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Morpho-physiological diversity of barley (*Hordeum vulgare* L.) germplasm for heat tolerance

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Abstract: The aim of the present study was to examine the diversity for heat tolerance in a set of 105 accessions using some key morpho-physiological attributes. The data was standardized by calculating percent change over control, and used for phonetic analysis and construction of dendograms based on distance matrix (all primary data is given as supplementary material listed in statistical analysis section of Materials and Methods). These distance matrices were then used for the unweighted pair group method with arithmetic mean (UPGMA) cluster analysis using the sequential agglomerative hierarchical nonoverlapping (*SAHN)* method. The cluster analysis indicated some potential attributes that could be effectively used to screen germplasm for heat tolerance. Among the growth attributes, shoot fresh and dry weight was a much stable indicator than root biomass. The alterations in the concentration of chlorophyll (Chl) *b* played a significantly better role in clustering of various genotypes than those in Chl *a*. Since leaf osmotic potential was fairly maintained in most of the barley accessions under study, the notable change in leaf water and osmotic potentials seemed to be the fundamental reason for the clustering pattern. The alterations in stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) were of foremost importance for clustering gas exchange parameters. The changes in electron transport rate and nonphotochemical quenching were of major importance for clustering based on chlorophyll fluorescence. Thus, it can be concluded that the thermo-tolerance and yield of barley when grown under high temperature stress can be enhanced by improving these attributes.

Key words: Barley, clustering, heat tolerance, physiological attributes, UPGMA, selection criteria

1. Introduction

The climatic changes due to global warming has caused temperature fluctuations in many regions of the world during the growing seasons of major agricultural crops (Jerry et al., 2015). Among these climatic adversaries, heat stress is of serious concern as it results in enormous crop losses in many areas of the world (Teixeira et al., 2013). Heat stress can affect key metabolic, physiological, and biochemical processes regulating plant growth and development which, in turn, markedly hamper crop productivity (Bahrami et al., 2019). It can also cause a multitude of harms to plants, i.e. scorching of plant parts, rapid senescence and abscission, retardation of plant growth, discoloration of fruits, and reduction in overall plant productivity (Abou-Elwafa and Amein, 2016; Riaz et al., 2021).

To cope with the adverse condition of heat stress, plants have developed the internal defensive system that generates signals to respond to the changes in plant metabolism. Plants produce compatible osmolytes that protect proteins and cell integrity, maintain turgor potential of cell by osmotic adjustment, and produce antioxidants that maintain cellular homeostasis (Hasanuzzaman et al., 2013). However, heat stressed plants adopt some metabolic means to counteract the deleterious impacts of ROS on cellular structures and macromolecules (Wang et al., 2018).

Heat stress has highly reduced the growth and productivity of barley (*Hordeum vulgare* L.). It grows in cool season at a temperature range of 15–30 °C (Nevo, 1992). Research has revealed that heat stress limits the photosynthesis in barley by damaging the activity of PS II and reducing the electron transport reaction and proton concentration in the thylakoid. Bahrami et al. (2019) demonstrated that terminal heat stress decreased photosynthetic performance (measured by gas exchange), PSII, and chlorophyll content in cultivated barley and to a much less degree in wild barley genotypes. Pshybytko et al. (2003) reported a decrease in nonphotochemical quenching (NPQ) values in heat-stressed barley leaves. Grain yield reductions due to high temperatures during terminal growth stages (anthesis and postanthesis) were

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recently demonstrated in such cereals as wheat (Mahdavi et al., 2021) and barley (Bahrami et al., 2021).

Breeding and development of new crop cultivars exhibiting heat tolerance is a major concern for researchers (Halford, 2009). Plants exhibit different response to heat stress reliant upon the intensity, period, and plant category. Moreover, the precise traits that induce heat tolerance in plants are not definable (Kosová et al., 2011). Various conventional and advanced techniques are being implemented to group available germplasm of crop plants with differential tolerance against environmental constrains. Among these, cluster analysis is a useful technique that can be used to select resistant genotypes within a set of large number of cultivars/accessions under study (Kalaji and Guo, 2008). Thus, we hypothesized that barley germplasm could be effectively screened for heat tolerance through the cluster analysis using data for key growth and physiological attributes.

The premier objective of the present study was to employ a suitable method to screen a large number of available barley germplasm (105 accessions) collected from diverse geographic origins for heat stress tolerance. The screening was based on some key physiological selection criteria such as growth parameters, water relations, photosynthetic pigments, and gas exchange and chlorophyll fluorescence attributes.

2. Materials and methods

The morphological and physiological response of 105 barley (*Hordeum vulgare* L.) accessions to heat stress was assessed in a pot experiment. The seeds of different barley accessions from Pakistan (50), Iran (11), Cyprus (4), and Japan (35) were obtained from the Institute of Agricultural Biotechnology and Genetic Resources, Pakistan Agricultural Research Council, Islamabad, while those of Saudi Arabian accessions (5) were provided by the King Saud University, Saudi Arabia. The details of the origin of these barley accessions are presented in Table S1.

2.1. Soil analysis and seed germination

Prior to sowing, a detailed analysis of soil used for the experiment was performed. The physico-chemical attributes were as follows: soil texture: clay-loam; saturation: 40.12%; field capacity: 19.56%; ECe: 0.207 dS m^{-1}; pH: 6.8. The barley seeds were surface-sterilized with 0.1% $HgCl_2$, and 10 seeds were sown in each pot containing 10 kg soil. Ten days after germination, the plants were thinned to six in each pot, and allowed to grow for 30 days under normal conditions. The average climatic conditions during the course of the experiment were as follows: photosynthetic active radiations (*PAR*): 1280 µmol m^{-2} s^{-1}; day length 11.5 h; relative humidity 59%; day temperature 25 °C, night temperature 11 °C; average ambient CO_2 concentration 350 mg L^{-1}.

2.2. Stress application

Heat stress was applied after 30 days of growth under the abovementioned conditions. A closed canopy of transparent plastic sheet was constructed with 9 × 3 × 1.5 m (length × width × height) dimensions. The average climatic conditions inside the canopy during the course of the experiment were recorded as: photosynthetic active radiations (*PAR*) 569.9 µmol m^{-2} s^{-1}; relative humidity 77%; day temperature 33 ± 6 °C; night temperature 13 ± 2 °C; average ambient CO_2 concentration 358 mg L^{-1}. The roof of the canopy was cleaned daily both inside and out to remove condensed moisture and dust particles to ensure consistent penetration of light inside the canopy. In addition, the canopy was opened daily for 30 min in the morning between 7.00 and 8.00 AM to maintain the CO_2 levels of canopy, equivalent to those of ambient air. The experiment was laid down in a randomized complete block design with three replications. The plants were grown for a further period of 45 days under the abovementioned conditions and data for various parameters were recorded.

2.3. Determination of morphological attributes

The data for shoot length, root length, and fresh weight of root and shoot were recorded. The plants were harvested and weighed using an analytical balance to obtain the fresh biomass of plants. The plants were dried at 65 °C for 72 h in an oven and their dry weights were determined.

2.4. Determination of chlorophyll concentration

Chlorophyll *a* and *b* were determined according to the procedure described by Arnon (1949). Fresh leaves (each sample of 0.5 g) were extracted with 80% acetone at 4 °C and then the extracts were centrifuged for 5 min at 10,000 × *g*. The optical densities were recorded from the supernatant at 645 and 663 nm on a spectrophotometer (IRMECO, U 2020) and the concentrations of both chlorophyll (Chl) *a* and *b* were calculated.

2.5. Determination of water relation attributes

A fully expanded second leaf was excised from each replicate at dawn and fitted in a Scholander type pressure chamber (Arimad-2-Japan) through the mid rib to determine its water potential. The same leaf was stored in a freezer at –20 °C. After 2 weeks, the frozen leaf was thawed and the sap was extracted by pressing it with a glass rod. The sap so extracted was directly used for the determination of osmotic potential using an osmometer (VAPRO, Model 5520, USA). The leaf turgor potential was calculated as the difference between water potential and osmotic potential values.

2.6. Determination of gas exchange characteristics

The LCA-4 ADC portable gas analyser (IRGA) was used to determine the net photosynthetic rate (*A*), transpiration rate (*E*), stomatal conductance (g_s), water use efficiency (*A*/*E*), and internal CO_2 /ambient CO_2 concentration (*Ci*/

Ca) of each plant. The second leaf from top was used to determine these characteristics. The specifications of the instrument were as follows: 403.3 mmol $m^{-2}s^{-1}$ molar flow of air, 99.9 kPa atmospheric pressure, 6.0 to 8.9 mbar water vapor pressure, 1311 µmol $m^{-2}s^{-1}$ *PAR*, 28.4 to 27.9 °C leaf temperature, and 352 µmol mol^{-1} ambient CO_2 concentration.

2.7. Determination of chlorophyll fluorescence characteristics

Before the measurements of chlorophyll fluorescence, leaves were kept in dark for 30 min, and data for different chlorophyll fluorescence attributes were determined according to the procedure described by Strasser and Strasser (1995); Strasser et al. (2000), and Misra et al. (2001). The chlorophyll fluorescence attributes such as electron transport rate (ETR), NPQ, and ratio of variable fluorescence to maximum fluorescence (F_v/F_m) were recorded using the OS5p Modulated Fluorometer (ADC BioScietific Ltd., Great Amwell Herts, UK).

2.8. Statistical analysis

In order to minimize the interference of unrelated parameters, all variables were divided into 5 distinct groups, i.e. morphological (root and shoot fresh and dry weights); chlorophyll concentration (Chl. *a*, Chl. *B*, and Chl *a*/*b* ratio); water relations (water, osmotic and turgor potentials), gas exchange attributes (*A, E, A/E,* g_s, C_i), and chlorophyll fluorescence (*Fv/Fm, ETR, NPQ*) (Barley heat stress raw data.xls: supplementary material). The percent change over control in all these attributes was calculated (Barley heat stress percent change.xls: supplementary material) and used for the phonetic analysis and construction of phylogenetic trees by using NtSys-pc program 2.11X (Rohlf, 2000). All 105 barley accessions were treated as operational taxonomic units (OTUs), and their morphological and physiological attributes were regarded as variable characters [(ntSYSpc analysis files.zip: supplementary material in ntSYSpc format) and (ntSYSpc analysis files.zip: supplementary material in CSV format)]. Pairwise distances on "interval data" of all pairs of taxa were computed using Euclidean (EUCLID) distance coefficients with the average taxonomic distance [(ntSYSpc distance matrixes.zip: supplementary material in ntSYSpc format) and (ntSYSpc distance matrixes.zip: supplementary material in CSV format)]. The distance matrix was used for unweighted pair group method with arithmetic mean (UPGMA) cluster analysis [(ntSYSpc tree calculations: supplementary material in ntSYSpc format) and (ntSYSpc tree calculations: supplementary material in CSV format)] using the sequential agglomerative hierarchical nonoverlapping (SAHN) method (Sneath and Sokal, 1973). The distinct branches of the trees so constructed were used to classify all 105 accessions into identifiable cluster, subclusters, subunits, and subgroups as summarized in Tables S2–S6 (Supplementary file: Barley summary of cluster analysis.doc and barley clustering of variables.xls).

3. Results and discussion

Climatic change has increased the already narrow genetic diversity in the crops. Thus, the identification or development of stress-resistant cultivars/lines of crops has got considerable importance. Efforts have been underway to develop suitable models using key biochemical and physiological markers for screening of available crop germplasm against a variety of abiotic stresses. This indeed helps the selection of suitable approaches and indicators for screening and selection of stress-tolerant cultivars/lines, particularly while screening a large number of genotypes/lines. Cluster analysis is one of the most popular methods for analysing multivariate data and for exploring the underlying structure of a given data set. Various techniques used for clustering include principal component analysis, principal coordinate analysis, redundancy analysis, and UPGMA (Sneath and Sokal, 1973; Anderson 2001; Chaum et al., 2010; Enyew et al., 2019). All these techniques employ mathematical algorithms and assemble large sample units in various groups and subgroups depending upon similarity or distance matrixes. In this study, the distance matrix was generated and used for UPGMA cluster analysis using SAHN method. The trees constructed were based on percent change in all five groups of variables and classified all 105 accessions into identifiable clusters.

The results revealed that all studied morphological attributes in barley genotypes showed a severe reduction under heat stress. These results are in accordance with those of a previous study, which reported that heat stress caused a significant decline in shoot dry mass, relative growth rate, net assimilation rate, leaf area, leaf numberü and the duration of vegetative growth in plants (Bahrami et al., 2019). The UPGMA analysis of all 105 barley accessions based on percent decrease in morphological attributes under heat stress is shown in Figure 1 and summarized in Table S2 (supplementary material). The cluster analysis of the data grouped different barley accessions into four identifiable clusters. Among these, cluster I was the largest cluster containing 60 accessions. It contained heat-tolerant accessions which exhibited the lowest decrease in their growth attributes. It was further subdivided into four subclusters A, B, C, and D. Of these, subcluster "A" contained 5 accessions exhibiting the least percent decrease in shoot and root fresh weights and shoot dry weight. However, the percent decrease in root dry weight of the accessions grouped in subcluster "A" was the highest as compared to that in the other three subclusters. Subcluster "B" contained 13 accessions which showed a greater decrease in shoot and root fresh weights than

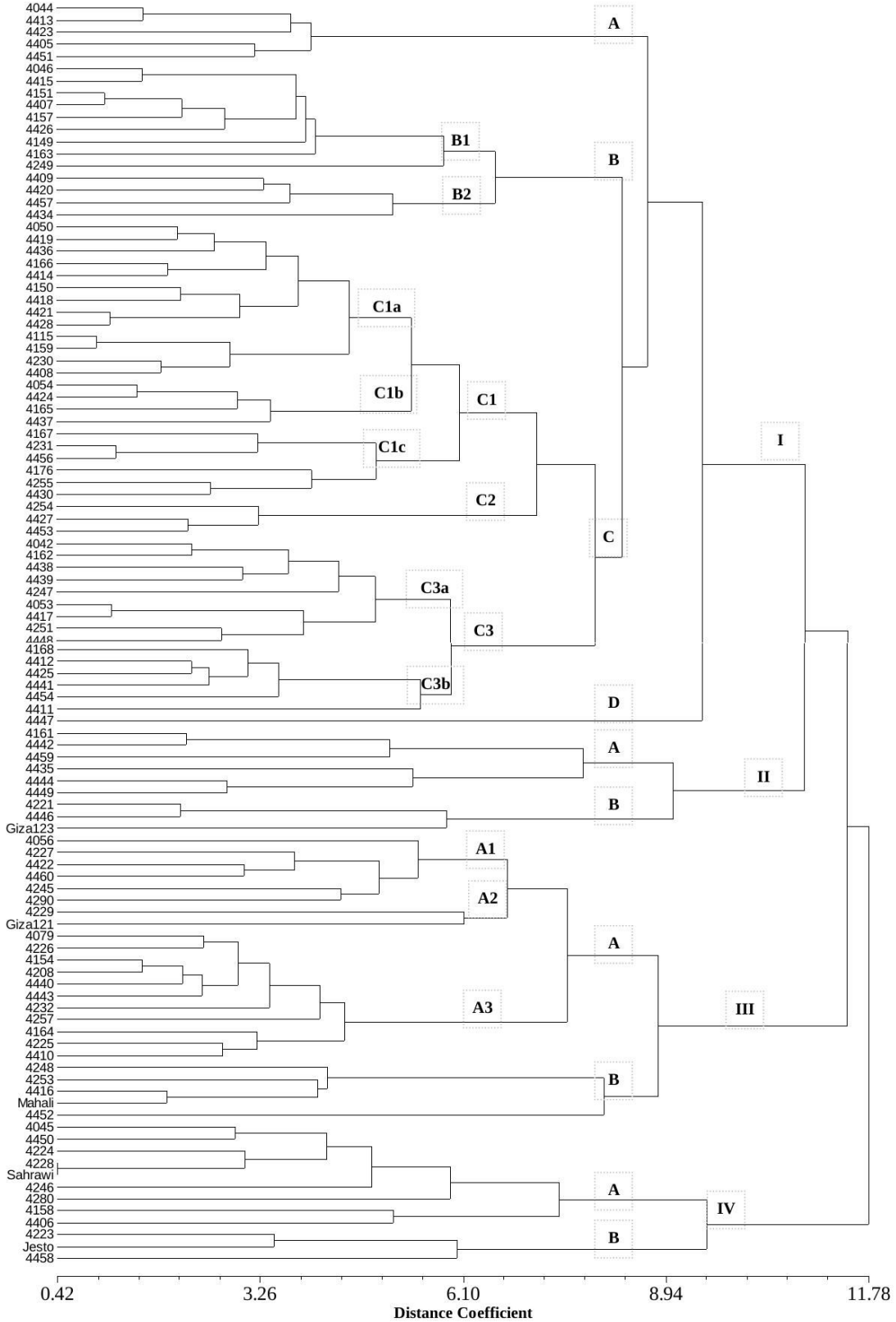


Figure 1. Results of cluster analysis (UPGMA) based on percent change in morphological attributes of 105 diverse barley accessions subjected to heat stress. The average taxonomic distances were calculated using ntSYSpc software (v 2.1). The similarity among accessions is indicated by distance coefficients and used to group accessions into independent clusters and subclusters in the phenogram.

that of the accessions grouped in subcluster "A". However, based on subcluster average, these accessions surprisingly exhibited the least decrease in shoot and root dry weights. Subcluster "C" was the largest subcluster containing 41 accessions which exhibited more decrease in all these growth attributes compared with the first two clusters. However, this was not true for root dry weight which showed the highest decrease in subcluster A. Subcluster "D" contained only one accession which was perhaps due to the maximum decrease in shoot dry weight (–22.3%) and root fresh wt. (–34.3%). Cluster II, comprising only 9 accessions, contained all those moderately heat-tolerant accessions which exhibited the maximum reduction in shoot fresh weight. This cluster was subdivided into two subclusters A and B. Subcluster "A" contained 6 accessions which showed an average decrease of 31.3% in shoot fresh weight. The three accessions grouped in subcluster "B" showed the second highest decrease (–24.6%) in shoot fresh weight and –28.4% in root dry weight. The 25 accessions in cluster III showed the highest decrease in all growth parameters except in shoot fresh weight which maximally decreased in the barley accessions grouped in cluster II and thus were moderately heat-sensitive. This cluster contained two subclusters. The average reduction in various growth parameters for 19 accessions grouped in subcluster "A" was –28.1%, –26.6%, and –33.5% for shoot dry, root fresh and dry weights, respectively. In comparison, the other six accessions grouped in subcluster "B" showed an average decrease of –24.8, –28.2, and –29.2% in growth attributes. This indicated that the accessions grouped in subcluster "B" were slightly more tolerant than those found in subcluster "A". The other 11 heat-sensitive barley accessions grouped in cluster IV were highly heat-sensitive, as they showed severe reduction in growth attributes under heat stress. Eight sensitive barley accessions grouped in subcluster "A" showed a moderate decrease in shoot fresh (subcluster average –9.5%) and dry weight (subcluster average –13.5%). However, the root growth of these accessions was severely inhibited under heat stress which showed decreases by –23.5 and –31.4% for root fresh and dry weights, respectively. In a similar manner, root fresh and dry weights in three accessions of subcluster "B" decreased by –40.0% and –33.4%, respectively. This was also the highest decrease in root growth in 104 barley accessions included in cluster IV. Hence, reduction in root growth under heat stress seemed to be a potential indicator for grouping these 11 accessions in cluster IV.

Thus, taking shoot fresh and dry weights into account as the key growth parameters of biomass production, the accessions grouped in clusters III and IV did not seem to be of much economic importance for cultivation in arid regions encountering high temperature stress. It also appeared that the maintenance of shoot fresh and dry weights and root fresh weight was the key reason for their clustering which is also one of the most important indicators of forage production of crop plants (Guenni et al., 2002). Though root growth in terms of fresh and dry weights substantially influenced shoot growth, it played a minor role in clustering except for root dry weight which was found to be a more stable attribute than the fresh weight (Manschadi et al., 2008).

The results showed that heat stress decreased the chlorophyll concentration in all studied accessions. It may be due to the reason that heat stress causes structural and functional disruptions of chloroplasts by producing high levels of reactive oxygen species causing lipid peroxidation of chloroplast and thylakoid membranes thereby reducing chlorophyll contents (Oukarroum et al., 2016). In the present study, it was observed that heat-tolerant barley genotypes showed high Chl *a/b* ratio in comparison to heat sensitive genotypes. These findings are in accordance with those of previous studies which reported that heat-tolerant genotypes exhibit high Chl *a/b* and chlorophyll/carotenoids ratios under high temperatures which is an important indicator of thermotolerance in most plants (Almeselmani et al., 2012; Bahrami, 2019). This leads to reduced rates of photosynthesis thereby imposing deleterious effects on plant growth under heat stress.

The UPGMA-based dendrogram divided all 105 barley accessions into three distinct clusters, i.e. Clusters I, II, and III containing 26, 22, and 57 accessions, respectively (Figure 2; Table S3: supplementary material). All heat tolerant genotypes were assembled into cluster I because these genotypes exhibited less reduction in Chl *a* and *b* contents (cluster average –20.98% and –21.47% for Chl *a* and *b*, respectively). Cluster 1 gave rise to two subclusters. Subcluster "A" contained 19 tolerant accessions while subcluster "B" had 7 accessions. The average reduction in chlorophyll contents of 7 accessions grouped in subcluster "B" was the minimum (i.e. –16.0% and –19.4% for Chl *a* and *b*) indicating it to be the most tolerant group. The 19 accessions grouped in subcluster "A" were second in tolerance as –26.0% and –23.5% reduction in Chl *a* and *b* content was recorded in this subcluster. Cluster II contained 22 moderately heat-tolerant barley accessions, which showed less reduction in concentration of photosynthetic pigments. It contained 3 subclusters. Subcluster "A" had 17 barley accessions while subcluster "B" had 4 accessions. Surprisingly, subcluster "C" contained only one accession (4114) which was also the most tolerant among all 105 accessions. This accession showed a minimal reduction in Chl *a* (–14.7%), and Chl *b* (–18.2%). On the other hand, the four accessions included in subcluster "B" showed the highest decrease in Chl *a* (–55.2%) and Chl *b* (–56.9%) indicating it to be highly heat-sensitive. The 17 accessions

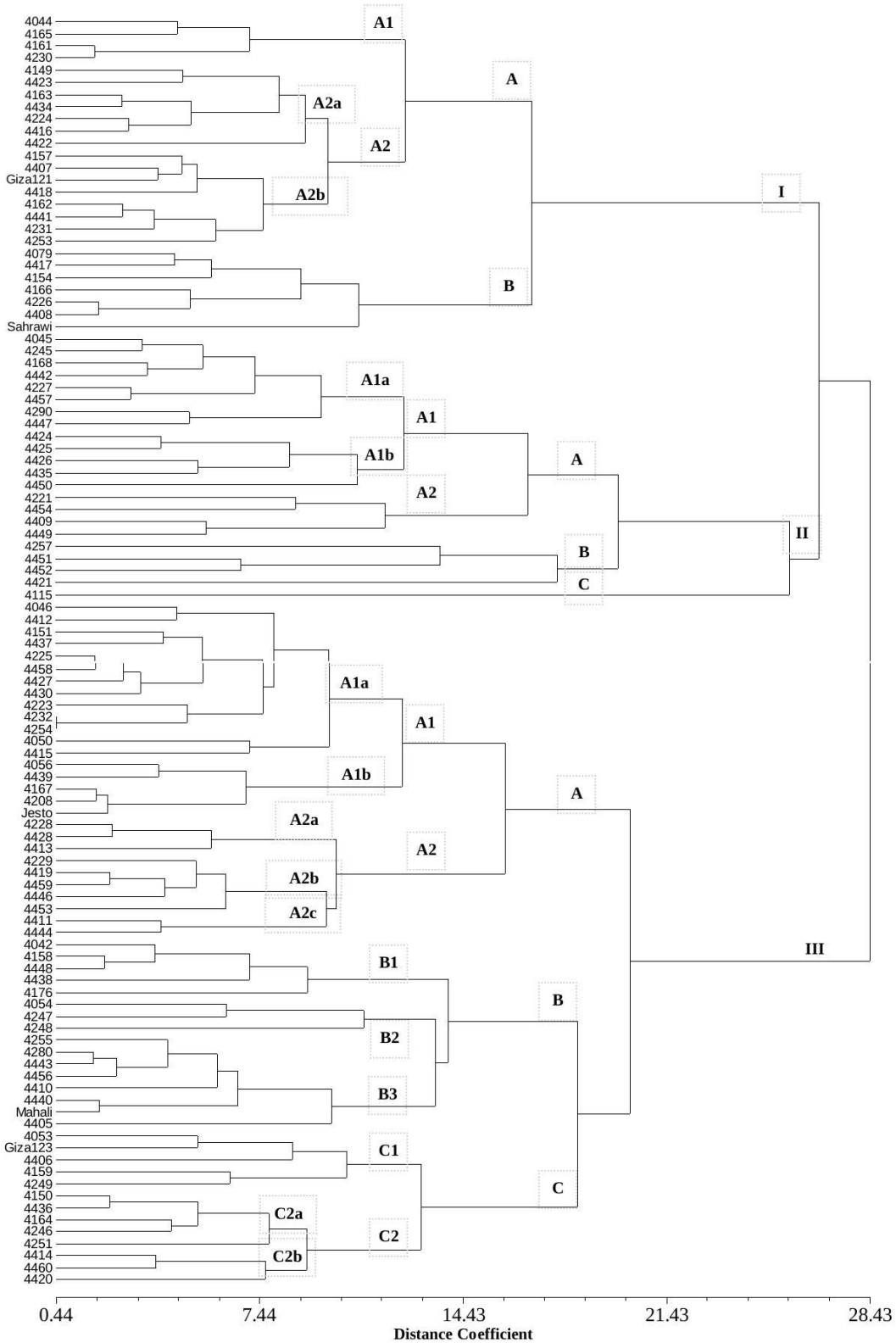


Figure 2. Results of cluster analysis (UPGMA) based on percent change in concentration of photosynthetic pigments of 105 diverse barley accessions subjected to heat stress. The average taxonomic distances were calculated using ntSYSpc software (v 2.1). The similarity among accessions is indicated by distance coefficients and used to group accessions into independent clusters and subclusters in the phenogram.

in subcluster A showed an average decrease of –40.9% and –38.2% in concentration of Chl *a* and *b*, respectively. In contrast, 57 heat-sensitive barley accessions were grouped in cluster III. This cluster contained three subclusters A, B, and C wherein 28, 16, and 13 accessions were present, respectively. The accessions grouped in subcluster "A" were comparatively were relatively less tolerant (–54.3% and –52.9% reduction in Chl *a* and *b*) compared with subcluster "B" (–49.5% and –55.0% reduction). The other 13 accessions in the subcluster "C" showed –41.2% and –33.3% reduction in Chl *a* and *b*, respectively.

From all this discussion, it is amply clear that the concentration of Chl *b* was less affected compared with Chl *a*, which seemed to be the fundamental reason for the clustering pattern of these accessions into various clusters and subclusters. Nevertheless, variation in concentration of Chl *a* under heat stress played a less significant role in clustering pattern. Thus, changes in the concentration of Chl *b*, being a somewhat stable attribute, can be used as a selection criterion for heat tolerance in plants (Ristic et al., 2007).

This study revealed that the changes in leaf water potential, osmotic potential, and turgor potential were significantly positive under heat stress as compared to those in control plants. Among these attributes, the percent change in water potential was abrupt as compared to osmotic potential which appeared to be a much consistent variable. Though turgor potential also showed a positive change under heat stress, it was the least changed attribute under heat stress (in terms of percent change). It has been reported that heat stress causes a rapid loss of leaf water content as a result of transpirational stress which may cause dehydration in plants (Saha et al., 2016; Sattar et al., 2020). The effects of high temperature worsen when high temperature is accompanied by soil drought, because it affects cell and tissue water content as well as all components of water potential to a greater degree (Lamaoui et al., 2018). Such transpiration-induced water deficiency in plants leads to perturbation in many physiological processes thereby resulting in growth reduction as observed in this study (Saha et al., 2016).

Figure 3 shows the clustering of different barley accessions based on their percent changes in water relation attributes under heat stress. The UPGMA analysis assembled all barley accessions into three clusters (Table S4: supplementary material). In cluster I, 61 barley accessions exhibiting a moderate change in water potential (average +60%) and for osmotic potential (average +50%) were assembled. This cluster exhibited moderate increase (percentage) in water relation attributes. Its subcluster "A" containing 10 accessions showed an average increase of 78.1%, 51.7%, and 31.2% in water potential (y_w), solute potential (y_s), and pressure potential (y_p), respectively. In comparison, its subcluster "B" contained only one moderately heat-tolerant accession 4221. Its subcluster "C" was the largest containing 50 barley accessions. The latter subcluster showed an average 65.2%, 36.6%, 15.5% increase in y_w, y_s, and y_p, respectively. Cluster II is comprised of 29 barley accessions which showed the lowest change in leaf water potential (average +30%) and osmotic potential (average +25%). The 25 accessions included in subcluster "A" showed an average increase of 40.6%, 28.2%, and 18.3% in y_w, y_s, and y_p, respectively. In comparison, the 4 accessions in subcluster "B" exhibited the lowest increase of 21.4%, 19.4%, and 18.0% for leaf y_w, y_s, and y_p, respectively, of these barley accessions. The 15 accessions in cluster III showed the greatest change of all in water potential (average +105%). The 8 accessions in subcluster "A" showed the highest increase in y_w (113.2%) and y_s (51.0%) while y_p did not show much increase (14.5%). In comparison, the other 7 accessions in subcluster "B" also showed significantly higher (92.7%) increase in y_w. However, y_s and y_p were not markedly increased (41.7% and 8.9%, respectively) in these accessions as compared to the accessions in the other clusters.

Since all accessions exhibited almost an equal change in turgor potential, it seemed to be a less significant indicator of screening barley accessions for heat tolerance as compared to water and osmotic potentials. The reason may be that the turgor is calculated as the difference between water and osmotic potentials (Lambers et al., 2008). Under heat stress, a substantial change in leaf water potential in different barley accessions was observed that may have been compensated by accumulation of different types of osmotica which reduced the leaf osmotic potential (Faralli et al., 2015). The maintenance of turgor potential under heat stress helped to sustain plant growth to some extent as reported earlier (Sattar et al., 2020). These results showed that water and osmotic potentials can be used as potential indicators of thermo-tolerance in barley.

The imposition of heat stress drastically reduced all gas exchange attributes including *A, E, A/E,* g_s, and C_i. It has already been reported that high temperature induced reduction in stomatal conductance that leads to reduced *Ci* and *E*. Bahrami et al. (2019) evaluated the effects of terminal heat stress on gas-exchange parameters, photosystem efficiency, proline accumulation, cell membrane leakage, and grain yield traits in wild and cultivated barleys. Thus, an increase in leaf temperature results in decreased photosynthetic efficiency of crop plants (Luo, 2011). Other reports indicate that the photosystem II reaction centre is the most receptive reaction centre in photosynthesis to temperature stress (Murata et al., 2007). Temperatures (35–40 °C) can directly injure the photosynthetic apparatus thereby affecting light energy capture, photosystem II efficiency, and electron

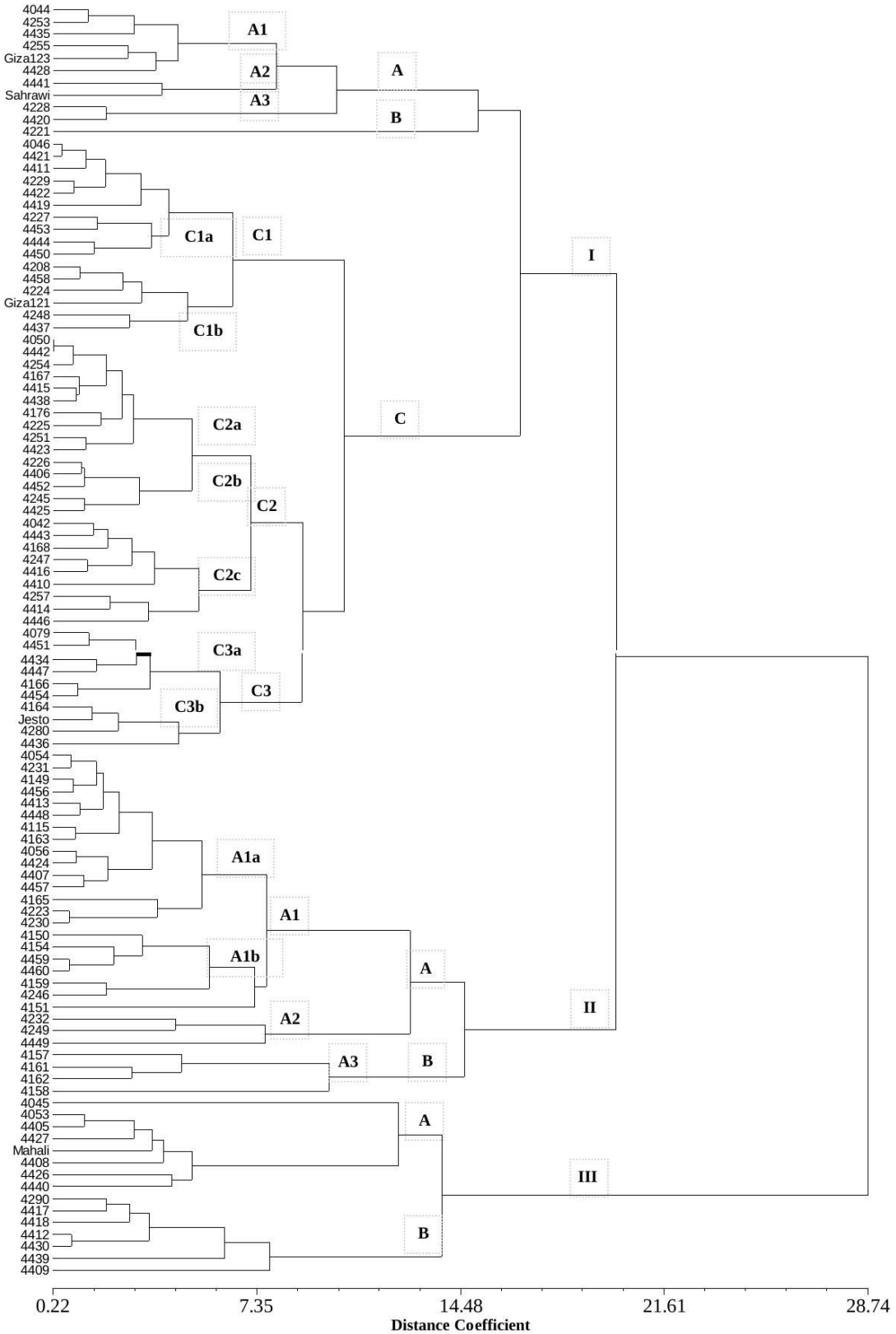


Figure 3. Results of cluster analysis (UPGMA) based on percent change in water relation parameters of 105 diverse barley accessions subjected to heat stress. The average taxonomic distances were calculated using ntSYSpc software (v 2.1). The similarity among accessions is indicated by distance coefficients and used to group accessions into independent clusters and subclusters in the phenogram.

transfer rate. Hence, they are considered the most sensitive components of the photosynthetic chain (Bahrami et al., 2019).

The cluster analysis grouped heat-sensitive and heat-tolerant genotypes into three distinct clusters (Figure 4; Table S5: supplementary material). Cluster I, being the largest of all, contained 53 barley accessions. This cluster was further divided into three subclusters depending on the extent of heat stress-induced reduction. The 32 barley accessions were grouped in subcluster "A" which exhibited the highest decrease in gas exchange attributes particularly g_s and C_i. The cluster average decrease for various gas exchange attributes was: *A* (–50.7%), *E* (–28.5%), *A/E* (–31.7%), g_s (–23.5%), and C_i (–29.7%). The 14 barley accessions present in subcluster "B" showed almost a parallel decrease in A (–52.2%), *E* (–28.5%), and *A/E* (–32.9%). However, these genotypes showed less effect of heat stress on g_s (–20.3%) and C_i (–15.1%). In comparison, the 7 genotypes grouped in subcluster "C" showed the least impact of heat stress on gas exchange attributes among all the subclusters. In this subcluster, an average decrease of 49.4%, 35.9%, 20.9%, 12.8%, and 19.8% was noted in *A, E, A/E,* g_s and C_i, respectively. Cluster II, containing 47 genotypes, was further subdivided into four subclusters containing 9, 22, 13, and 3 genotypes. Subcluster "A" contained 9 genotypes that exhibited the least cluster average for *A* (–35.2%), *E* (–23.1%), and *A/E* (–15.4%). However, these accessions showed the highest decrease in g_s (–24.7%) and C_i (–35.1%) of all subclusters. The 22 genotypes assembled in subcluster "B" showed a moderate decrease in *A* (–38.9%), *E* (–25.4%), and C_i (–28.1%) under heat stress. Nevertheless, *A/E* and g_s showed the least decrease in cluster average which was recorded to be –17.7% and –15.3%, respectively. The subcluster "C" grouped 13 genotypes having the second highest cluster average for *A* (–40.4%), but the lowest decrease was noted for C_i (–16.0%). Furthermore, a moderate decrease in *E* (–24.2%), *A/E* (–21.3%), and g_s (–25.3%) was found in this subcluster. Surprisingly, the subcluster "D" contained only three barley accessions exhibiting the highest decrease in all gas exchange attributes indicating their sensitivity to heat stress. Here, a decrease of –44.2%, –27.2%, –23.3%, and –39.1% was noted for *A, E, A/E*, and g_s, respectively. However, the decrease in C_i was the second lowest (–16.8%) among all subclusters in this cluster. Cluster III, which contained only 5 genotypes (Table S5: supplementary material), was highly heat-sensitive. It was further subdivided into two subclusters. Subcluster "A" contained two accessions which had a cluster average decrease of –47.1%, –36.5%, –37.7%, and –30.4% for *A, E,* g_s and C_i, respectively. However, *A/E* showed less decrease (–16.5%) under heat stress. Subcluster "B" assembled 3 barley accessions which exhibited marked sensitivity of gas exchange parameters and showed highest decrease of all clusters (–55.3%, –27.4%, –38.2%, –40.1%, and –26.2% for *A, E, A/E,* g_s and C_i, respectively)

The results indicated that the changes in *A, E*, and *A/E* played a significant role in clustering of different barley accessions into diverse clusters. Nevertheless, g_s and C_i seemed to be of utmost importance for their clustering pattern. As discussed earlier, *gs* is of crucial importance for entry of CO_2 into leaf tissue through stomata (C_i) and also for controlling transpiration rates (E) (Mathur et al., 2011). Thus, reduction in *Ci* as a result of stomatal closure reduced the photosynthesis (Sharma et al., 2015). These results suggest that the thermotolerance of crop plants can be enhanced by improving their g_s and consequently C_i. This will subsequently improve their *A* and *A/E* under high temperatures.

The results revealed that heat stress had a significantly adverse effect on chlorophyll fluorescence attributes of different barley accessions. It was observed that Fv/Fm and ETR decreased, while NPQ increased under heat stress. Chlorophyll fluorescence is used to assess changes in photosynthesis indirectly (Oukarroum et al., 2016). Heat stress causes damage to PS II activity that reduces the chlorophyll fluorescence. It is a reliable way to recognize and quantify the changes in photosynthetic apparatus due to heat stress (Sharma et al., 2015). Heat stress reduces the rate of electron transport and Fv/Fm of photosystem II which leads to reduce the growth of plants (Oukarroum et al., 2016)

The UPGMA cluster analysis for various chlorophyll fluorescence parameters is shown in Figure 5, while its summary is presented in Table S6 (supplementary material). Different barley accessions were grouped into five clusters. Cluster I assembled 46 barley accessions into two subclusters. Subcluster "A" contained 13 accessions which exhibited the lowest decrease in Fv/Fm (–13.5%) and ETR values (–9.8%). Furthermore, this group also showed the lowest increase in NPQ (+11.0%). In comparison, 33 barley accessions grouped into subcluster "B" showed a parallel change in chlorophyll fluorescence attributes (–13.1%, –10.8%, and +19.5%, respectively). Hence, it seemed that the change in ETR was the major criterion for clustering in this group. Though the 27 accessions grouped into cluster II exhibited a decrease in Fv/Fm values parallel to that of cluster I, the ETR and NPQ values showed a two-fold change which seemed to be the major cue of their clustering into this group. Cluster II was further divided into two subclusters with 13 and 14 accessions. The cluster average for barley accessions assembled in subcluster "A" was noted to be –15.3%, –19.2%, and +25.8% for Fv/Fm, ETR, and NPQ, respectively. In comparison, the accessions assembled in subcluster "B" showed an average change of 13.2%, –21.2%, and 18.2% in these attributes indicating

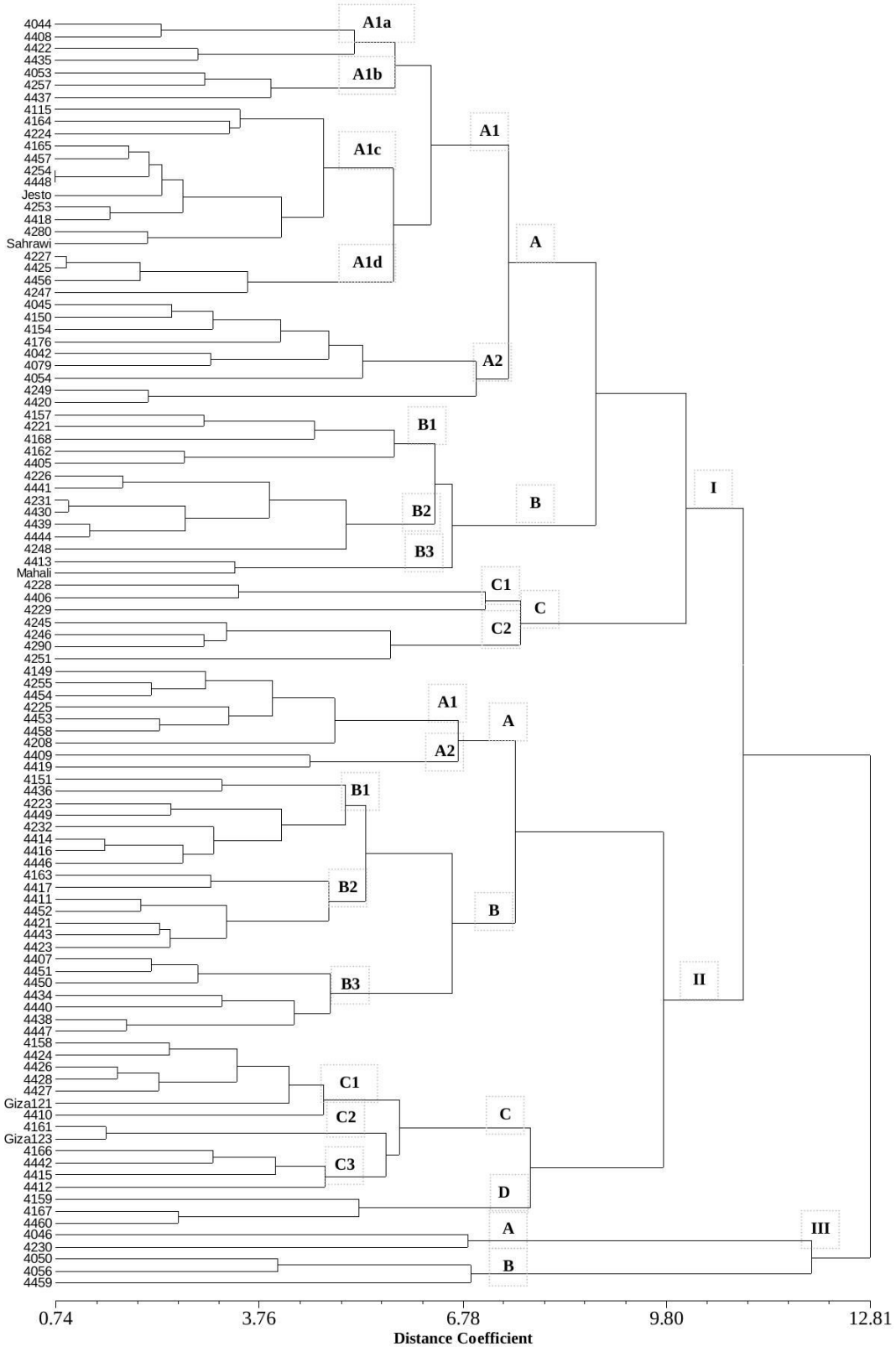


Figure 4. Results of cluster analysis (UPGMA) based on percent change in gas exchange attributes of 105 diverse barley accessions subjected to heat stress. The average taxonomic distances were calculated using ntSYSpc software (v 2.1). The similarity among accessions is indicated by distance coefficients and used to group accessions into independent clusters and subclusters in the phenogram.

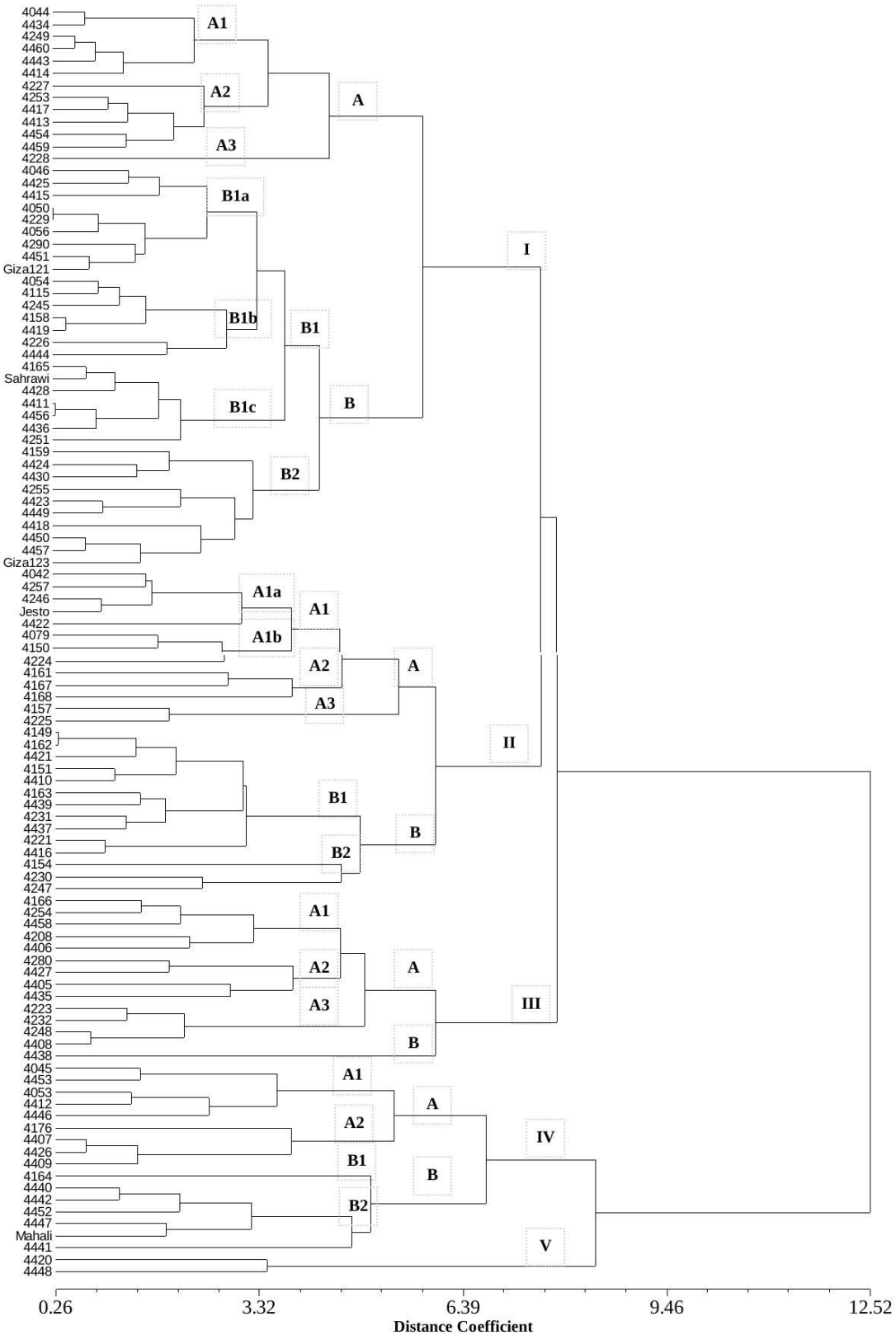


Figure 5. Results of cluster analysis (UPGMA) based on percent change in chlorophyll fluorescence of 105 diverse barley accessions subjected to heat stress. The average taxonomic distances were calculated using ntSYSpc software (v 2.1). The similarity among accessions is indicated by distance coefficients and used to group accessions into independent clusters and subclusters in the phenogram.

the greater sensitivity of ETR than that of Fv/Fm and NPQ to heat stress. Cluster III containing 14 barley accessions were divided into subcluster "A" containing 13 accessions and subcluster B with only one accession. The average change in subcluster "A" was found to be –15.1%, –18.6%, and +10.1% for Fv/Fm, ETR and NPQ, respectively. Cluster IV assembled 16 accessions showing almost a parallel change in Fv/Fm and ETR as occurred in the other clusters. However, the highest increase in NPQ seemed to be the main determinant for their assembly into this cluster. The 9 accessions in subcluster "A" showed an increase of 32.1% while the other 7 accessions assembled in subcluster "B" showed 36.4% increase in NPQ. Cluster V exhibited highest change in chlorophyll fluorescence attributes where a change of –20.1%, –14.4%, and 47.0% was noted in Fv/Fm, ETR, and NPQ respectively, which indicated their highest sensitivity to heat stress.

Surprisingly, a barley accession 4438 branched separately because of its highest decrease in Fv/Fm and ETR and lowest increase in NPQ values of all accessions. A similar pattern was observed for decrease in Fv/Fm value in those accessions clustered into clusters IV and V. However, 3- to 4-fold change in ETR and NPQ values was observed. From all this discussion, it is clear that the changes in ETR and NPQ values was the major cause of clustering pattern of various barley accessions under heat stress. A fast indicator of heat damages to photosynthetic apparatus is to check chlorophyll fluorescence (Oukarroum et al., 2016). Various studies have clarified the effect of heat stress on fluorescence (Bahar et al., 2011; Bahrami et al., 2019). Among all chlorophyll fluorescence parameters, Fv/Fm ratio is considered to be one of the important variables and is most widely studied in crop plants to assess the photoinhibition process under abiotic stresses (Dawood et al., 2020). In this study, Fv/Fm did not play a much significant role in clustering pattern of various barley genotypes grown under heat stress. Thus, change in NPQ and ETR seemed to be important indicators for selecting heat-resistant genotypes for cultivation in arid environments facing high-temperature stress.

4. Conclusion

Heat stress caused a severe reduction in growth of barley plants; associated with reduction in concentration of chlorophyll concentration, gas exchange parameters, and chlorophyll fluorescence attributes. Leaf water and osmotic potentials substantially decreased but maintenance of turgor helped barley accessions to cope with the deleterious impacts of heat stress. The UPGMA analysis revealed that maintenance of shoot fresh and dry weights was the key factor for clustering, while alteration in the concentration of Chl *b* played much significant role in clustering as compared to Chl *a*. Among water relation attributes, leaf water and osmotic potentials were the fundamental indicators for clustering. Other results showed that g_s and consequently C_i were critical parameters for the cluster analysis. Changes in ETR and NPQ values were a major cause of clustering for chlorophyll fluorescence attribute. Thus, all these indicators can be used as selection criteria for selecting thermo-tolerant genotypes for cultivation in areas encountering high temperature stress.

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Supplementary material

Table S1. The origin of barley (*Hordeum vulgare*) accessions used in the study.

Accessions	Collected from
4044, 4045, 4046, 4050, 4052, 4053, 4054, 4056, 4079, 4115, 4149, 4150, 4151, 4154, 4157, 4158, 4159, 4161, 4162, 4163, 4164, 4165, 4166, 4167, 4168, 4176, 4208, 4221, 4223, 4224, 4225, 4226, 4227, 4228, 4229, 4230, 4231, 4232, 4245, 4246, 4247, 4248, 4249, 4251, 4253, 4254, 4255, 4257, 4280, 4290	Pakistan
4405, 4406, 4407, 4408, 4409, 4410, 4411, 4412, 4413, 4414, 4415	Iran
4416, 4417, 4418, 4419	Cyprus
4420, 4421, 4422, 4423, 4424, 4425, 4426, 4427, 4428, 4430, 4434, 4435, 4436, 4437, 4438, 4439, 4440, 4441, 4442, 4443, 4444, 4446, 4447, 4448, 4449, 4450, 4451, 4452, 4453, 4454, 4456, 4457, 4458, 4459, 4460	Japan
Mahali, Giza123, Giza121, Sahrawi, Jesto	Saudi Arabia

Table S2. A summary of cluster analysis (UPGMA) based on percent change in morphological attributes of diverse barley accessions under heat stress.

Cluster	Subcluster	Subunit	Subgroup	No. of access.	Accessions
I	A	-	-	5	4044, 4413, 4423, 4405, 4451
	B	B1	-	9	4046, 4415, 4151, 4407, 4157, 4426, 4149, 4163, 4249
		B2	-	4	4409, 4420, 4457, 4434
	C	C1	C1a	13	4050, 4419, 4436, 4166, 4414, 4150, 4418, 4421, 4428, 4115, 4159, 4230, 4408
			C1b	4	4054, 4424, 4165, 4437
			C1c	6	4167, 4231, 4456, 4176, 4255, 4430
		C2	-	3	4254, 4427, 4453
		C3	C3a	9	4042, 4162, 4438, 4439, 4247, 4053, 4417, 4251, 4448
			C3b	6	4168, 4412, 4425, 4441, 4454, 4411
	D	-	-	1	4447
II	A	-	-	6	4161, 4442, 4459, 4435, 4444, 4449
	B	-	-	3	4221, 4446, Giza123
III	A	A1	-	6	4056, 4227, 4422, 4460, 4245, 4290
		A2	-	2	4229, Giza121
		A3	-	11	4079, 4226, 4154, 4208, 4440, 4443, 4232, 4257, 4164, 4225, 4410
	B	-	-	6	4248, 4228, 4253, 4416, Mahali, 4452
IV	A	-	-	8	4045, 4450, 4224, Sahrawi, 4246, 4280, 4158, 4406
	B	-	-	3	4223, Jesto, 4458
Total number of accessions				105	

Accessions 4044 to 4079, 4115 to 4176, and 4208 to 4290 are of Pakistan origin.
Accessions 4405 to 4415 are of Iran origin.
Accessions 4416 to 4419 are of Cyprus origin.
Accessions 4420 to 4460 are of Japan origin.
Accessions Mahali, Giza 123, Giza 121, Sahrawi, and Jesto are of Saudi Arabia origin.

Table S3. A summary of cluster analysis (UPGMA) based on percent change in concentration of photosynthetic pigments of diverse barley accessions under heat stress.

Cluster	Subcluster	Subunit	Subgroup	No. of access.	Accessions
I	A	A1	-	4	4044, 4165, 4161, 4230
		A2	A2a	7	4149, 4423, 4163, 4434, 4224, 4416, 4422
			A2b	8	4157, 4407, Giza 121, 4418, 4162, 4441, 4231, 4253
	B	-	-	7	4079, 4417, 4154, 4166, 4226, 4408, Sahrawi
II	A	A1	A1a	8	4045, 4245, 4168, 4442, 4227, 4457, 4290, 4447
			A1b	5	4424, 4425, 4426, 4435, 4450
		A2	-	4	4221, 4454, 4409, 4449
	B	-	-	4	4257, 4451, 4452, 4421
	C	-	-	1	4115
III	A	A1	A1a	13	4046, 4412, 4151, 4437, 4225, 4458, 4427, 4430, 4223, 4232, 4254, 4050, 4415
			A1b	5	4056, 4439, 4161, 4208, Jesto
		A2	A2a	3	4228, 4428, 4413
			A2b	5	4229, 4419, 4459, 4446, 4453
			A2c	2	4411, 4444
	B	B1	-	5	4042, 4158, 4448, 4438, 4176
		B2	-	3	4054, 4247, 4248
		B3	-	8	4255, 4280, 4443, 4456, 4410, 4440, Mahali, 4405
	C	C1	-	5	4053, Giza123, 4406, 4159, 4249
		C2	C2a	5	4150, 4436, 4164, 4246, 4251
			C2b	3	4414, 4460, 4420
Total number of accessions				105	

Accessions 4044 to 4079, 4115 to 4176, and 4208 to 4290 are of Pakistan origin.
Accessions 4405 to 4415 are of Iran origin.
Accessions 4416 to 4419 are of Cyprus origin.
Accessions 4420 to 4460 are of Japan origin.
Accessions Mahali, Giza 123, Giza 121, Sahrawi, and Jesto are of Saudi Arabia origin.

Table S4. A summary of cluster analysis (UPGMA) based on percent change in water relation parameters of diverse barley accessions under heat stress.

Cluster	Subcluster	Subunit	Subgroup	No. of access.	Accessions
I	A	A1	-	6	4044, 4253, 4435, 4255, Giza123, 4428
		A2	-	2	4441, Sahrawi
		A3	-	2	4228, 4420
	B	-	-	1	4221
	C	C1	C1a	10	4046, 4421, 4411, 4229, 4422, 4419, 4227, 4453, 4444, 4450
			C1b	6	4208, 4458, 4224, Giza121, 4248, 4437
		C2	C2a	10	4050, 4442, 4254, 4167, 4415, 4438, 4176, 4225, 4251, 4423
			C2b	5	4226, 4406, 4452, 4245, 4425
			C2c	9	4042, 4443, 4168, 4247, 4416, 4410, 4257, 4414, 4446
		C3	C3a	6	4079, 4451, 4434, 4447, 4166, 4454
			C3b	4	4164, Jesto, 4280, 4436
II	A	A1	A1a	12	4054, 4231, 4149, 4456, 4413, 4448, 4115, 4163, 4056, 4424, 4407, 4457
			A1b	3	4165, 4223, 4230
		A2	-	7	4150, 4154, 4459, 4460, 4159, 4246, 4151
		A3	-	3	4232, 4249, 4449
	B	-	-	4	4157, 4167, 4162, 4158
III	A	-	-	8	4045, 4053, 4405, 4407, Mhali, 4408, 4426, 4440
	B	-	-	7	4290, 4417, 4418, 4412, 4430, 4439, 4409
Total number of accessions				105	

Accessions 4044 to 4079, 4115 to 4176, and 4208 to 4290 are of Pakistan origin.
Accessions 4405 to 4415 are of Iran origin.
Accessions 4416 to 4419 are of Cyprus origin.
Accessions 4420 to 4460 are of Japan origin.
Accessions Mahali, Giza 123, Giza 121, Sahrawi, and Jesto are of Saudi Arabia origin.

Table S5. A summary of cluster analysis (UPGMA) based on percent change in gas exchange attributes of diverse barley accessions under heat stress.

Cluster	Subcluster	Subunit	Subgroup	No. of access.	Accessions
I	A	A1	A1a	4	4044, 4408, 4422, 4435
			A1b	3	4053, 4257, 4437
			A1c	12	4115, 4164, 4224, 4165, 4457, 4254, 4448, Jesto, 4253, 4418, 4280, Sahrawi
			A1d	4	4227, 4425, 4456, 4247
		A2	-	9	4045, 4150, 4154, 4176, 4042, 4079, 4054, 4249, 4420
	B	B1	-	5	4157, 4221, 4168, 4162, 4405
		B2	-	7	4226, 4441, 4231, 4430, 4439, 4444, 4248
		B3	-	2	4413, Mahali
	C	C1	-	3	4228, 4406, 4229
		C2	-	4	4245, 4246, 4290, 4251
II	A	A1	-	7	4149, 4255, 4454, 4225, 4453, 4458, 4208
		A2	-	2	4409, 4419
	B	B1	-	8	4151, 4436, 4223, 4449, 4232, 4414, 4416, 4446
		B2	-	7	4163, 4417, 4411, 4452, 4421, 4443, 4423
		B3	-	7	4407, 4451, 4450, 4434, 4440, 4438, 4447
	C	C1	-	7	4158, 4424, 4426, 4428, 4427, Giza121, 4410
		C2	-	2	4161, Giza123
		C3	-	4	4166, 4442, 4415, 4412
	D	-	-	3	4159, 4167, 4460
III	A	-	-	2	4046, 4230
	B	-	-	3	4050, 4056, 4459
Total number of accessions				105	

Accessions 4044 to 4079, 4115 to 4176, and 4208 to 4290 are of Pakistan origin.
Accessions 4405 to 4415 are of Iran origin.
Accessions 4416 to 4419 are of Cyprus origin.
Accessions 4420 to 4460 are of Japan origin.
Accessions Mahali, Giza 123, Giza 121, Sahrawi, and Jesto are of Saudi Arabia origin.

Table S6. A summary of cluster analysis (UPGMA) based on percent change in chlorophyll fluorescence of diverse barley accessions under heat stress.

Cluster	Subcluster	Subunit	Subgroup	No. of access.	Accessions
I	A	A1	-	6	4044, 4434, 4249, 4460, 4443, 4414
		A2	-	6	4227, 4253, 4417, 4413, 4454, 4459
		A3	-	1	4228
	B	B1	B1a	9	4046, 4425, 4415, 4050, 4229, 4056, 4290, 4451, Giza121
			B1b	7	4054, 4115, 4245, 4158, 4419, 4226, 4444
			B1c	7	4165, Sahwari, 4428, 4411, 4456, 4436, 4251
		B2	-	10	4159, 4424, 4430, 4255, 4423, 4449, 4418, 4450, 4457, Giza123
II	A	A1	A1a	5	4042, 4257, 4246, JESTO, 4422
			A1b	3	4079, 4150, 4224
		A2	-	3	4161, 4167, 4168
		A3	-	2	4157, 4225
	B	B1	-	11	4149, 4162, 4421, 4151, 4410, 4163, 4439, 4231, 4437, 4221, 4416
		B2	-	3	4154, 4230, 4247
III	A	A1	-	5	4166, 4254, 4458, 4208, 4406
		A2	-	4	4280, 4427, 4405, 4435
		A3	-	4	4223, 4232, 4248, 4408
	B	-	-	1	4438
IV	A	A1	-	5	4045, 4453, 4053, 4412, 4446
		A2	-	4	4176, 4407, 4426, 4409
	B	B1	-	1	4164
		B2	-	6	4440, 4442, 4452, 4447, Mahali, 4441
V	-	-	-	2	4420, 4448
Total number of accessions				105	

Accessions 4044 to 4079, 4115 to 4176, and 4208 to 4290 are of Pakistan origin.
Accessions 4405 to 4415 are of Iran origin.
Accessions 4416 to 4419 are of Cyprus origin.
Accessions 4420 to 4460 are of Japan origin.
Accessions Mahali, Giza 123, Giza 121, Sahrawi, and Jesto are of Saudi Arabia origin.