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IMPACT OF COMBINED WATER DEFICIT AND HIGH TEMPERATURE STRESS DURING EARLY SEEDLING STAGE OF FINGER MILLET [*ELEUSINE CORACANA* (L.) GAERTN.]

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ABSTRACT

The finger millet crop grown in summer season in the Indo-Gangetic plains of Indian subcontinent experience drought and heat stress which is detrimental for crop growth and yield. In the present investigation, effect of simultaneous water deficit (WD) and high temperature (HT) stress on germination and seedling growth of thirty finger millet genotypes was assessed. Polyethylene glycol (PEG) 6000 solution of 10% and 20% concentration was used to simulate water deficit stress, while high temperature stress was given at $37\pm 1^{\circ}\text{C}$. Germination percentage, Timson germination index, speed of germination, vigour indices I & II, plant height and dry matter stress indices were all positively correlated with each other while they all showed strong negative correlation with tolerance index. These parameters contributed the most to first principal component which explained 81.60% of total variance. Root shoot length ratio and dry weight ratio were mutually correlated and they contributed the most to second principal component which elucidated 15.15% of total variance. Among the genotypes under study, BR 407, RAU 3, RAU 8 and RAU F-13 exhibited the greatest tolerance, especially towards the combined stress conditions imposed. The findings of the study would aid in genetic enhancement of finger millet to develop higher resilience towards multiple abiotic stresses.

Key words : Finger millet, High temperature, Seedling stage, Stress tolerance indices, Vigour indices, Water deficit.

Introduction

The global mean temperature has risen by 1.1°C in the last decade of 2011-2020 as compared to the period of 1850-1900 due to unprecedented anthropogenic activities (Lee and Romero, 2023). Agricultural productivity of crops is dwindling due to various abiotic stresses among which heat and drought stresses are the most notorious and widespread of all (Sun *et al.*, 2020). In the Indo-Gangetic plains of Indian subcontinent, there is prevalence of heat and drought stress in the summer season (February-March to May-June), which affects the germination, growth and yield of crops grown in this season. Finger millet [*Eleusine coracana* (L.) Gaertn.]

is one such crop, which faces these stresses simultaneously, when grown in summer season in this region. Individual effect of drought (Bhatt *et al.*, 2011) and heat (Opole *et al.*, 2018) has been studied on this crop previously. However, the study of impact of combined heat and drought stress on finger millet would be more relatable and informative for the agriculturists and farming community. Thus, keeping the aforementioned facts in view, the present investigation was conducted to study the impact of simultaneous drought and heat stress on genotypes of finger millet at the seedling stage to assess the genotypic variation and stress tolerance. The findings of the study would be helpful for genetic enhancement

of finger millet for improving tolerance to these abiotic stresses.

Materials and Methods

Seeds of thirty genotypes of finger millet (BR 407, Rajendra Madua 1, RAU 3, RAU 8, RAU F-13, RAU F-15, RAU F-1, RAU F-2, RAU F-3, RAU F-4, RAU F-5, RAU F-6, RAU F-7, RAU F-8, RAU F-9, RAU F-10, RAUF-11, RAUF-12, STF-1, STF-2, STF-3, STF-4, STF-5, STF-6, STF-7, STF-8, STF-9, STF-10, STF-11, STF-12) were procured from Small Millets Section, RPCAU, Dholi, Muzaffarpur, Bihar, India. The laboratory experiment was conducted in the Department of Botany, Plant Physiology & Biochemistry, College of Basic Sciences & Humanities, RPCAU, Pusa, Samastipur, Bihar, India. The seeds were surface sterilized by application of 0.1% mercuric chloride (HgCl_2) solution for 2-3 minutes followed by washing in distilled water. Polyethylene glycol (PEG) 6000 10% and 20% solutions were used for inducing water deficit (WD) stress. High temperature (HT) stress was applied by keeping the seeds in seed germinator at a temperature of $37 \pm 1^\circ\text{C}$. Therefore, the treatments applied on the seeds were control (water at $27 \pm 1^\circ\text{C}$), WD PEG 10% (-41 kPa at $27 \pm 1^\circ\text{C}$), WD PEG 20% (-83 kPa at $27 \pm 1^\circ\text{C}$), HT (water at $37 \pm 1^\circ\text{C}$), HT+WD PEG 10% (-43 kPa at $37 \pm 1^\circ\text{C}$) and HT+WD PEG 20% (-86 kPa at $37 \pm 1^\circ\text{C}$) with three replications. The experiment was set up in petri plates with each replication having 10 seeds of finger millet. The germination percentage was recorded daily up to 15 days from the commencement date of experiment. Seedling growth parameters like root and shoot length and dry weight were recorded on the 15th day of experiment. Length was measured with the aid of scale ruler, while dry weight was measured on electronic weighing balance (with precision of 0.1 mg) after drying the seedlings in hot air oven at $65 \pm 2^\circ\text{C}$ for one day.

The parameters recorded from the experiment along with their formulae are as follows: Germination percentage (GP) [on 15th day] = [Number of germinated seeds/ Total number of seeds] \times 100; Timson germination index (TGI) = [GP on 1st day + GP on 2nd day + ... + GP on 15th day] / Number of days (Timson, 1965); Speed of germination (SOG) = [GP on 1st day/1] + [GP on 2nd day/2] + ... + [GP on 15th day/15] (Maguire, 1962); Root shoot length ratio (RSLR) = Root length [cm] / Shoot length [cm]; Root shoot dry weight ratio (RSDWR) = Root dry weight [mg] / Shoot dry weight [mg]; Vigour index I (VI_I) = Germination percentage \times Seedling length [cm] (Abdul-Baki and Anderson, 1973); Vigour index II (VI_II) = Germination percentage \times Seedling dry weight [mg]

(Abdul-Baki and Anderson, 1973); Plant height stress index (PHSI) = [Plant height of stressed seedling / Plant height of control seedling] \times 100 (Bousslama and Schapaugh, 1984); Dry matter stress index (DMSI) = [Dry weight of stressed seedling / Dry weight of control seedling] \times 100 (Bousslama and Schapaugh, 1984); Tolerance index (TOL) = Dry weight of control seedling [mg] – Dry weight of stressed seedling [mg] (Rosielle and Hamblin, 1981).

Factorial two-way analysis of variance was done in the Completely Randomized Design (CRD) and further principal component analysis (PCA) as well as Pearson correlation analysis at 0.05 level was done using KAU GRAPES software (Gopinath *et al.*, 2020).

Results and Discussion

The impact of water deficit and high temperature stress on the germination percentage, Timson germination index and speed of germination is shown in Fig. 1(a), 1(b) and 1(c), respectively. The values of these three attributes decreased under stress condition with the least reduction in WD PEG 10% treatment followed by HT, HT+WD PEG 10%, WD PEG 20% and the greatest reduction in HT+WD PEG 20%. For germination percentage, the reduction as compared to control were 9.69%, 64.03%, 22.10%, 44.95% and 86.66% for WD PEG 10%, WD PEG 20%, HT, HT+WD PEG 10% and HT+WD PEG 20%, respectively. As for Timson germination index, the decrement as compared to control were 26.14%, 70.55%, 34.24%, 53.98% and 91.40% for WD PEG 10%, WD PEG 20%, HT, HT+WD PEG 10% and HT+WD PEG 20%, respectively. Lastly for speed of germination, the diminution as compared to control were 29.52%, 72.72%, 37.11%, 56.90% and 93.06% for WD PEG 10%, WD PEG 20%, HT, HT+WD PEG 10% and HT+WD PEG 20%, respectively. Among the 30 genotypes, four genotypes namely BR 407, RAU 3, RAU 8 and RAU F-13 showed the highest values for these attributes in all the treatments, which indicate their better rate of germination under the stress conditions imposed. These three attributes showed strong positive correlation among themselves as well as with vigour indices I & II, plant height and dry matter stress indices. However, they all showed strong negative correlation with tolerance index. Mukami *et al.* (2019) reported reduction in germination percentage due to drought stress in finger millet. Under heat stress, there is decrease in germination percentage in pearl millet, which has been reported by Jacob *et al.* (2022). Decrement in germination percentage under combined heat and drought stress has been studied in pearl millet and sorghum by Smith *et al.* (1989).

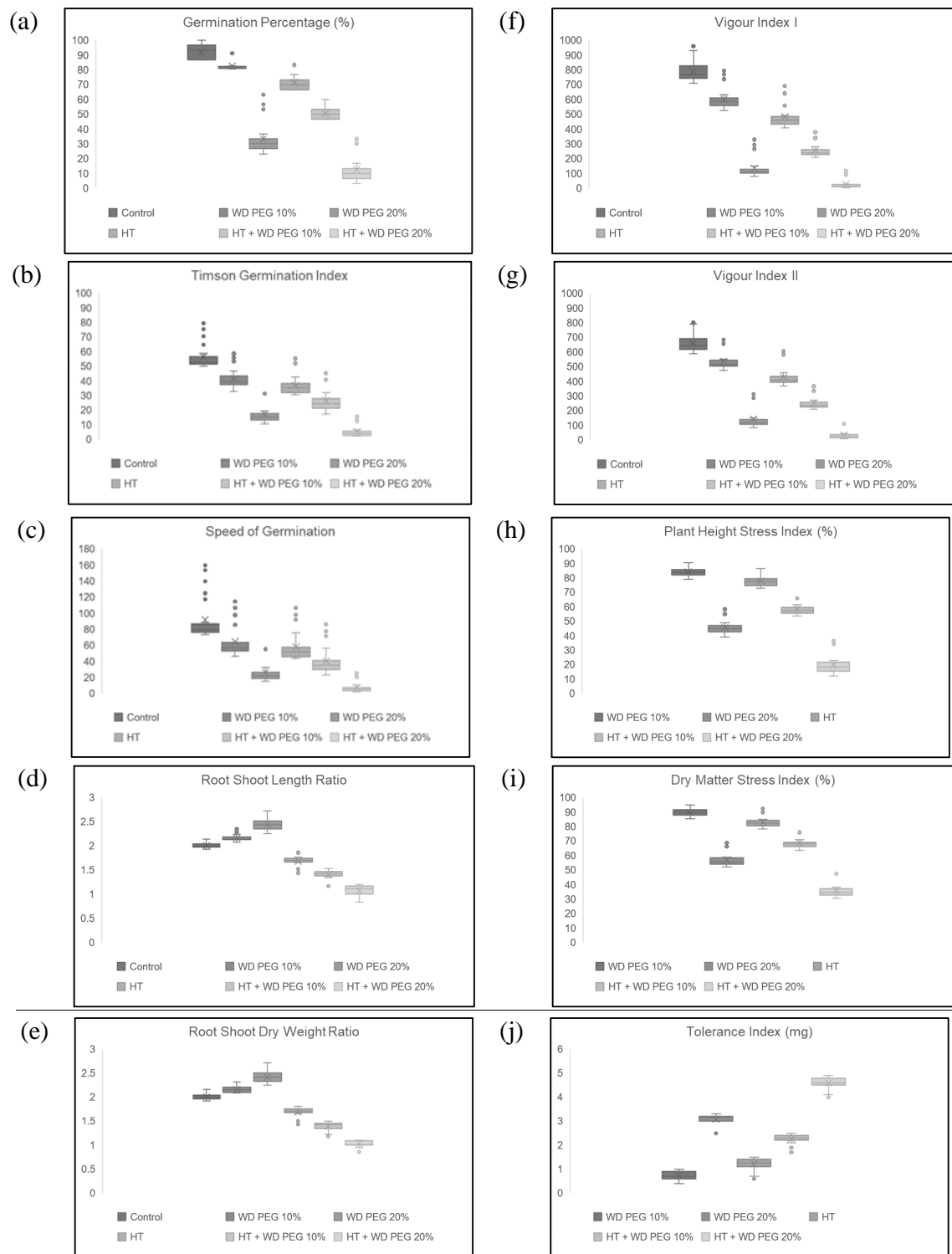


Fig. 1 : Box plots depicting the impact of water deficit and high temperature stress on various seedling parameters (× represents mean) – (a) Germination percentage (GP), (b) Timson germination index (TGI), (c) Speed of germination (SOG), (d) Root shoot length ratio (RSLR), (e) Root shoot dry weight ratio (RSDWR), (f) Vigour index I (VI_I), (g) Vigour index II (VI_II), (h) Plant height stress index (PHSI), (i) Dry matter stress index (DMSI), (j) Tolerance index (TOL).

The influence of water deficit and high temperature stress on the root shoot length ratio and dry weight ratio is shown in Fig. 1(d) and 1(e), respectively. For both the attributes, the values increased in case of WD PEG 10%

and WD PEG 20% treatments. This is because under water deficit condition, plants promote root growth to enhance water absorption to survive under limited water availability. The increment observed in root shoot length

ratio as compared to control were 7.61% and 21.35% for WD PEG 10% and WD PEG 20%, respectively. While, the increase in root shoot dry weight ratio as compared to control were 7.70% and 20.27% for WD PEG 10% and WD PEG 20%, respectively. Among the genotypes, the greatest increase in root growth was observed in case of BR 407, RAU 3, RAU 8 and RAU F-13. Thus, they showed the highest root shoot ratio for length as well as dry weight for water deficit treatments. However, for the treatments HT, HT+WD PEG 10% and HT+WD PEG 20%, the values for these two attributes decreased consistently. This is because under high temperature stress, plants promote shoot growth at the expense of root growth to dissipate heat via shoot system through transpiration. And in case of combined water deficit and high temperature stress, both root and shoot growth decreased drastically, however the negative effect on root growth was more than that on shoot growth which resulted in an overall decrease in root shoot ratio. The decrement observed in root shoot length ratio as compared to control were 16.20%, 29.97% and 46.81% for HT, HT+WD PEG 10% and HT+WD PEG 20%, respectively. While, the diminution in root shoot dry weight ratio as compared to control were 15.57%, 30.76% and 48.61% for HT, HT+WD PEG 10% and HT+WD PEG 20%, respectively. Among the genotypes, again the genotypes namely BR 407, RAU 3, RAU 8 and RAU F-13 showed the greatest increase in shoot growth for high temperature and combined stresses. Therefore, they showed the least values of root shoot ratio for these three treatments. Also, the extent of variation in root shoot ratio of these four genotypes under various stress conditions imposed depict the higher plasticity of these genotypes which make them tolerate these stresses. The root shoot length ratio and dry weight ratio showed strong positive correlation with each other. Mukami *et al.* (2019) reported that with increase in drought severity, root growth increased while shoot growth decreased in finger millet varieties under study, thus increasing the root shoot ratio. Significant reduction in root growth of foxtail millet under heat stress has been reported by Aidoo *et al.* (2016).

The impact of water deficit and high temperature on vigour indices I and II is presented in Fig. 1(f) and 1(g), respectively. Here also, the values decreased under stress condition with least reduction in case of WD PEG 10% followed by HT, HT+WD PEG 10%, WD PEG 20% and the highest reduction in HT+WD PEG 20%. For vigour index I, the reduction as compared to control were 23.95%, 83.23%, 39.26%, 67.81% and 96.93% for WD PEG 10%, WD PEG 20%, HT, HT+WD PEG 10% and HT+WD PEG 20%, respectively. As for vigour index II,

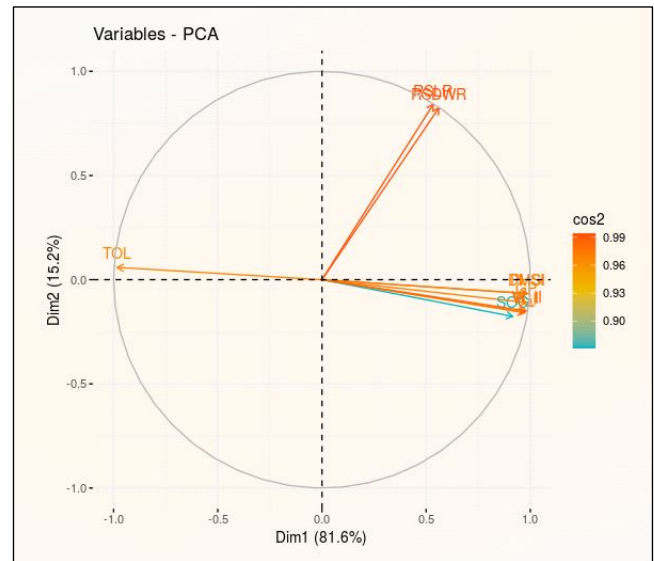


Fig. 2 : Principal component analysis of seedling parameters with depiction of first two principal components (Dim stands for PC).

the decrement observed as compared to control were 18.89%, 79.17%, 35.35%, 62.25% and 94.86% for WD PEG 10%, WD PEG 20%, HT, HT+WD PEG 10% and HT+WD PEG 20%, respectively. Among the genotypes, again BR 407, RAU 3, RAU 8 and RAU F-13 gave the highest values of the attributes under all the treatments. Both the vigour indices showed strong positive correlation with each other and as well as with germination percentage, Timson germination index, speed of germination, plant height and dry matter stress indices. However, they showed strong negative correlation with tolerance index. Anitha *et al.* (2020) reported that vigour index of finger millet seedlings decreased under drought stress. Yadav *et al.* (2013) determined from a study on pearl millet genotypes that better seedling vigour could aid in providing tolerance to heat stress which is supported by the genotypic variations found in the present investigation.

The influence of water deficit and high temperature on plant height and dry matter stress indices is shown in Fig. 1(h) and 1(i), respectively. The values decreased with increase in severity of the stress. Therefore, the highest values were obtained for WD PEG 10%, followed by HT, HT+WD PEG 10%, WD PEG 20% and the lowest values for HT+WD PEG 20%. The plant height stress index obtained for WD PEG 10%, WD PEG 20%, HT, HT+WD PEG 10% and HT+WD PEG 20% were 84.22%, 45.64%, 77.92%, 58.43% and 19.85%, respectively. Whereas, the dry matter stress index attained for WD PEG 10%, WD PEG 20%, HT, HT+WD PEG 10% and HT+WD PEG 20% were 89.84%, 56.97%, 82.92%, 68.51% and 36.02%, respectively.

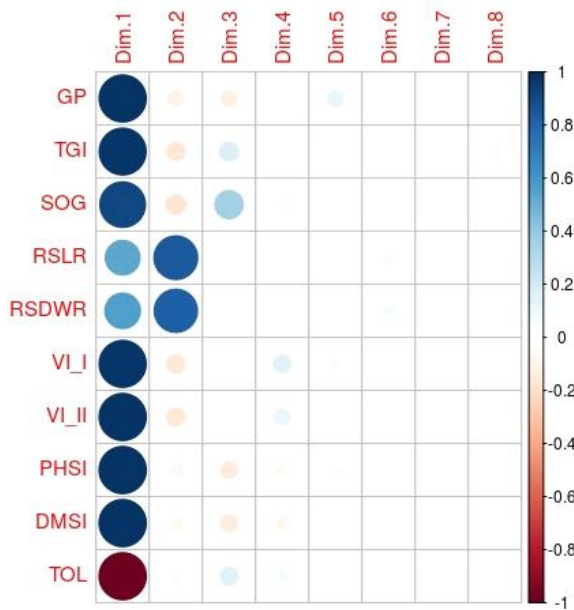


Fig. 3 : Correlation between seedling parameters and principal components (Dim stands for PC) – correlation values range from -1 (strong negative correlation) to +1 (strong positive correlation).

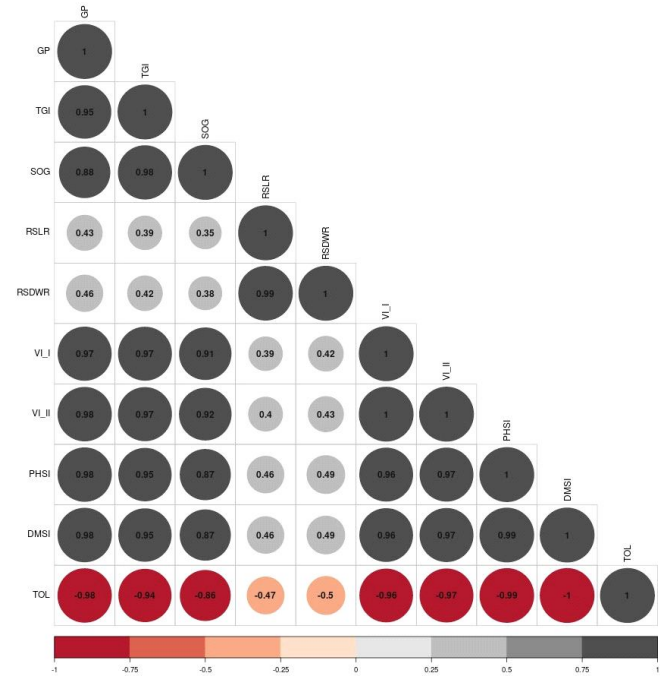


Fig. 4 : Correlation among seedling parameters – correlation values range from -1 (strong negative correlation) to +1 (strong positive correlation).

Among the genotypes, unquestionably BR 407, RAU 3, RAU 8 and RAU F-13 showed the highest values for these indices under all the stress treatments. Plant height stress index and dry matter stress index showed strong positive correlation with each other and as well as with germination percentage, Timson germination index, speed of germination and vigour indices I & II. But they showed strong negative correlation with tolerance index. Anitha

et al. (2020) also assessed plant height stress index in finger millet under drought stress condition, whose results corroborated the findings of the present study.

Lastly, the impact of water deficit and high temperature stress on tolerance index is presented in Fig. 1(j). Here, the least mean values were obtained for WD PEG 10% (0.7 mg), followed by HT (1.2 mg), HT+WD

Table 1 : Eigen values and percentage contribution of variables on principal components.

PC	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Eigen value	8.160	1.515	0.226	0.054	0.019	0.011	0.008	0.004	0.002	0.000
Percentage of variance	81.598	15.153	2.264	0.544	0.189	0.111	0.077	0.042	0.019	0.003
Cumulative percentage of variance	81.598	96.751	99.015	99.559	99.748	99.859	99.937	99.978	99.997	100.00
Percentage contribution of variables on PCs										
GP	11.771	0.797	5.652	1.918	68.223	0.095	8.306	0.130	-	-
TGI	11.540	1.665	12.226	4.008	1.293	0.002	3.420	65.728	-	-
SOG	10.286	2.055	55.406	4.961	1.966	0.000	0.721	24.164	-	-
RSLR	3.499	46.738	0.544	0.274	0.909	31.483	15.463	0.803	-	-
RSDWR	3.860	44.826	0.284	0.034	1.129	32.818	15.602	1.185	-	-
VI_I	11.667	1.621	0.008	37.325	14.794	0.851	0.133	0.305	-	-
VI_II	11.796	1.487	0.000	25.404	0.002	1.739	6.711	0.008	-	-
PHSI	11.858	0.290	7.837	6.723	10.306	20.337	26.490	7.402	-	-
DMSI	11.879	0.291	8.287	10.674	0.191	1.975	7.805	0.275	-	-
TOL	11.843	0.229	9.756	8.680	1.185	10.701	15.351	0.001	-	-

PEG 10% (2.3 mg), WD PEG 20% (3.1 mg) and the highest mean values for HT+WD PEG 20% (4.6 mg). Lesser the value for tolerance index, greater is the tolerance of genotype towards stress. Therefore, among the genotypes, again BR 407, RAU 3, RAU 8 and RAU F-13 showed the least values for the various stress conditions depicting their better tolerance towards them. Tolerance index showed strong negative correlation with germination index, Timson germination index, speed of germination, vigour indices I & II, plant height & dry matter stress indices.

The principal component analysis (Table 1 and Fig. 2) of the seedling parameters showed that out of ten PCs, only eight PCs were contributed by the seedling parameters and out of these eight, PC1 and PC2 had the greatest eigen values of 8.160 and 1.515, respectively. This meant that PC1 and PC2 accounted for 81.60% and 15.15% of the total variance respectively, which together explained 96.75% of variation in the data. Therefore, these two PCs are sufficient to explain the variation in seedling growth. The variables which contribute the most to PC1 are dry matter stress index, plant height stress index, tolerance index, vigour index II, germination percentage, vigour index I, Timson germination index and speed of germination. The remaining two variables namely root shoot length ratio and root shoot dry weight ratio contributed the most to PC2. The correlation between seedling parameters and principal components is shown in Fig. 3, while the correlation among the parameters is presented in Fig. 4.

Conclusion

The present study showed that simultaneous exposure to water deficit and high temperature stress is severely detrimental for finger millet growth. The rate of germination as well as seedling growth decreased under all the stress conditions. With respect to root shoot ratio, it was found that this ratio either increases (under drought stress) or decreases (heat and combined stress) in response to stress condition imposed. Assessment of finger millet genotypes determined that BR 407, RAU 3, RAU 8 and RAU F-13 have the greatest tolerance towards the combined stress among the thirty genotypes under study. These genotypes showed greater plasticity with respect to root shoot ratio, which depicts their tolerance to these abiotic stresses under study. The genetic variation obtained from the study would be helpful for further improvement of finger millet to attain greater heat and drought stress resilience through breeding programs.

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