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Research Article

Reselection within population for high grain iron density and its effects on agronomic traits in pearl millet

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Abstract

This study aimed to assess whether intra-population selection and its derived lines inter-mating for grain iron (Fe) has any associated changes in grain yield and other agronomic traits in two Open-Pollinated Varieties (OPVs) in pearl millet. The original (C0) and improved bulks (C1) were evaluated in two contrast seasons (referred to as environments). Result showed non-significant differences between C0 and C1 bulks for all the traits except 1000-grain mass in both the populations. This implied that the selection for higher Fe level did not cause any significant changes on grain yield and other agronomic traits. The S_1-based random mated bulks are generally more heterozygous as F_2 populations, thus, S_2-based population improvement would assemble large number of favorable additive allele to elevate Fe and Zn density which did happen marginally in this study. Interestingly, selection for high Fe significantly increased the 1000-grain mass by 4.8% and 14.2% in the AIMP 92901 and ICMR 312, respectively. One cycle of recurrent selection showed marginal improvement for grain Fe and Zn in C_1 over C_0 bulks of AIMP 92901 (2.4% more Fe and 7.9% more Zn) and ICMR 312 (8% more Fe and 5.4% more Zn). Nevertheless, these micronutrients are being additively controlled so population improvement is possible with increased cycle of selection and subsequent recombination to assemble more favorable alleles for significant difference from its original bulks. Thus, reselection was effective in improving the target traits with no correlated response on the yield traits.

Key words

Pearl millet, recurrent selection, iron, and zinc.

Introduction

Pearl millet is a highly cross-pollinated crop having more than 85% outcrossing probabilities Burton (1983), which makes pearl millet breeding system into two distinct cultivars breeding objectives, *i.e.* open-pollinated varieties (OPVs) and hybrids (single cross). In India, single cross hybrids account for more than 75% of the pearl millet area and improved OPVs are still occupying the remaining 25% of area, nevertheless, OPVs are almost exclusively cultivated in the Africa regions. Therefore, breeding OPVs and hybrids are equally important for global pearl millet improvement program. The objective of population improvement is to increase the frequency of favorable alleles while maintaining the genetic variation for that trait. These improved populations serve as variety for instance and later may serve as potential source for inbred development. Various forms of recurrent selection have been used to enhance intra- and inter-population improvements, mostly in cross pollinated crops Doerksen *et al.*(2003).

Pearl millet is amenable for conducting all forms of recurrent-selection methods Rai *et al.*(1999). Population improvement through selfing generation (S_1) is the result of direct selection favoring additive genetic effects because there is no masking effect of a tester Doerksen *et al.* (2003). S_1 progeny selection has been more frequently used than S_2 progeny selection because of extra generation per cycle is required with the latter approach. Earlier studies, compared S_1 and S_2 selection methods with full-sib (FS) and half-sib (HS) selection methods for grain yield in pearl millet, based on only one cycle of selection, showed that S_1 method is more effective than FS or HS methods Rai and Virk(1999).

Deficiencies of essential micronutrients are more widespread and their adverse health consequences are more severe, particularly, iron (Fe) and zinc (Zn) deficiencies has reported the most widespread, affecting more than two billion people worldwide, mostly in the low- and middle-income countries. Pearl millet has levels of protein with better amino acid balance than other major cereals such as rice, wheat and maize, and also has high levels of minerals, including iron and zinc. Research at the International Crops

Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, in alliance with the Harvest Plus Biofortification Program of the Consultative Group on International Agricultural Research (CGIAR), and in partnerships with several public and private sector pearl millet research programs, have shown large variability in released and commercial OPVs, both for iron and zinc content. Since iron deficiency is systematically found as a serious problem than zinc deficiency and found larger variability for iron than for zinc content, ICRISAT biofortification program has focused on genetic improvement for iron with zinc as an associated trait, due to its highly significant and positive association. Utilizing the large genetic variability for iron and zinc density with populations is an immediate objective of this program.

A preliminary study in pearl millet, based on performance *per se* of the populations across the two environments, C_1 bulks had 21-22% higher grain Fe than the C_0 bulks of both AIMP 92901 (66 ppm) and GB 8735 (62 ppm). Recurrent selection for high grain yield in pearl millet showed non significant difference between initial *vs.* advanced generation bulks of different composite populations for grain Fe. The advanced bulk (C_4) of Serere composite 1 had significantly lower grain Fe (18%) than the initial bulk, whereas, the advanced bulk (C_3) of smut resistant composite (C_0) showed significant improvement of grain Fe (9%) over its original bulk. hence, recurrent selection for high grain yield may not bring in the significant changes in grain Fe and Zn density in pearl millet Velu (2006). Although recurrent selection was undertaken in several OPVs (open-pollinated varieties) of pearl millet, to test if their Fe and Zn levels can be improved without any adverse effects on other traits, the most extensively studied of these is ICTP 8203, in which five high-Fe versions were developed by recombining various groups of its progenies Rai (2011). These were compared with the original (C_0) population of ICTP 8203 in multi-locational trials during 2010 rainy season and two improved versions yielded 1-2% less than the C_0 bulk (2330 kg ha^{-1}), while three versions yielded 8-11% more than the C_0 bulk. On the other hands, all the improved versions had 12-15% higher Fe density and 2-8% higher Zn density than the C_0 bulk (68 ppm Fe and 44 ppm Zn in the C_0 bulk) HarvestPlus (2011).

However, in most of the studies the discussion has focused only on grain micronutrient density, with little acknowledgement that grain yield may be an important factor influencing the differences in nutrient density. Pearl millet biofortification research at ICRISAT seeks to develop high-yielding hybrids with higher Fe and Zn density to demonstrate that Fe and Zn levels can be bred for without compromising on the other positive traits in hybrid cultivars for short and medium term. For the longer term, it envisages development of improved breeding population and hybrid parents with high Fe and Zn density combined with high yield potential and improved agronomic traits as an integral part of its mainstream breeding, hence improved version of adopted cultivars can significantly contribute to improved nutrition. This study aimed to assess whether intra-population selection and its derived lines inter-mating for grain iron (Fe) has any associated changes in grain yield and other agronomic traits in two Open-Pollinated Varieties (OPVs).

Materials and Methods

The materials consisted of two broad based populations, namely AIMP 92901 and ICMR 312. AIMP 92901 was jointly developed by ICRISAT and Marathwada Agricultural University, National Agricultural Research Project Station, Aurangabad, Maharashtra, by random mating 272 S_1 progenies from the C_5 cycle bulk of a Bold-Seeded Early Composite (BSEC) selected for agronomic traits at Aurangabad and the C_5 S_1 bulk of these selected progenies of BSEC that were found resistant to downy mildew (*Sclerospora graminicola* (Sacc. Schroet.) in screening at ICRISAT. AIMP 92901 was released in 2001 for cultivation in peninsular India. ICMR 312 was developed at ICRISAT by mass selection in BSEC with further progeny testing to improve its male fertility restoration ability and resistance to downy mildew. ICMR 312 is population pollen parent of a topcross hybrid ICMH 312 which was developed at ICRISAT and it was released in 1993 for cultivation in peninsular India.

Initially, 300 S_1's were produced from each population during summer 2009 by bagging and selfing the main panicle. All the 300 S_1's from each population were planted in two replications, spaced 75 cm between rows and 15 cm between the plants in rainy season 2009 at Patancheru. Approximately 5-8 main panicles were selfed to produce grain samples. Selfed panicles were harvested and threshed, and grain samples were analyzed for Fe and Zn in Waite analytical laboratory, Adelaide University, Australia (described in micronutrient analysis).

To develop a control (original or unimproved) population, equal quantity of seeds from each of the 300 S_1's were pooled and planted in 2010 summer season separately for each population. Each bulk had 15 rows (4 m length) for recombination by hand pollination (sib-mating) with bulk pollen collected from 30 - 50 plants (within population) and crossed on 15-20 plants of same population on each day. A total of 100-120 plants main panicles were crossed with bulk pollen in each population. The sib-mated panicles were harvested from each population and threshed separately as bulks to constitute the C_0 bulk of the two populations.

In order to evaluate for the second season and to select best progenies for recombination, the same trial (rainy 2009 trial) was repeated during summer 2010 and the data were recorded on self seed set percentage (SSS %) at maturity. The agronomic score (1-5) was given to each entry at the time of harvest. Based on the grain Fe density in rainy 2009 trial and good SSS % data from summer 2010 trial, top 20 S_2 progeny bulks were selected from each population and planted (4m-row) in late summer 2010 for recombination using full diallel mating design (Figure 1). The crossed panicles were harvested and threshed for each cross combination separately. Based on agronomic score, crosses involving entries that had ≥ 3 score (score 5: agronomically best and score 1: agronomically poor) were selected. Thus, crosses involving 13 S_2 progeny bulks from AIMP 92901 and 17 S_2 progeny bulks from ICMR 312 were selected. Equal quantity of seeds from these selected crosses was bulked to constitute C_1 bulks for each population (improved population).

The C_0 seed bulk (original) and C_1 seed bulk (improved) were grown in a trial replicate thrice in randomized complete block design (RCBD) during the 2010 rainy season at Patancheru. A four row plot of four meter length was adopted, with a spacing of 75 cm between rows and 15 cm between plants within a row. The recommended agronomic practices were followed to raise a good crop. The agronomic score and other plant traits (days to 50% flower, plant height, panicle length and 1000-grain mass) were recorded. After threshing, grains obtained from all productive tillers of an individual plot at optimum moisture level was weighed and recorded. Plot yield was converted into yield kg ha^{-1}.

The Fe and Zn estimation were done based on tri-acid mixture method at Central Analytical Laboratory, ICRISAT, Patancheru. The grain samples were finely ground (<60 mesh for grain samples) using cyclone mill and oven dried at 60°C for 48 h before analysis. Ground and dried grain samples of 0.5 g were transferred to 125 ml conical flasks. Twelve ml of tri-acid mixture of nitric acid, sulfuric acid and perchloric acid (9:2:1(v/v)) were added to the flasks. The flour samples were digested in a room temperature for 3 h followed by digestion for 2–3 h on a hot plate, until the digest was clear or colorless. The flasks were allowed to cool and density was diluted to an appropriate volume. The digests were used for Fe and Zn determination using Atomic Absorption Spectrophotometry (AAS).

Genetic gain per cycle was determined by direct comparison of cycles (C_0 and C_1) of both original and selected populations, and the gain realized in the selection was measured by the difference between the C_0 and C_1 populations (Keeling, 1982). Gain $cycle^{-1} = (\mu C_1 - \mu C_0) / \mu C_{0,}$ Where, μC_1 = mean of selected populations for trait 'x' evaluated; μC_0 = mean of original populations for trait 'x' evaluated.

Results and Discussion

This study conducted to assess whether selection for high grain Fe density through recurrent selection has any associated changes in grain yield and its component traits in initial (C_0) and advanced (C_1) generation cycle bulks, revealed non significant differences between initial / original bulks and their advanced bulks for grain yield, days to 50% flowering and plant height in two populations (Table 1). However, the significant improvement in panicle length was observed only in case of AIMP 92901. Despite both populations had shown almost similar gain for panicle length (9.1% in AIMP 92901 and 7.3% in ICMR 312). Interestingly, 1000-grain mass had significant differences in both the populations, whereas, panicle length significantly differed in AIMP 92901 and not in ICMR 312. In a previous study (Velu, 2006), evaluation of recurrent selection composite bulks revealed selection for grain yield during recurrent selection cycle did not cause any changes in the grain Fe and Zn density, this laid foundation for evaluation of single selection cycle of open pollinated varieties for high grain Fe and Zn density.

In the present study, though non significant differences between selection cycles, however selection increased the grain Fe and Zn density but there were differences among the populations. For instance, AIMP 92901 had 2.4% and 7.9% gain cycle^{-1} for Fe and Zn respectively; whereas ICMR 312 showed 8.0% and 5.4% gain cycle^{-1} for Fe and Zn respectively (Table 1). The non significant differences between selection cycles for grain Fe and Zn densitys may be due to reason that, we selected the progeny for recombination only based on single season evaluation, thus selection of progeny must be more than two season or environment evaluation would be rewarding. On the whole, two populations fairly exhibited increased density of grain Fe and Zn (Figure 2 and 3). A recent study reported the improvement of ICTP 8203 by a cycle of recombining 11 S3 progenies to develop ICTP 8203 Fe 10-2, which was released as Dhanashakti in India. Performance of Dhanashakti for Fe and Zn density, grain yield was superior to ICTP 8203 Rai *et al*. (2014). Therefore, present study was agreed with Byrne and Rasmusson, (1974) who reported each cycle of selection for high Strontium (Sr) content in wheat and barley had increased Sr content in advanced cycle and Velu (2006) who reported mean differences between initial and advanced cycle were non significant for grain Fe and Zn density over two environments.

While selecting for high grain Fe and Zn density, the significant mean differences were observed for 1000-grain mass by 4.8% and 14.2% in the AIMP 92901 and ICMR 312, respectively. This result agreed with Bidinger and Raju (2000). The negative but non significant mean differences and gain was observed for grain yield while, selection made for high grain Fe density. One cycle of selection may not be enough to ascertain whether any successful gain in improving grain Fe / Zn and its effect on grain yield. On the basis of the present study, S_2 recurrent selection could be useful in mean improvement of grain mass, Fe and Zn density. But this will be effective for the improvement of population *per se* when absence of over-dominance for that trait Lamkey(1992). However, in order to make progress, the genetic variances must be sufficient to allow for the gradual accumulation of favourable alleles. For long-term response, a larger population may be needed to maintain genetic variability and relaxed (>10%) selection intensity, but for a short-term response, selection programme use of a smaller effective population size (<30) would not compromise genetic progress.

The present study initially had very larger population ~300 S_1's with larger variability for Fe (38 - 117 mg kg^{-1} in AIMP 92901 and 42 - 100 mg kg^{-1} in ICMR 312) and Zn density (35 - 97 mg kg^{-1} in AIMP 92901 and 36 - 86 mg kg^{-1} in ICMR 312). Based on the grain Fe top 60 S_1's was selected in each population, further considering self seed set percentage only 13 and 17 S_2's were recombined with a selection intensity of 21.6% and 28.3% for AIMP 92901 and ICMR 312 respectively. These selection processes indicate that present study fulfilled the genetic expectations and assumptions (population size and selection intensity etc.,) since the magnitude of genetic drift for the population depends on the effective population size. Thus present study used early generation progenies for recombination and has less chance for genetic drift which causes reduction in genetic variation of the advanced generations. The mean performance and estimated gain cycle^{-1} for grain mass, Fe and Zn generally showed a favourable response to selection. An increase in the grain mass was more pronounced in both populations. Although enough choice of selection made for high Fe density, however, single selection cycle may not authentic for applied crop breeding; hence further work with increased cycle (2-3) of recombined selection with multi-environment/seasons evaluation may be required to ensure the gains made in this study which were stable over generations. In conclusion, present study showed genetic structure was changed with advance of selection thus reselection was effective in improving the target traits. This has no correlated response on the yield traits and this will contribute to general combing ability of lines/population. Therefore, continuing such breeding methods will be more effective on maintaining the genetic diversity of the population for a given trait.

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Table 1. Mean performance, test of significance and gain per cycle for grain Fe and Zn density and grain yield component estimated after one cycle of recurrent selection in two populations.

Cycle of selection	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Days to 50% flower	Plant height (cm)	Panicle length (cm)	1000-grain mass (g)	Grain yield (kg ha^{-1})
AIMP 92901							
C_0	68.3	46.3	42	187	21	13.6	2008
C_1	70.0	50.0	43	194	22	14.2	1942
P-value	0.82	0.45	0.42	0.30	0.05	0.05	0.22
% Gain cycle^{-1}	2.4	7.9	0.8	3.4	9.1	4.8	-3.3
LSD (5%)	27.5	16.9	1.4	19.7	1.9	0.6	161.5
C.V (%)	11.3	10.0	1.0	2.9	2.5	1.3	2.3
ICMR 312							
C_0	70.7	49.3	44	193	21	12.9	2140
C_1	76.3	52.0	43	191	23	14.7	1898
P-value	0.27	0.50	0.23	0.58	0.14	0.05	0.50
% Gain cycle^{-1}	8.0	5.4	-2.3	-1.3	7.3	14.2	-11.3
LSD (5%)	16.2	14.1	2.5	16.3	2.7	2.1	1262.3
C.V (%)	6.3	7.9	1.6	2.4	3.6	4.4	17.8

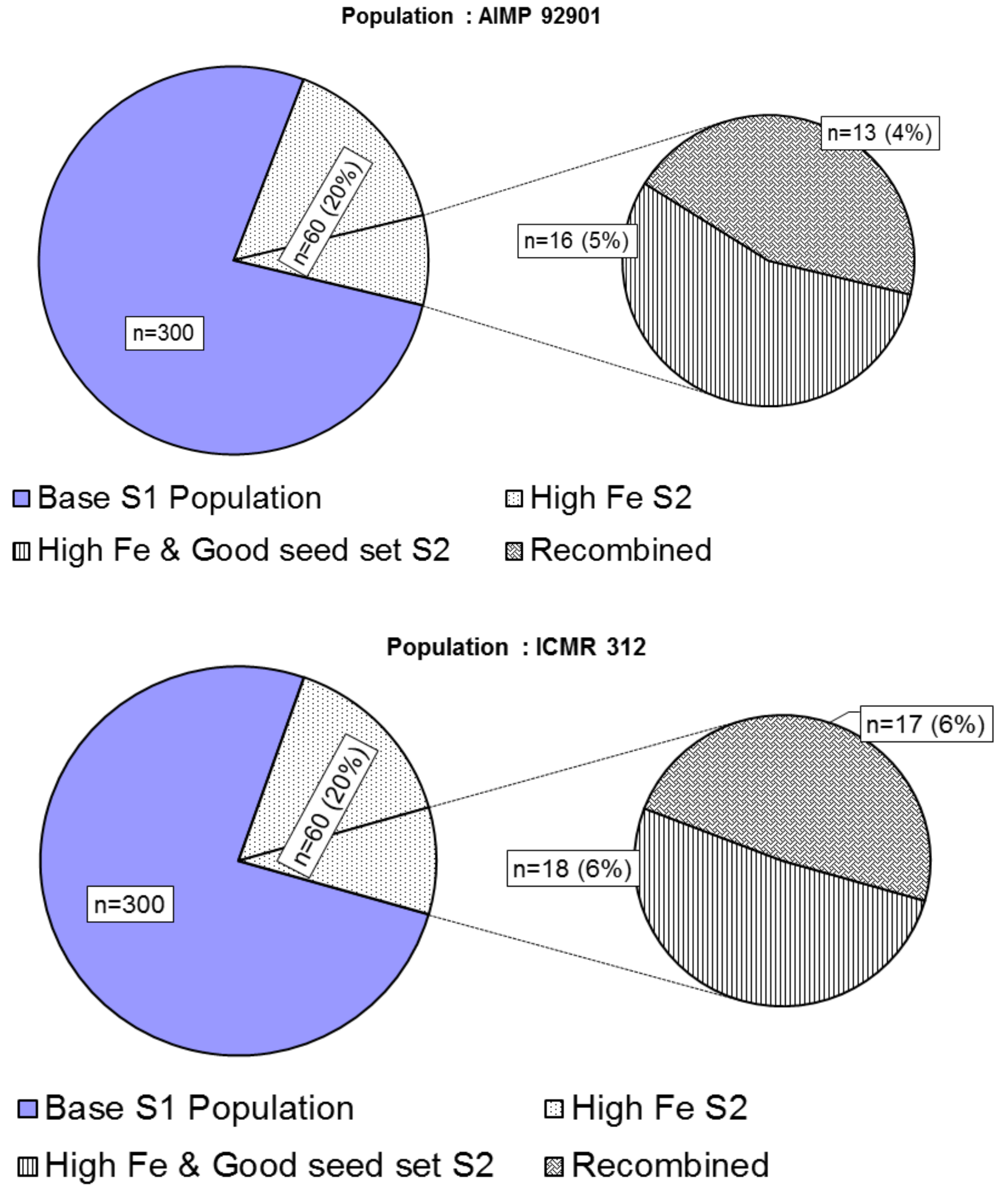

Fig. 1. High-iron population progenies (S0-S2) selction for recombination

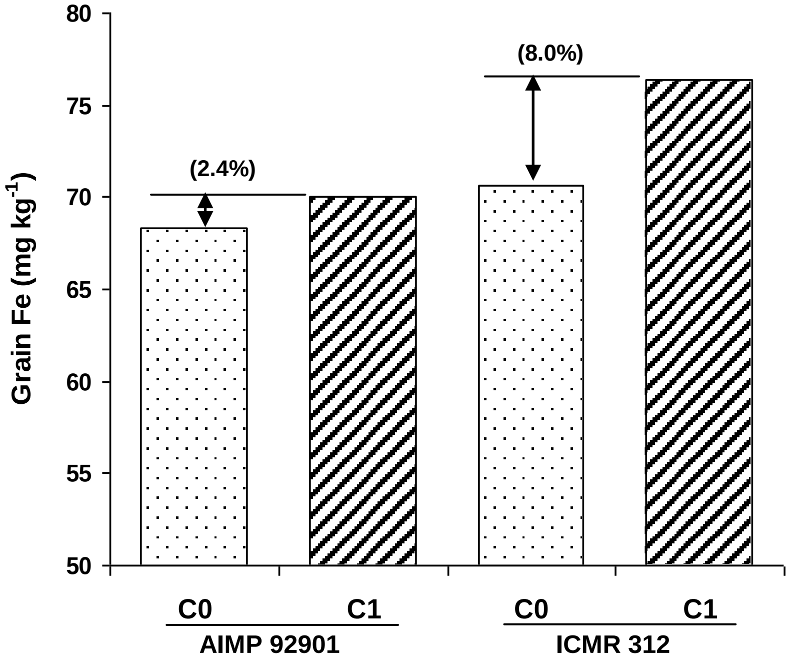

Fig. 2. Frequency distributions for grain Fe density from cycle 0 (C_0: dotted bar) to cycle 1 (C_1: crossed bar) of two populations (AIMP 92901 and ICMR 312). Values in the parentheses are percent of gain cycle^{-1}

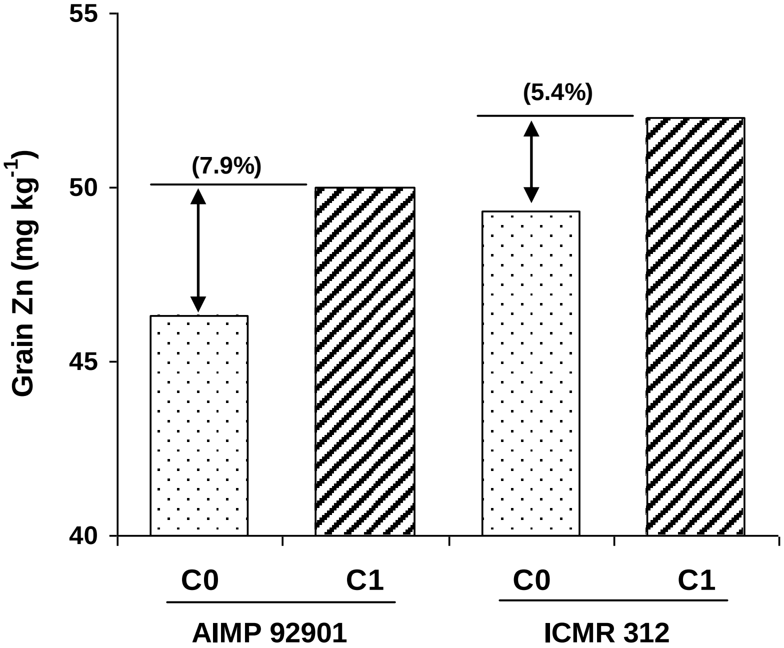

Fig. 3. Frequency distributions for grain Zn density from cycle 0 (C_0: dotted bar) to cycle 1 (C_1: crossed bar) of two populations (AIMP 92901 and ICMR 312). Values in the parentheses are percent of gain cycle^{-1}