

## Transfer of high grain zinc from *Triticum monococcum* and *Triticum boeoticum* to hexaploid wheat varieties and advanced lines using *T. durum* as a bridging species

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### Abstract

A set of 10 potential donors resulted from introgression of high grain zinc accessions of A genome diploid species in durum wheat background (*Triticum monococcum* acc. W49-27-1/2\*Aconchi89 and *T. boeoticum* acc. 4992/2\*PDW274) were crossed with 03 hexaploid wheat cultivars (WH 1105, DPW 621-50, HD 2967) and 02 advanced lines (PBW 698, PBW 703). A total of 1077 BC<sub>2</sub>F<sub>2</sub> derivatives from 33 of the all possible 50 crosses, were subjected to estimation of grain zinc and iron concentration, grain protein content, 1000-grain weight, plant height, days to heading, days to maturity, grain yield and significant genotypic variation was observed for all these traits. Twenty five lines from six crosses showed very high grain zinc concentration ranging from 84.1 to 93.7 ppm as compared to 53.26 to 64.50 ppm observed in the set of recipient parents. A further subset of 07 lines that displayed high grain iron concentration, protein content and 1000-grain weight, six of these lines were also found highly resistant to stripe rust and possessed desirable agronomic features and thus could be useful for further selection and direct use in varietal development. *T. monococcum* acc. W49-27-1/2\*Aconchi89 proved to be the better donor, while 02 advanced lines namely PBW 698 and PBW 703 possessing rust resistance genes *Lr24*, *Lr28*, *Yr10* and *Yr15* in PBW 343 background proved to be the most effective recipients.

**Keywords:** Grain micronutrient content, biofortification, introgression, grain protein content, backcross breeding

### 1. Introduction

Micronutrient deficiency is a public health issue which can be addressed to a significant extent by plant breeding strategies (Mishra, 2004); specially if these are applied to staple food crops like wheat. Wheat provides nearly 55 percent of carbohydrate and 20 percent of the food calories (Kumar *et al.*, 2010). Deficiencies for minerals and vitamins are widespread in the third world populations (WHO, 2002) therefore, planned improvement in two major cereals (wheat and rice) could serve as better sources of the nutritionally important micronutrients (e.g., zinc and iron) and vitamins. Biofortification is an important plant breeding objective in wheat and other important crops.

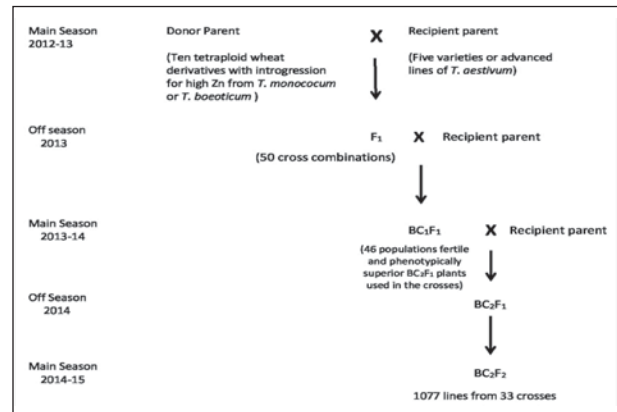
The present study focuses on grain zinc biofortification, owing to its role in nutrition as well as better amenability (as compared to iron) for genotypic analysis and transfer. An associated improvement for grain iron is generally expected (Cakmak *et al.*, 2004). Grain zinc concentration in commercial wheat cultivars generally ranges from 20-35 mg/kg (Rengel *et al.*, 1999; Cakmak *et al.*, 2004). For a measurable biological impact on human health, the concentration of zinc in whole wheat grain needs to be increased atleast by approximately 8 mg/kg, assuming a 400 g per day intake for adults in the countries where whole grain flour is used for making *chapatis* (Pfeiffer and McClafferty, 2007; <http://www.harvestplus.org>). Mega-

varieties like 'PBW 343' or currently popular varieties may be considered as suitable target material for such ameliorations (Velu *et al.*, 2012).

Identification of donor germplasm is a pre-requisite for a plant breeding intervention aimed at biofortification as cultivated wheats do not seem to possess the trait in transfer-worthy intensity. Chhuneja *et al.* (2006) reported higher grain zinc and iron concentrations in wheat wild relatives *Aegilops kotschyi* and *Ae. tauschii*. Further, Singh *et al.* (2008) observed that the diploid 'A' genome progenitor gene pool of wheat, comprising three closely related species *T. monococcum*, *T. boeoticum* and *T. urartu* harbours useful genes for many economically important traits including grain zinc. Transfer of this trait from diploid wild relatives to hexaploid wheat, however, is not a trivial task. As a first step in this process, high grain zinc from AA genome species needs to be transferred to *T. durum* (tetraploid wheat) which serves as a bridging species. Grain zinc enhanced tetraploid derivatives could then serve as suitable donors for improvement of grain zinc in hexaploid wheat varieties. Crossing with cultivated tetraploid wheat would not entail serious crossability barriers and  $F_1$  sterility, but monitoring of the trait in the segregating derivatives would again be required. The present study demonstrates these two steps and presents the outcome of this effort wherein large number of derivatives with high grain zinc concentration could be identified in agronomically promising rust resistant backgrounds.

## 2. Materials and methods

The introgression lines in tetraploid background were developed earlier (Chhuneja *et al.* unpublished) using high zinc *T. monococcum* and *T. boeoticum* accessions in crosses with durum wheat varieties. The set of  $BC_2F_7$  tetraploid lines includes eight lines (BF10, BF13, BF14, BF18, BF19, BF20, BF21 and BF22) derived from cross -*T. monococcum* acc. W49-27-1/2\*Aconchi89 and two lines BF25 and BF43) derived from cross *T. boeoticum* acc. 4992/2\*PDW274. The bridging recipients, Aconchi 89 and PDW 274 are durum wheat varieties developed at CIMMYT, Mexico and PAU, Ludhiana, respectively. These 10 introgression lines in tetraploid background were in turn used as donors for transferring high grain zinc trait to improved hexaploid wheat varieties (DPW 621-50, WH 1105 and HD 2967) and rust resistance gene pyramided version of a released variety PBW 343 (PBW 698 and PBW 703). The tetraploid donors and hexaploid recipients are listed in Table 1, along with their average grain zinc content. The breeding scheme followed to transfer high grain zinc to hexaploid wheats is given in Fig. 1. The breeding scheme entails the use of the tetraploid wheat lines as males in crosses with hexaploid wheat parents during 2012-13 crop season. The resulting pentaploid was back crossed to the hexaploid parent during the off season of 2013. Because of high sterility in pentaploid  $F_1$ , about 20 ears were used in the backcross and in most cases 25-30  $BC_1F_1$  plants became available



**Fig. 1** Strategy employed for material development and introgression of genes for grain zinc concentration from *T. durum* to *T. aestivum*

for further backcrossing. A second backcross was performed on these plants to obtain  $BC_2F_1$  generation, which was planted during off-season of 2014. A set of 1077 single plant progenies ( $BC_2F_2$ ) belonging to 33 crosses (out of 50 all possible crosses attempted between 10 tetraploid lines and five hexaploid parents) were planted during 2014-15 crop season in an augmented design based field layout. Each plot comprised of four rows of 1m length. The five hexaploid wheat recipient lines served as checks and were repeated in every block of 50 test lines.

**Table 1.** Parentage and average grain zinc concentration in the donor and recipient wheat lines used in the study

| S. No. | Donor line (Tetraploid) | Parentage                                 | Zinc conc. (ppm) |
|--------|-------------------------|---|------------------|
| 1      | BF10                    | <i>T. monococcum</i> W49-27-1/2*Aconchi89 | 66.59            |
| 2      | BF13                    | <i>T. monococcum</i> W49-27-1/2*Aconchi89 | 61.47            |
| 3      | BF14                    | <i>T. monococcum</i> W49-27-1/2*Aconchi89 | 64.91            |
| 4      | BF18                    | <i>T. monococcum</i> W49-27-1/2*Aconchi89 | 65.42            |
| 5      | BF19                    | <i>T. monococcum</i> W49-27-1/2*Aconchi89 | 56.50            |
| 6      | BF20                    | <i>T. monococcum</i> W49-27-1/2*Aconchi89 | 58.16            |
| 7      | BF21                    | <i>T. monococcum</i> W49-27-1/2*Aconchi89 | 57.40            |
| 8      | BF22                    | <i>T. monococcum</i> W49-27-1/2*Aconchi89 | 64.78            |
| 9      | BF25                    | <i>T. boeoticum</i> 4992/2*PDW274         | 54.72            |
| 10     | BF43                    | <i>T. boeoticum</i> 4992/2*PDW274         | 61.43            |

Contd.

| Recipient line (Hexaploid) |            |  |      |
|----------------------------|------------|--|------|
| 1                          | DPW 621-50 | KAUZ//ALTAR84/AOS/3/<br>MILAN/KAUZ/4/HUITES                              | 34.2 |
| 2                          | WH 1105    | MILAN/S87230//BABAX  | 40.1 |
| 3                          | PBW 698    | (PBW343+Lr24+Lr28)*3/<br>Yr10/6*AVOCET/3/<br>BW9250*3//<br>Yr15/6*AVOCET | 36.9 |
| 4                          | PBW 703    | (PBW343+Lr24+Lr28)*3/<br>Yr10/6*AVOCET/3/<br>BW9250*3//<br>Yr15/6*AVOCET | 37.1 |
| 5                          | HD 2967    | ALD/COC//URES/<br>HD2160M/HD2278   | 38.3 |

**2.1 Grain protein estimation:** Protein content was estimated using “Infratec1241” grain analyser supplied by M/S Foss Analytical AB, Sweden. The instrument uses the near infrared light transmitted through the grains. The grain samples were scanned in the range of 850 to 1050 nm with a bandwidth of 7 nm and there are 100 data points per scan. The results were displayed as percent protein content and percent moisture.

**2.2 Grain zinc and iron concentration estimation:** Grain zinc and iron concentration (ppm) were estimated using “Energy Dispersive X-ray Fluorescence” (EDXRF).

**2.3 Field observations:** Observations were recorded on days to heading, plant height (cm), days to maturity, number of tillers per metre row length, number of spikelets per spike, number of grains per spike, 1000 - grain weight (g) and plot yield. Analysis of variance for augmented block design was performed using OPSTAT statistical software.

### 3. Results and discussion

The frequency distribution of the set of 1077 BC<sub>2</sub>F<sub>2</sub> lines for grain zinc concentration (Fig. 2) shows a fairly normal distribution, (skewness (-0.032), kurtosis (0.478)). As shown in Table 1, the BC<sub>2</sub>F<sub>2</sub> progenies showed a wider spectrum

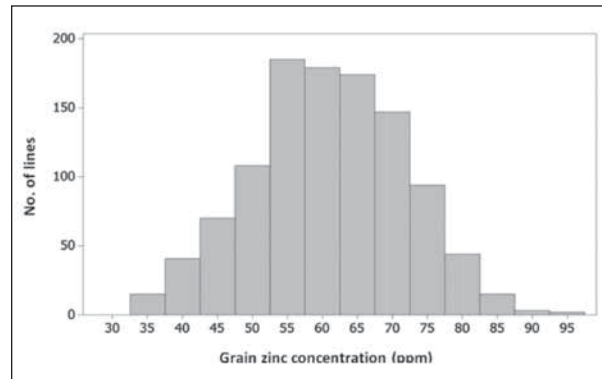


Fig. 2 Frequency distribution for grain zinc concentration in a set of 1077 BC<sub>2</sub>F<sub>2</sub> lines

of variation than the recipient parents for grain quality parameters. The protein content in the progenies ranged from 9.79 to 15.59 percent as compared to 11.18 to 12.77 percent in two hexaploid wheat parents. Similar situation was observed for the main target trait i.e., grain zinc concentration. The progenies with a range of 31.80 to 93.70 ppm for grain zinc showed clear improvement against highest value for recipient parent (66.90 ppm). Similar but moderate extension of the range in the favourable direction was also observed for grain iron concentration. Field observations on metric traits revealed significant expansion of the range in both directions for plant height, number of spikelets per spike, number of grains per spike and 1000 - grain weight (Table 2). The range for yield and yield components showed that a proportion of the derivatives were poorer than the recipient parent set. Large population size, however, provided adequate opportunity for selecting lines which combine high grain zinc concentration with other agronomic traits.

A subset of 25 lines possessing very high grain zinc concentration with grain zinc concentration cut off point of 84ppm is given in Table 3. The grain zinc concentration for these 25 lines ranged from 84.1 to 93.7 ppm. The parental checks also showed high value for zinc ranging from

**Table 2.** Agronomic and quality traits recorded on the set of BC<sub>2</sub>F<sub>2</sub> progenies

| Traits                         | BC <sub>2</sub> F <sub>2</sub> progenies |             |       | Recipient parent |      |
|--------------------------------|--|-------------|-------|------------------|------|
|                                | Range                                    | Mean        | CD    | Range            | Mean |
| Days to heading (days)         | 80-103                                   | 84.77±1.28  | 1.12  | 82-92            | 88   |
| Days to maturity (days)        | 120-126                                  | 127.45±0.39 | 0.68  | 122-132          | 127  |
| Plant height (cm)              | 48-97                                    | 82.39±0.75  | 1.72  | 68-92            | 83   |
| No. of tillers per meter       | 65-219                                   | 101±5.86    | 11.72 | 158-255          | 105  |
| No. of spikelets per spike     | 13-27                                    | 17.90±0.60  | 1.10  | 17-23            | 18   |
| No. of grains per spike        | 20-68                                    | 40.93±1.38  | 1.66  | 48-62            | 42   |
| 1000 - grain weight (g)        | 25-46                                    | 35.42±0.66  | 1.33  | 34-42            | 36   |
| Grain yield (g)                | 40-350                                   | 158.26±5.36 | 18.77 | 220-360          | 208  |
| Grain protein content          | 9.79-15.59                               | 12.58±0.15  | 0.31  | 11.18-12.77      | 12   |
| Grain zinc concentration (ppm) | 31.80-93.70                              | 60.89±1.44  | 3.12  | 31.00-66.90      | 61   |
| Grain iron concentration (ppm) | 29.40-61.00                              | 39.51±0.92  | 4.09  | 30.70-48.70      | 41   |

53.26 to 64.50 ppm. This is in contrast to the observations made in an earlier season, wherein the recipient parent set varied for grain zinc concentration from 32.4 to 40.1 ppm and the highest donor stock value was 66.59 ppm. The improvement over checks in both situations is of a similar order. The analysis of variance for grain zinc

content (based on repeats of the recipient parent set) is able to resolve fine genotypic differences and is indicative of a strong genotypic basis of the observed variation. High basal grain zinc levels in the main experiment is probably due to high zinc (ppm) in the soil and may not detract much from the genotypic rankings, critical for selection.

**Table 3.** Grain attributes of promising BC<sub>2</sub>F<sub>2</sub> progenies for grain zinc concentration

| Cross                 | Progeny no. | 1000-grain weight (g) | Protein (%) | Grain zinc conc. (ppm) | Grain iron conc. (ppm) |
|-----------------------|-------------|-----------------------|-------------|------------------------|------------------------|
| WH1105/BF10//2*WH1105 | 793         | 40                    | 13.11       | 85.3                   | 38.5                   |
| WH1105/BF20//2*WH1105 | 868         | 34                    | 15.69       | 90.8                   | 45.7                   |
|                       | 948         | 42                    | 12.96       | 84.1                   | 41.6                   |
| PBW698/BF10//2*PBW698 | 999         | 42                    | 12.71       | 86.7                   | 45.9                   |
|                       | 1000        | 45                    | 12.72       | 84.4                   | 52.6                   |
| PBW698/BF22//2*PBW698 | 1071        | 43                    | 12.30       | 86.3                   | 44.9                   |
|                       | 1083        | 45                    | 12.70       | 88.8                   | 44.7                   |
|                       | 1089        | 42                    | 13.00       | 89.8                   | 43.3                   |
|                       | 1093        | 43                    | 12.83       | 87.4                   | 40.9                   |
|                       | 1098        | 41                    | 12.64       | 84.4                   | 41.4                   |
|                       | 1133        | 40                    | 13.87       | 84.4                   | 43.7                   |
|                       | 1137        | 38                    | 13.91       | 84.9                   | 41.3                   |
|                       | 1138        | 47                    | 14.61       | 84.7                   | 44.5                   |
|                       | 1139        | 44                    | 14.66       | 86.4                   | 39.1                   |
|                       | 1149        | 43                    | 14.20       | 93.7                   | 47.4                   |
|                       | 1156        | 39                    | 14.20       | 86.1                   | 44.6                   |
|                       | 1157        | 40                    | 14.04       | 85.9                   | 43.8                   |
|                       | 1167        | 41                    | 13.89       | 84.2                   | 46.8                   |
|                       | 1169        | 41                    | 14.12       | 93.2                   | 48                     |
|                       | 1170        | 39                    | 14.64       | 84.9                   | 44.1                   |
|                       | 1171        | 41                    | 13.99       | 84.3                   | 42.7                   |
| PBW703/BF14//2*PBW703 | 1172        | 38                    | 14.57       | 84.2                   | 44.4                   |
|                       | 1176        | 45                    | 12.98       | 84.3                   | 40.5                   |
|                       | 1186        | 42                    | 14.16       | 89.7                   | 42.6                   |
| PBW703/BF21//2*PBW703 | 1190        | 42                    | 13.03       | 84.5                   | 45.6                   |
| Checks                | DPW621-50   | 35                    | 11.08       | 53.26                  | 40.40                  |
|                       | WH1105      | 36                    | 11.43       | 63.63                  | 41.61                  |
|                       | PBW698      | 38                    | 11.99       | 64.50                  | 42.12                  |
|                       | PBW703      | 36                    | 12.51       | 63.52                  | 42.10                  |
|                       | HD2967      | 38                    | 12.02       | 54.52                  | 38.48                  |
| CD (0.05)             |             | 1.33                  | 0.31        | 3.12                   | 4.09                   |

The overall worth of the lines with high grain zinc (>84 ppm) content can be assessed on the basis of other grain attributes as well as agronomic traits. High grain zinc must not be caused by grain shrivelling and it should be accompanied by high grain protein content as well as iron concentration. A few of the selected subset of lines possess this combination of traits (Table 3). Progeny number 999 and 1000 derived from cross PBW698/BF10//2\*PBW698 possess 47.4 and 52.6ppm grain iron concentration, 45g

and 43g 1000 - grain weight and a protein content of 14.20 and 12.72 percent, respectively. Similarly progeny numbers 1071 (44.9 ppm iron concentration, 43g 1000 - grain weight, 12.30 percent grain protein), 1083 (44.7ppm, 45g, 12.70percent), 1138 (44.5ppm, 47g, 14.61percent) and 1149 (47.4ppm, 43g, 14.2percent) carry a desirable combination of traits and emanate from the cross PBW698/BF22//2\*PBW698. Besides, progeny no. 1190 from PBW703/BF21//2\*PBW703 cross possess 45.6ppm

grain iron, 42g 1000 - grain weight and 13.3 percent grain protein content. All these lines except progeny number 1138 showed high level of resistance to stripe rust (Table 4). The plant height and days to maturity for selected lines

was in the acceptable range. Yield and yield components were not adversely affected in the demarcated progenies superior for grain micronutrients and protein content (Table 4).

**Table 4.** Resistance to stripe rust and other traits recorded on promising BC<sub>2</sub>F<sub>2</sub> progenies (with >84 ppm grain zinc concentration)

| Cross                 | Progeny No.           | Stripe rust score | Days to heading (days) | Plant height (cm) | No. of grains per spike | Grain yield (g/2 meters rows) |     |
|-----------------------|-----------------------|-------------------|------------------------|-------------------|-------------------------|-------------------------------|-----|
| WH1105/BF10//2*WH1105 | 793                   | 10S               | 84                     | 88                | 33                      | 180                           |     |
|                       | 868                   | tS                | 93                     | 87                | 35                      | 140                           |     |
| WH1105/BF20//2*WH1105 | 948                   | tS                | 91                     | 79                | 43                      | 190                           |     |
|                       | 999                   | tS                | 89                     | 87                | 47                      | 270                           |     |
| PBW698/BF10//2*PBW698 | 1000                  | tS                | 89                     | 79                | 37                      | 170                           |     |
|                       | 1071                  | tS                | 85                     | 78                | 31                      | 210                           |     |
|                       | 1083                  | tS                | 87                     | 77                | 35                      | 210                           |     |
|                       | 1089                  | tS                | 85                     | 86                | 50                      | 260                           |     |
|                       | 1093                  | tS                | 88                     | 76                | 33                      | 220                           |     |
|                       | 1098                  | tS                | 87                     | 80                | 43                      | 220                           |     |
|                       | 1133                  | 10S               | 87                     | 93                | 39                      | 160                           |     |
|                       | 1137                  | 10S               | 87                     | 87                | 39                      | 140                           |     |
|                       | PBW698/BF22//2*PBW698 | 1138              | 20S                    | 87                | 87                      | 38                            | 220 |
|                       |                       | 1139              | tS                     | 91                | 85                      | 38                            | 180 |
| 1149                  |                       | 5S                | 87                     | 88                | 48                      | 140                           |     |
| 1156                  |                       | 20S               | 87                     | 84                | 32                      | 180                           |     |
| 1157                  |                       | 20S               | 88                     | 86                | 37                      | 140                           |     |
| 1167                  |                       | tS                | 87                     | 81                | 40                      | 170                           |     |
| 1169                  |                       | tS                | 87                     | 79                | 43                      | 220                           |     |
| 1170                  |                       | tS                | 80                     | 82                | 46                      | 190                           |     |
| 1171                  |                       | tS                | 88                     | 80                | 36                      | 150                           |     |
| 1172                  |                       | tS                | 89                     | 82                | 37                      | 160                           |     |
| PBW703/BF14//2*PBW703 | 1176                  | 5S                | 87                     | 80                | 41                      | 250                           |     |
|                       | 1186                  | tS                | 88                     | 74                | 34                      | 140                           |     |
| PBW703/BF21//2*PBW703 | 1190                  | tS                | 89                     | 84                | 35                      | 155                           |     |
|                       | DPW621-50             | 60S               | 86                     | 82                | 37                      | 242                           |     |
|                       | WH1105                | 40S               | 86                     | 87                | 41                      | 237                           |     |
| Checks                | PBW698                | 5S                | 88                     | 77                | 36                      | 183                           |     |
|                       | PBW703                | tS                | 88                     | 78                | 41                      | 187                           |     |
|                       | HD2967                | 80S               | 87                     | 77                | 39                      | 230                           |     |
| CD (0.05)             |                       |                   | 1.12                   | 1.72              | 1.66                    | 5.97                          |     |

The correlation coefficients (Table 5) revealed that grain zinc concentration is significantly correlated with grain iron concentration (0.393\*\*). Correlations of grain zinc and iron with other traits show a parallelism.

A pattern of donors and recipients is evident in the progenies showing high grain micronutrient content. Out of the subset of 25 high value lines, 22 have the gene

pyramided versions of PBW 343 (i.e., PBW 698 and PBW 703) as the recipient parent. On the donor side, the entire selected subset *T. monococcum* introgressions rather than the *T. boeoticum* introgression lines. It is typical of crop improvement situations for almost all traits that some parental combinations do better than others. Thus the wide base represented by ten donor lines representing two crosses and five recipient lines representing four

**Table 5.** Phenotypic correlation coefficients among traits recorded on a set of 1077 BC<sub>2</sub>F<sub>2</sub> progenies

|                          | Days to heading | Days to maturity | No. of grains / spike | Plant height | No. of spikelets/ spike | 1000 - grain weight | Grain protein content | Grain zinc conc. | Grain iron conc. |
|--------------------------|-----------------|------------------|-----------------------|--------------|-------------------------|---------------------|-----------------------|------------------|------------------|
| Days to heading          | 1.00            | 0.002            | 0.01                  | -0.05        | -0.02                   | 0.17**              | 0.27**                | 0.15**           | 0.06*            |
| Days to maturity         |                 | 1.00             | 0.05                  | -0.04        | 0.07*                   | 0.02                | 0.20**                | 0.06             | -0.05            |
| No. of grains /spike     |                 |                  | 1.00                  | 0.10**       | 0.47**                  | -0.09**             | 0.009                 | 0.02             | -0.06*           |
| Plant height             |                 |                  |                       | 1.00         | 0.14**                  | -0.08*              | 0.05                  | -0.34**          | -0.17**          |
| No. of spikelets/ spike  |                 |                  |                       |              | 1.00                    | -0.12**             | -0.01                 | -0.16**          | -0.27**          |
| 1000 - grain weight      |                 |                  |                       |              |                         | 1.00                | 0.14**                | 0.40**           | 0.19**           |
| Grain protein content    |                 |                  |                       |              |                         |                     | 1.00                  | 0.26**           | 0.27**           |
| Grain zinc concentration |                 |                  |                       |              |                         |                     |                       | 1.00             | 0.39**           |
| Grain iron concentration |                 |                  |                       |              |                         |                     |                       |                  | 1.00             |

different crosses augured well for identification of promising derivatives. It was also imperative to generate a large progeny set and evaluate it at an appropriate stage of backcrossing. In the present study this was made possible by the rapid, non-destructive analysis using the XRF equipment. Grain zinc and iron concentrations represent traits which are genetically complex and environmentally labile. Nor do these offer ease of scale and screening associated with morphological or disease reaction traits. The genetic improvement for grain micronutrient is further complicated by the fact that the promising donors are mostly non adapted, wild or exotic materials. All these factors tend to make the progress for this trait very slow or even altogether elusive. Given this context, the present study covers only the initial but critical steps of bringing the donor complement into a commercially relevant background, besides sieving through a large population and narrowing down to a highly relevant subset. Smaller number and advancing homozygosity can now facilitate more intensive and accurate screening for traits of interest. A strong genetic element is evident from the genotypic pattern of donors and recipients. The large gap between grain zinc and iron enhancement is evident in the study and a matter of more intensive further investigation. Despite this gap the correlation matrix revealed an underlying unity with these two traits not only showing positive association with each other but also a close parallelism in their association with all other traits.

While wild progenitor species have been identified as potential donors (Chhuneja *et al.*, 2006, Singh *et al.*, 2008), relatively few studies which tap this potential are yet available. In one such study, *Ae. longissima* (2n=14, S<sub>1</sub>S<sub>1</sub>) accession 3506 was used as donor parent for transferring grain zinc concentration and other micronutrients to elite hexaploid wheat (*T. aestivum*) cultivars through wide hybridization (Kumari *et al.*, 2013). The progenitor wild species with wheat genomes (A, B/S, D) offer better

scope of transfer of this trait into agronomically desirable backgrounds on account of chromosomal homology and free recombination. The choice of A genome species donors in the present study is in line with this consideration. The use of a tetraploid bridge, representing an additional first step, is however almost unavoidable in light of extremely low success of crosses between diploid A genome species and hexaploid wheat.

The use of released, popular varieties as recipients is a common strategy but the entire derived material runs the risk of becoming susceptible in the event of emergence of new pathotypes of prevalent diseases. The present study thus used two rust resistant gene pyramided versions (PBW 698 and PBW 703) of cultivar PBW 343 along with other popular varieties as recipients. These recipient lines possess *Lr24*, *Lr 28*, *Yr10* and *Yr 15*. Two backcrosses are likely to have retained this gene combination in majority of the selected rust resistant lines which now need to be monitored through tagged molecular markers. The potential of gene pyramided lines as recipients is evident from the large proportion of their derivatives in the selected subset. This is also clear from the fact that none of the derivatives involving stripe rust susceptible recipients, DPW 621-50 and HD 2967 could make it to the selected subset. It is stated that, one or two further cycle of intense selection are likely to deliver competitive material with high grain micronutrient content.

## References

1. Cakmak I, A Torun, E Millet, M Feldman, T Fahima, A Korol, E Nevo, HJ Braun and H Ozkan. 2004. *Triticum dicoccoides*: an important genetic resource for increasing zinc and iron concentration in modern cultivated wheat. *Soil Science Plant Nutrition* 50: 1047-1054.
2. Chhuneja P, HS Dhaliwal, NS Bains and K Singh. 2006. *Aegilops kotschy* and *Aegilops tauschii* as sources

- for higher levels of grain Iron and Zinc. *Plant Breeding* **125**: 529-531.
3. Kumar P, RK Yadava, B Gollen, S Kumar, RK Verma and S Yadav. 2010. Nutritional Contents and Medicinal Properties of Wheat: A Review. *Life Science and Medicine Research* **2011**: 1-10.
  4. Kumari N, N Rawat, VK Tiwari, N Ghandhi, CA Patokar, S Kumar, SK Tripathi, GS Randhawa, R Prasad and HS Dhaliwal. 2013. Development and molecular characterization of wheat- *Aegilops longissima* derivatives with high grain micronutrients. *Australian Journal of Crop Science* **7**(4): 508-514.
  5. Mishra BK, RK Sharma and S Nagarajan. 2004. Plant breeding: A component of public health strategy. *Current Science* **86** (9): 1210-1215.
  6. Pfeiffer WH and B McClafferty. 2007. HarvestPlus: breeding crops for better nutrition. *Crop Science* **47**: 88-105.
  7. Rengel Z, GD Batten and DE Crowley. 1999. Agronomic approaches for improving the micronutrient density in edible portions of field crops. *Field Crops Research* **60**: 27-40.
  8. Singh K, P Chhuneja, S Kaur, S Kaur, T Garg, VK Tiwari, N Rawat, NS Bains, HS Dhaliwal and B Keller. 2008. *Triticum monococcum*: A source of novel genes for improving several traits in hexaploid wheat. Published In: Exploring and Exploiting Wheat Genetic Resources, The 11<sup>th</sup> International Wheat Genetics Symposium edited by Rudi Appels Russell Eastwood Evans Lagudah Peter Langridge Michael Mackay Lynne. Sydney University Press. <http://hdl.handle.net/2123/3430>.
  9. Velu G, RP Singh, H Espino, RJ Pena, AB Singh, A Mahendru, YM Mujahid, VS Sohu, G S Mavi, J Crossa, G Alvarado, AK Joshi and WH Pfeiffer. 2012. Performance of bio-fortified spring wheat genotypes in target environments for grain zinc and iron concentrations. *Field Crop Research* **137**: 261-267.
  10. WHO. 2002. Reducing risk and promoting healthy life. The world health report. *The world health organization*, Geneva, Switzerland.