction was recorded at 12 and 16EC (dS m^{-1}) (Fig 1).

Effect of salinity stress on different seed viability indices

The main aim of this study was to identify and characterize the best seed viability indices for salt tolerant and sensitive varieties of berseem during germination and early seedling growth stages to determine their potential for salt tolerance. Three seed viability indices *viz*., I, II and III, were calculated and recorded as per Azizi *et al*. (2011) and Dheeravathu *et al*., (2017) methodology. Seed viability index-III was standardize and found to be

TABLE 2

Effect of salt stress on seed germination percentage, seedling shoot length, seedling root length and root to shoot ratio

Varieties/ salinity	Wardan	BB2	BB3	JBSC-1	Wardan	BB2	BB3	JBSC-1	Wardan	BB2	BB3	JBSC-1	Wardan	BB2	BB3	JBSC-1
	Germination (%)				Shoot length (cm)				Root length (cm)				Root to shoot ratio			
Control	99^{a} +0.94	98^{a} +1.41	97^{a} +0.94	99^{a} +0.72	4.32^{a} +0.005	4.00^{a} +0.272	3.96^{a} +0.009	4.92^{a} +0.009	1.82^{a} +0.014	2.16^{a} +0.009	2.16^{a} +0.014	2.82^{a} +0.005	0.42	0.54	0.55	0.57
4EC	97^{b} +1.89	95^{b} +0.47	93^{b} +0.94	97^{b} +0.47	3.36^{b} +0.0189	3.72^{b} +0.0047	3.96^{b} +0.0094	3.94^{b} +0.0094	1.68^{b} +0.0189	2.08^{b} +0.0189	2.08^{b} +0.0141	2.48^{b} +0.0119	0.50	0.56	0.53	0.63
8EC	94^{c} +0.94	90^{c} +0.47	93^{c} +1.41	99^{c} +0.47	2.92^{c} +0.0094	3.38^{c} +0.0141	3.04^{c} +0.0094	3.12^{c} +0.0094	1.52^{b} +0.0047	2.12^{b} +0.0094	2.06^{b} +0.0141	2.41^{b} +0.0144	0.52	0.63	0.68	0.77
12EC	92^{c} +1.41	89^{c} +0.94	89^{c} +0.47	97^{c} +1.41	2.34^{d} +0.0141	2.22^{d} +0.0094	1.88^{d} +0.0047	2.30^{d} +0.0094	0.78^{c} +0.0094	1.20^{c} +0.1498	1.10^{c} +0.0047	1.86^{c} +0.0141	0.33	0.54	0.59	0.81
16EC	92^{d} +1.66	89^{d} +1.41	89^{d} +1.41	97^{d} +0.94	1.28^{e} +0.0141	1.28^{e} +0.0094	1.46^{e} +0.0119	1.65^{e} +0.0054	0.72^{d} +0.0047	1.12^{d} +0.0094	1.28^{d} +0.0189	1.56^{d} +0.0283	0.56	0.88	0.88	0.94
Mean % in stress	94	91	91	97	2.48	2.65	2.58	2.75	1.18	1.63	1.63	2.08	0.48	0.65	0.67	0.79
%roc	5.01	7.65	5.86	2.01	43	34	35	44	35	25	2.5	26				

Means followed by the same letter (s) in column (s) are not significantly different (p<.05), where letter "a" represents the least value,
The data are the mean ± SE (n=3); % ROC-% Reduction Over the Control

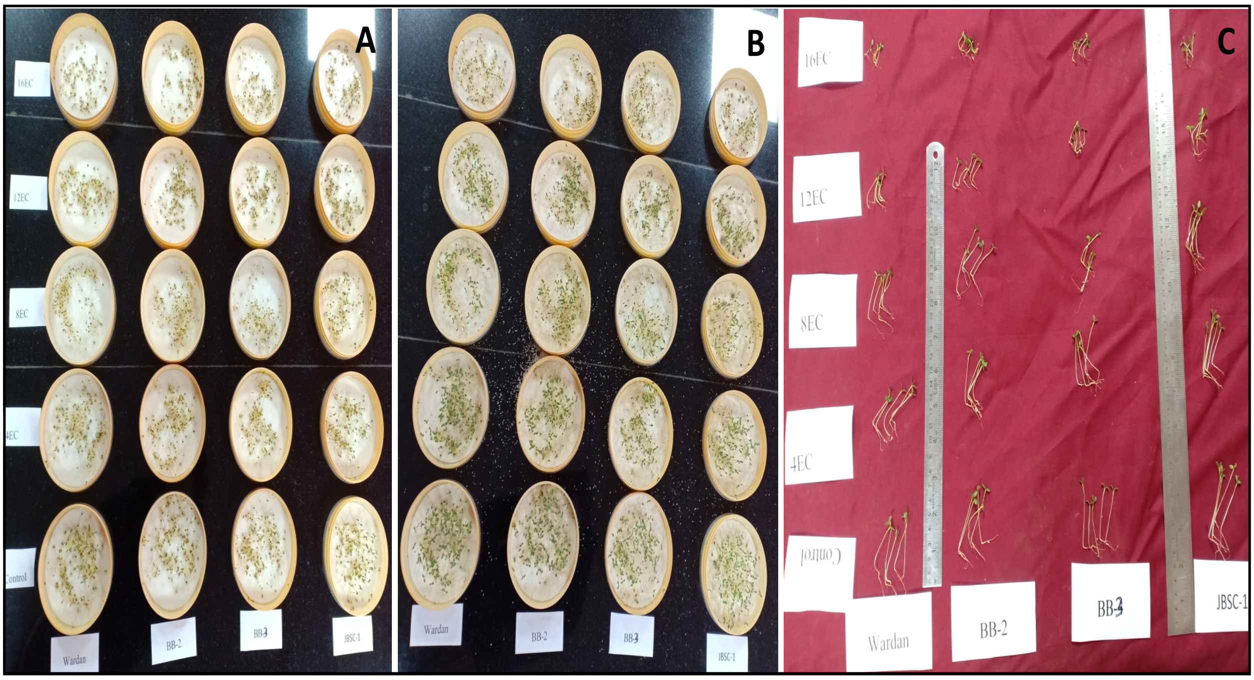
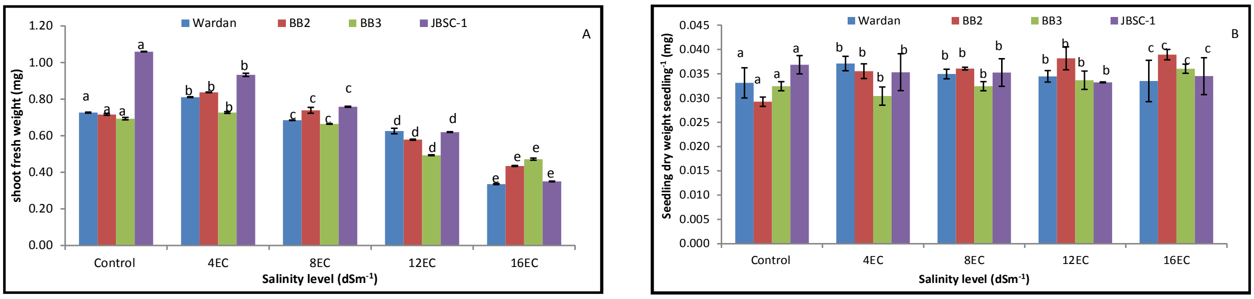
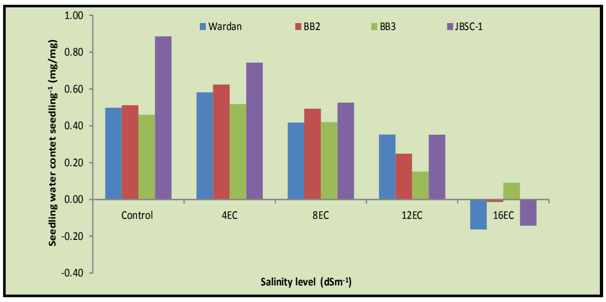


Fig. 1. Effect of salt stress on seed germination, seedling shoot length, root length (A-Seed germination before sand add, B-Sand added after seed germination, C-Seedling shoot lengths and root lengths).



Means followed by the same letter (s) on bar (s) are not significantly different (p<.05), where letter "a" represents the least value, The data are the mean ± SE (n=3)

Fig. 2. Effect of salt stress on berseem seedling fresh and dry weight (A - fresh weight, B - dry weight).



The data are the mean ± SE (n=3); $P < .05$

Fig. 3. Effect of salt stress on total seedling water content in berseem.

more effective for screening salinity stress tolerance in berseem (Table 3).

Based on the results, the berseem varieties varied in their response to salinity in germination and seedling characteristics. The results showed that with an increasing level of salt stress, GP, SL, RL, FW, SWC parameters of berseem varieties decreased, while DW increased compared to control. However, these parameters varied depending on the assessed multi cut and single cut berseem varieties. This is in line with our first assumption that the seeds that grew at different level of salinity levels had higher seedling fresh weight but lower dry weight in single cut and all the multi cut berseem varieties (Fig 1). In contrast to significant decrease in the fresh weight, the seedling dry weight increased as the stress concentrations increased. This indicated that the higher seedling fresh weight at control as well as at lower level of salinity stress was mainly due to an increase in the seedling tissue water content (Fig 3). These results were also in line with the results of Foolad and Lin (1997) obtained for tomato and Agarwal *et al.,* (2010) also

TABLE 3
Effect of salt stress on different seed viability indices in berseem varieties

Genotypes/Salinity	Wardan	BB2	BB3	JBSC-1
	seed viability index-I			
Control	6.06	5.71	5.92	7.69
4EC	4.87	5.49	5.64	6.20
8EC	4.17	4.95	4.76	5.46
12EC	2.87	3.15	2.64	4.02
16EC	1.84	2.15	2.43	3.13
	seed viability index-II			
Control	0.14	0.14	0.13	0.21
4EC	0.16	0.16	0.14	0.18
8EC	0.13	0.13	0.12	0.15
12EC	0.12	0.10	0.09	0.12
16EC	0.06	0.08	0.08	0.07
	seed viability index-III			
Control	0.03	0.03	0.03	0.04
4EC	0.04	0.04	0.03	0.04
8EC	0.03	0.04	0.03	0.04
12EC	0.03	0.04	0.03	0.03
16EC	0.03	0.04	0.04	0.03

TABLE 4
Correlation among different parameters up on salinity stress in berseem varieties

	S	R	R to S ratio	SVI-I	SVI-II	SVI-III
GP%	0.57**	0.27	-0.54*	0.54*	0.60**	0.36
S		.73**	-0.48*	0.97**	0.77**	-0.17
R			0.21	0.88**	0.67**	-0.17
R to S ratio				-0.26	-0.30	-0.09
SVI-I					0.80**	-0.15
SVI-II						0.27

**,*significant at the 0.01 and 0.05 levels respectively GP-germination percentage, SSL and SRL -seedling shoot and root length, SVI-I, II and III Seed viability indices

reported that on the basis of SSI, SHSI and seedling dry weight cv. BL 22 and Wardan have been proved as tolerant while BL 10 and JHB 051 have been proved as sensitive. Dheeravathu *et al*., (2017) identified the drought adaptive traits in berseem, The results revealed that germination percentage, seedling shoot and root length, seedling fresh and dry weight, total seedling water content and seed viability indices-I, II and III of berseem varieties *viz.,* Wardan, BB-2, BB-3 and JBSC-1 were strongly affected ($p< .05$) by all salt treatments (Table 2). Positive significant correlation was found between, seed viability index-I and II and germination percentage and shoot and root length and negative correlation ($p< .05$) was found between seed viability index-III, shoot and root length and root to shoot ratio (Table 4). Seed viability index-III is very difficult to assess in small seeded forage legumes and grasses, however the use of artificial sand in petri plates provided support for proper seedling growth. As this is a very simple and easy technique it may useful for *In- vitro* screening for salinity stress.

CONCLUSION

The current study indicated that increasing salinity levels affected germination percentage, seedling shoot and root length, seedling fresh and dry weight and seed viability index-I, II and III of three multi cut *viz.,* Wardan, BB-2, BB-3 and one single cut variety, JBSC-1. Significant differences in shoot length, root length, and seedling dry weight in 10 days old seedlings were observed among multi cut and single cut berseem varieties. From the overall observation of germination characters and early seedling growth, it was concluded that the single cut berseem showed better salt tolerance compared to control and multi cut varieties, We identified that seed viability index-III is the best screening technique for salinity stress tolerance than seed viability index-I and II. However, confirmations of these results have to be made in the field affected by salinity.

ACKNOWLEDGEMENTS

The authors acknowledge Indian Council of Agricultural Research (ICAR) and ICAR– Indian Grassland and Fodder Research Institute, Jhansi for financial support for conducting the experiment.

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