

HEAT TOLERANCE IN PEARL MILLET : A REVIEW

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(Received : 14 May 2016; Accepted : 30 June 2016)

SUMMARY

Global warming may cause serious problem in agricultural production, particularly in arid and semi-arid regions of the world. As the heat stress affects the seed germination, plant growth and development, resulting in drastic reduction in yield. Pearl millet is a multipurpose crop grown in these areas due to its tolerance to high temperature/drought. But, further improvement for thermo tolerance is of instant need, for economization of agriculture in these areas. Therefore, to develop/identify heat tolerant genotypes, knowledge of screening methods, heat tolerance mechanisms along with breeding techniques are important. Heat stress decreases the rate of germination and may also inhibit the germination depending on heat tolerance of the genotype. A lot of genetic variability is available for heat tolerance in pearl millet. The different screening techniques, viz. STI, SSTI, MTS, etc. may be utilized for screening the germplasm for heat tolerance. Breeding for heat tolerance information of genetic variability, gene action (additive and non-additive), heritability, stability and correlation in relation to heat tolerance is available. Gene interaction on heat tolerance showed its complex nature of inheritance. To overcome the heat stress various mechanisms such as maintenance of membrane stability, scavenging of reactive oxygen species (ROS), production of anti-oxidants, osmo-regulation of solutes and synthesis of heat shock proteins (HSPs) were used. Both conventional as well as molecular breeding techniques were utilized for genetic improvement for heat tolerance.

Key words : Heat tolerance, screening techniques, genetic improvement, pearl millet

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a multipurpose cereal grown for grain, stover and green fodder. It shall continue to play a prominent role in the integrated agricultural and livestock economy of the country particularly in rainfed areas due to its drought hardiness and tolerance to high temperature (Arya *et al.*, 2010). It has the virtue of having exceptionally highest productivity per day both for grain as well as fodder (Arya *et al.*, 2014).

Wahid *et al.* (2007) reported that heat stress due to increased temperature is an agricultural problem in many regions in the world. Transitory or constantly high temperatures cause an array of morpho-anatomical, physiological and biochemical changes in crop plants, which affect plant growth and development and may lead to a drastic reduction in economic yield.

As the temperature raises from optimum to the stress levels, there is a dramatic change in protein synthesis. A new set of proteins known as heat shock

proteins (HSPs) is produced and normal protein synthesis is greatly reduced (Lin *et al.*, 1984). The ability of crop plants to adapt to heat stress is potentially an important component of tolerance to heat stress under field conditions (Chen *et al.*, 1982).

The present review highlights the responses of potential genotypes of pearl millet for heat tolerance at germination, during growth and at maturity. The efforts were made during past in uncovering the mechanism of heat tolerance. Important selection criteria being used by plant breeders for screening and development of heat tolerant cultivars were also discussed.

Heat Stress Effect

Generally, the high temperature higher than the optimum shortened the growing period of crops, resulting in a shorter time of biomass accumulation, which ultimately responsible for low grain yield as well

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as low fodder yield (Arya *et al.*, 2010). Moreover, reduction in yield was not homogeneous and dependent on crop phenology, crop type or growing environment (Giannakopoulos *et al.*, 2009). The studies on stress revealed that changes in climate variability as can be expected in a warmer climate might have a more profound effect on yield than changes in climate. Furthermore, the changes in the frequency of extreme climatic events during the more sensitive growth stages have been recognized as a major yield determining factor for hot regions in the future (Arya *et al.*, 2010).

The crop plants production depends especially on the agro-climatic factor, which affects crop plants' life. It directly determines the days taken to complete different phenol-phases and consequently the rate and duration of growth. Any change in thermal regime from the optimum temperature range during plant growth period adversely affects the initiation and duration of phenophases and finally the yield of the crop due to changes in physiological processes (Kumar *et al.*, 2005).

The effect of stress at early stages of growth and development had more dangerous than the latter stages. Any kind of stress at early stages of plant growth reduces the number of tillering drastically and also provided stimulus to the plant for early flowering, resulting in reduction in plant's normal size, which ultimately produced small earheads, thus drastic reduction in grain yield along with fodder yield was noticed in pearl millet by Arya *et al.* (2010).

The rate of germination, coleoptiles elongation and rate of photosynthesis maximizing upto temperature of about 35°C and after that it inhibits the normal growth (Arya *et al.*, 2014).

Ashraf and Hafeez (2004) assessed thermo-tolerance of pearl millet cv. ICMV-94133 at germination and vegetative stage. Relative growth rate and net assimilation rate (NAR) increased significantly in pearl millet due to high temperature, but in maize, NAR was slightly reduced. N, P and K concentrations in the shoots of both the species increased at high temperature, but N accumulation was more pronounced in pearl millet than in maize. High temperature caused a marked increase in both the shoot and root Ca²⁺ concentration in maize, but it did not affect that of pearl millet. S concentration in the shoots of maize decreased significantly due to high temperature, whereas in pearl millet, it remained unaffected. Shoot Na⁺ concentration of both the species was not significantly affected by high temperature. High temperature caused a significant increase in N, P and K⁺

uptake in pearl millet, but the Ca²⁺, Mg²⁺, Na⁺ and S uptake remained unaffected in this species. In contrast, in maize, a significant increase in K⁺ and Ca²⁺ uptake, and a decrease in N, S, Mg²⁺ and Na⁺ uptake was found at high temperature. Overall, maize showed lower tolerance to high temperature compared with pearl millet.

Heat Tolerance

The temperature effect on germination, seedling establishment and early vegetative growth of pearl millet are well documented. The germination in pearl millet seeds linearly increased with the temperature from low up to optimal stage and declined with higher temperature was reported by Ong and Monteith (1982). The successful germination and seedling establishment under such environmental conditions will depend mainly on the use of genetically heat tolerant genotypes and appropriate management. Studies on four pearl millet landraces indicated that a high germination rate was an important trait (Mohamed, 1984).

Singh *et al.* (2003b) reported the TI values varied between 0.65 and 0.89 in experiment 1; 0.78 to 0.97 in experiment 2 and 0.43 to 0.59 in experiment 3. They suggested that poor stand/seedling establishment of some pearl millet genotypes might be due to low initial seedling emergence rather than subsequent seedling survival, thereby establishing the importance of environmental conditions before emergence.

Germination and Growth Parameters

Pearson (1975) also revealed that percentage seedling was independent of temperature between 15 and 33°C in the day time. Branching of roots was most prolific and production and expansion of new leaves were faster at high temperature. The dry weight accumulation was fastest at 35°C temperature. They also suggested that cool temperature depression of dry weight accumulation in pearl millet was due not only to lower rates of net photosynthesis but also to slower germination, slower and incomplete utilization of seed reserves, lower meristematic activity and top/root ratio and probably lower rates of carbohydrates translocation. Begg and Burton (1971) reported that rate of development to anthesis and leaf number was greater at the higher temperature, whereas dry weight was higher at the lower temperature. Most of the genotypes reached anthesis sooner at the higher temperature, and the

variation between genotypes averaged over all photoperiods was greater at the lower temperature. The effect of photoperiod tended to be greater at the higher temperature, and the temperature effect was greatest at 14 h. With the increase in photoperiod, there was also an increase in the number of tillers and the number of leaves on the main stem.

In the report of Launder (1971) it was stated that percentage of final emergence, rate of emergence, seedling survival and growth usually increased with temperature up to 33°C and declined at higher ones. It has been established that inhibition of seedling emergence is a common problem in pearl millet in semi-arid tropics (Soman *et al.*, 1987). The high soil temperatures have also been found to be one of the principal causes of poor seedling emergence and establishment (Rai and Kumar, 1994).

Joshi *et al.* (1997) reported that germination behaviour and seedling growth of pearl millet parents and hybrids were studied in a range of temperatures from 10 to 50°C under laboratory conditions. The optimum temperature for germination was found to be 30-35°C, while there was no germination at 10 and 50°C. The seeds kept at 10°C for a week, when transferred to the optimum temperature, resumed normal germination, thus exhibiting the chilling resistance. Hybrids performed better than the parents with respect to per cent germination, germination index and seedling growth in terms of length and dry weight at most of the temperatures studied. The hybrid GHB-15 exhibited better parental heterosis at all the temperatures and the magnitude of mid-parental heterosis was more at adverse temperatures than that at the optimum.

Soman (1981) and Peacock and Heinrich (1984) also reported that soil temperature above 45°C inhibited the emergence in pearl millet. The sensitivity of seeds to high temperature is likely to delay or prevent germination in many parts of the semi-arid tropics where pearl millet is grown. The very strong influence of timing of the period of high temperature suggested that seeds were most sensitive when they absorbed water. At some stage, near the end of imbibition, seeds appear to develop a partial immunity to high temperature; alternatively, cellular repair mechanisms may allow affected seeds to germinate after a slight delay. Therefore, seeds should be sown in the evening for better germination (Garcia-Huidobro *et al.*, 1985).

Gracia-Huidobro *et al.* (1982) stated that the rate

of germination increased linearly with temperature from a base T_b to a sharply defined optimum T_o beyond which the rate decreased linearly with temperature, reaching zero at T_m . Yadav *et al.* (2014b) also reported that alternating temperature had a small but systematic effect on germination rate.

Genetic Variability

Peacock *et al.* (1993) reported genetic variation in seedling emergence and survival was largely due to tolerance of high temperatures rather than tolerance of soil moisture deficit. The broad sense heritability ($h^2 = 0.82$) of the thermo-tolerance index (TI) trait was high. The genetic variability for thermo-tolerance at seedling stage was identified by Singh (1993) in a set of 38 genotypes, which included CMS lines, pollinators, inbreds, populations and hybrids. The hybrids HHB-67, HHB-68 and HHB-60 were identified among high tolerant types. The inbred lines H77/833-2, H90/4-5, and Togo-II also showed high tolerance traits. The hybrids and populations, in general, appeared more tolerant to drought stress than the CMS lines and pollinators. The rapid emergence and early vigour of hybrids could be the possible reason.

The studies of some other seedling characters for their effect and adaptation to high temperatures were undertaken. The investigation was extended to emergence rate, height of seedlings and number of leaves. A large magnitude of genotypic variability was noticed in respect of germination, thermo-tolerance index, emergence rate, seedling height and leaves/seedling. The criterion of seed to seedling thermo-tolerance index (SSTI) was used by Singh (1993) to evaluate the genotypes for tolerance to heat. This criterion takes into account the normal germination rate on the field as observed during monsoon.

Singh *et al.* (2001) studied genetic variability, phenotypic and genotypic coefficient of variation, heritability (broad sense) and expected genetic advance for seedling emergence and seedling heat tolerance index in 49 genotypes of pearl millet in Rajasthan, India. The analysis of variance revealed that significant differences were observed for both the characters. High estimates of phenotypic and genotypic coefficient of variation were also: estimated, thereby indicating that environment did not have major impact on the variability pattern of these traits. High heritability (broad sense) coupled with high genetic advance was also observed.

The genetic differences in tolerance to high temperature at both seedling and grain filling stage have been established. The germplasm and advance breeding material with higher degree of tolerance to high temperature have been identified in order to use them in breeding programs (Patil *et al.*, 2015).

Combining ability

Singh *et al.* (2003b) reported that in combining ability analysis the mean squares due to both *gca* and *sca* were significant indicating the importance of both additive and non-additive types of gene action for the inheritance of seedling heat tolerance. However, the narrow sense heritability was low ($h^2=0.25$), suggesting that simple mass selection would not be effective in improving the heat tolerance. Rather, reciprocal recurrent selection might be expected to be more rewarding. Environment did not have major impact on the variability pattern of seedling emergence and seedling heat tolerance index and they had high heritability (broad sense) coupled with high genetic advance (Singh *et al.*, 2000).

Singh and Sharma (2001) studied eight pearl millet inbred parental lines (HMS 1B, HMS 3B, RIB 335/74, D23, 20-K86, FTR 250-2-1, FTR 285 and FTR 336) with diverse origins for combining ability and heterotic pattern for seedling heat tolerance. Crosses were made in a diallel fashion excluding reciprocals, and the resulting 28 hybrids, eight inbred parents (selfed) and a control (heat tolerant hybrid HHB 67) were sown during the hot, dry, summer season (third week of May 1994) in Rajasthan, India. The maximum daily soil temperature range (at 5 mm soil depth) was 45.0 to 62.4°C and the maximum daily air temperature range was 37.0 to 45.5°C. Combining ability analysis revealed that general combining ability (*gca*) variance and specific combining ability (*sca*) variance were significant, indicating the importance of both additive and non-additive gene action in the inheritance of seedling heat tolerance index. However, narrow-sense heritability was low. Among the parents, D23 and FTR 250-2-1 showed significant positive *gca* effects. Further, significant *sca* effects for seedling heat tolerance index were found for crosses involving at least one poor general combiner indicating that SCA effects might be due to dominance or epistatic gene effects for this trait. HMS 3B x D 23 and HMS 3B x 20-K86 had the highest values of seedling survival percentage. These crosses also exhibited high

sca effects along with high estimates of heterosis.

Yadav *et al.*, (2006) revealed through the combining ability analysis that both additive (*gca*) and non-additive (*sca*) genetic variances played an important role in this material for most of the characters studied. The relative magnitudes of the respective variances, however, showed that the later was more important, particularly in case of characters such as seedling thermo-tolerance index (STI), seed to seedling thermo-tolerance index (SSTI), germination (%), emergence rate (ER), number of leaves/seedling, seedling height, seedling fresh weight (g), seedling dry weight (g), number of effective tillers/plant, plant height, ear weight (g)/plant, dry fodder yield (g)/plant, grain yield (g)/plant and total biological yield (g)/plant indicating that non-additive genetic variance (dominance variance) was mainly responsible in the inheritance of these characters. It may be mentioned that the regression analysis of Hayman also showed the preponderance of over dominance for most of the characters which confirms that non-additive component of variance was more important. However, the reverse was true for panicle emergence and ear length where there was a preponderance of additive genetic advance. Here the pedigree breeding for these characters would be expected to be more successful.

A consideration of the *gca* and *sca* effects might be of help in isolating suitable genotypes for more conventional breeding work. The *gca* analysis threw up some valuable parents. The parents (77/371× BSECT CP-1) and 96AC-93 were of particular importance for showing favourable response to heat tolerance in both the environments. The parents CVJ-2-5-3-1-3 and H77/833-2 were also good general combiners for heat tolerance as well as some other characters. Togo-II was the parent which combined well for grain yield and also the heat tolerance (Yadav, 2006).

The cross 1305 × 99HS-18 was the best specific combination with the cross H77/833-2 × 96AC-93 as close runner up. The best specific cross 1305 × 99HS-18 involved the one susceptible parent (1305). This parent also gave best specific cross for STI. It is a re-assertion of the fact the heat tolerant genes are also contributed by parents with poor performance *per se*. This indicates a complex nature of gene distribution and the control system for the trait. The contribution of positive genes by the poor performer has a special significance in plant breeding. We can expect the genes being disbursed in large germplasm and coming from any parent. This would also suggest that in absence of

particular sources, the trait could be tested in hybrids as a qualifying standard (Yadav, 2006).

Among the top hybrids selected on the basis of high sca for STI and SSTI seven cross combinations, namely, H77/833-2 × 96AC-93, 1305 × 96AC-93, (77/371 × BSECT CP-1) × Togo-II, H77/29-2 × CVJ-2-5-3-1-3, 1305 × 99HS-18, G73-107 × 77/245 and H77/833-2 × H77/29-2 were common. The 10 hybrids were also selected for the grain yield/plant on the basis of high sca. The hybrid (77/371 × BSECT CP-1) × Togo-II was common among the top hybrids for STI and SSTI as well as for grain yield. This hybrid was a tolerant × tolerant type.

Significant positive sca effects in all the three environments were exhibited by 11 cross combinations, namely, H77/833-2 × 1305, H77/833-2 × 99HS-18, H77/29-2 × 1305, H77/29-2 × 96AC-93, G73-107 × CVJ-2-5-3-1-3, G73-107 × 1305, G73-107 × Togo-II, 77/245 × CVJ-2-5-3-1-3, 77/245 × 96AC-93, CVJ-2-5-3-1-3 × (77/371 × BSECT CP-1) and 1305 × Togo-II. However, four crosses, namely, H77/833-2 × (77/371 × BSECT CP-1), H77/29-2 × Togo-II, 77/245 × 1305 and Togo-II × 99HS-18 in environments-I and II (stress environments), showed consistency for positive significant sca effects for grain yield (g)/plant (Yadav *et al.*, 2006).

Gene interactions

The analysis of $V_r - W_r$ graph indicated over dominance for emergence rate, number of leaves/seedling, seedling height (cm), fresh weight (g)/seedling, dry weight (g)/seedling, panicle emergence (days), number of effective tillers/plant, plant height (cm), ear weight (g)/plant, dry fodder yield (g)/plant, grain yield (g)/plant and total biological yield (g)/plant. Partial dominance was observed for seedling thermo-tolerance index and ear length (cm). The epistasis was found to be absent for emergence rate, seedling thermo-tolerance index, seed to seedling thermo-tolerance index, ear length (cm), seedling height (cm) and plant height (cm) (Yadav *et al.*, 2014a).

The parents 3, 5 (G73-107 and CVJ-2-5-3-1-3) appeared to have an excess of dominant genes for STI, while the parents 1, 6 (H77/833-2 and 1305) had maximum of recessive genes. The array points 10, 5 (99HS-18 and CVJ-2-5-3-1-3) appeared to possess maximum number of dominant genes for SSTI. The parents 1, 6 (H77/833-2 and 1305) possessed relatively

more number of recessive genes. The rest of the parents indicated to have almost equal proportion of dominant and recessive genes in them.

The additive effect (D) had a significant presence for STI and SSTI in both the stress environments. At the same time the dominance component of variance (H_1) for these characters was not only significant but also in higher magnitude. This effect of over dominance was also reflected by higher degree of overall dominance effect (h^2) for both these characters. The covariance between D and H_1 showed a symmetrical distribution of dominant and recessive alleles for SSTI which was, however, shown to be asymmetrical for STI due to its significant mean squares. The proportion of dominant genes was also higher over the recessive genes in parents for both STI and SSTI. It was also seen that proportion of dominant genes with positive effect was higher than those with negative effects in control of STI and SSTI. Only one dominant gene appeared to be responsible for expression of STI and SSTI. For other characters too the magnitude of H_1 was higher than that of D in all the environments. This indicated the preponderance of non-additive gene effects in the inheritance of these characters. Such a phenomenon was also found in combining ability analysis (Yadav *et al.*, 2014a).

The inter relationship of the H_1 and H_2 parameters was reflected in the allied genetic parameter $H_2/4H_1$ index that estimated the proportion of genes with positive/negative effects, showing dominance in the parents. The value of this index was less than the expected values of 0.25 for all the characters in all the three environments. This indicated that distribution of dominant genes having positive and negative effects in parents was generally asymmetrical. The ratio h^2/H_2 denoted the number of gene groups exhibiting dominance. The number of gene groups in control of dominance expression ranged from 1 to 5 for different characters. The heat tolerance was seen to be in the simplest control of one gene group. This would further strengthen our view that hybrid breeding for heat tolerance could be feasible and possible. Few other characters such as grain yield, total biological yield, plant height and ear length showed a complex control of 4 or 5 gene groups (Yadav, 2006).

Thermo-tolerance Index

Peacock *et al.* (1993) suggested that the poor

stand establishment of some pearl millet genotypes might be due to poor emergence rather than to subsequent seedling survival, and environmental conditions prior to emergence may be critical. They suggested that variation in emergence between genotypes could be reduced if all seeds were produced under similar conditions. Singh *et al.* (2003a) evaluated pearl millet hybrids and population lines for seedling establishment by having percent germination and thermo-tolerance index (TI) using ratio of seedlings surviving to total number of seedling emerged. The TI value was constantly higher in HHB-67 genotype in all the experiments. The results suggested that poor stand/seedling establishment of some pearl millet genotypes might be due to lower initial seedling emergence rather than subsequent seedling survival, thereby establishing the importance of environmental conditions before emergence. But in this context, SSTI worked by Singh (1993) assumes importance. This index takes into account the possible mortality of emerging seedlings before they come above the soil.

Rate of Germination

Khalifa and Ong (1990) also reported that the rate of germination of pearl millet cultivar increased linearly with increase in temperature from a base temperature (T_b) to an optimum value (T_o). Above T_o , rate of germination decreased linearly to zero at a maximum temperature (T_m) of 46-48°C depending on the genotype. They also reported that the short duration hybrids possessed a greater tolerance to high temperature than long duration hybrids. They indicated that faster germination rate of HHB-67 might have permitted it to escape the damaging effect of supra-optimal temperatures. The seeds which are slow to germinate are more sensitive to high temperature.

Development and Growth Stages

Ong (1984) reported the major influence of the temperature on stem elongation and crop canopy in a stand of pearl millet. The decrease of tiller number with increasing temperature was also reported by Pearson (1975). Ong (1983) explained this relationship by a shortening of tillering phase and an inhibition of secondary tillering with increasing temperature. The initial effect of high temperature was earlier tillering

whereas, at low temperature (19°C) tillering continued via basal tillers which produced secondary tillers, again at a rate which was closely related to the number of leaves present. Thus, the plants at 19°C produced two to five times more tillers than those at or above 25°C. Fussell *et al.* (1980) reported even more prolific tillering for the same species : 7.8 times more at 21°C compared to 30°C.

The rate of leaf extension and spikelet initiation was also a linear function of the temperature up to 34°C so that time needed to form complete canopy decreased with increasing temperature up to that limit was the major finding of Ong and Monteith (1982). Fussell *et al.* (1980) published that plant morphology, grain development and yield of pearl millet were markedly affected by temperature during three stages of plant growth such as vegetative, stem elongation and grain developments. The same report indicated that high temperature during all the three growth stages lowered the grain yield by reducing basal tillering, number of grains per inflorescence and grain weight. Inflorescence length, width and the density of spikelet also appeared to be determined by temperature regimes. The extremes of temperature before anthesis reduced the sites available for grain development and thus lowering the grain yield. There was no tillering response to different temperatures after the beginning of stem elongation, presumably because floral initiation had occurred before the start of the temperature treatments. After floral initiation, competition for assimilates was high, resulting in little new development of tiller initials. Plant development and flowering in most genotypes were also accelerated by increasing temperature up to 33°C.

Squire (1989) reported a marked effect of temperature range for growth processes in pearl millet. Heat stress not only affects the yield and its components but also to the isoenzyme composition of some crop plants (Iglesias, 1994). The response of crop plants to heat stress depends upon the nature of stress resistance and the phase of stress resistance in the plants.

Plant Physiology

Heat stress affects plant growth throughout its ontogeny, though heat-threshold level varies considerably at different developmental stages. For instance, during seed germination, high temperature may slow down or totally inhibit germination, depending on plant species and the intensity of the stress. At later stages, high temperature may adversely affect

photosynthesis, respiration, water relations and membrane stability, and also modulate levels of hormones and primary and secondary metabolites. Furthermore, throughout plant ontogeny, enhanced expression of a variety of heat shock proteins, other stress-related proteins, and production of reactive oxygen species (ROS) constitute major plant responses to heat stress (Wahid *et al.*, 2007).

The research findings on pearl millet revealed that the reproductive stages of crop plants were relatively more sensitive to high temperatures compared to vegetative stages (Prasad, 2013). High temperature during gametogenesis results in pollen/ovule sterility, which reduces per cent seed set and ultimately decreases the number of grains per ear head. Besides, high temperature during flowering stage decreases pollen shed, pollen germination, inhibits pollen tube growth and decreases fertilization and seed-set (Rao and Patil, 2015).

In several crops, pollen viability and pollen production begin to reach zero at T_{mix} of 39°C. However, pearl millet pollen showed T_{mix} value of >42°C (Prasad, 2013). Research on nature of gene action/genetic mechanism for high temperature tolerance or susceptibility and its association with grain yield and other related traits would help in the development of heat tolerant genotypes (Yadav *et al.*, 2013).

In order to survive under heat stress, plants implement various mechanisms, including maintenance of membrane stability, scavenging of ROS, production of antioxidants, accumulation and adjustment of compatible solutes, induction of mitogen-activated protein kinase (MAPK) and calcium-dependent protein kinase (CDPK) cascades, and, most importantly, chaperone signaling and transcriptional activation. All these mechanisms, which are regulated at the molecular level, enable plants to thrive under heat stress (Arya *et al.*, 2014).

Promising Genotypes

In this respect, ear length, ear weight and plant height could be important to study for stability as they showed greater association with yield in this experiment. The parent CVJ-2-5-3-1-3 was of particular value for its involvement in four of the seven stable hybrids in fertile environment. This parent was also tolerant to heat stress and was the best general combiner also. Another parent which could become the basis of further breeding programme were 1305 and H77/833-

2. The parent H77/833-2 is also a good combiner and performer for heat tolerance and is also one of the parents of world's earliest maturing hybrid HHB-67. The status of H77/833-2 in heat tolerance breeding is again authenticated in the present investigation. Conclusively, Yadav (2006) gave some reflection and hints for adoption of appropriate breeding strategy and screening methods for heat tolerance. The preponderance of dominance component for heat tolerance and other characters including grain yield suggested that hybrid breeding was most likely to succeed. There were, of course, indications for alternative approach for pedigree breeding for panicle emergence and ear length.

It was also seen that heat tolerant hybrid e. g. H77/29-2 × 1305 could be obtained from the involvement of both the susceptible parents. It suggested that genes for heat tolerance were widely distributed in complex combinations. It further suggested that we could resort to direct testing of hybrids and varieties before the final release.

Joshi *et al.* (2005) evaluated the performance of five pearl millet hybrids (GHB 558, GHB 559, GHB 316, GHB 538 and GHB 526) under water stress (soil moisture content of 14%) and heat (38-40°C) in a pot experiment. Water stress was imposed by withholding irrigation after emergence. Growth parameters and seedling survival were evaluated at 19-22 days after the last irrigation. GHB 526, specifically released for summer cultivation in Gujarat, India, was the most tolerant of drought and heat among the hybrids, as it recorded the greatest root mass (38.7 mg/plant), shoot dry mass (37.4 mg/plant), survival percentage (76.4%) and leaf elongation rate (0.59 cm/day). The root/shoot ratio and total dry mass did not significantly vary among the hybrids.

Maximum level of production and stability of yield are the two desired features in a commercial variety. Indeed, development of varieties showing wide adaptability has received increasing attention in recent years. Judging from the mean, linear regression and S^2_{di} estimates for grain yield, the hybrids H77/833-2 × 1305, H77/29-2 × 1305, H77/29-2 × 99HS-18, G73-107 × CVJ-2-5-3-1-3, 77/245 × CVJ-2-5-3-1-3, CVJ-2-5-3-1-3 × 1305 and CVJ-2-5-3-1-3 × (77/371 × BSECT CP-1) had high mean performance and above average responsiveness with stable performance and were thus, better suited to more favourable environments. While the hybrids H77/29-2 × 96AC-93, H77/29-2 × Togo-II, 77/245 × 1305, 77/245 × 96AC-93, 1305 × 99HS-18

and Togo-II × 99HS-18 showed high mean performance, below average responsiveness thus, suited to low input environmental conditions. Phenotypic stability of grain yield and one or more contributing traits could be considered as promising combinations but after evaluating them over the locations and years (Yadav *et al.*, 2009b and 2012b).

Biological Basis of Thermo Tolerance

Heat shock proteins (HSPs)

It has been shown that several species of higher plants including soybean, pea, tobacco, cotton, sorghum, maize and pearl millet respond to high temperature (39 to 45°C) stress. A characteristic set of proteins, known as heat shock proteins (HSPs) is synthesized a result of sudden high temperature stress (Nover, 1984). Two major categories of HSPs are detected; one has a high molecular weight and the other has low molecular weight. This phenomenon has been investigated in wide range of organisms both in animals and plants. According to Altschuler and Mascarenhas (1982), the similarity in response to heat shock in the range of 40 to 45°C suggested that there was fundamental role of HSPs in survival against high stress.

In pearl millet investigation on heat shock proteins mechanisms were studied (Howarth, 1994) for genotypic and developmental variation during seed germination. It was observed that millet seedlings were competent to synthesize the complete spectrum of HSPs during a two hour's heat shock of 45°C. Further, the investigation indicated that thermo-sensitivity of a genotype and HSPs synthesis was a complex response involving the transcriptional and translational control of RNA in the seed embryo.

In higher plants, the rate of synthesis of low molecular weight HSPs is correlated with acquired thermo-tolerance (Lin *et al.*, 1984). Accumulation of HSPs is important for protection from thermal killing but when the question comes whether quantity or quality of HSPs is important. Two cultivars which differ in thermo-tolerance normally do not differ in the quality of HSP induced at same temperature. Not only the HSP synthesis and acquired thermo-tolerance are highly coupled, but also the intra-specific differences in quality, quantity and the rate of accumulation of HSP are highly correlated with thermo-tolerance. Germinating pollen and early imbibing embryos (pre-torpedo stage) are not

capable to synthesis HSPs in response of heat shock.

At cellular levels, high temperature causes metabolic disturbances, depletion of respiratory substrates, reduction of photosynthetic activity, denaturation of proteins, inactivation of enzymes and damage to cellular structures. It is realized that a complex character like heat tolerance with respect to grain yield may not be linked to a single metabolic process. Plants have a multitude of mechanisms which help them to survive and propagate under high temperature stresses. Zhang *et al.* (1997) suggested that there was an association between HSPs and heat tolerance, drought tolerance as under heat shock conditions certain HSPs (SH1 and SH2) were enhanced as revealed by SDS-PAGE analysis.

Viswanathan and Khanna (1996) studied the occurrence of heat shock proteins (HSP) in plants, with reference to their mechanism of protection from high temperature stress, regulation of HSP expression, the role of HSPs in thermo-tolerance of higher plants, the correlation between HSP expression and thermo-tolerance, cellular localization of HSP, etc. in several crop species.

Mapping QTLs for Heat Tolerance

Howarth *et al.* (1994) reported that high temperature was a major cause of failure of seedling establishment in pearl millet. Heat tolerance was mapped in two crosses (ICMP451 × H77/833-2 and H77/833-2 × PRLT2/89-33) by determining the genotype of F₂ plants at loci spaced 10 cM apart over the genome and measuring thermo-tolerance in F₄ material derived from each F₂ plant. In the first cross, the selection of probes which show polymorphism among the F₂ is almost complete. These probes will be used to genotype individual plants. A sandbed screening system has been developed which measures seedling thermo-tolerance under simulated field conditions. It involves the use of electric cables buried to a constant depth in sand and electronically controlled to provide precise heating. This sandbed assay is being optimised to mimic field measurements of thermo-tolerance in 10 genotypes. It will then be used to measure the thermo-tolerance of F₄ seedlings to enable QTLs for this trait to be determined.

Hash and Witcombe (1994) in 1990 began the pearl millet breeding unit of the Cereals Program by creating segregating populations of pearl millet suitable for use in molecular mapping of the genome of this

highly cross-pollinated crop. This work has produced two populations derived from off-the-shelf crosses and three populations from planned crosses, intended to map quantitative trait loci (QTLs) contributing to resistance to downy mildew (incited by *Sclerospora graminicola*) or to serve as the base mapping population for pearl millet in Asia. Two populations from planned crosses, intended to map QTLs contributing to seedling heat tolerance, have also been produced. One of the two off-the-shelf populations, derived from the cross 700651-1 x 7042(S)-11, has proved unsuitable for molecular mapping. The parentages of the remaining six mapping populations are described in detail, along with the procedures being used in their multiplication. Traits that can be mapped in each of these populations are listed. Molecular marker maps of five of these populations are under construction.

Gene Encoding for Cytoplasmic Hsc70

Reddy *et al.* (2010) have shown molecular chaperones (Hsps) to facilitate protein folding or assembly under various developmental and adverse environmental conditions. They isolated a cDNA encoding a cytoplasmic Hsp70 (PgHsc70) from pearl millet by screening heat-stress cDNA library. PgHsc70 cDNA encoding 649 amino acids represents all conserved signature motifs characteristic of Hsp70s. The predicted molecular model of PgHsc70 protein suggests that the N-terminus ATP-binding region is evolutionarily conserved, in comparison to C-terminus peptide-binding domains. A single intron in ATPase domain coding region of PgHsc70 exhibited a high degree of conservation with respect to its position and phasing among other plant Hsp70 genes. Recombinant PgHsc70 protein purified from *E. coli* possessed *in vitro* chaperone activity and protected PgHsc70 expressing bacteria from damage caused by heat and salinity stress. Nucleotide sequence analysis of 5' flanking promoter region of PgHsc70 gene revealed a potential heat-shock element (HSE) and other putative stress-responsive transcription factor binding sites. Positive correlation existed between differentially up-regulated PgHsc70 transcript levels and the duration and intensity of different environmental stresses.

Gene Encoding for Cytosolic Hsc90

Heat shock protein 90 (Hsp90) has a crucial role in the folding of a set of proteins involved in the

regulation of many essential cellular pathways and also re-folding of stress-denatured polypeptides. Reddy *et al.* (2011) reported the full-length cDNA encoding for Hsp90 polypeptide and its corresponding gene was isolated from *Pennisetum glaucum* (designated PgHsp90). PgHsp90 cDNA encoded for a polypeptide of 698 amino acids with a predicted molecular mass of 80.3 kDa and shared a high sequence homology (97-81%) to other plant cytosolic Hsp90s and shared less sequence homology (40-45%) to organelle and endoplasmic reticulum specific Hsp90 isoforms. A deduced amino acid sequence possessed three structural domains: N-terminus (1-211) ATP binding domain, middle (281-540) client protein interacting domain and C-terminus (541-698) dimerization domain; the N-terminus and middle domain are linked by a charged linker domain (212-280). It possesses the five-conserved amino acid signature sequence motifs characteristic of the Hsp90 family and a C-terminus MEEVD pentapeptide characteristic of the cytosolic Hsp90 isoform. The predicted quaternary architecture generated for PgHsp90 through molecular modelling was globally akin to that of yeast Hsp90. The PgHsp90 gene consists of three exons and two introns. The quantitative up-regulation of PgHsp90 gene expression positively correlates in response to different stresses to meet the additional demand for protein folding support. Cumulatively, the *in vivo* and *in vitro* experiments indicated that PgHsp90 played an adaptive or protective role to counter the stress induced protein damage.

Screening Methods for Thermo-tolerance

Field Screening Technique

Measurements of germination in constant temperature incubators are not necessarily relevant to the field where soil temperatures vary diurnally. Observations with selected sorghum lines have shown that material which fails to germinate in the incubators at 40°C germinated and emerged when sown in soil of the same mean temperature (Soman and Peacock, 1985). Results of germination studies made at constant temperature do not necessarily reflect field germination where soil temperature at seed depth may fluctuate widely during the day (Singh *et al.*, 2003a).

Later in the row is a simple and effective field screening technique for pearl millet genotypes. The technique standardized by Peacock *et al.* (1993) was used

for pearl millet seedlings experiment conducted in Rajasthan during 1989 and 1990. The experiments were conducted during high temperature season of April and May. Environmental measurements were taken in respect of air temperature, soil surface temperature and soil moisture. A condition was created where the drought did not occur in the field. Recording of seedling emergence commenced immediately the first seedlings were seen and continued daily at 1700 local time until there was no further emergence. The number of live seedlings was counted daily and dead seedlings were marked with wooden sticks. This method provided a check against loss of seedlings by any other means, for example, removal by birds or rodents.

A TI was calculated as the ratio of seedling which survived to the total number of seedlings which emerged. The results of the experiment done with the above technique showed considerable genotypic variation in the TI which allowed the grouping of genotypes into categories of high and low thermo-tolerance. The technique is repeatable and allows a large number of genotypes to be screened at the same time.

Singh, (1993) using the same technique argued in favour of use of SSTI which proceeds on the basis of both, pre and post-emergence mortalities. The pre-emergence mortality was estimated as difference in germination under stress and normal conditions. This provides a good background work and knowledge to initiate programme of genetic studies and breeding for thermo-tolerance.

It was observed that alleles controlling the grain yield in stress and non-stress environment are partially different, therefore, need to select in target environments to improve the performance of the pearl millet genotypes (Patill and Jadeja, 2009; Yadav *et al.*, 2012a).

It was suggested that genes for heat tolerance were widely distributed in complex combinations. It further suggested that we could resort to direct testing of hybrids and varieties before the final release. It may be further stressed that method of SSTI should be preferred over STI for testing of varieties at least at final stage as this method would take care of under soil mortality (USM) of germinating seeds (Yadav *et al.*, 2013).

Laboratory-cum-Field Screening Technique

Genotypic response to temperature is difficult to study in the field because soil temperature varies with

the moisture status of the soil and the system becomes even more complex if a crust develops on the soil (Soman *et al.*, 1984). Laboratory techniques representing near-field condition have also been developed and used for sorghum and pearl millet.

Scheuring *et al.* (1978) recorded germination of the sorghum in wet sand in a warm water bath at a constant temperature for 72 h. The seeds were thus exposed to a continuous high temperature (maximum of 40°C). Wilson *et al.* (1982) varied soil temperature using two different surface colourants, charcoal and kaolin in small pots. Though the temperature regime obtained with their technique was adequate for screening for emergence, the water status of the soil could not be controlled. Similarly, Buckle and Grant (1974) grew maize seedling in soil in dark incubators. Mustain (1981) observed an effect of differing moisture levels on germination at high temperatures.

Soman and Peacock (1985) used clay pots filled with sieved top soil for germination of sorghum and pearl millet seedling, placed in a steel water tank so that only the top 7 cm of the pots was above the water level. The soil surface was 2 cm below the mouth of the pot. The surface of the water was covered with floating white plastic balls to reduce evaporation. The soil in pots was heated with infra red lamps (240-250 V, 250 W, Phillips, Type IRR) fitted to frame above the tank in two rows. The temperature of the soil surface could be altered by varying the height of the frame of infra red lamps above the soil surface, to obtain the temperatures of 35, 40, 45 and 50°C measured at 2 cm below the soil surface. Water was added to the tank daily to maintain a constant level, ensuring that the wet soil column (25 cm long) in the pots provided a steady water supply for the seedlings while allowing them to be affected by the temperature of the soil. The soil water content in the pots was measured gravimetrically. The experiment showed that a temperature of 45°C gave the best selection pressure and very few lines emerged at 50°C (Yadav *et al.*, 2006).

Laboratory techniques

Heat tolerance usually improves membrane stability under heat stress which can be determined as lipid fluidity or electrolyte leakage. Osmo-regulators like proline and glycine-betaine may have a protective role in heat stress. Sapra and Anaele (1990) screened the genotypes for heat tolerance in green house and field and the leaf samples taken at two weeks interval from

vegetative to reproductive stages were assayed for injury after treatment at 50 and 25°C. Considerable variation between genotypes and years was observed for both the traits. Chen *et al.* (1982) measured heat tolerance on the basis of 2, 3, 5-triphenyl tetrazolium chloride reduction and conductivity tests on leaf tissue. These methods were applied at early growth stages, following acclimatization at above 30°C for 12-24 h.

Many laboratory techniques have been developed by various scientists. One bioassay often used in characterizing high temperature tolerance is electrolyte leakage of leaves subjected to heat stress (Shanahan *et al.*, 1990). These are electrical conductivity methods (Nagarajan and Panda, 1980). Chlorophyll fluorescence method (Smillie and Gibbons, 1981; Moffat *et al.*, 1990) and embryo protein synthesis methods (Ougham *et al.*, 1988). Therefore, the membrane thermo-stability test would be a suitable procedure for selecting heat tolerant genotypes in a breeding programme.

Three screening techniques viz., (1) germinating seed in polyethylene glycol-600 (PEG) at -0.6 MPa osmotic pressure, (2) subjecting seedlings to PEG-600 at -0.06 MPa osmotic pressure in hydroponic solution for 14 days and (3) a heat tolerance test based on cellular membrane thermo-stability were compared by Bouslama and Schapaugh (1984). They observed highly significant correlations between the hydroponic seedlings and heat tolerance tests indicating that both drought and heat tolerance were identified in the same cultivars. The hydroponic seedling test was found most reliable and potentially useful for screening for drought tolerance in soybeans. Cellular membrane thermo-stability indicates the heat tolerance of different cultivars (Bouslama, 1983). Heat stress affects the physiological aspects e. g. membrane thermo-stability and chlorophyll fluorescence, regulation of compatible solutes as reported by Takeda *et al.* (1999). There was an accumulation of free proline in the leaves in response to high temperature.

Ibrahim and Quick (2001) found that the mean squares for *gca* were four times that of *sca*, indicating the importance of additive gene effects in acquired thermal tolerance. They expressed the membrane thermal stability (MTS) in per cent units as the reciprocal of the leakage : $MTS = (1 - T_1/T_2) \times 100$, where, T_1 is the conductivity reading after heat treatment, and T_2 is the conductivity reading after autoclaving.

High temperature disrupts water, ion and organic solute movement across plant membranes, which interferes with photosynthesis and respiration process.

Damage to membrane may be assayed by the MTS, which measures electrolyte leakage from leaves subjected to elevated temperatures (Sullivan, 1972). Although MTS is positively associated with yield performance under heat stressed conditions (Yadav *et al.*, 2009a).

Molecular Markers

Electrophoresis of isozymes

Molecular markers are being extensively used in crop improvement programmes to (1) speed up selection efficiency of breeding methods and (2) construct saturated linkage maps. Molecular markers are better from morphological markers because alleles at most molecular markers loci are usually co-dominant and thus all possible genotypes (homozygotes and heterozygotes) can be distinguished in segregating populations, any plant tissue can be used for study and in most molecular loci, polymorphism occurs naturally.

Isozymes, the multiple molecular forms of the same enzyme with the same substrate specificity (Markert and Moller, 1959) are usually detectable through electrophoresis due to differences in their net electrical charges. The differences in charge are because of the acidic and basic nature of different amino acids and the differences in electrophoretic mobility of enzymes are usually the result of changes in structural genes coding for polypeptide(s). Thus electrophoretic differences are the direct result of genetic differences. Detection of isozymes using electrophoretic techniques has been extensively used for the characterization and identification of species, inbred lines, isogenic lines and crosses in plant breeding studies. Hunter and Markert (1957) proposed the term "zymogram" to refer to the strips in which the enzyme location is demonstrated. Detection of isozyme using electrophoresis techniques has been extensively used for the characterization and identification of species, inbred lines, isogenic lines, and crosses in plant breeding studies. The information obtained by the analysis of isozyme extracted from seed/seedling by electrophoresis has been well established to be very useful for estimating genetic variability, identification of cultivars and confirming hybridity (Chhabra *et al.* 2002).

Isozymes patterns have been found useful for characterizing varieties of pearl millet (Chhabra, 1995, Chhabra *et al.* 2002). Peroxidase isoenzyme patterns

have been used to indicate characteristic genetic make up of different species of plants. Also, Inter-and intra-specific polymorphism is exhibited by peroxidases. The matching of bands among species indicates a closer genetic relationship between them (Chhabra *et al.* 1996).

The analysis of water-soluble seed esterases was found to be potentially useful which showed clear differences between the eight cultivars studied and good replication of protein patterns (but complex banding pattern) from within each cultivar. It has been shown (Varier *et al.*, 1992) that the expression of pearl millet esterases is unaffected by both, the site and season (year) of seed production in composite varieties. Shaista (1997) reported that isozymes of esterase, glutamate oxaloacetate transaminase and peroxidase were genotype-specific in pearl millet hybrids, restorers and male sterile lines. Gulati (1998) characterized 13 cytoplasmic-nuclear male sterile lines of pearl millet using isozymes profiles. Balma *et al.* (1996) conducted isozyme studies on 60 landraces of pearl millet representing genetic variability of eight countries of West Africa. Seed samples produced more variability and number of bands than leaf samples.

Feritar *et al.* (2002) used peroxidase (POX) isozyme for identification of 14 pearl millet accessions of African origin. Twelve isozyme systems, viz., alcohol dehydrogenase (ADH), catalase (CAT), esterase (EST), glutamate dehydrogenase (GDH), glutamate oxaloacetate transaminase (GOT), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), malic enzyme (ME), phosphoglucosomerase (PGI), 6-phosphogluconate dehydrogenase (6-PGD), Shikimate dehydrogenase (SKDH) and superoxide dismutase (SOD) were used to characterize NILs (near-isogenic lines) of pearl millet for height. Out of 12 enzyme systems six were identical for isolines of tall/dwarf near isogenic pairs and ADH (18 h imbibed seed) and EST (18 hours imbibed seed) were most effective in determining within and between pairs variation (Chhabra *et al.* 1996). Recently, the role of isozymes in pearl millet improvement has been reviewed nicely by Chhabra *et al.* (2002).

Malate Dehydrogenase Isozyme (MDH)

Gulisano *et al.* (1980) reported that there were changes in the activity of MDH and GDH under double stress conditions (dry heat and feed deprivation). MDH activity increased significantly under double stress, while under heat stress it increased slightly after 24 h and

decreased significantly after 72 h.

Schrader *et al.* (2004) reported that photosynthesis was inhibited by high temperatures because of increased thylakoid membrane ionic conductance and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) deactivation. To confirm the findings, a proxy for NADPH and stromal redox status the activation state of NADP-malate dehydrogenase (NADP-MDH) was measured and it was observed that sustained heating caused a decline in Rubisco activation and also oxidized the stroma by NADP-MDH activation and this was hypothesized to result from increased cyclic photophosphorylation, explaining the maintenance of ATP content in the face of increased thylakoid membrane ion leakage.

MDH was studied by Tostain and Riandey (1985) and Lavergne *et al.* (1986) and ADH was the first enzyme system whose genetics has been worked out in Pearl millet (Banuett-Bourillon, 1982a, b). Another enzyme system subjected to a detailed study is that of EST (Sandmeier *et al.*, 1981; Subba Rao *et al.*, 1989).

Superoxide dismutase enzyme (SOD)

Khanna-Chopra and Sabarinath (2004) reported that superoxide dismutase enzyme (SOD) was ubiquitous, being widely distributed among O_2 -consuming organisms and was the first line of defence against oxidative stress. The leaf protein extracts, thylakoidal and stromal fractions were subjected to elevated temperatures ranging from 50°C to boiling and it was observed that out of six SOD isoforms, SOD V showed stability even after boiling. A new SOD band with higher electrophoretic mobility appeared under high temperature treatment (>60°C). The inhibitor studies and sub-cellular analysis revealed that the SOD V isoform was a chloroplastic Cu/Zn SOD.

Rivero *et al.* (2004) studied the oxidative metabolism in tomato plants subjected to heat stress. The leaf concentrations of the antioxidant compounds (ASA) and activity of SOD were determined. High temperature stress caused (1) decreased shoot weight, (2) accumulation of H_2O_2 , (3) increased SOD activity and (4) increased level of antioxidant compounds. Heat stress occurred in tomato plants at 35°C and this temperature may have initially inhibited the ascorbate/glutathione cycle and then provoked an oxidative burst as indicated by foliar H_2O_2 accumulation.

The temperature determines the gas exchange

and chlorophyll fluorescence, as well as antioxidant response as expressed by SOD (Camejo *et al.*, 2002). Physiological and biochemical variables changed depending on the genotype and stress exposure time. Elongation of the radicle in the heat-sensitive cultivars was significantly inhibited by high temperature, while the radicle of heat tolerant cultivar grew normally. Low membrane permeability, high proline content and high SOD activity were observed in the heat tolerant cultivars both under normal and high temperatures.

Correlations

Yadav *et al.* (2006) calculated the correlations among the characters around heat tolerance indices STI and SSTI at two levels. One set of correlation was studied at the time of screening of the parents. These were limited to seedling characters only. The other set of correlation coefficients was calculated while evaluating the diallel progenies which also included the seedling characteristics and other morphological and agronomic characters.

It was observed that STI, SSTI and MTS recorded highest significant correlation among themselves. Here again the competence of all the three tests was established. The field based indices STI and SSTI showed significant correlation with other seedling traits e.g. emergence rate, height of seedling, number of leaves/seedling, fresh weight and dry weight/seedling (Yadav *et al.*, 2013). While evaluating the diallel progenies, it was seen that the heat tolerance indices STI and SSTI were not showing any perceptible correlation with either the developmental traits or the maturity characters. Since experiment proceeded without any stress after the establishment of seedling, the pearl millet plants showed great resilience and recovery. Hence, the early heat effects could have no impact on its later growth and development. The association of grain yield/plant and total biological yield was almost parallel with all the other traits. The most important character was dry fodder yield in both the cases. The other important characters were ear weight, ear length and plant height. The character of early vigour of seedling also contributed for good grain yield (Yadav *et al.*, 2012a).

The association of heat tolerance was particular witnessed with character related to seedling growth. These characters which could be said to be related to the early vigour of seedling including the speed of

germination could therefore be taken as indirect indicators of associated heat tolerance. The early vigour seedlings perhaps help the plants to escape the lethal effect of heat (Yadav *et al.*, 2009a and 2014b).

Breeding for heat tolerance

Pearl millet is an open cross-pollinated crop with 75-80 per cent out-crossing. Therefore, recurrent selection and pedigree selection are the two most common methods used for any kind of genetic improvement. As the natural out-crossing and protogynous flowering behaviour of pearl millet gives us an opportunity of random mating, which is the basis requirement for the implementation of recurrent selection in pearl millet. All the types of recurrent selection along with their modifications are applicable for genetic improvement in pearl millet (Sanjana, 2015).

Yadav *et al.* (2011) suggested that heterosis breeding could be successful in pearl millet for heat tolerance. The genotypes CVJ 2-5-3-1-3 and 77/371//BSECP CP-1 were identified as the best general combiners for heat tolerance indices. Seven hybrids viz. H77/833-2X96AC-93, 1305X96AC-93, 77/371//BSECT CP-1XTogo II, H77/29-2XCJVJ-2-5-3-1-3, 1305X99H3-18, G73-107X77/245 and H77/833-2XH77/29-2 were identified having high value of heat tolerance indices seedling thermo-tolerance index and seed to seedling thermo-tolerance index.

According to Wahid *et al.* (2007), there are a few examples of plants with improved heat tolerance through the use of traditional breeding protocols, the success of genetic transformation approach has been thus far limited. The latter is due to limited knowledge and availability of genes with known effects on plant heat-stress tolerance, though these may not be insurmountable in future. In addition to genetic approaches, crop heat tolerance can be enhanced by preconditioning of plants under different environmental stresses or exogenous application of osmoprotectants such as glycinebetaine and proline. Acquiring thermo-tolerance is an active process by which considerable amounts of plant resources are diverted to structural and functional maintenance to escape damages caused by heat stress. However, both conventional as well as molecular breeding techniques were utilized for genetic improvement for heat tolerance.

Although physiological, biochemical and molecular aspects of thermo-tolerance in plants are

relatively well understood, further studies focused on phenotypic flexibility and assimilate partitioning under heat stress and factors modulating crop heat tolerance are imperative. Such studies combined with genetic approaches to identify and map genes (or QTLs) conferring thermo-tolerance will not only facilitate marker-assisted breeding for heat tolerance but also pave the way for cloning and characterization of underlying genetic factors which could be useful for engineering plants with improved heat tolerance.

According to Patil *et al.* (2015) an integrated approach of genetic crop improvement backed with improved crop management, grain processing and food items development and policy support will enable pearl millet to play its rightful role in enhancing food nutritional security.

Future Thurst

In the era of global warming, for further improvement it is an urgent need to understand all kind of mechanisms (morphological, biochemical and physiological) related to heat tolerance. Moreover, there are only a limited efforts being made in this direction. As the genes for grain yield under heat stress exhibited very complex inheritance. However, with the help of modern screening techniques/tools, the genetic improvement against heat stress in pearl millet is possible. The genes for yield contributing traits and heat tolerance are available in the germplasm, these might be clubbed in one genotype by the use of conventional as well as molecular techniques in combination. Today, genetic engineering has the potential to incorporate the desirable genes of thermotolerance in pearl millet from the wild weedy species, proliferating in hot regions of the world.

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