Forage Res., **51**(1) : pp. 144-149 (2025) **http://forageresearch.in**

EFFECT OF HEAT STRESS ON SORGHUM GENOTYPES AT SEEDLING STAGE

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(Received: 5 June 2025; Accepted: 27 June 2025)

SUMMARY

Heat stress is a significant abiotic factor that negatively impacts morphological, physiological, and growth-related yield traits in plants. seedling shoot length (SSL), root length (SRL), and root-to-shoot ratio (RSR) across 42 sorghum genotypes, consistent with established thermos-inhibition responses. Notable genotypic variation emerged, with hybrids CSH 16 and CSH 45, *kharif* variety CSV 27, and rabi varieties CSV 22 and M35-1 exhibiting superior shoot and root growth. Genotypes CSH 45, CSV 49, and AKMS 14A showed the highest RSR (1.04–1.12), suggesting adaptive root allocation under stress. Biomass analysis revealed CSH 41, AKR 150, and AKMS 14B as top performers in fresh weight (4.82 g), while CSV 17 and NR 10-15 led in dry weight (1.32 g). Strong correlations were observed between root and shoot traits ($r = 0.68–0.91$, $p < 0.001$), highlighting growth interdependence. Water content analysis identified CSH 48, CSV 27, and AKMS 14B maintaining high shoot (84.85%) and root (85.45%) water content, critical for moisture-scarce environments. Significant correlations between water content and biomass ($r = 0.43–0.76$) underscore the role of hydraulic efficiency in stress adaptation.

Key words: High-temperature stress, root toshoot ratio, total shoot water content, total root water content

Global climate change poses a significant threat to agricultural productivity, with increasing temperatures and unpredictable weather patterns intensifying heat stress conditions (IPCC, 2021). Rising temperatures, coupled with erratic rainfall, exacerbate heat and drought stress, leading to substantial reductions in crop yield, forage quality, and nutritional value (Ostmeyer et al., 2020). The optimal temperature range for sorghum seedling establishment and growth is between 21°C and 34°C; however, temperatures exceeding this threshold severely impair germination, seedling vigor, and subsequent crop performance (Dheeravathu *et al.*, 2024). Projections from the Intergovernmental Panel on Climate Change (IPCC) indicate that global temperatures may rise by 1.5°C to 3.2°C by the end of the century, further aggravating heat stress impacts on crops (IPCC, 2021). At the seedling stage, HTS disrupts critical physiological processes, including photosynthesis, membrane stability, and enzyme activity, while inducing oxidative damage through the accumulation of reactive oxygen species (ROS) (Goyal *et al.*, 2023). These adverse effects lead to stunted growth, poor stand establishment, and ultimately, significant yield losses (Dheeravathu *et al.*, 2022b).

Sorghum [*Sorghum bicolor* (L.) Moench] stands as a vital cereal crop and climate-resilient C4 species, renowned for its ability to yield both grain and fodder under adverse conditions with low input demands and favorable economic returns (Hao *et al.*, 2021). Globally recognized as the fifth most important cereal after wheat, maize, rice and barley (Anon., 2020), it holds third position among India's primary food grain crops. Beyond serving as a crucial carbohydrate source for human nutrition, sorghum provides valuable livestock feed and demonstrates competitive production potential against crops like maize when provided with adequate moisture and inputs (Griebel *et al.*, 2019). This drought- and heat-tolerant crop exhibits remarkable adaptability to marginal environments, thriving where other food crops fail, while offering the dual advantage of rapid growth, substantial grain production, and abundant fodder output. Sorghum cultivation in India spans approximately 9 million hectares, yielding over 11 million tonnes annually (FAO, 2021). This crop holds particular significance for small-scale farmers in India's arid regions, where it serves primarily as a subsistence crop (Dheeravathu et al 2024). Global research indicates that climatic factors significantly influence both the yield and quality of fodder and fodder seed production. This deficit highlights the growing need for enhanced fodder seed production to meet current and future requirements. Consequently, a key challenge facing the seed sector involves developing improved cultivars capable of optimizing agricultural output under evolving climatic conditions (Dheeravathu et al 2024).

Climate resilient crops such as grasses: Guinea grass, Bajra -Napier hybrids and Tri-specific hybrids, Dinanath grass, (Singh *et al.*, 2021; Dheeravathu *et al.,* 2021a;Dheeravathu *et al.,* 2022a; Dheeravathu et al.,2022d, Dheeravathu *et al.,* 2022b), pulses: cowpea,berseem, clitoria, centrosema, siratro (Dheeravathu *et al.,* 2017a; Dheeravathu *et al.*, 2017 b; Dheeravathu *et al.*, 2021a; Dheeravathu *et al.*, 2021c; Dheeravathu *et al.*, 2022b; Dheeravathu *et al.*, 2022c, Dheeravathu *et al.*, 2023), forage cereals including millets: pearl millet, kodo millet and sorghum (Djanaguiraman, *et al.*, 2019;Dheeravathu *et al.,* 2022a, Dheeravathu *et al.*, 2024a and b), have been proven to be climate smart. Given these challenges, developing heat-tolerant sorghum varieties is essential to safeguarding food security under future climate scenarios (Nori et al., 2020). Recent studies highlight genetic variability in sorghum germplasm, with certain genotypes exhibiting enhanced thermotolerance through improved antioxidant defense mechanisms and osmotic adjustment (Dheeravathu *et al., 2022*). Identifying such resilient genotypes is crucial for breeding programs aimed at improving sorghum adaptation to rising temperatures. This study seeks to evaluate the physiological and morphological responses of different sorghum genotypes to heat stress at the seedling stage, providing insights into key traits associated with thermotolerance. The findings will contribute to the selection and development of improved sorghum varieties capable of sustaining productivity under increasing temperature stress.

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. The sorghum genotypes were procured from ICAR-IIMR, Hyderabad, India. A total of 42 sorghum genotypes which contain *hybrids*, *kharif*, *rabi* and *lines* 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. 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) data 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 42 sorghum genotypes replicated thrice.

Fifty polybags (22×15 cm) were filled with well dried soil collected from the field and FYM. The polybags were drilled at bottom for water drainage and side aeration holes. Five sorghum seeds of each genotype were sown directly in each polybag 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, shoot and root fresh weight, shoot and root 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 or root weight was measured on digital analytical balance. Dry shoot and root 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.

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

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

STATISTICAL ANALYSIS

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

TABLE 1

Performance of sorghum genotypes for morphological traits under high temperature stress condition

Name	Shoot Lt (cm)	Root Lt (cm)	Root: shoot ratio	TSWC (%)	TRWC (%)
CSH 14	26.90±0.71	16.70±0.44	0.62±0.02	64.35±1.70	34.29±0.91
CSH 16	29.50±1.06	23.00±0.83	0.78±0.03	74.11±2.67	49.21±1.77
CSH 25	26.90±0.17	21.00±0.56	0.78±0.02	72.37±1.91	68.75±1.82
CSH 30	31.50±0.83	22.00±0.58	0.70±0.02	34.48±0.91	55.00±1.46
CSH 41	28.00±0.74	20.50±0.54	0.73±0.02	47.66±1.26	82.81±2.19
CSH 45	28.50±0.57	29.50±0.59	1.04±0.02	72.99±1.46	65.43±1.31
CSH 48	25.00±0.66	23.00±0.61	0.92±0.02	84.85±2.24	85.45±2.26
CSV 17	31.00±0.82	29.50±0.78	0.95±0.03	78.03±2.06	67.18±1.78
CSV 20	24.00±0.87	22.50±0.81	0.94±0.03	67.62±2.44	65.12±2.35
CSV 27	33.60±0.89	24.65±0.65	0.73±0.02	83.97±2.22	70.94±1.88
CSV 36	29.00±0.77	22.00±0.58	0.76±0.02	75.00±1.98	58.90±1.56
CSV 39	27.00±0.71	28.50±0.75	1.06±0.03	80.53±2.13	60.00±1.59
CSV 41	18.55±0.37	20.75±0.41	1.12±0.02	79.44±1.59	57.28±1.15
CSV 22	39.90±1.06	29.00±0.77	0.73±0.02	79.20±2.10	75.71±2.00
CSV 23	32.00±1.15	21.00±0.76	0.66±0.02	80.00±2.88	65.49±2.36
CSV 26	21.80±0.58	24.25±0.64	1.11±0.03	56.92±1.51	65.85±1.74
CSV 29	34.50±0.91	20.50±0.54	0.59±0.02	80.00±2.12	46.97±1.24
M35-1	33.00±0.87	22.00±0.58	0.67±0.02	75.20±1.99	66.67±1.76
SPV 2758	36.00±0.72	24.00±0.48	0.67±0.01	82.21±1.64	65.57±1.31
SPV 2036	26.50±0.70	27.00±0.71	1.02±0.03	88.61±2.34	56.25±1.49
BJV 44	20.50±0.74	22.30±0.80	1.09±0.04	53.33±1.92	77.78±2.80
SPV 2217	32.00±0.85	16.50±0.44	0.52±0.01	94.44±2.50	40.00±1.06
CR 54	31.00±0.62	21.00±0.42	0.68±0.01	75.71±1.51	37.50±0.75
AKR 150	35.00±0.93	29.00±0.77	0.83±0.02	78.35±2.07	66.14±1.75
CB 33	34.00±1.23	24.00±0.87	0.71±0.03	78.99±2.85	62.90±2.27
C 43	31.00±.82	21.00±0.56	0.68±0.02	75.17±1.99	70.66±1.87
NR 10-15	33.00±0.87	22.00±0.58	0.67±0.02	78.00±2.06	48.39±1.28
NR 12-11	31.00±0.82	13.00±0.34	0.42±0.01	77.91±2.06	62.22±1.65
AKMS 14A	28.00±0.74	30.00±0.79	1.07±0.03	83.43±2.21	72.31±1.91
AKMS 14B	34.00±1.23	30.50±1.10	0.90±0.03	80.08±2.89	57.35±2.07
27A	31.00±0.82	32.00±0.85	1.03±0.03	78.15±2.07	64.04±1.69
27B	32.00±0.85	25.00±0.66	0.78±0.02	78.15±2.07	62.14±1.64
28A	32.00±0.85	24.00±0.63	0.75±0.02	75.09±1.99	69.00±1.83
28B	33.00±0.66	26.00±0.52	0.79±0.02	78.08±1.56	71.91±1.44
415A	20.40±0.54	16.00±0.42	0.78±0.02	79.37±2.10	47.06±1.25
415B	35.50±1.28	24.00±0.87	0.68±0.02	34.38±1.24	77.27±2.79
461A	35.00±0.91	17.30±0.46	0.05±0.00	71.14±1.88	64.81±1.71
461B	34.50±0.69	30.00±0.60	0.87±0.02	77.91±1.56	71.15±1.42
151A	35.00±0.93	20.00±0.53	0.57±0.02	80.91±2.14	71.57±1.89
151B	28.00±1.01	37.50±1.35	1.34±0.05	75.23±2.71	65.00±2.34
2219A	34.00±0.90	24.00±0.63	0.71±0.02	77.06±2.04	50.00±1.32
2219B	40.50±1.07	26.00±0.69	0.64±0.02	72.64±1.92	67.63±1.79

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.

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 [(35 °C) (Fig 1)]. Among the hybrids maximum shoot and root length were observed in CSH 16 and CSH 45 (29.5, 28.5; 23, 29.5), in kharif variety CSV 27 (36.6, 31), in rabi varieties CSV 22 and M35-1 (39.9, 33; 29, 22) in R and P lines AKR 150 and AKMS 14B (35, 34; 29, 30.5) respectively (Table-1). The present investigation revealed significant differences in shoot length among the accessions. The maximum root to shoot ratio was observed in CSH 45, CSV 49, CSV26, AKR 150 and AKMS 14A genotypes with the highest mean ratio of 1.04, 1.12, 1.11, 0.83 and 1.07 respectively (Table-1). Similar results were also reported by Wahid et al., (2007) who said that decline in seedling shoot length (SSL), seedling root length (SRL), and root-to-shoot ratio (RSR) under high-temperature stress in sorghum with established thermoinhibition responses in cereals, where elevated temperatures impair cellular elongation and division processes. Root Length was positively correlated with Shoot Length ($r = 0.68$, $p < 0.001$), Root Fresh Weight ($r = 0.35$, $p < 0.05$), and both Shoot Fresh and Dry Weights ($r = 0.32$ and $r = 0.29$, respectively, $p < 0.05$), indicating that overall plant growth traits are interrelated.

Shoot and root fresh weight and dry weight

Among the 42 sorghum genotypes CSH 41 and CSH 45 (2.21gm and 0.81 gm) recorded maximum root fresh weight in hybrid, CSV 27 (3.14 and 2.87) in kharif variety, M35-1, CSV 22 (1.20 and 0.70; 2.46 and 2.74 gm) in rabi variety, in R and P lines C 43 and AKMS 14A (1.27, 2.42); AKR 150 and AKMS 14B (4.25, 4.82) recorded the maximum root and shoot fresh weight compared with other genotypes whereas higher root and shoot dry weight was recorded by CSV 16 (0.3, 0.57 gm), CSV 17 (0.4, 0.69 gm), M35-1 (0.4, 0.61 gm), NR 10-15 (0.64, 1.32 gm) and AKMS 14B (0.58, 0.96 gm) respectively (Table 2). These results corroborate previous studies demonstrating reduced fresh and dry weights in seedlings subjected to abiotic stress, as observed in berseem (*Trifolium alexandrinum*) (Dheeravathu et al., 2021a). A highly significant and strong positive correlation was observed between Root Fresh Weight (Root.F.WT) and Shoot Fresh Weight (Shoot.F.WT) ($r = 0.91$, $p < 0.001$), as well as with Shoot Dry Weight (Shoot.Dry.WT) ($r = 0.87$, $p < 0.001$), indicating that plants with greater root biomass also tend to have more shoot biomass (Fig 2).

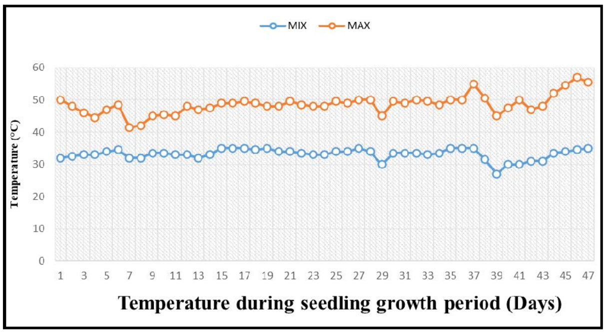

Fig. 1. Temperature during seedling growth period.

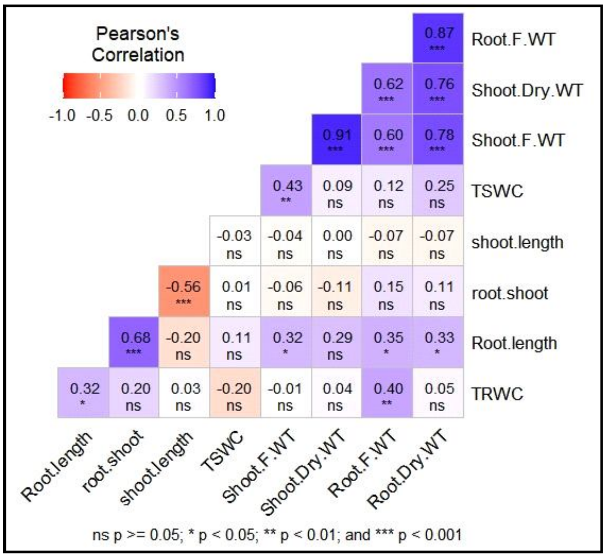

Fig. 2. Correlation among different parameters upon temperature stress in sorghum accessions.

Parameters: Root Length; 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; TSWC: Total shoot water content; TRWC: Total root water content.

Total shoot and root water content

The traits like total shoot water content and total shoot water content benefit the genotypes to survive in hotter and drier growing seasons. Among

TABLE 2

Performance of sorghum genotypes for shoot and root fresh and dry weight under high temperature stress conditions

Name	Root Fresh wt. (g)	Shoot Fresh wt. (g)	Root dry wt. (g)	Shoot dry wt. (g)
CSH 14	0.35±0.01	1.15±0.03	0.2±0.01	0.41±0.01
CSH 16	0.63±0.02	2.24±0.08	0.3±0.01	0.58±0.02
CSH 25	0.32±0.01	0.76±0.02	0.1±0.00	0.21±0.01
CSH 30	0.20±0.01	0.58±0.02	0.1±0.00	0.38±0.01
CSH 41	2.21±0.06	1.07±0.03	0.4±0.01	0.56±0.01
CSH 45	0.81±0.02	2.11±0.04	0.3±0.01	0.57±0.01
CSH 48	0.55±0.01	0.99±0.03	0.1±0.00	0.15±0.00
CSV 17	1.31±0.03	3.14±0.08	0.4±0.01	0.69±0.02
CSV 20	0.43±0.02	1.05±0.04	0.2±0.01	0.34±0.01
CSV 27	1.17±0.03	2.87±0.08	0.3±0.01	0.46±0.01
CSV 36	0.73±0.02	1.72±0.05	0.3±0.01	0.43±0.01
CSV 39	0.35±0.01	1.13±0.03	0.1±0.00	0.22±0.01
CSV 41	1.03±0.02	1.80±0.04	0.4±0.01	0.37±0.01
CSV 22	0.70±0.02	2.74±0.07	0.2±0.00	0.57±0.02
CSV 23	1.13±0.04	5.20±0.19	0.4±0.01	1.04±0.04
CSV 26	0.41±0.01	0.65±0.02	0.1±0.00	0.28±0.01
CSV 29	0.66±0.02	1.45±0.04	0.4±0.01	0.29±0.01
M35-1	1.20±0.03	2.46±0.07	0. ±40.01	0.61±0.02
SPV 2758	0.61±0.01	2.53±0.05	0.2±0.00	0.45±0.01
SPV 2036	0.48±0.01	1.58±0.04	0.2±0.01	0.18±0.00
BJV 44	0.27±0.01	0.15±0.01	0.1±0.00	0.07±0.00
SPV 2217	0.15±0.00	1.26±0.03	0.1±0.00	0.07±0.00
CR 54	0.32±0.01	2.10±0.04	0.2±0.00	0.51±0.01
AKR 150	1.27±0.01	4.25±0.11	0.4±0.01	0.92±0.02
CB 33	1.24±0.03	3.95±0.14	0.5±0.02	0.83±0.03
C 43	1.67±0.02	3.02±0.08	0.5±0.01	0.75±0.02
NR 10-15	1.24±0.03	6.00±0.15	0.64±0.02	1.32±0.03
NR 12-11	0.45±0.00	1.72±0.04	0.17±0.00	0.38±0.01
AKMS 14A	2.42±0.06	3.56±0.09	0.67±0.02	0.59±0.02
AKMS 14B	1.36±0.05	4.82±0.17	0.58±0.02	0.96±0.03
27A	1.14±0.03	2.38±0.06	0.41±0.01	0.52±0.01
27B	1.03±0.03	2.38±0.06	0.39±0.01	0.52±0.01
28A	1.00±0.03	2.65±0.07	0.31±0.01	0.66±0.02
28B	0.89±0.02	2.19±0.04	0.25±0.01	0.48±0.01
415A	0.17±0.00	0.63±0.02	0.09±0.00	0.13±0.00
415B	0.44±0.02	0.64±0.02	0.1±0.00	0.42±0.02
461A	0.54±0.01	1.49±0.04	0.19±0.01	0.43±0.01
461B	0.52±0.01	2.58±0.05	0.15±0.00	0.57±0.01
151A	1.02±0.03	2.41±0.09	0.29±0.01	0.46±0.01
151B	1.20±0.04	2.22±0.11	0.42±0.02	0.55±0.02
2219A	0.14±0.00	1.70±0.10	0.07±0.00	0.39±0.01
2219B	1.39±0.04	3.29±0.10	0.45±0.01	0.9±0.02

the genotypes, CSH 48 (84.85, 85.45), CSV 27 (83.97, 70.94), CSV 22 (79.2, 75.71), AKR 150 (78.35, 66.14) and AKMS 14B (80.08, 72.31) respectively (Table-1) from hybrid, kharif, rabi, R and P-lines recorded the maximum shoot and root water content. This indicates that these cultivars are likely to perform well under moisture-limiting environments. Root water content is especially crucial for extracting deeper soil moisture, thereby supporting transpiration and nutrient uptake during dry spells (Dheeravathu et al 2023). Shoot Fresh Weight showed a significant positive correlation with Shoot Dry Weight ($r = 0.76$, $p < 0.001$) and Total Shoot Water Content (TSWC) ($r = 0.60$, $p < 0.001$). A moderate yet significant correlation was also found between TSWC and Root Fresh Weight ($r = 0.43$, $p < 0.01$), suggesting that higher shoot water content is associated with increased root biomass.

CONCLUSIONS

High-temperature stress poses significant threats to global agricultural output, leading to substantial yield reductions. Plant resilience to such abiotic stresses is governed by intricate physiological and morphological adaptations mediated by multiple genetic factors. The study demonstrates that high-temperature stress significantly reduces seedling shoot length (SSL), root length (SRL), and root-to-shoot ratio (RSR) across all sorghum genotypes. Despite the reduction, genotypic variability was evident, with hybrids (CSH 16, CSH 45), kharif (CSV 27, and rabi (CSV 22, M35-1) varieties showing superior shoot and root growth, suggesting inherent thermotolerance. Notably, genotypes with higher RSR (CSH 45, CSV 49, AKMS 14A) exhibited adaptive root allocation, potentially enhancing water and nutrient uptake under stress. Biomass accumulation further highlighted stress resilience, with CSH 41, KMS 14B recording high fresh and dry weights. Collectively, these findings identify promising genotypes for breeding programs aimed at enhancing sorghum resilience to heat, addressing climate change challenges in arid agroecosystems.

ACKNOWLEDGEMENTS

The authors acknowledge Acharya N. G. Ranga Agricultural University (ANGRAU), Lam, Guntur and Indian Council of Agricultural Research (ICAR)-ICAR-Indian Institute of Millets Research, Hyderabad for support and carrying out the research work.

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