Received: 11 Jul 2023 | Accepted: 28 Aug 2023 | Online: 31 Aug 2023

Electronic Journal of Plant Breeding

Research Article

Heterosis, character association and path analysis for grain protein content in rice (*Oryza sativa L.*)

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Abstract
Rice grain protein content had many unique activities like hypo-allergenic, anticancer properties *etc*. helps in nourishing protein energy malnutrition. The knowledge on the magnitude of genetic effects and association of traits helps in identifying desirable superior hybrids to enhance protein content coupled with yield. The experimental material consisted of 30 hybrids generated in full diallel mating design by hybridizing six parents differing in protein content. The promising hybrid combination IG74 / Gandhasala, its reciprocal cross and IG74 / TKM13 exhibited significant standard heterosis over the high protein check IG74 and the crosses TKM13 / CO52, CO52 / Vellai chithiraikar and CO52 / Gandhasala were adjudged as heterotic promising hybrids for the yield when compared with the check CO52. The grain protein content was found to be negatively associated with grain yield but had positive association with thousand grain weight and number of productive tillers per plant. Path analysis indicated that protein content had a negligible direct effect over grain yield and had positive indirect effect through thousand grain weight whereas having negative indirect effect through number of filled grains. Therefore, it is suggested that selection of parents for high test weight would help to identify good genotypes with high grain yield and protein content.

Keywords: Rice, diallel analysis, protein content, heterosis, correlation and path analysis.

INTRODUCTION

Rice (*Oryza sativa* L.) is recognized as the "grain of life" (Singh *et al*., 2020) as it serves as most important and staple food for more than half of the global population, enabling it as a critical factor of food security. India is one of the world's largest countries by area, which ranked the second and next to China. According to the Directorate of Economics and Statistics (D&ES, 2019- 20), India, the second largest country had 43.8 million hectares of area, with production of 118.4 million tonnes, and beholding productivity of 2705 kg per hectare. People usually consume polished milled rice which mostly represents endosperm as both the embryo and bran layers were removed off during polishing. Hence, the nutritional value of polished rice by endosperm comprised of (70- 80%) carbohydrates but quite low in protein content (about 7% at 14% moisture) whereas dehulled brown rice contains around 8% protein (Aiyswaraya *et al*., 2017). Protein energy undernourishment affects 25 percent of children whose diet consists primarily of rice and staple food crops that contain an insufficient amount of vital amino acids for whom the recommended intake of protein is 13-19g/day/child (Gearing, 2015). Infact rice protein were the best among other cereal proteins due to their better balance of essential amino acid and higher digestibility. Rice protein possess unique anti oxidative, hypo-allergenic, anti-obesity and anticancer properties. Also, protein influences structural, functional, nutritional and grain quality in rice (Amagliani *et al.*, 2017). The protein digestibility corrected amino acid score (PDCAAS) indicates the existence of essential amino acid and overall protein quality are higher in rice (0.55) compared to other popular cereals like wheat (0.40). Therefore, development of rice varieties with increased protein content is essential to meet demands of population. Screening and exploitation of landraces which possess valuable resources for genetic improvement of protein and other essential micronutrients can eradicate malnutrition. Development of rice varieties with high protein content either through conventional method or by marker assisted introgression holds a great potential for sustainable food-based solution (Graham *et al.*, 2001). The development of protein rich rice lines, CRDhan 310(10.3%) and CRDhan 311(10.1%) by ICAR-National Rice Research Institute, Cuttack brought a hope to plant breeders to develop more desirable protein rich rice varieties similar to this. For development of any hybrids, the primary step is to determine the nature and magnitude of heterosis for the desirable component traits. Genetically diverse parents are likely to express hybrid vigour in higher magnitude. Earlier reports revealed that grain protein content governed by both additive (Jinbao *et al.*, 2014; Bassuony and Wissa, 2015) and non-additive (Anyanwu and Obi, 2015; Singh *et al.*, 2019 and Lingaiah *et al.*, 2021) gene effects. The superior parental and hybrids combinations can be recognized using standard heterosis, mid parent and better parent heterosis. Correlation studies deal with informations regarding inter-relationships between the yield and its attributing traits and helps in determining magnitude of association among the traits. Path coefficient analyses helps to assess the direct and indirect contributions of each attributing traits to yield which could be helpful in picking up desirable traits for indirect selection (Rasel *et al.*, 2018). With this information the current study is attempted to sort out superior hybrids and to determine inter-relationships and cause effects for grain protein content.

MATERIALS AND METHODS

The experiment was carried out at Department of Rice, TNAU, Coimbatore. The experimental material included six parents based on protein content namely high category (greater than >10%) IG74 (11.16%) and Gandhasala (13.17%); medium category (between 8-10%) CO52 (8.06%) and low category (lesser than <8%) CRDhan315 (6.88%), TKM13 (7.74%) and Vellai chithiraikar (7.73%). Crosses were effected among the parents by following full diallel mating design including reciprocals and 30 hybrids were developed. The six parental lines and 30 crosses were evaluated during *Rabi* 2021 in a Randomized Block Design (RBD) with two replications. The grain protein content was determined using micro-kjeldahl analysis suggested by Somichi *et al.* (1972). Through this method initially, nitrogen content was estimated and then it was converted to protein by multiplying the nitrogen value with conversion factor of 6.25. Ten biometrical observations and protein content (PC) were recorded as per Standard Evaluation System (IRRI, Philippines) from five randomly selected plants in each genotype. Standard agronomical practices were followed during the complete crop growth period. The data collected were subjected to estimate heterosis, correlation and path analysis to determine gene action and interrelationships among protein content and yield traits.

Standard heterosis was calculated over two check varieties *viz*., IG74 and CO 52 for grain protein content and yield traits respectively. The Relative heterosis (mid parental heterosis), Heterobeltiosis (better parental heterosis) and standard heterosis were determined

Analysis of Variance (ANOVA) for RBD was calculated at 5 % level of significance. The estimation of heterosis, correlation and path analysis were done through TNAUSTAT statistical package.

RESULTS AND DISCUSSION

Analysis of variance revealed highly significant variations among the genotypes, including parents and hybrids, for all parameters, including grain protein content, indicating the presence of sufficient genetic variability among the genotypes for all the traits studied (**Table 1**). Results on relative heterosis, heterobeltiosis and standard heterosis

Table 1. Mean sum of squares of six parents and thirty F_1 s in rice for yield and grain protein content

Source of variance	Df	DFF	PH	NPT	PL	FLL	FLW	NFG	TGW	SPY	TPDB	PC
Replication	1	8.00	43.55	0.89	5.12	16.43	0.01	190.13	0.09	4.12	13.00	0.06
Genotypes	35	146.97**	923.86**	9.19**	34.04**	60.29**	0.15**	5942.51**	2.78**	45.38**	2873.61**	4.16**
Error	35	2.43	27.58	2.52	1.85	5.39	0.03	174.67	0.35	4.53	34.60	0.11

* Significant at 5% level, ** Significant at 1% level. Df = Degrees of freedom DFF=Days to 50% flowering, PH=plant height, NPT=number of productive tillers, PL=panicle length, FLL=flag leaf length, FLW=flag leaf width, NFG=number of filled grains per panicle, TGW=1000 grain weight, SPY=single plant yield, TPDB=total plant dry biomass, PC=protein content

https://doi.org/10.37992/2023.1403.100

are presented in **Table 2, 3 and 4.** For both grain protein content and yield, among the 30 hybrids, significant and positive relative heterosis (0.17% - 21.48%) was observed in thirteen crosses. The crosses IG74 / TKM13 and CRDhan315 / CO52 recorded significant positive heterosis for protein content coupled with yield related traits like 1000 grain weight and number of filled grains. Similar significant results for protein trait were reported by Rathava *et al.* (2019) and Patil *et al.* (2012). In contrast Solanke *et al.* (2019) revealed negative heterosis for protein content along with yield. In case of other yield related traits, six hybrids were found to be positively significant for number of productive tillers and two hybrids were found to be significant in desirable direction for plant height as detailed in **Table 2**.

In the present study semi dwarf hybrids CRDhan315 / Vellaichithiraikar (15.49% for protein content) and Vellaichithiraikar / CRDhan315 (5.99%) were found to be superior as they had significantly positive heterobeltiosis

Table 2. Estimates of Mid parental heterosis for grain protein content and yield traits (in percent)

Crosses	DFF	PH	NPT	PL	FLL	FLW	NFG	TGW	SPY	TPDB	PC
IG74/CO52	-14.21**	0.95**	6.00	25.17**	1.13	-5.26	-18.83	-2.80	8.96**	-1.48**	-8.87
IG74/TKM13	-18.13**	2.98	8.91	32.61**	-2.29**	-44.68	-18.79	6.24	12.23*	-6.46	21.48**
IG74/CRDhan315	-15.87**	4.89**	10.42	15.31**	3.47	-15.73	-0.71	3.90**	-7.78*	-42.48**	8.31**
IG74/ Vellai chithiraikar	-15.24**	-10.7*	22.73**	9.91**	1.06	-5.56	-24.18	3.68*	19.53**	-8.22	-15.25**
IG74/Gandhasala	-6.49	-7.83	3.09	-14.16**	-8.09**	2.22	-38.76	4.03	4.77**	-30.41	-2.49
CO52 /IG74	-2.11**	23.67**	10.00	19.40**	-10.3	10.53	0.00	5.59	15.72**	45.03**	5.49
CO52/ TKM13	-9.05**	8.77	-10.68**	11.99*	61.23**	-16.98	63.29**	4.18*	9.15**	-23.95*	3.19*
CO52/ CRDhan315	-8.25*	13.97*	12.24	36.51**	33.49**	39.42**	118.82**	7.64*	11.34*	14.21**	0.17**
CO52/ Vellai chithiraikar	-1.27	4.15	11.11	9.26**	1.53	-14.29	35.76**	8.92**	36.01**	42.63**	12.98**
CO52/Gandhasala	4.95	-4.83	3.03	22.06**	10.83**	5.88	53.88**	2.19	34.29**	46.25**	-5.84
TKM13/ IG74	5.70**	-14.29	12.87	-2.05**	-29.8**	27.66	42.95	-1.45	8.19*	3.47	5.49**
TKM13 /CO52	0.00	-4.36	-30.1**	16.65*	13.72**	24.53	74.68**	6.65*	35.01**	9.46*	11.39*
TKM13/ CRDhan315	2.39	-7.64	7.07	-14.08	-7.81	-24.90	73.64**	10.12**	29.47**	-4.52**	15.34**
TKM13/ Vellai chithiraikar	5.24	-14.00	20.88**	14.62**	20.27*	-25.49**	26.20	-0.37	8.18	15.42**	7.79
TKM13/Gandhasala	5.85	-12.22	0.00	12.79**	13.90	-26.67*	41.81*	2.25	12.10	-13.02	-8.71**
CRDhan315 /IG74	-1.06**	16.30**	2.08	14.69**	2.79	12.36	8.79	14.29**	27.03*	-24.33**	10.48**
CRDhan315/ CO52	2.91*	5.07*	10.20	22.82**	20.26**	53.85**	88.62**	2.40*	11.69*	12.49**	20.39**
CRDhan315/ TKM13	-1.44	3.38	11.11	11.82	15.37	34.39	48.27**	7.90**	11.02**	20.78**	14.45**
CRDhan315/ Vellai chithiraikar	-1.78	-6.15	18.60**	-1.81	-6.06*	16.16	20.85	11.03**	22.05**	12.31**	19.89**
CRDhan315/Gandhasala	3.98	-3.30	11.58	13.76**	5.90	-5.35	37.66**	3.58	15.39*	27.33*	4.42
Vellai chithiraikar/IG74	-0.28**	-3.92*	11.36**	28.56**	-5.69	33.33	12.85	6.44*	21.94**	13.20	-6.38**
Vellai chithiraikar/CO52	-1.77	-1.95	13.33	16.91**	17.02	9.52	26.87**	10.78**	17.45**	1.87**	11.18**
Vellai chithiraikar/TKM13	-2.74	15.78	7.69*	23.52**	5.86*	-37.25**	-2.64	3.93	9.05	17.73**	6.67
Vellai chithiraikar/ CRDhan315	-2.80	14.25	23.26**	12.66	33.10*	1.01	11.91	9.39**	24.76**	5.52**	10.03**
Vellai chithiraikar/ Gandhasala	12.21**	7.69	3.45	18.98**	28.82**	-14.29	-6.59	8.76**	19.78**	36.57**	-10.49
Gandhasala/ IG74	1.08	2.10	9.28	-16.19**	-32.19**	-28.89	36.62	2.68	23.05**	32.82	0.27
Gandhasala/CO52	-1.98	9.36	9.09	6.26**	25.02**	-33.33	55.86**	6.08	13.54**	6.25**	-2.87
Gandhasala/ TKM13	-1.95	7.44	2.00	19.63**	10.40	-16.67*	-8.42*	6.27	2.24	5.69	-6.90**
Gandhasala/CRDhan315	-2.49	7.16	7.37	16.88**	15.40	27.57	14.64**	3.58	4.51*	-11.19*	-5.21
Gandhasala/ Vellaichithiraikar	0.26**	-3.07	12.64	13.91**	12.37**	-2.04	7.75	11.43**	13.73**	20.65**	1.41

DFF=Days to 50% flowering, PH=plant height, NPT=number of productive tillers, PL=panicle length, FLL=flag leaf length, FLW=flag leaf width, NFG=number of filled grains per panicle, TGW=1000 grain weight, SPY=single plant yield, TPDB=total plant dry biomass, PC=protein content

for not only grain protein content but also for single plant yield and thousand grain weight. Sixteen hybrids showed positive significant better parental heterosis for single plant yield, six hybrids for 1000 grain weight, eleven hybrids for number of filled grains. None of the hybrids showed significant heterobeltiosis for number of productive tillers. This non realization of heterobeltiosis for productive tillers may be due to the fact of influence of additive gene action. Significant relative heterosis and heterobeltiosis for grain protein content and yield associated characters suggested that the presence of greater genetic diversity among the parental lines and also reveals the unidirectional distribution of interallelic constitution resulting contribution towards desirable hybrid vigour in the present material (Rathava *et al.,* 2019).

Significant standard heterosis for protein content was obtained in three hybrids namely IG74 / Gandhasala (6.27%), Gandhasala / IG74 (9.27%) and IG74 / TKM13 (5.14%) for protein content. Dodake *et al.* (2022) have also reported significant positive standard heterosis for protein content. For yield and yield attributing traits

Table 3. Estimates of Better parental heterosis for grain protein content and yield traits (in percent)

Crosses	DFF	PH	NPT	PL	FLL	FLW	NFG	1000GW	SPY	TPDB	PC
IG74/CO52	-21.26**	-4.61	3.92	21.25**	-12.99**	-18.18	-24.90	-5.23	7.86*	-7.57**	-21.55**
IG74/TKM13	-25.82**	-9.73**	5.77	31.5*	-17.23**	-58.06**	-38.11*	0.45	11.46	-10.24	3.14
IG74/CRDhan315	-22.44**	4.63*	8.16	0.71	-7.77	-23.47	-3.24	0.00	-10.07	-51.62**	-12.46**
IG74/ Vellai chithiraikar	-18.62**	-23.3**	10.2	-6.67	-1.94	-15.00	-31.50	3.10	17.33**	-19.16*	-29.44**
IG74/Gandhasala	-12.18**	-10.05	2.04	-18.32**	-12.50**	-20.69**	-45.42	-0.23	-1.65	-38.50**	-9.91**
CO52 /IG74	-10.14**	16.86	7.84	15.66**	-22.82**	-4.55	-7.47	2.95	14.55*	36.07**	-9.18**
CO52/ TKM13	-10.33**	0.43	-11.54**	7.61	58.25**	-29.03	31.97**	0.96	7.31**	-31.34**	1.49
CO52/ CRDhan315	-8.70*	7.44	7.84	22.64**	28.22**	31.82*	107.47**	6.22	7.50	1.55	-7.14
CO52/ Vellai chithiraikar	-5.80**	-14.73**	-1.96	-4.67	-10.32	-18.18	32.28**	5.62*	32.18**	18.92	8.57
CO52/Gandhasala	2.42	-12.12	0.00	19.83*	-0.40	-6.90*	47.71**	0.48	24.87**	22.31	-24.12**
TKM13/ IG74	-4.23**	-24.87**	9.62	-2.86*	-40.53**	-3.23**	8.95*	-6.82	7.45	-0.71	-10.44
TKM13 /CO52	-1.41**	-11.70	-30.77**	12.08	11.62**	6.45	41.18**	3.35	32.74**	-1.18**	9.56
TKM13/ CRDhan315	0.47	-19.20**	1.92	-25.49**	-13.02	-38.71	34.78**	8.11*	27.10**	-22.33**	8.60
TKM13/ Vellai chithiraikar	-0.94**	-33.80**	5.77	-3.33	4.52	-38.71**	4.09	-6.29	6.91	5.53	5.26
TKM13/Gandhasala	1.88	-24.65**	-3.85	6.47	0.67	-29.03*	18.41	0.74	5.91	-20.20**	-27.35**
CRDhan315 /IG74	-8.78**	16.02*	0.00	0.18	-8.37	2.04	6.02	10.00	23.86	-36.36**	-10.71**
CRDhan315/ CO52	2.42*	-0.95	5.88	10.34**	15.50**	45.45*	78.84**	1.05	7.83	0.03	11.61
CRDhan315/ TKM13	-3.29	-9.56**	5.77	-3.03**	8.84	9.68	15.09**	5.92**	8.99**	-1.75**	7.77
CRDhan315/ Vellai chithiraikar	-5.85**	-19.29**	8.51	-5.00	-13.94	15.00	11.81	6.29*	21.23**	-14.67**	15.49*
CRDhan315/Gandhasala	1.95	-5.41	10.42	3.92	-1.21	-20.69	25.57	3.19	10.97	-3.01**	-20.51**
Vellai chithiraikar/IG74	-4.26**	-17.54**	0.00	9.17	-8.50	20.00	1.97	5.84	19.70**	-0.29*	-22.04**
Vellai chithiraikar/CO52	-6.28**	-19.73**	0.00	2.00	3.35	4.55	23.62**	7.42*	14.14**	-15.06	6.83
Vellai chithiraikar/TKM13	-8.45**	-10.87**	-5.77	4.17	-8.00	-48.39**	-19.69	-2.25	7.77	7.65	4.17
Vellai chithiraikar/ CRDhan315	-6.83**	-1.75**	12.77	9.00	21.94	0.00	3.54	4.72*	23.92**	-19.83**	5.99*
Vellai chithiraikar/ Gandhasala	9.64*	-5.63**	-6.25	5.50	26.32**	-27.59	-8.02	3.75	14.44*	36.06**	-30.00**
Gandhasala/ IG74	-5.08**	-0.36	8.16	-20.26**	-35.44**	-44.83**	21.76	-1.52	15.50	17.38**	-7.37**
Gandhasala/CO52	-4.35	0.98	5.88	4.31*	12.35	-41.38*	49.62**	4.31	5.57**	-11.15	-21.72**
Gandhasala/ TKM13	-5.63	-7.77**	-1.92	12.93	-2.42	-19.35*	-23.53	4.70	-3.40	-3.03**	-25.90**
Gandhasala/CRDhan315	-4.39	4.83	6.25	6.77	7.65	6.90	4.58	3.19	0.51	-32.35**	-27.84**
Gandhasala/ Vellaichithiraikar	-2.03*	-15.05**	2.08	1.00	10.19**	-17.24	6.11	6.29	8.67*	20.20**	-20.70**

DFF=Days to 50% flowering, PH=plant height, NPT=number of productive tillers, PL=panicle length, FLL=flag leaf length, FLW=flag leaf width, NFG=number of filled grains per panicle, TGW=1000 grain weight, SPY=single plant yield, TPDB=total plant dry biomass, PC=protein content

Table 4. Estimates of standard heterosis for grain protein content and yield traits (in percent)

Crosses	DFF	PH	NPT	PL	FLL	FLW	NFG	TGW	SPY	TPDB	PC
IG74/IG74	-16.43**	12.39**	-3.92	-6.26	38.72**	-27.27	-14.94	5.26	-2.02	-12.36**	0.00
IG74/CO52	-21.26**	7.20	3.92	21.25**	20.71*	-18.18	-24.9*	-0.24	7.86	-7.57*	-21.55**
IG74/TKM13	-23.67**	1.46	7.84	23.27**	14.81	-40.91**	0.41	5.74*	9.20*	-21.33**	5.14**
IG74/CRDhan315	-23.19**	18.17**	3.92	26.4**	27.95**	-31.82*	-13.28	5.26	-11.89*	-37.85**	-12.46**
IG74/ Vellai chithiraikar	-26.09**	20.23**	5.88	25.28**	36.03**	-22.73	-27.80*	9.81**	14.95**	-29.15**	-29.44**
IG74/Gandhasala	-16.43**	6.22	-1.96	-15.21*	21.38**	4.55	-40.66**	5.02	-3.64	-46.10**	6.27*
CO52/IG74	-10.14**	31.33**	7.84	15.66**	7.07	-4.55	-7.47	8.37**	14.55**	36.07**	-9.18**
CO52/CO52	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-27.82**
CO52/ TKM13	-7.73**	0.43	-9.80	7.61	58.25**	0.00	114.11**	0.96	7.31	-31.34**	-26.75**
CO52/ CRDhan315	-8.70**	21.35**	7.84	53.91**	39.23**	31.82*	107.47**	6.22*	7.50	30.47**	-32.97**
CO52/ Vellai chithiraikar	-5.80**	33.78**	-1.96	27.96**	17.00*	-18.18	39.42**	12.44**	32.18**	18.92**	-21.64**
CO52/Gandhasala	2.42	3.77	0.00	24.38**	24.92**	22.73	60.58**	0.48	24.87**	22.31**	-10.48**
TKM13/ IG74	-1.45	-15.56**	11.76	-8.95	-17.51*	36.36*	76.76**	-1.91	5.27	-12.98**	-10.44**
TKM13 /CO52	1.45	-11.70*	-29.41**	12.08	11.62	50.00**	129.05**	3.35	32.74**	-1.18	-20.92**
TKM13/ TKM13	2.90	-15.35**	1.96	-7.83	-3.70	40.91**	62.24**	-6.22*	-3.37	-19.44	-30.20**
TKM13/ CRDhan315	3.38*	-8.74	3.92	-6.49	-5.56	-13.64	118.67**	5.26	22.82**	-0.21	-24.19**
TKM13/ Vellai chithiraikar	1.93	3.86	7.84	29.75**	36.36**	-13.64	68.88**	-0.24	3.31	-14.99**	-26.52**
TKM13/Gandhasala	4.83**	-11.02*	-1.96	10.51	26.26**	0.00	92.12**	-2.63	2.35	-35.72**	-14.29**
CRDhan315 /IG74	-9.66**	31.03**	-3.92	25.73**	27.10**	-9.09	-4.98	15.79**	21.36**	-18.24**	-10.71**
CRDhan315/ CO52	2.42	11.87*	5.88	38.48**	25.42**	45.45**	78.84**	0.96	7.83	28.51**	-19.44**
CRDhan315/ TKM13	-0.48	2.14	7.84	21.70**	18.18*	54.55**	86.72**	3.11	5.32	26.23**	-24.78**
CRDhan315/ CRDhan315	-0.97	12.94**	-7.84	25.50**	8.59	-10.91	-10.37	-2.63	-6.90	28.48**	-38.35**
CRDhan315/ Vellai chithiraikar	-6.76**	26.62**	0.00	27.52**	12.29	4.55	17.84	13.16**	14.4**	9.63**	-23.16**
CRDhan315/Gandhasala	0.97	11.70*	3.92	30.43**	23.91**	4.55	36.51**	0.48	3.31	24.61**	-6.23**
Vellai chithiraikar/IG74	-13.04**	29.36**	-3.92	46.53**	26.94**	9.09	7.47	12.68**	17.27**	-12.61**	-22.04**
Vellai chithiraikar/CO52	-6.28**	25.93**	0.00	36.91**	34.85**	4.55	30.29**	14.35**	14.14**	-15.06**	-22.89**
Vellai chithiraikar/TKM13	-5.80**	39.82**	-3.92	39.82**	20.03*	-27.27	30.29**	4.07	4.14	-13.29**	-27.28**
Vellai chithiraikar/ CRDhan315	-7.73**	54.14**	3.92	46.31**	59.09**	-9.09	9.13	11.48**	16.94**	3.00	-29.48**
Vellai chithiraikar/ Vellai chithiraikar	-9.18**	56.88**	-23.53**	34.23**	30.47**	-9.09	5.39	6.46*	-5.63	-33.25**	-33.47**
Vellai chithiraikar/ Gandhasala	4.35**	48.05**	-11.76	41.61**	64.81**	-4.55	0.00	10.53**	8.00	-8.49*	-17.43**
Gandhasala/ IG74	-9.66**	17.66**	3.92	-17.23**	-10.44	-27.27	32.37**	3.59	13.17*	2.87	9.27**
Gandhasala/CO52	-4.35**	19.25**	5.88	8.28	40.91**	-22.73	62.66**	4.31	5.57	-11.15**	-7.66*
Gandhasala/ TKM13	-2.90	8.92	0.00	17.23**	22.39**	13.64	24.07*	1.20	-6.65	-21.89**	-12.59**
Gandhasala/CRDhan315	-5.31**	23.79**	0.00	34.00**	35.02**	40.91**	13.69	0.48	-6.43	-13.08**	-14.87**
Gandhasala/ Vellaichithiraikar	-6.76**	33.26**	-3.92	35.57**	43.77**	9.09	15.35	13.16**	2.55	-19.16**	-6.45**
Gandhasala/Gandhasala	-4.83**	18.09**	-5.88	3.80	25.42**	31.82*	8.71	-3.35	-14.04**	-32.74**	17.97**

DFF=Days to 50% flowering, PH=plant height, NPT=number of productive tillers, PL=panicle length, FLL=flag leaf length, FLW=flag leaf width, NFG=number of filled grains per panicle, TGW=1000 grain weight, SPY=single plant yield, TPDB=total plant dry biomass, PC=protein content

compared with the check CO52, eight crosses were found to be positively significant. The hybrids TKM13 / CO52 (32.74%), CO52 / Vellai chithiraikar (32.18%) and CO52 / Gandhasala (24.87%) possessed significant economic heterosis for single plant yield. These hybrids also possessed highly positive significant for 1000 grain weight besides early duration (**Table 4**). Hussein, 2021 found significant standard heterosis in six crosses for number of filled grains together with yield. In our study two hybrid combinations Gandhasala / IG74 and IG74 / TKM13 were found to have significant positive standard heterosis for grain protein content and grain yield. This may be due to the influence of non-additive gene action and specific combining ability. Hence a careful selection of the parents helps in improving the yield along with grain protein content.

Genotypic correlation **Table 5** revealed that grain protein content had a significant and negative correlation with single plant yield but positive association with 1000 grain weight and number of productive tillers. Similar type of association was obtained by Sheju *et al.* (2013) and Thuy *et al*. (2023). Inverse relationship between grain protein content and yield resulted due to environmental influence, source-sink interactions and dilution of the protein by non-protein compounds. (Mahesh *et al.,* 2022 and Kibite and Evans, 1984). Interestingly, significant positive correlation was observed between protein content and thousand grain weight as well as number of productive tillers. Single plant yield was found significant and positively correlated with number of filled grains per panicle, thousand grain weight and panicle length. These traits were used as selection criteria for grain yield improvement. These observations were supported by earlier findings of Arunkumar *et al*. (2022) and Jangala *et al*. (2022) for the number of filled grains and Prasannakumari *et al*. (2020) for grain weight. Panicle height and flag leaf width were found to have negative significant correlation with grain yield. For other major yield traits, number of productive tillers per plant displayed positive association with flag leaf length, 1000 grain weight and protein content and exhibited negative correlation with number of filled grains per panicle. These results indicated that indirect selection for 1000 grain weight will help in simultaneous improvement of yield and protein content. Pujar *et al.* (2020) reported similar significant correlation between 1000 grain weight and protein content, suggesting the possibility of simultaneous improvement.

The results of path coefficient analysis for single plant yield and grain protein content are presented in **Table 6**. The path coefficient analysis revealed that grain protein content had a negligible direct effect (0.013) on single plant yield indicating that direct selection of protein may not be useful for improving yield. In contrast high direct effect on grain yield by protein content was recorded by Patel *et al*. (2016). In the present study, high positive direct effect on grain yield was contributed by 1000 grain weight, total plant biomass and number of filled grains per panicle. Whereas, a negative direct effect on grain yield was observed by plant height, flag leaf width, number of productive tillers, days to fifty percent flowering, panicle length and flag leaf length. The grain protein content had a negative indirect effect *via* number of filled grains number of productive tillers on single plant yield which were in accordance with findings of Sheju *et al.* (2013). While considering the indirect effects of different traits on yield it was observed that plant height had positive indirect effect *via* thousand grain weight (0.365) and number of productive tillers influence *via* thousand grain weight (0.254) and days to fifty percent flowering *via* number of filled grains (0.212).
It was concluded that protein content was governed

Table 5. Genotypic correlation coefficient between single plant yield with yield components and grain protein content

	DFF	PH	NPT	PL	FLL	FLW	NFG	TGW	TPDB	PC	SPY
DFF	1.000										
PH	-0.241	1.000									
NPT	-0.145	-0.576*	1.000								
PL	-0.037	0.605*	-0.030	1.000							
FLL	-0.123	-0.595*	0.766**	0.525*	1.000						
FLW	0.466	-0.299	-0.044	0.024	-0.070	1.000					
NFG	0.603*	-0.483	-0.732**	-0.076	-0.128	0.429	1.000				
1000GW	-0.336	-0.612*	0.686**	0.425	0.307	-0.285	-0.270	1.000			
TPDB	0.344	0.049	0.223	0.558*	-0.203	0.223	0.241	0.006	1.000		
PC	-0.205	-0.105	0.538*	-0.439	-0.105	-0.086	-0.242	0.590*	-0.252	1.000	
SPY	0.092	-0.531*	0.488	0.731**	-0.367	-0.669*	0.899**	0.615*	0.382	-0.157*	1.000

DFF=Days to 50% flowering, PH=plant height, NPT=number of productive tillers, PL=panicle length, FLL=flag leaf length, FLW=flag leaf width, NFG=number of filled grains per panicle, TGW=1000 grain weight, SPY=single plant yield, TPDB=total plant dry biomass, PC=protein content

Table 6. Path coefficients between single plant yield with yield components and grain protein content

	DFF	PH	NPT	PL	FLL	FLW	NFG	TGW	TPDB	PC	SPY
DFF	**-0.049**	0.059	0.020	0.001	0.003	-0.091	0.212	-0.201	0.139	-0.011	0.092
PH	0.012	**-0.244**	0.025	-0.024	-0.013	0.059	-0.169	0.365	0.020	0.001	0.531
NPT	0.007	0.043	**-0.141**	0.001	0.004	0.009	-0.011	0.254	0.090	0.005	0.488
PL	0.002	-0.147	0.004	**-0.039**	-0.014	-0.005	-0.026	-0.051	0.104	-0.010	0.731
FLL	0.006	-0.121	0.023	-0.020	**-0.027**	0.014	-0.045	0.183	-0.082	0.000	-0.069
FLW	-0.023	0.073	0.006	-0.001	0.002	**-0.196**	0.150	-0.170	0.090	0.000	-0.069
NFG	-0.030	0.118	0.005	0.003	0.003	-0.084	**0.349**	-0.161	0.097	-0.008	0.899
1000GW	0.016	-0.149	-0.211	-0.017	-0.008	0.056	-0.094	**0.597**	0.003	0.000	0.615
TPDB	-0.017	-0.012	-0.031	-0.010	0.005	-0.044	0.084	0.004	**0.404**	-0.001	0.382
PC	0.010	0.026	-0.017	0.017	0.003	0.017	-0.084	0.030	-0.102	**0.013**	-0.157

Residual effect: 0.312

DFF=Days to 50% flowering, PH=plant height, NPT=number of productive tillers, PL=panicle length, FLL=flag leaf length, FLW=flag leaf width, NFG=number of filled grains per panicle, TGW=1000 grain weight, SPY=single plant yield, TPDB=total plant dry biomass, PC=protein content

both by additive and with the predominant non additive effects as the significance was observed on relative heterosis, heterobeltiosis and standard heterosis. The two promising heterotic hybrids Gandhasala / IG74 and IG74 / TKM13 was found to have higher significantly positive standard heterosis for both protein content and plant yield. So, the careful selection of parents will help in concurrent improvement of plant yield coupled with grain protein content. Henceforth, these hybrids could be utilised for further heterosis breeding programme, where the selection can be deferred to future generations. The grain protein content had negative interrelation with grain yield and had a positive association with thousand grain weight and number of productive tillers per plant. Hence, selection of parents for high test weight would help to identify good genotypes with high grain yield and protein content.

ACKNOWLEDGMENT

We would like to acknowledge Department of Rice, TNAU for providing field and lab facilities to conduct the research.

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