Forage Res., **51**(2) : pp. 273-279 (2025) **http://forageresearch.in**

PHYSIOLOGICAL STUDY OF FORAGE SORGHUM UNDER IMPOSED SALINITY STRESS AT SEEDLING STAGE

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(Received: 10 August 2025; Accepted: 20 September 2025)

SUMMARY

Salinity stress is one of the major abiotic factor that significantly impairs sorghum germination and growth of the seedling. In the present investigation, ten sorghum varieties were tested for salinity stress tolerance at seedling stage. Under control conditions exhibited high germination rates (up to 97% in CSV-21 and CSV-38F), germination percentage declined progressively with rising salinity, with no variety achieving 100% germination at 200 mM. Root-to-shoot ratio was also affected, peaking in CSV-32F (3.86) and CSV-38F (2.85) at 150 mM. Shoot and root lengths decreased significantly under salt stress, with CSV-21 exhibiting maximum shoot length (14.6 cm) and PC-615 recording the highest root length (5.83 cm) in controls. Seedling biomass was severely impacted, with fresh weight (0.23 g in CSV-38F) and dry weight (0.09 g in CSV-32F) declining markedly at 200 mM. Varieties CSV-38F and CSV-32F demonstrated relative tolerance, suggesting potential for cultivation in saline-prone areas. These insights underscore the need for salt-tolerant sorghum genotypes to sustain productivity under abiotic stress.

Key words: Germination percentage, root to shoot ratio, salinity, sorghum seedling vigour index

Salinity stress is a critical environmental challenge that severely limits agricultural productivity, particularly in arid and semi-arid regions where soil salt accumulation is prevalent (Munns and Tester, 2008). Salinity continued to pose significant challenges to global agricultural productivity, affecting approximately 20% of the world's irrigated croplands (FAO, 2022). The United Nations reported that over 62 million hectares of agricultural land were salt-affected, leading to estimated annual economic losses exceeding $31 billion due to reduced crop yields (UNCCD, 2022). Salinity has significantly impacted Indian agriculture, degrading approximately 5.95 million hectares of land and causing an estimated annual production loss of 6.2 million metric tons (Singh et al., 2020). The spatial distribution of salt-affected soils shows particular concentration, with nearly half (48%) of the nation's affected areas located in just five states: Gujarat, Rajasthan, Punjab, Haryana, and Andhra Pradesh (Mandal et al., 2010). Major staple crops were particularly vulnerable, with yield reductions of 40-60% in high-salinity regions, while even relatively salt-tolerant crops like sorghum exhibited 20-30% of reduction in productivity (ICARDA, 2022).

Sorghum (*Sorghum bicolor* L.) is a cereal crop widely cultivated in arid and semi-arid regions across the tropics and subtropics. As an indigenous crop to Africa, it has become a staple in India, primarily grown in Maharashtra, Karnataka, Andhra Pradesh, Telangana, Madhya Pradesh, Rajasthan, and Uttar Pradesh. In India, sorghum occupies approximately 4.5 million hectares (2020-21), producing about 4.8 million tonnes of grain with an average productivity of 1,067 kg/ha (ICAR-IIMR, 2021). Sorghum demonstrates numerous agronomically valuable characteristics, including deep root systems, waxy leaf coatings, heat tolerance, and efficient water-use mechanisms. The crop exhibits remarkable adaptability to poor soils with low fertility and can withstand prolonged dry spells.

However, its seedling stage is a crucial phase for crop establishment which is highly vulnerable to salt stress, leading to poor germination, stunted growth, and even plant death (Hadebe *et al.*, 2017). The detrimental effects of salinity arise from osmotic stress, which reduces water uptake, and ionic toxicity, disrupting cellular functions primarily due to excessive sodium (Naz) and chloride (Cl) accumulation leading to nutrient imbalances and reduction in growth of seedling (Zhu, 2016). These physiological traits contribute to its climate resilience and nutritional security value, maintaining its importance in both traditional and modern cropping systems (Reddy *et al.*, 2022). Sorghum's dual-purpose utility for grain and fodder, along with its gluten-free nutritional profile, ensures its continued relevance in sustainable agriculture amid changing climatic conditions.

Climate resilient crops: The climate resilient crops such as grasses: guineagrass, bajra -napier hybrids and tri-specific hybrids, dinanath grass, (Singh *et al.*, 2010, Singh *et al.,* 2020, Dheeravathu *et al.*, 2021a, Dheeravathu *et al.*, 2021b, Dheeravathu *et al.*, 2021c., Dheeravathu *et al.*, 2022a, Dheeravathu *et al.,* 2022b, Amrutha *et al.,* 2023), forage cereals including millets: pearl millet sorghum, kodo millet, (Malathi *et al.*, 2022, Dheeravathu and Vadithe, 2024a, Dheeravathu and Vadithe, 2024b, Dheeravathu *et al.,* 2024b, Sravanthi *et al.,* 2024a and b, Sravanthi et al., 2025), pulses: cowpea, berseem, clitoria, centrosema, siratro (Dheeravathu *et al.,* 2017 a and b, Dheeravathu *et al.,* 2022c., Dheeravathu *et al.,* 2023), have been proven to be climate smart. Optimizing water use for crops and forage crops by improving WUE is a challenge for securing agricultural sustainability in arid and semi-arid regions. Considering the adverse effects of accumulated salts in soil and irrigation water, elevated CO_2 and temperature on soil health, forage yield and productivity, it is high time for an in-depth understanding of physiological and biochemical changes in forage crop varieties/ genotypes/lines in response to climate change.

MATERIALS AND METHODS

The study was conducted in Plant physiology laboratory at ICAR- Indian Institute of Millets Research (IIMR), Rajendranagar, Hyderabad. It is located at Southern Telangana Zone of Telangana state. It is located at 17 19' 40.9" N latitude, 78 23' 38.2" E longitude, at an altitude of 542 meters above mean sea level.

Seed materials

The seeds were procured from ICAR-IIMR, Hyderabad, India. A total of ten sorghum varieties were evaluated in this study. Sorghum seeds were surface sterilized (disinfected) with sodium hypochlorite (Na_2HCl) solution for 3 min at room temperature and then thoroughly washed for 5 min with distilled water and these seeds were used for this experiment.

Experimental details and Design

The experiment was carried out with ten sorghum varieties *vi.,* CSV-21, CSV-32F, CSV-38F, CSV-33MF, CSH-24MF, PC-6, PC-615, MP-CHARI, CO31TFL and HJ-541 with different salinity levels *i.e.* 0 as control (DDH_2O), 50, 100,150 and 200 mM as prepared by mixing required NaCl with distilled water according to Dheeravathu *et al.,* (2018). For each treatment, 25 seeds from each variety are utilized. 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 respective salt concentration. Small amount (10 g) 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 (Fig.1).

Observations were recorded on root length (RL), shoot length (SL), shoot fresh weight (SFW), root fresh weight (RFW), seedling dry weight (SDW), Seed salinity vigour Indices-I, II, IV and V.

The number of germinated seeds was counted every day up to 10 days and the seeds were considered germinated when the radicle emerged. At the 10th day (starting of germination), five seedlings were randomly selected and seedling shoot and root length with their fresh weight and dry weight were measured, the fresh weight and dry weight of seedling were obtained after taking the length. For dry weight recording, the seedlings were wrapped in brown paper bags and placed in oven at 80^{o}C for 24 h. The dry weight of the seedlings was recorded.

Determination of germination percent was computed as per the following equations Germination percent = S/T×100.

This formula S is the number of germinated seeds; T is the total number of seeds.

Seed salinity vigour Indices-I, II, IV and V were computed as per Dheeravathu *et al*., (2021a) methodology with the following equations:

Seed salinity vigour Index-I = Final germination (%) × average seedling length [(shoot + root length (cm)].

Seed salinity vigour index-II = Final germination (%) × fresh weight of seedling [(shoot + root (mg)].

Seed salinity vigour Index-IV = Final germination (%) × average shoot length (cm)

Seed salinity vigour Index-V = Final germination (%) × average root length (cm)

STATISTICAL ANALYSIS

Analysis of data was performed with Microsoft Excel using factorial completely randomized design and R for correlation analysis.

RESULTS AND DISCUSSION

Germination percentage and root to shoot ratio

Germination was greatly reduced at the highest level of salt treatment (Table.1). The percent germination recorded above 80% in controls was observed in CSV-21(97%) and CSV-38F (97%) and no variety recorded 100% germination (Table-1). Among all the treatments highest % germination was observed in CSV-38F in all the treatments. 'The data indicated a progressive decline in germination percentage with each increasing level of salinity up to 200 mM over control. . Root to shoot ratio was also affected by salinity. CSV-32F and CSV-38F (3.86 and 2.85) recorded the highest root to shoot ratio at 150 mM salt concentration respectively. Root-to-shoot ratio displayed consistent negative correlations with shoot length (r = –0.47), SVI-1 (r = –0.36), SVI-2 (r = –0.23), and SVI-4 (r = –0.41), indicating that a higher root-to-shoot ratio, often interpreted as a stress-adaptive trait, may be inversely associated with traits representing overall vigor under optimal or controlled conditions. The results were similar with Dheeravathu et al., (2021a) who reported that increase in salinity leads to decrease in germination percentage.

Shoot and root length

Among the ten varieties studied, all varieties showed significant difference with others in terms of their shoot and root length (Table 1). Varieties, CSV-21 showed maximum shoot length followed by PC-615; CSV-32F CO31TFL and CSV-38F (14.6 cm; 10.3 cm; 10.1 cm; 8.6 cm) and maximum root length was observed in PC-615 (5.83 cm) followed by MP-CHARI, CSV-32F, CSV-38F (4.93 cm; 4.47 cm; 4.00 cm); HJ-541 showed minimum shoot and root length (1.7 cm; 0.73 cm) respectively in case of control. shoot length showed a very strong positive relationship with SVI-1 (r = 0.94), SVI-4 (r = 0.95), and root length (r = 0.79), suggesting that shoot elongation plays a dominant role in determining seedling vigor as reflected in these indices. Root length, in turn, exhibited a high positive correlation with SVI-5 (r = 0.92), indicating that root development also contributes notably to the overall seedling performance (Fig.2). Shoot and root length decreased in all varieties with increase in salt concentration to all the treatments.

Seedling fresh and dry weight

Salinity strongly affected the fresh weight and dry weight of the seedlings of all ten sorghum varieties. Maximum seedling fresh weight was observed in CSV-38F (0.23 g) and dry weight was observed in CSV-32F (0.09 g) followed by CSH-24MF (0.09 g). At high salinity levels (200 mM), seedling fresh weight significantly decreased, with the most pronounced reduction observed across all varieties at this concentration. The decrease in fresh weight at 50 mM salinity was comparable to that at 100 mM. Similarly, dry weight declined at higher salinity levels (100 and 200 mM), though the reduction in fresh weight was more pronounced than that of dry weight as salinity increased. These results corroborate with Dheeravathu, S. N., *et al*., (2021) who reported that increasing salinity levels lead to a decline in seedling fresh and dry weight.

Effect of salinity on different seedling vigour indices

Average mean seedling vigour Index-I, II, IV, V was observed more in control CSV-21 (1729.12, 19.88, 1441.21 and 287.65) followed by CSV-38F (1224.93, 22.01, 837.22 and 387.84) and CSV-32F (925.06, 18.06, 642.31, and 283.01) in control respectively(table-1). SVI-1 and SVI-2 showed a perfect correlation (r = 1.00), which may be attributed to the fact that both indices are derived from similar input variables such as seedling length and germination

TABLE 1

Performance of ten sorghum varieties for morphological traits under salinity stress condition.

Variety	Control	50	100	150	200
Germination %					
Concentration(mM)					
CSV-21	97±3.5	88±3.2	76±2.7	52±1.8	36±1.3
CSV-32F	63±3.2	60±3.0	56±2.8	52±2.6	48±2.4
CSV-38F	97±5.6	96±5.4	96±5.4	88±4.2	80±3.5
CSV-33MF	36±1.6	28±1.3	28±1.2	28±1.2	24±1.1
CSH-24MF	32±4.3	24±3.2	12±1.6	12±1.6	12±1.6
PC-6	48±3.0	24±1.5	24±1.4	16±0.9	16±1.0
PC-615	53±9.8	48±10.8	44±8.9	40±8.0	35±7.1
MP-CHARI	64±1.0	53±0.8	53±0.8	36±0.5	32±0.5
CO31TFL	69±9.0	60±8.7	48±6.9	36±5.2	16±2.3
HJ-541	60±8.1	32±4.8	28±4.2	24±3.6	16±2.4
Mean shoot length (cm)					
CSV-21	14.9±2.6	1.8±0.4	0.6±0.1	0.62±0.2	0.10±0.0
CSV-32F	10.1±3.2	1.1±0.1	1.1±0.3	0.23±0.1	0.17±0.1
CSV-38F	8.6±1.9	2.0±0.5	0.9±0.1	0.41±0.1	0.30±0.0
CSV-33MF	1.2±0.7	0.4±0.0	0.3±0.7	0.13±0.1	0.13±0.1
CSH-24MF	2.4±0.6	0.7±0.2	0.17±0.3	0.14±0.0	0.10±0.1
PC-6	3.8±1.1	1.0±0.6	0.8±0.1	0.35±0.0	0.27±0.1
PC-615	10.3±3.2	7.8±2.3	2.2±1.6	0.42±0.1	0.00±0.0
MP-CHARI	9.1±1.5	8.0±1.3	6.7±0.9	1.41±0.6	1.17±0.3
CO31TFL	10.0±1.5	8.1±1.4	5.1±0.2	3.50±0.4	0.30±0.2
HJ-541	1.7±0.2	0.2±0.0	0.2±0.1	0.00±0.0	0.00±0.0
Mean Root length (cm)					
CSV-21	2.97±2.1	2.33±0.6	0.97±0.2	0.93±0.5	0.13±0.1
CSV-32F	4.47±2.8	2.27±0.2	2.10±0.8	0.90±0.4	0.43±0.1
CSV-38F	4.00±0.1	2.23±0.2	1.93±0.3	1.23±0.2	0.67±0.2
CSV-33MF	1.83±1.2	1.03±0.1	0.60±0.2	0.23±0.1	0.13±0.1
CSH-24MF	1.80±0.7	1.43±0.9	0.33±0.1	0.30±0.1	0.23±0.1
PC-6	1.50±0.1	1.20±0.2	0.40±0.1	0.40±0.0	0.20±0.1
PC-615	5.83±6.7	2.30±1.1	1.20±0.4	1.03±0.1	0.00±0.0
MP-CHARI	4.93±0.5	2.10±0.5	1.67±0.4	1.17±0.6	0.47±0.2
CO31TFL	3.37±1.5	3.30±1.0	1.43±0.7	0.73±0.4	0.50±0.3
HJ-541	0.73±0.3	0.20±0.1	0.13±0.1	0.00±0.0	0.00±0.0
Seedling Fresh wt (g)					
CSV-21	0.21±0.1	0.10±0.0	0.10±0.0	0.07±0.	0.07±0.0
CSV-32F	0.29±0.1	0.12±0.0	0.10±0.0	0.10±0.0	0.09±0.0
CSV-38F	0.23±0.1	0.12±0.0	0.09±0.0	0.09±0.0	0.08±0.0
CSV-33MF	0.03±0.0	0.03±0.0	0.02±0.0	0.02±0.0	0.02±0.0
CSH-24MF	0.13±0.0	0.11±0.0	0.01±0.0	0.10±0.0	0.08±0.0
PC-6	0.90±0.1	0.06±0.0	0.06±0.0	0.05±0.0	0.05±0.0
PC-615	0.17±0.0	0.13±0.0	0.08±0.0	0.06±0.0	0.04±0.0
MP-CHARI	0.13±0.0	0.07±0.0	0.07±0.0	0.07±0.0	0.04±0.0
CO31TFL	0.06±0.0	0.06±0.0	0.05±0.0	0.05±0.0	0.03±0.0
HJ-541	0.12±0.0	0.11±0.0	0.10±0.0	0.09±0.0	0.09±0.1
Seedling dry weight (g)					
CSV-21	0.07±0.1	0.06±0.1	0.05±0.0	0.04±0.0	0.04±0.0
CSV-32F	0.09±0.1	0.08±0.2	0.07±0.0	0.07±0.1	0.07±0.1
CSV-38F	0.06±0.1	0.05±0.1	0.05±0.0	0.05±0.0	0.04±0.0
CSV-33MF	0.02±0.1	0.02±0.0	0.01±0.0	0.01±0.0	0.01±0.0
CSH-24MF	0.09±0.1	0.09±0.2	0.08±0.0	0.04±0.0	0.04±0.0
PC-6	0.05±0.0	0.05±0.1	0.05±0.0	0.04±0.0	0.03±0.0
PC-615	0.05±0.0	0.04±0.1	0.04±0.0	0.03±0.0	0.03±0.0
MP-CHARI	0.04±0.0	0.04±0.1	0.04±0.0	0.03±0.0	0.02±0.0
CO31TFL	0.02±0.0	0.02±0.0	0.02±0.0	0.01±0.0	0.01±0.0
HJ-541	0.10±0.1	0.08±0.2	0.08±0.0	0.08±0.1	0.01±0.0

contd...

TABLE 1 Contd.

Root: shoot ratio

CSV-21	1.61±0.7	1.56±0.4	1.33±0.2	1.30±0.0	0.20±0.2
CSV-32F	0.44±0.2	2.06±0.1	1.91±0.5	3.86±0.1	2.60±0.1
CSV-38F	0.46±0.7	0.95±0.1	2.15±0.3	2.85±0.4	2.22±0.3
CSV-33MF	1.57±0.1	4.89±0.2	1.50±0.5	1.00±0.4	1.75±0.1
CSH-24MF	0.75±01	1.55±0.1	1.70±0.0	3.00±0.1	1.40±0.1
PC-6	0.13±0.0	2.00±0.1	0.52±0.3	0.67±0.4	1.50±0.2
PC-615	0.63±0.1	0.20±0.0	0.61±0.1	3.12±0.1	0.00±0.0
MP-CHARI	0.55±0.0	0.28±0.3	0.32±0.0	0.82±0.6	0.41±0.1
CO31TFL	0.33±0.4	0.31±0.3	0.28±0.2	0.21±0.1	1.67±0.2
HJ-541	0.42±0.5	0.83±0.1	0.62±0.4	0.00±0.3	0.00±0.0

Seedling salinity vigour index - I

Concentration (mM)

Variety	Control	50	100	150	200
CSV-21	1729.12±62.3	363.73±13.1	119.07±4.2	79.73±2.8	8.40±0.3
CSV-32F	925.06±46.2	202.00±10.1	179.20±8.9	58.93±2.9	28.80±1.4
CSV-38F	1224.93±97.1	374.40±52.1	272.00±19.7	146.67±23.6	77.33±12.4
CSV-33MF	107.28±4.9	49.47±2.7	28.00±1.2	8.47±0.3	7.80±0.4
CSH-24MF	135.74±18.2	44.80±6.3	4.80±0.9	4.80±0.6	4.40±0.6
PC-6	206.17±12.8	69.60±4.3	28.00±1.7	10.00±0.5	8.67±0.6
PC-615	867.98±75.6	544.43±21.1	165.42±33.4	61.91±12.5	0.00±0.0
MP-CHARI	910.85±13.7	542.86±8.1	468.10±7.6	96.11±1.5	53.67±0.8
CO31TFL	915.73±33.2	636.00±92.1	312.00±45.3	152.40±22.1	12.80±1.8
HJ-541	147.01±22.2	11.73±17.1	0.00±0.0	0.00±0.0	0.00±0.0
Seedling salinity vigour index -II					
CSV-21	19.88±0.7	9.15±0.3	5.62±0.2	5.30±0.2	2.66±0.1
CSV-32F	18.06±0.9	7.44±0.4	5.82±03	4.58±0.	4.99±0.2
CSV-38F	22.01±3.5	11.14±1.8	8.83±1.4	7.28±1.2	7.22±1.2
CSV-33MF	0.76±0.0	0.68±0.0	0.62±0.0	0.50±0.0	0.42±0.0
CSH-24MF	4.10±0.6	2.64±0.4	1.37±0.1	1.25±0.2	0.98±0.2
PC-6	43.49±2.7	1.54±0.1	1.49±0.1	0.86±0.1	0.85±0.1
PC-615	9.07±1.8	7.78±1.6	3.88±0.8	2.32±0.3	0.05±0.5
MP-CHARI	8.65±0.1	3.84±0.1	3.47±0.1	2.59±0.0	1.35±0.0
CO31TFL	4.12±0.6	3.42±0.5	2.30±0.3	1.84±0.3	0.54±0.1
HJ-541	7.00±1.1	2.90±0.1	2.71±0.4	2.46±0.4	1.52±0.2
Seedling salinity vigour index - IV					
CSV-21	1441.11±52.0	158.21±5.7	46.12±1.6	31.22±1.1	4.51±0.1
CSV-32F	642.11±32.1	66.23±3.3	62.11±3.1	12.23±0.6	8.61±0.4
CSV-38F	837.12±34.7	192.32±21.1	86.14±3.9	38.21±6.1	24.62±3.9
CSV-33MF	42.14±1.9	11.34±0.4	8.15±0.5	4.25±0.2	3.52±0.1
CSH-24MF	78.14±10.4	18.41±2.4	2.31±0.2	2.24±0.2	1.53±0.3
PC-6	182.21±11.4	23.42±1.4	18.35±1.1	5.21±0.3	4.84±0.3
PC-615	555.22±12.9	460.21±13.2	106.32±2.5	16.23±3.3	0.00±0.0
MP-CHARI	590.21±8.5	425.12±6.4	356.33±5.4	53.22±0.8	38.27±0.6
CO31TFL	689.31±25.1	484.15±7.3	243.31±5.3	126.21±8.3	5.32±0.2
HJ-541	103.13±15.6	6.12±1.0	5.21±0.2	0.00±0.0	0.00±0.0
Seedling salinity vigour index - V					
CSV-21	287.65±10.4	205.33±7.4	73.47±2.6	48.53±1.7	4.80±0.2
CSV-32F	283.01±14.2	136.00±6.8	117.60±5.9	46.80±2.3	20.80±1.0
CSV-38F	387.84±16.4	182.40±19.4	185.60±9.9	108.53±7.5	53.33±8.6
CSV-33MF	65.56±3.0	41.07±1.9	16.80±0.8	5.60±0.2	3.73±0.3
CSH-24MF	58.18±7.8	27.20±3.7	3.60±0.4	2.80±0.5	2.80±0.4
PC-6	46.40±1.5	24.16±2.9	9.60±0.6	6.40±0.2	3.20±0.4
PC-615	313.19±16.4	84.51±17.1	58.96±1.9	45.70±9.2	0.00±0.0
MP-CHARI	320.97±4.8	117.47±1.8	112.13±1.7	43.13±0.7	15.33±0.2
CO31TFL	226.64±13.9	152.00±22.1	68.80±2.5	26.40±3.8	8.00±1.2
HJ-541	43.71±6.6	5.33±0.8	3.73±0.2	0.00±0.0	0.00±0.0

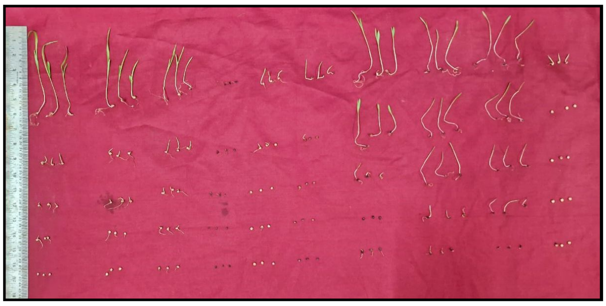

Fig. 1. Ten sorghum varieties subjected to salinity stress.

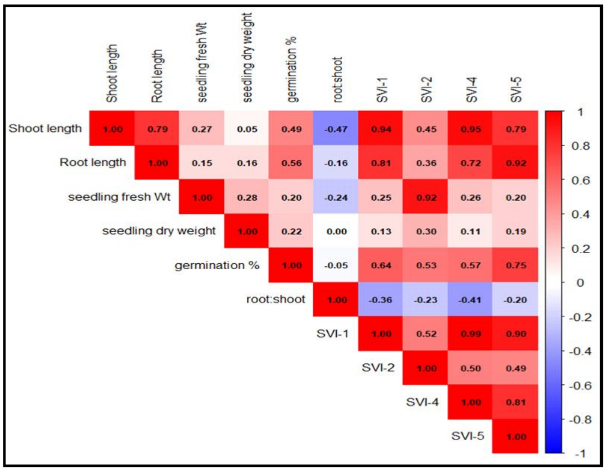

Fig. 2. Correlation matrix for sorghum varieties subjected to salinity stress.

percentage, thereby capturing overlapping aspects of vigor. The results indicated that morphological and physiological characteristics viz. germination %, shoot length, root length, root fresh weight, shoot fresh weight and seedling dry weight are adversely affected by salinity stress. Similar results were observed by Almodares *et al* (2007) and Rehman *et al.*, (2000) who reported that salinity has a negative correlation with germination percentage, germination index, and vigour index in sorghum.

CONCLUSIONS

Salinity stress poses a significant threat to global agriculture production and productivity by adversely affecting various growth stages and physiological processes. salinity stress adversely affected germination, seedling growth, and vigor, with CSV-21, CSV-32F, and CSV-38F showing relatively better tolerance. These findings align with previous studies, confirming that salinity reduces germination percentage, seedling growth, and biomass in sorghum. Further research could focus on identifying salt-tolerant genotypes for cultivation in saline-prone areas.

ACKNOWLEDGEMENT

The authors sincerely acknowledge the ICAR–Indian Institute of Millets Research, Hyderabad, Global Centre of Excellence on Millets (Shree Anna) project and Science and Engineering Research Board (SERB)-Core Research Grant (CRG), New Delhi for support and carrying out the research work for their support and facilitation in carrying out the present research work.

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