

# Unlocking the potential of ancient hexaploid Indian dwarf wheat, *Triticum sphaerococcum* for grain quality improvement

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Wild and ancient wheat are considered to be rich source of nutrients and better stress tolerant, and hence being re-emphasized for mainstreaming its cultivation by the farmers and bringing back to the food basket. In the present study, thirty-four diverse accessions of Indian dwarf wheat, *Triticum sphaerococcum* conserved in the Indian National Genebank were evaluated for thirteen grain quality parameters namely thousand grain weight (TGW), hectolitre weight (HW), sedimentation value (Sed), grain hardness index (HI), protein (Pro), albumin (Alb), globulin (Glo), gliadin (Gli), glutenin (Glu), gluten, lysine (Lys), Fe<sup>2+</sup> and Zn<sup>2+</sup> content, and four antioxidant enzymes activities. Substantial variations were recorded for studied traits. Thousand grain weight, HW, Sed, HI, Pro, Alb, Glo, Gli, Glu, Gluten, Lys, Fe<sup>2+</sup>, and Zn<sup>2+</sup> varied from 26.50-45.55g, 70.50-86.00kg/hl, 24.00-38.00ml, 40.49-104.90, 15.34-19.35%, 17.60-40.31mg/g, 10.75-16.56mg/g, 26.35-44.94mg/g, 24.47-39.56mg/g, 55.33-75.06mg/g, 0.04-0.29%, 42.72-90.72ppm, and 11.45-25.70ppm, respectively. Among antioxidants, peroxidase (POX), catalase (CAT), glutathione reductase (GR), and superoxide dismutase (SOD) activity ranged from 0.06-0.60unit/ml, 0.02-0.61unit/ml, 0.11-2.26unit/ml, and 0.14-0.97unit/ml, respectively. Hardness Index was positively associated with Pro and Zn<sup>2+</sup> content whereas Lys was negatively associated with gluten content. Likewise, gluten and Fe<sup>2+</sup> content had positive association with major protein fraction *i.e.* Gli and Glu. Hierarchical cluster analysis grouped 34 accessions into 4 clusters and the major group had 9 indigenous and 8 exotic accessions. We also validated high GPC accessions and EC182958 (17.16%), EC187176 and EC182945 (16.16%), EC613057 (15.79%), IC634028 (15.72%) and IC533826 (15.01%) were confirmed with more than 15 % GPC. Also, superior trait specific accessions namely, EC187167, IC534021, EC613055, EC180066, EC182959 for low gluten content and IC384530, EC313761, EC180063,

IC397363, EC10494 for high iron content ( $>76.51$ ) were identified that may be used in wheat quality improvement for nutritional security of mankind.

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## Abstract

Wild and ancient wheat are considered to be rich source of nutrients and better stress <sup>tolerance</sup> tolerant, and hence being <sup>re-considered</sup> re-emphasized for mainstreaming its cultivation by the farmers and <sup>bringing it</sup> bringing it back to the food basket. In the present study, thirty-four diverse accessions of Indian dwarf wheat, *Triticum sphaerococcum* conserved in the Indian National Genebank were evaluated for thirteen grain quality parameters namely thousand grain weight (TGW), hectolitre weight (HW), sedimentation value (Sed), grain hardness index (HI), protein (Pro), albumin (Alb), globulin (Glo), gliadin (Gli), glutenin (Glu), gluten, lysine (Lys), Fe<sup>2+</sup> and Zn<sup>2+</sup> content, and four antioxidant enzymes activities. Substantial variations were recorded for studied traits. Thousand grain weight, HW, Sed, HI, Pro, Alb, Glo, Gli, Glu, Gluten, Lys, Fe<sup>2+</sup>, and Zn<sup>2+</sup> varied from 26.50-45.55g, 70.50-86.00kg/hl, 24.00-38.00ml, 40.49-104.90, 15.34-19.35%, 17.60-40.31mg/g, 10.75-16.56mg/g, 26.35-44.94mg/g, 24.47-39.56mg/g, 55.33-75.06mg/g, 0.04-0.29%, 42.72-90.72ppm, and 11.45-25.70ppm, respectively. Among antioxidants, peroxidase (POX), catalase (CAT), glutathione reductase (GR), and superoxide dismutase (SOD) activity ranged from 0.06-0.60unit/ml, 0.02-0.61unit/ml, 0.11-2.26unit/ml, and 0.14-0.97unit/ml, respectively. Hardness Index was positively associated with Pro and Zn<sup>2+</sup> content whereas Lys was negatively associated with gluten content. Likewise, gluten and Fe<sup>2+</sup> content had positive association with <sup>with the</sup> major protein fraction *i.e.* Gli and Glu. Hierarchical cluster analysis grouped 34 accessions into 4 clusters and the major group had 9 indigenous and 8 exotic accessions. We also validated high GPC accessions and EC182958 (17.16%), EC187176 and EC182945 (16.16%), EC613057 (15.79%), IC634028 (15.72%) and IC533826 (15.01%) were confirmed with more than 15 % GPC. Also, superior trait specific accessions namely, EC187167, IC534021, EC613055, EC180066, EC182959 for low gluten content and IC384530, EC313761, EC180063, IC397363,

EC10494 for high iron content ( $>76.51$ ) were identified that may be used in wheat quality improvement for nutritional security of mankind.

## Introduction

Worldwide wheat is the most important cereal crop, contributing 55% of total carbohydrate, 21% of the total proteins, and 19-20% calories demand of around half of the world population (Shiferaw et al. 2013). Along with carbohydrates, proteins, dietary fibre and micronutrients, wheat also contains minerals, vitamins and fats (Sarwar et al. 2013). Grain quality consists of various characters that together are responsible for determining end use of grains (Rondanini et al. 2013). Chemical composition of grain *i.e.* starch content is considered important for ethanol production whereas protein and carbohydrate content for food and feed production. The major component of wheat endosperm is starch and it also consists of glucose polymers, amylase, amylopectin and small amount of protein and lipids (Fasahat et al. 2015). The wheat grains milled into flour is used as raw material for different foods such as chapatti, bread, cakes, biscuits, muffins, pasta, noodles, sweets and pastries (Uthayakumaran and Wrigley 2017). The utilization of wheat grain in different forms mainly depends on protein content and gluten strength.

In bread wheat (*Triticum aestivum*), protein content varies from ~9-13 % (Žilić et al. 2011; Mohan and Gupta 2013; Punia et al. 2019). Wheat protein comprised of four fractions (Osborne 1907) which includes albumins (water soluble), globulins (salt soluble), gliadins (alcohol soluble), and glutenins (diluted acid or base soluble). The 25% fraction of total wheat protein is contributed by albumin and globulin components (Belderok et al. 2000). Rest two protein fractions, gliadins and glutenins are collectively called gluten and shares 75% of total protein and provide visco-elastic properties to wheat dough. Therefore, the proportions of these four components are responsible for dough making and baking quality of flour (Vigni et al. 2013). Sedimentation value provides an idea about content and quality of gluten as well as loaf volume (Pasha et al. 2007) and thus is helpful in classification of wheat in different categories for their utilization. Grain hardness is influenced by protein content and moisture content (Turnbull and Rahman 2002) and cause notable effects on milling as well as baking quality (Bettge et al. 1995). Many critical factors influence the quality hence it is important to assess parameters for It is a critical factor for deciding quality of wheat end-product hence important parameter for global trade. On the basis of grain hardness, wheat is grouped into two categories *i.e.* soft wheat and hard wheat (Pomeranz and Williams 1990).

Free radicals and reactive oxygen species (ROS) cause harmful effects <sup>on the</sup> cell membrane, enzymes, DNA and maintenance of DNA. Oxidative damage to DNA and enzymes <sup>can result</sup> in some serious health issues such as cardiovascular diseases, carcinogenesis, type II diabetes, and obesity. Free radicals have been linked to over 60 kinds of diseases. This includes cancer, Alzheimer's disease, Parkinson's disease and cataracts. Enzymatic antioxidants such as glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) (Narwal et al. 2014) directly reacts with ROS (Lobo et al. 2010) and consequently prevents oxidative damage <sup>resulting</sup> in reduced risk of oxidative-stress related chronic diseases. Enzymatic antioxidants help in breakdown of free radicals by seeking out oxidative products and <sup>converting</sup> convert them into hydrogen peroxide, and then into the water. In addition, they also act as metal chelators, and terminate the oxidative enzyme inhibitors hence ROS reactions (Karadag et al. 2009). Hence improved concentration of antioxidant helps in disease prevention & health promotion.

Cereals and their products contribute approximately 50% of  $\text{Fe}^{2+}$  and 20% of  $\text{Zn}^{2+}$  in Western diet (Velu et al. 2017). <sup>In the case</sup> In case of wheat,  $\text{Fe}^{2+}$  and  $\text{Zn}^{2+}$  content was reported to be low <sup>that too was situated</sup> and that too situated in outer layers of grains. Presently around three billion people are malnourished due to non-availability of micronutrients as well as vitamins. Less diverse diet leads to deficiency of minerals, among which  $\text{Fe}^{2+}$  and  $\text{Zn}^{2+}$  deficiency causes more pronounced <sup>effects</sup> effect due to involvement in various metabolic processes. Micronutrient enrichment of major food crops through breeding is one of the sustainable <sup>ways to reduce</sup> ways out to reduce malnutrition among <sup>groups</sup> higher risk group (resource-poor women, infants and children) (Šramková et al. 2009). It has been proven by various researchers <sup>that there are wheat</sup> that in wheat genome traits related to micro-nutrient <sup>which are available that</sup> enrichment are available that could facilitate significant improvement in vitamins and mineral as well as growth promoting components without any adverse effect on yield (Rose et al. 2002; Šramková et al. 2009). <sup>During the course of the</sup> During course of domestication and selection process, desirable allelic <sup>combinations were selected which led to the evolution</sup> combination was selected leading to evolution of modern crops that feed the world today but at the cost of reduced variation resulting into narrowing down of genetic base of crop species (van Heerwarden et al. 2011) <sup>and is referred</sup> and referred as domestication bottleneck (Hammer 1984). For development of high yielding wheat varieties with improved quality for targeting specific product, a number of breeding programme have been initiated. As a consequence of this, wheat

cultivation is based on genetically similar cultivars which resulted in compromised genetic base due to dependency on limited number of parents (Shuaib et al. 2010).

Wheat related species are considered potential sources for grain yield and quality traits for which these are not fully explored. Therefore, there is urgent need of characterization of these species with the aim of identification of promising lines for improved quality traits. Among ancient wheat, *Triticum sphaerococcum* (AABBDD,  $2n = 6x = 42$ ) is a landrace of wheat, and is known as the Indian dwarf wheat. It has several advantageous features such as, short and strong culms, hemispherical grains, higher protein content compared to bread wheat, and resistance to biotic and abiotic stresses, however *sphaerococcum* wheat is poorly studied (Josekutty 2008, Matsuoka 2011). Being a hexaploid species, it may be easily exploited for bread wheat improvement to achieve food and nutritional security to fulfill United Nations' sustainable development goal. Therefore, the main objectives of this study were (1) to evaluate 34 accessions of *T. sphaerococcum* for important quality traits and identification of superior line for individual traits and (2) diversity assessment of *T. sphaerococcum* accessions on the basis of quality traits.

## Materials & Methods

The experimental material for present investigation comprised of diverse set of thirty-four accessions of *T. sphaerococcum* conserved in the national genebank namely IC384530, IC397363, IC533826, IC53387, IC534021, IC534522, IC212160, IC534882, IC420038, EC313761, EC541164, EC576654, EC613055, EC613057, EC187172, EC187182, EC187181, EC180062, EC187183, EC187167, EC182959, EC182945, EC10494, EC10492, EC182958, EC180041, EC180061, EC180067, EC182947, EC180066, EC182956, EC180063, EC187176, IC634028. The grain harvest was obtained from the field trial grown during *Rabi* 2019-20 at ICAR-National Bureau of Plant Genetic Resources (NBPGR), Issapur farm (76°50' E longitude and 28°40' N latitude, 223 m MSL). The soil of the farm was sandy loam in texture having organic carbon 0.7%, available N 297.6 kg/ha, available P 26.7 kg/ha and available K 196.0 kg/ha with soil pH of 6.5. Recommended agronomic practices, irrigation and fertilizers (120 kg N; 60 kg  $P_2O_5$  and 40 kg  $K_2O$  ha<sup>-1</sup>) were followed throughout the experiments. Complete doses of  $K_2O$  and  $P_2O_5$  were applied at the time of sowing. Nitrogen was supplied in three split doses, 60 kg N ha<sup>-1</sup> at sowing, 30 kg N ha<sup>-1</sup> at crown root initiation and 30 kg N ha<sup>-1</sup> at booting stages. The experiment was adequately irrigated with five irrigations during the crop growth. Weeding was done manually. Spray of fungicide, tilt was done to avoid the infection of rust diseases.

These accessions were evaluated biochemically following completely randomized design with three replications for seventeen quality parameters namely thousand grain weight (TGW), hectolitre weight (HW), sedimentation value (Sed), grain hardness index (HI), protein content

(Pro), albumin content (Alb), globulin content (Glo), gliadin content (Gli), glutenin content (Glu), gluten content, lysine content (Lys),  $\text{Fe}^{2+}$  and  $\text{Zn}^{2+}$  content, peroxidase activity (POX), catalase activity (CAT), glutathione reductase activity (GR) and superoxide dismutase activity (SOD). The grain material was sun dried. Grain moisture content and TGW was recorded before the biochemical estimation. For HW and HI whole grain was analysed whereas for rest of the traits flours were utilized. The flour of seeds of each accession was prepared by grinding these seeds in cyclotec laboratory flour mill and was tested in three replicates. HW was estimated by using hectolitre apparatus. Sodium dodecyl sulphate (SDS Solution) sedimentation volume of each sample was estimated by the method suggested by Axford et al. (1978). HI and moisture content was analyzed using the single-kernel characterization system (SKCS) model 4100 (Perten Instruments North America, Inc., Reno, NV). Lysine was calculated by dye binding method as suggested by Udy (1956). Total protein content was estimated on wet basis following Micro-Kjeldahl Method (Kjeldahl 1883). Following the sequential Osborne extraction procedure (Osborne 1907) the proportion of Alb, Glo, Gli, and Glu was extracted as described by Mughal et al. (2020). As gluten is composed of Gli and Glu therefore it was derived by summing up of estimation of these two contributing components.  $\text{Fe}^{2+}$  and  $\text{Zn}^{2+}$  content was estimated by wet digestion (15ml acid mixture (9 concentrated nitric acid: 2 sulfuric acid: 1 perchloric acid + 0.5g wheat flour) followed by atomic absorption spectroscopy (Jackson 1973). Quantification of extracted proteins (Pro, Alb, Glo, Gli, and Glu) was conducted using a spectrophotometer, and BSA (Bovine Serum Albumin) was used as a standard. The enzymatic antioxidants were estimated by using methodology as suggested by Todorova et al. (2021). Eight promising accessions for grain protein content were validated by growing consecutively at NBPGR Pusa Farm and NBPGR, Issapur Farm, New Delhi during year 2019-20, 2020-21 and 2021-22.

## Statistical analysis

All data were subjected to analysis of variance (ANOVA) using completely randomized design (CRD) and comparison of treatment means was performed using Duncan's Multiple Range Test (DMRT) at  $P < 0.05$  using IBM SPSS Statistics software. The mean values of individual genotypes for each trait were subsequently used for analysing summary statistics and graphs using MS-Excel software. Histogram and correlation among traits were analysed by using IBM SPSS Statistics software. Hierarchical cluster analysis was performed using Euclidean distance measure following Unweighted Pair Group Method with Arithmetic Averages (UPGMA) method by using PAleontological STatistics (PAST) software (Hammer et al. 2001). Flow of work done is depicted in Supplementary Fig.1.

## Results

### Variation for quality characteristics among *T. sphaerococcum* accessions

Analysis of variance revealed significant variance for all the characters at 1% level of significance (Table 1). Further to find out the significant differences among genotypes, Post Hoc test, Duncan's Multiple Range Test (DMRT) was performed which resulted in grouping of accessions in larger number of subgroups 8 (TGW), 17 (HW), 13 (Sed), 18 (HI), 13 (Pro), 14 (Alb), 14 (Glo), 20 (Gli), 18 (Glu), 19 (Gluten), 19 (Lys), 18 (Fe<sup>2+</sup>), 13 (Zn<sup>2+</sup>), 19 (PER), 16 (CAT), 25 (GR), 13 (SOD), which indicates existence of sufficient variation. Likewise, existence of wider variation was also supported by other parameters (Table 2). It was also depicted pictorially by histogram (Supplementary Fig. 2) and bar diagram (Fig. 1-4). All the traits followed normal distribution except Alb, CAT and SOD which were positively skewed.

### **Hectolitre weight (HW), SDS sedimentation (Sed) test and Grain hardness (HI):**

Hectolitre weight (HW) ranged from 70.50kg/hl (EC187182) to 86.00kg/hl (EC180066) with the mean of 81.25kg/hl among studied *T. sphaerococcum* accessions (Supplementary Fig. 2a). High HW along with high TKW (26.05-45.55g) (Supplementary Fig. 2b) indicated high milling yield potential of Indian dwarf wheat.

Gluten quality which is estimated in terms of Sed varied from 24.00ml (EC182945) to 38.00ml (EC180063) with an average of 30.38ml (Supplementary Fig. 2c and Fig. 1). Based on Sed, wheat can be classified into three categories [ $<48$ ml (weak), 48-68ml (medium strong),  $>68$ ml (strong)] but all 34 accessions of *T. sphaerococcum* showed Sed less than 48.00ml therefore grouped in weak gluten class due to its low hydration capacity. Further, they have been classified in three categories based on their suitability for making different products (Table 3). For making good quality bread, chapatti and biscuit, the required Sed are  $>60$ ml, 30-60ml and  $<30$ ml, respectively. Further, non-significant but positive estimates of correlation coefficient (+0.18) was found between Sed and Protein (Table 4).

Grain hardness ranged from 40.49 (EC182958) to 104.92 (EC182959) with an average of 82.03 (Supplementary Fig. 2d and Fig. 1). On the basis of HI, accessions were classified in four categories *i.e.*  $<33$  (Soft), 34-46 (Medium soft), 47-59 (Medium hard), and  $>60$  (Hard) according to Morris et al. (2001). Majority of accessions (82.35%) were categorized as hard wheat and five accessions EC187176, EC187182, EC182945, EC10492, and EC10494 were grouped in medium hard category. Only one accession EC182958 was reported medium soft with HI of 40.49. In our experiment, we observed significant positive correlation ( $r = +0.37^*$ ) between Pro and HI, however, HI and TGW were negatively associated ( $-0.35^*$ ) (Table 4).



## **Total Protein content (Pro), protein fraction and lysine content (Lys) estimation**

Accessions significantly differed from each other for Protein content and ranged from 15.34-19.35% with an average of 17.62% (Supplementary Fig. 2e and Fig. 2). Maximum Protein content, 19.35% was reported in accession, EC613057 followed by 19.24 % in EC10494.

Proportion of four protein fractions among 34 accessions of *T. sphaerococcum* are shown in Fig. 2. The concentration of Alb varied from 17.60mg/g to 40.31mg/g with the mean of 23.91mg/g (Supplementary Fig. 2f). Out of 34 accessions, only one IC534522 exhibited moderate concentration of Alb (40.32mg/g) whereas rest consisted of low concentration of Alb ranging from 17.60mg/g to 30.00mg/g (Table 5). As far as Glo is concerned, 18 accessions showed moderate concentration and it ranged from 14.00mg/g-16.56mg/g with the highest concentration of Glo (16.56mg/g) in accession EC182945 (Supplementary Fig.2g). For Gli content, all the accessions belonged to low category with the range of 26.35mg/g-44.94mg/g (Supplementary Fig.2h). In contrast, all accessions were grouped in high Glu content class with the mean of 31.00mg/g and ranged from 24.47mg/g-39.56mg/g (Supplementary Fig.2i). Gli and Glu are collectively called gluten which shares 85% of total protein (Fisher and Halton 1936) and are responsible for visco-elastic property to wheat dough. Therefore, the proportion of these four components are responsible for dough making and baking quality of flour (Vigni et al. 2013). By summing up of these two fractions of protein, we got rough estimate of gluten content. With an average of 64.97mg/g proportion of gluten among accessions, it varied from 55.33-75.06mg/g (Supplementary Fig.2j). Significant positive correlation of gluten with Gli ( $r=+0.73^{**}$ ) and Glu ( $r=+0.60^{**}$ ) depicted collective contribution of these components in gluten estimation. Regarding lysine content, *T. sphaerococcum* wheat had an average of 0.15% of sample Lys with range of 0.04% (EC187172) to 0.29% (EC10492) (Supplementary Fig.2k). We also observed non-significant and negative correlation ( $r=-0.03$ ) between lysine and protein whereas significant negative ( $-0.35^{*}$ ) association was found with gluten (Table 4).

## **Fe<sup>2+</sup> and Zn<sup>2+</sup> content**

Among micronutrients, *T. sphaerococcum* accessions had the mean of 65.11 ppm Fe<sup>2+</sup> content with the range of 42.72ppm (EC180066) to 90.72ppm (IC384530) (Supplementary Fig.2l and Fig. 3), whereas slightly narrow range were observed for Zn<sup>2+</sup> content *i.e.*, from 11.45ppm to 25.70ppm in accession EC182956 and EC10492 respectively (Supplementary Fig.2m). Fe<sup>2+</sup> content showed highly significant positive correlation with major protein fraction *i.e.* Gli

( $r=+0.48^{**}$ ) and Glu ( $r=+0.52^{**}$ ) (Table 4). Likewise,  $Zn^{2+}$  content was positively ( $+0.55^{**}$ ) associated with HI.  $Zn^{2+}$  concentration showed a non-significant weak correlation with TGW (0.22).

### Enzymatic antioxidant activity

Among four studied antioxidants, POX activity ranged from 0.06-0.60unit/ml with average of 0.22unit/ml (Supplementary Fig.2n and Fig. 4). The accession “EC541164” possessed the highest POX activity. POX is considered important from quality point of view due to its involvement in reduction of dough adhesiveness. The CAT, GR and SOD activity varied from 0.02unit/ml-0.60unit/ml, 0.11unit/ml-2.26unit/ml, and 0.14unit/ml-0.97unit/ml respectively (Supplementary Fig.2o-2q). Correlation analysis results indicated that only GR and POX showed positive correlation with HI. Whereas negative relation was observed between POX and Pro, GR and HW, CAT with Sed and Alb, and SOD with  $Zn^{2+}$  content.

### Cluster analysis

Thirty-four *T. sphaerococcum* accessions were subjected to cluster analysis by employing unweighted pair group method with arithmetic average (UPGMA) and Euclidean distance measure. Euclidean distance matrix depicts extant of genetic dissimilarity among accessions. The maximum distance of 86.22 was observed between EC613055 which belonged to cluster B and IC384530 which is clustered independently in cluster D from rest of the accessions. After that EC187172 and IC384530 with 75.97 euclidean distance were grouped in cluster B and D respectively. EC187183 and IC420038 were most similar due to lowest euclidean distance of 6.57 and both of them belonged to cluster C. Following this, pair of EC613055/ EC576654 clustered in group B with a distance of 7.02. Clustering pattern of Indian dwarf wheat accessions based on quality data and is presented in Fig.5 and Table 6. Based on quality parameters, at a Euclidean distance of 30, 34 accessions of Indian dwarf wheat were grouped into four main clusters A, B, C and D. Further main cluster A, B and C is divided into two sub-clusters each whereas only one accession was grouped in main cluster D. The distribution pattern revealed maximum number of genotypes i.e., 17 in cluster C followed by cluster B having 10 genotypes. Cophenetic correlation coefficient was estimated as 0.86 for checking agreement between phenograms and dissimilarity matrices for accuracy of dendrogram. Mean performance of different clusters for the studied quality traits was shown in Table 6. Minimum mean Sed

(29.45ml) was observed among accessions of cluster B. In addition to this, cluster B was also characterized with low Gli (31.40mg/g), Glu (30.04mg/g), gluten content (61.44mg/g) and high HI (90.24). Cluster B also showed high mean value for POX (0.26unit/ml) and GR (0.78unit/ml) activity hence could be targeted for wheat nutritional quality improvement. For flour yield improvement accessions clustered in cluster C could be targeted due to maximum mean HW (81.82kg/hl). Accessions grouped in cluster C also showed high mean value Alb (24.96mg/g) and Lys (0.19%), whereas cluster D was characterized by high Glo (14.17mg/g). In addition to high Glo accession of cluster D also showed high Sed (32.00ml), Gli (41.78mg/g), gluten (72.68mg/g), Fe<sup>2+</sup> (90.71ppm), and SOD activity (0.63unit/ml). Cluster A demonstrated high Glu (33.26mg/g), Zn<sup>2+</sup> (22.56ppm) content, CAT (0.16unit/ml) and GR (0.78unit/ml) activity. To understand and confirm the dynamics of the *sphaerococcum* accessions, we have further carried out principal coordinate analysis (PCoA). Based on the pair wise Euclidean distance matrix among the 34 *sphaerococcum* accession, clear distinction could be visualized, and this was in concordance with the results of the biplot (Fig. 6).

### Validation of high grain protein accessions

Eight *sphaerococcum* accessions with high grain protein content (GPC) were validated under multi location and multi-year trial by growing consecutively at NBPGR Pusa Farm and NBPGR, Issapur Farm, New Delhi during year 2019-20, 2020-21 and 2021-22. Their comparative performance along with overall mean is presented in Fig. 7. Top performing accessions with more than 15 % GPC were confirmed as EC182958 (17.16%), EC187176 and EC182945 (16.16%), EC613057 (15.79%), IC634028 (15.72%) and IC533826 (15.01%) which can be used for introgression purpose and bread wheat quality improvement.

## Discussion

Wild relatives and ancient wheat serve as the most important genetic resources for numerous valuable traits and possess wider variation for quality traits (Zelbig et al. 2021). In comparison to bread wheat (*T. aestivum*), emmer wheat (*T. dicoccoides*) is reported to possess more lysine and isoleucine content (Nevo and Beiles 1992). Similarly, as appose to cultivated wheat, wild relatives; primitive cultivars are considered better resources for zinc and iron fortifications (Cakmak 2008; Velu et al. 2011; Xu et al. 2011). Extensive characterization of genetic resources is preliminary step for designing any quality breeding programme. The ancient wheat, *T.*

*sphaerococcum*, endemic to north-western India and southern Pakistan was withdrawn from cultivation during the early 20<sup>th</sup> century due to introduction of modern wheat cultivar as the result of green revolution (Mori et al. 2013). Indian dwarf wheat is least exploited hexaploid species for quality assessment. Here we explored *T. sphaerococcum* germplasm for their nutritional quality traits so that to enhance their potential utilization for bread wheat improvement.

In our study, nearly similar range of HW was observed in *T. sphaerococcum* as reported by Mohan and Gupta (2013) in bread wheat cultivars. Higher the HW means better milling quality in terms of flour yield (Martin et al. 2021) and thus accessions coming under the category of higher HW may result in better flour yield. As, HW is affected by grain shape, size and density, therefore the possible reason for higher value is their round grain shape. In fact, Sed recorded among these accessions was much lower than Indian bread wheat varieties (35-60ml, Mohan and Gupta, 2013) and (33-52ml, Panghal et al. 2018). Whereas, regarding hardness index, most of the studied accessions belonged to hard category. However, relatively wide range (29-94 ) for HI in Indian bread wheat varieties were reported by Mohan and Gupta, 2013. It has been observed that wheat with high protein tends to be hard whereas soft grain with low protein.

Wheat constitutes approximately 80% of the total cereal intake, and it contributes 50% of total energy and 60% of total protein intake. Based on our findings, *Sphaerococcum* wheat grain accumulated more protein in contrast to narrow range (9.40-13.50) of protein in Indian bread wheat varieties reported by Mohan and Gupta, 2013. We have also confirmed the high GPC accessions by growing in different environments. However, Ciaffi et al. (1992) found large variation in *T. dicoccoides*, ranging from 16.00-27.00%. Similarly, in related species of wheat, mean Pro was reported as 16.67% (Jiang et al. 2008) confirming the finding that wild and primitive wheat have a wider range of variation for grain protein than cultivated ones. The Alb and Glo contribute 15-20% share of wheat flour protein. These non-prolamin protein fractions are considered nutritionally better due to higher proportion of lysine and methionine as compared to other proteins in wheat (Lasztity 1986). In addition to this, these proteins are also considered important for germinating and protecting embryos from insects and pathogens (Dupont and Altenbach 2003). In our study, we have identified one accession for moderate Alb and eighteen for moderate Glo. These accessions were considered good from nutritional point of view and could be utilized in wheat quality improvement. The Gli and Glu ratio in present study ranged

from 0.80 to 1.50 which is lower than as observed in case of common wheat (1.60-3.80) (Geisslitz et al. 2019). It has been proven that low Gli:Glu ratio is linked with good baking quality (Thanhaeuser et al. 2015). Therefore, these accessions are also considered good for baking purposes. Cereal based diet and food items are poor with respect to essential amino acid such as lysine and methionine (Smriga et al. 2004). Inadequate intake of Lys results in a syndrome called 'protein energy malnutrition' which is suspected to be more prevalent in developing countries. Manipulation of cereal proteins and amino acid profile will definitely cause significant impact in the life of hundreds of millions of poor people. Despite of extensive research aimed at identification of Lys rich wheat accessions; the high Lys mutants have not been discovered yet. Accessions with higher Lys estimates (EC10492, EC187176, EC182945, IC534522, EC313761) found in our study could be utilized in wheat quality improvement.

The iron content in *T. sphaerococcum* accession were much higher than those obtained by Zhao et al. (2009); Mohan and Gupta (2013); Goel et al. (2018) and Khokhar et al. (2020). However, variation for Zn content is less as appose to previous findings (Goel et al. 2018; Khokhar et al. 2020) whereas in agreement with the findings of Zhao et al. (2009) . It has been observed that grains with high Pro accumulates more micronutrient, which may be due to co-localisation of Pro,  $Zn^{2+}$  and  $Fe^{2+}$  in embryo and aleurone layer of grain (Khokhar et al. 2020).  $Zn^{2+}$  concentration showed a non-significant weak correlation with TGW (0.22) which is in close agreement with the findings of Khokhar et al. (2020). A significant positive association (+0.43\*) between  $Fe^{2+}$  and  $Zn^{2+}$  contents indicated the possibility of simultaneous improvement of both the micronutrients (Goel et al. 2018). It was also stipulated that there is involvement of common mechanism for accumulation of both micronutrients (Hernandez et al. 2020). Likewise, significant positive association between  $Fe^{2+}$  and  $Zn^{2+}$  was observed by various researchers (Velu et al. 2017; Hernandez et al. 2020; Khokhar et al. 2020). Further, the probable reason of association between  $Fe^{2+}$  and  $Zn^{2+}$  content was found to be co-localization of quantitative trait loci present at chromosome 3B governing these traits (Velu et al. 2017). Due to lack of ample genetic variation in cross compatible species, the traditional breeding for micronutrient enhancement was non-exploitable. Henceforth hexaploid species *T. sphaerococcum* could be exploited in this direction.

For antioxidants, maximum CAT, GR, and SOD activity were recorded in case of accessions EC18294, EC180063 and EC182956 respectively. CAT was reported to be involved

in oxidative reaction during bread making and its highest activity were recorded during kernel development stage and was responsible for detoxification of  $H_2O_2$  (Kruger and Leberg 1974). Likewise, SOD protects the oxidation of cellular components through reactive oxygen species (Mughal et al. 2020). Hence accessions showing high activity for antioxidants could be targeted for wheat quality improvement in terms of nutrition. GR may control the ratio of GSSG (oxidised glutathione)/ GSH (reduced glutathione) in wheat flour and play a role in determining the physical properties of wheat flour doughs (Every et al. 2006).

Based on association analysis, there were non-significant positive estimates of correlation coefficient between Sed and Pro; however, it was significantly correlated as per findings of Drikvand et al. 2013. Further, significant positive correlation was observed between Pro and HI which is supported by the finding of Drikvand et al. 2013. HI and TGW were negatively associated at 0.05% level of significance, likewise non-significant but negative correlation estimates was observed by Abdipour et al. 2016. Also, it has been proven that the Lys is negatively correlated with Pro as reported by Rharrabti et al. 2001.

The clustering pattern based on quality parameters revealed 17 accessions in cluster C followed by cluster B having 10 genotypes. Further cophenetic correlation coefficient was estimated as 0.86 which is greater than acceptable level of 0.85 (Stuessy 1909). Minimum mean Sed was observed among accessions of cluster B. In addition to this, cluster B was also characterized with low Gli ,Glu, gluten content and high HI . Cluster B also showed high mean value for POX and GR activity, hence, could be targeted for wheat nutritional quality improvement. For flour yield improvement, accessions clustered in cluster C could be targeted due to maximum mean HW. Accessions grouped in cluster C also showed high mean value of Alb and Lys , whereas cluster D was characterized by high Glo. As Alb and Glo protein is reported nutritionally better due to higher proportion of lysine and methionine (Lasztity 1984) therefore these accessions could be utilized in common wheat improvement for essential amino acids. In addition to high Glo, accession of cluster D also showed high Sed, Gli , gluten ,  $Fe^{2+}$ , and SOD activity . Also, accession of cluster D could be targeted for enhancement of protein content in common wheat as they are characterized with high protein content. Cluster A demonstrated high Glu ,  $Zn^{2+}$  content, CAT and GR activity henceforth could be exploited in improvement of respective traits.

## Future implications

Based on comprehensive estimation of grain quality parameters, our study revealed that the primitive wheat landrace, Indian dwarf wheat *T. sphaerococcum* are rich source of grain protein content, lysine content, iron and zinc content and antioxidant activity. It also has low gluten content which is a major dietary requirement based on consumer preference. Therefore exploration, conservation and use may be promoted to gain maximum benefit from this species. Among the studied accessions, EC10492, EC313761 (from USSR) were found superior for maximum seven traits followed by EC180063 and EC182958 for five traits. We also validated high GPC accessions such as EC182958 (from Canada), EC187176, EC182945, EC613057 (from France), IC634028 (Kathod Gehun from Tapi district of Gujarat) and IC533826 with more than 15 % grain protein content.

## Conclusions

Wild relatives, ancient species and landraces are rich valuable genetic resources for abiotic and biotic stress tolerance, and nutritional qualities and play major role in wheat improvement. In the present study, we analysed 34 *T. sphaerococcum*, Indian dwarf wheat accessions conserved in the national genebank of India for seventeen grain quality parameters. We found sufficient variation among accessions for all studied quality traits and accessions with superior quality traits were identified. Among 34 accessions, EC10492, EC313761 were found superior for maximum seven traits followed by EC180063 and EC182958 for five traits. We also validated high GPC accessions and EC182958 (17.16%), EC187176 and EC182945 (16.16%), EC613057 (15.79%), IC634028 (15.72%) and IC533826 (15.01%) were confirmed with more than 15 % GPC. The superior accessions identified in the present study could be the potential resource for a breeder to improve the wheat nutritional quality. The *sphaerococcum* wheat should be promoted for cultivation on the farmers' field considering its richness in grain nutrient and end use quality with low gluten, high protein, high iron and zinc content and high antioxidant activity. Further, the germplasm explorer should prioritize for the collection of this species from the unexplored areas to harness its nutritional richness for alleviating nutritional imbalance and improving health of mankind. The results of present study showed the possibility of utilization of Indian dwarf wheat in wheat quality breeding aiming for nutritional security under United Nation's sustainable development goals (SDG).

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 581

# **Table 1**(on next page)

Analysis of variance (ANOVA) for different quality characters in *T. sphaerococcum* accessions

**Table 1.** Analysis of variance (ANOVA) for different quality characters in *T. sphaerococcum* accessions

Source	d · f.	Mean Sum of Squares																
		TGW	HW	Sed	HI	Pro	Alb	Glo	Gli	Glu	Glute n	Lys	Fe+2	Zn+2	PER	CAT	GR	SO D
Treatm ent	3 3	51.57 2**	33.69 3**	35.68 4**	774.83 1**	4.080 **	53.22 1**	8.061 **	47.59 6**	34.72 4**	71.92 4**	0.015 **	906.09 7**	36.21 3**	0.049 **	0.038 **	0.971 **	.077 **
Error	6 8	13.10 9	0.250	0.250	0.757	0.342	0.469	0.200	0.587	0.594	2.375	0.001	19.974	6.970	0.001	0.001	0.001	.001

TGW-Thousand grain weight, HW-Hectolitre weight, Sed-Sedimentation value, HI-Hardness index, Pro- Protein content, Alb-Albumin content, Glo-Globulin content, Gli-Gliadin content, Glu-Glutenin content, Lys-Lysine content, Fe<sup>+2</sup>-Iron Content, Zn<sup>+2</sup>- Zinc content, POX-Peroxidase, CAT-Catalase, GR-Glutathione reductase, SOD-Superoxide dismutase

## Table 2 (on next page)

Mean performance and variability for grain quality traits among 34 accessions of *T. sphaerococcum*



**Table 2.** Mean performance and variability for grain quality traits among 34 accessions of *T. sphaerococcum*

S.No.	Quality trait	Mean	Range	SE	CV (%)	SED	Top five accessions along with DUNCAN group
1	TGW	32.85	26.50-45.55	0.71	12.61	0.23	IC420038 <sup>A</sup> IC534021 <sup>B</sup> EC187167 <sup>B</sup> EC180067 <sup>B,C</sup> EC613057 <sup>B,C,D</sup> (>36.62)
2	HW (kg/hl)	81.25	70.50-86.00	0.57	4.12	0.25	EC180066 <sup>A</sup> EC541164 <sup>B</sup> IC534522 <sup>B</sup> IC212160 <sup>B,C</sup> EC613057 (>84)
3	Sed (ml)	30.38	24.00-38.00	0.59	11.35	0.24	EC180063 <sup>A</sup> EC182958 <sup>B</sup> EC10492 <sup>B,C</sup> IC534021 <sup>C</sup> EC187176 <sup>C</sup> (>33)
4	HI	82.14	40.49-104.91	2.76	19.57	0.75	EC182959 <sup>A</sup> IC533826 <sup>B</sup> IC634028 <sup>B,C</sup> EC182956 <sup>C,D</sup> EC313761 <sup>D,E</sup> (>94)
5	Pro (%)	17.62	15.34-19.35	0.20	6.62	0.34	EC613057 <sup>A</sup> EC182958 <sup>B,C</sup> EC187176 <sup>B,C,D</sup> IC634028 <sup>B,C,D</sup> EC182945 <sup>B,C,D</sup> (>19.70)
6	Alb (mg/g)	23.91	17.60-40.31	0.72	17.62	0.46	IC534522 <sup>A</sup> EC10492 <sup>B</sup> EC10494 <sup>B</sup> IC53387 <sup>C</sup> EC187182 <sup>C,D</sup> (>27.80)

7	Glo (mg/g)	13.35	10.75-16.56	0.28	12.28	0.20	EC182945 <sup>A</sup> EC180062 <sup>B,C</sup> EC180063 <sup>B,C</sup> EC187172 <sup>B,C</sup> IC534021 <sup>C,D</sup> (>15.62)
8	Gli (mg/g)	33.97	26.35-44.94	0.68	11.73	0.58	EC313761 <sup>A</sup> IC384530 <sup>B</sup> EC187182 <sup>C</sup> IC533826 <sup>C,D</sup> IC397363 <sup>C,D</sup> (>38.42)
9	Glu (mg/g)	31.00	24.47-39.56	0.58	10.97	0.59	EC182958 <sup>A</sup> EC10494 <sup>B</sup> EC180067 <sup>B,C</sup> EC10492 <sup>C,D</sup> IC634028 <sup>D,E</sup> (34.64)
10	Gluten (mg/g)	64.97	55.33-75.06	0.84	7.54	2.37	EC313761 <sup>A</sup> EC10492 <sup>A,B</sup> IC384530 <sup>A,B,C</sup> IC533826 <sup>B,C,D</sup> EC182958 <sup>B,C,D</sup> (>71.44)
11	Lys (% of sample)	00.15	0.04-0.29	0.01	47.90	0.001	EC10492 <sup>A</sup> EC187176 <sup>A</sup> EC182945 <sup>B</sup> IC534522 <sup>C</sup> EC313761 <sup>C,D</sup> (>0.24)
12	Fe <sup>2+</sup> Content (ppm)	65.11	42.72-90.72	1.70	15.25	6.97	IC384530 <sup>A</sup> EC313761 <sup>B</sup> EC180063 <sup>B,C</sup> IC397363 <sup>B,C</sup> EC10494 <sup>B,C</sup> (>76.51)
13	Zn <sup>2+</sup> content (ppm)	19.00	11.45-25.70	0.60	18.29	3.10	EC10492 <sup>A</sup> EC187182 <sup>A,B</sup> EC182958 <sup>A,B,C</sup> EC180041 <sup>A,B,C</sup> EC313761 <sup>A,B,C</sup> (>23.35)

14	POX (unit/ml)	0.22	0.06- 0.60	0.02	30.46	0.001	EC541164 <sup>A</sup> IC534882 <sup>B</sup> EC187167 <sup>C</sup> EC313761 <sup>C,D</sup> EC576654 <sup>D,E</sup> (>0.34)
15	CAT (unit/ml)	0.12	0.02- 0.61	0.02	42.32	0.002	EC182945 <sup>A</sup> EC613057 <sup>B</sup> EC180061 <sup>C</sup> IC53387 <sup>D</sup> EC180041 <sup>E</sup> (>0.20)
16	GR (unit/ml)	0.70	0.11- 2.26	0.10	18.56	0.001	EC180063 <sup>A</sup> EC180066 <sup>B</sup> EC182956 <sup>C</sup> EC10494 <sup>D</sup> EC187182 <sup>E</sup> (>1.45)
17	SOD (unit/ml)	0.31	0.14- 0.97	0.03	26.37	0.001	EC182956 <sup>A</sup> IC384530 <sup>B</sup> EC180062 <sup>C</sup> EC180067 <sup>C</sup> EC182947 <sup>C</sup> (>0.48)

TGW-Thousand grain weight, HW-Hectolitre weight, Sed-Sedimentation value, HI-Hardness index, Pro- Protein content, Alb-Albumin content, Glo-Globulin content, Gli-Gliadin content, Glu-Glutenin content, Lys-Lysine content, Fe<sup>+2</sup>-Iron Content, Zn<sup>+2</sup>- Zinc contents, POX-Peroxidase, CAT-Catalase, GR-Glutathione reductase, SOD-Superoxide dismutase, SE-Standard Error, CV-Coefficient of variation

# **Table 3**(on next page)

Classification of *T. sphaerococcum* accessions based on their sedimentation value and suitability for products

1 **Table 3.** Classification of *T. sphaerococcum* accessions based on their sedimentation value and  
 2 suitability for products

<b>Sedimentation value (cc)</b>	<b>Suitable for</b>	<b><i>T. sphaerococcum</i> accessions</b>
>60ml	Bread	-
30-60ml	Chapatti	IC384530, IC397363, IC533826, IC53387, IC534021, IC534522, IC212160, IC534882, IC420038, EC10494, EC313761, EC541164, EC576654, EC613055, EC613057, EC187172, EC187182, EC187181, EC180062, EC187183, EC187167, EC182959, EC182945, EC10492, EC182958, EC180041, EC180061, EC180067
<30ml	Biscuit	EC182947, EC180066, EC182956, EC180063, EC187176, IC634028

3

# **Table 4**(on next page)

Pearson correlation matrix among seventeen grain quality traits including TGW

**Table 4.** Pearson correlation matrix among seventeen grain quality traits including TGW

	HW	Sed	HI	Pro	Alb	Glo	Gli	Glu	Gluten	Lys	Fe <sup>+2</sup>	Zn <sup>+2</sup>	TGW	POX	CAT	GR	SOD
<b>HW</b>	1																
<b>Sed</b>	-0.18	1.00															
<b>HI</b>	0.19	-0.23	1.00														
<b>Pro</b>	0.06	0.18	0.37*	1.00													
<b>Alb</b>	-0.03	0.01	0.07	-0.27	1.00												
<b>Glo</b>	-0.16	0.00	-0.12	0.16	-0.16	1.00											
<b>Gli</b>	-0.06	-0.05	-0.12	0.04	0.17	0.25	1.00										
<b>Glu</b>	0.11	0.00	-0.30	0.15	-0.03	-0.08	-0.13	1.00									
<b>Gluten</b>	0.03	-0.04	-0.31	0.14	0.12	0.15	0.73**	0.60**	1.00								
<b>Lys</b>	0.12	0.02	-0.20	-0.03	0.15	0.03	0.25	0.21	-0.35*	1.00							
<b>Fe<sup>+2</sup></b>	-0.13	0.17	-0.23	0.27	0.08	0.13	0.48**	0.18	0.52**	0.28	1.00						
<b>Zn<sup>+2</sup></b>	-0.22	0.10	0.55**	0.05	0.25	0.07	0.19	0.22	0.31	0.05	0.43*	1.00					
<b>TGW</b>	0.00	0.19	-0.35*	0.06	0.17	0.31	-0.03	0.03	0.00	0.06	0.06	0.22	1.00				
<b>POX</b>	0.00	-0.30	0.40*	-0.38*	0.14	-0.11	-0.02	-0.21	-0.16	-0.25	-0.26	-0.12	-0.29	1.00			
<b>CAT</b>	0.18	-0.34*	-0.20	0.23	-0.33*	0.22	0.06	-0.07	0.00	0.08	0.01	0.04	-0.03	-0.28	1.00		
<b>GR</b>	-0.37*	0.01	0.05	0.25	0.11	0.25	-0.02	-0.13	-0.10	0.03	-0.09	-0.22	0.03	-0.12	-0.27	1.00	
<b>SOD</b>	-0.17	-0.12	0.23	0.17	-0.32	0.21	-0.04	0.06	0.01	-0.24	0.05	-0.33*	0.03	0.20	-0.13	0.23	1.00

TGW-Thousand grain weight, HW-Hectolitre weight, Sed-Sedimentation value, HI-Hardness index, Pro- Protein content, Alb-Albumin content, Glo-Globulin content, Gli-Gliadin content, Glu-Glutenin content, Lys-Lysine content, Fe<sup>+2</sup>-Iron Content, Zn<sup>+2</sup> - Zinc content, POX-Peroxidase, CAT-Catalase, GR-Glutathione reductase, SOD-Superoxide dismutase

# **Table 5**(on next page)

Categorization of *T. Sphaerococcum* accessions in low, medium, and high category for protein fractions



**Table 5.** Categorization of *T. Sphaerococcum* accessions in low, medium, and high category for protein fractions

Accessions	Alb(mg/g)	Glo(mg/g)	Gli(mg/g)	Glu(mg/g)
IC384530	L	M	L	H
IC397363	L	L	L	H
IC533826	L	L	L	H
IC53387	L	M	L	H
IC534021	L	M	L	H
IC534522	M	L	L	H
IC212160	L	M	L	H
IC534882	L	L	L	H
IC420038	L	L	L	H
EC313761	L	M	L	H
EC541164	L	L	L	H
EC576654	L	M	L	H
EC613055	L	L	L	H
EC613057	L	L	L	H
EC187172	L	M	L	H
EC187182	L	M	L	H
EC187181	L	M	L	H
EC180062	L	M	L	H
EC187183	L	L	L	H
EC187167	L	L	L	H
EC182959	L	L	L	H
EC182945	L	M	L	H
EC10494	L	M	L	H
EC10492	L	M	L	H
EC182958	L	L	L	H
EC180041	L	L	L	H
EC180061	L	M	L	H
EC180067	L	L	L	H
EC182947	L	M	L	H
EC180066	L	L	L	H
EC182956	L	M	L	H
EC180063	L	M	L	H
EC187176	L	L	L	H
IC634028	L	M	L	H

Alb-Albumin: Low = <40mg/g.wt., Medium = 40.1-50mg/g.wt., high = 50.1-55 mg/g.wt.

Glo-Globulin: Low = <13 mg/g.wt., Medium = 13.1-17 mg/g.wt., High = 17.1-21mg/g.wt.

Gli-Gliadins: Low = <55 mg/g.wt., Medium = 55.1-80 mg/g.wt., High = 80.1-93 mg/g.wt.

Glu-Glutenin: Low = <12 mg/ g.wt., Medium = 12.1-16 mg/g.wt., High = 16.1-20 mg/g.wt

# **Table 6**(on next page)

. Four clusters constituting of different genotypes along with mean values of clusters for seventeen quality traits

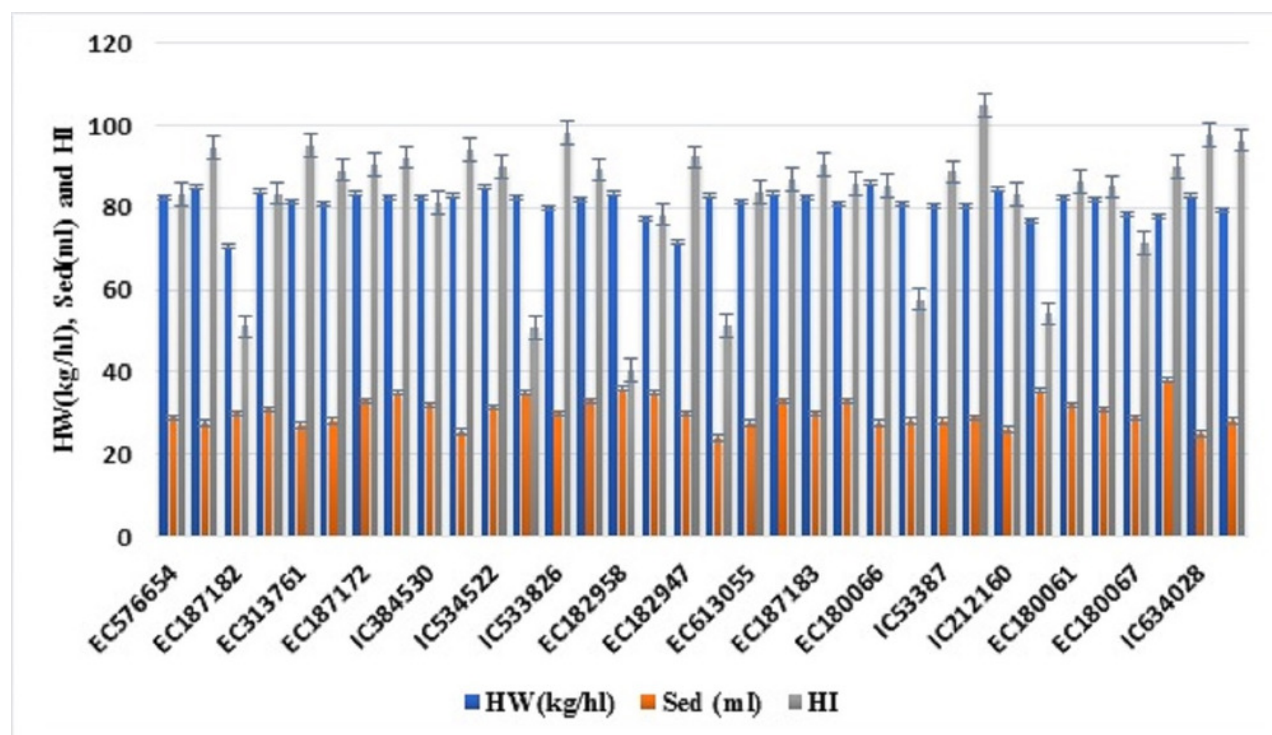
1 **Table 6.** Four clusters constituting of different genotypes along with mean values of clusters for  
2 seventeen grain quality traits

<b>Cluster</b>		<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>
<b>No. of genotypes</b>		<b>6</b>	<b>10</b>	<b>17</b>	<b>1</b>
Genotypes		EC187176, EC182945, EC182958, EC10494, EC187182, EC10492	EC182959, EC182956, EC187172, EC180061, EC613055, EC576654, EC187181, EC187167, EC182947, EC180066	EC180063, EC313761, IC397363, IC533826, IC534021, IC420038, EC187183, IC53387, EC541164, IC534882, EC613057, EC180062, EC180041, IC212160, IC634028, IC534522, EC180067	IC384530
Mean values of clusters for seventeen quality traits	TGW(g)	35.30	31.84	32.70	31.11
	HW (kg/hl)	79.58	81.15	81.82	82.50
	Sed (ml)	31.42	29.45	30.47	32.00
	HI	50.92	90.24	88.45	81.07
	Pro (%)	18.38	17.20	17.51	18.90
	Alb (mg/g)	24.12	22.39	24.96	20.13
	Glo (mg/g)	14.10	13.29	13.07	14.17
	Gli (mg/g)	35.02	31.40	34.65	41.78
	Glu (mg/g)	33.26	30.04	30.78	30.90
	Gluten (mg/g)	68.28	61.44	65.42	72.68
	Lys (% of sample)	00.10	0.13	0.19	0.16
	Fe <sup>2+</sup> (ppm)	65.43	65.10	65.40	90.71
	Zn <sup>2+</sup> (ppm)	22.56	17.22	18.95	16.25
	POX (unit/ml)	0.11	0.26	0.23	0.12
	CAT(unit/ml)	0.16	0.10	0.10	0.15
	GR(unit/ml)	0.78	0.78	0.66	0.11
	SOD (unit/ml)	0.22	0.36	0.28	0.63

3    TGW-Thousand grain weight, HW-Hectolitre weight, Sed-Sedimentation value, HI-Hardness index, Pro- Protein content, Alb-Albumin content,  
 4    Glo-Globulin content, Gli-Gliadin content, Glu-Glutenin content, Lys-Lysine content, POX-Peroxidase, CAT-Catalase, GR-Glutathione reductase,  
 5    SOD-Superoxide dismutase  
 6

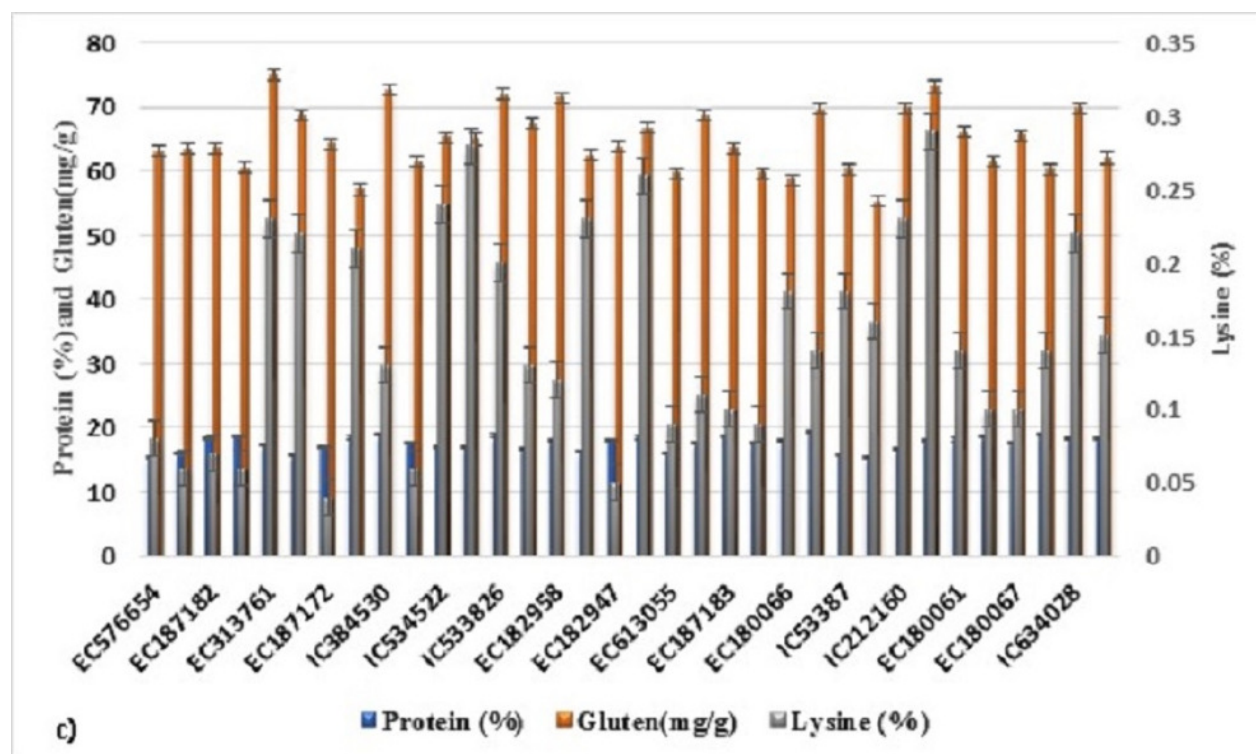
# Figure 1

Variability of various grain quality traits among 34 accessions of *T. sphaerococcum* for Hectolitre weight, Sedimentation value and Hardness index



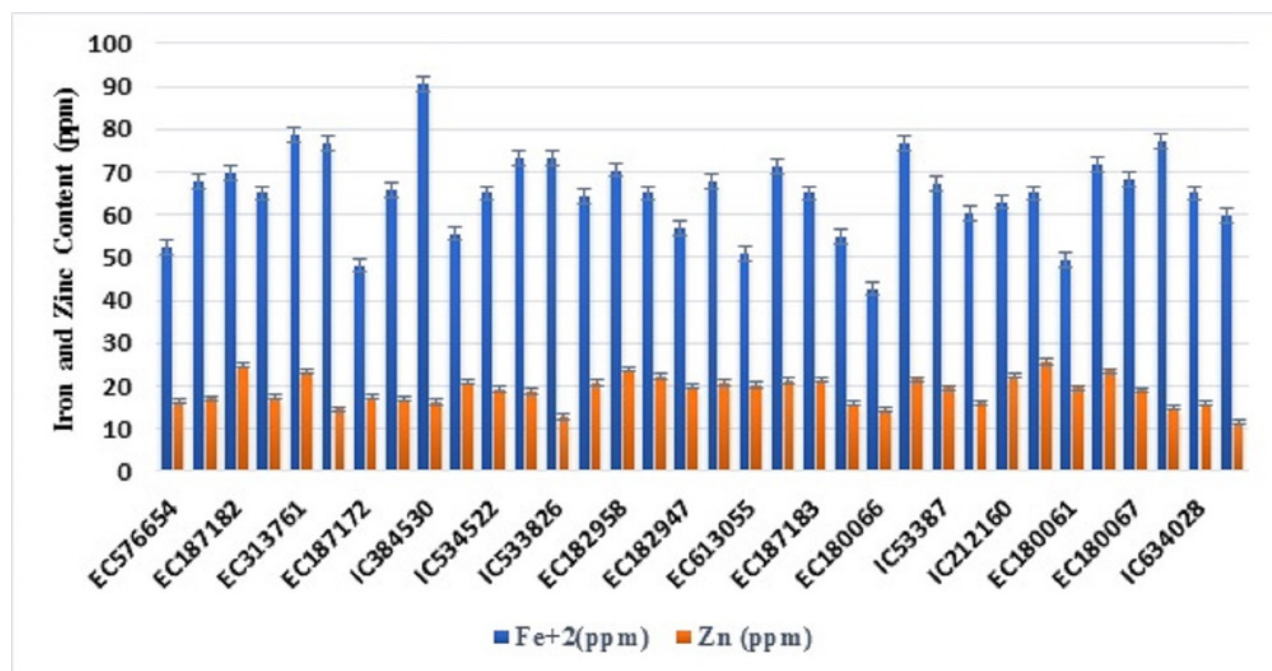
# Figure 2

Variability of various grain quality traits among 34 accessions of *T. sphaerococcum* for Protein, Gluten and Lysine content



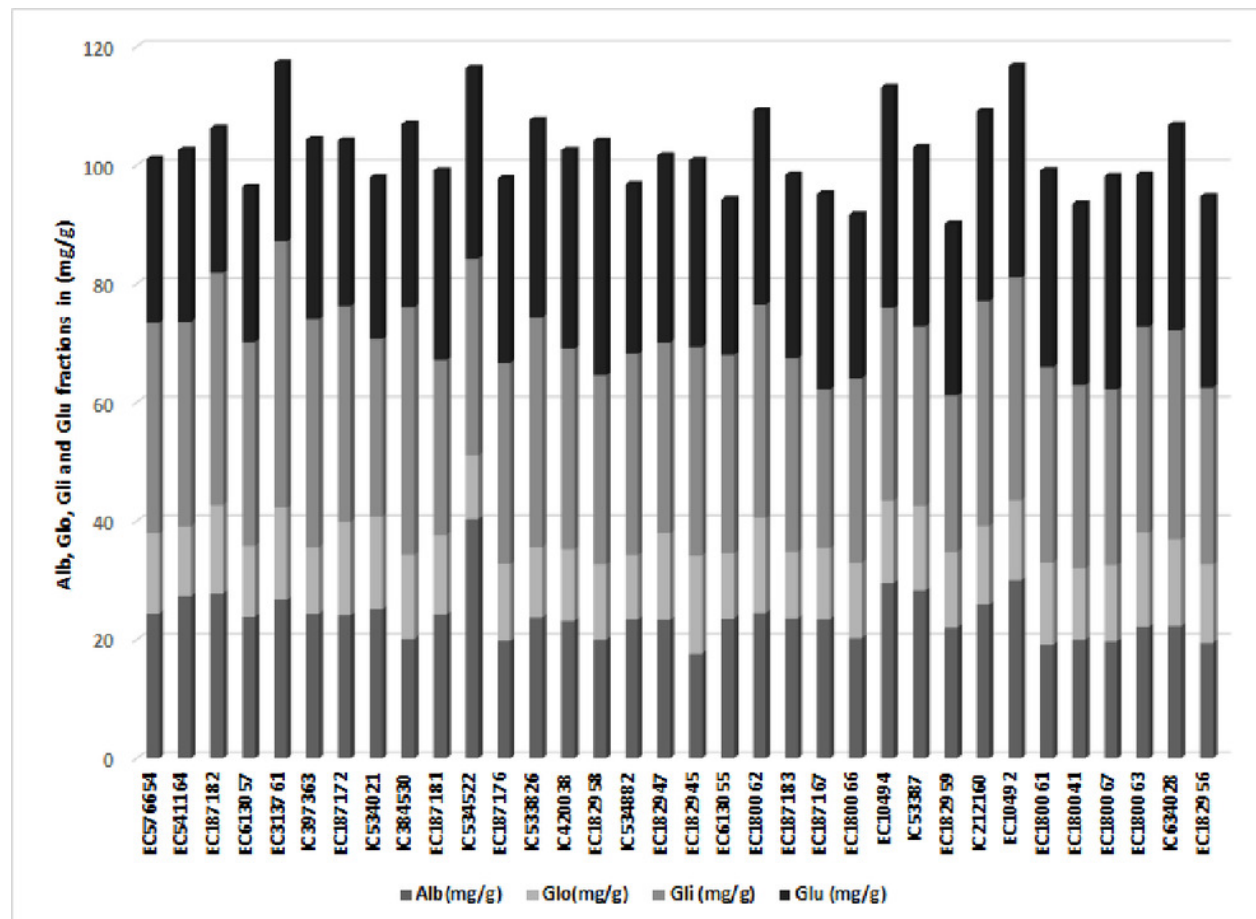
# Figure 3

Variability of various grain quality traits among 34 accessions of *T. sphaerococcum* for Iron and Zinc content



# Figure 4

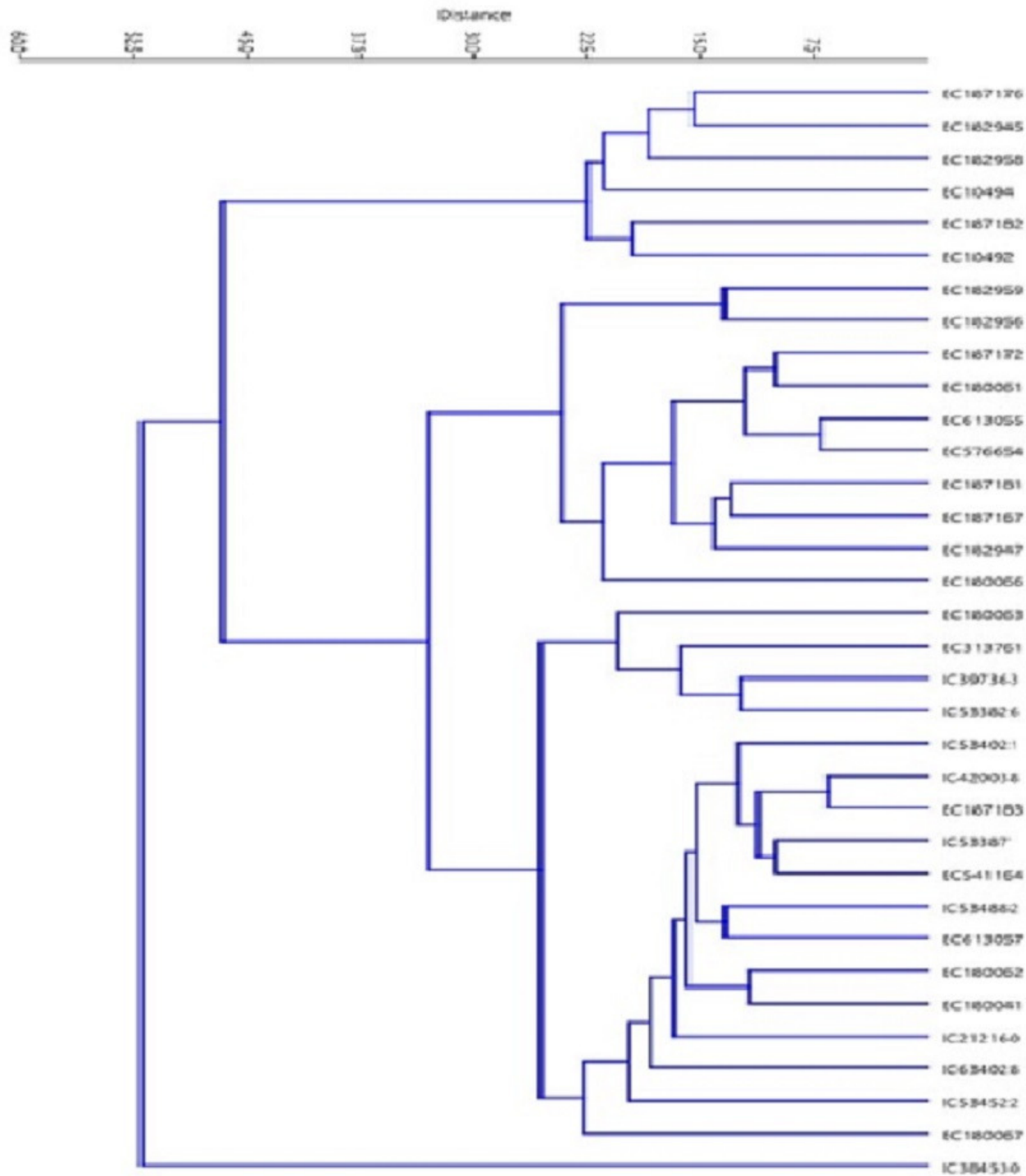
Proportion of four protein fractions among 34 accessions of *T. sphaerococcum*



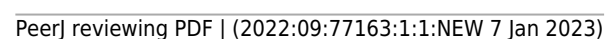


# Figure 5

Clustering pattern of 34 *T. sphaerococcum* accessions based on eighteen quality traits



Biplot representing distribution pattern of 34 *T. sphaerococcum* accessions



# Figure 7

Depiction of mean performance of eight high grain protein accessions over the locations and years through line diagram

