Malnutrition is a major threat to the world, especially for zinc (Zn) and iron (Fe). Breeding of wheat with increased grain Zn and Fe levels is a cost-effective, sustainable solution to malnutrition issues. Modern wheat varieties have limited variation in grain Zn and Fe. Among the wheat species, *T. dicoccum* exhibits high micronutrient variability that can be conveniently explored to improve other cultivated wheat species. Hence, the magnitude of variability for grain nutrients was studied in dicoccum wheat germplasm accessions of the local collection in Peninsular India. Grain Zn concentration ranged from 35.2 ppm to 54.0 ppm, while Fe concentration ranged from 33.8 ppm to 48.5 ppm. Wide variability was also reported for protein content (14.8% to 16.9%) and sedimentation value (22.8 ml to 41.3 ml). Moderate phenotypic and genotypic coefficients of variation were observed for the number of grains per spike, thousand grain weight, and yellow pigment. The heritability and genetic advance over mean were moderate to low for grain nutrients. In the tested material, there is a possibility to improve both Zn and Fe simultaneously as indicated by correlation values. Thus, the present study provides valuable genetic resources for grain quality parameters improvement which are associated with the quality of the end-products in Indian wheat.

Keywords: Iron, *T. dicoccum*, Variability, Zinc

1. Introduction

Micronutrient malnutrition, affects over 40 percent of the world's population, especially in many developing nations (Welch and Graham, 2002) [27]. Around one-third of humans in all age groups and populations, particularly in developing countries, especially women and children, are severely affected by a deficiency of key micronutrients, such as iron (Fe), zinc (Zn), and vitamin A (Ghandilyan et al., 2006) [6]. Traditional efforts to address the micronutrient deficiency problem have concentrated on micronutrient supplementation and food fortification (White and Broadley, 2005 and Ghandilyan et al., 2006) [28, 6]. These methods, however, have not proved sustainable, especially in developing countries where people are unable to afford high micronutrient content animal and fishery products. Instead, most people in these regions are eating cereals as the staple food that only offers a limited amount of micronutrients. A solution to mineral malnutrition called "biofortification" has been suggested in recent years (Singh et al., 2005) [21]. Bio-fortification is a process of increasing bioavailable concentrations of essential elements in the edible portions of crops through agronomic intervention or genetic selection (White and Broadley, 2005) [29].

Wheat is an important food crop grown in developed as well as developing nations (Joshi, 2007) [10]. The three species of wheat *Triticum aestivum*, *Triticum durum*, and *Triticum dicoccum* are cultivated in the country. The bread wheat is the most important species as it covers more than 90 percent of the wheat cultivated area. In general, bread wheat exhibits narrow genetic variability for grain nutrients. Tetraploids considered as one of the most promising donors to improve Zn and Fe concentrations of wheat (Cakmak et al., 2000) [4]. Durum wheat has also higher protein content than bread wheat with grain protein representing a sink for Zn and other micronutrients. On the contrary, durum wheat has limited variability. Interestingly, *Triticum dicoccum* proved to be a very good source of mineral nutrients content (Cakmak et al., 2004) [3]. This natural variation can be utilized to biofortify bread and durum wheat for Zn and Fe. In addition to this, consumer preference for richness, diversity, and
high-quality food products has increased interest in dicoccum wheat and its products (Annapurna, 2000) [1]. The products of dicoccum are more tasty and soft, have potential baking, parboiling, and popping quality. It also possesses higher content of lysine, crude fiber, and minerals concentration than bread and durum wheat (Zaharieva et al., 2010) [20]. As a consequence of these nutritional characteristics, the demand for dicoccum grain has increased over the last decade.

Genetic enhancement of crop cultivars with elevated levels of these micronutrients would be cost-effectively sustainable in solving global micronutrient malnutrition problem. Consequently, under ICAR funded project, the work was initiated to identify new genetic sources in the local collection of dicoccum germplasm for grain nutrients that are being unexplored. Further, such information may be great information to set the future path for the bio-fortification breeding program of cultivated species of wheat.

2. Materials and methods

2.1 Field Experiment

The present study included pre-tested 56 dicoccum wheat germplasm accessions, out of which, 34 local germplasm accessions, 13 advanced breeding lines, and nine checks (Table-1) were evaluated in alpha lattice design with four blocks and two replications. Each block consisted of 14 genotypes with two rows per genotype and 3-meter length with spacing of 20 cm between rows. The experiment was conducted at three distinct environments namely, All India Coordinated Wheat Improvement Project, University of Agricultural Sciences, Dharwad, Agriculture Research Station, Agricultural Research Station, Kalloli and Ugar Sugars Ltd., Ugar Khurd which are characterized by different agro-climatic zone, soil types, nutritional status and locations for studying the genetic variability for yield.

Table 1: List of Dicoccum wheat germplasms used in the investigation

| No. | Germplasm lines | S. No. | Germplasm lines | S. No. | Advance breeding lines |
|-----|-----------------|-------|-----------------|-------|-----------------------|
| 1   | DDK-50371       | 21    | DDK-50366       | 40    | DDK-50529             |
| 2   | DDK-50415       | 22    | DDK-50367       | 41    | DDK-50530             |
| 3   | DDK-50344       | 23    | DDK-50379       | 42    | DDK-50531             |
| 4   | DDK-50373       | 24    | DDK-50416       | 43    | DDK-50532             |
| 5   | DDK-50382       | 25    | DDK-50364       | 44    | DDK-50533             |
| 6   | DDK-50400       | 26    | DDK-50377       | 45    | DDK-50534             |
| 7   | DDK-50383       | 27    | DDK-50342       | 46    | DDK-50428             |
| 8   | DDK-50347       | 28    | DDK-50363       | 47    | HI 8627(d)            |
| 9   | DDK-50391       | 29    | DDK-50381       | DDK-50381 |
| 10  | DDK-50319       | 30    | DDK-50406       | 48    | DDK1025               |
| 11  | DDK-50402       | 31    | DDK-50422       | 49    | DDK 1001              |
| 12  | DDK-50380       | 32    | DDK-50388       | 50    | DDK 1029              |
| 13  | DDK-50384       | 33    | DDK-50390       | 51    | NP200                 |
| 14  | DDK-50407       | 34    | DDK-50404       | 52    | HW1098                |
| 15  | DDK-50596       | Advanced breeding lines | DKK-50396 |
| 16  | DDK-50419       | 35    | DDK-50526       | 53    | DWR 1006              |
| 17  | DDK-50420       | 36    | DDK-50330       | 54    | Bijaga yellow         |
| 18  | DDK-50370       | 37    | DDK-50426       | 55    | UAS 428               |
| 19  | DDK-50393       | 38    | DDK-50527       | 56    | Amruth                |
| 20  | DDK-50385       | 39    | DDK-50528       |       |                       |

2.2 Observations recorded

Yield, yield attributing traits viz., days to fifty percent flowering, days to maturity, number of productive tillers per meter row, plant height (cm), spike length (cm), number of grains per spike and thousand-grain weight (g), quality parameters like protein content (%), yellow pigment (ppm) and sedimentation value (ml) and micronutrient such as iron and zinc content (ppm).

2.3 Procedure for estimation of quality parameters and micronutrients (Fe and Zn)

2.3.1 Protein content (%): Protein content of the grain was analyzed by a non-destructive method using near-infrared transmittance based protein analyzer.

2.3.2 Micronutrient analysis of grains: Zn and Fe content was determined by using Atomic Absorption Spectrophotometer and expressed the concentration in ppm.

2.3.3 Sedimentation value (ml): The sedimentation value of the grain was estimated by sodium dodecyl sulfate (SDS) test by following the standard analytical procedure as described by Mishra and Gupta (1995) [15].

2.3.4 Yellow pigment (ppm): The yellow pigment in wheat grain was analyzed by the procedure as described by Mishra and Gupta (1995) [15].

2.4 Estimation of micro and macronutrients in the soil before sowing and after harvesting

The soil from each experimental plot was collected at the depth of 0 to 30 cm from three locations separately in separate bags and then 10 gm of soil from each treatment used for estimation of micro and macronutrients before sowing and after harvest of the crop. The data obtained from three locations were pooled and subjected to the biometrical analysis that included heritability and genetic advance in percent mean. Genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), broad-sense heritability (h²b) and genetic advance over a mean (GAM) were estimated by the formula suggested by Burton and De Vane (1953), Johnson et al. (1955) and Hanson et al. (1956) [2, 9, 7]. The estimate of GCV and PCV were classified as low, medium, and high (Sivasubramanian and Menon, 1973) [22]. The heritability was categorized as suggested by Robinson et al. (1949) [19]. Further, genetic advance in percent mean was classified by adopting the method of Johnson et al. (1955) [9].

3. Result and discussion

The hulled wheat, *Triticum dicoccum*, is one of the first cereals to be domesticated. By the early 20th century, high-yielding wheat varieties had almost everywhere replaced the emmer. *Triticum dicoccum* represents a very promising genetic source to increase concentrations of Zn and Fe in modern wheat cultivars. It is also a rich source of genetic diversity for some agronomically and nutritionally valuable traits, especially for amino acids and proteins (Cakmak et al. 2004) [3]. Variability study is limited in dicoccum wheat (*Triticum dicoccum*) in general, micronutrients in specific. Variability particularly decides the effectiveness of selection (Subhasschandra et al., 2009) [23]. Higher variation paves the way for crop improvement. Genetic information such as heritability and genetic advance over mean for various quality and yield contributing traits will be of great value to allow the breeder to use the best genetic stock to improve the breeding program (Kyosev and Desheva, 2015) [11].
It is interesting to note that germplasm exhibited wide variation for all the grain nutrients and agronomic traits indicating the existence of useful genetic variability among the entries studied (Table 2). Grain Zn varied by 35.21 to 54.04 ppm with a mean of 44.9 ppm while grain Fe content ranged from 33.79 to 48.53 ppm with a mean of 41.12 ppm (Table 3). Around 12 percent of the genotypes recorded higher Zn content (Fig.1a) than the best check while only 2 percent for Fe content (Fig.1b). W.r.t. to protein content, more than 50 percent of genotypes were superior to check (Fig.1c).

Further, the tested materials found to be the most potential donors for sedimentation value which is an indirect measure of gluten strength (Fig.1d) and yellow pigment (Fig.1e) most desired parameter from the consumer preference point of view. These results suggest that the studied germplasms may be a good source for grain nutrients to improve varieties with agronomic performance. However, none of the entries were having a good agronomic background. The highest Zn containing genotypes such as DDK 50388 and DDK 50344 while, DDK 50366 are being used as potential donors for tetraploid wheat improvement in UAS, Dharwad.

**Table 2: Pooled ANOVA for alpha lattice design for different quantitative traits**

| S. No. | Particulars                              | df | SS          | MSS         | F          | Mean   | SD    | SE   |
|--------|-----------------------------------------|----|-------------|-------------|------------|--------|-------|------|
| 1      | Days to 50 % flowering                  | 55 | 1281.52     | 23.3        | 21.26**    | 70.11  | 3.49  | 0.47 |
| 2      | Days to maturity                         | 55 | 1546.39     | 28.12       | 13.8**     | 103.21 | 3.96  | 0.53 |
| 3      | Plant height (cm)                        | 55 | 3549.64     | 64.54       | 33.26**    | 96.92  | 5.74  | 0.77 |
| 4      | Spike length (cm)                        | 55 | 42.61       | 0.77        | 12.4**     | 8.16   | 0.65  | 0.09 |
| 5      | No of tillers per meter row              | 55 | 14476.18    | 263.2       | 77.99***   | 132.02 | 12.57 | 1.68 |
| 6      | No of grains per spike                   | 55 | 3015.74     | 54.83       | 8.48**     | 42.42  | 5.57  | 0.74 |
| 7      | Grain yield (kg ha⁻¹)                    | 55 | 12248995    | 222709      | 53.9**     | 3791.08| 335.31| 44.81|
| 8      | Thousand grain weight (g)                | 55 | 2910.03     | 52.91       | 27.17**    | 44.27  | 5.22  | 0.70 |
| 9      | Sedimentation value (ml)                 | 55 | 1254.5      | 22.81       | 55.6**     | 33.56  | 3.39  | 0.45 |
### Table 3: Pooled mean, range, and genetic parameters for yield, yield traits and quality parameters in dicoccum wheat germplasm

| Character                 | Mean   | Range    | PCV (%) | GCV (%) | h² (%) | GA as per cent of mean |
|---------------------------|--------|----------|---------|---------|--------|------------------------|
| Days to 50% flowering     | 70.11  | 61.00 - 75.00 | 6.09    | 4.58    | 56.00  | 7.10                   |
| Days to maturity           | 103.21 | 96.50 - 113.50 | 4.48    | 3.54    | 65.00  | 5.77                   |
| Plant height (cm)          | 96.92  | 80.83 - 107.00 | 7.33    | 5.51    | 56.00  | 8.55                   |
| Spike length (cm)          | 8.16   | 6.27 - 9.40     | 12.12   | 6.37    | 27.00  | 6.91                   |
| No of tillers per row length | 132.00 | 106.00 - 168.66 | 11.01   | 9.16    | 69.00  | 15.71                  |
| Thousand grain weight (g) | 42.42  | 36.50 - 56.08   | 14.78   | 11.93   | 65.00  | 19.84                  |
| Grain yield (kg ha⁻¹)      | 44.27  | 36.50 - 61.71   | 12.69   | 11.38   | 80.00  | 21.65                  |
| Sedimentation value (ml)   | 3791.08| 3230.00 - 4608.00 | 11.30  | 8.20    | 52.00  | 12.23                  |
| Yellow pigment (ppm)       | 4.75   | 3.48 - 6.27     | 14.59   | 14.42   | 97.00  | 29.38                  |
| Protein (%)                | 16.16  | 14.78 - 16.85   | 5.42    | 2.30    | 18.00  | 2.01                   |
| Fe content (ppm)           | 41.12  | 33.79 - 48.53   | 8.13    | 5.71    | 49.00  | 8.27                   |
| Zn content (ppm)           | 44.92  | 35.21 - 54.04   | 14.29   | 8.80    | 37.00  | 11.64                  |

* Significance at 5 % level of significance ** Significant at 1 % level of significance

### 3.1 Coefficient of Variation
The range in mean values does not reflect the total variance in the material studied. Hence, the actual variance has to be estimated for the characters to know the extent of existing variability. So, the coefficient of variation (PCV and GCV) which is calculated by considering the respective means have been used for the comparison. High values of these parameters indicate wider variability and vice versa.

The quality traits like micronutrients like iron and zinc, sedimentation value, protein content, yellow pigment and other yield attributes viz., spike length, number of productive tillers per meter row length and grain yield, exhibited low to moderate PCV and low GCV indicating their lesser amenability for selection in advanced generations. This narrow variability is of course due to the selection of pretested material for high nutrients in the previous season. Similar findings were reported by earlier workers like Nazar et al. (2006), Mecha et al. (2016), Hokrani et al. (2013), Tsegaye et al. (2012), and Wahidy et al. (2016) [16, 14, 8, 25, 26].

The influence of the environment was significant in an expression of micronutrients and protein content and yield attributes such as spike length, the number of grains per spike and grain yield as revealed by wider differences between PCV and GCV. These findings are following the findings of Gashaw et al. (2010) [8], Tsegaye et al. (2012) [25], and Nakasani et al. (2013) [17]. Shimelis et al. (2016) [20] found low values for GCV and the higher difference in magnitude with the corresponding protein content in durum wheat and he suggested that trait is difficult to improve. Overall, the coefficient of variation indicated a moderate amount of variability for most of the traits except for a few traits (Table 3).

### 3.2 Heritability and genetic advance over mean
Broad sense heritability gives an idea about the portion of observed variability attributable to genetic differences. According to Johnson et al. (1955) [8], heritability estimates along with genetic gain would be more useful than the former alone in predicting the effectiveness of selecting the best individuals. Therefore, it is essential to consider the predicted genetic advance over mean along with heritability estimate as a tool in the selection program for better efficiency.

In the current study, high heritability coupled with high genetic advance over mean was recorded for quality traits like yellow pigment and yield attribute like thousand-grain weight. This indicates that there was a low environmental influence on the expression of these characters and these attributes were extremely heritable hence, one can practice selection in early generations. High heritability and genetic advance over mean for these traits were earlier reported by Tsegaye et al. (2012) [25] in durum wheat genotypes, while Mecha et al. (2016) [14] in bread wheat genotypes.

High heritability coupled with moderate genetic advance observed for a quality parameter like sedimentation value and yield attributes like the number of tillers per meter row length and grains per spike. Similar results were reported for the number of tillers per meter row length by Wahidy et al. (2016) [26], Mecha et al. (2016) [14], for sedimentation value by Nakasani et al. (2013) [17], and Hokrani et al. (2013) [8], for grains per spike by Mecha et al. (2016) [14]. However, the results are in contrast with observations made by Nazar et al. (2006) [16] and moderate heritability for the number of grains per spike was reported by Tanzeen et al. (2009) and the moderate heritability for the trait number of tillers per row meter length was reported by Manal (2009) [13].

Low to moderate heritability and low to moderate genetic advance were noticed for important quality parameters like protein content, micronutrients (iron and zinc content of the grains), and yield traits like spike length and grain yield. These results are as per Shimelis et al. (2016) [20]. Therefore, selection based on phenotypic observation alone may not be very effective for these traits. It is worthier to mention here that both Zn and Fe can be simultaneously improved as revealed by correlation value (Fig 2). A strong association of the protein with Zn and Fe was also shown by earlier worker Ortiz-Monasterial et al. (2007) [18] indicating grain protein may be a sink for Zn and Fe (Table 3).
4. Conclusions
It is concluded from the present study that the dicoccum germplasm can serve as the most potential donors for all the quality parameters including micronutrients. Further, few promising accession can be registered as national genetic stocks or identified as varieties. This is a kind of study, which indicates the possibility of exploration of the unrealized potential of ancestral species like Khapli wheat to address the global issue of malnutrition through bio-fortification.

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