Mysore J. Agric. Sci., 52 (2) : 308-315, 2018

Identification of Dolichos Bean (*Lablab purpureus* L. Sweet) Recombinant Inbred Lines Rich in Micronutrients

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ABSTRACT

Analysis, detection and exploitation of genetic variability within the breeding populations and released varieties is the short-term strategy for identification and delivery of micronutrients-rich crop cultivars in high yielding genetic background to cater to the immediate needs of the target population suffering from micronutrients malnutrition. Ninety-seven dolichos bean recombinant inbred lines (RILs) along with their parents and two released varieties were evaluated for fresh pod and seed weight plant^{-1} and grain micronutrients such as iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn) were estimated. The results revealed substantial variability among RILs for all the four grain micronutrient contents. The extent of variability was higher for Fe and Cu than Zn and Mn contents. The best four RILs and the top best RIL were/was better than their better parent and released varieties by two-folds for Fe and Cu and by one and a half times for Zn and Mn, respectively. The appearance of high frequency of transgressive RILs despite no/marginal differences between their parents suggested dispersion of increasing and decreasing effect alleles between the parents at the loci controlling all the four micronutrients. Three RILs which were significantly rich in combination of at least two micronutrients would serve as potential donors for developing micronutrients-dense cultivars. Positively significant and a fairly high magnitude of correlation coefficients between Zn and Fe, and Zn and Cu and their poor correlation with fresh pod and seed weight plant^{-1} suggested possibility of breeding Zn, Fe and Cu rich cultivars without compromising their economic product yield potential in dolichos bean.

Keywords : Genetics, micronutrients, transgression, variability

IN human beings micronutrients (Fe, Zn, Cu and Mn) deficiency, known as hidden hunger affects about 38 per cent of pregnant women and 43 per cent of pre-school children worldwide. Crop products are the primary source of these micronutrients to human beings, especially to those living in developing countries and depend on vegetarian diet (Bouis and Saltzman, 2017).

The delivery of crop varieties selected and / or bred for enhanced grain micronutrients through plant breeding is considered as the most cost-effective and sustainable approach to combat micronutrients malnutrition (Bouis and Saltzman, 2017). It is possible to increase micro-nutrient contents in edible portions of staple crops to target levels required for reducing micronutrient malnutrition without compromising yield and / or farmer-preferred agronomic traits through plant breeding (Bouis and Saltzman, 2017).

In India, especially in southern states, dolichos bean is one of the popular grain legume-cum-vegetable crops produced and consumed as a staple cereal food complement by millions of people in large quantities. It is predominantly grown for fresh beans for use as a vegetable. Fresh pods are the harvestable and marketable economic products. It serves as one of the major sources of protein for people who depend on vegetarian diet. It is expected to reduce malnutrition in target population at least by 50 per cent through regular and large-scale intake of micronutrients-dense diets based on the products of crops (Bouis and Saltzman, 2017) such as dolichos bean.

Exploitation of genetic variability within the breeding populations such as advanced breeding lines, di-haploids, recombinant inbred lines (RILs), etc., is the short-term strategy for identification and delivery of micronutrients-rich crop cultivars in high yielding genetic background to cater to the immediate needs of the farmers for production and end-users/target population for consumption. The objectives of the present investigation were to (1) assess genetic variability and identify micronutrients-rich RILs, (2) explore genetic control of micronutrients contents and (3) assess association among micronutrients and with fresh pod and seed weight plant^{-1}.

Material and Methods

The material of the study consisted of 97 RILs derived from HA 10-8 × RIL 3-180. While HA 10-8 is a determinate photoperiod insensitive (PIS) advanced breeding line, RIL 3-180, is a determinate PIS RIL derived from HA 4 × CPI 31113 cross (Shivakumar *et al.,* 2016).

Field evaluation of RILs : The RILs, their parents along with HA 3 and HA 4 (released varieties) were evaluated in augmented design at four locations, Gandhi Krishi Vignana Kendra (GKVK), Bangalore, Agricultural Research Station (ARS), Chintamani and Zonal Agricultural Research station (ZARS), Mandya and experimental plots of College of Agriculture, Hassan during 2017 rainy season. The entries were sown in single rows of 2.5 m length with a row to row spacing of 0.45 m and 0.3 m between plants within a row in five blocks, each containing 21 entries. A basal dose of 25:50:25 kg ha^{-1} NPK (nitrogen: phosphorous: potassium) was applied to the experimental plots. Recommended package of practices were followed to raise a healthy crop under rain fed condition.

Sampling of plants and data collection: Data were recorded on five randomly tagged plants on fresh pod and seed weight following the descriptors (Byregowda *et al.,* 2015).

Sample preparation and grain micronutrients estimation: The grains sampled from the RILs, their parents and released varieties (HA 3 & HA 4) evaluated at GKVK, Bangalore were used for estimation of Zn, Fe, Cu and Mn contents. Dry pods from selfed plants from each entry were harvested manually and hand-threshed. The grains were sun-dried and washed with double distilled water to remove any surface contaminants and dried in hot-air oven at 70 ^{0}C for 72 h. Washed and fine powdered 0.5 g of grain samples were digested in a mixture of hydrochloric acid and perchloric acids. The digested samples were used for estimation of Zn, Fe, Cu and Mn contents using atomic absorption spectro-photometry (AAS) (Lindsay and Norvell, 1978) and their concentrations were expressed in parts per million (ppm).

Statistical analysis: The computed mean values of data recorded on randomly sampled plants of RILs evaluated in four locations for fresh pod and seed weights and the mean values of grain micronutrients contents were used for the statistical analysis. Absolute range (AR) = (highest – lowest), standardized range (SR) = [(highest–lowest)/mean] and phenotypic coefficient of variability (PCV) = [phenotypic standard deviation/experimental mean] × 100 for grain micronutrient contents were estimated. The correlation coefficients among grain micronutrients and correlation of micronutrients with fresh pod and seed weight plant^{-1} were estimated.

Exploring genetics of micronutrients contents: Skewness, the third degree statistics (K_3) and Kurtosis, the fourth degree statistics (K_4) were estimated to explore the nature of genetic control of grain micro-nutrient contents. The significance of the estimates of K_3 and K_4 was examined following students' 't' test using the standard errors (SE) estimated as SE_{K3} = "[6n (n-1)/(n-2) (n+1) (n+3)] and SE_{K4} = "[24 $(n-1)^2$ / (n-2) (n-3) (n+3) (n+5)], respectively, where, n= number of RILs (Fisher, 1950). Positively and negatively skewed distributions of RILs indicate complementary and duplicate epistasis of decreasing and increasing effect genes controlling the target traits, respectively. Mesokurtic (K_4=0), leptokurtic (K_4>3) and platykurtic (K_4<3) distributions of RILs indicate involvement of moderate, fewer and large number of genes controlling the target traits. While K_4=0 or K_4<3 in the absence of epistasis, K_4 is always >3 in the presence of epistasis. All the statistical analyses were implemented using SPSS software.

Results and Discussion

Genetic variability: RILs are valuable sources for identifying high yielding pure-lines for use as varieties for commercial production in predominantly

TABLE I

Descriptive statistics for grain micronutrients contents in Recombinant Inbred Lines (RILs) derived from HA 10-8 × RIL 3-180

Micronutrient	Mean	Maximum	Minimum	Standardized range	Phenotypic coefficient of variation
Zinc (ppm)	73.27 ± 1.15	132.00	44.00	1.20	15.72
Iron (ppm)	102.32 ± 3.51	284.00	44.00	2.35	34.49
Copper (ppm)	3.60 ± 0.12	06.00	00.00	1.67	32.38
Manganese (ppm)	28.51 ± 0.56	42.00	02.40	1.39	19.74

self-pollinated crops like dolichos bean. Although RILs are derived using single seed descent method without selection, it is possible to recover homozygous genotypes as productive as those which can be identified through pedigree selection for target traits including grain micronutrients and transgressive segregation can be fixed. In the present study, the RILs differed widely for grain Fe, Zn, Mn and Cu contents in that order as quantified by estimates of AR (Table I). However, differences among the RILs were greater for grain Fe and Cu than the other two micronutrients as indicated by the estimates of SR and PCV (Table I). Blair *et al.* (2009) among common bean RILs and Khazaei *et al.* (2017) among lentil diverse germplasm accessions also reported nearly two-fold difference in the range of grain Zn and Fe contents. Substantial variability suggests ample scope for selecting the RILs rich in grain micronutrient contents in dolichos bean.

RILs rich in individual and combination of grain micronutrients: The six best RILs rich in each of the grain micronutrient contents were identified (Tables II, III, IV and V). RILs significantly surpassed the better parent as well as released varieties. The top six best RILs rich in Zn contents were relatively better fresh pod and seed yielders than those rich in Fe and Mn contents and comparable to those rich in Cu. Further, the top best four RILs and the top best RIL were / was better than their better parents by two folds for Fe and Cu and by one and a half folds for Zn and Mn, respectively. Three RILs (RIL 17, RIL 24 and RIL 28) were significantly better than their better parent and released varieties for combination of atleast two micronutrients (Table VI). These RILs could be used to investigate genetic and physiological basis of accumulation of micronutrients and as potential donors for breeding cultivars rich in different combinations of four micronutrients in high yielding genetic background. Hacisalihoglu *et al.* (2005) identified and reported a core set of six RILs with

TABLE II

Performance of six best Zn rich dolichos bean Recombinant Inbred Lines (RILs) derived from HA 10-8 × RIL 3-180 for selected agronomic traits

RILs	Zinc (ppm)	Fresh pod weight plant^{-1} (g)	Fresh seed weight plant^{-1} (g)
RIL-24	132.00	66.72	33.70
RIL-17	98.00	53.83	28.75
RIL-28	90.00	47.09	25.48
RIL-43	90.00	70.43	35.53
RIL-50	90.00	48.13	25.49
RIL-52	90.00	69.62	36.59
Parents			
HA 10-8	76.00	93.56	50.56
RIL 3-180	58.00	42.30	18.40
Released varieties			
HA 3	52.00	80.08	44.85
HA 4	58.00	89.39	50.06
SEm ±	1.15	1.00	0.55
CD @P=0.05	3.18	2.78	1.53

TABLE III

Performance of 10 best Fe rich dolichos bean recombinant inbred lines (RILs) derived from HA 10-8 × RIL 3-180 for selected agronomic traits

RILs	Iron (ppm)	Fresh pod weight plant^{-1} (g)	Fresh seed weight plant^{-1} (g)
RIL-24	284.00	66.72	33.70
RIL-81	222.00	51.83	25.70
RIL-87	212.00	55.78	29.73
RIL-22	204.00	58.66	31.35
RIL-82	190.00	60.95	32.59
RIL-17	158.00	53.83	28.75
Parents			
HA 10-8	76.00	93.56	50.56
RIL 3-180	104.00	78.54	42.30
Released varieties			
HA 3	74.00	80.08	44.85
HA 4	76.00	89.39	50.06
SEm±	3.51	1.00	0.55
CD @P=0.05	9.73	2.78	1.53

TABLE IV

Performance of six best Cu rich dolichos bean recombinant inbred lines (RILs) derived from HA 10-8 × RIL 3-180 for selected agronomic traits

RILs	Copper (ppm)	Fresh pod weight plant^{-1} (g)	Fresh seed weight plant^{-1} (g)
RIL-01	6.00	79.47	41.87
RIL-09	6.00	60.90	32.85
RIL-10	6.00	53.50	27.82
RIL-17	6.00	53.83	28.75
RIL-22	6.00	58.66	31.35
RIL-28	6.00	47.09	25.48
Parents			
HA 10-8	2.00	93.56	50.56
RIL 3-180	2.00	78.54	42.30
Released varieties			
HA 3	2.00	80.08	44.85
HA 4	2.00	89.39	50.06
SEm±	0.12	1.00	0.55
CD @P=0.05	0.32	2.78	1.53

TABLE V

Performance of six best Mn rich dolichos bean recombinant inbred lines (RILs) derived from HA 10-8 × RIL 3-180 for selected agronomic traits

RILs	Copper (ppm)	Fresh pod weight plant^{-1} (g)	Fresh seed weight plant^{-1} (g)
RIL-81	42.00	51.83	25.70
RIL-28	40.00	47.09	25.48
RIL-58	38.00	49.71	24.92
RIL-86	38.00	51.25	27.22
RIL-33	36.00	59.46	30.08
RIL-56	36.00	54.32	29.01
Parents			
HA 10-8	28.00	93.56	50.56
RIL 3-180	26.00	78.54	42.30
Released varieties			
HA 3	34.00	80.08	44.85
HA 4	28.00	89.39	50.06
SE±	0.56	1.00	0.55
CD @P=0.05	1.55	2.78	1.53

TABLE VI

RILs significantly better than their parents and released varieties for combination of at least two grain micronutrients

RILs	Zn (ppm)	Fe (ppm)	Cu (ppm)	Mn (ppm)
RIL 01	88.00	-	6.00	-
RIL 17	98.00	158.00	6.00	-
RIL 23	86.00	150.00	-	-
RIL 24	132.00	284.00	-	-
RIL 28	90.00	-	6.00	40.00
Parents				
HA 10-8	76.00	76.00	2.00	28.00
RIL 3-180	58.00	104.00	2.00	26.00
Released varieties				
HA 3	52.00	74.00	2.00	34.00
HA 4	58.00	76.00	2.00	28.00
SEm ±	1.15	3.51	0.12	0.56
CD @ P=0.05	3.18	9.73	0.32	1.55

grain Zn and Fe contents significantly better than their better parent for use in strategic and applied breeding for Zn and Fe rich common bean cultivars.

Transgressive segregation: A high frequency of RILs transgressed their parental limits for all the micronutrients and the frequency of transgressed RILs was higher for Cu and Mn than that for Zn and Fe (Table VII and Fig. 1 to 4). Further, there were either

TABLE VII

Estimates of frequencies of transgressive segregants for grain micronutrient contents in recombinant inbred lines (RILs) derived from HA 10-8 × RIL 3-180

Grain micronutrient	Frequency of RILs surpassing	
	Higher parent	Lower parent
Zinc	0.25	0.03
Iron	0.23	0.04
Copper	0.76	0.01
Manganese	0.43	0.22

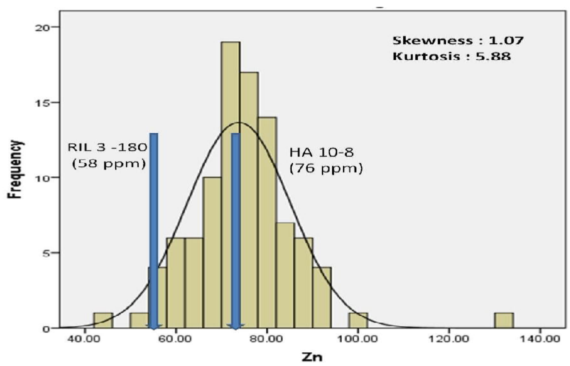

Fig. 1: Graph depicting frequency distribution for Zn content in RILs derived from HA 10-8 × RIL 3-180

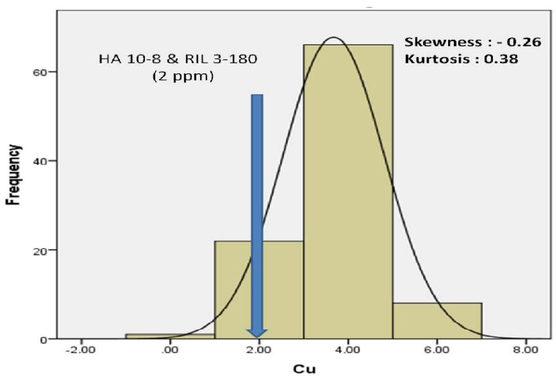

Fig. 3: Graph depicting frequency distribution for Cu content in RILs derived from HA 10-8 × RIL 3-180.

subtle differences (for Cu and Mn) or marginal differences (for Zn and Fe) between the parents of the RILs. Appearance of a large frequency of transgressive segregants despite absence of/marginal differences between their parents could be attributed to dispersion of complementary dominant increasing and decreasing effect alleles between the parents (Kuczynska, 2007 and Koide *et al.*, 2018) at the loci controlling all the grain micronutrients contents. Blair *et al.* (2009), Blair *et al.* (2010) and Blair *et al.* (2011) in common bean RIL populations, Santos and Bioteux (2015) in cowpea and Zemolin *et al.* (2016) in common bean F_2 populations discovered transgressive segregants for Zn and Fe contents. In the present study, it is likely that the dolichos bean RILs that received increasing and decreasing alleles from both the parents (as a result of several cycles of recombination during the process of their development) transgressed their parents.

Third and fourth degree statistics-based genetics of micronutrients: Most researchers aimed at understanding the genetics of quantitative traits (QTs) have used either first degree statistics (means) or

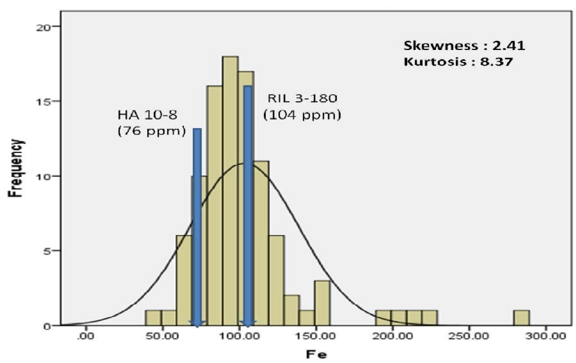

Fig. 2: Graph depicting frequency distribution for Fe content in RILs derived from HA 10-8 × RIL 3-180.

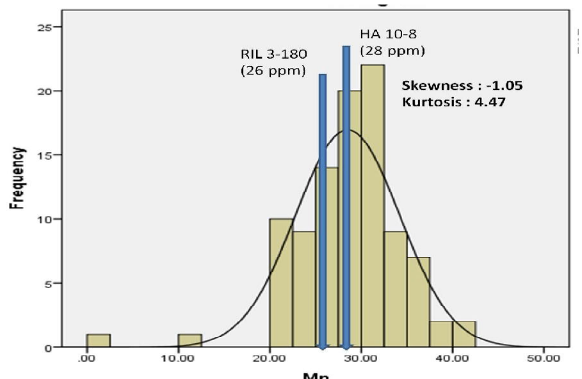

Fig. 4: Graph depicting frequency distribution for Mn content in RILs derived from HA 10-8 × RIL 3-180.

second degree statistics (variances) and their derivatives and a few have used both. However, seldom there have been reported attempts on the use of third degree statistics (skewness) and fourth degree statistics (kurtosis) to explore the genetics of QTs. Further, skewness and kurtosis are more powerful statistics to investigate genetics of QTs than analysis of variance especially for detecting and characterizing epistasis. The greatest advantage of using skewness and kurtosis is that it is not necessary to develop structured populations such as half-sibs and full-sibs, which is both time and resources demanding. The available QTs' phenotype data of stabilized populations such as RILs, which are now routinely developed in crop breeding programmes is all that is necessary to elicit insights into the possible nature of genetic control of QTs, including grain micronutrients contents based on the magnitude and direction of estimates of skewness and kurtosis.

In the present study, leptokurtic positively skewed distribution of RILs (Fig. 1 & 2) indicated that Zn and Fe contents are controlled by fewer genes with majority of them displaying complementary epistatic decreasing effects. Inheritance of Mn content is also likely to be controlled by fewer genes but with majority of them displaying duplicate epistatic increasing effects as indicated by leptokurtic negatively skewed distribution of RILs (Fig. 4). It is likely that complementary and duplicate epistasis of genes controlling Zn and Fe, and Mn, respectively is the result of their repulsion and coupling phase linkages, respectively. Contrary to Zn, Fe and Mn, platykurtic near-symmetrical distribution of RILs (Fig. 3) suggested the possible involvement of a large number of non-epistatic genes controlling the inheritance of Cu with increasing and decreasing effect alleles are being equally frequent. Intense and mild selection should be practiced to realize rapid genetic gain in Zn, Fe and Cu, and Mn, respectively. The skewness- and kurtosis-based inferences on the genetic control of grain micronutrients contents in dolichos bean are only indicative and need to be confirmed through the combination of first and second degree statistics.

Association among grain micronutrients contents: The correlation coefficients among the target traits are reliable, if they are estimated in closed / full-sib populations such as RILs which are derived from parents differing for the genes controlling the target traits, as linkage disequilibrium (LD) is created in such populations. A fairly high magnitude of correlation coefficients between Zn & Fe and Zn & Cu were detected in dolichos bean (Table VIII). These results and those of Hacisalihoglu *et al.* (2005) and

TABLE VIII

Estimates of correlation coefficients among grain micronutrients contents in the Recombinant Inbred Lines (RILs) derived from HA 10-8 × RIL 3-180

Micronutrient	Zinc	Iron	Copper	Manganese
RIL 01	88.00	-	6.00	-
Zinc	1.00			
Iron	0.49 **	1.00		
Copper	0.48 **	0.30 **	1.00	
Manganese	0.20 *	0.34 **	0.31 **	1.00

Khazaei *et al.* (2017) who also reported significant positive correlation of grain Zn with Fe and Cu contents in common bean and lentil, respectively suggested the possibility of linkage between the genes controlling Zn, Fe and Cu in dolichos bean. Empirical evidences on the co-localization / overlapping of several quantitative traits loci (QTLs) controlling Fe and Zn in common bean (Blair *et al.*, 2009; Blair *et al.*, 2010 and Blair *et al.*, 2011) would lend adequate support to indirect evidence for the linkage of genes controlling grain Fe and Zn contents in dolichos bean, which however needs to be confirmed. Significant correlation of Zn with Fe and Cu in dolichos bean could be attributed to common mechanism of uptake and metabolism or to common transporters controlling the movement of these micronutrients within the plants (Ghandilyan *et al.*, 2006). Further, significant and low magnitude of correlation between Mn with other micronutrients and between Cu and Mn suggest that genes controlling Mn are likely to be loosely linked to those controlling the other three micronutrients. It is possible that the genes controlling micronutrient contents could be linked in repulsion phase considering that the increasing and decreasing effect alleles controlling micronutrient contents are dispersed between the parents of RILs. The likelihood of either strong / loose linkage of genes suggests possibility of combining all the micronutrients in a single cultivar.

Association of grain micronutrients with fresh pod and seed weight: Maximum impact of micronutrients can be realized if and only if high micronutrients are delivered in high yielding cultivars with farmers' and end-user preferred pod quality evident traits such as high pod fragrance in dolichos bean. Although the top six best RILs rich in four micronutrients contents were not significantly superior to their better parent and released varieties, a very poor correlation coefficient of any of the four micronutrients with fresh pod and fresh seed weight plant^{-1} (Fig. 5 to 8) suggest possibility of combining high micronutrients contents in high yielding background. It is reported that genotypes rich in grain Fe and Zn contents exhibit high seedling vigour, especially in low-fertile soils, high levels of resilience to disease and moisture stresses, all of which are advantages in regions with nutrient poor soils and erratic rainfall (Bouis and Saltzman, 2017) where dolichos bean is predominantly grown.

Considerable variation was evident for all the micronutrients contents among the RILs. Appearance of large frequency of transgressive segregants among RILs suggested dispersion of dominant increasing and decreasing alleles between their parents at the loci controlling all the micronutrients. Inheritance of grain Zn and Fe, and Mn contents is likely to be controlled by fewer genes displaying complementary and duplicate epistasis with decreasing and increasing effects, respectively. On the contrary, inheritance of grain Cu content is controlled by a large numbers of equally frequent non-epistatic decreasing and increasing effects genes. Three RILs (RIL 17, RIL 24 and RIL 28) which were significantly better than their better parent and released varieties (HA 3 and HA 4)

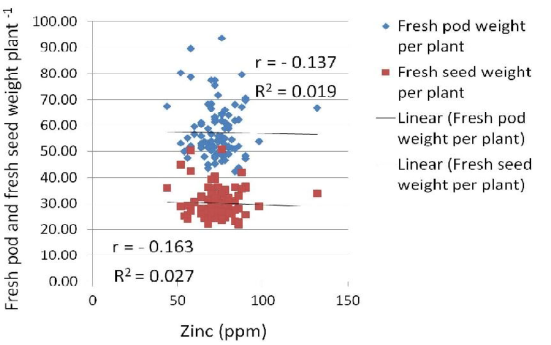

Fig. 5: Graph depicting estimates of correlation between grain Zn and the fresh pod and fresh seed weight plant^{-1} in RILs derived from HA 10-8 × RIL 3-180

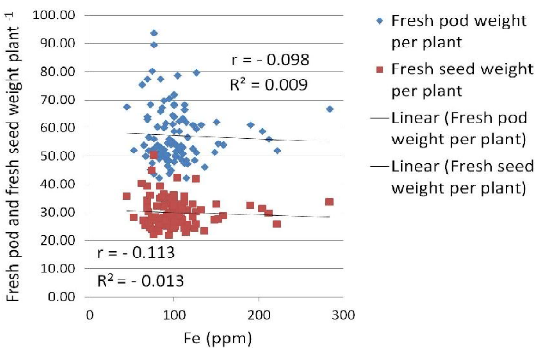

Fig. 6: Graph depicting estimates of correlation between Fe and the fresh pod and fresh seed weight plant^{-1} in RILs derived from HA 10-8 × RIL 3-180

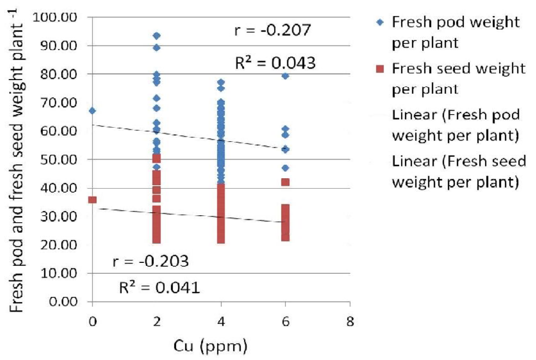

Fig. 7: Graph depicting estimates of correlation between Cu and the fresh pod and fresh seed weight plant^{-1} in RILs derived from HA 10-8 × RIL 3-180

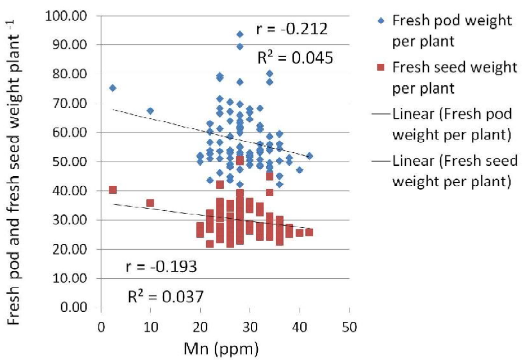

Fig. 8: Graph depicting estimates of correlation between Mn and the fresh pod and fresh seed weight plant^{-1} in RILs derived from HA 10-8 × RIL 3-180

for combination of at least two micronutrients, could be used as potential donors for breeding cultivars rich in different combinations of four micronutrients. Poor correlation of all the four micronutrients contents with fresh pod and seed weight plant^{-1} suggested developing micronutrients-rich cultivars without compromise in economic product yield potential in dolichos bean.

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(*Received :* May, 2018 *Accepted :* June, 2018)