



Evaluation of pearl millet (*Pennisetum glaucum*) genotypes and validation of screening methods for supra-optimal temperature tolerance at seedling stage

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ABSTRACT

The supra-optimal temperatures at seedling stage in pearl millet are known to be the most important cause of poor crop stands at farmers' field which can be managed through developing high temperature tolerant pearl millet [*Pennisetum glaucum* (L.) R. Br emend Stuntz] varieties. Identification of the superior germplasm for heat tolerance is essential for effective manipulation through breeding. Field screening techniques and laboratory screening technique were simultaneously used for the evaluation of pearl millet genotypes. In the field conditions, screening for heat tolerance was done using Seedling Thermo-tolerance Index (STI) and Seed to Seedling Thermo-tolerance Index (SSTI), whereas membrane thermo-stability (MTS) was taken as index for heat tolerance in the laboratory technique. These three indices were highly correlated among themselves. STI values were invariably high, whereas SSTI has lower values, as it also covers the effect of under soil mortality (USM). The field based thermo-tolerance indexes also showed significant correlation with other growth characteristics of seedling. Based on our results, it could be suggested that membrane thermo-stability test (MTS) may be used for screening large number of genotypes or germplasm accessions. We also suggest, the field based screening techniques, indices STI and SSTI, should be performed for evaluation of germplasm before they are released. Cluster analysis and correlation showed that growth characteristics of seedling can also provide certain indications of heat tolerance in genotypes.

Key words: Membrane thermo-stability, Pearl millet, *Pennisetum glaucum*, Seed to seedling thermo-tolerance index, Seedling thermo-tolerance index, Supra-optimal temperature tolerance

Pearl millet [*Pennisetum glaucum* (L.) R. Br emend Stuntz] is one of the most important staple crop for millions of people in the semi-arid and arid regions of Asia and sub-Saharan Africa (Arya and Yadav 2009). With its ability to adapt to diverse agro-ecological conditions, pearl millet may have unique position in the world agriculture (Govila 2001). It is a multipurpose cereal grown for grain and stover. It shall continue to play a prominent role in the integrated agricultural and livestock economy, particularly in rainfed areas due to its drought hardiness and tolerance to high temperature (Singh 1993). Stand establishment of pearl millet continues to be a severe constraint to successful production under subsistence farming conditions in the semi-arid tropics and with mechanized farming practices (Soman *et al.* 1987). Failure to obtain adequate plant populations is often associated with adverse weather conditions occurring at critical times during

the germination and seedling establishment process. The temperature is one of the key climatic factors and has profound effect on the growth and development of the pearl millet (Fussel *et al.* 1980, Squire 1989, Khalifa and Ong 1990, Yadav *et al.* 2006). The soil temperatures in farmers' field in India and Africa commonly exceed 45°C and the temperatures >60°C have occasionally been measured (Soman *et al.* 1981, Singh 1993, Yadav *et al.* 2006) and as high as 68°C have been recorded (Peacock 1977). Under such conditions heat stress is often accompanied by drought stress and there are usually interactions within the plant to these two stresses (Sullivan *et al.* 1977, Peacock and Heinrich 1984).

The supra-optimal temperatures are known to be the most important cause of poor crop stands in farmer's fields of such areas (Ong 1983, Rai and Anand 1994). In pearl millet, germination increases linearly with temperature from a base of 10°C to 12°C to optimum at 30°C to 34°C and declined to zero at about 45°C to 47°C (Soman and Peacock 1985, Peacock and Heinrich 1984). Ten days after emergence, pearl millet seedlings get established properly and the seedling mortality due to high field temperature stopped meaning

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thereby that the effect of temperature gets to its minimal at this stage (Peacock *et al.* 1993, Singh 1993, Yadav *et al.* 2006). The temperature sensitivity of higher plants is not constant throughout their life cycle (Levitt 1980), with the seed germination and seedling establishment being especially susceptible (Howarth *et al.* 1997). Genetic variability for adaptation to high temperature exists in crop plants (Howarth *et al.* 1997, Yadav *et al.* 2009). Pearl millet growing environments are characterized by low, erratic rainfall and high temperature. High temperature being solely a climatic factor, which cannot be controlled by any artificial means, therefore, the modification of pearl millet varieties is required in the response to high temperature.

Identification of the superior germplasm for supra-optimal temperature tolerance is essential for effective manipulation through breeding techniques. A major challenge in conventional breeding for heat tolerance is the identification of reliable and effective screening methods to facilitate detection of heat-tolerant plants (Mohammadi *et al.* 2007, Wahid *et al.* 2007). Several screening methods and selection

criteria have been developed/proposed by different researchers (Bravo *et al.* 2011). Field screening methods include seedling thermo-tolerance index (STI) (Peacock *et al.* 1993), seed to seedling thermo-tolerance index (SSTI) (Singh 1993, Yadav *et al.* 2006, 2011), heat tolerance index (HTI) as growth recovery after heat exposure in sorghum (Young *et al.* 2001). Thermo-tolerance screening at germination and early vegetative stage is effective for pearl millet and maize (Ashraf and Hafeez 2004). The objective of this study was the validation of screening methods for supra-optimal temperature tolerance and to identify superior heat tolerant genotypes for further use in breeding programmes.

MATERIALS AND METHODS

Forty-six genotypes of pearl millet which included advanced inbred lines (35) including four male sterile maintainer lines and hybrids (11) were screened for supra-optimal temperature tolerance (Table 1). A field screening technique, i.e. thermo-tolerance index (Peacock *et al.* 1993) was applied to screen the pearl millet genotypes for heat

Table 1 List of pearl millet genotypes screened for thermo-tolerance at seeding stage

Genotypes	Description	Origin and characteristics
CSSC-46-2	Inbred	Bred at CCS Haryana Agricultural University, Hisar by selfing and selection. It is medium late maturity, stay-green type, bold grain, compact ear head and resistant to downy mildew and blast.
G73-107	Inbred	Bred at CCS Haryana Agricultural University, Hisar. It has profuse tillering, narrow leaves, typical orange brown anthers and resistant to major diseases and pests.
H77/29-2	Inbred	Bred at CCS Haryana Agricultural University, Hisar by selfing and selection. It is medium dwarf, nodal pigmentation - violet brown, resistant to downy mildew, shoot fly, grey weevil, chaffer beetle and leaf roller.
78/711	Inbred	Bred at CCS Haryana Agricultural University, Hisar.
96AC-93	Inbred	Resistant to downy mildew; bred at CCS Haryana Agricultural University, Hisar by selfing and selection.
HTP-94/54	Inbred	Bred at CCS Haryana Agricultural University, Hisar. It has nodal pigmentation – light violet, bold grains with whitish grey grain colour, very tall; resistant to downy mildew and tolerant to moisture stress.
ICMR-01004	Inbred	Bred by marker-assisted backcrossing at ICRISAT-Patancheru using ICMP 451-P6 as donor and H77/833-2 as recurrent parent; moderately susceptible to rust.
ICMR-01007	Inbred	Bred by marker-assisted backcrossing at ICRISAT-Patancheru using ICMP 451-P6 as donor and H 77/833-2 as recurrent parent; resistant to rust.
H90/4-5	Inbred	Bred at CCS Haryana Agricultural University, Hisar by selfing and selection. It is an advance line with dwarf, stay-green, compact lanceolate earhead characters and good combiner.
INB 87/74-3-20	Inbred	Bred at CCS Haryana Agricultural University, Hisar.
VCF ₄ -2359	Inbred	Bred at CCS Haryana Agricultural University, Hisar.
MRC	Inbred	Advance line at CCS Haryana Agricultural University, Hisar; selection from Mandore restorer composite material from ICRISAT-Patancheru
72-2-2-98K-1	Inbred	Bred at CCS Haryana Agricultural University, Hisar.
1448-98K-1	Inbred	Bred at CCS Haryana Agricultural University, Hisar.
1250	Inbred	Bred at CCS Haryana Agricultural University, Hisar by selfing and selection; stay-green type with resistant to downy mildew and blast.
HTP-31	Inbred	Bred at CCS Haryana Agricultural University, Hisar by selfing and selection from top cross hybrids.
77/667	Inbred	Bred at CCS Haryana Agricultural University, Hisar
1305	Inbred	Bred at CCS Haryana Agricultural University, Hisar by selfing and selection. It is late flowering, medium dwarf and has semi compact, cylindrical earhead.
HTP-92/80	Inbred	Bred at CCS Haryana Agricultural University, Hisar by selfing and selection from top cross hybrids
96AC-82	Inbred	Bred at CCS Haryana Agricultural University, Hisar

(Contd.)

Table 1 *Contd.*

Genotypes	Description	Origin and characteristics
S-97/120	Inbred	Bred at CCS Haryana Agricultural University, Hisar by selfing and selection; tall and stay-green type.
99HS-18	Inbred	Bred at CCS Haryana Agricultural University, Hisar
Togo-II	Inbred	Selection from ICRISAT material- Togo; bred at CCS Haryana Agricultural University, Hisar.
HTP-88	Inbred	Bred by random mating of 70 S1 progenies from Togo populations
ICR-161	Inbred	Stay green advance line from ICRISAT, Patancheru
77/245	Inbred	Bred at CCS Haryana Agricultural University, Hisar by selfing and selection. It is late flowering and high tillering.
77/371	Inbred	Bred at CCS Haryana Agricultural University, Hisar by selfing and selection; high tillering and stay-green type.
CVJ-2-5-3-1-3	Inbred	Dwarf, developed at ICRISAT, Patancheru
99/57-1	Inbred	Bred at CCS Haryana Agricultural University, Hisar
77/273	Inbred	Bred at CCS Haryana Agricultural University, Hisar by selfing and selection. It has compact earhead.
H77/833-2	Inbred	Bred at CCS Haryana Agricultural University, Hisar by selfing and selection within a Rajasthani landrace population
7B	Inbred	HMS 7B is a male sterile maintainer line of HMS 7A. Bred at CCS Haryana Agricultural University, Hisar in 1990 from inbred 35 by selfing and selection.
18B	Inbred	HMS 18B is a male sterile maintainer line of HMS 18A. Bred at CCS Haryana Agricultural University, Hisar in 1999 from 854 by selfing and selection.
36B	Inbred	HMS 36B is a male sterile maintainer line of HMS 36A ₄ . Bred at CCS Haryana Agricultural University, Hisar in 2002 from HB98/213 by selfing and selection.
94222B	Inbred	Male sterile maintainer line of 94222A, developed at ICRISAT, Patancheru
HHB-67-1	Hybrid	Released hybrid among ICMA 843-22 × H77/833-2-202; improved version of HHB-67, developed by crossing downy mildew resistant parents suitable for early, normal and late sown conditions. Highly resistant to moisture stress, extra early maturing and 5% more grain yield than HHB- 67. Both parents developed in ICRISAT, Patancheru - CCS HAU, Hisar collaboration project.
HHB-67-2	Hybrid	Released hybrid among ICMA 843-22 × H77/833-2-146; improved version of HHB-67, highly resistant to downy mildew, high stable yield, tolerant to drought, small bristles. Male parent H77/833-2-146 developed by backcrossing ICMP 451 with H77/833-2 as recurrent parent through MAS and female parent ICMA 843-22 developed by backcrossing of MS 843A. Both parents developed in ICRISAT, Patancheru - CCS HAU, Hisar collaboration project.
HHB-94	Hybrid	Hybrid among ICMA 89111A × G73-107; highly resistant to downy mildew and tolerant to smut, resistant to shoot fly and leaf rollers. CCS Haryana Agricultural University, Hisar released this hybrid in 1998.
HHB-117	Hybrid	Hybrid among HMS7A × 77/29-2; female parent HMS7A was developed by 81A × 35 and maintainer 7B developed by repeated backcrossing with 35. It has a dual purpose hybrid with stay green character for general cultivation in Haryana. Resistant to downy mildew and pests. CCS Haryana Agricultural University, Hisar released this hybrid in 2003.
HHB-146	Hybrid	Hybrid among ICMA 95222 × HTP-94/54; dual purpose hybrid, highly resistant to downy mildew extra ordinarily tall; CCS Haryana Agricultural University, Hisar released this hybrid in 2002.
HHB-162	Hybrid	ICMA 94111A × H77/833-2; besides extra early maturity like HHB-67 combines high grain yield and dry fodder yield, highly resistant to downy mildew; bred at CCS Haryana Agricultural University, Hisar.
HHB-176	Hybrid	HMS 94555A × H77/833-2; bred at CCS Haryana Agricultural University, Hisar for general cultivation in Haryana.
HHB-181	Hybrid	HMS 89111A × H77/833-2; late flowering, high grain yield, highly resistant to downy mildew and tolerant to smut, resistant to shoot fly and leaf rollers. Bred at CCS Haryana Agricultural University, Hisar for general cultivation in Haryana.
G73-107× BSECTCP-1	Hybrid	Bred at CCS Haryana Agricultural University, Hisar using BSECT CP-1 from ICRISAT, Patancheru as male parent.
77/371× BSECTCP-1	Hybrid	Bred at CCS Haryana Agricultural University, Hisar using BSECT CP-1 from ICRISAT, Patancheru as male parent.
841B×90/4-5	Hybrid	Bred at CCS Haryana Agricultural University, Hisar using 841B from IARI, New Delhi as female parent.

tolerance at seedling stage. This technique could be used in the hottest months (May-June) with no rainfall. Since the technique was applicable to the very young seedlings of pearl millet, it was also of concern to test the association of seedling thermo-tolerance to other phenotypic characteristics of seedlings. The membrane thermo stability (based on quantification of electrolyte leakage) was also conducted for screening of the pearl millet genotypes at seedling stage (Ibrahim and Quick 2001).

The experiment was conducted at the research area of Department of Genetics and Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar (Lat: 29°10'N, Long: 75°46'E, and 215.2m above mean sea level), located in subtropical region of Haryana, India. There were 46 genotypes tested for heat tolerance and related traits in three replicated plots under supra-optimal temperature exposure at seedling stage in two stress environments (environment-1 and environment-2) created through different dates of sowing on 10th May and 1st June, so as to conduct the experiment under different range of temperatures with no rainfall. Each genotype was grown in three-rows each of 3 m length spaced 30 cm apart with 10 cm plant to plant spacing (plant density 33.3 plants/m²). The field was given medium light irrigation through flooding and was precisely levelled before sowing. The seeds were sown manually.

The soil of experimental field was Alluvial soil, order-inceptisols (according to USDA soil classification), sandy loam in texture, calcareous and hyperthermic. The water holding capacity, field capacity and permanent wilting point of the experimental field were 40, 17 and 6%, respectively (Singh 2007). Irrigation was withheld during the course of experiment, however, drought stress-free conditions were ensured through monitoring at regular interval (on alternate days) by measuring the moisture status of the soil by gravimetric method. The soil samples of about 50 g were taken up to 20 cm depth from the experimental plot by using soil sampling auger (Dirksen 1999). The soil samples were collected immediately in the leak-proof, seamless, pre-weighed aluminium containers and placed in oven at 105°C until the mass stabilizes at a constant value (Gardner *et al.* 2001). The samples and containers were weighed in the laboratory both before and after drying, the difference being the mass of water originally in the sample. The moisture content on dry weight basis may be calculated (Black 1965) using the following formula:

$$\text{Soil moisture (\%)} = \frac{(\text{wt. of wet soil} - \text{wt. of dry soil})}{\text{wt. of dry soil}} \times 100$$

The experiment was terminated before reaching the permanent wilting point. A condition of no-drought was maintained in order to determine the exclusive effect of temperature at seedling stage. This was maintained by measuring the moisture status of the soil by gravimetric method on alternate days. The soil surface temperature was

measured by a soil thermometer at soil surface, temperature at 5 cm and 100 cm (1 m) above the soil surface were recorded between 2:00 pm and 2:30 pm daily.

The emphasis was given to the observation of germination, survival and mortality of the seedlings. The seedlings were inspected every day in the morning. The seedlings which had died due to heat stress were noted in each genotype on daily basis. Such seedlings were marked by fixing a small wooden stick along their side as a check against the loss of dead seedlings. Observations continued till the surviving seedlings were established (15 DAE) and there was no further mortality.

Thermo-tolerance characteristics, viz. seedling thermo-tolerance index (STI), seed to seedling thermo-tolerance index (SSTI) and membrane thermo-stability (MTS) were recorded. Seedling thermo-tolerance index (STI) was calculated as the ratio of number of seedling survived to the number of seedling emergence, expressed in percentage. Seed to seedling thermo-tolerance index (SSTI) was calculated as the ratio of seedling survival to the number of seedling expected to emerge, expressed in percentage (Singh 1993, Yadav *et al.* 2006, 2011). The SSTI is an extension of STI by taking expected germination into account. It was necessary to correct the effect of under soil mortality (USM). The loss of germination due to heat (i.e. under soil mortality) can be measured only after a standard germination test (Singh 1993). Therefore, the germination of seed from the same lot under monsoon environment (main pearl millet growing season, where no under soil mortality occurred due to heat stress) was taken as expected germination for calculation of SSTI.

These genotypes were also screened through laboratory technique (membrane thermo-stability: Ibrahim and Quick 2001, Martineau *et al.* 1979a & b, Yadav *et al.* 2009). The young leaves of 21 days old seedlings of each genotype in three replication plots were used for membrane thermo-stability test. A middle portion of 10cm from each fully expanded youngest leaf from top of the plant was taken and the midrib was removed. Each half was taken to provide replication. Each leaf portion was cut into pieces and thoroughly washed with distilled water to remove the electrolyte released from the cutting of plant tissue and put them into test tubes with maintaining sufficient water to prevent desiccation of plant material during heat treatment. These test tubes were covered with aluminium foil and incubated in water bath at 55°C for 15 minutes. After this heat treatment, 10 ml of distilled water was added to each test tube and kept at 10°C in a refrigerated incubator shaker (Innova-4230, New Brunswick Scientific, USA) for overnight to allow diffusion of electrolytes from plant material. Following morning, these test tubes were brought out of refrigerated incubator shaker and T₁ (initial electrical conductivity) was recorded with electrical conductivity meter (CyberScan PC-510, Eutech Instruments). Then these test

tubes were autoclaved at 15 psi for 2 minutes for measurement of complete electrolyte leakage from the leaf samples and final electrical conductivity (T_2) was recorded. Membrane thermo-stability (MTS) was calculated according to Ibrahim and Quick (2001) and Yadav *et al.* (2009) as follows:

Membrane thermo stability (MTS) = $(1 - T_1/T_2) \times 100$
where, T_1 , conductivity after heat treatment; T_2 , conductivity after autoclaving.

The growth characteristics, viz. germination (%), emergence rate (ER), number of leaves/seedling (two weeks stage), seedling height (two weeks stage), seedling fresh weight (g) (four weeks stage) and seedling dry weight (g) (four weeks stage) were recorded. The data for seedling height and number of leaves per seedling was recorded on 10 random seedlings per plot. Emergence rate (an index for speed of germination) can be used as a tool in pearl millet breeding programmes for evaluation of seedling vigour. Speed of germination is one of the oldest concepts of seedling vigour. The emergence rate (ER) was calculated by dividing the number of normal seedlings obtained at each counting by the number of days seeds had been in the seed bed (DAS). The values obtained at each count were then summed at the end of the germination test to obtain the emergence rate (Maguire 1962, Czabator 1962).

$$\text{Emergence Rate (ER)} = \frac{\text{Seeding count}}{\text{Days to first count}} + \dots + \frac{\text{Seeding count}}{\text{Days to first count}}$$

Analysis of variance for randomized block design (RBD) for field traits, i.e. germination, emergence rate, number of leaves/seedling, seedling height, seedling fresh weight, seedling dry weight and Completely Randomized Design (CRD) for membrane thermo-stability test (Table 2) was conducted under each environment on mean basis (Panse and Sushatme 1967). Correlations among the various characters studied were calculated to establish the nature and magnitude of associations among the traits (Fisher and Yates 1963). Multivariate clustering was done based on paired group cluster method using similarity coefficient under Past 1.40 software (Hammer *et al.* 2001). Multivariate analysis based on the Euclidean distance matrix was estimated among genotypes to distinguish genotypes from each other. The

grouping of genotypes was generated using paired group cluster method.

RESULTS AND DISCUSSION

Environmental conditions

The maximum soil temperature rose up to 63.5°C in E_2 , was about 18.5°C higher than the normal atmospheric temperature measured at 1 m above the soil surface (Fig 1a). This excessive amount of heat was the main cause of seedling mortality. Many a seedlings were found desiccated at the point of contact with soil besides the desiccation at the tips. The temperature around the tip level, i.e. 5cm above the soil surface also reached up to an extent of 50°C. It could therefore be concluded that the seedlings had to survive in temperatures as high as 50°C at tip level and 63°C at soil contact level (Soman *et al.* 1981, Singh 1993, Yadav *et al.* 2006). Simultaneously, the moisture level remained above the permanent wilting point (Fig 1b), therefore drought effect was not relevant.

Thermo-tolerance screening

Heat tolerance field screening techniques and laboratory screening technique were simultaneously used. The heat tolerance was measured as seedling thermo-tolerance index (STI) through the field screening technique according to Peacock *et al.* (1993). Another index known as seed to seedling thermo-tolerance index (SSTI) (Singh 1993, Yadav *et al.* 2006, 2011) was also calculated in addition to STI, whereas membrane thermo-stability (MTS) was examined as index for heat tolerance in the laboratory technique (Yadav *et al.* 2009). Membrane thermo-stability (MTS) is a well-established and validated technique used by many workers in different crops. The analysis of variance (RBD) for eight different characters and CRD analysis for membrane thermo-stability in two different stress environments was carried out and the results presented in Table 2 showed significant genotypic differences for all the characters in both environments which justified further analysis of the data. Similar intra-specific variation for heat tolerance was also reported for sorghum (Sullivan and Ross 1979), soybean (Martineau *et al.* 1979 a & b), wheat (Blum and Ebercon

Table 2 Analysis of variance (ANOVA) for traits related to pearl millet heat tolerance screened in two environments (E_1 , E_2)

Environment	Source of Variation	D.F	STI	SSTI	Germination	Rate of emergence	No. of leaves	Seedling height	Fresh weight	Dry weight
E_1	Replication	2	5.765	7.02	12.36	41.99	0.155	0.185	0.36	0.03
	Treatment	45	250.53**	208.98**	88.24**	154.51**	5.44**	13.29**	30.75**	1.33**
	Error	90	7.18	7.78	3.67	11.19	0.75	3.29	1.44	0.07
E_2	Replication	2	1.61	19.86	12.98	263.18	0.84	8.56	1.57	0.05
	Treatment	45	315.04**	260.78**	94.08**	65.33**	4.98**	16.64**	39.51**	1.53**
	Error	90	6.63	7.38	4.83	20.83	0.87	2.92	2.35	0.11

* Significant at P = 0.05, ** significant at P = 0.01

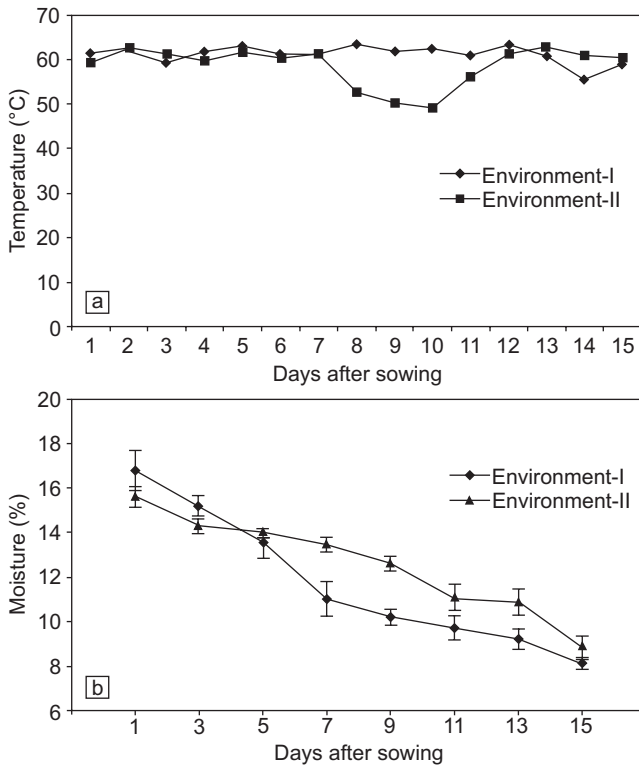


Fig 1 a) Observations recorded for surface temperature of soil b) soil moisture content measured in the root zone of pearl millet seedlings (up to 15cm depth) in two environments during experiment

1981), *Dactylis* spp. (Premachandra and Shimada 1987) and maize (Premachandra *et al.* 1989).

Evaluation of genotypes based on thermo-tolerance indexes (STI, SSTI, MTS)

The analysis of variance (RBD) for field thermo-tolerance indexes (STI and SSTI) and CRD analysis for membrane thermo-stability (laboratory experiment) in two different stress environments was carried out and the results presented in Table 2 showed significant genotypic differences which justified further analysis of the data. The genotypes (inbreds) were evaluated on the basis of mean performance for various characters in each environment (Tables 3). On the pooled basis seedling thermo-tolerance index (STI) value of inbreds ranged from 41.09 to 77.84 with an average of 55.66. The genotype H77/833-2 attained the highest STI score followed by 99HS-18, CVJ-2-5-3-1-3, 96AC-93, Togo-II and G73-107. Based on STI, these seven genotypes (inbreds) were classified as the tolerant for heat stress while 1305, 77/245 and H77/29-2 were recognized as the susceptible to heat stress. Seed to seedling thermo-tolerance index (SSTI) is expected to be more rigorous compared to STI as the SSTI also considers the under surface mortality (USM). The genotype 96AC-93 attained the highest SSTI value followed by 99HS-18, G73-107, H77/833-2, CVJ-2-5-3-1-3 and Togo-

II based on environmental mean basis (Table 3). The genotype 1305 had the lowest SSTI value followed by two genotypes 77/245 and H77/29-2. The values of SSTI of inbreds ranged from 31.47 to 64.79 with an average of 44.69.

Seedling thermo-tolerance index (STI) values of hybrids ranged from 46.29 to 71.18 with an average of 61.32, whereas SSTI values ranged from 35.21 to 62.36 with hybrid mean of 48.09. The average STI and SSTI value of hybrids were higher than the inbreds with narrow range. The hybrid 77/371 × BSECT CP-1 attain the highest STI as well as SSTI score followed by hybrid HHB-67-2 and HHB-146, respectively. Other two hybrids 841B × 90/4-5 and G73-107×BSECT CP-1 showed low STI as well as SSTI values.

In general the STI values were invariably higher compared to SSTI and the reduction in SSTI values were not in strict proportion to the STI (Fig 2). The difference between STI and SSTI values depend on thermo-sensitivity of seed germination of particular genotype.

Some inbreds (e.g 36B, 77/273, 96AC-82) and hybrids (e.g G73-107 × BSECT CP-1, HHB-117, HHB-181) showed poor germination and high STI values, therefore, a caution is needed in evaluating such genotypes for STI. The inbreds 77/273, 36B, 96AC-82 and hybrids (G73-107 × BSECT CP-1), HHB-117, HHB-181 had STI values 58.00, 53.89, 50.59, .64, 64.39, 63.90, respectively and SSTI values 44.25, 35.43, 36.79, 44.13, 46.43, 46.82, respectively. This changed the whole status of the genotypes in respect to thermo-tolerance. The SSTI index had a highly reduced value shifting these genotypes towards the susceptible side. It may be concluded therefore, that SSTI is more reliable tool for screening and evaluating the genotypes for heat tolerance.

For membrane thermo stability (MTS), inbred CVJ-2-5-3-1-3 attained the highest MTS value (75.05) and inbred 1305 scored lowest (52.35). Among the hybrids, 77/371 × BSECT CP-1 scored highest MTS value (73.12) and hybrid 841B × 90/4-5 had the lowest value (60.96). Among the hybrids the variability was less compared to inbreds for heat tolerance and related traits as observed from range of all the characters.

Peacock *et al.* (1993) in his experiment had evaluated the materials on the basis of survival of young seedlings only and there was no consideration of pre-emergence heat effect. However, along with seedling survival for thermo-tolerance, seed germination thermo-sensitivity is also equally important (Garcia-Huidbro *et al.* 1985, Howarth 1989, Khalifa and Ong 1990). It is imperative, therefore, to study the germination along seedling response to understand the effect of temperature on stand establishment of pearl millet. Out of top ten genotypes selected on the mean basis for thermo-tolerance using different screening techniques (STI, SSTI and MTS), nine genotypes were found common.

Evaluation of genotypes based on morphological traits

The analysis of variance (RBD) for other seedling traits

Table 3 Mean performance of the genotype during screening for different characters

Genotypes	STI (%)*		SSTI (%)*		Germination (%)*		Emergence rate (ER)		No. of leaves/seedling		Seedling height (cm)		Fresh weight (g)/seedling		Dry weight (g)/seedling		Membrane thermo stability*										
	E ₁	E ₂	Mean	E ₁	E ₂	Mean	E ₁	E ₂	Mean	E ₁	E ₂	Mean	E ₁	E ₂	Mean	E ₁	E ₂	Mean									
CSSC-46-2	64.73	71.04	67.89	52.88	64.60	58.74	42.53	44.75	43.64	48.92	41.25	45.09	6.20	6.53	6.37	10.00	11.87	10.93	10.47	15.25	11.42	1.88	2.85	2.37	70.35	76.25	73.30
G73-107	64.72	71.23	67.97	54.97	65.82	60.39	48.32	52.76	50.54	50.81	40.50	45.66	6.30	6.90	6.60	11.63	12.43	12.03	9.67	12.22	10.18	2.98	3.64	3.31	71.78	73.59	72.69
H77/29-2	44.21	44.52	44.36	31.60	36.34	33.97	42.40	47.95	45.18	38.09	37.77	37.93	3.14	3.80	3.47	11.37	12.70	12.03	3.37	5.68	3.83	1.03	1.67	1.35	54.51	56.03	55.27
78/711	47.84	53.99	50.91	38.21	46.92	42.56	40.53	46.48	43.50	33.76	37.01	35.39	4.34	5.09	4.72	11.20	12.10	11.65	6.76	7.44	6.90	1.32	1.54	1.43	61.95	62.91	62.43
96AC-93	70.84	72.73	71.78	63.01	66.56	64.79	55.99	49.82	52.90	40.03	37.83	38.93	6.60	6.70	6.65	12.90	13.60	13.25	10.33	13.10	10.89	2.41	2.77	2.59	70.80	72.87	71.83
HTP-94/54	54.62	51.58	53.10	48.39	39.77	44.08	42.01	32.95	37.48	34.05	35.04	34.55	3.39	4.07	3.73	13.43	12.00	12.72	10.78	12.40	11.10	2.06	2.21	2.14	59.62	61.66	60.64
ICMR-01004	50.83	54.37	52.60	39.13	47.54	43.34	42.17	48.40	45.28	47.77	40.23	44.00	5.28	5.87	5.58	7.47	9.40	8.43	6.19	9.75	6.90	1.63	2.41	2.02	61.13	61.39	61.26
ICMR-01007	55.38	59.60	57.49	49.49	53.25	51.37	58.52	59.10	58.81	41.83	39.52	40.68	2.59	3.16	2.88	8.33	10.10	9.22	3.62	4.35	3.77	1.09	1.30	1.20	66.68	67.99	67.33
H90/4-5	54.59	59.63	57.11	49.65	53.49	51.57	54.31	54.06	54.19	48.84	42.81	45.82	5.77	5.51	5.64	7.33	7.67	7.50	4.08	4.41	4.14	1.43	1.49	1.46	66.03	66.49	66.26
(G73-107 × BSECT CP-1)	59.10	54.17	56.64	49.51	38.75	44.13	45.75	38.74	42.24	40.29	46.52	43.40	5.73	6.07	5.90	11.50	10.23	10.87	6.64	8.81	7.08	1.60	2.24	1.92	61.50	62.67	62.08
INB 87/74-3-20	51.44	58.09	54.77	41.70	47.90	44.80	45.56	47.15	46.35	38.45	41.17	39.81	5.78	5.57	5.67	9.63	9.07	9.35	5.61	5.65	5.62	1.22	1.15	1.19	64.83	66.92	65.87
VCF ₄ -2359	46.04	47.33	46.69	38.02	40.96	39.49	40.97	42.95	41.96	38.82	37.11	37.96	5.97	6.43	6.20	12.30	12.03	12.17	13.27	16.40	13.89	2.38	3.25	2.82	57.63	61.16	59.39
MRC	47.54	45.06	46.30	40.39	39.09	39.74	44.31	42.00	43.15	45.87	36.81	41.34	5.40	5.73	5.57	9.43	10.53	9.98	6.00	8.83	6.57	1.02	1.66	1.34	57.97	58.32	58.14
72-2-2-98K-1	50.29	59.51	54.90	36.70	47.58	42.14	40.29	46.10	43.19	39.67	40.98	40.32	5.87	5.98	5.93	9.73	10.83	10.28	6.37	7.65	6.62	1.26	1.31	1.29	65.52	65.37	65.45
1448-98K-1	54.02	52.54	53.28	40.72	45.35	43.04	36.99	42.21	39.60	47.70	34.36	41.03	4.53	4.83	4.68	10.13	10.80	10.47	9.07	11.53	9.56	1.75	2.25	2.00	61.00	60.32	60.66
(77/371 × BSECT CP-1)	70.31	74.04	72.18	55.54	69.19	62.36	47.97	55.54	51.76	40.11	39.47	39.79	5.80	5.37	5.58	13.80	15.17	14.48	9.57	13.55	10.36	2.07	2.81	2.44	70.81	75.43	73.12
1250	52.32	56.88	54.60	36.34	47.82	42.08	42.14	54.66	48.40	53.40	35.74	44.57	4.73	4.77	4.75	9.17	8.50	8.83	6.38	8.71	6.84	1.26	1.81	1.53	58.97	62.78	60.87
HTP-31	51.06	56.78	53.92	36.05	44.83	40.44	41.15	51.20	46.17	49.13	42.27	45.70	3.02	3.29	3.16	7.97	7.67	7.82	4.89	5.68	5.04	1.20	1.47	1.34	58.31	58.39	58.35
77/667	44.41	48.70	46.56	38.18	39.21	38.70	28.60	38.09	33.34	42.77	28.19	35.48	3.63	3.47	3.55	8.20	8.13	8.17	6.73	9.51	7.29	1.37	2.06	1.72	62.82	60.46	61.64
1305	40.33	41.85	41.09	27.84	35.10	31.47	44.64	50.88	47.76	47.75	39.43	43.59	2.97	3.59	3.28	8.53	10.60	9.57	5.51	8.67	6.14	1.11	1.82	1.46	52.08	52.62	52.35
HTP-92/80	49.63	53.64	51.63	33.44	46.57	40.01	39.44	52.26	45.85	38.40	38.41	38.41	5.02	5.13	5.08	12.37	14.47	13.42	3.10	5.75	3.63	0.84	1.45	1.14	63.53	62.76	63.14
96AC-82	48.81	52.38	50.59	34.86	38.72	36.79	43.26	45.40	44.33	39.59	36.14	37.87	4.60	5.27	4.93	6.57	9.03	7.80	4.09	5.04	4.28	1.17	1.32	1.24	65.66	62.69	64.18
S-97/120	50.21	52.52	51.36	34.73	43.05	38.89	41.69	50.54	46.11	40.39	35.14	37.77	4.97	5.57	5.27	8.07	9.97	9.02	2.94	5.74	3.50	1.04	1.81	1.42	61.39	57.99	59.69
99HS-18	78.56	76.26	77.41	57.94	63.26	60.60	48.34	50.11	49.23	47.99	34.13	41.06	6.83	6.99	6.91	12.37	14.07	13.22	14.36	17.59	15.00	3.02	3.26	3.14	72.88	77.05	74.97
Togo-II	71.00	72.29	71.64	52.23	58.27	55.25	47.09	48.39	47.74	55.13	36.20	45.66	6.95	7.11	7.03	10.97	13.43	12.20	9.38	12.85	10.07	2.02	2.67	2.35	72.65	75.51	74.08
HTP-88	55.37	59.84	57.61	40.23	50.92	45.57	46.93	58.13	52.53	47.90	39.78	43.84	4.30	5.11	4.71	13.93	15.67	14.80	9.92	12.43	10.42	1.74	2.35	2.05	67.06	66.86	66.96
ICR-161	43.72	49.69	46.70	32.75	38.48	35.61	41.03	50.88	45.95	47.37	30.89	39.13	5.63	5.93	5.78	9.77	11.20	10.48	7.27	7.63	7.34	1.71	1.63	1.67	56.99	55.52	56.26
77/245	42.31	44.12	43.22	29.36	38.37	33.87	44.22	47.53	45.87	52.44	36.63	44.54	4.03	4.90	4.47	13.57	15.37	14.47	5.93	7.59	6.26	1.43	1.78	1.60	53.30	54.73	54.02
77/371	53.24	60.34	56.79	42.55	54.29	48.42	46.70	53.61	50.16	54.98	39.87	47.42	4.81	5.24	5.02	7.62	6.77	7.19	6.61	9.17	7.12	1.42	1.75	1.58	64.00	64.89	64.44
CVJ-2-5-3-1-3	69.80	78.76	74.28	55.71	60.55	58.13	48.43	49.15	48.79	44.39	38.44	41.41	7.63	7.80	7.72	11.87	13.37	12.62	11.12	14.67	11.83	3.01	3.74	3.38	74.60	75.49	75.05
99/57-1	49.20	56.14	52.67	39.29	43.59	41.44	43.26	42.86	43.06	31.59	41.86	36.72	5.17	5.31	5.24	9.20	8.23	8.72	3.40	4.63	3.65	0.85	1.16	1.00	63.00	64.47	63.73
77/273	55.22	60.78	58.00	38.38	50.11	44.25	39.05	47.11	43.08	46.79	32.69	39.74	5.37	5.70	5.54	8.23	7.83	8.03	8.32	10.68	8.79	1.54	2.04	1.79	67.92	65.44	66.68
841B × 90/4-5	47.76	44.83	46.29	35.55	34.86	35.21	43.11	44.83	43.97	33.55	38.85	36.20	5.08	5.65	5.36	10.10	11.50	10.80	9.59	13.60	10.39	1.88	2.50	2.19	62.35	59.57	60.96

Table 3 Contd.

Genotypes	STI (%)*			SSTI (%)*			Germination (%)*			Emergence rate (ER)			No. of leaves/seedling			Seedling height (cm)			Fresh weight (g)/seedling			Dry weight (g)/seedling			Membrane thermo stability*		
	E ₁	E ₂	Mean	E ₁	E ₂	Mean	E ₁	E ₂	Mean	E ₁	E ₂	Mean	E ₁	E ₂	Mean	E ₁	E ₂	Mean	E ₁	E ₂	Mean	E ₁	E ₂	Mean	E ₁	E ₂	Mean
7B	53.32	48.47	50.89	37.66	45.67	41.66	40.69	54.96	47.82	46.41	34.85	40.63	4.47	5.44	4.95	12.17	13.50	12.83	5.50	5.56	5.51	1.36	1.31	1.33	70.33	69.84	70.08
18B	47.85	42.49	45.17	34.80	33.96	34.38	38.78	42.30	40.54	35.50	32.46	33.98	3.24	3.52	3.38	7.53	9.33	8.43	6.27	7.55	6.52	1.42	1.55	1.48	66.61	64.61	65.61
36B	49.92	57.86	53.89	35.10	35.75	35.43	41.98	37.92	39.95	30.56	28.20	29.38	5.55	6.47	6.01	8.77	8.43	8.60	12.24	12.70	12.34	2.37	2.34	2.36	66.37	65.38	65.88
94222B	49.79	56.19	52.99	38.04	45.08	41.56	39.85	42.63	41.24	29.55	34.80	32.18	5.11	5.87	5.49	9.43	11.10	10.27	7.90	11.23	8.57	1.67	2.34	2.00	69.48	66.61	68.05
HHB-67-1	56.49	59.13	57.81	44.93	47.32	46.12	48.55	49.33	48.94	41.33	44.46	42.89	8.10	8.21	8.16	12.53	14.03	13.28	12.17	15.38	12.81	1.94	2.69	2.31	63.12	64.98	64.05
HHB-67-2	68.29	67.81	68.05	48.56	56.03	52.30	51.90	54.28	53.09	45.71	44.34	45.03	8.23	8.37	8.30	12.17	14.00	13.08	13.03	15.55	13.54	3.11	3.51	3.31	71.62	70.27	70.95
HHB-94	62.21	65.87	64.04	50.28	52.05	51.17	54.44	53.84	54.14	40.60	40.09	40.35	5.38	5.77	5.57	10.33	12.07	11.20	10.11	11.17	10.32	1.94	2.09	2.02	68.86	69.58	69.22
HHB-117	59.88	68.90	64.39	43.85	49.00	46.43	48.28	49.09	48.68	38.16	41.72	39.94	6.56	6.97	6.76	10.10	9.77	9.93	12.50	12.50	12.50	2.20	2.43	2.31	68.64	70.55	69.59
HHB-146	61.26	66.50	63.88	48.75	56.99	52.87	48.59	53.18	50.89	43.38	43.21	43.29	7.31	8.37	7.84	12.83	13.07	12.95	9.93	11.43	10.23	1.90	2.11	2.00	68.24	70.65	69.44
HHB-162	57.40	58.28	57.84	45.37	47.07	46.22	49.15	50.54	49.84	34.41	45.89	40.15	6.51	6.67	6.59	14.10	14.20	14.15	14.23	14.57	14.30	2.52	2.64	2.58	65.09	63.63	64.36
HHB-176	58.33	60.59	59.46	45.25	45.56	45.40	44.37	43.49	43.93	28.08	44.04	36.06	5.88	6.25	6.07	12.50	12.23	12.37	9.59	10.58	9.79	1.73	1.99	1.86	63.03	59.34	61.19
HHB-181	61.74	66.07	63.90	46.15	47.49	46.82	49.95	48.95	49.45	34.37	39.11	36.74	6.43	6.40	6.42	13.13	13.70	13.42	12.72	14.36	13.05	3.69	3.74	3.72	68.42	70.23	69.33
H77/833-2	75.24	80.45	77.84	56.32	62.83	59.58	39.75	43.20	41.47	56.35	52.33	54.34	6.46	6.84	6.65	8.60	9.47	9.03	9.02	10.59	9.33	2.65	2.89	2.77	71.75	75.42	73.58
Mean	55.46	58.39	56.92	42.84	48.17	45.50	44.65	48.05	46.35	42.46	38.66	40.56	5.36	5.73	5.55	10.50	11.33	10.91	8.19	10.22	8.59	1.79	2.19	1.99	64.81	65.38	65.10
SE	2.188	2.102		2.277	2.218		1.57	1.79		2.73	3.73		0.71	0.76		1.48	1.39		0.98	1.25		0.22	0.27		2.26	2.07	
CD	4.355	4.184		4.531	4.414		3.12	3.57		5.44	7.42		1.41	1.51		2.95	2.78		1.95	2.49		0.44	0.54		4.57	4.17	

in two stress environments was also carried out (Table 2) indicating significant genotypic differences which justified further analysis of the data. The environment (E₁) had the highest range of temperature with a high average which caused lower germination in E₁ than in E₂ resulting lower SSTI values. Genotype-wise, highest germination (58.81) was recorded for the inbred ICMR-01007 over both the environments, which provides a preliminary indication of heat resistance. The inbred 77/667 had the lowest germination capacity (33.34) and also showed low STI and SSTI values. Likewise, the hybrid HHB-94 attained the highest germination among the hybrids, whereas G73-107 × BSECT CP-1 had the lowest germination. On the mean basis over the environments, H77/833-2 was evaluated as the inbred with fastest emergence of seedlings. The inbred 36B showed the lowest emergence rate of the seedlings. Among the hybrids HHB-67-2 was observed as fastest growing hybrid with maximum number of leaves/seedling. HHB-67-2 is also the earliest maturing hybrid. Number of leaves/seedling is an attribute of initial vigour and growth of the plant. The genotype HHB-67-2 had the highest number of leaves/seedling among hybrids and inbreds while the genotype ICMR- 01007 had the lowest value. The number of leaves/seedling among the hybrids ranged from 5.36 to 8.30 with a mean of 6.60 which was higher than the mean of inbreds (5.22). The genotypes showing high vigour are apparently showing better tolerance to high temperature. It could be attributed to the better utilization of seed resources (Kapoor 1972).

Seedling height is an indication of vigour and speed of growth of seedling at an early stage. The genotype HTP-88 was the fastest growing genotype and 77/371 was identified as the slowest growing genotype as indicated by mean of both the environments. Seedling height of inbreds ranged from 7.19 cm to 14.8 cm, whereas range of hybrids was 9.93 cm to 14.48 cm. The average height of hybrids (12.41cm) was higher than inbreds (10.44cm). The inbred 99HS-18 had the highest fresh weight/seedling (15.00 g), while the inbred S-97/120 had the lowest fresh weight/seedling (3.50 g). The average fresh weight/seedling of hybrids (11.31 g) was higher than inbreds (7.74 g). E₁ had the lower dry weight/seedling than E₂ indicating the more heat stress during the E₁. The hybrid HHB-181 had the highest seedling dry weight among inbreds and hybrids while the inbred 99/57-1 had the lowest dry weight/seedling.

Correlation coefficient analysis

Three measured indexes of thermo-tolerance (STI, SSTI and MTS) were found highly correlated among themselves (Table 4). The laboratory tests like MTS could be used to screen a large number of genotypes with all-time ease. Nonetheless, the MTS test should be probably combined with SSTI as it provides a direct evidence of heat tolerance of seeds and seedlings. Therefore, the reliable screening for heat tolerance could be also done by laboratory test of MTS

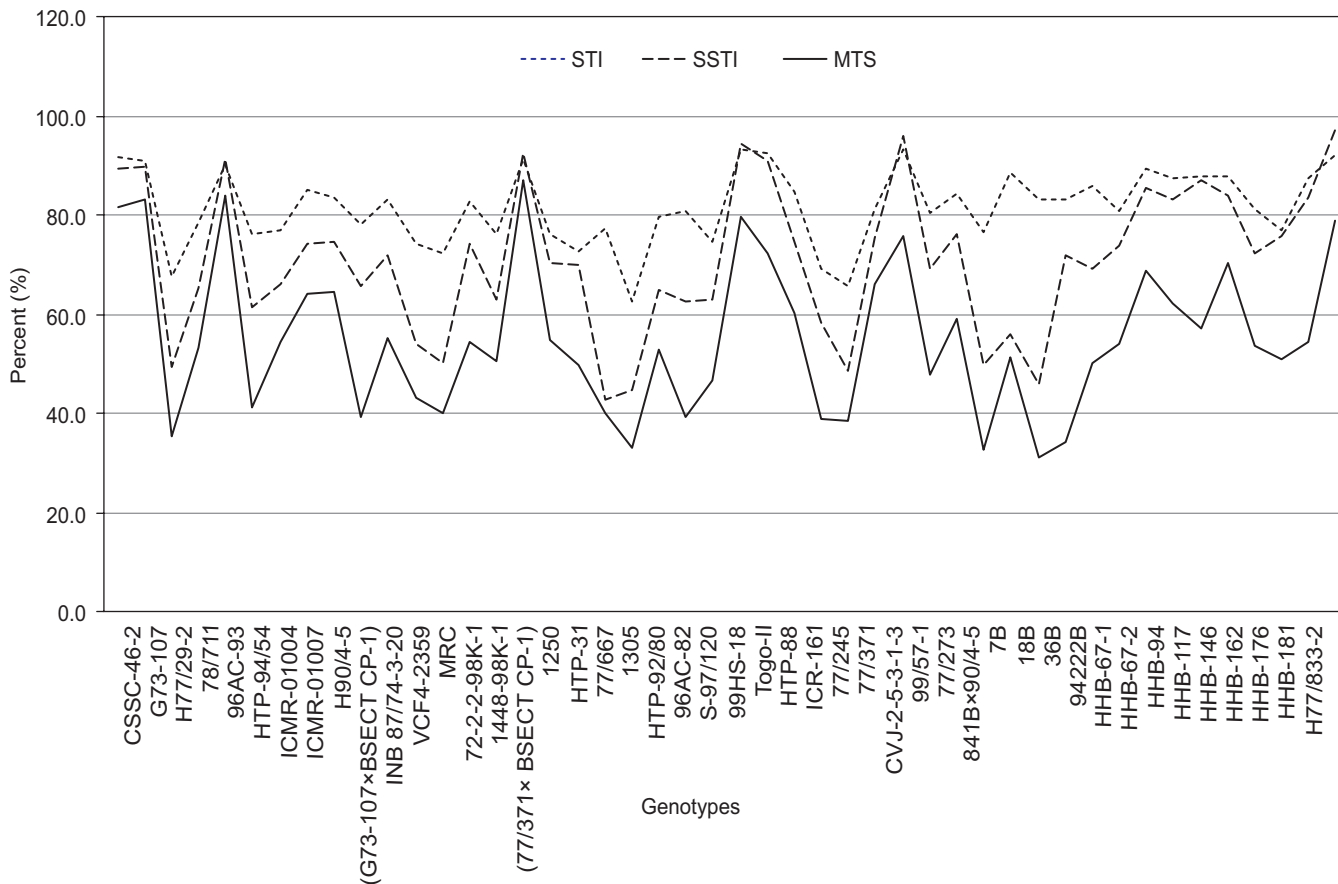


Fig 2 Values of STI, SSTI and Membrane thermo-stability

Table 4 Correlation coefficients among various traists related to pearl millet heat tolerance performed across two experiments (E₁, E₂)

Characters	STI	SSTI	Germination	Rate of emergence	No. of leaves	Seedling height	Fresh weight	Dry weight	MTS
STI	1.000	0.915**	0.498**	0.245	0.655**	0.357*	0.564**	0.692**	0.807**
	1.000	0.902**	0.288*	0.391**	0.620**	0.224	0.494**	0.594**	0.881**
SSTI		1.000	0.582**	0.165	0.581**	0.337*	0.504**	0.622**	0.727**
		1.000	0.465**	0.363*	0.493**	0.297*	0.402*	0.504**	0.848**
Germination			1.000	0.050	0.371*	0.286	0.262	0.357*	0.362*
			1.000	0.232	0.091	0.283	-0.093	0.001	0.255
Rate of emergence				1.000	0.056	-0.176	-0.103	0.021	0.045
				1.000	0.360*	0.126	0.073	0.167	0.241
No. of leaves/ seedling					1.000	0.368*	0.635**	0.652**	0.576**
					1.000	0.377**	0.627**	0.627**	0.557**
Seedling height						1.000	0.550**	0.474**	0.149
						1.000	0.481**	0.460**	0.266
Fresh weight /seedling							1.000	0.859**	0.436**
							1.000	0.881**	0.472**
Dry weight /seedling								1.000	0.568**
								1.000	0.541**
Membrane thermo stability									1.000
									1.000

* Significant at P = 0.05, ** significant at P = 0.01
Upper values for E1 and lower values for E2

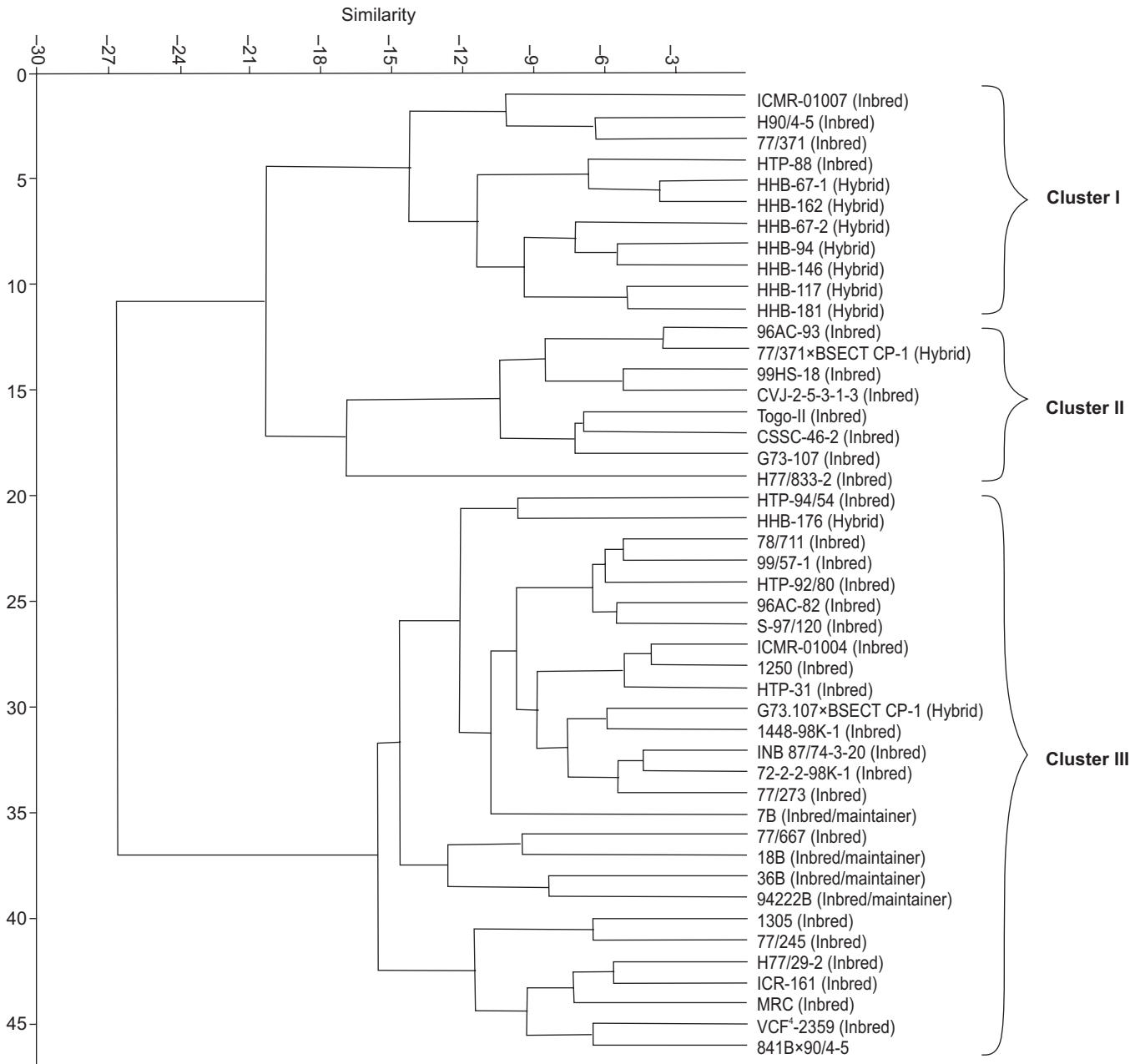


Fig 3 Dendrogram representing similarity among pearl millet genotypes based on PAST cluster analysis of Euclidean distance based on phenotypic traits.

(Oosterhuis *et al.* 2008). The field based indices STI and SSTI also showed significant correlation with other growth traits of seedling, e.g germination, emergence rate, height of seedling, number of leaves/seedling, fresh weight and dry weight/seedling. These growth characters of seedling can also give some credible indications for heat tolerance of genotypes (Peacock *et al.* 1993, Kumar 2007, Singh 1993). The faster germination and early vigour of seedling could act as physical indicators for heat tolerance which could be recognized for their efficiency to escape the lethal effect of heat.

Cluster analysis

Cluster analysis of the data (including all the characters) based on similarity among genotypes indicated the relative position of genotypes in each group (Fig 3). The pearl millet genotypes were broadly grouped into three clusters, i.e cluster-I (ICMR-01007, H90/4-5, 77/371, HTP-88, HHB-67-1, HHB-162, HHB-67-2, HHB-94, HHB-146, HHB-117, HHB-181), cluster-II (96AC-93, (77/371 × BSECT CP-1), 99HS-18, CVJ-2-5-3-1-3, Togo-II, CSSC-46-2, G73-107, H77/833-2) and cluster-III (HTP-94/54, HHB-176, 78/711, 99/57-1, HTP-92/80, 96AC-82, S-97/120, ICMR-01004, 1250, HTP-31,

(G73-107×BSECT CP-1), 1448-98K-1, INB 87/74-3-20, 72-2-2-98K-1, 77/273, 7B, 77/667, 18B, 36B, 94222B, 1305, 77/245, H77/29-2, ICR-161, MRC, VCF₄-2359, 841B×90/4-5). Cluster-II consists generally heat tolerant genotypes. Within this group, H77/833-2 has shown distinctness from other genotypes. This genotype is an elite pollinator, which has been used in many commercially released early maturing hybrids (Divedi *et al.* 2012). It is a male parent of earliest maturing commercial hybrid HHB-67. Almost all the hybrids are grouped in cluster-I. Generally hybrids are superior for morphological traits as compared to inbred due to heterotic effects.

The contrasting pairs of high heat tolerant genotypes (e.g. H77/833-2, G73-107, CVJ-2-5-3-1-3, 77/371 × BSECT CP-1, 96AC-93, Togo-II, 99HS-18) and susceptible genotypes (e.g. H77/29-2, 77/245, 1305) have potential use as parents in breeding experiments. The resulting populations from selective crosses will be useful for deriving quantitative data which can be applied to quantitative genetics in order to understand the inheritance and basis of thermo-tolerance expression in pearl millet. On similar lines quantification of thermo-tolerance variations in pearl millet will contribute in understanding inheritance of thermo-tolerance expression at seedling stage through use of data in quantitative genetics.

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