Breeding pearl millet cultivars for high iron density with zinc density as an associated trait

KN Rai*, OP Yadav2, BS Rajpurohit3, HT Patil1, M Govindaraj4, IS Khairwal4, AS Rao5, H Shivade6, VY Pawar7 and MP Kulkarni8

1. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India
2. All India Coordinated Pearl Millet Improvement Project (AICPMIP), Mandor 342 304, Rajasthan, India
3. College of Agriculture, Mahatma Phule Krishi Vidyapeeth, Dhule 424 002, Jalgaon, Maharashtra, India
4. Shakti Vardhak Seeds Pvt. Ltd., Tilak Bazar, Hisar 125 001, Haryana, India
5. Nirmal Seeds Pvt. Ltd., Pachora 424 201, Jalgaon, Maharashtra, India

*Corresponding author: k.rai@cgiar.org

Abstract

Pearl millet, as a species, has higher levels of iron (Fe) and zinc (Zn) densities than other major cereal crops. However, this study showed the existence of about twofold variability for Fe density (31–61 ppm) and zinc density (32–54 ppm) among 122 commercial and pipeline hybrids developed in India. Thus, there is a need to increase the cultivation of hybrids having higher Fe and Zn levels and enhance their consumption to better address various health problems associated with the deficiencies of these micronutrients. High-yielding open-pollinated varieties (OPVs) and hybrids with higher levels of Fe and Zn densities than those found in most of the commercial cultivars otherwise not bred for these micronutrients as target traits have been developed and are available for commercialization. Breeding lines and germplasm with still higher levels of Fe and Zn densities have been identified. Their utilization in breeding has the potential to enable development of hybrids with >75 ppm Fe density and >55 ppm Zn density. The primary focus of pearl millet biofortification is on improving Fe density with Zn density as an associated trait. Depending on the genotypic composition of the trials, moderate to high correlations between Fe and Zn densities have been observed, indicating good prospects of simultaneous genetic improvement for both traits, but perhaps also the need to make conscious selection for Zn density along with Fe density. Lack of association of Fe and Zn densities with grain size showed that both micronutrients can be improved without compromising on seed size. The association of Fe and Zn densities with grain yield was weak and negative, but not always significant, indicating that both micronutrients can be improved without significantly compromising grain yield by using large segregating populations. This, however, is one area that merits further research.

Introduction

Micronutrient malnutrition is increasingly being recognized as a serious public health problem affecting more than half of the population worldwide, with preschool children and women in the developing countries being most vulnerable (UN SCN 2004). Most widespread of these are dietary deficiencies in iron (Fe), zinc (Zn) and vitamin A. Among the 20 major risk factors of the global burden of disease estimates, Fe deficiency ranks 9th and Zn deficiency ranks 11th, while in the high mortality countries, including India, they rank 6th and 5th, respectively (WHO 2002). Iron deficiency is the principal cause of anemia that leads to morbidity and mortality of mother and child at childbirth, and impairs cognitive skills and physical activity (Scrimshaw 1984, Hercberg et al. 1987, Welch 2002), while Zn deficiency leads to immune system dysfunctions and high susceptibility to infectious diseases, retardation of mental development, altered reproductive biology, gastrointestinal problems and stunted growth of children (Prasad 1996, Solomons 2003, Black et al. 2008). These nutritional deficiency problems have enormous socioeconomic impacts at the individual, community and national levels (Darnton-Hill et al. 2005, Stein 2010). India annually loses about 4 million disability-adjusted life years (DALYs) due to Fe deficiency and another 2.8
millions of DALYs due to Zn deficiency (Qaim et al. 2007). Pharmaceutical supplementation, industrial food fortification, and agricultural approaches of dietary diversification and biofortification have been suggested to address these problems. Biofortification of staple crops is most cost-effective, sustainable, consumer-acceptable, pro-rural and pro-poor intervention.

Pearl millet (*Pennisetum glaucum*), grown as a major warm-season cereal crop on about 28 million ha in the arid and semi-arid regions of Asia and Africa (Yadav et al. 2012), is highly nutritious with high protein content and dietary fiber, more balanced amino acid profile and high mineral content (Andrews and Anand Kumar 1992). A recent study showed that pearl millet accounts for 19–63% of the total Fe intake and 16–56% of the total Zn intake from all food sources in some parts of the major pearl millet growing states of India such as Maharashtra, Gujarat and Rajasthan (Parthasarathy Rao et al. 2006). It is also the cheapest source of Fe and Zn as compared to other cereals and vegetables. Large variability for Fe and Zn density has been reported in a diverse range of breeding lines, improved populations and germplasm (Velu et al. 2007, 2008, Rai et al. 2012), indicating that cultivars with higher levels of these micronutrients can be developed in pearl millet. Pearl millet biofortification program at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), supported by HarvestPlus Challenge Program of the Consultative Group on International Agricultural Research (CGIAR), has initiated a major effort towards the development of high-yielding cultivars with high Fe and Zn density. In this paper, we report on pearl millet biofortification strategy, variability for Fe and Zn density in commercial and pipeline cultivars as well as in breeding lines, association between micronutrient densities and productivity, and prospects of breeding high-yielding cultivars with high levels of these micronutrients.

**Pearl millet biofortification strategy**

The thrust of ICRISAT’s pearl millet improvement program at Patancheru, India is on the development and dissemination of a wide range of improved breeding lines, populations and hybrid parents for utilization by public and private sector pearl millet programs, largely targeted for India, which has the largest pearl millet area (>9 million ha) in the world. As a consequence of this strategy, a large number and diverse range of germplasm (mostly from the western African regions) has been used, and diversified breeding products have been developed and disseminated. These have been extensively used both in the public and private sector to develop a large number of hybrids. In view of the potential large diversity among the hybrids, it was considered to examine hybrids under cultivation and those in the pipeline for release with respect to variability for Fe and Zn density, and identify those with high levels of these micronutrients to enhance their adoption. Open-pollinated varieties (OPVs) were also evaluated for Fe and Zn density to identify those with high levels of these micronutrients for use in further biofortification research and possible development of their improved versions. Parental lines of potential hybrids and improved breeding lines were also evaluated for variability for Fe and Zn density to identify those for use in biofortified hybrid and hybrid parent development. For the longer term, these micronutrient-dense breeding lines, hybrid parents and lines derived from populations identified for high Fe and Zn densities will be used in hybridization programs and population breeding to develop a diverse range of high-yielding and disease resistant breeding lines and hybrid parents with higher levels of Fe and Zn densities in the context of Fe and Zn density being used as core traits and integral part of pearl millet improvement. Thus, in search for additional sources of high Fe and Zn density for sustained long-term genetic improvement, germplasm evaluation has been undertaken.

Since almost all the high-Fe breeding lines and populations were reported to be entirely or largely based on *innadi* germplasm (Velu et al. 2007, 2008), which are specifically adapted to peninsular India, the initial thrust in pearl millet biofortification research has been targeted to this region. These studies also showed that there is larger variability for Fe density than for Zn density, and there is high positive association between these two traits. Also, Fe deficiency is a more serious problem (both in extent and intensity) than Zn deficiency. Thus, higher priority in the program was given to genetic improvement of Fe density, with Zn density as an associated trait. In terms of breeding approach, the total efforts have been through conventional breeding than genominc approaches due to former’s greater cost-effectiveness and short time frame for biofortified cultivar development and delivery. Considering the quantitative inheritance of Fe and Zn density and hence the resultant genotype × environment interaction, a broad-based partnership with the public and private sector breeding programs was developed for multilocation testing to accelerate the biofortification breeding process.

**Variability in commercial and pipeline hybrids**

A trial of 122 hybrids (21 hybrids from 9 public sector research organizations, including ICRISAT; and 101 hybrids from 33 seed companies) that had been released or were either marketed as truthfully labeled seed by the private sector, or were in the pipeline, were evaluated in a
investigations in six pearl millet biofortification hybrid
would improve as an associated trait. Further
Fe density as the priority trait in pearl millet, Zn density
(2013). This would indicate that while breeding for high
other studies (Velu et al. 2007, 2011, Govindaraj et al.
Fe and Zn density in pearl millet have been reported in
hybrids had not been bred for these micronutrients as
target traits. Similar high positive correlations between
Fe and Zn density in pearl millet have been reported in
other studies (Velu et al. 2007, 2011, Govindaraj et al.
2013). This would indicate that while breeding for high
Fe density as the priority trait in pearl millet, Zn density
would improve as an associated trait. Further
investigations in six pearl millet biofortification hybrid
3-replication trial of 1-row plot of 4 m length in a
randomized complete block design at AICPMIP (All
India Coordinated Pearl Millet Improvement Project)
Center, Mandor, Rajasthan in northwestern India and at
Patancheru, Andhra Pradesh in peninsular India during the
2011 rainy season. ICTP 8203 was used as a high-Fe
control, which is an OPV developed at ICRISAT by
direct selection in an iniadi landrace from northern Togo
(Rai et al. 1990) and had been found having the highest
level of Fe density among several populations in a
preliminary test (Velu et al. 2008) and in several trials
that had followed over the years.
Large variability was observed both for Fe and Zn
density among the hybrids (Table 1). Ninety-five (78%)
hybrids had 36–50 ppm Fe density and 92 (75%) hybrids
had 36–45 ppm Zn density. Nine hybrids had high Fe
density (56–65 ppm) and Zn density (46–55 ppm), which
were far higher than densities observed in improved
wheat (Triticum aestivum) varieties (25–35 ppm Fe and
24–31 ppm Zn) (G Velu, CIMMYT, personal
communication). ICTP 8203 had the highest Fe density
(71 ppm) as well as Zn density (58 ppm). Shradha,
developed from a cross between RHRBH1A and RHRBI
138 at Mahatma Phule Krishi Vidyapeeth, Rahuri,
Maharashtra, is a large-seeded and dark gray color
hybrid, typical of iniadi landrace (Andrews and Anand
Kumar 1996). The remaining eight hybrids are from
private seed companies. The hybrid 86 M 86 has been
developed by Pioneer Seed Company and it has iniadi
germplasm in both its male and female parents (personal
communication, RS Mahala, Pioneer Overseas
Corporation, India). While the broad nature of
germplasm involved in the parental lines of other hybrids
is not known, hybrids PAC 903 and PAC 982 also have
iniadi germplasm in their parentage. Shradha is
specifically adapted to Peninsular India while the other
three hybrids, 86 M 86, PAC 903 and PAC 982, have
wider adaptation with 86 M 86 among these being
cultivated most widely.

Character association
There was a highly significant and high positive
correlation (r=0.78; P<0.01) between Fe and Zn density
in hybrids tested in the hybrid trial (Fig. 1) although these
hybrids had not been bred for these micronutrients as
target traits. Similar high positive correlations between
Fe and Zn density in pearl millet have been reported in
other studies (Velu et al. 2007, 2011, Govindaraj et al.
2013). This would indicate that while breeding for high
Fe density as the priority trait in pearl millet, Zn density
would improve as an associated trait. Further
investigations in six pearl millet biofortification hybrid
trials have shown highly significant but moderate to high
positive correlations between Fe and Zn density, ranging
from 0.49 to 0.71 (P<0.01) (Table 2). Thus, it would
appear that for significant genetic gains to be made for Zn
density, conscious selection for it along with Fe density
will also have to be made, and simultaneous selection for
both micronutrients should be effective.

Breeding for high Fe and Zn density must not
compromise with grain yield and grain size. Any adverse
association of Fe and Zn density with grain yield (dilution
effect) and grain size (concentration effect) would impact
the efficiency of breeding for these micronutrients in
high-yielding and large grain size backgrounds. Results
of the six hybrid trials mentioned above showed weak to
moderate negative correlations between Fe density and
grain yield, although not always significant, and mostly
negative and weak but statistically non-significant
correlations between Zn density and grain yield (Table
2). It would appear that the effect of such weak and
variable associations can be circumvented, especially for
Zn density, using large segregating populations. Both Fe
and Zn densities had non-significant weak and often
positive correlations with grain size, indicating that
breeding for these micronutrients in large seed size
background would require no additional resources.
Further research using random sets of lines derived from
random-mated populations constituted from crosses
between high-Fe/Zn and low-Fe/Zn lines is required to
examine the magnitude and direction of association of
these micronutrients with grain yield and seed size.

Prospects of breeding high-yielding cultivars
with high Fe and Zn density
Large intra-population variability for Fe and Zn densities
had been reported in several populations, including ICTP
8203 (Velu et al. 2008). ICTP 8203 is a popular OPV in
peninsular India, grown on about 800,000 ha in
Maharashtra alone at the peak of its adoption in 1995
(Bantilan et al. 1998). It is still cultivated on about
150,000 ha in this region and parts of Rajasthan and Uttar
Pradesh in northern India. It has been observed that Fe
and Zn densities are largely under additive genetic
control (Velu et al. 2011, Govindaraj et al. 2013),
implying effective intra-population improvement for Fe
and Zn density. Thus, four improved versions of ICTP
8203 based on S3 progeny testing were developed in 2009
and further three versions of it, based on S3 progeny
testing, were developed in 2010. Four of these (two from
each year) had been evaluated in 42 common trials (10
trials in 2010 and 32 trials in 2011) across India. One
version, designated as ICTP 8203 Fe 10-2, which had
been developed by recombining 11 S3 progenies, had on
Table 1. Distribution of released, commercial and pipeline pearl millet hybrids in various grain iron (Fe) and zinc (Zn) density classes, 2011 rainy season.

<table>
<thead>
<tr>
<th>Fe density class (ppm)</th>
<th>31–35</th>
<th>36–40</th>
<th>41–45</th>
<th>46–50</th>
<th>51–55</th>
<th>No. of hybrids (Zn)</th>
<th>No. of hybrids (Fe)</th>
</tr>
</thead>
<tbody>
<tr>
<td>31–35</td>
<td>HBB 197, ABH 999, Bio 70</td>
<td>GHB 719, Mahodaya 325, RBH 173, RBH 121, GHB 538</td>
<td>HBB 223</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36–40</td>
<td>N 61, RHB 154, Hi Pearl 51, Bio 8494, 86 M 66</td>
<td>Shanti, GHB 744, Mukhia, GK 1135, Mahodaya 318, PHB 2168, JKBH 1103, NPH 1651, GHB 732, NPH 2798, Bio 8141, HBB 94, MPMH 17, RBH 177, Proagro 9-444</td>
<td>NBH 1188, KBH 261</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>41–45</td>
<td>HTBH 4201, VBBH 3028, Mahodaya 331</td>
<td>AB 1, NBH 1717, Bisco 5151, NBBH 411, X 7, KBH 9119, Bio 13, NBH 5464, JKBH 1096, TEJAS, Biogene 33+, Navbharat Gavari, HTBH 4202, RBTH 10-007, VBBH 444, VBBH 456, M 64, Nu 306, Kaveri Boss 65</td>
<td>KBH 11, NPH 3685, Ajeet 37, S 368, VBBH 3087, GHB 558, Mahodaya 330, Gangothy 67, NBBH 908, KH 302, Bisco 5141, B 2301, 86 M 88, Solid 78, GK 1104, Euro 1133, MRE 204, NPH 2475, Kaveri Super Boss</td>
<td>86 M 01, Hi Pearl 138</td>
<td>43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>46–50</td>
<td>Sujlam 68, Hi Pearl 91, VIRAT, Kaneri x 563, GK 1116, B 2195, S 361, KBH 7206, N 68</td>
<td>MRB 2210, TNBH 0642, S 362, Ajeet 35, RBH 1818, Proagro 9450, Nu 310, VBBH 3115, PAC 909, RBTH 10-005, NBH 1024, GK 1090, Hi Pearl 130, KBH 2360, JKBH 1097</td>
<td>MRB 287-36, Sanjivani 333, MBH 3125, JKBH 676</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>51–55</td>
<td>HTBH 4203</td>
<td>Navbharat Banas express, PAC 931, KBH 7201, NBH 1134, ICMH 356</td>
<td>Saburi, Sanjivani 2312, RBH 9</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>56–60</td>
<td>HTBH 4204</td>
<td>PAC 982, Ajeet 39, XL 51, 86 M 86, PAC 903</td>
<td>Shradha</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>61–65</td>
<td></td>
<td>Ajeet 38</td>
<td>Sanjivani 222</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Mean of two locations.
Open-pollinated variety ICTP 8203, used as a control, had 71 ppm Fe density and 58 ppm Zn density.
an average, 71 ppm Fe density (9% more than ICTP 8203), 38 ppm Zn density (comparable to ICTP 8203), and 2.2 t ha\(^{-1}\) grain yield (11% more than ICTP 8203) (Table 3). It was also the most farmer-preferred variety in on-farm trials conducted by Nirmal Seeds Pvt. Ltd. in Maharashtra. A similar intra-population selection for Fe density was carried out in another commercial OPV (ICMV 221). Based on X-Ray Fluorescence Spectrometry (XRF) results of limited 4-environment trials at Patancheru, a high-Fe version of it, designated as ICMV 221 Fe-11-1, had 87 ppm Fe density (22% more than ICMV 221), 62 ppm Zn density (16% more than ICMV 221) and 4.02 t ha\(^{-1}\) grain yield (2% higher than ICMV 221. Multilocational evaluation of these two varieties is underway. The two most popular and widely cultivated hybrids are 86 M 86 and 9444. While both are equally high-yielding, 9444 was among those having the lowest levels of Fe and Zn density and 86 M 86 was among those few with the highest levels of Fe and Zn densities (Fig. 1). All these results further indicate that it is possible to combine high grain yield with high levels of Fe and Zn densities.

Table 2. Correlation among grain iron (Fe) and zinc (Zn) density, grain yield (GY) and 1000-seed weight (SW) in four initial hybrid trials (IHTs) and two advanced hybrid trials (AHTs) of pearl millet, 2011 rainy season, Patancheru, India\(^{1}\).

<table>
<thead>
<tr>
<th>Trial</th>
<th>No. of entries</th>
<th>Fe vs. Zn</th>
<th>Fe vs. GY</th>
<th>Zn vs. GY</th>
<th>Fe vs. SW</th>
<th>Zn vs. SW</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHT-A</td>
<td>24</td>
<td>0.53**</td>
<td>−0.13</td>
<td>0.17</td>
<td>−0.01</td>
<td>0.05</td>
</tr>
<tr>
<td>AHT-B</td>
<td>28</td>
<td>0.49***</td>
<td>−0.24</td>
<td>−0.32</td>
<td>0.21</td>
<td>0.02</td>
</tr>
<tr>
<td>IHT-A1</td>
<td>30</td>
<td>0.67***</td>
<td>−0.58**</td>
<td>−0.24</td>
<td>0.32</td>
<td>0.22</td>
</tr>
<tr>
<td>IHT-A2</td>
<td>26</td>
<td>0.67***</td>
<td>−0.39*</td>
<td>−0.04</td>
<td>0.12</td>
<td>−0.05</td>
</tr>
<tr>
<td>IHT-B1</td>
<td>36</td>
<td>0.71**</td>
<td>−0.27</td>
<td>−0.18</td>
<td>0.04</td>
<td>0.29</td>
</tr>
<tr>
<td>IHT-B2</td>
<td>34</td>
<td>0.58***</td>
<td>−0.48**</td>
<td>−0.28</td>
<td>−0.15</td>
<td>0.07</td>
</tr>
</tbody>
</table>

2. * = Significant at 5% level; ** = Significant at 1% level.

Table 3. Grain iron (Fe) and zinc (Zn) density, grain yield and time to 50% flower of biofortified pearl millet cultivars.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Fe (ppm)</th>
<th>Zn (ppm)</th>
<th>Grain yield (t ha(^{-1}))</th>
<th>Time to 50% flower(^{1}) (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variety trial(^{2})</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICTP 8203 Fe 10-2</td>
<td>71</td>
<td>49–106</td>
<td>38</td>
<td>18–71</td>
</tr>
<tr>
<td>Hybrid trial(^{3})</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICMH 1201</td>
<td>76</td>
<td>47–97</td>
<td>40</td>
<td>21–58</td>
</tr>
<tr>
<td>86 M 86</td>
<td>57</td>
<td>42–71</td>
<td>38</td>
<td>22–61</td>
</tr>
<tr>
<td>ICTP 8203</td>
<td>74</td>
<td>50–102</td>
<td>42</td>
<td>23–75</td>
</tr>
</tbody>
</table>

1. Mean of 25 trials for variety trial.
2. Conducted in 42 locations × year environments during 2010 and 2011.
As mentioned before, the thrust of pearl millet biofortification program at ICRISAT, Patancheru is towards hybrid development. The most extensive multilocation field trials of biofortified hybrids, for the first time, were initiated in 2011. Based on the mean performance in 31 trials conducted in 2011 and 2012, an experimental hybrid, designated as ICMH 1201, was identified that had 76 ppm Fe density and 40 ppm Zn density (both comparable to those in ICTP 8203), but its yield was 3.7 t ha\(^{-1}\) of grain (38% higher than ICTP 8203). This hybrid flowered only 3 days later than ICTP 8203, so it fits well in the same early maturity group. ICTP 8203 is particularly adapted to marginal environments associated with low productivity. Since the productivity levels of the environments in this trial had a wide range (2.28–2.83 t ha\(^{-1}\)), we examined if there existed any relationship between the productivity levels of the environments and relative yield advantage of ICMH 1201 over ICTP 8203. At each productivity level within this range, ICMH 1201 had higher grain yield than ICTP 8203, and there was no relationship between the productivity level of the environments and grain yield advantage of ICMH 1201 over ICTP 8203. This experimental hybrid and ICTP 8203 are being evaluated in on-farm trials in Maharashtra. Further, both Fe and Zn densities in this hybrid were not associated with grain yield levels as observed in various trials (Fig. 3).

So far, biofortified hybrids for high Fe and Zn densities had been developed using parental lines identified having higher levels of these micronutrients from amongst those that had not been actually bred for Fe and Zn density as target traits in the mainstream breeding. Use of such lines would continue until parental lines with higher Fe and Zn densities are developed through targeted breeding for these micronutrients in high-yielding backgrounds. Large variability for both Fe and Zn density in advanced breeding lines not specifically bred for these micronutrients has been observed (Table 4). Progenies derived from some of the OPVs identified for high Fe density have been found having >100 ppm Fe density and >60 ppm Zn density. Further evaluation and utilization of these materials and those being identified in the germplasm will provide additional genetic resources to breed parental lines that should enable breeding high-

![Figure 2. Grain yield superiority of high-Fe pearl millet hybrid (ICMH 1201) over ICTP 8203 at various productivity levels.](image)

![Figure 3. Grain iron (Fe) or zinc (Zn) density (ppm) and grain yield of high Fe hybrid (ICMH 1201) (Note: Means of 28 locations).](image)

<table>
<thead>
<tr>
<th>Material</th>
<th>No. of entries</th>
<th>&lt;40</th>
<th>41–60</th>
<th>61–80</th>
<th>81–100</th>
<th>101–120</th>
<th>&gt;121</th>
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<tbody>
<tr>
<td><strong>Fe density</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Advanced breeding lines</td>
<td>386</td>
<td>25</td>
<td>47</td>
<td>23</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Population progenies</td>
<td>232</td>
<td>0</td>
<td>2</td>
<td>28</td>
<td>50</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td><strong>Zn density</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Advanced breeding lines</td>
<td>386</td>
<td>48</td>
<td>49</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Population progenies</td>
<td>232</td>
<td>0</td>
<td>50</td>
<td>46</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

yielding hybrids with >75 ppm Fe density and >55 ppm Zn density.

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References


