Development and molecular characterization of wheat- *Aegilops longissima* derivatives with high grain micronutrients

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**Abstract**

Developing food crops with enhanced mineral concentrations is one of the most sustainable and cost effective approaches for alleviation of micronutrient. This article aims at development and molecular characterization of wheat- *Aegilops longissima* derivatives with high grain micronutrients (iron, zinc, copper, manganese, calcium, magnesium and potassium). *Aegilops longissima* (2n=14, SS) accession 3506 with high grain micronutrients was used for transferring these traits to elite wheat (*Triticum aestivum*) cultivars through wide hybridization. The fertile HD2687/L3506/WL711 BC₂F₂ derivatives were developed through selfing and selection for chromosome constitution, meiotic stability and micronutrient concentrations was done at each generation. Sixteen derivatives were finally selected and characterized. The selected backcross derivatives showed enhanced grain iron, zinc, copper, manganese, calcium, magnesium and potassium concentrations over the parental wheat cultivars by up to 183.6%, 243.6%, 135.18%, 160.42%, 223.29%, 43.90% and 35.05%, respectively. Introggression of chromosomes 2, 7 and 1 from *Ae. longissima*, confirmed by plant waxiness, GISH, anchored wheat SSR markers and HMW glutenin subunit profiling and was found to be associated with enhanced micronutrients in the derivatives.  

**Key words:** Alien introgression; Biofortification; Grain iron; Grain zinc; Protein.

**Abbreviations:** IARC- International Agricultural Research Centers, CGIAR- Consultative Group Of International Agricultural Research, DAPI- 4-, 6-diamidino- 2-phenylindole, GISH- genomic *in situ* hybridization.

**Introduction**

More than two billion people in the developing countries, depending largely on cereal and tuber crops as their staple food, suffer from iron and zinc deficiency commonly called as “hidden hunger” (Welch and Graham, 2004; White and Broadley, 2009). The micronutrient malnutrition results in poor mental and physical development and increased rate of mortality and morbidity (Holz and Brown, 2004; Cakmak, 2008). Among various approaches, biofortification is considered as the most promising, cost effective and sustainable approach for alleviation of micronutrient malnutrition (Ortiz-Monasterio et al., 2007; Bouis and Welch, 2010). Various collaborative projects for the enhancement of grain micronutrients of rice, wheat, maize, cassava, beans and sweet potato have been initiated by HarvestPlus (www.harvestplus.org), IARCs of CGIAR (www.cgiar.org), and national organizations in different parts of the world (Giménez-Galera et al., 2010). Wheat is the second most important cereal crop in terms of area and food source providing approximately 60% of the daily calorie intake in several developing countries of the world (FAOSTAT 2008; http://faostat.fao.org. Chatzav et al., 2010). Therefore, biofortification of minerals within wheat grain itself will have a positive impact on human health. Most of the wheat cultivars have very low grain micronutrient concentration and genetic variability (Monasterio and Graham, 2000; Peleg et al., 2009; Rawat et al., 2009a, b). However, a wide range of variation for grain micronutrients has been observed among wild relatives of wheat (Cakmak et al., 2004; Chhuneja et al., 2006; Chatzav et al., 2010). The useful variability for grain micronutrients has recently been transferred from some non- progenitor *Aegilops* species to elite wheat cultivars (Neelam et al., 2010; Tiwari et al., 2010). This article deals with the introgression and molecular characterization of wheat- *Ae. longissima* derivatives with high grain micronutrients.

**Materials and methods**

**Plant Materials**

*Aegilops longissima* (S¹S²) accession 3506 with very high grain iron (59.1 mg/kg) and zinc (45.0 mg/kg) concentrations (Table 2, Rawat et al., 2009a) was obtained from the wheat germplasm collection of Punjab Agricultural University,
Ludhiana, India. It was crossed as male parent with an elite wheat (Triticum aestivum L.) cultivar HD2687 as the female parent in 2006-07. The F1 hybrids were partially fertile due to unreduced gamete formation giving a spontaneously developed octoploid amphiphil (AABBDDS\(^5\)). The amphiphil was backcrossed with another wheat cultivar WL 711 to get two viable BC\(_1\) seeds in 2007-08. The BC\(_1\) plants (AABBDDS\(^5\)) were allowed to self to have eleven BC\(_2\)F\(_2\) and sixteen BC\(_2\)F\(_3\) backcross derivatives. The BC\(_2\)F\(_3\) progenies were grown along with their parents in the field at Indian Institute of Technology, Roorkee in 2009-10, in rows of 2 m length, with plant to plant distance of 10 cm and row to row spacing of 30 cm with recommended fertilizer and irrigation practices as that of wheat cultivars (Package of Practices, PAU, Ludhiana). The BC\(_2\)F\(_3\) derivatives (Table 1, Table2) were analyzed (in six replications) for grain micronutrient concentrations and characterized for morphological traits, chromosome number and pairing, GISH, and SSR markers. The details of the plant materials used are given in Table1.

**Morphological traits**

The data on morphological traits on 3-22 plants of different BC\(_2\)F\(_1\) derivatives such as plant waxiness, head type, rachis toughness, grain color, number of seeds per spike was recorded in the field (Table 1).

**Cytological studies**

Comprehensive meiotic analysis of BC\(_1\)F\(_2\) and BC\(_2\)F\(_1\) plants for chromosome number and pairing was done according to the method described by Rawat et al. (2009a).

**Micronutrients analysis**

For micronutrient analysis, whole grain samples of BC\(_2\)F\(_3\) derivatives were digested and analyzed according to the protocol described by Rawat et al. (2009a). A minimum of three replications of micronutrient analysis were made for each of the derivatives, cultivars and Ae. longissima accession and ultrapure grade chemicals for digestion after standard. All of the standards used in this study were from Merck, Germany.

**Statistical analysis**

Student t- test was applied for testing the significance of differences among means of cultivars, Ae. longissima and BC\(_2\)F\(_3\) derivatives.

**In situ hybridization**

Three of the selected BC\(_2\)F\(_3\) derivatives viz. 79-2-1-4, 79-2-1-25 and 79-1-5-5 were subjected to GISH analysis for characterization of alien introgression. Actively growing root tips from germinating seeds were treated for 24 h with ice water to accumulate metaphases and then fixed in 3:1 ethanol: glacial acetic acid. The root tips were stained in 1% aceticarmine and squashed in 45% acetic acid. The genomic probe for subsequent use in genomic in situ hybridization (GISH) experiments was prepared using sheared genomic DNA (0.2–0.6 kb) of Ae. longissima (SISI). The S\(^2\)-genome genomic DNA was labeled with tetramethylrhodamine-5-dUTP (red) (Roche Applied Science, Indianapolis, IN) using nick translation following manufacturer’s direction. Labeled probes were purified using QIAquick Nucleotide Removal Kit (Qiagen, Valencia, CA). In order to prevent the hybridization of labeled genomic probe with wheat chromosomes, unlabeled sheared genomic DNA of Chinese Spring wheat (100 bp–1 kb) was used as blocking DNA in a ratio of 1 ng labeled probe (S\(^2\)-genome): 100 ng of blocking DNA. Hybridization conditions, post-hybridization washes and imaging were as described by Zhang et al. (2001). Chromosomes were counterstained with 4-, 6-diamidino-2-phenylindole (DAPI). Slides were analyzed with an epifluorescence Zeiss Axioimager M1 microscope.

**Molecular analysis**

The DNA was extracted from 4-5 gram of young leaves of the parents and selected BC\(_2\)F\(_1\) plants during early tillering stage and PCR was carried out according to Tiwari et al. (2010). For the characterization of alien introgression, the wheat anchored microsatellite markers at the distal positions of each of the 42 chromosome arms, transferable and polymorphic between the T. aestivum parents and Ae. longissima 3506 were applied on the selected derivatives with high grain micronutrients content. Further, Chromosome arm specific molecular markers gwm102 (2DS), gdm 148 (2DL) and gdm 539 (2DL) and wmc 479 (7AS), gwm 350 (7AS), wmc 139 (7AL), wmc 809 (7AL) were applied for confirming the introgression of alien group 2 and 7 chromosomes of Ae. longissima in the selected derivatives.

**HMW glutenin subunit profiling**

SDS–PAGE of high molecular weight (HMW) glutenin subunits of endosperm proteins of mature and dried seeds of parents and the selected derivatives was done using 10% acrylamide following the method of Smith and Payne (1984).

**Results**

**Morphological traits**

The data of various morphological traits of parental lines and sixteen fertile BC\(_2\)F\(_3\) derivatives is given in Table 1. All of the selected plants were non-waxy with spelta head except the BC\(_2\)F\(_3\) derivatives 79-2-1-14 and 79-1-6-5 which had square heads. The BC\(_2\)F\(_3\) derivatives 79-2-1-24, 79-2-1-25, 79-1-5-5 and 79-1-5-6 had brittle rachis and red seed color whereas all other 12 derivatives had tough rachis and amber colored grains.

**Cytological analysis**

The F1 hybrids between T. aestivum cv. HD2687 and Ae. longissima 3506 were partially fertile (2n=28) with limited homoeologous chromosome pairing. The subsequent backcross with WL711 led to the development of fertile BC\(_2\)F\(_2\) and BC\(_3\)F\(_3\) derivatives. The BC\(_1\)F\(_2\) plants with 2 to 3 fold higher grain micronutrient concentrations than those of control cultivars and nearly 18-21 bivalents were selected for further studies. The increase in bivalent frequency was observed in the selected BC\(_2\)F\(_2\) and BC\(_3\)F\(_3\) derivatives. The bivalent frequency in the BC\(_2\)F\(_2\) derivatives varied from sixteen (79-2-1) to twenty-two (79-1-6) whereas the univalent frequency ranged from one (79-1-6) to seven (79-1-5, 79-2-4) with occasional trivalent and quadriivalent (Fig. 1a-d). Most of the finally selected BC\(_2\)F\(_3\) derivatives showed 41-43 chromosomes with 18-21 bivalents and 1-6 univalents (Table 3, Fig. 1e-h).

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Table 1. Morphological characteristics of the three parents and 16 selected BC₁F₃ wheat - *Ae. longissima* derivatives in field testing during 2010-11.

<table>
<thead>
<tr>
<th>Parents/ Derivatives</th>
<th>Derivatives pedigree</th>
<th>Waxiness</th>
<th>Head type</th>
<th>Rachis</th>
<th>Grain color</th>
<th>No. of seeds per spike</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-3506</td>
<td>S308/CHR//KAL released in 1977</td>
<td>Non Waxy</td>
<td>Spelta</td>
<td>Brittle</td>
<td>Red</td>
<td>35.5</td>
</tr>
<tr>
<td>WL-711</td>
<td>CPAN2009/HD2329 released in 1999</td>
<td>Waxy</td>
<td>Square</td>
<td>Tough</td>
<td>Amber</td>
<td>48.3</td>
</tr>
<tr>
<td>HD-2687</td>
<td>BC₁F₃ HD2687/L 3506//WL711-1-2-1-14</td>
<td>Non Waxy</td>
<td>Spelta</td>
<td>Tough</td>
<td>Amber</td>
<td>15.4</td>
</tr>
<tr>
<td>79-2-1-4</td>
<td>BC₁F₃ HD2687/L 3506//WL711-1-2-1-19</td>
<td>Non Waxy</td>
<td>Spelta</td>
<td>Tough</td>
<td>Amber</td>
<td>7.5</td>
</tr>
<tr>
<td>79-2-1-24</td>
<td>BC₁F₃ HD2687/L 3506//WL711-1-2-24</td>
<td>Brittle</td>
<td>Spelta</td>
<td>Brittle</td>
<td>Red</td>
<td>9.0</td>
</tr>
<tr>
<td>79-2-1-25</td>
<td>BC₁F₃ HD2687/L 3506//WL711-1-2-25</td>
<td>Non Waxy</td>
<td>Spelta</td>
<td>Brittle</td>
<td>Red</td>
<td>7.2</td>
</tr>
<tr>
<td>79-2-4-5</td>
<td>BC₁F₃ HD2687/L 3506//WL711-1-4-2</td>
<td>Non Waxy</td>
<td>Spelta</td>
<td>Tough</td>
<td>Amber</td>
<td>23.5</td>
</tr>
<tr>
<td>79-1-4-6</td>
<td>BC₁F₃ HD2687/L 3506//WL711-1-4-6</td>
<td>Non Waxy</td>
<td>Spelta</td>
<td>Brittle</td>
<td>Amber</td>
<td>9.5</td>
</tr>
<tr>
<td>79-1-4-8</td>
<td>BC₁F₃ HD2687/L 3506//WL711-1-4-8</td>
<td>Non Waxy</td>
<td>Spelta</td>
<td>Tough</td>
<td>Amber</td>
<td>8.4</td>
</tr>
<tr>
<td>79-1-5-5</td>
<td>BC₁F₃ HD2687/L 3506//WL711-1-5-5</td>
<td>Non Waxy</td>
<td>Spelta</td>
<td>Brittle</td>
<td>Red</td>
<td>13.5</td>
</tr>
<tr>
<td>79-1-5-6</td>
<td>BC₁F₃ HD2687/L 3506//WL711-1-5-6</td>
<td>Non Waxy</td>
<td>Spelta</td>
<td>Brittle</td>
<td>Red</td>
<td>10.5</td>
</tr>
<tr>
<td>79-1-5-7</td>
<td>BC₁F₃ HD2687/L 3506//WL711-1-5-7</td>
<td>Non Waxy</td>
<td>Spelta</td>
<td>Tough</td>
<td>Amber</td>
<td>10.0</td>
</tr>
<tr>
<td>79-1-6-1</td>
<td>BC₁F₃ HD2687/L 3506//WL711-1-6-1</td>
<td>Non Waxy</td>
<td>Spelta</td>
<td>Tough</td>
<td>Amber</td>
<td>14.6</td>
</tr>
<tr>
<td>79-1-6-5</td>
<td>BC₁F₃ HD2687/L 3506//WL711-1-6-5</td>
<td>Non Waxy</td>
<td>Spelta</td>
<td>Tough</td>
<td>Amber</td>
<td>17.9</td>
</tr>
<tr>
<td>79-1-6-10</td>
<td>BC₁F₃ HD2687/L 3506//WL711-1-6-10</td>
<td>Non Waxy</td>
<td>Spelta</td>
<td>Tough</td>
<td>Amber</td>
<td>12.7</td>
</tr>
<tr>
<td>79-1-8-3</td>
<td>BC₁F₃ HD2687/L 3506//WL711-1-8-3</td>
<td>Non Waxy</td>
<td>Spelta</td>
<td>Tough</td>
<td>Amber</td>
<td>9.6</td>
</tr>
</tbody>
</table>

Table 2. Grain micronutrient concentrations of parents and the selected BC₁F₃ derivatives.

<table>
<thead>
<tr>
<th>Parents/ derivatives</th>
<th>Fe (mg/kg) ± S.E</th>
<th>Zn (mg/kg) ± S.E</th>
<th>Cu (mg/kg) ± S.E</th>
<th>Mn (mg/kg) ± S.E</th>
<th>Ca (mg/kg) ± S.E</th>
<th>Mg (mg/kg) ± S.E</th>
<th>K (mg/kg) ± S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>WL-711</td>
<td>29.4±1.2</td>
<td>26.4±0.8</td>
<td>5.1±0.9</td>
<td>16.1±1.3</td>
<td>140.2±1.2</td>
<td>495.2±1.3</td>
<td>2010.4±1.6</td>
</tr>
<tr>
<td>HD-2687</td>
<td>31.7±1.2</td>
<td>28.6±1.2</td>
<td>5.7±1.2</td>
<td>16.2±0.6</td>
<td>100.3±1.4</td>
<td>520.1±2.0</td>
<td>1884.5±1.2</td>
</tr>
<tr>
<td>L-3506</td>
<td>59.1±0.8</td>
<td>45.0±1.1</td>
<td>8.0±1.5</td>
<td>25.0±1.3</td>
<td>348.2±0.7</td>
<td>630.5±0.9</td>
<td>2604.1±0.8</td>
</tr>
<tr>
<td>79-2-1-4</td>
<td>57.5±0.2</td>
<td>60.3±1.6</td>
<td>8.4±0.9</td>
<td>34.0±2.4</td>
<td>204.4±1.6</td>
<td>647.4±1.2</td>
<td>2440.2±1.0</td>
</tr>
<tr>
<td>79-2-1-14</td>
<td>77.4±1.3</td>
<td>70.6±1.2</td>
<td>9.2±1.0</td>
<td>36.2±0.6</td>
<td>200.1±0.9</td>
<td>650.0±1.4</td>
<td>2309.3±0.9</td>
</tr>
<tr>
<td>79-2-1-19</td>
<td>65.8±1.0</td>
<td>69.4±1.0</td>
<td>12.7±2.0</td>
<td>42.0±1.3</td>
<td>210.8±0.5</td>
<td>687.3±1.5</td>
<td>2104.0±1.5</td>
</tr>
<tr>
<td>79-2-1-24</td>
<td>64.6±1.4</td>
<td>94.0±1.5</td>
<td>8.2±1.2</td>
<td>36.0±1.6</td>
<td>208.3±0.7</td>
<td>600.1±1.6</td>
<td>2502.1±2.3</td>
</tr>
<tr>
<td>79-2-1-25</td>
<td>40.5±1.8</td>
<td>74.1±1.8</td>
<td>7.8±1.4</td>
<td>40.1±0.8</td>
<td>220.4±1.4</td>
<td>652.0±1.7</td>
<td>2293.8±2.1</td>
</tr>
<tr>
<td>79-2-4-5</td>
<td>75.7±2.0</td>
<td>68.8±1.4</td>
<td>13.6±1.6</td>
<td>36.2±1.2</td>
<td>388.2±0.9</td>
<td>689.5±1.0</td>
<td>2156.2±1.7</td>
</tr>
<tr>
<td>79-1-4-2</td>
<td>55.3±1.3</td>
<td>83.8±1.7</td>
<td>9.3±0.9</td>
<td>36.4±1.5</td>
<td>250.6±1.2</td>
<td>625.1±1.3</td>
<td>1896.3±0.8</td>
</tr>
<tr>
<td>79-1-4-6</td>
<td>67.0±0.8</td>
<td>87.0±0.9</td>
<td>8.0±0.8</td>
<td>38.6±2.0</td>
<td>224.3±0.8</td>
<td>639.0±0.7</td>
<td>1927.5±1.3</td>
</tr>
<tr>
<td>79-1-4-8</td>
<td>48.6±1.2</td>
<td>66.1±1.0</td>
<td>7.8±0.9</td>
<td>40.3±2.4</td>
<td>209.4±0.9</td>
<td>630.5±1.6</td>
<td>2128.0±1.4</td>
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<tr>
<td>79-1-5-5</td>
<td>86.1±1.0</td>
<td>75.0±1.6</td>
<td>12.2±1.2</td>
<td>38.5±1.0</td>
<td>352.1±0.7</td>
<td>678.0±1.0</td>
<td>2403.5±0.9</td>
</tr>
<tr>
<td>79-1-5-6</td>
<td>56.5±1.4</td>
<td>86.3±2.1</td>
<td>11.8±1.4</td>
<td>37.1±0.8</td>
<td>342.7±1.2</td>
<td>710.1±2.1</td>
<td>2630.1±1.8</td>
</tr>
<tr>
<td>79-1-5-7</td>
<td>61.2±1.6</td>
<td>69.8±1.3</td>
<td>12.0±1.9</td>
<td>36.4±0.7</td>
<td>316.5±1.2</td>
<td>663.4±1.7</td>
<td>2506.2±1.6</td>
</tr>
<tr>
<td>79-1-6-1</td>
<td>72.5±0.9</td>
<td>78.2±0.9</td>
<td>8.4±2.3</td>
<td>35.3±1.3</td>
<td>348.1±1.7</td>
<td>683.0±0.7</td>
<td>2400.8±1.3</td>
</tr>
<tr>
<td>79-1-6-5</td>
<td>67.0±0.6</td>
<td>70.9±1.0</td>
<td>9.2±1.8</td>
<td>42.2±1.2</td>
<td>300.4±0.8</td>
<td>696.3±1.2</td>
<td>2387.0±1.0</td>
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<td>79-1-6-10</td>
<td>77.5±0.7</td>
<td>94.9±0.7</td>
<td>9.4±0.9</td>
<td>38.6±2.0</td>
<td>333.6±1.4</td>
<td>709.5±1.6</td>
<td>2392.1±0.7</td>
</tr>
<tr>
<td>79-1-8-3</td>
<td>76.6±0.9</td>
<td>93.7±1.5</td>
<td>12.7±1.7</td>
<td>41.1±0.9</td>
<td>352.1±1.2</td>
<td>730.2±2.0</td>
<td>2600.3±1.2</td>
</tr>
</tbody>
</table>

Notes: Different superscript letters on mean values denote significant differences among derivatives and control as based on t-test.
whereas the highest concentrations for grain zinc, copper, calcium, manganese, magnesium and potassium were observed in the derivatives 79-1-6-10 (241%), 79-2-4-5 (151%, 223%), 79-1-6-5 (162%), 79-1-8-3 (43.9%) and 79-1-5-6 (35.0%), respectively.

**Characterization of introgression lines by in situ hybridization**

Introgresion of a pair of *Ae. longissima* chromosomes was observed in the BC_{1}F_{1} derivative 79-2-1-4 (2n=44, Fig. 2a). In 79-2-1-25 derivative (2n=46), the *Ae. longissima* probe strongly hybridized with two pairs of chromosomes. The presence of a satellite in the short arm of a pair of chromosomes and its comparison with the standard karyotype indicated that it could be 1S' of *Ae. longissima* (Fig. 2b). In 79-1-5-5, hybridization with S genome probe showed the introgression of a pair of S' chromosomes and two other S' chromosomes (Fig. 2c).

**Molecular characterization**

On the basis of molecular marker analysis, it was found that chromosome 2S’ of *Ae. longissima* 3506 was present in all of the selected derivatives whereas the introgression of 7S’ chromosome was found in 11 out of 16 selected derivatives (Fig. 3). The introgression of 4S’ chromosome was observed only in BC_{1}F_{1} 79-1-6-5 whereas two of the selected derivatives 79-2-1-4 and 79-2-1-19 showed the presence of 5S’ chromosome of *Ae. longissima* (Fig. 3). There was no introgression of 3S’ and 6S’ chromosomes in any of the selected derivatives. Only the complete introgression of 2S’ and 7S’ chromosomes not involving any translocation was found in the derivatives as the markers of both short and long arms were present in them. This was also confirmed by in situ hybridization analysis (Fig. 2). It was observed that the derivatives with only introgression of group 2 and 7 chromosomes of *Ae. longissima* (79-2-4-5, 79-1-4-2, 79-1-5-5, 79-1-5-6, 79-1-6-1, 79-1-6-5, 79-1-6-10, 79-1-8-3) had higher grain micronutrient concentrations than the derivatives with introgression of other chromosomes and their combinations (Table 3, and Fig. 3). The derivatives with 1S’, 2S’ or only 2S’ chromosome also showed comparable grain zinc concentration to that of the derivatives with 2S’ and 7S’ chromosomes (Fig. 3).

**High molecular weight glutenin subunit (HMW-GS) profile**

High molecular weight glutenin subunit (HMW-GS) profiles of some of the selected BC_{1}F_{1} derivatives along with both parents are shown in Fig. 4. The HMW glutenin subunits of *Ae. longissima* (1S’) had lower electrophoretic mobility than the wheat HMW subunits controlled by Glu A1, Glu B1 and Glu D1 loci. Therefore comparative analysis of HMW-GS could provide useful information regarding the introgression of group 1 chromosome of *Ae. longissima*. The HMW-GS of the *Ae. longissima* showed two migrating zones. The one with slower electrophoretic mobility was located above the Glu D1 subunit 2 of *T. aestivum* HD2687 and WL711 whereas the other band with faster electrophoretic mobility was located between the Glu D1 subunit 2 and Glu B1 subunit 7 of the wheat parents. The group 1 of *Ae. longissima* was found to be present in five of the selected derivatives (Table 3, Fig. 4). The BC_{1}F_{1} 79-2-1-4 and 79-2-1-14 showed the addition of *Ae. longissima* subunits in the wheat background whereas the selected derivatives 79-2-1-19, 79-2-1-24 and 79-2-1-25 showed the loss of Glu D1 subunit 12 of wheat parents (Fig. 4) suggesting the substitution of 1S’ for

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**Fig 1.** Chromosome pairing at metaphase-I of PMCs of some BC_{1}F_{2} and BC_{2}F_{1} derivatives. a. BC_{1}F_{2} 79-2-1 (2n=47, 1I’+2 II’+16II+5I), b. BC_{1}F_{2} 79-2-4 (2n=48, 1III’+19II’+7II’+7I’), c. BC_{2}F_{2} 79-1-4 (2n=43, 18 II’+7I), d. BC_{2}F_{2} 79-1-6 (2n=45, 22 II+1I), e. BC_{2}F_{2} 79-1-4-6 (2n=41, 20II+1I), f. BC_{1}F_{1} 79-2-4-5 (2n=45, 22 II+1I), g. BC_{1}F_{1} 79-1-4-8 (2n=44, 21II+2I), h. BC_{1}F_{1} 79-1-5-6 (2n=43, 1III+19II+2I).

**Fig 2.** Genomic in situ hybridization pattern of root tip cells at mitotic metaphase of three wheat-*Ae longissima* acc. 3506 derivatives a. BC_{1}F_{1} 79-2-1-4 with one pair of a S’ chromosome (red), b. BC_{1}F_{1} 79-2-1-25 with one pair of a satellite S’ and one pair of a S’ chromosomes (red) c. BC_{1}F_{1} 79-1-5-5 with a pair of S’ chromosomes and two other S’ chromosomes.

**Grain micronutrients concentrations**

The wheat cultivars *T. aestivum* WL711 and HD2687 had very low grain micronutrient concentrations (Table 2). *Ae. longissima* 3506 had almost 2.2 fold higher grain iron concentration and 3.4 fold higher grain calcium concentration whereas for most of the other minerals studied it showed nearly 1.4 fold higher concentrations than the wheat cultivars. All of the selected derivatives showed significant differences in grain micronutrient concentration over both of the wheat cultivars and also among themselves. A wide range of variation was found for concentrations of grain iron (40.5-86.1 mg/kg), zinc (60.3-94.9 mg/kg), copper (7.8-13.6 mg/kg), manganese (35.3-42.2 mg/kg), calcium (200.1-352.1 mg/kg) and magnesium (600.1-730.2 mg/kg) except for potassium. The highest increase in grain iron concentration was observed in the BC_{1}F_{1} derivative 79-1-5-5 (186.04 %)


1D. No introgression of group 1 chromosome of Ae. longissima was found in the other selected derivatives (data not shown).

Discussion

All of the selected derivatives showed non- waxy leaf sheaths as that of Ae. longissima parent. The plant non- waxiness in wheat is controlled by a dominant gene which is epispastic to the waxiness and is controlled by homoeologous group 2 chromosomes (Liu et al., 2006). Therefore, the presence of non-waxiness in all of the derivatives indicated the introgression of group 2 chromosome of Ae. longissima. The four BC1F1 derivatives 79-2-1-24, 79-2-1-25, 79-1-5-5 and 79-1-5-6 had brittle rachis and red grain color as that of Ae. longissima parent. A dominant gene for red grain color and brittleness are known to be present on homoeologous group 3 of wheat (Metzger and Silbaugh, 1970; Watanabe et al., 2002, Himi et al. 2011). Thus, these derivatives might have had the introgression of chromosome 3S from Ae. longissima. The reasons for the failure of polymorphic SSR markers to detect the introgression of 3S was however, not clear. Ae. longissima acc. 3506 had nearly 1.4 to 3.4 fold higher grain micronutrient concentrations than the wheat.
The presence of two co-located QTL for grain zinc concentration and content was observed on barley (*Hordeum vulgare* L.) chromosome 2H (Loneragan et al., 2009) which is syntenic to its wheat homoeologue (Deynze et al., 1995; Cho et al., 2006). All of these findings strongly support our results that the *Ae. longissima* 2S and 7S chromosomes possess orthologs for grain micronutrient concentrations. Addition of more than one chromosome has been observed in most of the backcross derivatives which led to extensive linkage drag and reduced fertility and harvest index. For commercial exploitation of biofortified wheat, recombinants of these addition/substitution lines of group 1, 2 or 7 chromosomes with fine transfers in elite wheat cultivars will be required. The work on homoeologous chromosome paring and irradiation induced fine compensating transfers from the derivatives in elite wheat cultivar background without transfer of disease resistance by various workers (McIntosh, 1991; Ceoloni et al., 1992).

Most of the selected derivatives had higher grain micronutrients Fe, Zn, Cu and Mn concentrations than both the wheat as well as *Ae. longissima* parents whereas the concentrations of Mg, Ca and K was as high as that of the better parent *Ae. longissima*. The isolation of transgressive derivatives for the micronutrient concentrations indicated additive action of the genes of the parental species for these nutrients. These derivatives also had bolder grains further supporting the proof of the concept that *Ae. longissima* possesses superior genetic system for micronutrient biofortification in the wheat background which may not be due to the usual concentration effect. On the basis of GISH analysis, molecular markers and HWM GS profiling, most of the derivatives with high grain micronutrients concentrations showed the introgression of 2S followed by 7S and 1S chromosomes. One or two species of the tetraploid *Aegilops* species have been effectively used for the transfer of disease resistance by various workers (McIntosh, 1991; Ceoloni et al., 1992).

References


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