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Research Note

Studies on character association and path analysis studies for yield, grain quality and nutritional traits in F_2 population of rice (*Oryza sativa* L.)

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Abstract
Rice is a major cereal crop from the consumption point of view, dietary energy and nutrition source for more than half of the world's population. Rice is rich in starch but deficient in major micronutrients. Present experiment consists of F_2 mapping population for high grain zinc content, with 312 entries studied for 15 different traits. Correlation study indicated that, grain zinc and iron content were significant and positive. So there is a scope for simultaneous improvement of both traits. Grain yield per plant showed a non-significant and negative association with zinc and iron content. Plant height, total tillers per plant, the number of effective tillers per plant, panicle length, 1000 grain weight, fertile grains per panicle, kernel breadth, L/B ratio has significant and positive correlation and positive direct effects with grain yield per plant.

Key words
Grain zinc, correlation, rice, mapping population, path coefficient

Rice is the most important food crop and energy source for more than half of world's population. It is a crop of economic significance not only across South East Asia but also the globe. Rise in global demand is imposed by increasing population which necessitate 40% more rice production by 2030 (Khush, 2005). Rice is rich in carbohydrates and starch but poor in micronutrients such as Zinc and Iron. Present day high yielding rice varieties are low in mineral content in brown rice and lower in polished form (Garcia-Oliveira *et al*. 2008). Population solely depend on rice based food suffers from micronutrient deficiency or hidden hunger. Zinc and iron deficiencies are most prevalent and affecting around 2 billion people and causing more than 0.8 million deaths annually (WHO, 2002).

Zinc is an important micronutrient in human health, a cofactor for more than 300 enzymes, plays an important role in protein structure, transcription and different cellular processes (Ishimaru *et al*. 2011). Deficiency of the same results in diarrhoea, pneumonia, stunting and child mortality (Prasad, 2004). Fe is an being important component of haemoglobin and its deficiency results in anemia and reduced growth. Biofortification is a genetic approach which aims at enrichment of food with vital nutrients at biological and genetic level (Bouis, 2002). It is a sustainable approach and successful strategy if assisted and implemented with molecular breeding. Breeding efforts from last few years have resulted in development of biofortified crops including rice, with increased micronutrient content (Harvest Plus, 2014). Increasing bio available zinc in rice endosperm is an important target of bio fortification efforts. In order to identify genes responsible for high grain zinc content and to know genetic basis of grain zinc mechanism, development of mapping population and QTL mapping is prerequisite. Although there are several types of mapping populations, segregating mapping population like F_2 or $F_{2:3}$ are quick and easy to develop as they permit mapping QTLs (Singh and Singh, 2015). Complexity of grain yield and its related traits have been reported by several studies. Although yield is the primary goal of a plant breeder, importance can be given to secondary traitsthat have positive correlation with yield. There are several character association studies carried using genotypes or varieties which are homozygous. However, inferences derived from correlation studies in segregating populations can be more meaningful as they are based on individual plant observations. Segregating populations can be subjected to selection based on early generation testing (Chahota *et al*. 2007).

Studies of correlation in segregating populations have been done by Zeng, 2004; Nandeshwar *et al*. 2010; Barman and Borah, 2012; Sala and Geetha, 2015; Dharwal *et al*. 2017; Abhilash *et al*. 2018; Thuy *et al*. 2018 and Seneega, *et al*. 2019. Correlation studies can help breeder to identify various traits and trait combination to act upon by selection for the yield. This correlation can be attributed to linkage or pleiotropy effect of genes (Singh and Choudhary, 1985) or their physiological and developmental relationship or environmental effect and also, it can be a combined output (Oad *et al*. 2002).

Path analysis leads to partitioning of correlation into various components predicting direct effect by independent variables on response variable along with indirect effects of these predictors via other predictable variables (Dewey and Lu, 1959). Such study of direct and indirect effect aids in effective selection by the plant breeder (Priya and Joel, 2009.). Simultaneous improvement of grain nutritional status without compromising its yield and quality has always been the target of plant breeders. Grain dimension traits like kernel breadth kernel length, kernel length to breadth ratio (L/B ratio) have direct impact on consumer acceptance and commercial success of any new variety. So, studying the inter relationship of grain quality traits, nutrient content and yield contributing traits is important. Possibility of simultaneous improvement of high yield and high zinc has earlier been proposed (HarvestPlus, 2014). Hence the objective of present study was to find out association of different traits (including yield contributing traits, grain quality and nutritional traits) in segregating population was undertaken to study correlation and path analysis.

The present experiment was carried in *Kharif-2018*, at Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. Based on grain zinc content data available, parents having contrast grain zinc content were chosen and crossed and mapping population was developed. Rajendra kasturi (Short grain aromatic variety with low grain zinc content) and URG-30 (a local rice genotype) of Eastern Uttar Pradesh, identified as High Zinc donor were crossed. In F_2 312 plants were raised with parents following recommended package of practices. Each F_2 plant was harvested separately. Observations for morphological traits like days to flowering, plant height (cm), total tillers per plant, the number of effective tillers per plant and yield related traits *viz* panicle length (cm), total grains per panicle (number), fertile grains per panicle, spikelet fertility (%), grain yield per plant (grams), 1000 grain weight (grams) were recorded. After harvest, grain samples were prepared for Zinc and Iron analysis. Grain Zinc (in ppm) and Iron content (in ppm) were analyzed at IRRI South Asia Hub, with XRF machine facility available at Harvest Plus Lab, ICRISAT Hyderabad. Observation for grain quality traits like, kernel length, kernel breadth, kernel length to breadth ratio (L/B ratio) were also recorded.

Data recorded was subjected to statistical analysis. Character correlation analysis was performed using RStudio software with Corrplot package (R studio,2015). Path analysis was done as per Dewey and Lu, (1959) using OPSTAT (Sheoran, 1998), using all traits as independent variables except grain yield per plant, grain yield per plant was used as dependent variable.

Plant height, total tillers per plant, the number of effective tillers per plant, panicle length, grains per panicle, fertile grains per panicle, 1000 grain weight and kernel breadth showed highly significant and positive correlation with grain yield per plant (**Table 1 and Fig. 1**). Similarly positive correlation between panicle length and grain yield per plant has been reported (Reddy *et al*. 2013). Positive correlation of plant height, total tillers per plant, thet number of effective tillers per plant, panicle length, grains per panicle, fertile grains per panicle, 1000 grain weight, kernel breadth with grain yield per plant has been reported in many studies (Zeng 2004; Immanuel *et al*. 2011; Rajamadhan *et al*. 2011; Ramya *et al*. 2012; Seyoum *et al*. 2012; Jambhulkar and Bose, 2014; Sudeepthi *et al*. 2020). This indicated that these traits can be considered for improvement of grain yield per plant.

Plant height showed significant and positive correlation with number of effective tillers per plant. It showed a non significant and negative correlation with grain zinc content which is similar to the report by Dharwal *et al*. 2017. Correlation of grain yield per plant with days to flowering was highly significant and negative as studied by Abhilash *et al*. 2018. Spikelet fertility, kernel length, L/B ratio exhibited non significant and positive correlation with grain yield per plant. However Abhilash *et al*. 2018, reported a negative correlation between spikelet fertility and grain yield per plant. Negative correlation of spikelet fertility on 1000 grain weight has been reported by Karim *et al*. 2014, which is similar to the present study. Non significant and negative correlation of 1000 grain weight with the number of effective tillers per plant, spikelet fertility were explained on ground of source and sink relationship (Zeng, 2004) and due to nutrient competition (Sudeepthi *et al*. 2020).

Table 1. Correlation matrix of all traits

Traits	DTF	Ht	Ti	ETi	PnL	NoG	NoFG	SpFert	TGW	Zinc	Iron	KL	KB	L/B	GYld
DTF	1														
Ht	0.129*	1													
Ti	0.157**	0.298**	1												
ETi	0.139*	0.338**	0.971**	1											
PnL	-0.016	0.517**	0.045	0.078	1										
NoG	0.065	0.286**	-0.028	-0.027	0.449**	1									
NoFG	0.029	0.268**	-0.022	-0.025	0.434**	0.959**	1								
SpFert	-0.108	-0.045	0.008	-0.006	-0.035	-0.052	0.226**	1							
TGW	-0.187**	0.064	-0.056	-0.049	0.113*	-0.224**	-0.234**	-0.053	1						
Zinc	-0.306**	-0.034	-0.068	-0.065	-0.058	-0.325**	-0.277**	0.162**	0.071	1					
Iron	-0.181**	-0.112*	0.016	0.004	-0.085	-0.039	-0.012	0.105	-0.190**	0.353**	1				
KL	-0.088	-0.038	-0.007	-0.028	0.045	-0.259**	-0.312**	-0.202**	0.632**	-0.110	-0.258**	1			
KB	-0.142*	0.075	-0.030	-0.038	0.079	-0.191**	-0.189**	-0.001	0.368**	0.205**	-0.092	0.219**	1		
L/B	-0.022	-0.075	0.008	-0.010	0.005	-0.169**	-0.221**	-0.192**	0.448**	-0.201**	-0.212**	0.886**	-0.254**	1	
GYld	-0.190**	0.556**	0.578**	0.604**	0.347**	0.219**	0.230**	0.036	0.115*	-0.066	-0.037	0.061	0.116*	0.011	1

*- significant at 1%, ** - significant at 5%

DTF- days to flowering, Ht-Plant height, Ti-Total tillers per plant, ETi-Number of Effective tillers per plant, PnL-panicle length, NoG-total grains per panicle, NoFG-Number of fertile grains per panicle, SpFert-spikelet fertility, TGW-1000 grain weight, Zinc-Grain zinc content, Iron-Grain iron content, KL-Kernel length, KB-Kernel breadth, L/B-Kernel length to kernel breadth ratio, GYld-Grain yield per plant.

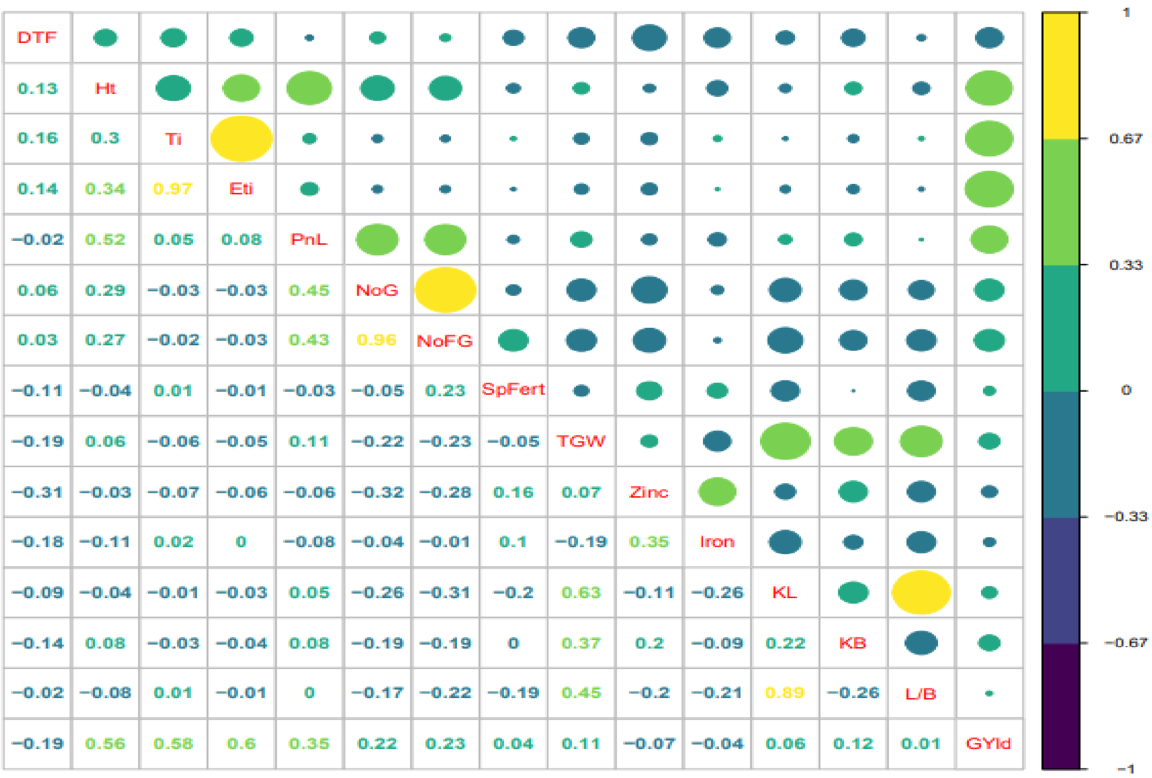

Fig.1. Correlation matrix of all traits by R

DTF- days to flowering, Ht-Plant height, Ti-Total tillers per plant, ETi-Number of Effective tillers per plant, PnL-panicle length, NoG-total grains per panicle, NoFG-Number of fertile grains per panicle, SpFert-spikelet fertility, TGW-1000 grain weight, Zinc-Grain zinc content, Iron-Grain iron content, KL-Kernel length, KB-Kernel breadth, L/B-Kernel length to kernel breadth ratio, GYld-Grain yield per plant.

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Positive correlation between grain zinc content with 1000 grain weight, kernel breadth, spikelet fertility was found and such positive correlation between grain zinc and the grain characteristics such as grain weight, grain width has been detected by Zhang *et al*. 2004, also. Kernel length had negative correlation with grain zinc content while positive with kernel breadth. The results are in line with Zhang *et al*. 2004.

Grain Zinc content has highly significant and positive correlation with grain iron content. Such positive correlation between grain zinc and grain iron content has been also reported (Patil *et al*. 2015; Dharwal *et al*. 2017 and Shivani *et al*. 2018). It indicate co segregation of genetic factors responsible for increment in both minerals (Gregorio 2002), and it has also been validated by co localized QTLs for both traits (Swamy *et al*. 2016). This revealed that there are scope for simultaneous improvement of both traits from bio fortification aspects. Grain zinc had significant and negative correlation with days to flowering, total grains per panicle, fertile grains per panicle, L/B ratio and non significant and negative correlation with grain yield per plant, kernel length, plant height, total tillers per plant, the number of effective tillers per plant and panicle length. Thuy et al., 2018 reported positive correlation of zinc with these traits which are contrast to our finding. Spikelet fertility showed a significant and positive correlation with grain zinc content and non significant and positive correlation with grain iron content and grain yield per plant.

Table 2. Direct and indirect effects of different traits as partitioned by path analysis

Character	DTF	Ht	Ti	ETi	PnL	NoG	NoFG	SpFert	TGW	Zinc	Iron	KL	KB	L/B	r
DTF	**-0.311**	0.046	0.022	0.055	-0.001	-0.030	0.018	0.014	-0.007	0.026	-0.002	0.105	-0.097	-0.028	-0.190**
Ht	-0.040	**0.355**	0.041	0.133	0.017	-0.132	0.170	0.006	0.003	0.003	-0.001	0.045	0.051	-0.096	0.556**
Ti	-0.049	0.106	**0.139**	0.382	0.002	0.013	-0.014	-0.001	-0.002	0.006	0.000	0.008	-0.021	0.010	0.578**
ETi	-0.043	0.120	0.135	**0.393**	0.003	0.012	-0.016	0.001	-0.002	0.006	0.000	0.033	-0.026	-0.012	0.604**
PnL	0.005	0.184	0.006	0.031	**0.034**	-0.207	0.275	0.004	0.004	0.005	-0.001	-0.054	0.054	0.006	0.347**
NoG	-0.020	0.102	-0.004	-0.011	0.015	**-0.460**	0.608	0.007	-0.009	0.028	-0.000	0.310	-0.130	-0.215	0.219**
NoFG	-0.009	0.095	-0.003	-0.010	0.015	-0.441	**0.634**	-0.029	-0.009	0.024	-0.000	0.373	-0.129	-0.281	0.230**
SpFert	0.034	-0.016	0.001	-0.002	-0.001	0.024	0.143	**-0.127**	-0.002	-0.014	0.001	0.241	-0.001	-0.244	0.036
TGW	0.058	0.023	-0.008	-0.019	0.004	0.103	-0.148	0.007	**0.040**	-0.006	-0.002	-0.757	0.251	0.570	0.115*
Zinc	0.095	-0.012	-0.010	-0.026	-0.002	0.149	-0.176	-0.021	0.003	**-0.087**	0.004	0.131	0.140	-0.256	-0.066
Iron	0.056	-0.040	0.002	0.002	-0.003	0.018	-0.008	-0.013	-0.008	-0.031	**0.010**	0.309	-0.063	-0.269	-0.037
KL	0.027	-0.013	-0.001	-0.011	0.002	0.119	-0.198	0.026	0.025	0.010	-0.003	**-1.197**	0.149	1.126	0.061
KB	0.044	0.027	-0.004	-0.015	0.003	0.088	-0.120	0.000	0.015	-0.018	-0.001	-0.262	**0.682**	-0.323	0.116*
L/B	0.007	-0.027	0.001	-0.004	0.000	0.078	-0.140	0.024	0.018	0.017	-0.002	-1.060	-0.173	**1.271**	0.011

Residual Effect=0.34

Diagonal values in represents direct effect of variables on grain yield per plant.

r-Correlation coefficient of each trait with grain yield per plant

DTF- days to flowering, Ht-Plant height, Ti-Total tillers per plant, ETi-Number of Effective tillers per plant, PnL-panicle length, NoG-total grains per panicle, NoFG-Number of fertile grains per panicle, SpFert-spikelet fertility, TGW-1000 grain weight, Zinc-Grain zinc content, Iron-Grain iron content, KL-Kernel length, KB-Kernel breadth, L/B -Kernel length to kernel breadth ratio, GYld-Grain yield per plant.

Grain iron content a had significant and positive correlation with grain zinc content and non significant and positive correlation with total tillers per plant, the number of effective tillers per plant, spikelet fertility. It was significant and negatively correlated with days to flowering, plant height, 1000 grain weight, kernel length, L/B ratio and non significant with grain yield per plant, panicle length, total grains per panicle, fertile grains per panicle and kernel breadth. Grain yield per plant reported to have a non significant and negative correlation with grain iron and zinc content. Significant and negative correlation of grain zinc content with grain yield per plant has been already reported by Patil *et al*. 2015 and Shivani *et al*. 2018. where as it was significant and positive for grain iron content with grain yield in the studies conducted by Patil *et al*. 2015 and Sala and Geetha, 2015.

Similar to the present study positive correlation of kernel length with 1000 grain weight was observed by Seneega *et al*. 2019, plant height with the number of effective tillers per plant and fertile grains with spikelet fertility by Kole *et al*. 2008, and plant height with panicle length by Pandey *et al*. 2018.

Path coefficient analysis was carried out using grain yield per plant as dependent variable and 14 traits as independent variables by partitioning correlation coefficients into direct and indirect effects (**Table 2**). Path analysis is helpful for deciding relative importance of different factors for yield improvement.

Path coefficient study revealed that maximum positive direct effect on grain yield per plant has been exhibited by

L/B ratio (1.271) followed by kernel breadth (0.68), fertile grains per panicle (0.63). However the direct effect was also observed for panicle length (Nandeshwar *et al*. 2010), the number of effective tillers per plant (Ramya *et al*. 2012; Seneega *et al*. 2019; Sudeepthi *et al*. 2020), grains per panicle (Seyoum *et al*. 2012), plant height (Jambhulkar and Bose, 2014), 1000 grain weight (Immanuel, *et al*. 2011; Karim *et al*. 2014), total tillers per plant (Pandey et al., 2018). It was observed that kernel length had a maximum negative direct effect on grain yield per plant (-1.19), followed by grains per panicle (-0.46), and days to flowering (-0.311).

Traits like plant height, total tillers per plant, the number of effective tillers per plant, panicle length, fertile grains per panicle, 1000 grain weight, kernel breadth, recorded highly significant and positive correlation coupled with positive direct effect on grain yield per plant. Kernel length to breadth ratio had a non significant positive correlation and positive direct effect on grain yield per plant. It indicated that respective direct effects of this all traits on grain yield per plant accounted for major segment of total correlation between them. So direct selection using this all traits would be rewarding and effective. Similar trend of relationship (positive direct effect and positive correlation) of majority of this traits on grain yield per plant has been reported (Immanuel *et al*. 2011; Rajamadhan *et al*. 2011; Ramya *et al*. 2012 ; Jambhulkar and Bose, 2014; Karim *et al*. 2014 and Sudeepthi *et al*. 2020). Positive direct effect by effective tillers per plant, 1000 grain weight and plant height has been concluded by Patil *et al*. 2015.

Grain zinc content exhibited a negative direct effect and non significant and negative correlation with grain yield per plant. It had negative indirect effects *via* plant height, total tillers per plant, the number of effective tillers per plant, panicle length, fertile grains per panicle, spikelet fertility, L/B ratio. But it had indirect positive effect *via* days to flowering, total grains per panicle, 1000 grain weight, grain iron content, kernel length and kernel breadth. Hence, the direct selection based on zinc content for yield is not recommended.

Considering the fact about, grain iron content exhibiting negative correlation and positive direct effect, restricted simultaneous selection model are recommended where restrictions must be imposed to nullify factors contributing negative indirect effects.

Residual effect of 0.34 indicated the characters included in the study contributed more than 65% of variability in grain yield per plant. However, a high residual effect (0.48 to 0.7) (Ramya *et al*. 2012, Jambhulkar and Bose, 2014 and Sudeepthi *et al*. 2020) has been reported.

Present study provided background knowledge about almost all major traits which must be considered while improving yield along with grain mineral content not only in homozygous breeding material but also in segregating mapping population. Although grain yield as well as grain zinc and iron content are complex traits for study, we can simplify them by targeting simultaneously with other correlated traits. Plant height, total tillers per plant, the number of effective tillers per plant, panicle length, 1000 grain weight, fertile grains per panicle, kernel breadth, L/B ratio has significant and positive correlation and positive direct effects with grain yield per plant. So these traits combination can be considered for yield improvement.

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Conflict of interest disclosure

The authors declare that there is no conflict of interest.

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