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SCREENING OF SORGHUM GERMPLASM FOR HIGH TEMPERATURE STRESS TOLERANCE AT SEEDLING STAGE

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SUMMARY

Heat stress is one of the major abiotic stresses that adversely affect morpho-physiological and growth yield traits. In the present investigation, fifty sorghum accessions were tested for high temperature stress tolerance at seedling stage. Shoot length, shoot, and root fresh weight, shoot, root dry weight and, total shoot and root water content were significantly (p<0.001) affected by high temperature stress. The maximum root to shoot ratio was observed in five accessions *viz*., ECO533402(1.8), ICO319906 (1.5), ICO333362 (1.4), ICO332479 (1.4) and ICO333372 (1.4). Highest total shoot and root fresh weight was recorded in ICO305901(89g; 85g), ICO333356 (84g; 88g) and ICO305887 (83g; 82g) accessions. Among the fifty accessions, five (ICO305910, ICO305903, ICO305887, ECO533402 and ICO305887) accessions were found to exhibit heat stress tolerance compared to other accessions at high temperature (35°C). Positive significant correlation was found between root to shoot ratio (p<0.001).

Key words: Heat stress, sorghum, root to shoot ratio, total shoot water content, total root water content

Global climate change poses significant challenges to crop productivity, particularly under heat stress conditions (IPCC, 2013). Rising temperature and altered precipitation patterns exacerbate heat and drought stress, leading to reduced forage yield, quality, and nutritional value (Ostmeyer *et al*., 2020). Global temperatures are expected to rise by 1.5°C to 3.2°C by 2100, according to the Intergovernmental Panel on Climate Change (IPCC), which would aggravate HTS on crops even more (IPCC, 2013). HTS can reduce productivity and impact food security by causing oxidative stress, interfering with photosynthesis, and hindering the growth of seedlings (Goyal *et al*., 2023; Gao *et al*., 2023). To mitigate the impacts of HTS, identifying and utilizing heat-tolerant sorghum germplasm is crucial (Nori *et al*., 2019).

Sorghum [*Sorghum bicolor* (L.) Moench] is one of the staple cereals and a climate-smart C_4 crop with the ability to produce grain and fodder in challenging conditions with minimal input requirements and high net returns (Hao *et al*., 2021). In terms of production and consumption, it ranks as the fifth most significant cereal crop globally after wheat, maize, rice and barley (Anon., 2020). It ranks third among the major food grain crops of India. Apart from being a major source of carbohydrates for humans, it also serves as a source of cattle feed and has a high potential to compete with crops like maize under better moisture and inputs conditions. It is resilient to diverse environmental conditions with a forte to perform well in marginal conditions under water and temperature constraints without competing with other food crops (Griebel *et al*., 2019). It makes comparatively quick growth and gives not only a decent grain yield but also large quantities of fodder.

In India, sorghum is grown over 9 million ha with a production of over 11 million tonnes (FAO, 2021). It is particularly important in the dryland regions of India for the small-holder farmers and is often grown as a subsistence crop (Anbazhagan *et al*., 2022). Many experiments conducted worldwide suggested that climate has a marked effect on fodder and fodder seed productivity and quality. Currently seed availability of forage crops is only 15- 20%. There is high demand for fodder seed production at present and future as well. A major challenge ahead for those involved in the seed industry, therefore, it is cruvial to provide cultivars that can maximize future crop production in a changing climate (Ainsworth *et al.*, 2008; Bruins 2009; Ceccarelli *et al.*, 2010).

The pros and cons of climate change on cereal crops will have a tremendous influence on food security and climate resilient crops such as grasses: guinea grass, bajra -napier hybrids and tri-specific hybrids, dinanath grass, (Dheeravathu *et al.*, 2018, Singh *et al.*, 2020, Dheeravathu *et al.*, 2021a, Dheeravathu *et al.*, 2021b, Dheeravathu *et al.*, 2021c., Dheeravathu *et a*l., 2022a, Antony *et al.*,2021, Dheeravathu *et al.*, 2022b), pulses: cow pea, berseem, clitoria, centrosema, siratro (Dheeravathuet *et al.*, 2017a, Dheeravathu *et al.*, 2017 b, Dheeravathu *et al.*, 2022c, Dheeravathu *et al.,* 2023), forage cereals including millets: pearl millet and sorghum (Singh *et al.*, 2010, Malathi *et al.*,2022, Amrutha, *et al*, 2023, Dheeravathu and Vadithe, 2024), have been proven to be climate smart. Reduced forage productivity, quality, and nutritional value resulting from heat and drought stress are being made worse by rising temperatures and changing precipitation patterns (Nori *et al.*, 2017). One of the most reliable measures of HTS tolerance is morphological parameters, such as shoot and root length (Goyal *et al.*, 2023). During the seedling stage, the mean optimum temperature range is 21°C to 34°C which is crucial for establishment and growth of sorghum seedling (Singh *et al.*, 2015). High temperature stress (HTS) poses an imminent threat to sorghum productivity. Screening sorghum accessions for HTS tolerance at the seedling stage can help breeders develop heat stress tolerance varieties.

MATERIALS AND METHODS

The study was conducted in research farm 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 germplasm was procured from Gene bank ICAR-IIMR, Hyderabad, India. A total of 50 sorghum accessions were evaluated in this study. Sorghum seeds were surface sterilized (disinfected) with sodium hypochlorite (NaOCl) solution for 3 min and then thoroughly washed for 5 min with distilled water and these seeds were used for the experiments.

Experimental details and Design

The study was carried out from 2nd week of February, 2024 to 3rd week of March, 2024, to screen the accessions for heat stress tolerance in summer season for high temperature. Meteorological weather data (mean high and low temperature) was collected from, Meteorological Weather Division, Agricultural Research Institute (ARI), PJTSAU, Rajendranagar, Hyderabad. (Fig. 1). The experiment was conducted in completely randomized design (CRD) with 50 sorghum genotypes replicated thrice.

Fifty polybags (22×15 cm) were filled with well dried soil collected from the field. The polybags were made small holes to have bottom water drainage and side aeration holes. Five sorghum seeds of each genotype were sown directly at 2 cm depth in each polybag. The excess seedlings were thinned out and two seedlings were maintained in each polybag after emergence. The polybags were irrigated according to the crop's requirement.

Observations were recorded on root length, shoot length, root fresh weight TSWC and TRWC, shoot fresh weight, root dry weight and shoot dry weight. After 35 days of sowing, two seedlings from each replication were taken for recording the observations and the mean was calculated. The shoot of each plant was separated by cutting at the base of the stem. To retrieve the intact root system, the soil was removed with a very low speed water stream and root was washed carefully to remove any adhering soil without harming the root system. The washed seedlings were dried on paper towels and data regarding root length was measured from the tip of the primary root to base of hypocotyl and the shoot length was measured form the tip of the primary leaf to the base of the hypocotyl with the help of a scale and was expressed in centimetre.

Fresh shoot and root weight was measured on digital analytical balance. Shoot and root dry weight was measured after putting shoots and roots in kraft paper bags separately and drying in the oven at 70ºC for constant dry weight (Dheeravathu *et al.,* 2021). The average dry shoot and root weight was then calculated. Total root water content (TRWC) and total shoot water content (TSWC) was calculated by following the method described by Dheeravathu *et al.*, (2018, 2021a) using the below mentioned formula.

$$\text{Total shoot water content (TSWC\%)} : \frac{\text{Shoot Fresh Weight} - \text{Shoot Dry Weight}}{\text{Shoot Fresh weight}} \times 100$$

TABLE 1
Performance of 50 sorghum accessions for morphological traits under high temperature stress condition

Name	Root Lt (cm)	Shoot Lt (cm)	Root: shoot ratio	TSWC (%)	TRWC (%)
ICO305887	25+ 0.66	47 +1.24	0.5+0.01	83+2.2	82+2.2
ICO305891	25+ 0.90	33 +1.19	0.8+0.03	77+2.8	67+2.4
ICO305893	32±0.85	30+0.79	1.1+0.03	762.0	67+1.8
ICO305894	29±0.77	59+1.56	0.5+0.01	81+2.1	74+2.0
ICO305901	26±0.69	42+1.11	0.6+0.02	89+2.4	85+2.3
ICO305902	27±0.54	45+0.90	0.6+0.01	95+1.9	42+0.8
ICO305903	28±0.74	50+1.32	0.6+0.01	80+2.1	74+1.9
ICO305910	28±1.01	60+2.16	0.5+0.02	74+2.7	67+2.4
ICO305912	33±0.87	43+1.14	0.8+0.02	80+2.1	67+1.8
ICO305920	21±0.42	36+0.72	0.6+0.01	74+1.5	47+0.9
ICO305921	27±0.72	35+0.91	0.8+0.02	76+2.0	68+1.8
ICO319848	19±0.69	34+1.23	0.6+0.02	80+2.9	74+2.7
ICO319852	23±0.61	24+0.63	1.0+0.03	80+2.1	70+1.8
ICO319871	22±0.57	52+1.38	0.4+0.01	81+2.2	75+2.0
ICO319873	11±0.28	34+0.90	0.3+0.01	77+2.0	72+1.9
ICO319884	23±0.46	48+0.96	0.5+0.01	75+1.5	48+1.0
ICO319891	30±0.80	24+0.62	1.3+0.03	77+2.0	73+1.9
ICO319894	26±0.92	34+1.21	0.8+0.03	72+2.6	67+2.4
ICO319898	21±0.56	35+0.91	0.6+0.02	80+2.1	79+2.1
ICO319900	20±0.40	19+0.37	1.1+0.02	74+1.5	54+1.1
ICO319904	22±0.57	26+0.69	0.8+0.02	77+2.0	79+2.1
ICO319905	22±0.79	26+0.94	0.8+0.03	71+2.6	77+2.8
ICO319906	39±1.02	25+0.66	1.5+0.04	82+2.2	70+1.9
ICO319907	22±0.58	22+0.58	1.0+0.03	81+2.1	76+2.0
ICO332474	25±0.65	18+0.48	1.4+0.04	83+2.2	81+2.1
ICO332479	26±0.53	19+0.37	1.4+0.03	57+1.1	65+1.3
ICO333353	30±0.78	33+0.87	0.9+0.02	73+1.9	73+1.9
ICO333356	18±0.66	23+0.84	0.8+0.03	84+3.0	88+3.2
ICO333362	37±0.97	26+0.67	1.4+0.04	76+2.0	73+1.9
ICO333366	23±0.45	38+0.75	0.6+0.01	77+1.5	74+1.5
ICO333372	29±0.77	21+0.56	1.4+0.04	83+2.2	76+2.0
ICO333390	32±1.14	25+0.90	1.3+0.05	67+2.4	75+2.7
ICO333391	19±0.50	30+0.79	0.6+0.02	70+1.8	79+2.1
ECO532932	15±0.40	20+0.53	0.8+0.02	74+2.0	55+1.5
ECO532949	21±0.56	29+0.76	0.7+0.02	78+2.1	66+1.7
ECO533109	28±0.56	36+0.72	0.8+0.02	76+1.5	52+1.0
ECO533130	29±0.75	29+0.77	1.0+0.03	74+2.0	57+1.5
ECO533131	25±0.90	27+0.97	0.9+0.03	72+2.6	76+2.7
ECO533132	27±0.71	26+0.68	1.1+0.03	76+2.0	56+1.5
ECO533133	20±0.40	30+0.60	0.7+0.01	73+1.5	51+1.0
ECO533136	28±0.75	38+0.99	0.8+0.02	71+1.9	56+1.5
ECO533141	18±0.66	21+0.76	0.9+0.03	74+2.7	50+1.8
ECO533163	30±0.80	33+0.87	0.9+0.02	77+2.0	64+1.7
ECO533164	23±0.62	30+0.79	0.8+0.02	73+1.9	71+1.9
ECO533167	22±0.58	28+0.73	0.8+0.02	81+2.1	84+2.2
ECO533173	31±0.63	24+0.47	1.3+0.03	74+1.5	57+1.1
ECO533174	18±0.76	26+1.00	0.7+0.02	65+1.7	41+1.1
ECO533196	20±0.72	32+1.15	0.6+0.02	76+2.7	67+2.4
ECO533402	44±1.16	25+0.66	1.8+0.05	78+2.1	76+2.0
ECO533413	21±0.41	21+0.41	1.0+0.02	74+1.5	69+1.4

$$\text{Total root water content (TRWC\%)} : \frac{\text{Root Fresh Weight} - \text{Root Dry Weight}}{\text{Root Fresh weight}} \times 100$$

STATISTICAL ANALYSIS

Analysis of data was performed with Microsoft Excel and SAS 9.3 statistical program using completely randomized design.

RESULTS AND DISCUSSION

The results indicated that morphological characteristics viz. shoot length, root length, root fresh weight, shoot fresh weight, TSWC, TRWC, root to shoot ratio ($p<0.05$) were significantly affected by high temperature stress. The five sorghum accessions viz., ICO305887, ICO305901, ICO305910, ICO305903 and ECO533402 performed well at high temperature stress conditions while lowest was measured in ECO533174 among the 50 sorghum accessions. Strong and significant positive correlations were observed among most biomass-related traits (e.g., Root Fresh Weight and Root Dry Weight, r = 0.819, $p < 0.001$) Fig 2.

Effect of high temperature on seedling shoot and root length and root to shoot ratio

Seedling shoot (SSL), seedling root length (SRL) and root to shoot length ratio (RSR) decreased for all accessions at high temperature (Fig 1). Among the 50 sorghum accessions the maximum shoot and root length were observed in these accessions ICO305910 (60cm), ICO305894 (59cm), ICO319871 (52 cm), ICO305903 (50 cm) and ICO319884 (48 cm); ECO533402 (44 cm),, ICO319906 (38.5 cm), ICO333362 (36.5 cm), ICO305912 (33 cm), and ICO305893(32 cm), respectively (Table- 1).Shoot Length (Shoot Lt) showed a strong positive correlation with Shoot Fresh Weight (Shoot F Wt) (r = 0.819, $p < 0.001$), Fig.2, reflecting a robust association between shoot elongation and biomass. The present investigation revealed significant differences in shoot length among the accessions. The maximum root to shoot ratio was observed in ECO533402, ICO319906 and ICO333362 accessions with the highest mean length of 1.8 cm, 1.5 cm and 1.4 cm, respectively (Table 1). Similar result was also reported by Shinde *et al*., (2013).

Effect of high temperature on shoot and root fresh weight

Among the 50 sorghum accessions, 5 accessions (ICO305910, ICO305887, ICO305903, ICO319871 and ICO305894) recorded higher mean root fresh weight of 11 g, 6.09 g, 4.55 g, 4.50 g and 4.44 g, respectively compared with other accessions whereas higher mean root fresh weight was recorded by ICO305910, ECO533402, ICO305903, ICO305887 and ICO319848 with mean values of 4.18 g, 3.52 g, 2.58 g, 1.92 g and 1.91 g, respectively (Table-2). The correlation between Root Fresh Weight (Root F Wt) and Shoot Fresh Weight (Shoot F Wt) is found to be

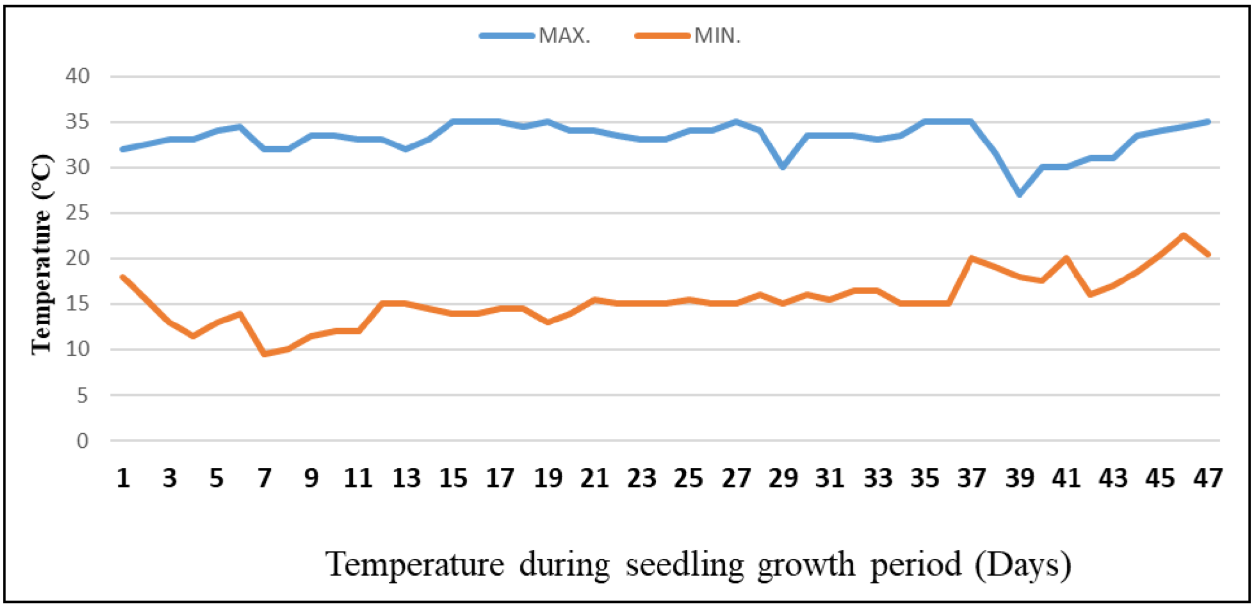

Fig. 1. Temperature during seedling growth period.

TABLE 2
Performance of 50 sorghum accessions for root and shoot fresh and dry weight under high temperature stress conditions.

Name	Root F Wt (g)	Shoot F Wt (g)	Root dry wt (g)	Shoot dry wt (g)
ICO305887	1.92+0.051	6.09+0.16	0.34+0.01	1.01+0.03
ICO305891	0.66+0.024	1.70+0.06	0.22+0.01	0.39+0.01
ICO305893	0.64+0.017	1.31+0.03	0.21+0.01	0.31+0.01
ICO305894	1.58+0.042	4.44+0.12	0.41+0.01	0.86+0.02
ICO305901	0.68+0.018	4.02+0.11	0.10+0.00	0.44+0.01
ICO305902	1.39+0.028	4.09+0.08	0.81 +0.06	0.22+0.00
ICO305903	2.58+0.068	4.55+0.12	0.68+0.02	0.90+0.02
ICO305910	4.18+0.151	11.00+0.40	1.37+0.05	2.82+0.10
ICO305912	1.15+0.030	3.00+0.08	0.38+0.01	0.61+0.02
ICO305920	0.30+0.006	2.10+0.04	0.160+0.00	0.54+0.01
ICO305921	1.10+0.029	2.47+0.07	0.35+0.01	0.60+0.02
ICO319848	1.91+0.069	4.00+0.14	0.49+0.02	0.79+0.03
ICO319852	0.63+0.017	0.70+0.02	0.19+0.01	0.14+0.00
ICO319871	1.66+0.044	4.50+0.12	0.41+0.01	0.84+0.02
ICO319873	0.25+0.007	0.96+0.03	0.07+0.00	0.22+0.01
ICO319884	0.58+0.012	2.45+0.05	0.30+0.01	0.61+0.01
ICO319891	0.75+0.020	1.45+0.04	0.20+0.01	0.33+0.01
ICO319894	1.32+0.048	1.86+0.07	0.43+0.02	0.53+0.02
ICO319898	1.68+0.044	2.87+0.08	0.36+0.01	0.57+0.02
ICO319900	0.26+0.005	1.38+0.03	0.12+0.00	0.36+0.01
ICO319904	0.99+0.026	1.31+0.03	0.21+0.01	0.30+0.01
ICO319905	0.99+0.036	1.01+0.04	0.23+0.01	0.29+0.01
ICO319906	1.40+0.037	2.64+0.07	0.42+0.01	0.48+0.01
ICO319907	0.78+0.021	1.24+0.03	0.19+0.01	0.24+0.01
ICO332474	0.74+0.020	0.93+0.02	0.14+0.00	0.16+0.00
ICO332479	0.20+0.004	0.23+0.00	0.07+0.00	0.10+0.00
ICO333353	1.81+0.048	2.25+0.06	0.48+0.01	0.61+0.02
ICO333356	0.25+0.009	0.37+0.01	0.03+0.00	0.06+0.00
ICO333362	1.28+0.034	0.82+0.02	0.35+0.01	0.20+0.01
ICO333366	0.87+0.017	1.99+0.04	0.23+0.00	0.46+0.01
ICO333372	0.99+0.026	1.18+0.03	0.24+0.01	0.20+0.01
ICO333390	1.58+0.057	1.60+0.06	0.39+0.01	0.53+0.02
ICO333391	0.63+0.017	1.05+0.03	0.13+0.00	0.32+0.01
ECO532932	0.20+0.005	0.70+0.02	0.09+0.00	0.18+0.00
ECO532949	0.47+0.012	1.57+0.04	0.16+0.00	0.34+0.01
ECO533109	0.54+0.011	2.74+0.05	0.26+0.01	0.66+0.01
ECO533130	0.56+0.015	3.14+0.08	0.24+0.01	0.81+0.02
ECO533131	1.19+0.043	1.13+0.04	0.29+0.01	0.32+0.01
ECO533132	0.55+0.015	1.34+0.04	0.24+0.01	0.32+0.01
ECO533133	1.00+0.020	1.75+0.04	0.49+0.01	0.48+0.01
ECO533136	0.45+0.012	2.40+0.06	0.20+0.01	0.69+0.02
ECO533141	0.20+0.007	0.68+0.02	0.10+0.01	0.18+0.01
ECO533163	0.76+0.020	1.64+0.04	0.27+0.01	0.38+0.01
ECO533164	0.91+0.024	1.96+0.05	0.26+0.01	0.52+0.01
ECO533167	1.28+0.034	2.18+0.06	0.20+0.01	0.41+0.01
ECO533173	0.47+0.009	1.00+0.02	0.20+0.00	0.26+0.01
ECO533174	0.17+0.030	1.20+0.20	0.10+0.01	0.42+0.03
ECO533196	0.45+0.016	1.89+0.07	0.15+0.01	0.45+0.02
ECO533402	3.52+0.093	1.59+0.04	0.83+0.02	0.35+0.01
ECO533413	0.64+0.013	1.08+0.02	0.20+0.00	0.28+0.01

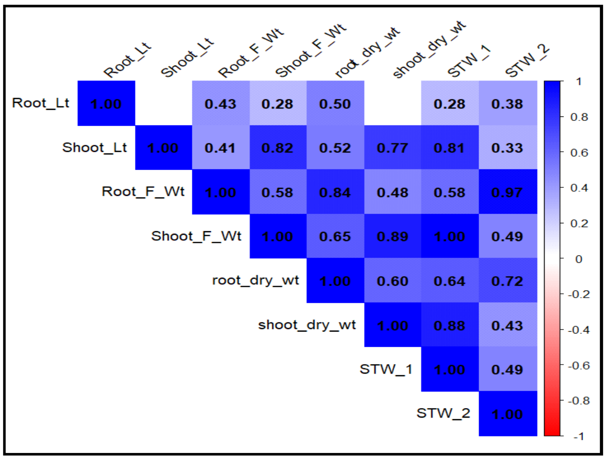

Fig. 2. Correlation among different parameters upon temperature stress in sorghum accessions.

Parameters: Root_Lt: Root length; Shoot_Lt: Shoot length; Root_F_Wt: Root fresh weight; root_dry_Wt: Root dry weight; Shoot_F_Wt: Root fresh weight; shoot_dry_Wt: Shoot dry weight; STW_1: Total shoot water content; STW_2: Total root water content

substantial (r = 0.708, $p < 0.001$), Fig. 2, indicating a significant positive relationship and suggesting that increased root biomass is associated with greater shoot biomass accumulation. These findings support prior research showing that seedlings exposed to abiotic stress have lower fresh and dry weight in berseem and sweet William (Dianthus barbatus) Azizi *et al.,* 2011; Dheeravathu *et al.*, 2021a).

Effect of high temperature on shoot and root dry weight

Deshmukh *et al.*, (2024) in their study reported that total biomass influences the development and physiological maturity of the genotypes. The data revealed that 5 accessions (ICO305910, ICO305887, ICO305903, ICO305894 and ICO319871) recorded higher mean shoot dry weight of 2.82 g, 1.01 g, 0.90 g, 0.86 g and 0.84 g, respectively compared with other accessions. Higher mean root dry weight was recorded in ICO305910, ECO533402, ICO305902, ICO305903 and ICO319848 accessions with values of 1.37 g, 0.83 g, 0.81 g, 0.68 g and 0.49 g, respectively (Table 2) compared with other accessions. The correlation between Root Fresh Weight (Root F Wt) and Shoot Fresh Weight (Shoot F Wt) is substantial (r = 0.708, $p < 0.001$), indicating a significant positive relationship Fig. 2 and suggesting that increased root biomass is associated with greater shoot biomass accumulation.

Effect of high temperature on total water content in shoot and root

The traits like total shoot water content and total root water content benefit the accessions to survive in hotter and drier growing seasons. Among the accessions, ICO305902 recorded the highest total shoot water content of 95%, where ICO305901, ICO333356 and ICO305887 recorded the high root and shoot water content with the per cent of 89, 84 and 83; 89, 88 and 82 respectively (Table 1). Rapid water loss from the plant surface due to high temperature stress is often associated with dehydration which leads to a decrease in the total root and shoot water content. Our results are in conformity with (Machado and Paulsen, 2001) who reported that heat stress results in rapid water loss from plant surface.

High temperature stress adversely affects the plant processes like germination, growth and establishment of the sorghum seedlings. Our results showed that shoot and root dry weight, declined for all accessions. These results agreed with prasad *et al.* (2021) who reported that HT stress has a major detrimental impacts on various morphological and physiological changes that effects sorghum seedling growth.

CONCLUSIONS

High temperature stress causes huge loses to global agriculture production and productivity. Plant abiotic stress tolerance is a complex trait that involves various morphological, physiological and biochemical mechanisms involving various genes. The results obtained in this study suggests the importance of high temperature stress on morpho-physiological traits of sorghum accessions at seedling stage. All the tested attributes were significantly affected by high temperature stress. High temperature reduces the shoot length, shoot dry and fresh weight, TSWC, TRWC, shoot to root ratio, root length, root fresh and dry weight during the seedling stage. Five sorghum accessions *viz*., ICO305887, ICO305901, ICO305910, ICO305903 and ECO533402 performed well at high temperature stress conditions among 50 sorghum accessions. The strong correlations among fresh and dry weights across both root and shoot systems emphasize the close coupling of tissue moisture content and biomass in plant development. Considering the superiority of these identified accessions at seedling stage, they may be used for further detail study for high temperature tolerance mechanisms and varietal development in sorghum.

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