Unlocking the potential of ancient hexaploid Indian dwarf wheat, *Tritium 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^{2+} and Zn^{2+} 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^{2+}, and Zn^{2+} 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^{2+} content whereas Lys was negatively associated with gluten content. Likewise, gluten and Fe^{2+} 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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2   **for grain quality improvement**

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Abstract

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19 hence being re-considered for mainstreaming its cultivation by the farmers and bringing it back
20 to the food basket. In the present study, thirty-four diverse accessions of Indian dwarf wheat,
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22 grain quality parameters namely thousand-grain weight (TGW), hectolitre weight (HW),
23 sedimentation value (Sed), grain hardness index (HI), protein (Pro), albumin (Alb), globulin
24 (Glo), gliadin (Gli), glutenin (Glu), gluten, lysine (Lys), Fe^{2+} and Zn^{2+} content, and four
25 antioxidant enzymes activities. Substantial variations were recorded for studied traits. TGW,
26 HW, Sed, HI, Pro, Alb, Glo, Gli, Glu, Gluten, Lys, Fe^{2+}, and Zn^{2+} varied from 26.50-45.55g,
27 70.50-86.00kg/hl, 24.00-38.00ml, 40.49-104.90, 15.34-19.35%, 17.60-40.31mg/g, 10.75-
28 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,
29 and 11.45-25.70ppm, respectively. Among antioxidants, peroxidase (POX), catalase (CAT),
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39 EC180066, and EC182959 for low gluten content and IC384530, EC313761, EC180063,
40 IC397363, EC10494 for high iron content (>76.51) were identified that may be used in wheat
41 quality improvement for nutritional security of mankind.

Introduction

44 Worldwide wheat is the most important cereal crop, contributing 55% of total carbohydrates,
45 21% of the total proteins and 19-20% of the calories demand of around half of the world's
46 population (Shiferaw et al. 2013). Along with carbohydrates, proteins, dietary fiber, and
47 micronutrients, wheat also contains minerals, vitamins, and fats (Sarwar et al. 2013). Grain
48 quality consists of various characteristics that together are responsible for determining the end
49 use of grains (Rondanini et al. 2013). The chemical composition of grain *i.e.* starch content is
50 considered important for ethanol production whereas protein and carbohydrate content for food
51 and feed production. The major component of wheat endosperm is starch and it also consists of
52 glucose polymers, amylase, amylopectin, and a small amount of protein and lipids (Fasahat et al.
53 2015). The wheat grains milled into flour is used as raw material for different foods such as
54 chapatti, bread, cakes, biscuits, muffins, pasta, noodles, sweets, and pastries (Uthayakumaran
55 and Wrigley 2017). The utilization of wheat grain in different forms mainly depends on protein
56 content and gluten strength.

57   In bread wheat (*Triticum aestivum*), protein content varies from ~9-13 % (Žilić et al.
58 2011; Mohan and Gupta 2013; Punia et al. 2019). Wheat protein is comprised of four fractions
59 (Osborne 1907) which include albumins (water soluble), globulins (salt soluble), gliadins
60 (alcohol soluble), and glutenins (diluted acid or base soluble). The 25% fraction of total wheat
61 protein is contributed by albumin and globulin components (Belderok et al. 2000). The
62 remaining two protein fractions, gliadins, and glutenins are collectively called gluten and share
63 75% of total protein and provide visco-elastic properties to the wheat dough. Therefore, the
64 proportions of these four components are responsible for dough making and baking quality of
65 flour (Vigni et al. 2013). Sedimentation value provides an idea about the content and quality of
66 gluten as well as loaf volume (Pasha et al. 2007) and thus is helpful in the classification of wheat
67 in different categories for their utilization. Grain hardness is influenced by protein content and
68 moisture content (Turnbull and Rahman 2002) and causes notable effects on milling as well as
69 baking quality (Bettge et al. 1995). Many critical factors influence the quality of wheat end-
70 product hence it is important to assess parameters for global trade. Based on grain hardness,
71 wheat is grouped into two categories *i.e.* soft wheat and hard wheat (Pomeranz and Williams
72 1990).

73   Free radicals and reactive oxygen species (ROS) cause harmful effects on the cell

74 membrane, enzymes, DNA, and maintenance of DNA. Oxidative damage to DNA and enzymes

75 can result in some serious health issues such as cardiovascular diseases, carcinogenesis, type II

76 diabetes, and obesity. Free radicals have been linked to over 60 kinds of diseases. This includes

77 cancer, Alzheimer's disease, Parkinson's disease, and cataracts. Enzymatic antioxidants such as

78 glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX)

79 (Narwal et al. 2014) directly react with ROS (Lobo et al. 2010) and consequently prevents

80 oxidative damage resulting in reduced risk of oxidative-stress related chronic diseases.

81   Enzymatic antioxidants help in the breakdown of free radicals by seeking out oxidative

82 products and converting them into hydrogen peroxide, and then into the water. In addition, they

83 also act as metal chelators and terminate the oxidative enzyme inhibitors hence ROS reactions

84 (Karadag et al. 2009). Hence the improved concentration of antioxidants helps in disease

85 prevention & health promotion. Further, regarding mineral content, cereals and their products

86 contribute approximately 50% of Fe^{2+} and 20% of Zn^{2+} in the Western diet (Velu et al. 2017). In

87 case of wheat, Fe^{2+} and Zn^{2+} content was reported to be low and that too was situated in the outer

88 layers of grains. Presently around three billion people are malnourished due to the non-

89 availability of micronutrients as well as vitamins. A less diverse diet leads to deficiency of

90 minerals, among which Fe^{2+} and Zn^{2+} deficiency causes more pronounced effects due to

91 involvement in various metabolic processes. Micronutrient enrichment of major food crops

92 through breeding is one of the sustainable ways to reduce malnutrition among higher-risk groups

93 (resource-poor women, infants, and children) (Šramková et al. 2009). It has been proven by

94 various researchers that in the wheat genome traits related to micro-nutrient enrichment are

95 available that could facilitate significant improvement in vitamins and minerals as well as

96 growth-promoting components without any adverse effect on yield (Rose et al. 2002; Šramková

97 et al. 2009). During the course of the domestication and selection process, desirable allelic

98 combinations were selected which led to the evolution of modern crops that feed the world today

99 but at the cost of reduced variation resulting in the narrowing down of the genetic base of crop

100 species (van Heerwarden et al. 2011) and is referred as domestication bottleneck (Hammer

101 1984). For the development of high-yielding wheat varieties with improved quality for targeting

102 specific products, several breeding programs have been initiated. As a consequence of this,

103   wheat cultivation is based on genetically similar cultivars which resulted in a compromised

104   genetic base due to dependency on the limited number of parents (Shuaib et al. 2010).

105   Wheat-related species are considered potential sources for grain yield and quality traits

106   for which these are not fully explored. Therefore, an urgent need was sought for the

107   characterization of these species with the aim of identification of promising lines for improved

108   quality traits. Among ancient wheat, *Triticum sphaerococcum* (AABBDD, 2n = 6x = 42) is a

109   landrace of wheat and is known as the Indian dwarf wheat. It has several advantageous features

110   such as short and strong culms, hemispherical grains, higher protein content compared to bread

111   wheat, and resistance to biotic and abiotic stresses, however, sphaerococcum wheat is poorly

112   studied (Josekutty 2008, Matsuoka 2011). Being a hexaploid species, it may be easily exploited

113   for bread wheat improvement to achieve food and nutritional security to fulfill United Nations'

114   sustainable development goal. Therefore, the main objectives of this study were (1) to evaluate

115   34 accessions of *T. sphaerococcum* for important quality traits and identification of superior line

116   for individual traits and (2) diversity assessment of *T. sphaerococcum* accessions based on

117   quality traits.

Materials & Methods

119   The experimental material for the present investigation comprised a diverse set of thirty-four

120   accessions of *T. sphaerococcum* conserved in the national genebank namely IC384530,

121   IC397363, IC533826, IC53387, IC534021, IC534522, IC212160, IC534882, IC420038,

122   EC313761, EC541164, EC576654, EC613055, EC613057, EC187172, EC187182, EC187181,

123   EC180062, EC187183, EC187167, EC182959, EC182945, EC10494, EC10492, EC182958,

124   EC180041, EC180061, EC180067, EC182947, EC180066, EC182956, EC180063, EC187176,

125   IC634028. The grain harvest was obtained from the field trial grown during *Rabi* 2019-20 at

126   ICAR-National Bureau of Plant Genetic Resources (NBPGR), Issapur farm (7650' E longitude

127   and 2840' N latitude, 223 m MSL). The soil of the farm was sandy loam in texture having

128   organic carbon 0.7%, available N 297.6 kg/ha, available P 26.7 kg/ha, and available K 196.0

129   kg/ha with soil pH of 6.5. Recommended agronomic practices, irrigation, and fertilizers (120 kg

130   N; 60 kg P_2O_5 and 40 kg K_2O ha^{-1}) were followed throughout the experiments. Complete doses

131   of K2O and P2O5 were applied at the time of sowing. Nitrogen was supplied in three split doses,

132   60 kg N ha^{-1} at sowing, 30 kg N ha^{-1} at crown root initiation, and 30 kg N ha^{-1} at booting stages.

133   The experiment was adequately irrigated with five irrigations during the crop growth. Weeding

134   was done manually. The spray of fungicide, tilt was done to avoid the infection of rust diseases.

135   These accessions were evaluated biochemically following a completely randomized design with

136   three replications for seventeen quality parameters namely thousand-grain weight (TGW),

137   hectolitre weight (HW), sedimentation value (Sed), grain hardness index (HI), protein content

138   (Pro), albumin content (Alb), globulin content (Glo), gliadin content (Gli), glutenin content

139   (Glu), gluten content, lysine content (Lys), Fe^{2+} and Zn^{2+} content, peroxidase activity (POX),

140   catalase activity (CAT), glutathione reductase activity (GR) and superoxide dismutase activity

141   (SOD). The grain material was sun-dried. Grain moisture content and TGW were recorded

142   before the biochemical estimation. For HW and HI whole grain was analyzed whereas, for the

143   rest of the traits, flours were utilized. The flour of seeds of each accession was prepared by

144   grinding these seeds in a cyclotec laboratory flour mill and was tested in three replicates. HW

145   was estimated by using hectolitre apparatus. The sodium dodecyl sulphate (SDS Solution)

146   sedimentation volume of each sample was estimated by the method suggested by Axford et al.

147   (1978). HI and moisture content was analyzed using the single-kernel characterization system

148   (SKCS) model 4100 (Perten Instruments North America, Inc., Reno, NV). Lysine was calculated

149   by the dye-binding method as suggested by Udy (1956). Total protein content was estimated on a

150   wet basis following Micro-Kjeldahl Method (Kjeldahl 1883). Following the sequential Osborne

151   extraction procedure (Osborne 1907) the proportion of Alb, Glo, Gli, and Glu was extracted as

152   described by Mughal et al. (2020). As gluten is composed of Gli and Glu therefore it was derived

153   by summing up of estimation of these two contributing components. Fe^{2+} and Zn^{2+} content was

154   estimated by wet digestion (15ml acid mixture (9 concentrated nitric acid: 2 sulfuric acid: 1

155   perchloric acid + 0.5g wheat flour) followed by atomic absorption spectroscopy (Jackson 1973).

156   Quantification of extracted proteins (Pro, Alb, Glo, Gli, and Glu) was conducted using a

157   spectrophotometer, and BSA (Bovine Serum Albumin) was used as a standard. The enzymatic

158   antioxidants were estimated by using the methodology suggested by Todorova et al. (2021).

159   Eight promising accessions for grain protein content were validated by growing consecutively at

160   NBPGR Pusa Farm and NBPGR, Issapur Farm, New Delhi during the year 2019-20, 2020-21,

161   and 2021-22.

162   **Statistical analysis**

163   All data were subjected to analysis of variance (ANOVA) using completely randomized design

164   (CRD) and a comparison of treatment means was performed using Duncan's Multiple Range

165   Test (DMRT) at P < 0.05 using IBM SPSS Statistics software. The mean values of individual

166   genotypes for each trait were subsequently used for analyzing summary statistics and graphs

167   using MS Excel software. Histogram and correlation among traits were analyzed by using IBM

168   SPSS Statistics software. Hierarchical cluster analysis was performed using Euclidean distance

169   measure following Unweighted Pair Group Method with Arithmetic Averages (UPGMA)

170   method by using PAleontological STatistics (PAST) software (Hammer et al. 2001). The flow of

171   work done is depicted in Supplementary Fig.1.

Results

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

174   Analysis of variance revealed significant variance for all the characters at 1% level of

175   significance (Table 1). Further to find out the significant differences among genotypes, a Post

176   Hoc test, Duncan's Multiple Range Test (DMRT) was performed which resulted in the grouping

177   of accessions in a larger number of subgroups 8 (TGW), 17 (HW), 13 (Sed), 18 (HI), 13 (Pro),

178   14 (Alb), 14 (Glo), 20 (Gli), 18 (Glu), 19 (Gluten), 19 (Lys), 18 (Fe^{2+}), 13 (Zn^{2+}), 19 (PER), 16

179   (CAT), 25 (GR), 13 (SOD), which indicates the existence of sufficient variation. Likewise, the

180   existence of wider variation was also supported by other parameters (Table 2). It was also

181   depicted pictorially by the histogram (Supplementary Fig. 2) and bar diagram (Fig. 1-4). All the

182   traits followed normal distribution except Alb, CAT, and SOD which were positively skewed.

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

184   Hectolitre weight (HW) ranged from 70.50kg/hl (EC187182) to 86.00kg/hl (EC180066) with the

185   mean of 81.25kg/hl among studied *T. sphaerococcum* accessions (Supplementary Fig. 2a). High

186   HW along with high TKW (26.05-45.55g) (Supplementary Fig. 2b) indicated high milling yield

187   potential of Indian dwarf wheat. Gluten quality which is estimated in terms of Sed varied from

188   24.00ml (EC182945) to 38.00ml (EC180063) with an average of 30.38ml (Supplementary Fig.

189   2c and Fig. 1). Based on Sed, wheat can be classified into three categories [<48ml (weak), 48-

190   68ml (medium strong), >68ml (strong)] but all 34 accessions of *T. sphaerococcum* showed Sed

191   less than 48.00ml therefore grouped in weak gluten class due to its low hydration capacity.

192   Further, they have been classified into three categories based on their suitability for making

193   different products (Table 3). For making good quality bread, chapatti, and biscuit, required Sed

194   are >60ml, 30-60ml, and <30ml, respectively. Further, non-significant but positive estimates of

195   correlation coefficient (+0.18) were found between Sed and Protein (Table 4).

196   Grain hardness ranged from 40.49 (EC182958) to 104.92 (EC182959) with an average of 82.03

197   (Supplementary Fig. 2d and Fig. 1). Based on HI, accessions were classified in four categories

198   *i.e.* <33 (Soft), 34–46 (Medium soft), 47–59 (Medium-hard), and >60 (Hard) according to Morris

199   et al. (2001). The majority of accessions (82.35%) were categorized as hard wheat and five

200   accessions EC187176, EC187182, EC182945, EC10492, and EC10494 were grouped in the

201   medium hard category. Only one accession EC182958 was reported medium soft with HI of

202   40.49. In our experiment, we observed a significant positive correlation (r= +0.37*) between Pro

203   and HI, however, HI and TGW were negatively associated (-0.35*) (Table 4).

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

205   Accessions significantly differed from each other for Protein content and ranged from 15.34-

206   19.35% with an average of 17.62% (Supplementary Fig. 2e and Fig. 2). Maximum Protein

207   content, 19.35% was reported in accession, EC613057 followed by 19.24% in EC10494. The

208   proportion of four protein fractions among 34 accessions of *T. sphaerococcum* is shown in Fig.

209   2. The concentration of Alb varied from 17.60mg/g to 40.31mg/g with the mean of 23.91mg/g

210   (Supplementary Fig. 2f). Only one IC534522 exhibited a moderate concentration of Alb

211   (40.32mg/g) whereas rest consisted of low concentration of Alb ranging from 17.60mg/g to

212   30.00mg/g (Table 5). As far as Glo is concerned, 18 accessions showed moderate concentration

213   and it ranged from 14.00mg/g-16.56mg/g with the highest concentration of Glo (16.56mg/g) in

214   accession EC182945 (Supplementary Fig.2g). For Gli content, all the accessions belonged to low

215   category with the range of 26.35mg/g-44.94mg/g (Supplementary Fig.2h). In contrast, all

216   accessions were grouped in high Glu content class with the mean of 31.00mg/g and ranged from

217   24.47mg/g-39.56mg/g (Supplementary Fig.2i). Gli and Glu are collectively called gluten which

218   shares 85% of total protein (Fisher and Halton 1936) and is responsible for visco-elastic property

219   to the wheat dough. By summing up these two fractions of protein, we got a rough estimate of

220   gluten content. With an average of 64.97mg/g proportion of gluten among accessions, it varied

221   from 55.33-75.06mg/g (Supplementary Fig.2j). Significant positive correlation of gluten with Gli

222   (r=+0.73**) and Glu (r=+0.60**) depicted collective contribution of these components in gluten

223   estimation. Regarding lysine content, *T. sphaerococcum* wheat had an average of 0.15% of

224   sample Lys with a range of 0.04% (EC187172) to 0.29% (EC10492) (Supplementary Fig.2k).

225 We also observed non-significant and negative correlation (r=-0.03) between lysine and protein

226 whereas significant negative (-0.35*) association was found with gluten (Table 4).

227 **Fe^{2+} and Zn^{2+} content**

228 Among micronutrients, *T. sphaerococcum* accessions had a mean of 65.11 ppm Fe^{2+} content with

229 a range of 42.72ppm (EC180066) to 90.72ppm (IC384530) (Supplementary Fig.2l and Fig. 3),

230 whereas slightly narrow range was observed for Zn^{2+} content i.e., from 11.45ppm to 25.70ppm in

231 accession EC182956 and EC10492 respectively (Supplementary Fig.2m). Fe^{2+} content showed a

232 highly significant positive correlation with major protein fraction *i.e.* Gli (r=+0.48**) and Glu

233 (r=+0.52**) (Table 4). Likewise, Zn^{2+} content was positively (+0.55**) associated with HI. Zn^{2+}

234 concentration showed a non-significant weak correlation with TGW (0.22).

235 **Enzymatic antioxidant activity**

236 Among four studied antioxidants, POX activity ranged from 0.06-0.60 units/ml with an average

237 of 0.22 units/ml (Supplementary Fig.2n and Fig. 4). The accession "EC541164" possessed the

238 highest POX activity. POX is considered important from the quality point of view due to its

239 involvement in the reduction of dough adhesiveness. The CAT, GR and SOD activity varied

240 from 0.02unit/ml-0.60unit/ml, 0.11unit/ml-2.26unit/ml, and 0.14unit/ml-0.97unit/ml respectively

241 (Supplementary Fig.2o-2q). Correlation analysis results indicated that only GR and POX showed

242 a positive correlation with HI. Whereas negative relation was observed between POX and Pro,

243 GR and HW, CAT with Sed and Alb, and SOD with Zn^{2+}content.

244 **Cluster analysis**

245 Thirty-four *T. sphaerococcum* accessions were subjected to cluster analysis by employing an

246 unweighted pair group method with arithmetic average (UPGMA) and Euclidean distance

247 measure. Euclidean distance matrix depicts the extent of genetic dissimilarity among accessions.

248 The maximum distance of 86.22 was observed between EC613055 which belonged to cluster B

249 and IC384530 which is clustered independently in cluster D from the rest of the accessions.

250 After that EC187172 and IC384530 with 75.97 euclidean distances were grouped in clusters B

251 and D respectively. EC187183 and IC420038 were most similar due to the lowest euclidean

252 distance of 6.57 and both of them belonged to cluster C. Following this, pair of EC613055/

253 EC576654 clustered in group B with a distance of 7.02. The clustering pattern of Indian dwarf

254 wheat accessions is based on quality data and is presented in Fig.5 and Table 6. Based on quality

255 parameters, at a Euclidean distance of 30, 34 accessions of Indian dwarf wheat were grouped

256   into four main clusters A, B, C, and D. Further main cluster A, B, and C is divided into two sub-

257   clusters each whereas only one accession was grouped in main cluster D. The distribution pattern

258   revealed the maximum number of genotypes i.e., 17 in cluster C followed by cluster B having 10

259   genotypes. The cophenetic correlation coefficient was estimated as 0.86 for checking the

260   agreement between phenograms and dissimilarity matrices for the accuracy of the dendrogram.

261   The mean performance of different clusters for the studied quality traits was shown in Table 6.

262   Minimum mean Sed (29.45ml) was observed among accessions of cluster B. In addition to this,

263   cluster B was also characterized by low Gli (31.40mg/g), Glu (30.04mg/g), gluten content

264   (61.44mg/g), and high HI (90.24). Cluster B also showed a high mean value for POX (0.26

265   unit/ml) and GR (0.78 unit/ml) activity and hence could be targeted for wheat nutritional quality

266   improvement. For flour yield improvement accessions clustered in cluster C could be targeted

267   due to the maximum mean HW (81.82kg/hl). Accessions grouped in cluster C also showed high

268   mean values Alb (24.96mg/g) and Lys (0.19%), whereas cluster D was characterized by high Glo

269   (14.17mg/g). In addition to high Glo accession of cluster D also showed high Sed (32.00ml), Gli

270   (41.78mg/g), gluten (72.68mg/g), Fe^{2+} (90.71ppm), and SOD activity (0.63unit/ml). Cluster A

271   demonstrated high Glu (33.26mg/g), Zn^{2+} (22.56ppm) content, CAT (0.16unit/ml), and GR

272   (0.78unit/ml) activity. To understand and confirm the dynamics of the sphaerococcum

273   accessions, we have further carried out principal coordinate analysis (PCoA). Based on the

274   pairwise Euclidean distance matrix, the clear distinction could be visualized among these

275   accessions, which was in concordance with the results of the biplot (Fig. 6).

276   **Validation of high grain protein accessions**

277   Eight sphaerococcum accessions with high grain protein content (GPC) were validated under

278   multi-location and multi-year trials by growing consecutively at NBPGR, Pusa Farm and

279   NBPGR, Issapur Farm, New Delhi during the year 2019-20, 2020-21, and 2021-22. Their

280   comparative performance along with the overall mean is presented in Fig. 7. Top performing

281   accessions with more than 15 % GPC were confirmed as EC182958 (17.16%), EC187176, and

282   EC182945 (16.16%), EC613057 (15.79%), IC634028 (15.72%) and IC533826 (15.01%) which

283   can be used for introgression purpose and bread wheat quality improvement.

284   # Discussion

285   Wild relatives and ancient wheat serve as the most important genetic resources for numerous

286   valuable traits and possess wider variation for quality traits (Zelbig et al. 2021). In comparison to

287   bread wheat (*T. aestivum*), emmer wheat (*T. dicoccoides*) is reported to possess more lysine and

288   isoleucine content (Nevo and Beiles 1992). Similarly, as appose to cultivated wheat, and wild

289   relatives; primitive cultivars are considered better resources for zinc and iron fortifications

290   (Cakmak 2008; Velu et al. 2011; Xu et al. 2011). Extensive characterization of genetic resources

291   is a preliminary step for designing any quality breeding program. The ancient wheat, *T.*

292   *sphaerococcum*, endemic to north-western India and southern Pakistan was withdrawn from

293   cultivation during the early 20th century due to the introduction of the modern wheat cultivar as

294   the result of the green revolution (Mori et al. 2013). Indian dwarf wheat is the least exploited

295   hexaploid species for quality assessment. Here we explored *T. sphaerococcum* germplasm for

296   their nutritional quality traits to enhance their potential utilization for bread wheat improvement.

297   In our study, a nearly similar range of HW was observed in *T. sphaerococcum* as reported by

298   Mohan and Gupta (2013) in bread wheat cultivars. Higher HW means better milling quality in

299   terms of flour yield (Martin et al. 2021) and thus accessions coming under the category of higher

300   HW may result in better flour yield. As, HW is affected by grain shape, size, and density,

301   therefore the possible reason for the higher value is their round grain shape. Sed recorded among

302   these accessions was much lower than Indian bread wheat varieties (35-60ml, Mohan and Gupta,

303   2013) and (33-52ml, Panghal et al. 2018). Whereas, regarding the hardness index, most of the

304   studied accessions belonged to the hard category. However, a relatively wide range (29-94) for

305   HI in Indian bread wheat varieties was reported by Mohan and Gupta, 2013. It has been observed

306   that wheat with high protein tends to be hard whereas soft grain with low protein.

307   Wheat constitutes approximately 80% of the total cereal intake, and it contributes 50% of

308   total energy and 60% of the total protein intake. Based on our findings, sphaerococcum wheat

309   grain accumulated more protein in contrast to the narrow range (9.40-13.50) of protein in Indian

310   bread wheat varieties reported by Mohan and Gupta, 2013. We have also confirmed the high

311   GPC accessions by growing in different environments. However, Ciaffi et al. (1992) found a

312   large variation in *T. dicoccoides*, ranging from 16.00-27.00%. Similarly, in related species of

313   wheat, mean Pro was reported as 16.67% (Jiang et al. 2008) confirming the finding that wild and

314   primitive wheat have a wider range of variation for grain protein than cultivated ones. The Alb

315   and Glo contribute 15-20% share of wheat flour protein. These non-prolamin protein fractions

316   are considered nutritionally better due to a higher proportion of lysine and methionine as

317   compared to other proteins in wheat (Lasztity 1986). In addition to this, these proteins are also

318 considered important for germinating and protecting embryos from insects and pathogens

319 (Dupont and Altenbach 2003). In our study, we have identified one accession for moderate Alb

320 and eighteen for moderate Glo. These accessions were considered good from the nutritional point

321 of view and could be utilized in wheat quality improvement. The Gli and Glu ratio in the present

322 study ranged from 0.80 to 1.50 which is lower than as observed in the case of common wheat

323 (1.60-3.80) (Geisslitz et al. 2019). It has been proven that a low Gli: Glu ratio is linked with good

324 baking quality (Thanhaeuser et al. 2015). Therefore, these accessions are also considered good

325 for baking purposes. Cereal-based diet and food items are poor concerning essential amino acids

326 such as lysine and methionine (Smriga et al. 2004). Inadequate intake of Lys results in a

327 syndrome called 'protein energy malnutrition' which is suspected to be more prevalent in

328 developing countries. Manipulation of cereal proteins and amino acid profiles will cause a

329 significant impact on the life of hundreds of millions of poor people. Despite extensive research

330 aimed at the identification of Lys-rich wheat accessions; the high Lys mutants have not been

331 discovered yet. Accessions with higher Lys estimates (EC10492, EC187176, EC182945,

332 IC534522, and EC313761) found in our study could be utilized in wheat quality improvement.

333 The iron content in *T. sphaerococcum* accession was much higher than those obtained by Zhao et

334 al. (2009); Mohan and Gupta (2013); Goel et al. (2018) and Khokhar et al. (2020). However,

335 variation for Zn content is less as appose to previous findings (Goel et al. 2018; Khokhar et al.

336 2020) whereas in agreement with the findings of Zhao et al. (2009). It has been observed that

337 grains with high Pro accumulate more micronutrients, which may be due to the co-localization of

338 Pro, Zn^{2+}, and Fe^{2+} in the embryo and the aleurone layer of the grain (Khokhar et al. 2020). Zn^{2+}

339 concentration showed a non-significant weak correlation with TGW (0.22) which is in close

340 agreement with the findings of Khokhar et al. (2020). A significant positive association (+0.43*)

341 between Fe^{2+} and Zn^{2+} contents indicated the possibility of simultaneous improvement of both

342 micronutrients (Goel et al. 2018). It was also stipulated that there is the involvement of a

343 common mechanism for the accumulation of both micronutrients (Hernandez et al. 2020).

344 Likewise, a significant positive association between Fe^{2+} and Zn^{2+} was observed by various

345 researchers (Velu et al. 2017; Hernandez et al. 2020; Khokhar et al. 2020). Further, the probable

346 reason for the association between Fe^{2+} and Zn^{2+} content was found to be the co-localization of

347 quantitative trait loci present at chromosome 3B governing these traits (Velu et al. 2017). Due to

348 the lack of ample genetic variation in cross-compatible species, the traditional breeding for

349 micronutrient enhancement was non-exploitable. Henceforth hexaploid species *T.*
350 *sphaerococcum* could be exploited in this direction. For antioxidants, maximum CAT, GR, and
351 SOD activity were recorded in the case of accessions EC18294, EC180063, and EC182956
352 respectively. CAT was reported to be involved in oxidative reaction during bread making and its
353 highest activity was recorded during the kernel development stage and was responsible for the
354 detoxification of H_2O_2 (Kruger and Leberg 1974). Likewise, SOD protects the oxidation of
355 cellular components through reactive oxygen species (Mughal et al. 2020). Hence accessions
356 showing high activity for antioxidants could be targeted for wheat quality improvement in terms
357 of nutrition. GR may control the ratio of GSSG (oxidized glutathione)/ GSH (reduced
358 glutathione) in wheat flour and play a role in determining the physical properties of wheat flour
359 doughs (Every et al. 2006).

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

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

380 characterized by high protein content. Cluster A demonstrated high Glu, Zn^{2+} content, CAT, and

381 GR activity henceforth could be exploited in the improvement of respective traits.

Future implications

383 Based on the comprehensive estimation of grain quality parameters, our study revealed that the

384 primitive wheat landrace, Indian dwarf wheat *T. sphaerococcum* is a rich source of grain protein

385 content, lysine content, iron and zinc content, and antioxidant activity. It also has low gluten

386 content which is a major dietary requirement based on consumer preference. Therefore

387 exploration, conservation, and use of collections belonging to this species may be promoted to

388 gain maximum benefit for the well-being of mankind. Among the studied accessions, EC10492,

389 and EC313761 (from USSR) were found superior for a maximum of seven traits followed by

390 EC180063 and EC182958 for five traits. We also validated high GPC accessions such as

391 EC182958 (from Canada), EC187176, EC182945, EC613057 (from France), IC634028 (Kathod

392 Gehun from Tapi district of Gujarat), and IC533826 with more than 15 % grain protein content.

Conclusions

394 Wild relatives, ancient species, and landraces are rich valuable genetic resources for abiotic and

395 biotic stress tolerance, and nutritional qualities and play a major role in wheat improvement. In

396 the present study, we analyzed 34 *T. sphaerococcum*, Indian dwarf wheat accessions conserved

397 in the national genebank of India for seventeen-grain quality parameters. We found sufficient

398 variation among accessions for all studied quality traits and accessions with superior quality

399 traits were identified. Among these accessions, EC10492, and EC313761 were found superior

400 for a maximum of seven traits followed by EC180063 and EC182958 for five traits. We also

401 validated high GPC accessions and EC182958 (17.16%), EC187176 and EC182945 (16.16%),

402 EC613057 (15.79%), IC634028 (15.72%) and IC533826 (15.01%) were confirmed with more

403 than 15 % GPC. The superior accessions identified in the present study could be the potential

404 genetic resource for a breeder to improve the wheat's nutritional quality. The sphaerococcum

405 wheat should be promoted for cultivation on the farmers' field considering its richness in grain

406 nutrients and end-use quality with low gluten, high protein, high iron, and zinc content, and high

407 antioxidant activity. Further, the germplasm explorer should prioritize the collection of this

408 species from unexplored areas to harness its nutritional richness for alleviating nutritional

409 imbalance and improving the health of mankind. The results of the present study showed the

410   possibility of utilization of Indian dwarf wheat in wheat quality breeding aiming for nutritional

411   security under the United Nation's sustainable development goals (SDG).

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572

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

1   **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	Gluten	Lys	Fe+2	Zn+2	PER	CAT	GR	SOD
Treatment	33	51.572**	33.693**	35.684**	774.831**	4.080**	53.221**	8.061**	47.596**	34.724**	71.924**	0.015**	906.097**	36.213**	0.049**	0.038**	0.971**	.077**
Error	68	13.109	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

3   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

4   content, Lys-Lysine content, Fe^{+2}-Iron Content, Zn^{+2}- Zinc content, POX-Peroxidase, CAT-Catalase, GR-Glutathione reductase, SOD-Superoxide dismutase

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

1 **Table 2.** Mean performance and variability for grain quality traits among 34 accessions of *T.*
2 *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[A] IC534021[B] EC187167[B] EC180067[B,C] EC613057 [B,C,D] (>36.62)
2	HW (kg/hl)	81.25	70.50-86.00	0.57	4.12	0.25	EC180066[A] EC541164[B] IC534522[B] IC212160[B,C] EC613057 (>84)
3	Sed (ml)	30.38	24.00-38.00	0.59	11.35	0.24	EC180063[A] EC182958[B] EC10492[B,C] IC534021[C] EC187176 [C] (>33)
4	HI	82.14	40.49-104.91	2.76	19.57	0.75	EC182959[A] IC533826[B] IC634028 [B,C] EC182956[C,D] EC313761 [D,E] (>94)
5	Pro (%)	17.62	15.34-19.35	0.20	6.62	0.34	EC613057[A] EC182958[B,C] EC187176[B,C,D] IC634028[B,C,D] EC182945 [B,C,D] (>19.70)
6	Alb (mg/g)	23.91	17.60-40.31	0.72	17.62	0.46	IC534522[A] EC10492[B] EC10494[B] IC53387 [C] EC187182 [C,D] (>27.80)

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

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

5 TGW-Thousand grain weight, HW-Hectolitre weight, Sed-Sedimentation value, HI-Hardness index, Pro- Protein content, Alb-Albumin content,

6 Glo-Globulin content, Gli-Gliadin content, Glu-Glutenin content, Lys-Lysine content, Fe^{+2}-Iron Content, Zn^{+2}- Zinc content, POX-Peroxidase,

7 CAT-Catalase, GR-Glutathione reductase, SOD-Superoxide dismutase, SE-Standard Error, CV-Coefficient of variation

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

Sedimentation value (cc)	Suitable for	*T. sphaerococcum* accessions
>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

Pearson correlation matrix among seventeen grain quality traits including TGW

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

	HW	Sed	HI	Pro	Alb	Glo	Gli	Glu	Gluten	Lys	Fe^{+2}	Zn^{+2}	TGW	POX	CAT	GR	SOD
HW	1																
Sed	-0.18	1.00															
HI	0.19	-0.23	1.00														
Pro	0.06	0.18	0.37*	1.00													
Alb	-0.03	0.01	0.07	-0.27	1.00												
Glo	-0.16	0.00	-0.12	0.16	-0.16	1.00											
Gli	-0.06	-0.05	-0.12	0.04	0.17	0.25	1.00										
Glu	0.11	0.00	-0.30	0.15	-0.03	-0.08	-0.13	1.00									
Gluten	0.03	-0.04	-0.31	0.14	0.12	0.15	0.73**	0.60**	1.00								
Lys	0.12	0.02	-0.20	-0.03	0.15	0.03	0.25	0.21	-0.35*	1.00							
Fe^{+2}	-0.13	0.17	-0.23	0.27	0.08	0.13	0.48**	0.18	0.52**	0.28	1.00						
Zn^{+2}	-0.22	0.10	0.55**	0.05	0.25	0.07	0.19	0.22	0.31	0.05	0.43*	1.00					
TGW	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				
POX	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			
CAT	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		
GR	-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	
SOD	-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

3 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

4 content, Lys-Lysine content, Fe^{+2}-Iron Content, Zn^{+2}- Zinc content,POX-Peroxidase, CAT-Catalase, GR-Glutathione reductase, SOD-Superoxide dismutase

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

1 **Table 5.** Categorization of *T. Sphaerococcum* accessions in low, medium, and high category for
2 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

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

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

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

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

. 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

Cluster	A	B	C	D
No. of genotypes	6	10	17	1
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
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^{2+} (ppm)	65.43	65.10	65.40	90.71
Zn^{2+} (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

Mean values of clusters for seventeen quality traits

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

Figure 1

Variability of various grain quality traits among 34 accessions of *T. sphaerococcum* for Hectolitre weight, Sedimentation value and Hardness index
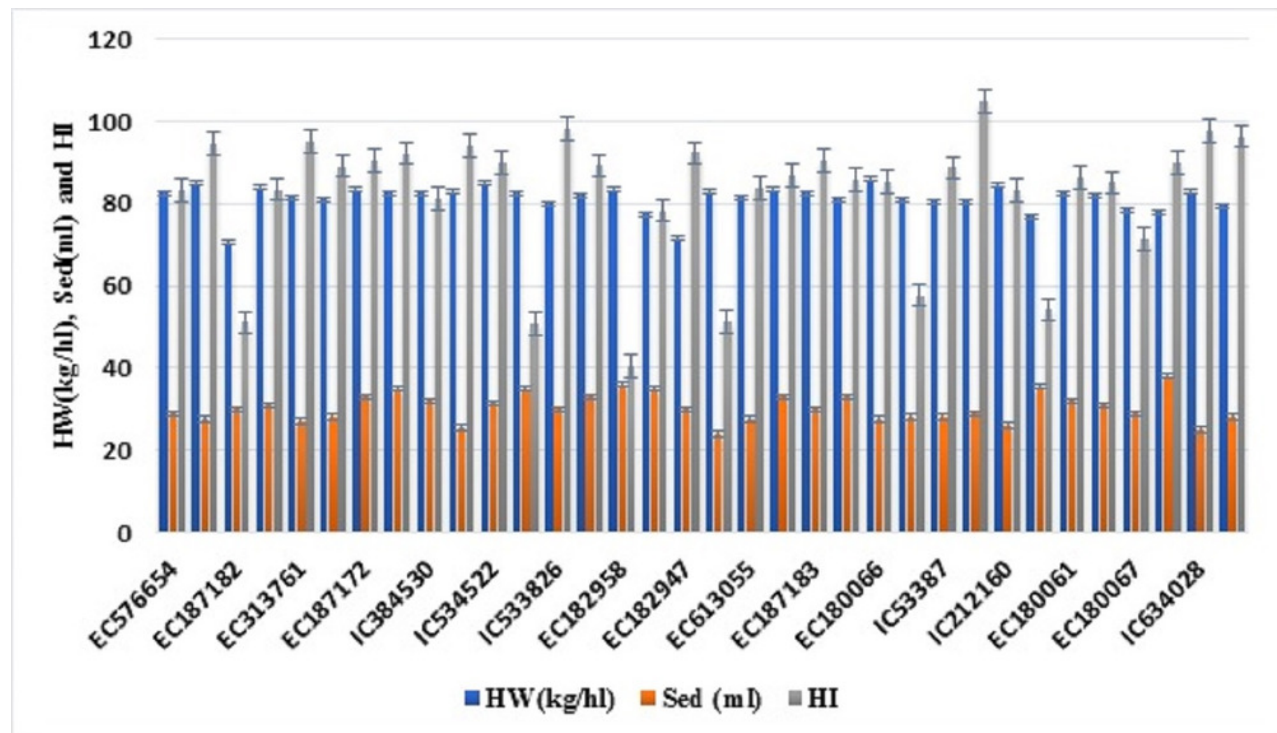
Figure 2

Variability of various grain quality traits among 34 accessions of *T. sphaerococcum* for Protein, Gluten and Lysine content
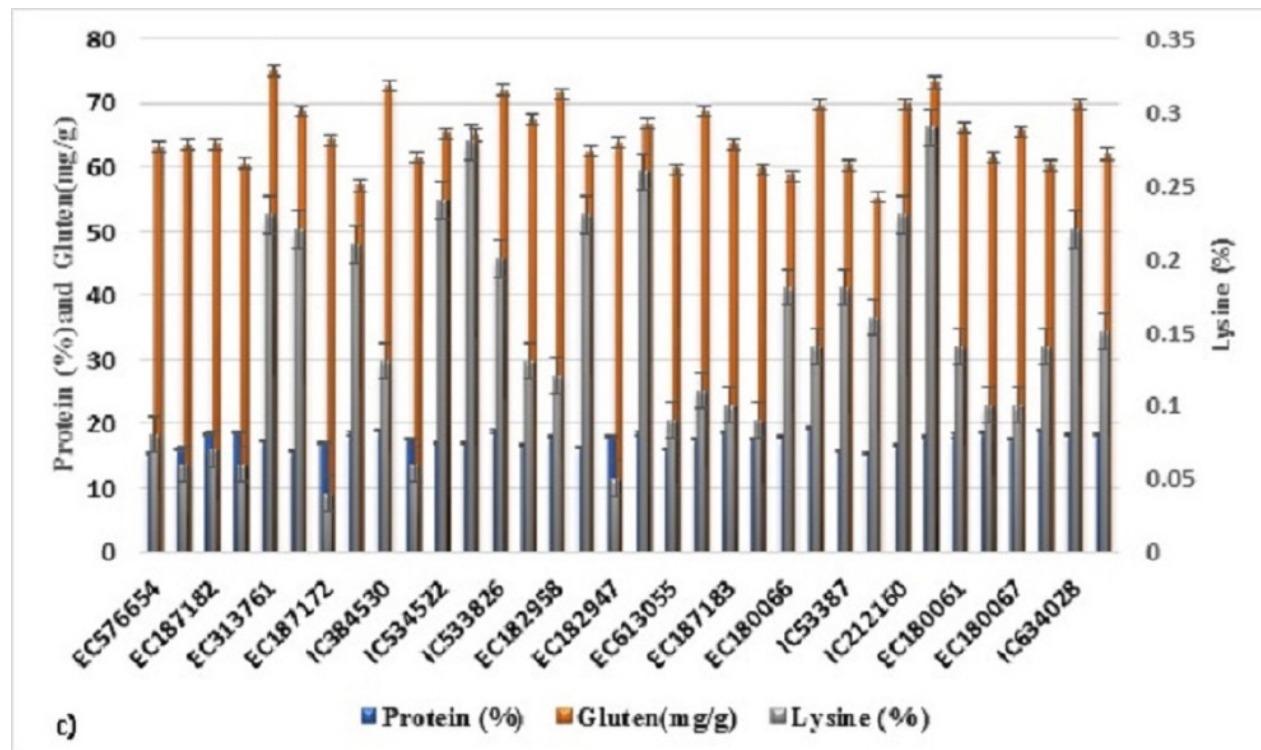
Figure 3

Variability of various grain quality traits among 34 accessions of *T. sphaerococcum* for Iron and Zinc content
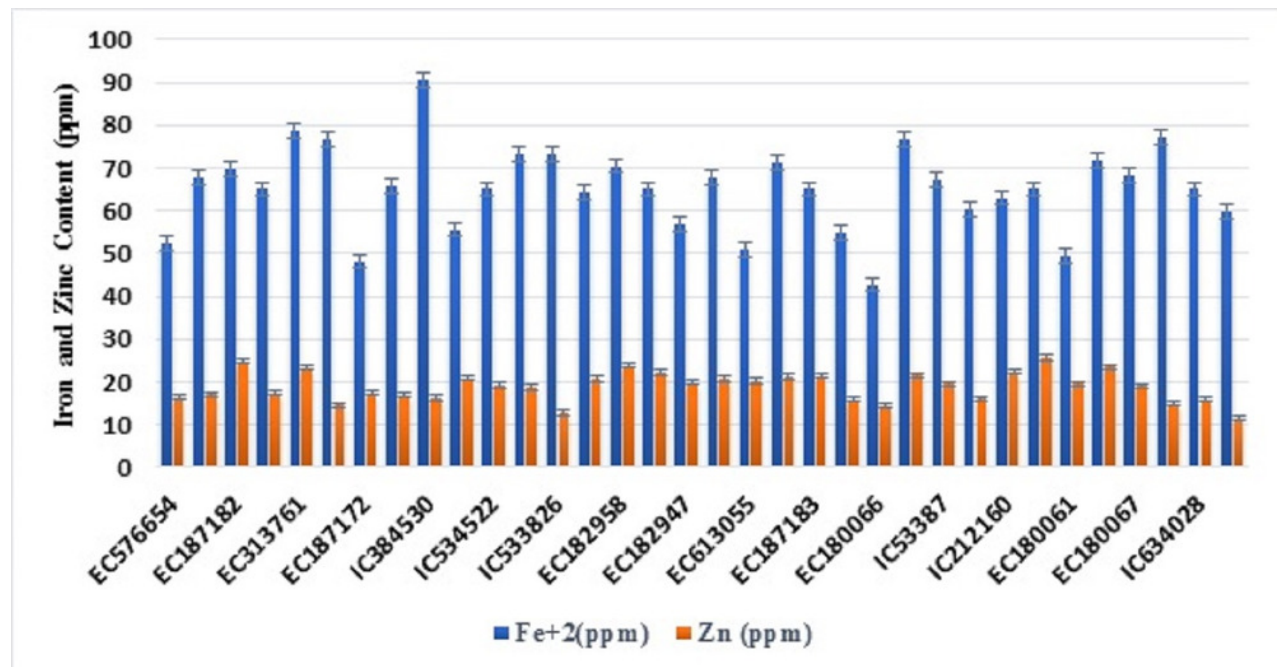
Figure 4

Proportion of four protein fractions among 34 accessions of *T. sphaerococcum*
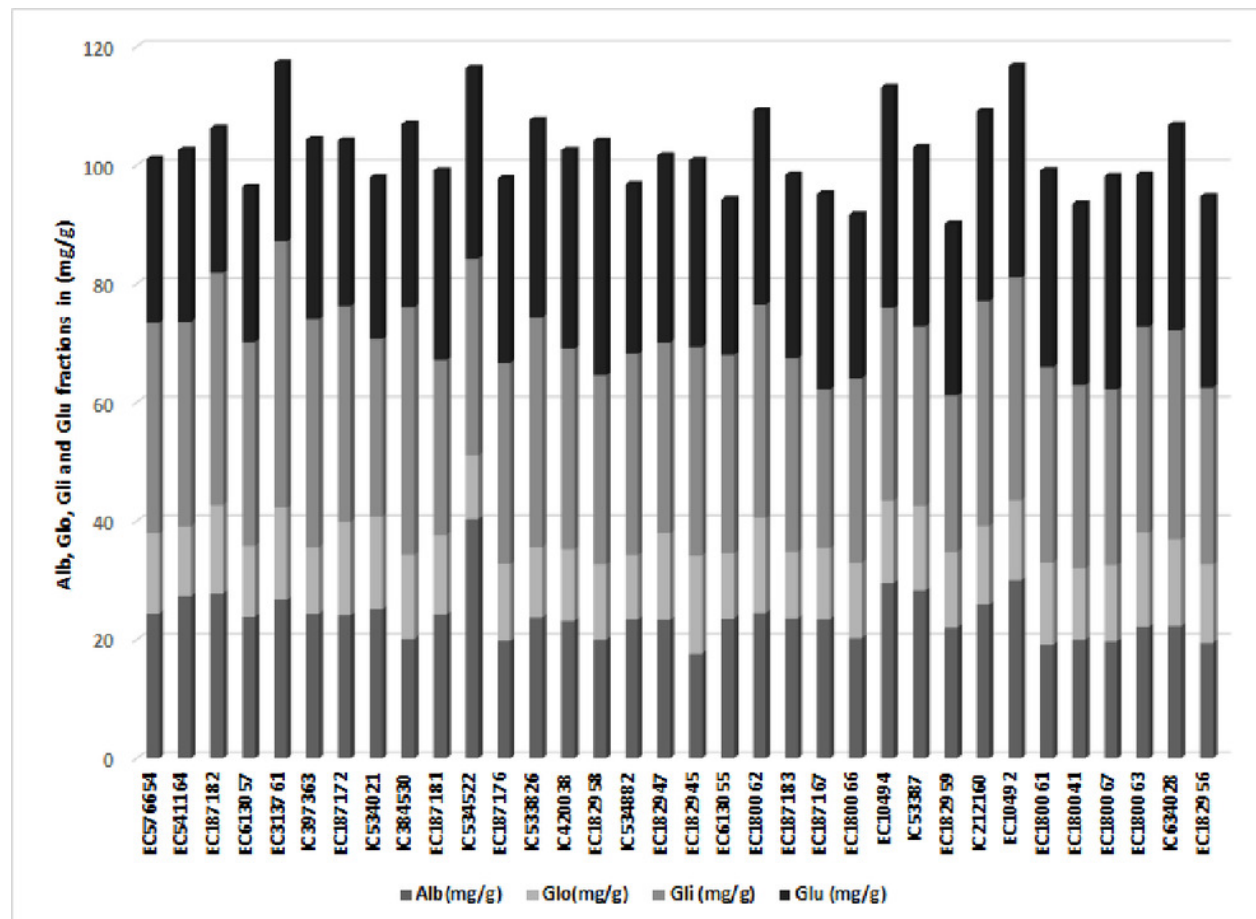
Figure 5

Clustering pattern of 34 *T. sphaerococcum* accessions based on eighteen quality traits
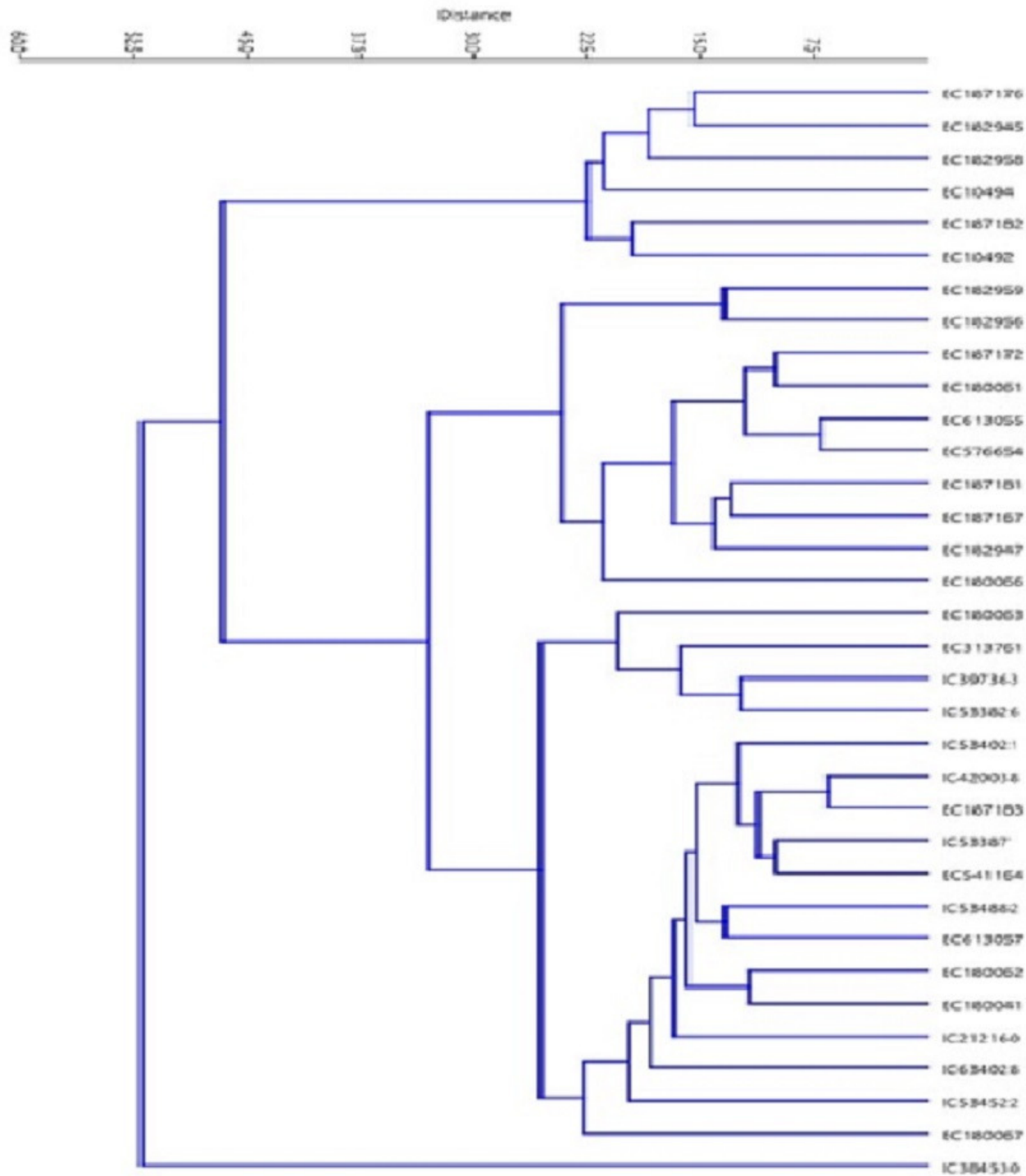
Figure 6

Biplot representing distribution pattern of 34 *T. sphaerococcum* accessions
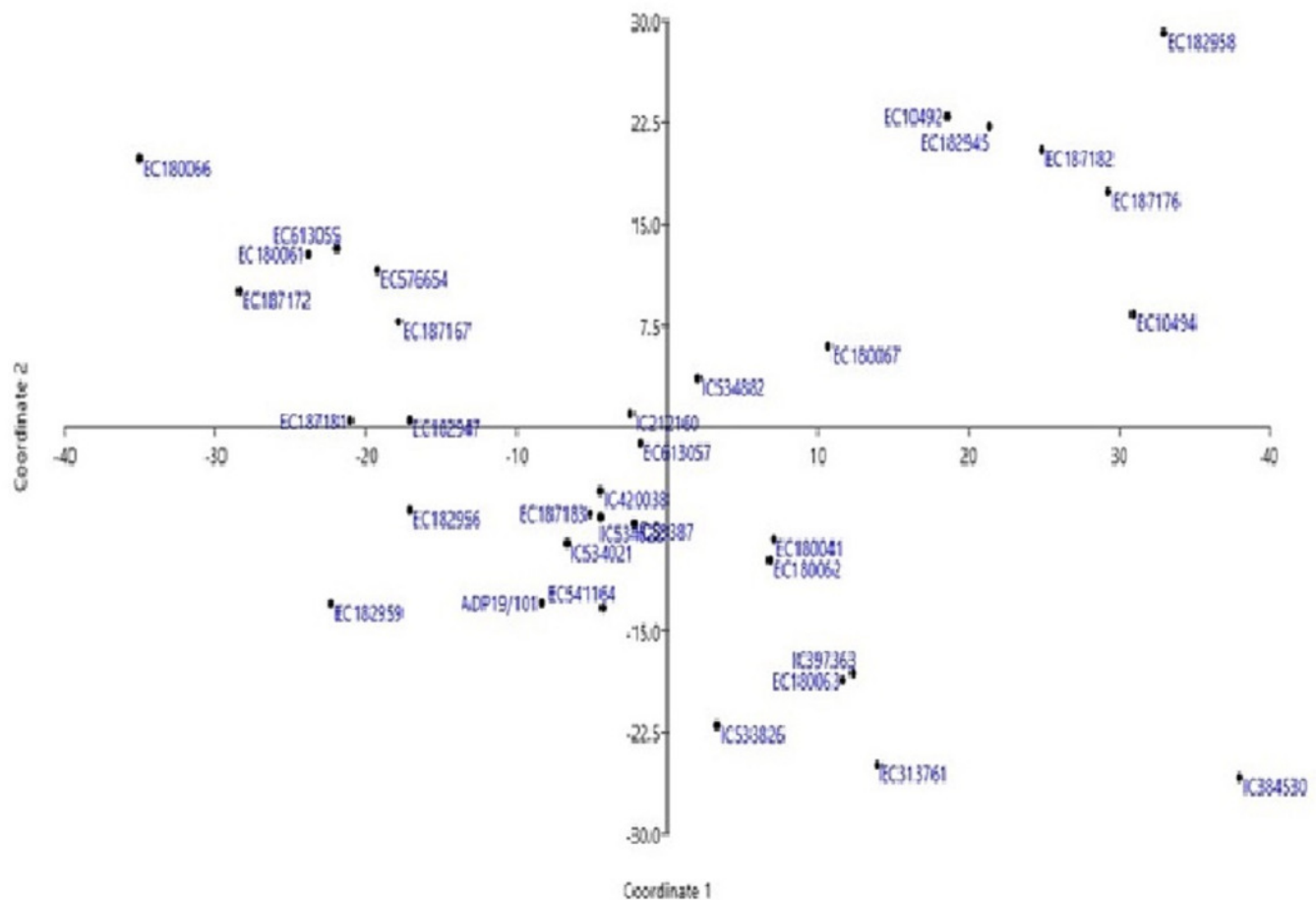
Figure 7

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