Association Analysis in Germplasm and F₂ Segregating Population of Barnyard Millet (*Echinochloa frumentacea* Roxb. Link) for Biometrical and Nutritional Traits

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**A B S T R A C T**

An investigation was carried out to assess the character association in forty germplasm and two F₂ population for biometrical and nutritional traits in barnyard millet during *Rabi*, 2014 and *Rabi*, 2015 respectively. Both F₂ population exhibited wide variation and comparatively better than their respective parental genotypes for yield related traits and micronutrient content. From association analysis, there was alteration/shift in the association of few character pairs in germplasm and two segregating population, meaning that the traits might be unlinked due to weak or non-significant association or variability level. Path analysis in germplasm revealed that stem girth, single ear head weight, ear head breadth, number of racemes, zinc content, number of productive tillers, flag leaf length, thousand grain weight and plant height had high positive direct effect on grain yield. It is surprising to note that the association between Fe and Zn content in grains was positive but non-significant association with grain yield would create the possibility of selecting the plant which high micronutrient content with moderate yield or hybridization followed by selection for enhancement of iron and zinc content in barnyard millet.

**Keywords** Barnyard millet, Correlation, Path analysis, Micronutrient.

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**Introduction**

The increasing lifestyle diseases among the population due to eating of the unavoidable foods like rice and wheat, nature also given an alternate nutri-cereals for mankind’s are small seeded grains called small millets. Of them, barnyard millet (*Echinochloa frumentacea*), a highly self-pollinated crop which belongs *Poaceae* family has ability to persist under severe drought, salinity, heat, floods and have excellent climate resilient capacity compared to other cereal crops. Grains are cultivated mainly for food and the straw has a good fodder value (Gopalan *et al.*, 2008). The carbohydrate, protein content and good amount of dietary fibre makes slow digestion of food by the way the amount of glucose moving in the blood per second also slow, hence it said to be low glycemic food that is favorable for the diabetic person and who is engaged in sedentary activities. The
micronutrients, iron and zinc content in the grains also found to be maximum among the cereals, those that are currently deficient in children’s, anemic patient’s especially pregnant women’s who are living in the developing countries such as India. In any crop improvement programme, once the variability is determined in germplasm and segregating population, the estimation of association among the traits is crucial in order to decide the selection criteria that improve yield components and nutritional traits. These may be variable in different segregating population. The $F_2$ population of different crosses was studied to select the appropriate segregating population for further improvement (Laila et al., 2014). Therefore the objective of the present study is to evaluate the forty barnyard millet germplasm and two $F_2$ population for their natural variability and variability created by crossing, to identify the strongly associated traits and separate the direct effects from the indirect effects through other related characters that gives clues in a selection programme for improvement of grain yield along with micronutrient.

Materials and Methods

The study was conducted during 2014 (germplasm) and 2015 (parents, $F_2$ population) at the Research field of Department of Plant Breeding and Genetics, Agricultural College & Research Institute, Madurai, Tamil Nadu, India. The experiments material included, forty germplasm from various sources, three parental materials ACM 331, ACM 333 and MA 10 that utilized for hybridization were based on variation in iron and zinc content (ACM 331 low in Fe and Zn, where ACM 333 and MA 10 are rich in both), and $F_2$ segregating population of ACM 331 x ACM 333 and ACM 331x MA 10 material. In both the trials, materials were evaluated with two replications in a Randomized Complete Block Design. The germplasm was sown in two rows of 3 m length and $F_2$ segregating population in 40 rows for each replication. Seeds sown with the spacing of 30 x 15 cm and recommended agronomic practices were followed during the crop period. The observations were recorded on five plants randomly in each germplasm and 200 $F_2$ plants of each replication for 15 biometrical and nutritional traits viz., plant height, flag leaf length, flag leaf breadth, ear head length, ear head breadth, number of racemes, single ear head weight, length of lower raceme, number of leaves per tiller, stem girth, thousand grain weight, single plant yield, iron (Fe) and zinc (Zn) content. The Fe and Zn content was estimated on the grains of main tiller of the individual plant. The estimation of micronutrient content was done through Atomic Absorption Spectrophotometer. Correlation coefficients were further partitioned into components of direct and indirect effects by path analysis (Wright, 1921; Dewey and Lu, 1959) that was done through software GENRES, 1994.

Results and Discussion

Relationship among traits

The selection process is complicated and incomplete, unless the breeders knowledge of association between interplaying components with yield and nutritional characters known. Therefore, a better understanding of the contribution of each trait in building up the genetic makeup of the any crop may be obtained through correlation studies (Guddadamath et al., 2011 in Okra). The relationship between yield and its main contributing traits, in homogenous population, has been studied by several researchers, Gupta et al., (2009); Channappagoudar et al., (2008) and Mohan et al., (2006) in barnyard millet but none on segregating population so far. Estimates of simple phenotypic
correlation coefficients (Pearson) among the fifteen biometrical and nutritional characters in germplasm and two F₂ segregating population are depicted in table 1. As ample of evidence from earlier reports, associations of traits is variable from species to species or even in non –segregating and segregating generations of same population. Shift in association between these characters in germplasm and non-segregating population might be of weak association or shuffling of gene during meiotic event (Guddadamath et al., 2011) and also the more variability generated through segregation in F₂ than parents (Karладee, 1980).

From the correlation studies, it is known that flag leaf breadth, ear head length and ear head breadth are strongly associated traits due to correlated expression both in germplasm and two segregating populations meaning that the gene for these traits may be tightly linked or pleiotropic effect. This is an agreement with previously mentioned barnyard millet researchers in this paper elsewhere. The trait, number of raceme per inflorescence is closely associated with single ear head weight while, the number of productive tillers and flag leaf breadth were significantly associated trait with grain yield per plant. It is physiologically proven concept where, flag leaves are the major source of phloem-delivered photo assimilates during the grain-filling stage (Farrar, 1996) and productive tillers, another important agronomic characters for grain yield per plant because, the tiller number per plant determines the panicle number, a crucial factor of grain yield (Smith and Dilday, 2003 and Yan et al., 1998). All other traits are inconsistent in showing association in all the population, it shows their nondependent with each other or unlinked or distance between the genes are larger or variability confounded in germplasm and F₂ population.

An interested finding that, though Fe and Zn content are positive but non-significant association with number of leaves per tiller and grain yield per plant in all the population both of them highly correlated among selves. The high positive significant association between Fe and Zn densities has been reported in pearl millet (Rai et al., 2014 and Govindaraj et al., 2013), sorghum (Ashok Kumar et al., 2013 and 2010), rice (Anandan et al., 2011), wheat (Velu et al., 2011 and Zhang et al., 2010) and finger millet (Upadhyaya et al., 2011). As well as non-significant association of grain Fe and Zn densities with grain weight (Rai et al., 2012 and Gupta et al., 2009).

**Path coefficient analysis**

Path coefficient analysis is unavoidable for plant breeders to improve the selection efficiency by pinpointing the traits with significant effects on yield or yield components (Toebe and Filho, 2013). In this study, for germplasm plant height, flag leaf breadth, ear head breadth, number of raceme, single ear head weight, number of productive tillers, thousand grain weight, number of leaves/tiller, stem girth and zinc content had direct positive effect on grain yield (Table 2). Of them, single ear head height employed high direct positive effect (0.473) and positively associated with grain yield per plant as well as indirect positive effects of remaining traits, however single ear head weight was lessened by flag leaf length, ear head length, no. of productive tillers and iron content.

It is accordance with Prakash and Vanniarajan (2015), where single ear head weight had maximum association and direct effect with yield. Though Fe and Zn content were highly associated, they could not be ideal for direct selection of grain yield due to negative direct effect of Fe, however it is possible by selecting the traits higher in number of raceme, single ear head weight, number of leaves/tiller, stem girth and Zn content that had indirect effect.
Table 1: Correlation coefficients for fifteen biometrical and nutritional characters for 40 germplasm and F2 of cross I & cross II in barnyard millet

| Variables                  | PH   | FL   | FB   | EL   | EB   | NR   | SEW  | NPT  | TW   | NOL  | LR   | SG   | Fe   | Zn   | GYP |
|----------------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|-----|
| **Plant height**           |      |      |      |      |      |      |      |      |      |      |      |      |      |      |     |
| Germplasm                  | 1.000| 0.595*| 0.623*| 0.761*| 0.546*| 0.468*| 0.594*| 0.291| 0.293| 0.721*| 0.206| 0.517*| 0.082| 0.117| 0.631*|
| Cross I                    | 1.000| 0.282| 0.239| 0.599*| 0.107| -0.058| -0.124| 0.210| 0.207| 0.318| 0.250| 0.264| -0.135| 0.026| 0.164|
| Cross II                   | 1.000| -0.024| -0.112| 0.055| -0.060| 0.066| -0.043| 0.057| -0.248| 0.110| -0.047| -0.033| 0.110| 0.150| 0.104|
| **Flag leaf length**       |      |      |      |      |      |      |      |      |      |      |      |      |      |      |     |
| Germplasm                  | 1.000| 0.799*| 0.565*| 0.345*| 0.538*| 0.593*| 0.365*| 0.285| 0.589*| 0.049| 0.614*| 0.272| 0.341*| 0.620*|
| Cross I                    | 1.000| 0.599*| 0.406*| 0.394*| 0.293| 0.165| 0.045| -0.048| 0.382*| 0.421*| -0.113| 0.071| 0.209| 0.214|
| Cross II                   | 1.000| 0.616*| 0.604*| 0.583*| 0.412*| 0.622*| 0.199| -0.043| -0.158| 0.453*| 0.090| -0.258| -0.373*| 0.244|
| **Flag leaf breadth**      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |     |
| Germplasm                  | 1.000| 0.588*| 0.302| 0.651*| 0.736*| 0.465*| 0.493*| 0.683*| -0.009| 0.537*| 0.294| 0.322*| 0.731*|
| Cross I                    | 1.000| 0.386*| 0.179| 0.387*| 0.100| 0.314| 0.258| 0.150| 0.098| -0.080| 0.061| -0.023| 0.549*|
| Cross II                   | 1.000| 0.358*| 0.579*| 0.107| 0.463*| 0.325*| -0.003| -0.002| 0.151| 0.209| -0.066| -0.177| 0.436*|
| **Ear head length**        |      |      |      |      |      |      |      |      |      |      |      |      |      |      |     |
| Germplasm                  | 1.000| 0.638*| 0.668*| 0.593*| 0.427*| 0.531*| 0.603*| 0.115| 0.107| 0.660*|
| Cross I                    | 1.000| 0.563*| 0.571*| 0.464*| 0.157| -0.004| -0.047| 0.325*| 0.069| -0.276| -0.197| 0.051|
| Cross II                   | 1.000| 0.298| 0.026| 0.152| 0.066| 0.212| 0.361*| 0.424*| 0.027| -0.006| 0.038| 0.131|
| **Ear head breadth**       |      |      |      |      |      |      |      |      |      |      |      |      |      |      |     |
| Germplasm                  | 1.000| 0.381*| 0.386*| 0.016| 0.210| 0.225| 0.527*| 0.233| 0.262| 0.299| 0.492*|
| Cross I                    | 1.000| -0.187| -0.094| 0.204| 0.303| 0.232| 0.713*| 0.202| 0.111| 0.096| 0.143|
| Cross II                   | 1.000| 0.231| 0.433*| 0.276| 0.030| -0.122| 0.343*| 0.130| -0.180| -0.349*| 0.267|
| **No. of raceme**          |      |      |      |      |      |      |      |      |      |      |      |      |      |      |     |
| Germplasm                  | 1.000| 0.785*| 0.317*| 0.480*| 0.572*| -0.020| 0.492*| 0.151| 0.133| 0.804*|
| Cross I                    | 1.000| 0.581*| 0.329| -0.289| 0.094| -0.239| 0.062| 0.091| 0.022| 0.319|
| Cross II                   | 1.000| 0.358*| 0.011| 0.036| 0.080| 0.127| 0.096| -0.154| -0.307*| -0.187|
| **Single ear head weight** |      |      |      |      |      |      |      |      |      |      |      |      |      |      |     |
| Germplasm                  | 1.000| 0.311| 0.502*| 0.718*| -0.055| 0.367*| 0.218| 0.232| 0.838*|
| Cross I                    | 1.000| 0.158| -0.422*| 0.189| -0.132| -0.139| 0.374*| 0.280| 0.036|
| Cross II                   | 1.000| 0.199| -0.081| 0.411*| 0.384*| 0.139| -0.194| -0.226| 0.247|
| **No. of productive tillers** |      |      |      |      |      |      |      |      |      |      |      |      |      |      |     |
| Germplasm                  | 1.000| 0.158| 0.550*| -0.038| 0.355*| 0.019| 0.023| 0.423*|
| Cross I                    | 1.000| 0.193| 0.037| -0.078| 0.189| 0.039| -0.118| 0.450*|
| Cross II                   | 1.000| -0.212| 0.389*| 0.218| 0.065| 0.144| 0.080| 0.764*|
| **Thousand grain weight**  |      |      |      |      |      |      |      |      |      |      |      |      |      |      |     |
| Germplasm                  | 1.000| 0.423*| -0.132| 0.336*| 0.117| 0.030| 0.583*|
| Cross I                    | 1.000| -0.154| 0.125| 0.151| -0.076| -0.197| 0.300|
| Cross II                   | 1.000| -0.245| -0.021| -0.131| 0.200| -0.024| -0.161|
| **No. of leaves/tiller**   |      |      |      |      |      |      |      |      |      |      |      |      |      |      |     |
| Germplasm                  | 1.000| -0.097| 0.511*| 0.081| 0.091| 0.699*|

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| Germplasm | PH | FL | FB | EL | EB | NR | SEW | NT | TW | NOL | LR | SG | Fe | Zn | GYP |
|-----------|----|----|----|----|----|----|-----|----|----|-----|----|----|----|----|-----|
| Cross I   | 0.100 | -0.115 | 0.096 | -0.247 | 0.193 | 0.105 | 0.283 | -0.029 | 0.035 | 0.009 | -0.046 | 0.284 | -0.019 | 0.023 | 0.631* |
| Cross II  | 0.060 | -0.193 | 0.121 | -0.180 | 0.123 | 0.121 | 0.284 | -0.038 | 0.034 | 0.008 | -0.009 | 0.326 | -0.066 | 0.069 | 0.620* |
| Cross I   | 0.064 | -0.156 | 0.150 | -0.187 | 0.110 | 0.129 | 0.346 | -0.045 | 0.056 | 0.008 | -0.002 | 0.268 | -0.079 | 0.075 | 0.731* |
| Cross II  | 0.077 | -0.109 | 0.088 | -0.319 | 0.224 | 0.148 | 0.278 | -0.025 | 0.051 | 0.008 | -0.042 | 0.322 | -0.027 | 0.022 | 0.660* |
| Cross I   | 0.056 | -0.069 | 0.044 | -0.206 | 0.347 | 0.084 | 0.182 | -0.002 | 0.025 | 0.004 | -0.076 | 0.125 | -0.063 | 0.061 | 0.492* |
| Cross II  | 0.047 | -0.105 | 0.095 | -0.214 | 0.133 | 0.221 | 0.373 | -0.031 | 0.056 | 0.007 | 0.002 | 0.260 | -0.036 | 0.027 | 0.804* |
| Cross I   | 0.060 | -0.116 | 0.110 | -0.187 | 0.134 | 0.174 | 0.473 | -0.031 | 0.059 | 0.009 | 0.006 | 0.196 | -0.052 | 0.048 | 0.838* |
| Cross II  | 0.073 | -0.118 | 0.090 | -0.186 | 0.103 | 0.125 | 0.334 | -0.040 | 0.032 | 0.013 | -0.006 | 0.278 | -0.046 | 0.044 | 0.699* |
| Cross I   | 0.034 | -0.012 | 0.002 | -0.098 | 0.192 | -0.003 | -0.021 | -0.004 | -0.015 | 0.001 | -0.138 | 0.143 | -0.063 | 0.054 | 0.067 |
| Cross II  | 0.056 | -0.123 | 0.079 | -0.200 | 0.085 | 0.112 | 0.181 | -0.042 | 0.040 | 0.007 | -0.038 | 0.512 | -0.039 | 0.022 | 0.571* |
| Cross I   | 0.008 | -0.053 | 0.050 | -0.037 | 0.092 | 0.033 | 0.105 | -0.002 | 0.014 | 0.003 | -0.036 | 0.084 | -0.237 | 0.189 | 0.203 |
| Cross II  | 0.011 | -0.067 | 0.056 | -0.035 | 0.105 | 0.030 | 0.113 | -0.002 | 0.003 | 0.003 | -0.037 | 0.056 | -0.224 | 0.200 | 0.061 |

Bold values are direct effect for Germplasm, cross I and cross II. Residual effect = 0.55, plant height (PH), flag leaf length (FL), flag leaf breadth (FB), ear head length (EL), ear head breadth (EB), number of racemes (NR), single ear head weight (SEW), length of lower raceme (LR), number of leaves per tiller (NOL), stem girth (SG), thousand grain weight (TW) iron (Fe), zinc (Zn) and single plant yield (GYP).

Additional Notes: Significant levels: 5 percent, plant height (PH), flag leaf length (FL), flag leaf breadth (FB), ear head length (EL), ear head breadth (EB), number of racemes (NR), single ear head weight (SEW), length of lower raceme (LR), number of leaves per tiller (NOL), stem girth (SG), thousand grain weight (TW) iron (Fe), zinc (Zn) and single plant yield (GYP).
It is because of significant genotypic association and direct positive effects of these traits on grain yield per plant, direct selection of these traits would be effective to enhance yield in germplasm of the current study.

Thus, in present study it could be concluded that, to breed improved cultivars with elevated levels of these micronutrients through conventional breeding approaches, the emphasis for these traits stem girth, single ear head weight, ear head breadth, number of raceme, Zn, number of productive tillers, flag leaf breadth, thousand grain weight and plant height in germplasm, flag leaf breadth, single ear head weight, number of productive tillers, number of leaves tillers, thousand grain weight and Zn in ACM 331 x ACM 333 and plant height, flag leaf breadth, single ear head weight, number of productive tillers, number of leaves tillers and Fe content in ACM 331 x MA 10 would be rewarding in selection programme for maximum improvement in yield of barnyard millet.

When the enhancement of micronutrients (Fe & Zn) are concern, it is possible to improve both simultaneously (strong association) though not had direct effect in selection but positive non-significant with grain yield indicated that the breeders without compromising on grain yield could develop the maximum micronutrient rich variety. It is concomitant with Govindaraj et al., (2013) and Rai et al., (2014) in pearl millet. This is what essential in current scenario of any biofortification programme. An addition additive (fixable) nature of genetic variance for most of these traits due to high heritability and high genetic advance in germplasm allows the breeders to develop, a high yielding genotype possessing good quality.

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