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Association Analysis of Protein and Yield Related Traits in F_3 Population of Rice (*Oryza sativa* L.) Crosses

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A B S T R A C T

Analysis of variance indicated the existence of significant differences among the progenies for all the traits. Correlation co-efficients among grain yield and its fourteen attributing traits were estimated in two hundred F_3 population of rice. The study revealed significant positive correlation of number of filled grains per panicle, spikelet fertility, total number grains per panicle, number of productive tillers per plant and 1000-grain weight with grain yield. Hence, the above correlated traits can be effectively utilized in formulating indirect selection schemes. Path analysis indicated maximum positive direct effect of total number of grains per panicle followed by spikelet fertility on grain yield, while protein content had direct negative phenotypic effect on grain yield and its correlation with grain yield was also negative and non-significant. Further, most of the other traits also exhibited positive indirect effect *via* plant height, productive tillers per plant and days to maturity but negative indirect effect *via* 1000-grain weight, number of filled grains per panicle and L/B ratio. Selection of plants based on total number of grains per panicle with maximum productive tillers and spikelet fertility is likely to yield productive desirable segregants in these populations.

Keywords

F_3 population, ANOVA, Correlation, Path analysis, Rice protein.

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Introduction

Rice is one of the principle food crops and one third of the world population and two thirds of the Indian population is utilizing rice as staple food. It contributes 43 per cent of caloric requirement and 20-25% of agricultural income. In India, rice is grown in an area of 43.5 million ha (23% of gross cropped area) with an annual production of 90 million tons (Viraktamath and Sundaram, 2010). Yield of paddy is controlled by many genes interacting with environment. Selection of parents on the basis of yield is often misleading. Hence, it is imperative to know the cause and effect relationship between yield and yield attributing characters for an efficient selection strategy for the plant breeders. While selecting for yield, the nature of correlation between yield and component traits and their direct and indirect effects on yield in the segregating generation of different cross combinations are of great significance in any breeding programme (Dewey and Lu, 1959). The present investigation was therefore undertaken with a view to assess the nature of correlation of grain yield with its components as well as direct and indirect effect of component traits on grain yield in two hundred F_3 population of rice. Rice is a major source of food protein in Asia and other countries where the daily intake of rice is high. Its value as a protein source is enhanced by its high lysine content relative to other cereal grains. The main limitation of rice as a protein source is its less protein content (6 to 8 %). Any increase in protein content would result in substantial increase in protein intake by large number of consumers provided the quality of the protein is not impaired. In the last two decades, new research findings generated by the nutritionists have brought to light the importance of vitamins, minerals and proteins in maintaining good health, adequate growth and even acceptable levels of cognitive ability apart from the problem of protein energy**,** malnutrition. Even though the levels of carbohydrates are adequate in rice, parallel analysis of the levels and bioavailability of the other micronutrients in rice revealed that the levels are very low and consumption of rice alone cannot meet the Recommended Daily Allowance (RDA) for a range of vitamins, minerals and proteins.

Materials and Methods

Experimental material consisted of F_3 progenies belonging to four F_2 population selected based on yield superiority. A total of 200 progenies were studied in F_3 generation during *kharif*, 2014, at Indian institute of Rice Research (IIRR) Farm, ICRISAT Campus, Patancheru, Hyderabad, Telangana, All the 200 entries were sown separately in a well prepared nursery bed. Twenty seven day's old seedlings of each plant were transplanted in two rows of 4.5 m length by adopting a spacing of 20 cm between rows and 15 cm between plants in a randomized block design, replicating twice. All the recommended crop management practices and plant protection measures were followed for raising a healthy crop. Observations were recorded on five randomly selected plants from each replication for plant height, number of productive tillers per plant, panicle length, number of filled grains/panicle, total number of grains/panicle, spikelet fertility, kernel length, kernel breadth, L/B ratio and grain yield per plant. However the observations on days to 50% flowering, days to maturity, 1000- grain weight and protein content were recorded on whole plot basis. The correlation coefficients were computed according to Falconer (1964) and path analysis were worked out following the methods suggested by Wright (1921) as illustrated by Dewey and Lu (1959) respectively. Analysis of variance was computed based on randomized block design for each of the character separately as per standard statistical procedure (Panse and Sukhatme, 1985).

For the estimation of protein content, Nitrogen content of rice was determined by Micro-Kjheldahl distillation method (Piper, 1966). Powdered seed sample of 100mg was taken in 100ml Micro-Kjheldahl flask to which three ml of concentrated H_2SO_4 was added and left over night for digestion and later heated at low temperature, H_2O_2 was added until the digested sample became clear. After digestion, the contents were transferred to Micro-Kjheldahl nitrogen distillation set. Boric acid (4%) was taken in a 150ml beaker to which 1-2 drops of mixed indicator was added. The tip of the condenser was dipped into the solution which facilitates the absorption of released NH_3. NaOH (40%) was added @ 10ml to distillation flask and distillation was continued till NH_3 was completely released. Test was carried out to know whether all the NH_3 was released by introducing a piece of red litmus paper at the condenser nozzle. The distillation was continued until the red litmus turned to blue which indicated the release of NH_3. The distillation was stopped when there was no release of NH_3 as and when red litmus remains unchanged. The contents of the beaker were titrated against 0.02N H_2SO_4 and nitrogen percentage was calculated by using the following formula.

Calculation

$$\text{Nitrogen (\%)} = \frac{\text{Titer value x 0.02 N x 1.401}}{\text{Weight of the sample}}$$

Protein content was calculated by multiplying nitrogen content of seed with correction factor (Sadasivam and Manickam, 1996).

Protein content (%) = Nitrogen content (%) x 6.25 (Factor).

Results and Discussion

The analysis of variance revealed highly significant differences among the 200 progenies of rice for all the characters under study indicating considerable genetic variation in the material (Table 1).

The correlation coefficients between various pairs of characters are presented in (Table 2). Phenotypic correlations revealed that grain yield per plant had significant positive association with number of filled grains per panicle, spikelet fertility, total number grains per panicle, number of productive tillers per plant and 1000-grain weight.

Hence, these characters could be considered as criteria for selection for higher yield as these were mutually and directly associated with grain yield. Whereas the characters panicle length, kernel length, plant height, days to maturity, kernel breadth, L/B ratio and days to 50% flowering, showed non-significant positive association with grain yield per plant, while it was showing negative and non-significant association with protein content.

The characters plant height, panicle length, kernel length, kernel breadth, L/B ratio, days to 50% flowering and days to maturity also positively associated with grain yield per plant it indicates that this character also consider for selection for higher yield.

Similar kind of association was revealed by Sanghera *et al.,* (2013) and Imad Naseem *et al.,* (2014) for productive tillers per plant and 1000 grain weight, Nagaraju *et al.,* (2013) for number of filled grains per panicle, Nandan and Sweta Singh (2010) for plant height and days to 50% flowering, Ganapati *et al.,* (2014) for panicle length, Madavilatha (2005) for kernel length, L/B ratio and days to maturity, Balvir lodhi *et al.,* (2014) for protein content.

However, days to 50% flowering recorded a significant positive phenotypic correlation with days to maturity. Plant height recorded highest significant positive phenotypic correlation with panicle length followed by total number of grains per panicle, number of filled grains per panicle and kernel breadth. Whereas panicle length exhibited positive significant correlation with plant height.

Number of filled grains per panicle exhibited highest significant positive phenotypic correlation with total number grain per panicle followed by spikelet fertility, plant height and kernel breadth. Spikelet fertility exhibited a significant positive phenotypic correlation with number of filled grains per panicle and total number of grains per panicle. Similar results were reported by Ganapati *et al.,* (2014) and Imad Naseem *et al.,* (2014) for days to maturity,

Table.1 Analysis of variance for fourteen characters in F_3 generation of rice (*Oryza sativa* L.)

S. No.	Character	Mean sum of squares		
		Replications (d.f.=1)	**Treatments (d.f.=199)**	**Error (d.f.=199)**
1.	Days to 50% flowering	0.09	145.80 **	1.99
2.	Days to maturity	2.72	100.31 **	1.72
3.	Plant height (cm)	0.11	770.96 **	6.70
4.	Number of productive tillers per plant	0.006	7.87 **	0.74
5.	Panicle length(cm)	0.009	7.63 **	0.54
6.	Number of filled grains per panicle	0.024	4253.87 **	102.19
7.	Total no of grains per panicle	0.022	3855.40 **	106.23
8.	Spikelet fertility (%)	1.30	201.14 **	43.75
9.	Kernel length (mm)	0.02	1.17 **	0.04
10.	Kernel breadth (mm)	0.01	0.26 **	0.07
11.	L/B ratio	0.10	0.40 **	0.11
12.	1000-grain weight (g)	0.03	26.31 **	0.77
13.	Grain yield per plant (g)	0.02	65.51 **	1.39
14.	Protein content (%)	3.495**	1.866 **	0.382

Table.2 Estimation of genotypic (G) and phenotypic (P) correlation coefficient between yield and its component characters in F3 generation of rice (*Oryza sativa* L.)

Character		DFF	PTH	N0.PTT	PL	No.FGP	T N0.GP	SF	KL	KB	L/B	DM	1000 GW	PROTEIN	GYP
DFF	**G**	**10000**	-0.0378	-0.0570	-0.1626**	-0.0198	-0.0313	0.1009*	-0.0810	-0.0863	0.1082*	0.5607**	0.0015	-0.0805	0.0057
	P	**1.0000**	-0.0374	-0.0559	-0.1517**	-0.0176	-0.0300	0.0790	-0.0739	-0.0664	0.0780	0.5538**	0.0056	-0.0634	0.0042
PTH	**G**		**1.0000**	0.0214	0.5259**	0.1086*	0.1530**	-0.0118	-0.1213*	-0.1360**	0.0312	0.0694	0.0059	-0.0906	0.0691
	P		**1.0000**	0.0192	0.4864**	0.1077*	0.1502**	0.0078	-0.1203*	0.1041*	-0.0224	0.0669	0.0053	-0.0787	0.0680
N0.PTT	**G**			**1.0000**	0.0432	0.0543	0.0405	0.0656	0.0351	0.0435	-0.0112	-0.0198	-0.2975**	-0.1160	0.4528**
	P			**1.0000**	0.0434	0.0571	0.0443	0.0592	0.0332	0.0321	-0.0005	-0.0193	-0.2646**	-0.1167*	0.4019**
PL	**G**				**1.0000**	0.0948	0.0882	0.0900	-0.1327**	-0.2254**	0.0338	-0.0980	-0.0139	0.0522	0.0905
	P				**1.0000**	0.0868	0.0856	0.0621	-0.1223*	-0.1483**	0.0217	-0.0814	-0.0184	0.0269	0.0891
No.FGP	**G**					**1.0000**	0.9453**	0.7957**	0.0600	0.1353**	-0.0889	0.0536	-0.6573**	- 0.0128	0.5833**
	P		`			**1.0000**	0.9019**	0.7205**	0.0507	0.1028*	-0.0803	0.0549	-0.6179**	-0.0183	0.5599**
T N0.GP	**G**						**1.0000**	0.5686**	0.0316	0.1393**	-0.1577**	0.0636	-0.6290**	0.0327	0.4848**
	P						**1.0000**	0.3649**	0.0322	0.0999*	-0.1140*	0.0607	-0.5925**	0.0391	0.4608**
SF	**G**							**1.0000**	0.1014*	0.0590	0.1086*	0.0269	-0.5117**	-0.0896	0.6689**
	P							**1.0000**	0.0591	0.0484	0.0233	0.0321	-0.3897**	-0.0982	0.5312**
KL	**G**								**1.0000**	0.5831**	0.3581**	-0.0536	-0.0392	0.0228	0.0786
	P								**1.0000**	0.4038**	0.3461**	-0.0508	-0.0374	0.0186	0.0768
KB	**G**									**1.0000**	-0.5144**	-0.0496	-0.1943**	0.0422	0.0296
	P									**1.0000**	0.6120**	-0.0390	-0.1212*	0.0209	0.0359
L/B	**G**										**1.0000**	0.0081	0.1298**	-0.0720	0.0499
	P										**1.0000**	0.0043	0.0858	-0.0374	0.0387
DM	**G**											**1.0000**	0.0075	-0.0867	0.0583
	P											**1.0000**	0.0086	-0.0842	0.0556
1000GW	**G**												**1.0000**	0.0377	0.1091*
	P												**1.0000**	0.0340	0.1085*
PROTEIN	**G**													**1.0000**	-0.0773
	P													**1.0000**	-0.0738

*** Significant at 5 per cent level; ** Significant at 1 per cent level**

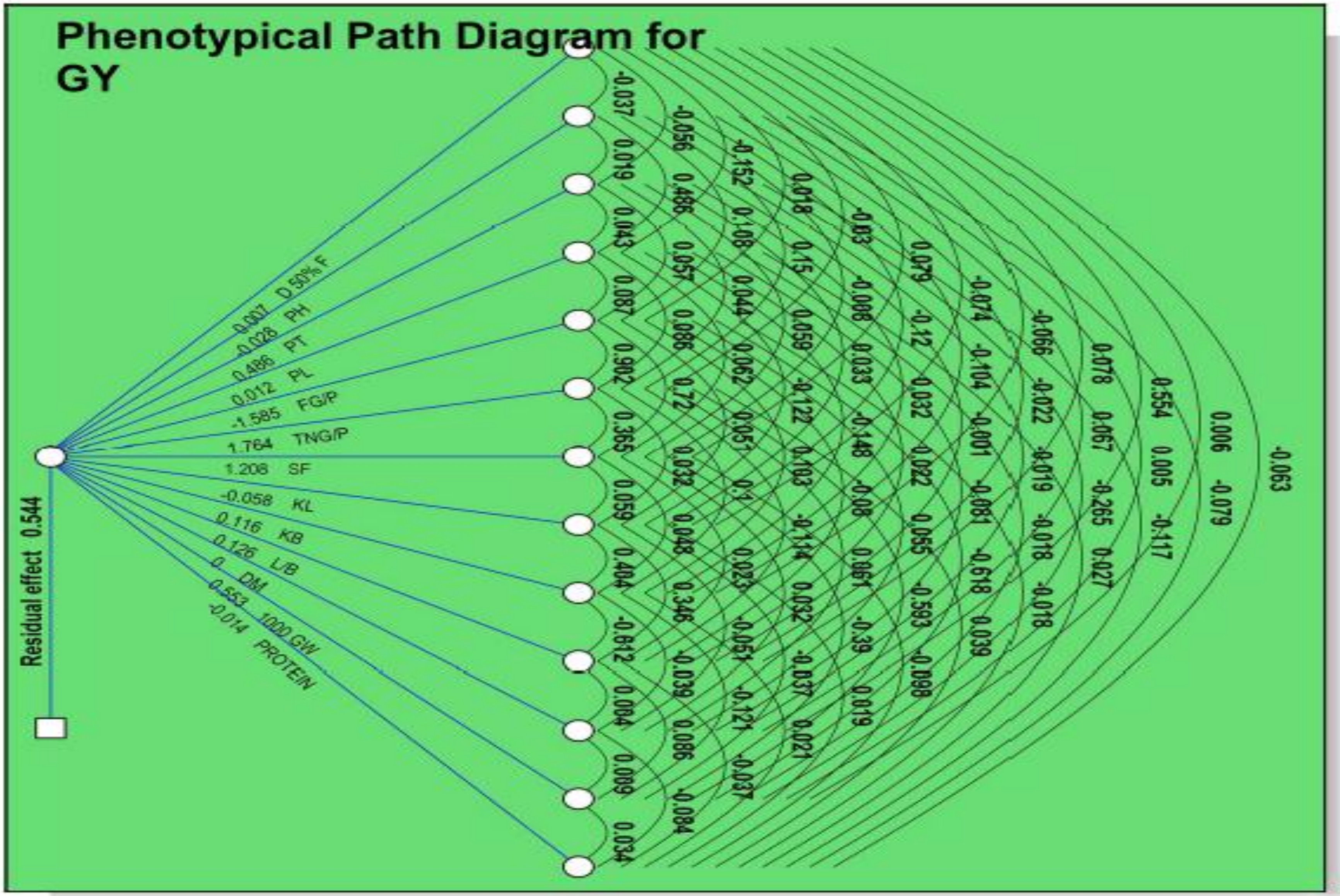
DFF: Days to 50% flowering, DM: Days to maturity, PTH: Plant height(cm), No.PTT: Number of productive tillers/plant, PL: Panicle length(cm), No.FGP: Number of filled grains/panicle, T N0.GP:Total no of grains/panicle, SF: Spikelet fertility, KL: Kernal length(mm), KB: Kernal breadth(mm), L/B: Length Breadth Ratio, 1000 GW: 1000 Grain weight, GYP: Grain yield/ plant(g)

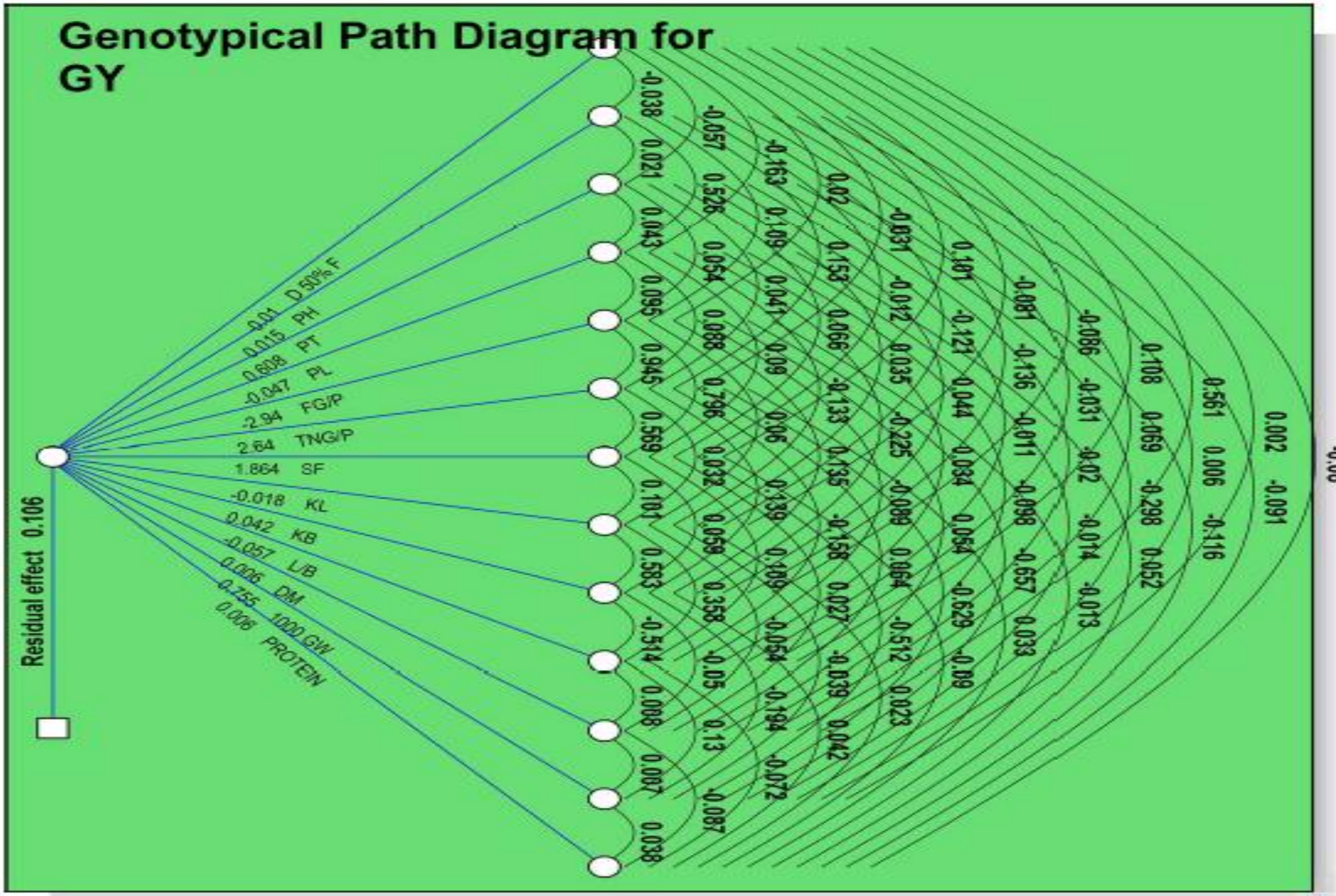
P -represents phenotypic correlation coefficient; G- represents genotypic correlation coefficient

Table.3 Estimation of genotypic (G) and phenotypic (P) direct and indirect effects between yield and yield attributing characters in two hundred progenies of rice (*Oryza sativa* L.) in F_3 generation

Character		DFF	PTH	No.PTT	PL	No.FGP	T N0.GP	SF	KL	KB	L/B	DM	1000 GW	Protein	GYP
DFF	G	**-0.0099**	-0.0006	-0.0346	0.0077	-0.0581	-0.0828	0.1881	0.0015	-0.0036	-0.0062	0.0035	0.0011	-0.0005	0.0057
	P	**0.0070**	0.0010	-0.0272	-0.0019	-0.0279	-0.0529	0.0954	0.0043	-0.0077	0.0098	0.0002	0.0031	0.0009	0.0042
PTH	G	0.0004	**0.0152**	0.0130	-0.0250	-0.3193	0.4041	-0.0220	0.0022	-0.0057	0.0018	0.0004	0.0045	-0.0005	0.0691
	P	-0.0003	**-0.0279**	0.0093	0.0059	-0.1706	0.2649	-0.0095	0.0070	-0.0121	-0.0028	0.0000	0.0029	0.0011	0.0680
No.PTT	G	0.0006	0.0003	**0.6076**	-0.0021	-0.1596	0.1071	0.1223	-0.0006	0.0018	0.0006	-0.0001	-0.2245	-0.0007	0.4528
	P	-0.0004	-0.0005	**0.4861**	0.0005	-0.0905	0.0782	0.0715	-0.0019	0.0037	-0.0001	0.0000	-0.1462	0.0016	0.4019
PL	G	0.0016	0.0080	0.0263	**-0.0475**	-0.2786	0.2328	0.1677	0.0024	-0.0094	-0.0019	-0.0006	-0.0105	0.0003	0.0905
	P	-0.0011	-0.0136	0.0211	**0.0122**	-0.1377	0.1510	0.0750	0.0071	-0.0172	0.0027	0.0000	-0.0102	-0.0004	0.0891
No.FGP	G	-0.0002	0.0016	0.0330	-0.0045	**-2.9397**	2.4958	1.4835	-0.0011	0.0056	0.0051	0.0003	-0.4961	-0.0001	0.5833
	P	0.0001	-0.0030	0.0278	0.0011	**-1.5851**	1.5909	0.8704	-0.0029	0.0119	-0.0101	0.0000	-0.3414	0.0003	0.5599
T N0.GP	G	0.0003	0.0023	0.0246	-0.0042	-2.7788	**2.6403**	1.0601	-0.0006	0.0058	0.0090	0.0004	-0.4747	0.0002	0.4848
	P	-0.0002	-0.0042	0.0215	0.0010	-1.4296	**1.7640**	0.4408	-0.0019	0.0116	-0.0144	0.0000	-0.3274	-0.0005	0.4608
SF	G	-0.0010	-0.0002	0.0399	-0.0043	-2.3391	1.5012	**1.8644**	-0.0018	0.0025	-0.0062	0.0002	-0.3862	-0.0005	0.6889
	P	0.0006	0.0002	0.0288	0.0008	-1.1420	0.6437	**1.2081**	-0.0034	0.0056	0.0029	0.0000	-0.2153	0.0014	0.5312
KL	G	0.0008	-0.0018	0.0213	0.0063	-0.1763	0.0834	0.1890	**-0.0182**	0.0243	-0.0205	-0.0003	-0.0296	0.0001	0.0786
	P	-0.0005	0.0034	0.0161	-0.0015	-0.0804	0.0568	0.0714	**-0.0580**	0.0468	0.0437	0.0000	-0.0207	-0.0003	0.0768
KB	G	0.0009	-0.0021	0.0264	0.0107	-0.3977	0.3678	0.1099	-0.0106	**0.0417**	0.0294	-0.0003	-0.1466	0.0002	0.0296
	P	-0.0005	0.0029	0.0156	-0.0018	-0.1630	0.1762	0.0585	-0.0234	**0.1159**	-0.0772	0.0000	-0.0670	-0.0003	0.0359
L/B	G	-0.0011	-0.0005	-0.0068	-0.0016	0.2612	-0.4163	0.2024	0.0065	-0.0214	**-0.0571**	0.0001	0.0980	-0.0004	0.0499
	P	0.0005	0.0006	-0.0003	0.0003	0.1272	-0.2010	0.0281	-0.0201	-0.0709	**0.1262**	0.0000	0.0474	0.0005	0.0387
DM	G	-0.0055	0.0011	-0.0120	0.0047	-0.1576	0.1679	0.0501	0.0010	-0.0021	-0.0005	**0.0063**	0.0056	-0.0005	0.0583
	P	0.0039	-0.0019	-0.0094	-0.0010	-0.0871	0.1070	0.0388	0.0029	-0.0045	0.0005	**0.0004**	0.0047	0.0012	0.0556
1000 GW	G	0.0097	0.0078	-0.1808	0.0355	1.9322	-1.5607	-0.9540	0.0455	-0.0081	-0.0074	0.0345	**0.7547**	0.0002	0.1091
	P	0.0056	0.0089	-0.0286	-0.0322	0.9795	-1.0452	-0.4708	0.0414	-0.0140	0.0543	0.0566	**0.5525**	0.0005	0.1085
Protein	G	0.0008	-0.0014	-0.0705	-0.0025	0.0376	0.0865	-0.1671	-0.0004	0.0018	0.0041	-0.0005	0.0285	**0.0059**	-0.0773
	P	-0.0004	0.0022	-0.0567	0.0003	0.0290	0.0690	-0.1186	-0.0011	0.0024	-0.0047	0.0000	0.0188	**-0.0139**	-0.0738
Genotypic Residual effect = 0.1050 Phenotypic Residual effect = 0.5434 Bold values are direct effects															

Fig.1 Phenotypical and genotypical path diagram for 14 characters of rice




Genotypical Path Diagram for GY
Residual effect 0.106
0.01 D 50% F
-0.015 PH
0.608 PT
-0.047 PL
-2.94 FG/P
2.64 TNG/P
1.864 SF
-0.018 KL
0.042 KB
-0.057 L/B
0.006 DM
0.255 1000 GW
0.008 PROTEIN

The study of phenotypic correlation studies revealed that selection of plants with more number of filled grains per panicle, total number grains per panicle, spikelet fertility, more number of productive tillers per plant and 1000-grain weight would result in improvement of yield.

The association of different component characters among themselves and with yield is quite important for devising an efficient selection criterion for yield. The total correlation between yield and component characters may be some times misleading, as it might be an over-estimate or under-estimate because of its association with other characters.

Hence, indirect selection by correlated response may not be some times fruitful. When many characters are affecting a given character, splitting the total correlation into direct and indirect effects of cause as devised by Wright (1921) would give more meaningful interpretation to the cause of association between the dependent variable like yield and independent variables like yield components (Table 3 and Fig. 1). This kind of information will be helpful in formulating the selection criteria. Indicating the selection for these characters is likely to bring about on overall improvement in single plant yield directly.

The present study indicated that the grain yield can be improved by following indirect selection in advance generations based on total number of grains per panicle exerted the highest positive direct effect on grain yield followed by spikelet fertility, 1000-grain weight, number of productive tillers per plant, L/B ratio, kernel breadth, panicle length, days to 50% flowering, days to maturity indicating that the selection for these characters was likely to bring about an overall improvement in grain yield per plant directly. Similar results reported by Seyoum *et al.*, (2012) for total number of grains per panicle and spikelet fertility, Zulqarnain *et al.*, (2012) for 1000-grain weight and Imad Naseem *et al.*, (2014) for productive tillers per plant. Therefore, it is suggested that preference should be given to these characters in the selection programme to isolate superior lines with genetic potentiality for higher yield in rice genotypes. However, negative direct effects on grain yield were exhibited by number of filled grains per panicle, kernel length, protein content, in decreasing order, respectively.

In plant breeding, it is very difficult to have complete knowledge of all component traits of yield. The residual effect permits precise explanation about the pattern of interaction of other possible components of yield. Under this study value of genotypical and phenotypical residual effects is 0.105, 0.543 respectively. So that other possible independent variables effects on dependent variable i.e. grain yield per plant is also there.

Protein content had direct negative phenotypic effect on grain yield per plant and its correlation with grain yield was also negative and non-significant.

The correlation between protein content and grain yield per plant was negative and non-significant mainly due to negative indirect effect influence through spikelet fertility, kernel breadth, number of filled grains per panicle, L/B ratio and plant height.

Whereas significant positive correlation with number of productive tillers per plant, days to 50% flowering, plant height, total number of grains per panicle, panicle length, number of filled grains per panicle. Similar results were reported by Mohan Y.C *et al.*, (2002), Balvir lodhi *et al.*, (2013) and Gangadhara *et al.*, (2012) for negative correlation between grain yield and protein content.

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