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Genetic Diversity in Bread Wheat for Heat Tolerance

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ABSTRACT

Heat stress is a major yield limiting factor of wheat productivity in India. Therefore, development of high temperature tolerant wheat genotype is an important objective of wheat breeding. Significant differences for the years, genotypes and their interactions indicated that the responses of genotypes under heat stress varied not only among themselves, but also over the years. This suggested the need for evaluation of wheat genotypes over a wide range of environments/years for obtaining consistent expression for heat tolerance. There was considerable influence of high temperature stress in both years as grain yield was reduced nearly about 50% under late sown experiment as compared to that of timely sown conditions. Only one genotype, i.e., Raj 3765 have shown significantly high yield under both conditions indicating a complementation of high yield and heat resistance genes. The genotypes DBW 88, HD 2967, HI 1563, HI 1571, NIAW 1951, Raj 4083 and UP 2425 were with significantly high yield under timely sown, while HD 2932, PBW1 75, PBW 373, Raj 3765, UAS 320, WH1124, WH 1142 and WH1164 were promising for grain yield under heat stress and for physiological parameters undertaken. Indices of heat tolerance, i.e., heat response index (HRI) and heat susceptibility index (HSI) were the most promising traits because of their strong associations with heat tolerance parameters, namely, tetrazolium triphenyle chloride test (TTC) and cell membrane stability (CMS), and due to their significance for their mean values for majority of the genotypes under heat stress conditions. In addition, the above traits had a contribution of about 70% of the total variation for heat tolerance as revealed by principal component analysis in the material under study. The diversity and spatial analysis revealed that the contrasting genotypes in terms of heat tolerance and spatial distribution may generate the desirable segregating material for improvement of heat tolerance in bread wheat.

Keywords: Bread wheat, genetic diversity, heat tolerance

Introduction

Globally, high temperature stress is one of the major constraints in wheat productivity. The report of the Intergovernmental Panel on Climatic Change (IPCC) indicate that global mean temperature will rise 0.3 ^{0}C per decade reaching to approximately 1 and 3 ^{0}C above the present value by the years 2025 and 2100, respectively, (Hays *et al*., 2007; Asseng *et al*.,2011; Singh and Dwivedi, 2015). Therefore, the understanding of mechanisms of heat tolerance in view of crop improvement under heat stress has become an important objective. High temperature stress is indicated by increase in temperature above a threshold for a period of time which may cause irreversible damage to plant growth and physiological development (Wahid *et al*., 2007; Sareen *et al*., 2015). High temperature stress is a complicated mechanism comprising heat intensity (temperature degrees days), heat duration and fluctuations in high temperature affecting the key physiological processes, namely, photosynthesis, translocation and assimilate partitioning to the grain. The influence of heat stress is more visible when the temperature increases more than the threshold level influencing fluidity and viability of cell membranes leading to membrane injury and even up to death of the organ. (Schoffl *et al*., 1999).

Heat stress reduces growth and development of plants by disturbing lipid structure and reducing the membrane lipid, resulting in to loss of membrane integrity and irreversible damage of cell membranes

(Gigon *et al*., 2004, Harb *et al*., 2010). Increase in the fluidity of membranes resulting into disintegration of lipid bilayer membranes and membrane stability is considered as one of the reliable parameter of heat tolerance (Blum *et al*., 2001; Magdalena *et al*., 2015). It was also observed that lipid peroxidation resulted in to polyunsaturated precursors with hydrocarbon components, namely, ketones, malondialdehyde etc. (Garg and Manchanda 2009). Malondialdehyde constitutes a three carbon dialdehydes which is highly reactive and formed as result of metabolism of polyunsaturated and arachidonic fatty acid metabolism. (Hameed *et al.,* 2012). Membranes are sensitive to heat stress because the increase in temperatures alter the integrity and functions of membranes resulting into tertiary and quaternary structure of proteins. As the membranes contain proteins in a moving mosaic form and lipids sway between their monolayers and rotate around their axes and carbon bonds with acyl chains. The protein conformation is influence by both increase and decrease in the temperature resulting into unfolding of proteins (Pastore *et al.,* 2007). Membrane thermosability of wheat inbred lines has been utilized as the parameter of heat tolerance (Gupta *et al*., 2013). Genetic variability for membrane thermostability have been reported by various workers (Dhanda and Munjal, 2006;Dhanda and Munjal, 2012; Munjal and Dhanda, 2016) which may be used for improvement of wheat for heat tolerance.

Membrane based damage are mainly observed in crop plants as cell membrane stability for injury in plasma membrane, as chlorophyll fluorescence for injury in thylakoid membrane, and as viability test for stability of mitochondrial membrane (Essemine *et al.,* 2011; Jha *et al.*, 2014). These tests are also being used in various crops for quantification for the level of acquired high temperature tolerance in plants (Wahid *et al*, 2007). For example, the 2, 3, 5 triphenyl tetrazolium chloride test is used to measure reduction of TTC by electrons from mitochondrial electron transport chain (Towill and Mazur, 1974). The inhibition of triphenyl tetrazolium chloride reduction is an indicator of respiratory enzyme inactivation or mitochondrial dysfunction which reflects the relative level of cell viability. Lipid peroxidation in membranes is presumed as one of the important cause for alteration of fluidity in membranes due to heat stress on plants (Mirza *et al.*, 2013). The extent of membrane fluidity increases with increase in temperature stress and membrane composition. The lipids having comparatively more unsaturated fatty acids with short fatty acid chains or with low sterol content were generally more fluid and had better stability under heat stress (Allakhverdiev *et al.*, 2008).

Heat stress has a major impact on chloroplasts, metabolism of carbon, stroma and on thylakoid lamellae during photochemical reactions. (Wang *et al*., 2009). The chloroplasts are the primary site of injury resulting in to distorted structure and organization of thylakoid membranes, reduction and swelling of grana (Rodríguez *et al*., 2005). Thus, the activity of the photosystem II is highly influenced and may even stop under severe high temperature stress (Morales *et al*., 2003). High temperature stress also influences soluble proteins, Rubisco binding proteins, activity of key enzymes involved in photosynthesis, namely, sucrose phosphate synthase, ADP-glucose pyrophosphorylase, and invertase resulting into reduced synthesis of starch and sucrose (Hasanuzzaman *et al*., 2013). Thylakoid membrane is measured in terms of chlorophyll fluorescence and highly susceptible to heat stress. Chlorophyll fluorescence related with photosystem II, reflected the extent of photosynthesis and has become one of the most reliable methods to determine photosynthetic efficiency under heat stress in crop plants (Kornyeyev *et al*., 2003).

During prolonged heat stress conditions, plant indicates the mechanisms which help in adaptations in short-term avoidance of heat stress through transpirational cooling, earlier flowering and other physiological mechanisms. In well irrigated conditions, high transpiration rate may cause to lower the canopy temperature from the ambient temperature up to 6°C (Fitter and Hay, 2002). Dense Root system of plant also contributes towards heat avoidance through obtaining more moisture through deep penetration and wide coverage in the soil (Fisher *et al*., 1982). High temperature also enhances the rate of reproductive development resulting into shorter time to photosynthesis contributing towards grain yield (Xue *et al*., 2004) as plant growth and flowering depend on accumulate degree days in temperature (Penuelas and Fillela, 2001). The short duration genotypes may escape from heat stress at later stages of plant growth, but the genotypes with high grain yield and comparatively longer duration under heat stress may possess the genes conferring protective mechanisms. Thus, identification of heat tolerant genotypes at different stages of plant growth for various traits related to heat stress and may help to combine heat tolerant genes in desired background. Therefore, the objectives of the present investigations are to determine the mean performance and genetic diversity of wheat genotypes for heat tolerance and the relative contribution of the physiological traits under heat stress conditions in wheat bread wheat.

Materials and Methods

Fifty-six genotypes of wheat (*Triticum aestivum* L.) differing in their performance under heat stress were grown under normal (second week of November) and heat stress environments (last week of December) during the years 2012-2013 and 2013-2014 under field conditions at the experimental area of Wheat and Barley Section, Department of Genetics and Plant Breeding, CCS HAU, Hisar India. In order to create heat stress at anthesis and the reproductive stages, the sowing of the heat stress experiment was delayed by about 5 weeks. The experiments were conducted in a randomized complete block design with three replications for both environments and with a plot size of a 2 rows of 1.5m length with a 20×5 cm spacing within rows and between plants. Data on average of five competitive plants selected randomly from each row were recorded for grain yield per plant, days to 50% heading and physiological parameters as given below. The data for physiological traits was recorded reproductive stage of plant growth after 1st week of April in the heat stress experiment when the maximum day time ambient temperature was above 33°C in both years (Figure 4).

Chlorophyll fluorescence:

The Chlorophyll fluorescence measurements, F0, Fm, and Fv/Fm were taken 4 cm from the base of abaxial surface of flag leaves using a chlorophyll fluorometer, model 0S-30p Opti-Sciences under late sown environments at post anthesis stage. Measurements were taken on five randomly selected plants in each three replications. The fully expanded leaves were first acclimated to the dark for 15 minutes by fixing clips. The dark-adapted samples were continuously irradiated for 1second, provided by an array of 3 light emitting diodes in sensor. The florescence signals were detected as F0, Fm, and Fv/Fm.

Cell membrane stability:

Membrane thermostability was measured by the method of Sullivan (1972), modified later on by Ibrahim and Quick, (2001) was followed. A random sample of flag leaf from 3 plants from each replication was collected at post anthesis stage. At field, each sample was collected in sealed plastic bags and immediately kept in ice boxes. At laboratory all the samples were thoroughly rinsed twice in deionised water. The mid rib of flag leaves were removed gently by hand and about 5 cm portion from central flag leaf area was excised and cut in to 4 equal parts. Leaf was taken in glass test tubes. The test tubes samples were tightly covered with aluminum foil and submerged in water bath (maintained at 45°C) to depth equal to height of water in test tubes for 30-minute time periods after the treatment 10 ml deionised water was added and the test tubes were held overnight at 40°C in refrigerator. The samples were brought to room temperature and conductance was measured with an electrical conductivity meter after calibration with a standardized KCl solution (T1). The test tubes samples were then autoclaved at pressure of 0.10 Mpa for 10 minutes to completely kill plant tissues and release all the electrolytes. The conductance (T2) was measured again after autoclaved. Membrane thermostability was expressed in %. Membrane thermostability was measured by the formula given below.

Membrane thermostability= $1- (T_1/T_2)\times100$

Where

T1 = conductivity reading after heat treatment

T2 = conductivity reading after autoclaving

Canopy temperature depression:

A hand held infrared thermometer (Everest Interscience Inc.), model6110.4ZL, temp range low, USA for rapid indirect determination was used for instantaneous measurement of canopy temperature depression. Canopy temperature depression, the difference between air temperature (Ta) and canopy temperature (Tc), Measurement were taken when infrared thermometer viewed 100 per cent canopy cover and held at an angle of 30°C, approximately 50 cm above the canopy from horizontal and at 1 m distance from the edge of the plot end. Data was recorded between 12:00 hrs to 14:00 hrs. Measurements was taken when the sky was clear and there was little or no wind.

Tetrazolium Triphenyl Chloride Assay:

TTC assay was done following the method of Ibrahim and Quick (2001). Two sets of long flag leaves (3.5 cm) were excised, rinsed in deionized water, and placed singly in a test tube with 0.1 ml of deionized water. Out of two sets, the first set was kept at 25° C for 90 min as control set, and the second set was placed in a water bath at 49°C for 90 min. Immediately following the 25°C and 49°C treatments 10 ml of TTC solution (0.8% TTC in 0.05 M NaPO4 buffer, pH 7.4, and 0.5 ml/l Tween 20) was added per tube and vacuum-infiltrated for 10 min. The leaves were incubated in the TTC solution for 24 h at 25°C in dark. After incubation, the leaves were removed and rinsed with distilled water, placed individually in separate tubes containing 2 ml of 95% ethanol, and submerged for 24 h at 25°C in the dark. The level of acquired high temperature tolerance was determined by measuring the percentage reduction of TTC to formazan using the following formula.

TTC= (ODh/ ODc)×100.

Where ODh referred to the mean optical density (530 nm) values for the heat-stressed set (49°C for 90 min), and OD referred to the mean optical density for the control set (25°C for 90 min).

Heat susceptibility index for grain yield (HSI): Heat susceptibility index (HSI) was calculated over stress and non-stress environment. The HSI of individual genotype was calculated by the method suggested by Fischer and Maurer (1978) with the following formula:

HSI for grain yield = 1-(Y/YP)/D.

Where, D = 1- (X/XP), Y and YP is grain yield for individual genotypes under heat stress and normal environment, respectively. X and XP represents mean grain yields of all genotypes under heat stress and normal environment, respectively.

Heat Response Index for grain yield (HRI):

The heat tolerance of individual genotype was computed using the formula given by Bidinger *et al*., (1987) as HRI = (Ya- Yest)/SES

Where, Yest and Ya are the estimated yields by regression and actual yields, respectively, and SES is the standard error of the dependent trait, significant positive values of HRI denote heat tolerance, while significant negative values denote heat susceptibility of genotype negative values denote heat susceptibility of genotype.

Statistical Analysis:

Simple correlation coefficients among various traits were calculated by the method given by Snedecor and Cochran (1981). Principal components analysis was performed by the method given by (Everitt and Dunn, 1992). The genotypes were further subjected to cluster analysis by following the method of (Everitt, 1993; Eisen *et al*., 1998). The statistical analysis was performed by using the softwares, namely, OPSTAT and SPSS 19.

Results

Analysis of variance and mean performance

The mean sum of squares due to genotypes, years and for the interactions between genotype and years were significant for almost all the traits under heat stress conditions, but nonsignificant under timely sown conditions except for grain yield (Table 2). Therefore, under timely sown conditions the data for all the traits expect for grain yield was excluded for further analysis. The considerable differences for the genotypes among themselves and over the years existed and to get consistent performance these should be evaluated over some more number of years. The genotype Raj 3765 was significantly higher yielder than overall mean values under both timely (16.03*) and late sown (11.46**) conditions along with other heat stress related traits indicating a desirable combination high yielding and heat tolerant genes in this genotype which may be exploited for obtaining high grain yields under heat stress affected environments (Table 3). On the other hand, the genotypes DBW 88 (17.30**), HD 2967 (17.18**), HI 1571 (18.08), NIAW 1951 (16.12*), Raj 4083 (18.27**), UP 2425 (18.91**), were significantly higher yielder under timely sown conditions and may serve as the good source of high yielding attributes.

The mean values of other genotypes, namely, HD 2932 (12.41**), PBW 373 (10.51*), UAS 320 (11.02*), WH1124 (10.28*), WH 1142 (10.83*) and WH1164 (12.75**) were significantly higher than overall mean for grain yield and one or more heat stress related traits, under heat stress conditions. The better performance of above genotypes may be exploited for incorporation of heat stress tolerant genes in high yielding background. The information from the parental details revealed that the heat tolerance in above genotypes might have been contributed by the renowned stress tolerant varieties like Kauz, Veery, GW 322, *Ae. Squrossa* and Pastor (Table 1). The genotypes HD 3059, NIAW 34, Sonalika, WH 1021, WH 1123, and WH 730 were also significantly superior to the of the physiological traits, namely, mitochondrial viability test TTC, CMS, efficiency of photosystem II (chlorophyll fluorescence) and high rate of transpiration (CTD) under heat stress, but not for grain yield. Such types of material may be specifically used for improvement of heat tolerant traits.

The early heading genotype may escape heat stress particularly in the areas subjected to under late heat stress. Thus, the genotypes DBW 16 (86.00**), HD 2851 (83.00**), HI 1563 (84.72**) MP 3379 (86.00**), NW 5054 (85.39**), NI 5439 (86.06*), PBW 590 (85.00**), PBW 688 (83.33**), UP 2425 (85.61*), WH 1021 (83.67**), WH 147 (82.33**) and WH 730 (85.33**) tended to escape from heat stress as they were among the entries which were significantly earlier in heading than overall mean (90.36±3.69). Heat sensitivity index (HSI) and heat response index (HRI) are based on relative reduction of grain yield from normal to heat stress environments. HSI provides the relative reduction irrespective of effects of other variables, while HRI can remove the intervening influence of other traits namely, grain yield potential and escape mechanism due to early heading etc. The genotypes DBW 90, MP 3336, NI 5439, Raj 3765, Sonalika, WH 730, WH 021, WH 1123, WH 1124 and DBW 16 performed significantly better over their mean values for both i.e. HSI and HRI, while, NIAW 34, HPW 251, UP 2845, UP 2843, WH 1080 were better for either of these traits. Thus, based on grain yield, physiological traits and heat tolerance indices the genotypes Raj 3765, NI 5439, WH 730, WH 1021, WH 1123, WH 1124 and WH 1142 may be termed as heat resistant /tolerant, on the other hand the genotypes HD 2985, HD 3043, HD 3090, HUW 667, K 1114, NIAW 1951, PBW 343, Raj 4083, UP 2425, UP 2844, WH 147 and WH 542 showed heat susceptible response.

Correlations

Correlation coefficient analysis revealed that increased grain yield under heat stress conditions was significantly contributed by TTC (0.314*) CMS (0.365**) late heading (0.446**), chlorophyll fluorescence (0.330*), CTD (0.539**), HSI (-0.661**) and HRI (0.668**) (Table 4). This suggested the considerable role of above traits selecting for higher grain yield under heat stress. The positive correlations of TTC with other physiological traits, chlorophyll fluorescence (0.504**), HSI (-0.391**) and HRI (0.394**) indicated that heat resistant genotypes, in general, had better viability of mitochondrial cell viability as well better stability of thylakoid membrane. Days to heading appeared to be independent of physiological traits except with CTD (0.274*) indicating that heat tolerant plant may be of both types, i.e., early and late heading, but about transpiration rate the genotypes with delayed heading had higher rate of transpiration. The HRI appeared to be the most promising trait followed by HSI in terms of association with other traits and as an index of heat tolerance, because this index is free from influences of intervening variables such as escape from heat stress and high yielding genes. The strong association of HSI with HRI (-0.847**) suggested that the HSI was also effective to that of HRI for categorization of genotypes in their response towards heat stress in present set of material.

Principal components

To find out a desirable combination of few important traits required for high grain yield under heat stress conditions and to reduce the redundancy of the variables for simplification the selection process principal component analysis was performed (Table 5). The scree plot of principal components (Figure 1) indicated that first four principal components accumulated for 86.6% of the total variation. The first component accounted the highest contribution (47.25%) in total variation for heat tolerance, followed by the second (19.55%) and third (12.41%). This revealed that HSI (-0.907) followed by HRI (0.877) appeared to the important traits with a contribution of 47.25% of the total variation under heat stress conditions indicated by first component. The second component revealed that the physiological traits, namely, CMS (0.983) and TTC test (0.851) had a contribution of 20% towards total variation. While days to heading (0.963) in third component and chlorophyll fluorescence (0.989) in fourth component were a part of 12.41% and 7.33% of total variation under heat stress. Canopy temperature depression, however was the last contributing trait (1.003) in total variation due to heat stress with a contribution of 6.82%. Thus, HSI followed by HRI, CMS and TTC were the important yield determines of grain yield under heat stress conditions in present study.

Cluster analysis

To find out diversity for heat tolerance all the genotypes were classified into six different clusters (Figure 2). First cluster comprised a group of 6 entries, namely, Sonalika, WH 1179, WH 730, DBW 16, WH 1021 and HP 1744. Majority of these genotypes were significant for one or more physiological traits including heat tolerant resistance/ tolerant indices indicating heat tolerance response. The second cluster consisted of a group of 8 entries having moderate heat tolerant response. Among these the genotypes NW 2036, WH 1124, NIAW 34 and Raj 3765 showed significance for HRI and or HSI in addition to other physiological parameters. The remaining genotypes in this group were also better for one or more parameters of heat stress. The third cluster comprised a group of 11 genotypes with mix responses towards heat stress. The genotypes WH 1123, WH 1164, DBW 90 and HD 2932 indicated significance for one or more heat tolerant traits, while DBW 88, WH 1126, WH 542, HD 3043 and PBW 343 had sensitive type response. Group 4 and 5 comprised majority of heat sensitive genotypes except few genotypes indicating heat tolerant response in one or more characters. In group 6 all the genotypes, in general, had heat sensitive response except NW 2036, HPW 251 and MP 3379 which were significantly better at least one character related to heat tolerance. Thus, heat stress revealed considerable influence on grouping of genotypes, for example, first and second group consisted heat tolerant genotypes, third and fourth groups contained mixed type of genotypes having moderately heat tolerant to heat sensitive genotypes. In fifth and sixth group the response of genotypes was in general heat sensitive.

Scatter plot of genotypes

In addition to the diversity analysis which indicates arbitrary distances within and among the groups of genotypes, the scatter plot of the genotypes further elaborated their diversity in terms of spatial distance (Figure 3). Genotypes plotted on scatter plot according to PC 1 and PC 2 depicting 66.8% of total variation. PC1 constituted 47.25% of the total variation loaded with HSI and HRI involving as major components of heat tolerance, while PC2 represented 19.55% of the total variation loaded with CMS and TTC. the genotypes, Raj 3765, WH 1179, NIAW 34, WH 730, UP 2843, WH 1124, PBW 343 and WH 1021were better for both axis and showed a desirable combination of heat tolerance indices (HSI and HRI) and other heat tolerant traits namely, CMS and TTC as these genotypes had better performance for on both axis, while the genotypes PBW 373, DBW 90, PBW 373 WH 1164, WH 1123, HPW 251, MP 3336, UP 2845 and WH 1142 had better performance for the traits loaded on PC1, i.e., HSI, and HRI. The genotypes DL 153-2, HI 1571, PBW 644, HD 3059 and HP 1744, HD 3059, NW 2036, Sonalika, WH 542 and DBW 16 were better only CMS and TTC loaded on PC 2. This may probably due to their inherent characteristics as most of, many of above genotypes had one or more parent as stress tolerant in their pedigrees, for example, the varieties PRL "S", VE#5 'S', Pastor, Parula, Raj 3765, GW 173, PBW 175, Kauz and *Ae. Sq* (Taus) are the promising stress tolerant varieties of wheat which are one or more parent in the pedigrees of above genotypes (Table 1). The other genotypes which were near to origin or lower position in graph indicated moderately heat tolerant to heat sensitive response.

Discussion

It is now well established that various important cellular functions depend on proper working various membrane related activities, involved in photosynthesis, respiration and cellular activities for nutrient transport. (Vigh *et al*., 1998; Kültz 2005; Vigh *et al*., 2007; Crul *et al*., 2013). Due to high temperature stress the leakage of the plasma membrane causes severe protein denaturation. Lipid peroxidation is also one of the most deleterious processes occur under severe heat stress conditions in the membranes resulting into distortion of fluidity in membranes. The deleterious effects on membrane-based processes may be on plasmalemma, thylakoid membranes and mitochondrial membranes. Heat stress increases the fluidity in the membranes leading to disintegration of the lipid bilayer and proteins. Damage in the membranes is generally taken as a parameter for heat stress tolerance in terms of lipid destruction (Asthir, 2015). Lipid denaturation are mainly formed due to polyunsaturated precursors including some hydrocarbon components, namely, ketones, malondialdehyde and other related products (Garg & Manchanda, 2009). Malondialdehyde is a three carbon compound produced as a result of peroxidation of polyunsaturated fatty acids and metabolism of arachidonic acid. Some of these products are used to produce coloured reaction to find out the extent of damage in the membranes (Hameed *et al*., 2012). Increase in fluidity of cell themembrane resulted in activation of signal which are lipid-based cascades and increased cyto-skeletal reorganization (Bita and Gerates, 2013).

Light energy may be utilized by the plants in three different ways, for example, in photosynthesis, dissipated as heat energy and may be re-emitted as light in terms of chlorophyll fluorescence. As these processes occur in competition, therefore by measuring one process, i.e. chlorophyll fluorescence the extent of photosynthetic capacity in photosystem II (PSII) may be determined which has become one of the reliable tools for measuring photosynthesis under stress conditions (Czyczyło *et al.,* 2013; Georg *et al.*, 2013, Hemantaranjan *et al*., 2014). Therefore, the genotypes PBW 373, Raj 3765, UAS 320, WH 1124, WH 1142, and WH 1164 may be utilised not only for improvement of grain yield, but also for majority of heat tolerance related traits under present study. This indicated complementation of high yielding genes with heat tolerance/resistance genes in these varieties which may provide adaptability over wider area under heat stress conditions. The varieties PBW 373 and Raj 3765 are old varieties released for cultivations under heat stressed areas of India, while the variety WH 1124 and WH 1142 were recently released for cultivation under late sown and drought prone areas of northern India, respectively (Anonymous, 2015). Among other genotypes which performed better for physiological traits and indices of heat tolerance, WH 730 is registered for late heat tolerance, while HD 3059, Sonalika, WH 1021 are widely cultivated under the areas subjected under heat stress in India (Anonymous, 2014). The above genotypes along with UP 2845, NW 2036, etc. which had also better performance for physiological traits and indices of heat tolerance indicating a higher proportion of genes contributing towards heat tolerance than grain yield potential may be used for incorporation of these desirable traits in existing wheat germplasm.

Various research workers revealed that formazan production was comparatively more in heat tolerant genotypes than in heat sensitive (Wahid *et al*., 2007; Varshney *et al*., 2011). This may probably due to because the heat sensitive cultivar has poor tolerance mechanisms to face the initial moderate level of heat stress and consequently the genotype may not develop the adaptive mechanism to face the severe stress conditions. While the tolerant plants could activate the adaptive mechanisms during initial moderate heat stress and become capable to tolerate the severe heat stress. This may be due to increased formazan production during heat stress (Block and Brouwer, 2002). The tetrazolium test is based on the fact that viable cells can reduce tetrazolium salts into soluble formazans under heat stress (Berridge *et al.,* 1967). This assay indicates the ability of a plant tissue to continue the electron transport and the inhibition of tetrazolium tetrazolium triphenyl chloride results into inactivation of dehydrogenase thus producing less formazan production under heat stress (Chen *et al*., 1982). The amount of tetrazolium triphenyl chloride is decreased by the mitochondrial dehydrogenases and the production of formazan is regulated by cytochrome oxidase in the mitochondria (Rich *et al*., 2001). Thus, cell viability test is being widely for quantification of acquired thermal tolerance in plants (Farooq *et al*., 2011), because, inhibition of tetrazolium triphenyl chloride reduction is an indicator of mitochondrial dysfunction or inactivation of respiratory enzymes resulting in to the variable response of cell viability. The genotypes, namely, NIAW 34, Raj 3765 and WH 1021, having high values of formazan production or better mitochondrial viability also better performance for heat stress related traits may be used as the source for improvement of heat tolerance in bread wheat.

To have effective selection for heat tolerance the number of characters as well as the genotypes need to be reduced in order of their merit. Thus, the extent of genetic diversity is a pre-requisite for selection and improvement of heat tolerance (Parker *et al.,* 2002; Sharma *et al.,* 2014). The spatial diversity for heat tolerance may further help to may provide beneficial alleles for improvement of heat tolerance and yield potential (Dodig *et al*., 2010; Sun *et al*., 2013). The heat tolerant includes the genotypes like Sonalika which is an old, widely grown variety under heat stress and released for cultivation under heat stress areas of India since 1966. The other varieties, namely, WH 1124 and HD 3059 are recent release, while DBW 16, WH 1021, NIAW 34, PBW 644, NI 5439, Raj 3765 are old varieties released for cultivation under heat/drought stress areas of India (Anonymous, 2015). The genotype WH 730 has unique characteristics for heat tolerance in addition to its higher yields under heat stress for which it was registered for heat tolerance with the National Bureau of Plant Genetic Resource, New Delhi. The resistance in these varieties was primarily contributed by the parents, namely, Kauz, Pastor, *Aegilops squrossa*, and some already very popular varieties cultivated under heat/drought stress areas of India, namely, GW 322, Lok 1 etc. The genotypes may be used with high yielding well adapted genotypes to develop a desirable material under heat stress conditions.

Conclusions

The genotypes Raj 3765, NIAW 34, Sonlika, WH 730, PBW 373, WH 1124, WH 1164, WH 1123, MP 3336, UP 2845, WH 1142 etc. were important for low reduction of grain yield under heat stress, while DL 153-2, HI 1571, HD 3059, HP 1744, NW 5054, DBW 16 etc. were important for better performance of physiological traits, namely, CMS, TTC and CTD. In addition, the genotypes Raj 3765, UAS 120, WH 1124 and WH 1164 etc. indicated a desirable combination resistance potential with yield potential which may provide higher grain yield over the wide range of area, while the genotypes DBW 88, HD 2967, HI 1563, HI 1571, NIAW 1951, Raj 4083 and UP 2425 were significantly higher yielder under timely sown conditions. HRI followed by HIS, TTC and CMS were the most promising traits because of their strong associations with other heat tolerance parameters and significance for their mean values for majority of the genotypes under heat stress conditions. The diversity analysis further revealed that the contrasting genotypes in terms of heat tolerance which may be used for improvement of heat tolerance in bread wheat.

Table 1. Pedigrees bread wheat of the genotypes

Sr.	Genotypes	Parentage details	Sr.	Genotypes	Parentage details
1	DBW 16	RAJ 3765/WR 484//HUW 468	29	PBW 343	ND/VG1944//KAL//BB/3/YACO‘S’/4/ VEE#5‘S’
2	DBW 88	KAUZ//ALTAR84/AOS/3/MILAN/ KAUZ/4/HUITES	30	PBW 373	ND/VG9144//KAL/BB/3/YACO‘S’/4/ VEE#5‘S’
3	DBW 90	HUW468/WH730	31	PBW 590	WH594/RAJ3814//WH485
4	DBW 95	K9908/PBW534	32	PBW 644	PBW175/HD2643
5	DL 153-2	TANORI 71/NP 890	33	PBW 660	WG6761/WG6798
6	HD 2733	ATTILA/3/TUI/CARC//CHEN/CHTO/4/ ATTILA	34	PBW 688	W7561/HD2808
7	HD 2851	CPAN3004/WR426/HW2007	35	RAJ 3765	HD2402/VL639
8	HD 2932	KAUZ/STAR//HD2643	36	RAJ 4083	PBW343/UP2442/WR258/UP2425
9	HD 2967	ALD/CUC//URES/HD2160M/HD2278	37	Sonalika	1154.388/AN/3/YT54/N 10B/LR 64
10	HD 2985	PBW 343/PASTOR	38	UAS 320	UAS257/GW322//DWR195
11	HD 3043	PJN/BOW//OPATA*2/3CROC_1/A. Squarrosa(224) //OPATA	39	UP 2425	HD 2320/UP 2263
12	HD 3059	KAUZ//ALTAR84/AOS/3/MILAN/ KAUZ/4/HUITES	40	UP 2843	CPAN073/UP2382//OPATA/RAYON// KAUZ
13	HD 3090	SFW/VAISHALI//UP2425	41	UP 2844	HD2844/FRTL/AGRI//NAC
14	HI 1563	MACS2496*2/MC10	42	UP 2845	CPAN4022/UP2382//KAUZ//BOW/NKT
15	HI 1571	Raj 3077/WLT277//HW2045	43	WH 730	CPAN 2092/Improved Lok-1
16	HPW 251	WW24/LEHMIP2-U149	44	WH 1021	GW296/SONAK
17	HUW 667	RAJ1972/HUW468	45	WH 1080	PRL/*2PASTOR
18	K 1114	HP1731/HUW234	46	WH 1105	MILAN/S87230//BABAX
19	MP 3304	GW322/J485	47	WH 1123	NI5663/RAJ3765//K9330
20	MP 3336	HD2402/GW173	48	WH 1124	MUNIA/CHTO//AMSEL
21	MP 3379	Raj 3077/DL788-2	49	WH 1126	WBLL1*2/VIVITSI
22	NW 2036	BOW/CROW/BUC/PVN	50	HP 1744	CIANO/PARULA//CHILERE/GARUDA
23	NIAW 34	CNO 79/PRL “S”	51	WH 1142	OEN/Ae. Sq. (TAUS)/FCT/3/2*WEAVER
24	NIAW 1951	HD2781/NIAW301	52	WH 1164	RL6043/4*NAC//2*PASTOR
25	NW 2036	BOW/CROW/BUC/PVN	53	WH 1179	OASIS/SKAUZ//4*BCN/3/3*PASTOR
26	NW 5054	THELIN//2*ATTILA*2/PASTOR	54	WH 147	E4870/C303//5339/PV18
27	NI 5439	NI8883/MP1055	55	WH 542	JUPATECO/BLUE JAY//URES
28	PBW 175	HD 2160/WG 1025	56	WH 711	ALD‘S’HUAC//HD2285/3/HFW 17

Table 2. Mean sum of squares of wheat genotypes for various traits under heat stress conditions

Source of Variation	**DF**	**Grain yield (TS)**	**Grain yield (LS)**	**TTC (LS)**	**CMS (LS)**	**Days to 50% heading (TS)**	**Days to 50% heading (LS)**	**CFL (LS)**	**CTD (LS)**
Replication	4	6.41	3.82	116.63	13.30	0.82	1.43	0.0005	0.43
Year (Y)	1	161.35	541.95*	0.95	826.98*	1.01	137.52	0.0000	25.07
Genotype (G)	55	26.61	7.92	144.43	95.77	49.50	45.88	0.0086	2.50
Interaction (Y×G)	55	0.33	1.48	89.26	104.41	8.62	11.87	0.0001	1.78
Error	220	3.36	1.82	12.97	14.30	2.48	2.60	0.0007	0.15
Total	335								

*, **: Significant at 5% and 1% level of significance, TS: Timely Sown conditions, LS: Late Sown conditions, TTC: Tetrazoliom Tripenyl Chloride test, CMS: Cell Membrane Stability index, CFL: Chlorophyll Fluorescence , CTD: Canopy Temperapture Depression.

Table 3. Mean performance of bread wheat genotypes for various traits under heat stress conditions

Sr	Genotype	Grain yield under normal	Grain yield under stress	Tetrazoliom tripenyl chloride test	Cell membrane stability index	Days to 50% heading	Chlorophyll fluorescence	Canopy temperapture depression	Heat sensitivity index	Heat response index
1	DBW 16	10.25	5.45	59.40*	62.84	86.00*	0.77*	5.90	1.07	0.41
2	DBW 88	17.30**	5.28	54.64	58.92	89.61	0.72	5.80	1.32	-1.45
3	DBW 90	13.30	9.31	52.27	58.85	91.67	0.71	7.50	0.68**	1.79
4	DBW 95	6.25	4.12	36.6	36.18	90.28	0.62	2.33	1.11	-0.97
5	DL 153-2	8.14	3.35	62.94**	66.19*	93.67	0.66	3.23	1.34	-2.46
6	HD 2733	10.42	4.15	48.00	52.16	95.17	0.70	3.67	1.37	-2.93
7	HD 2851	13.02	4.35	45.98	54.51	83.00**	0.68	4.17	1.52	-1.19
8	HD 2932	12.75	12.41**	53.16	59.28	91.17	0.71	6.93**	1.13	-0.77
9	HD 2967	17.18**	4.46	43.32	50.23	84.61**	0.62	3.37	1.33	-0.44
10	HD 2985	9.64	5.45	47.42	51.24	92.00	0.69	6.17	0.99	-0.62
11	HD 3043	12.42	7.55	55.33	59.75	94.67	0.65	6.23*	0.89	-0.24
12	HD 3059	13.22	7.65	65.20**	67.85*	89.67	0.81**	6.47*	0.96	0.60
13	HD 3090	10.05	5.61	40.62	44.45	93.00	0.69	5.33	1.01	-0.85
14	HI 1563	16.22*	4.07	50.94	58.08	84.72**	0.73	2.70	1.53	-1.88
15	HI 1571	18.08**	6.07	50.57	69.19**	89.94	0.62	6.23	1.52	-3.05
16	HPW 251	7.62	5.31	43.36	52.86	86.28*	0.63	5.43	0.69**	1.3

Continuing table 3

Sr	Genotype	Grain yield under normal	Grain yield under stress	Tetrazoliom tripenyl chloride test	Cell membrane stability index	Days to 50% heading	Chlorophyll fluorescence	Canopy temperapture depression	Heat sensitivity index	Heat response index
17	HUW 667	10.98	4.11	40.41	46.92	87.00	0.70	3.03	1.36	-1.11
18	K 1114	14.42	7.78	42.78	62.59	92.00	0.65	5.43	1.05	-0.27
19	MP 3304	11.35	3.63	43.49	49.09	87.67	0.65	5.37	1.28	-0.88
20	MP 3336	12.73	9.38	52.13	47.99	86.67	0.69	6.47*	0.60**	3.17**
21	MP 3379	11.05	4.45	36.74	45.56	86.00*	0.71	7.60*	1.36	-0.92
22	NW 2036	13.08	8.75	61.31*	65.52*	95.00	0.78**	5.43	0.76*	0.61
23	NAIW 34	12.52	9.38	63.35**	69.75**	90.00	0.77*	6.23	0.57**	2.55**
24	NIAW1951	16.12*	8.25	37.1	51.56	89.39	0.64	6.03	1.11	0.06
25	NW 2036	14.75	8.36	39.65	53.15	90.06	0.73	6.67	0.99	0.59
26	NW 5054	14.90	4.38	53.73	57.1	85.39*	0.63	3.60	1.61	-2.45
27	NI 5439	11.25	5.22	41.58	49.77	86.06*	0.73	6.20	1.22	-0.25
28	PBW 175	12.42	3.25	53.52	56.56	88.00	0.65	3.47	1.68	-3.12
29	PBW 343	11.34	9.5	55.56	64.55	96.00	0.67	6.03	0.57**	0.87
30	PBW 373	15.38	10.51*	51.98	60.81	98.00	0.62	6.93**	0.72**	0.78
31	PBW 590	10.29	5.48	49.81	55.61	85.00**	0.71	4.87	1.07	0.64
32	PBW 644	13.49	7.25	67.73**	66.11	88.33	0.75	5.20	1.06	0.37

Continuing table 3

Sr	Genotype	Grain yield under normal	Grain yield under stress	Tetrazoliom tripenyl chloride test	Cell membrane stability index	Days to 50% heading	Chlorophyll fluorescence	Canopy temperapture depression	Heat sensitivity index	Heat response index
33	PBW 660	15.31	5.28	35.40	40.58	88.06	0.70	5.90	1.49	-2.29
34	PBW 688	10.79	5.12	59.58*	57.02	83.33**	0.69	5.07	1.20	0.43
35	Raj 3765	16.03*	11.46**	64.45**	69.72**	93.67	0.75	6.90**	0.65**	2.39**
36	RAJ 4083	18.27**	7.67	35.81	52.59	88.72	0.74	4.07	1.32	-1.27
37	Sonalika	12.62	8.47	58.58	66.91*	90.00	0.70	5.77	0.75**	1.59*
38	UAS 320	14.40	11.02*	43.45	55.55	99.33	0.65	6.53*	0.72**	0.24
39	UP 2425	18.91**	9.35	51.35	61.05	85.61*	0.71	5.13	1.15	0.81
40	UP 2844	13.35	6.21	47.98	52.87	98.00	0.63	6.43**	1.22	-2.68
41	UP 2845	10.48	8.45	42.23	50.09	97.67	0.65	5.33	0.44**	0.82
42	WH 1021	5.86	4.58	60.30*	65.00	83.67**	0.75	7.23**	0.50**	1.85**
43	WH 1080	9.62	6.4	46.15	40.41	99.33	0.65	5.47	0.76**	-1.24
44	WH 1105	11.38	6.88	51.41	46.25	96.00	0.76	6.80**	0.90	-0.77
45	WH 1123	13.57	11.2	59.61*	58.45	92.00	0.77*	6.40	0.56**	2.55**
46	WH 1124	11.91	10.28*	64.82**	63.85	95.83	0.77*	6.93**	0.31**	2.45**
47	WH 1126	10.05	6.2	58.04	59.37	90.50	0.79**	5.30	0.87	0.28
48	HP 1744	5.73	4.88	61.67*	64.72	88.67	0.75	5.73	0.95	-0.26

Continuing table 3

Sr	Genotype	Grain yield under normal	Grain yield under stress	Tetrazoliom tripenyl chloride test	Cell membrane stability index	Days to 50% heading	Chlorophyll fluorescence	Canopy temperapture depression	Heat sensitivity index	Heat response index
49	WH 1142	15.28	10.83*	49.79	47.25	93.00	0.80**	6.37	0.66**	2.21**
50	WH 1164	15.12	12.75**	56.98	63.52	91.00	0.79**	6.53*	0.55**	3.38**
51	WH 1179	15.35	9.48	58.67	68.59**	90.06	0.68	5.27	0.87	1.46**
52	WH 147	13.98	5.58	39.92	53.19	82.33**	0.65	6.20	1.37	-0.22
53	WH 542	7.18	8.34	57.04	62.07	90.33	0.75	3.67	1.22	-1.36
54	WH 711	8.48	4.45	42.68	46.51	94.67	0.64	6.17	1.08	-1.72
55	WH 730	8.29	6.62	55.57	70.91**	85.33**	0.75	5.50	0.46**	2.53**
56	UP 2843	12.72	9.35	61.59*	67.49*	97.00	0.69	6.10	0.60**	0.93
Mean		**12.44**	**7.06**	**51.14**	**56.95**	**90.36**	**0.70**	**5.55**	**1.00**	**0.00**
SE(m)		1.497	1.10	2.94	3.09	1.32	0.02	0.32	-	-
SE(d)		2.117	1.56	4.16	4.37	1.86	0.03	0.45	0.09	0.45

*, **: Significant at 5% and 1% level of significance

Table 4. Correlation coefficients of bread wheat genotypes for among various traits under heat stress conditions

Character	**TTC**	**CMS**	**Days to 50% heading**	**CFL**	**CTD**	**HSI**	**HRI**
Grain yield	0.314*	0.365**	0.446**	0.330*	0.539**	0-.661**	0.668**
Tetrazoliom tripenyl chloride test (TTC)	1.000	0.797**	0.101	0.504**	.159	-0.391**	0.394**
Cell membrane (CMS) stability index		1.000	-.007	0.342*	0.182	-0.298*	0.361**
Days to 50% heading			1.000	-.095	0.274	-0.438**	0.000
Chlorophyll Fluorescence (CFL)				1.000	0.255	-0.342*	0.468**
Canopy temperature depression (CTD)					1.000	-0.549**	0.498**
Heat sensitivity index (HSI)						1.000	-0.847**

*, **: Significant at 5% and 1% level of significance; HRI: Heat Respone Index

Table 5. Principal components of various traits in bread wheat for heat tolerance

Character	**PC 1**	**PC 2**	**PC 3**	**PC 4**	**PC 5**
Heat sensitivity index	-0.907	-0.023	-0.223	0.023	-0.069
Heat response index	0.877	0.022	-0.258	0.056	0.032
Cell membrane stability index	-0.058	0.983	-0.097	-0.091	0.048
Tetrazoliom tripenyl chloride test	0.124	0.851	0.132	0.191	-0.048
Days to 50% heading	0.00	-0.013	0.963	-0.042	0.035
Chlorophyll Fluorescence	-0.02	0.002	-0.034	0.989	0.023
Canopy temperapture depression	0.003	0.000	-0.001	0.012	1.003
Grain yield	0.15	0.067	0.197	0.109	0.066
Eigen value	3.78	1.564	0.993	0.587	0.545
Variance	47.251	19.555	12.412	7.334	6.818
Cumulative variance	47.251	66.806	79.218	86.552	93.37

Figure 1. Scree Plot principal components for various traits in bread wheat

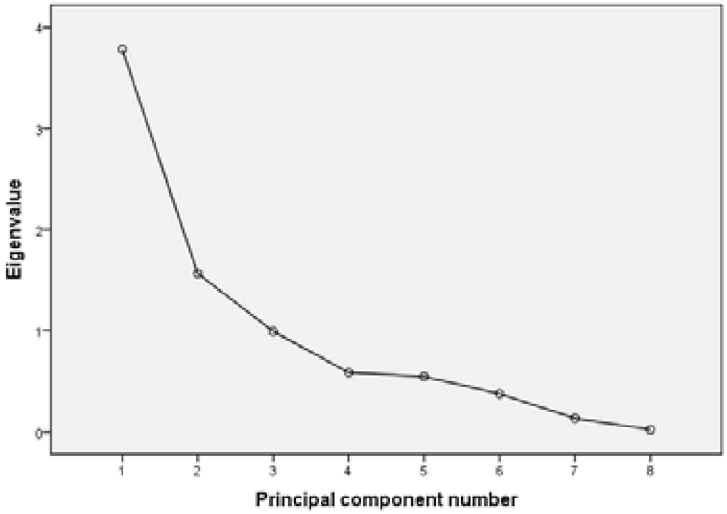


Figure 3. Scatter diagram of bread wheat genotypes under heat stress conditions

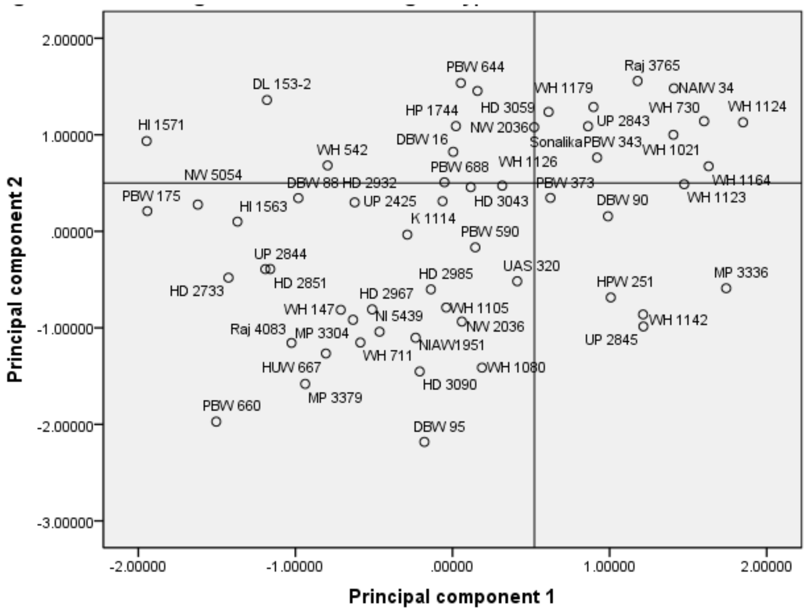


Figure 4. Weekly maximum and minimum temperature during the growth season

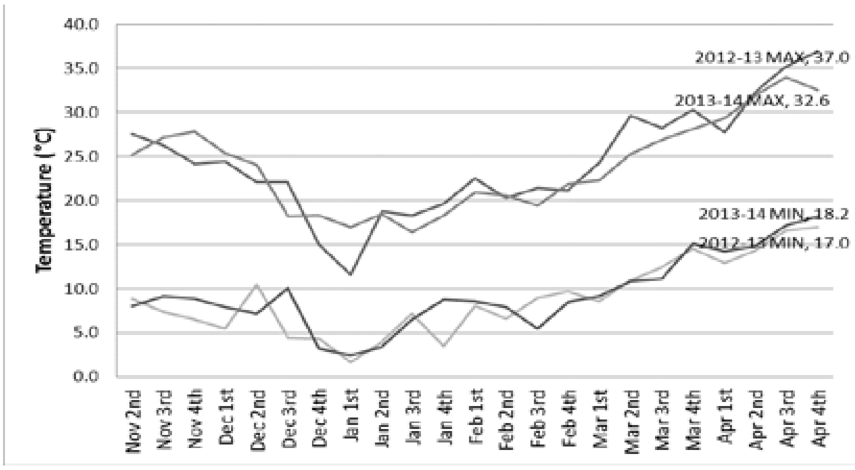
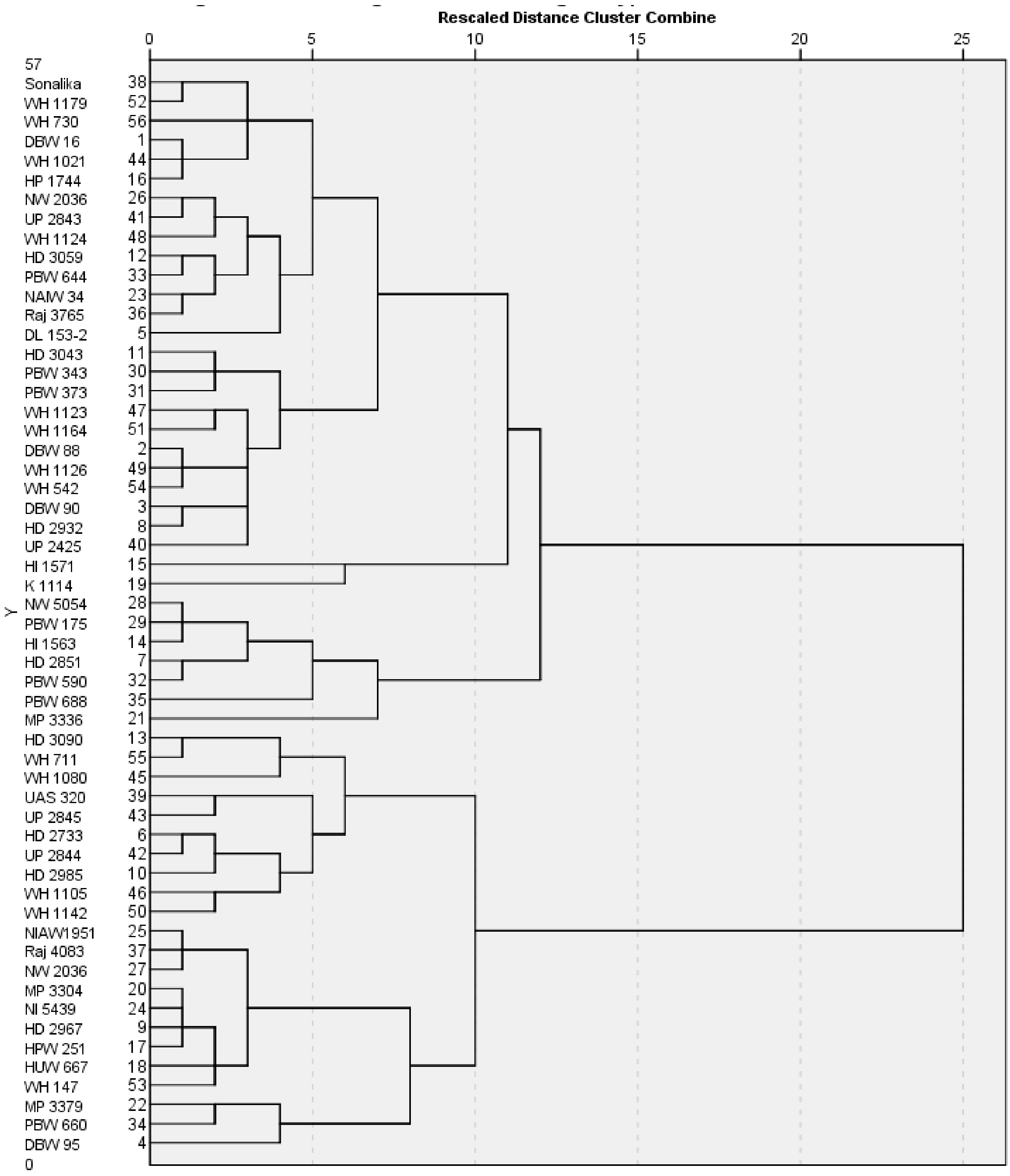


Figure 2. Clustering of bread wheat genotypes under heat stress conditions



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