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Heat Stress Tolerance Indices and Their Contribution in Identifying Heat Tolerant Bottle Gourd [*Lagenaria siceraria* (Molina) Standl.] Genotypes

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ABSTRACT

Background: Bottle gourd, a popular winter vegetable in Bangladesh, is experiencing a year-round increase in demand. High temperature stress disrupts physiological structure, fruit quality and crop yields, limiting the year-round cultivation of bottle gourd. The research aimed to identify heat stress-tolerant genotypes to extend growing seasons and increase production among varieties and breeding materials.

Methods: The study conducted at Vegetable Research Center, Dinajpur of BRAC Seed and Agro Enterprise, Bangladesh consisting 58 bottle gourd genotypes under normal and heat stress conditions using augmented block design. Meteorological data showed heat stress resulted ~5-7°C higher air temperatures than normal sowing environments.

Result: Heat stress tolerance indices indicated that the genotypes with a combination of greater values of MP, GMP, HM, YI and STI and lower values of SSI and TOL are high performed and productive breeding material. Yield based stress indices SSI and TOL had a negative association with fruit yield under heat stress (Ys), while strong positive correlations with STI, MP, GMP, HM and YI. The cluster analysis classified bottle gourd genotypes as tolerant, medium tolerant and susceptible to heat stress. Heat stress tolerance indices could be effective tools for identifying heat tolerant bottle gourd genotypes in field. The genotypes BG-45, BG-02, BG-57, BG-39 and BG-43 determined as high performing and stable breeding material under both normal and heat stress environments and might potentially be employed in future breeding programs.

Key words: Bottle gourd, Correlation analysis, Heat stress tolerance indices, Heat tolerant variety, Principal component analysis.

INTRODUCTION

Bottle gourd [*Lagenaria siceraria* (Molina.) Standl.] is a popular winter vegetable crop grown in Bangladesh, belong to the Cucurbitaceae family (Bose and Som, 1986). Its demand for fresh young fruits, shoots and succulent leaves is increasing year-round for their high water content, fibers, carbs, vitamins, minerals and amino acids (Milind and Satbir, 2011). It have a wide range of genetic diversity, heritability and genetic advance (Sharma and Sengupta, 2012).

The global climate has steadily changed during the last century (Zhang *et al.,* 2019). Climate change, global warming and weather uncertainty have raised serious concerns regarding agriculture and crop productivity (Lesk *et al.,* 2016). Climate change is elevating global surface temperatures, inducing heat stress in plants and having a negative impact on their development, growth and yield, altered rainfall patterns and shorter winter seasons (Sultan *et al.,* 2019). Rising global temperatures are threatening human survival by decreasing crop productivity and increasing the global population, which is predicted to exceed nine billion by 2050, resulting in increased food demand (Cole *et al.,* 2018).

Cucurbits is a perishable food item, serve various functions in meals but are sensitive to unstable climatic changes. It is predicted to reduce cucurbit production by 15 % due to climate change (Olarewaju *et al.,* 2023). The ideal temperature for bottle gourd growing is 25-30°C (Kumar and Reddy, 2021). High temperatures (>30°C) disrupt cucurbits physiological structure and fruit shapes, crucial for market quality (Saroj and choudhary, 2020). Yield levels are vital role for assessing heat tolerance and growing under normal and heat-stress conditions is a standard method for selecting heat-tolerant genotypes. A number of stress tolerance indices are effective for identifying heat-tolerant bottle gourd genotypes, making them the most credible indicators (Lamba *et al.,* 2023;

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Poudel *et al.,* 2024). Therefore, present research was carried out to assess the stress indices in identifying high yielding with heat-tolerance bottle gourd genotypes.

MATERIALS AND METHODS

The research was carried out at Vegetable research center, Dinajpur of BRAC Seed and Agro Enterprise in the old Himalayan piedmont plain agro-ecological zone of Bangladesh. The study assessed 58 diversified bottle gourd genotypes including registered varieties, advanced breeding lines and local land races collected from Agro-ecological zones of Bangladesh, India, Thailand, China and seed companies (Fig 1). Among the examined genotypes, registered varieties Khetlau (BG-50), BARI Lau-4 (BG-51) and BARI Lau-5 (BG-52), BU Lau-1 (BG-53) and BU Lau-2 (BG-54) were also employed. The experimental genotypes were cultivated for non-stress (winter season) during October 2022-February 2023 and heat stress (summer) in April 2023-August 2023. The genotypes were planted using an augmented block design. Ten plants of each genotype were planted with 2-meter space between rows and plants in each setups. With the exception of sowing time, every other aspect of the two trials (agronomic package and practices) were similar.

Data collection

Weather data were collected from the Bangladesh Meteorological Department, Dinajpur. The high temperature stress was recorded nearly 5-7°C higher than normal, with mean maximum temperatures reaching 29.66°C in Rabi 2022-23 while 34.62°C in summer, 2023 (Fig 2). Various morphological and yield data were collected from each genotype under both heat stressed and normal environments.

Statistical analysis

MS Excel 2016 were utilized for data entry, processing and stress index calculation. The stress indices, PCA analysis and biplot were analysed using Plant Abiotic Stress Index Calculator (Pour-Aboughadareh *et al.,* 2019). Correlation and cluster analysis were performed using Python using Jupyter 7.0.8.

The yield data were utilized in equations for following indices to calculate genotypic mean values for fruit yield under heat stress (Ys) and non-stress (Yp). Xs and Xp represent the average yield of all genotypes under heat stress and non-stress environments, respectively (Porch, 2006).

Tolerance (TOL) = Yp - Ys (Rosielle and Hamblin, 1981)

$$\text{Mean productivity (MP)} = \frac{Yp + Ys}{2}$$

(Rosielle and Hamblin, 1981)

$$\text{Harmonic mean (HM)} = 2\,\frac{Ys \times Yp}{Ys + Yp}$$

(Bidinger *et al.,* 1987)

$$\text{Geometric mean productivity (GMP)} = \sqrt{Yp \times Ys}$$

(Ramirez and Kelly, 1998)

$$\text{Stress susceptibility index (SSI)} = \frac{1-(Ys / Yp)}{1-(Xs / Xp)}$$

(Fischer and Maurer, 1978)

$$\text{Stress tolerance index (STI)} = \frac{(Ys \times Yp)}{(Xp)^2}$$

(Fernandez, 1992)

Yield index (YI) = Ys/Xs (Gavuzzi *et al.,* 1997)

Yield stability index (YSI) = Ys/Yp

(Bouslama and Schapaugh, 1984)

Relative stress index (RSI) = (Ys / Yp) / (Xs / Xp)

(Fischer and Wood, 1979)

RESULTS AND DISCUSSION

Yield based indices of heat tolerance

Yield-based stress indices TOL, MP, GMP, HM, SSI, STI, YI, YSI and RSI were determined for studied bottle gourd genotypes using yield under both normal (Yp) and heat stress (Ys) conditions (Table 1). The genetic variation among genotypes defined by different heat stress indices are depicted in figures (Fig 3-5). Nineteen bottle gourd genotypes possessed higher TOL, SSI, YSI and RSI and were marked as heat-susceptible genotypes because they recorded high yield under normal environments but zero yield when the plant died under heat stress and hence, these genotypes are only appropriate for normal environments.

The lowest value of TOL, SSI, YSI and RSI was exhibited in genotypes BG-42 followed by BG-18, BG-35 and BG-39 denoted as tolerant to heat stress. These genotypes performed less under both conditions due to less yield differential, implying that low values do not always indicate high performance and emphasising the relevance of yield as a crucial factor. Lamba *et al.* (2023) suggested to determined heat tolerant genotypes considering lower TOL, SSPI, YSI and RSI which supported the current findings. Superior genotype selection has also been reported based on lower SSI and TOL in chickpeas (Jha *et al.,* 2018), common bean (Porch, 2006) and bottle gourd (Mashilo *et al.,* 2017). Based on greater values of MP, GMP, HM, STI and YI and lower TOL and SSI combinations genotype BG-02 were considered to be most stable and productive genotypes followed by BG-43, BG-57, BG-01 and BG-45 identified among all the genotypes under both conditions as heat tolerant. Sofi *et al.* (2018) suggested classifying genotypes into four groups based on their resilience and productivity in both stress and non-stress environments. These indices were effective in identifying high-yielding cum heat-tolerant, which identical to the findings reported by Poudel *et al.* (2024) and Jha *et al.* (2018).

Correlation between fruit yield and stress tolerance indices

The indices that showed a strong relationship with fruit yield in both environments were chosen as best in selecting genotypes with high yield in both conditions (Table 2). Fruit yield under non-stress (Yp) was significant positive association (0.369) with fruit yield under heat stress (Ys) suggested that these might be utilized to select high yielding bottle gourd genotypes. Fruit yield (Ys) had positive and significant relationship with MP (0.904), GMP (0.983), HM (0.991), STI (0.968), YI (1) and YSI (0.952) but highly negative significant correlation with TOL (-0.796) and SSI (-0.952). Whereas, Yp demonstrated a significant and positive relationship with TOL (0.268), MP (0.732), GMP (0.405), HM (0.396), STI (0.514) and YI (0.369). Fruit yield (Ys) was negatively correlated with TOL (-0.796) while positively correlated (0.268) under normal conditions; thus, selection based on these indices will yield more fruit under normal conditions but less under heat stress (Lamba *et al.,* 2023). Yield (Yp and Ys) were determined positive association with MP, GMP, HM, STI and negatively correlated with TOL and SSI for heat tolerance in wheat (Poudel *et al.,* 2024), chickpeas (Jha *et al.,* 2018) and water stress in cow pea (Gull *et al.,* 2019) which matched with current findings. Kumar *et al.* (2020) reported that yield (both normal and stress environments) in mungbean was positively associated with all stress tolerance indices except SSI and Superiority measure. Both TOL and SSI had substantial negative associations with all stress tolerance indices, whereas SSI and TOL demonstrated a strong

Fig 1: Morphological variations of studied 58 bottle gourd genotypes.

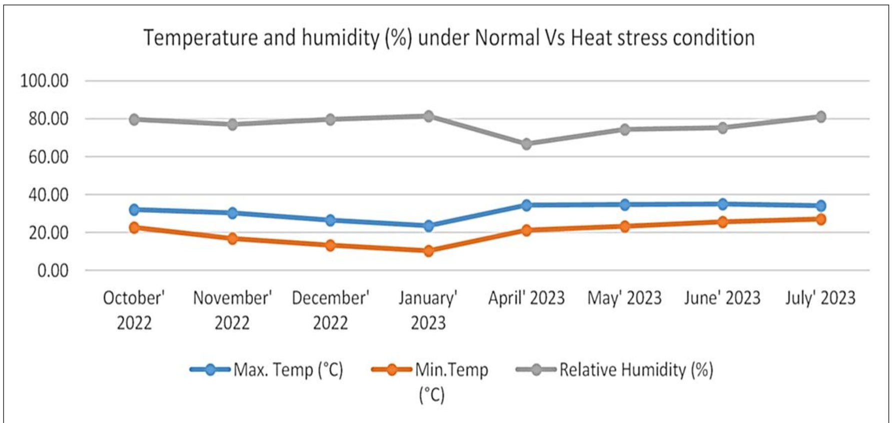

Fig 2: Monthly temperature and relative humidity (%) during non-stress and heat stress period.

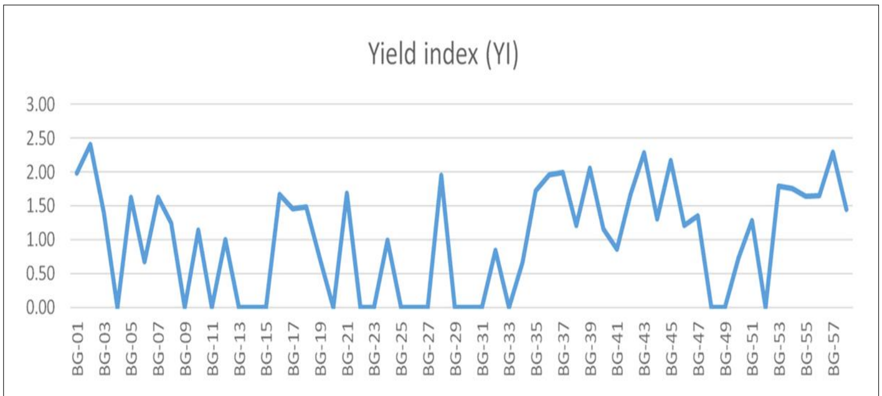

Fig 3: Variability of YI under non-stress and heat stress environments.

Table 1: Fruit yield (Yp and Ys t ha^{-1}) and stress tolerance indices, their mean rank and standard deviation of 58 bottle gourd genotypes.

Genotype	Yp	Ys	TOL	MP	GMP	HM	SSI	STI	YI	YSI	RSI	R±SDR
BG-01	45.94	33.65	12.29	39.80	39.32	38.85	0.50	1.14	1.98	0.73	1.59	10.09±6.55
BG-02	51.25	40.73	10.52	45.99	45.69	45.39	0.38	1.53	2.40	0.79	1.73	5.73±6.40
BG-03	42.33	23.59	18.74	32.96	31.60	30.30	0.82	0.73	1.39	0.56	1.21	22.18±5.27
BG-04	45.56	0.00	45.56	22.78	0.00	0.00	1.85	0.00	0.00	0.00	0.00	38.45±10.50
BG-05	36.66	27.56	9.10	32.11	31.79	31.47	0.46	0.74	1.63	0.75	1.64	17.18±5.56
BG-06	21.12	11.37	9.75	16.25	15.50	14.78	0.85	0.18	0.67	0.54	1.17	36.18±11.68
BG-07	37.88	27.53	10.35	32.71	32.29	31.89	0.51	0.77	1.62	0.73	1.58	18.45±3.93
BG-08	31.53	21.05	10.48	26.29	25.76	25.25	0.62	0.49	1.24	0.67	1.45	27.64±5.78
BG-09	27.51	0.00	27.51	13.76	0.00	0.00	1.85	0.00	0.00	0.00	0.00	42.18±4.14
BG-10	26.54	19.38	7.16	22.96	22.68	22.40	0.50	0.38	1.14	0.73	1.59	28.09±11.75
BG-11	23.71	0.00	23.71	11.86	0.00	0.00	1.85	0.00	0.00	0.00	0.00	42.55±6.47
BG-12	28.40	16.88	11.52	22.64	21.90	21.17	0.75	0.35	1.00	0.59	1.29	31.64±6.95
BG-13	50.74	0.00	50.74	25.37	0.00	0.00	1.85	0.00	0.00	0.00	0.00	37.82±12.24
BG-14	28.80	0.00	28.80	14.40	0.00	0.00	1.85	0.00	0.00	0.00	0.00	41.91±3.65
BG-15	27.23	0.00	27.23	13.62	0.00	0.00	1.85	0.00	0.00	0.00	0.00	42.36±4.88
BG-16	41.22	28.25	12.97	34.74	34.12	33.52	0.58	0.86	1.67	0.69	1.49	17.09±5.52
BG-17	35.56	24.67	10.89	30.12	29.62	29.13	0.57	0.64	1.45	0.69	1.51	23.82±3.71
BG-18	27.90	25.06	2.84	26.48	26.44	26.40	0.19	0.51	1.48	0.90	1.95	19.18±15.31
BG-19	25.51	12.32	13.19	18.92	17.73	16.62	0.96	0.23	0.73	0.48	1.05	36.36±7.15
BG-20	23.36	0.00	23.36	11.68	0.00	0.00	1.85	0.00	0.00	0.00	0.00	42.73±7.46
BG-21	39.26	28.54	10.72	33.90	33.47	33.05	0.51	0.82	1.68	0.73	1.58	16.00±3.95
BG-22	35.32	0.00	35.32	17.66	0.00	0.00	1.85	0.00	0.00	0.00	0.00	41.00±4.27
BG-23	27.25	0.00	27.25	13.63	0.00	0.00	1.85	0.00	0.00	0.00	0.00	42.27±4.50
BG-24	50.91	16.85	34.06	33.88	29.29	25.32	1.24	0.63	0.99	0.33	0.72	29.91±12.19
BG-25	24.68	0.00	24.68	12.34	0.00	0.00	1.85	0.00	0.00	0.00	0.00	42.64±5.87
BG-26	48.12	0.00	48.12	24.06	0.00	0.00	1.85	0.00	0.00	0.00	0.00	38.18±11.22
BG-27	29.63	0.00	29.63	14.82	0.00	0.00	1.85	0.00	0.00	0.00	0.00	41.82±3.49
BG-28	38.88	32.98	5.90	35.93	35.81	35.69	0.28	0.94	1.94	0.85	1.85	9.73±5.64
BG-29	38.20	0.00	38.20	19.10	0.00	0.00	1.85	0.00	0.00	0.00	0.00	40.18±5.84
BG-30	38.08	0.00	38.08	19.04	0.00	0.00	1.85	0.00	0.00	0.00	0.00	40.27±5.44
BG-31	35.18	0.00	35.18	17.59	0.00	0.00	1.85	0.00	0.00	0.00	0.00	41.18±3.82
BG-32	29.86	14.26	15.60	22.06	20.64	19.30	0.97	0.31	0.84	0.48	1.04	34.91±3.73
BG-33	23.54	0.00	23.54	11.77	0.00	0.00	1.85	0.00	0.00	0.00	0.00	42.64±6.96
BG-34	26.38	11.30	15.08	18.84	17.27	15.82	1.06	0.22	0.67	0.43	0.93	38.64±5.95
BG-35	32.45	29.09	3.36	30.77	30.72	30.68	0.19	0.69	1.72	0.90	1.95	14.45±11.53
BG-36	40.66	33.14	7.52	36.90	36.71	36.52	0.34	0.99	1.95	0.82	1.77	9.82±3.82

Table 1: **Continue....**

Table 1: **Continue....**

BG-37	45.18	33.74	11.44	39.46	39.04	38.63	0.47	1.12	1.99	0.75	1.62	10.27±5.52
BG-38	44.23	20.46	23.77	32.35	30.08	27.98	0.99	0.66	1.21	0.46	1.01	28.00±7.58
BG-39	39.36	34.74	4.62	37.05	36.98	36.91	0.22	1.00	2.05	0.88	1.92	7.09±5.77
BG-40	41.45	19.61	21.84	30.53	28.51	26.62	0.97	0.60	1.16	0.47	1.03	29.18±4.85
BG-41	36.73	14.50	22.23	25.62	23.08	20.79	1.12	0.39	0.86	0.39	0.86	34.09±2.12
BG-42	30.51	28.23	2.28	29.37	29.35	29.33	0.14	0.63	1.66	0.93	2.01	16.09±13.81
BG-43	51.84	38.72	13.12	45.28	44.80	44.33	0.47	1.47	2.28	0.75	1.63	7.64±8.13
BG-44	42.61	22.16	20.45	32.39	30.73	29.16	0.89	0.69	1.31	0.52	1.13	24.09±5.47
BG-45	41.88	36.70	5.18	39.29	39.20	39.12	0.23	1.13	2.16	0.88	1.91	6.00±3.71
BG-46	28.40	20.50	7.90	24.45	24.13	23.81	0.51	0.43	1.21	0.72	1.57	27.36±8.99
BG-47	39.82	22.88	16.94	31.35	30.18	29.06	0.79	0.67	1.35	0.57	1.25	24.64±2.34
BG-48	53.96	0.00	53.96	26.98	0.00	0.00	1.85	0.00	0.00	0.00	0.00	37.09±13.69
BG-49	40.97	0.00	40.97	20.49	0.00	0.00	1.85	0.00	0.00	0.00	0.00	39.55±7.71
BG-50	40.13	12.32	27.81	26.23	22.24	18.85	1.28	0.36	0.73	0.31	0.67	35.64±5.71
BG-51	45.52	21.71	23.81	33.62	31.44	29.40	0.97	0.73	1.28	0.48	1.04	24.91±8.84
BG-52	35.20	0.00	35.20	17.60	0.00	0.00	1.85	0.00	0.00	0.00	0.00	41.09±4.01
BG-53	36.92	30.33	6.59	33.63	33.46	33.30	0.33	0.82	1.79	0.82	1.79	12.45±6.41
BG-54	42.36	29.77	12.59	36.07	35.51	34.97	0.55	0.93	1.76	0.70	1.53	14.91±5.66
BG-55	32.76	27.76	5.00	30.26	30.16	30.05	0.28	0.67	1.64	0.85	1.84	17.27±10.23
BG-56	35.13	28.00	7.13	31.57	31.36	31.16	0.38	0.72	1.65	0.80	1.73	17.09±7.79
BG-57	48.62	38.80	9.82	43.71	43.43	43.16	0.37	1.39	2.29	0.80	1.74	6.18±4.45
BG-58	49.42	24.46	24.96	36.94	34.77	32.72	0.93	0.89	1.44	0.49	1.08	20.45±11.33
SE	1.12	1.79	1.73	1.22	2.06	2.03	0.08	0.06	0.11	0.05	0.10	
SD	8.56	13.63	13.15	9.29	15.70	15.44	0.64	0.44	0.80	0.34	0.75	
CV	0.23	0.80	0.65	0.34	0.75	0.76	0.62	0.89	0.80	0.76	0.76	

positive connection (0.886). High positive associations with SSI and TOL but negative correlations with other heat stress indices were also reported by Jha *et al.* (2018). The findings indicated that these parameters were more effective in selecting genotypes with high yields under different environments.

Principal component and biplot analysis

The first two principal components (PCs) with an Eigen value >1.0 generated variations by 83.89% and 14.50% of PC1 and PC2, respectively, for heat tolerance indices (Table 3) which contributed highest variation (98.39%) of the six components. Seepal *et al.* (2025), Mashilo *et al.* (2017) and Farshadfar *et al.* (2013) reported 97.46%, 99.72% and 99.60% of total variations in field pea, bottle gourd and wheat respectively. PC1 explained significant positive association with Ys, MP, GMP, HM, STI, YI and YSI but had a negative relationship with TOL and SSI. Thus, PC1 regarded as stress tolerance component. PC2 were identified as heat susceptibility component since it explained a greater correlation with TOL, SSI and Yp. This method also utilized by Poudel *et al.* (2024) and Devi *et al.* (2021) to categorize the components of wheat under heat stress. Ullah *et al.* (2022) suggested that most appropriate criteria are to select stable genotypes with low PC2 and greater PC1 values and vice versa. Thus, genotypes BG-45, BG-57, BG-02 and BG-43 were found higher PC1 values with lower PC2 values regarded as superior with stable and tolerant genotypes under both heat stress and non-stress environments and might recommended for cultivation, Lamba *et al.* (2023) employed this approach to identify stable heat tolerant genotypes.

The biplot depicted that Yp and Ys were positively connected with MP, GMP, HM, STI, YI and YSI, while Ys was negatively associated with TOL and SSI, as evidenced by the obtuse and acute angles between their vectors respectively (Fig 6). Lamba *et al.* (2023) and Jha *et al.* (2018) displayed these types of relationship among indices using biplots for heat tolerance in wheat and chickpeas, respectively. Devi *et al.* (2021) similarly illustrated positive relationship with MP, GMP and STI as acute angles between their vectors while negative with TOL and SSI as an obtuse angle in Ys.

Cluster analysis

The cluster analysis categorized genotypes into three clusters based on stress tolerance indices trends. Among the clusters, cluster 2 possessed the twenty-one genotypes with highest MP, GMP, STI, YI and YSI, indicating strong performance and desirable traits categorized as tolerant.

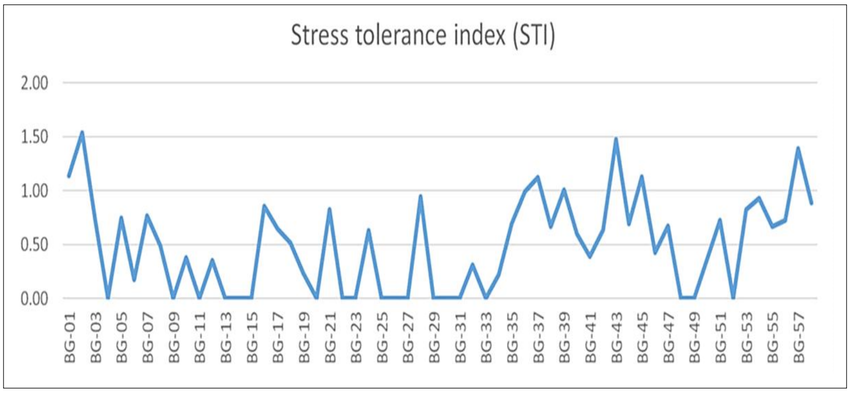

Fig 4: Variability of STI under non-stress and heat stress environments.

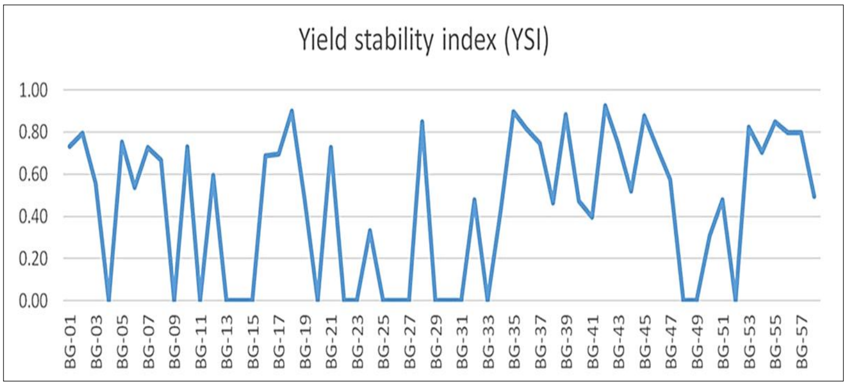

Fig 5: Variability of YSI under non-stress and heat stress environments.

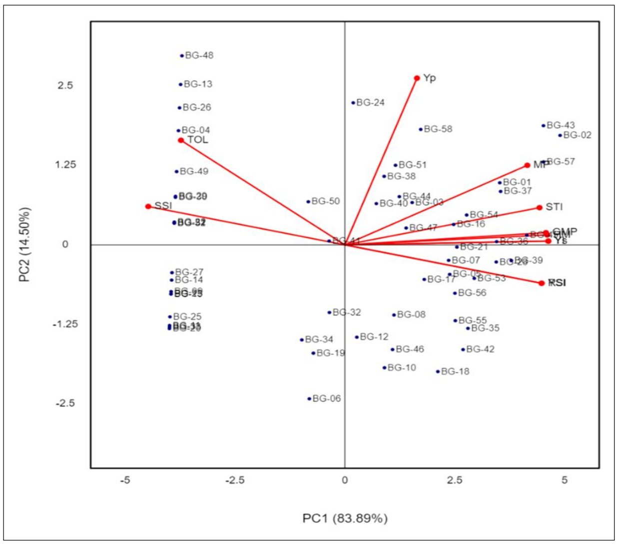

Fig 6: Biplot displayed relationship among heat stress indices of 58 genotypes.

Table 2: Correlation coefficient between fruit yield (Yp and Ys) and stress tolerance indices.

	Yp	Ys	TOL	MP	GMP	HM	SSI	STI	YI	YSI	RSI
Yp	1										
Ys	0.369**	1									
TOL	0.268*	-0.796**	1								
MP	0.732**	0.904**	-0.461**	1							
GMP	0.405**	0.983**	-0.755**	0.908**	1						
HM	0.396**	0.991**	-0.769**	0.909**	0.999**	1					
SSI	-0.156^{NS}	-0.952**	0.886**	-0.77**	-0.927**	-0.936**	1				
STI	0.514**	0.968**	-0.669**	0.946**	0.960**	0.966**	-0.847**	1			
YI	0.369**	1.000**	-0.796**	0.904**	0.983**	0.991**	-0.953**	0.967**	1		
YSI	0.155^{NS}	0.952**	-0.886**	0.77**	0.926**	0.936**	-1**	0.846**	0.952**	1	
RSI	0.156^{NS}	0.953**	-0.886**	0.771**	0.927**	0.937**	-1**	0.847**	0.953**	1**	1

*= Significant at 5%, **= Significant at 1% and NS= Non-significant.

Table 3: Principal component analysis showing eigenvectors, eigenvalues and variability.

Factors	PC1	PC2
Yp	0.116	0.737
Ys	0.329	0.017
TOL	-0.265	0.462
MP	0.295	0.352
GMP	0.325	0.054
HM	0.327	0.044
SSI	-0.318	0.170
STI	0.314	0.165
YI	0.329	0.017
YSI	0.318	-0.170
RSI	0.318	-0.170
Eigenvalue	9.23	1.60
Variability	83.89	14.50
Cumulative	83.89	98.39

Similarly, cluster 3 exhibited medium values of heat tolerance indices and consisted 18 genotypes classified as semi-tolerant. Consequently, cluster 1 had the highest TOL and SSI values but least MP, GMP and YI values, with 19 genotypes suited only for normal condition, indicating that they were susceptible genotypes (Fig 7). Naghavi *et al.* (2013) categorized genotypes into three clusters for drought tolerance which validated selection through clustering of studied genotypes.

Ranking of genotypes

The ranking system was employed to determine the overall performance of genotypes. In order to identify the most heat-tolerant genotype based on all indicators, mean rank (R) and SDR of all indices were estimated. The genotypes BG-02, BG-45 and BG-57 were identified as most heat-tolerant with low rank values based on all heat stress tolerance indices (Table 1). Mashilo *et al.* (2017) and Naghavi *et al.* (2013) utilized ranking method

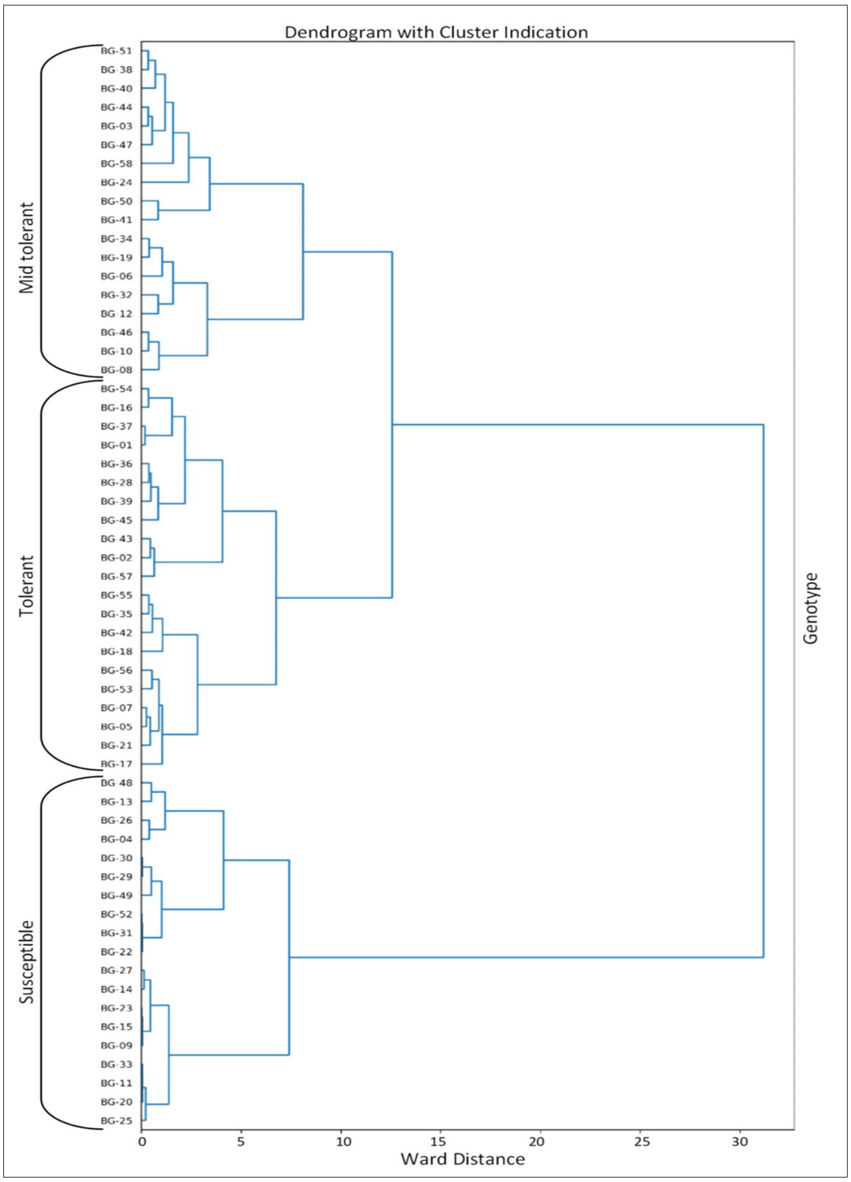

Fig 7: Dendrogram showing classification of genotypes based on heat tolerance.

for screening corn and bottle gourd genotypes, respectively.

CONCLUSION

The correlation coefficient analysis of heat stress indices revealed that MP, GMP, HM and STI had positive association with Yp and Ys, but TOL and SSI had negative correlation with Ys. Thus, these indices might be utilized for identification heat tolerant bottle gourd genotypes. PCA analysis revealed 98.39% of total genetic variation employed in PC1 and PC2, which classified genotypes as tolerant and susceptible, respectively. Cluster analysis illustrated the genotypes more clearly for tolerant, mid-tolerant and susceptible grouped for heat stress conditions. The genotypes BG-45, BG-02, BG-57, BG-39 and BG-43 were determined to be heat tolerant by evaluating their contributions of stress indices by PCA, biplot and ranking. Further, the heat stress tolerance indices TOL, SSI, MP, GMP, STI and YI are the most appropriate indicators to determine heat tolerance genotypes.

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Conflict of interest

The authors declare no conflict of interest exists.

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