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Studying the genetic basis of pod physical and biochemical traits in Rajmash (*Phaseolus vulgaris* L.)

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Abstract
The efficiency and efficacy of the parent selection programmes in most crops including Rajmash can be significantly improved by estimating the combining ability and effects. In the present study ten parents were used in a crossing program in line × tester mating design to study combining ability for pod physical and biochemical traits in Rajmash. Parental lines and their crosses were evaluated for nutritional parameters as well as pod physical components.The results showed that pod length, seed index, protein content and anthocyanine content were under control of additive genetic effect while number of seed per pod and total phenol content were controlled by non-additive genetic effect. TRCR 3, TRCR 2 possessed highly significant GCA effect for pod length and seed index. IPR-205-19 was good combiner for number of seed per pod. TRCR 2 was good combiner for biochemical traits *viz*., protein content, anthocyanine content and total phenol content.

Keywords: pod, TRCR, physical, Rajmash, *Phaseolus vulgaris* L.

Introduction

Catering to the food needs of the rapidly growing population presents a significant obstacle across the Asian continent. Agriculture takes a prominent position on both national and international agendas, aiming to establish food security and effective management of natural resources. The global agricultural sector faces an enormous challenge, marked by the escalating impact of projected climate fluctuations and the ever-increasing population pressure, prompting serious concerns among policymakers and the scientific community. As the world grapples with the challenge of feeding a burgeoning population, pulses emerge as an invaluable solution due to their nutritional richness, environmental benefits, and potential to improve global food security. Their incorporation into diets can play a pivotal role in ensuring that the growing population receives the essential nutrients necessary for a healthy and prosperous future.

Pulses constitute a valuable source of protein, calcium (Ca), iron (Fe), thiamine, and riboflavin in economically challenged regions worldwide. (Norton *et al*., 1985) [16]. For this reason, across numerous global regions, particularly within tropical climates, the consumption of pulses is often linked with economic limitations. However, in India, a distinct perspective emerges, where the consumption of pulses finds acceptance due to religious practices or local customs that discourage the intake of meat or dairy products. Pulses, including beans, are important sources of protein in many regions of the world (Ceyhan, 2006, Patel *et al*. 2021 and Sharma *et al.*, 2022) [4, 21, 30]. Pulses are also an excellent source of dietary fiber, vitamins, photochemicals (phenolic acid, anthocyanins), and minerals (Akçin, 1988) [1]. The Rajmash, *Phaseolus vulgaris* L., holds a significant role in global human nutrition. Recently, the consumption of legumes, especially dry beans, has experienced growth. This shift can be attributed to a heightened awareness among consumers regarding the nutritional attributes present in foods. (Peksen and Artik, 2005) [20]. Rajmash (also known as garden, green, string, or French beans) are a form of common bean. As opposed to dry bean, in which the mature seeds are eaten, the immature pods and seeds of snap beans are consumed as a vegetable. Rajmashis known as a “super food” due to its protein, dietary fiber, and mineral content, and due to it being a daily food for more than 300 million people all over the world (Saleh *et al*., 2012) [25].

The presence of phytochemicals such as polyphenolic compounds prevents various disorders like cardiovascular disease, blood glucose, obesity and colon cancer; in addition they show high antioxidant activity (Pinheiro *et al*., 2010; Hayat *et al*., 2014) [23, 10].

The color variation is especially great in Rajmash, with red, black, brown, and white colors are common. Among these colors, red and black color pigments in the seed coats of rajmash are an attractive potential source for natural food colorants. Anthocyanins are commonly used in food supplements and nutraceuticals because of their beneficial effects on humans. Anthocyanins exhibit a broad range of pharmacological activities and they have antioxidant, anti-inflammatory, anticancer, anti-ulcer, Cardioprotective, antidiabetic, and neuroprotective properties (Husain *et al*. 2022) [11]. Anthocyanins in the seed coat of were first reported by Feenstrain 1960, and subsequently several investigators have identified different kinds of anthocyanins from diverse Rajmash varieties. Feenstra 1960 found four anthocyanins, malvidin 3-glucoside, petunidin 3-glucoside, delphinidin 3-glucoside, and delphinidin 3,5-diglucoside, in black violet beans (*P. vulgaris* L.). Another anthocyanin, malvidin 3,5-diglucoside, together with delphinidin 3-glucoside, petunidin 3-glucoside, and malvidin 3-glucoside, was reported in the black bean cultivar Kurodanekinugasa by Okita *et al*. 1972 [18]. In another study, Stanton and Francis 1966 reported that delphinidin 3-glucoside was the major anthocyanin in the Canadian Wonder cultivar.

Total phenolic content has a strong antioxidant activity in legumes. As a rule, the antioxidant capacity of bean seeds arises from their content of phenolic compounds. These are natural antioxidants, able to protect against reactive oxygen species, which are responsible for reactions underlying many serious diseases (Amarowicz and Weidner, 2009; Reynoso-Comacho *et al*., 2006) [24]. Nyau, Prakash, Rodrigues, and Farrant (2016) [17] have demonstrated that red beans are the richest in total polyphenols, followed by grey mottled, brown and white beans, and that there is a strong positive correlation between antioxidant activity and total polyphenol content.

Rajmash grown for processing *vs*. the fresh market have different quality characteristics that may affect TPC. Processors require that cultivars be white-seeded to ensure that no water-soluble flavonoids are produced in any plant parts including the pods as they will leach into the liquor in canned beans and present as a colored ring in the testa in cut frozen beans, thereby reducing quality (Myers JR, *et al.* 1999) [13]. The criteria for fresh market beans are comparatively less strict. Although beans with colored seeds are permissible, the predominant seed color is typically brown, stemming from flavonols, rather than black or purple, which arise from anthocyanins. This preference for brown seeds is due to their lesser visibility within the pod. Varieties with brown seeds usually exhibit green-looking pods, while those with purple or black seeds might feature either entirely purple or striped purple and green pods. (Myers JR, *et al.*, 2019) [14]. Rajmash, hailed for its antioxidant properties and health benefits, faces the challenge of a yield plateau that necessitates a focus on overcoming bottlenecks to maintain a consistent supply of this valuable crop. In the pursuit of harnessing the nutritional and economic benefits of Rajmash, tackling the yield plateau becomes essential to meet the demands of a growing population and ensure food security.

Developing high yielding hybrid or variety with adaptability to environment fluctuations is the ultimate aim of all the crop improvement programmes (Patel *et al* 2021 and Sharma *et al* 2022) [21, 22]. Though the latest technologies like genetic engineering and recombinant DNA techniques are being used in the crop improvement programme, yet hybridization method has its own advantage and frequently used in breeding programme aimed to improve the yield (Sharma and Sridevi 2016 [31], Sharma and Shadakshari 2021 and Sharma *et al* 2022). Combining ability studies are useful not only in analysing the genetic architecture of the characters under study but also in ranking the parental line on the basis of their performances in the crosses, the information thus obtained helps in designing suitable breeding procedure for genetic amelioration of the crop and selection of suitable parents which when crossed will give rise to more desirable segregates. The objective of the present study is to understand the inheritance of physical and biochemical quality characteristics identified as important for Rajmash production.

Materials and Methods

The material for study consists of 7 phenotypically diverse lines TRCR 3, TRCR 2, IPR-205-19, IPR-277-19, SKAU-R-19, RR-21-01 and RR-21-12 (used as female) and three tester GR-1,RKR 1033 and HUR (used as male) crossed in line × tester mating design (Kempthorne, 1957) [12] to synthesize 21 hybrids during rabi 2021-22. The hybrid so produced were evaluated along with their parents at Pulses Research Station, S. D. A.U, Gujarat in randomized block design with three replication during rabi 2022-23. In each treatment, five competitive plants were randomly selected to record the observations for various quantitative and biochemical traits. The seeds extracted from matured pods were powdered using grinding machine with 0.2 mm size mesh and the seed powder was used for biochemical analysis. All the biochemical estimations were done in triplicates. Protein content was estimated by near infrared analyzer, total anthocynine content from dried seed powder was estimated following procedure suggested by Swain and Hillis (1959) [36] and total phenol content from dried seed powder was estimated following procedure suggested by Bray and Thorpe (1954) [3]. Variance analysis was done by using the means of investigated traits. The line × tester method (Kempthorne, 1957 [12]; Singh and Chaudhary, 1979) [33] was followed to analyze genetic components of each characteristic.

Results and Discussion

The analysis of variance (Table 1) indicated that the mean squares of genotypes for all characters investigated were significantly different, indicating the presence of variability among hybrids and their parents, hence later analysis for combining ability was worth full. The results revealed that the magnitude of GCA variance was greater than SCA variance for the four characters *viz*., pod length, seed index, protein content and anthocyanin content which indicates predominance of additive gene action, other traits showed higher SCA variance than GCA variance *viz*., number of seed per pod and total phenol content indicated the predominance of non-additive gene action governing the inheritance of these characters. Similar results have been reported by Gandhi *et al* (2024) [7] and Selvakumar *et al*. (2014) [26] for the importance of additive gene action for different yield attributing traits. Similarly additive effects were reported for seed index and protein content by Nath *et al*. (2018) [15] and Sharma and Mehta (2014) [27]. The preponderance of non-additive variance for number of seed per pod. The gca effects of parents are presented in table. 2. with regards to estimates for gca effects for pod length, line TRCR 3 (1.72) and TRCR 2 (1.49) showed significant positive gca effect for pod length, if pod length increases, the number of seeds per pod also increases thus in turn contributing to increased seed yield so, these lines were high utility in the breeding programme. The seed weight of a genotype serves as an indicator to the expression of end product. The highest significant positive gca effect for seed index was registered by the female parent TRCR 3 (4.41), SKAU-R-19 (3.93), TRCR 2 (3.55) and the male parent GR 1 (2.07). These parents possessed genes for enhancing plants seed weight. Hence, these lines and testers showing positive gca effects could be used in hybridization programme to develop hybrids with high seed weight. Given the nutritional significance of protein content in pulses, positive combining ability effects are sought after. Impressively, the lines TRCR 2 (0.49) and IPR-277-19 (0.84) exhibited positively significant gca effects for protein content. This signifies their potential as favorable candidates for hybridization aimed at enhancing protein content in the resulting hybrids. Anthocyanin content, with its various implications, found notable general combiner candidates. Specifically, the lines TRCR 2 (1.08), SKAU-R-19 (3.1), and RR-21-01 (0.87) emerged as promising general combiners for anthocyanin content. Their positive GCA effects point towards their potential contribution to offspring with elevated anthocyanin content. The quest for improved taste in Rajmash underscored the importance of phenol content. Interestingly, several lines demonstrated negatively significant gca effects for phenol content. Notably, TRCR 3 (-0.28), TRCR 2 (-0.77), IPR-205-19 (-0.46) and GR 1 (-0.20) showcased traits conducive to reduced phenol content, thus aligning with the goal of enhancing rajmash taste. As in the present study highly significant gca effects for pod length, number of seed per pod, and seed index were obtained by Selvakumar *et al*. (2014) [26], for protein content by Ceyhan, E *et al*. (2014) [5].

The SCA effects of the crosses for of pod physical and biochemical quality traits were presented in table.3. Positive and significant specific combining ability effects for pod length was observed by hybrid IPR-277-19 × GR 1, suggesting a synergistic combination that promotes elongated pod structures. Among the 21 crosses evaluated, only one combination, TRCR 2 × GR 1, exhibited significant and positive SCA effects for the number of seeds per pod. This particular cross underscores the potential for specific interactions leading to an enhanced seed count per pod. The crosses involving RR-21-12 × GR 1 displayed positive and significant SCA effects, aligning favorably with desirable directions. This particular combination emerged as a promising specific combiner for anthocyanine content, implying the potential for progeny with heightened anthocyanin levels. A subset of six hybrids, namely RR-21-12 × HUR, TRCR 2 × HUR, SKAU-R-19 × GR 1, SKAU-R-19 × RKR 1033, IPR-205-19 × RKR 1033, and PR-277-19 × GR 1, exhibited significant negative SCA effects for phenol content. Similar results for biomass yield and harvest index in Rajmash were observed by Gandhi *et al* (2024) [7]. These findings point to the possibility of reducing phenol content through specific interactions among these hybrid combinations, thereby contributing to improved taste in kidney beans.

Table 1: Analysis of variance for different characters in Rajmash

Source of variation	D.F.	Pod length	Number of seed per pod	Seed index	Protein content	Anthocyanine content	Total phenol content
Replications	2	1.70	0.20	0.54	0.02	1.47	0.13
Genotypes	30	4.25**	0.81**	76.16**	2.05**	26.93**	1.58**
Parents	9	4.30**	0.53*	84.74**	3.30**	19.90**	1.58**
Lines	6	6.38**	0.74**	79.23**	2.90**	21.28**	0.37*
Testers	2	0.23	0.11	75.01**	5.82**	8.98**	2.21**
Lines *vs*. Testers	1	0.02	0.08	137.21*	0.63	33.45*	7.60**
Crosses	20	4.57**	1.01**	76.94**	1.72**	21.33**	1.70**
Parents *vs*. Crosses	1	5.40	0.66	12.90	0.79	217.29**	0.01
Error	60	4.25	0.23	15.12	0.25	0.25	0.08
σ^2GCA		0.41**	0.04	7.09**	0.20**	1.86**	0.06
σ^2SCA		0.15	0.12*	2.71	0.01	0.82*	0.34**
σ^2GCA/ σ^2SCA		2.65	0.34	2.61	20.13	2.26	0.17

*and** indicate significant at 5 percent and 1 percent levels of significance, respectively σ2GCA= Variance GCA, σ2SCA=Variance SCA

Table 2: Estimates of general combining ability effects for different traits in Rajmash

Parent genotype	Physical quality traits			Biochemical quality traits		
	Pod length	Number of seed per pod	Seed index	Protein content	Anthocyanine content	Total phenol content
Lines						
TRCR 3	1.72**	0.27	4.41**	0.25	0.20	-0.28**
TRCR 2	1.49**	0.35	3.55*	0.49*	1.08*	-0.77**
IPR-205-19	-0.16	0.54**	-1.64	-0.07	0.29	-0.46**
IPR-277-19	-0.57*	-0.73**	-1.57	0.84**	-5.39**	0.43**
SKAU-R-19	-0.09	-0.44*	3.93**	0.23	3.1**	1.02**
RR-21-01	-0.85**	-0.03	-0.75	-0.70**	0.87*	-0.10
RR-21-12	-1.54**	0.06	-7.92**	-1.05**	-0.17	0.16
Testers						
GR 1	0.03	-0.12	2.07*	0.22	0.17	-0.20**
RKR 1033	-0.25	-0.09	-2.09*	-0.43**	-0.41	0.03
HUR	0.22	0.21	0.01	0.21	0.24	0.17**

*and** indicate significant at 5 percent and 1 percent levels of significance, respectively

Table 3: Estimates of specific combining ability effects of twenty-one cross combinations in Rajmash.

Crosses	Pod length	Number of seed per pod	Seed index	Protein content	Anthocyanin content	Total phenol content
TRCR 3 × GR 1	-0.89*	0.12	-1.78	-0.38	0.68	0.20
TRCR 3 × RKR1033	0.12	-0.02	-1.98	0.10	-1.03	0.01
TRCR 3 × HUR	0.77	-0.10	3.76	0.28	0.35	-0.21
TRCR 2 × GR1	0.20	0.64*	-0.14	0.02	0.60	-0.01
TRCR 2 × RKR 1033	-0.07	-0.70*	-0.05	-0.03	-0.53	0.66**
TRCR2 × HUR	-0.14	0.06	0.19	0	-0.07	-0.65**
IPR-205-19 × GR 1	-0.32	-0.33	0.42	0.37	-0.35	0.46**
IPR-205-19 × RKR1033	0.09	0.04	1.39	-0.05	-0.02	-0.49**
IPR-205-19 × HUR	0.23	0.29	-1.81	-0.32	0.37	0.02
IPR-277-19 × GR 1	0.85*	-0.28	-0.62	-0.19	-0.65	-0.49**
IPR-277-19 × RKR 1033	0	0.28	1.93	-0.16	0.77	0.13
IPR-277-19 × HUR	-0.84*	0.01	-1.31	0.35	-0.12	0.36*
SKAU-R-19 × GR 1	-0.16	0.46	4.50	0.18	-0.63	-0.47**
SKAU-R-19 ×RKR 1033	-0.32	0.12	-3.78	-0.47	-0.47	-0.53**
SKAU-R-19 × HUR	0.48	-0.59	-0.72	0.29	1.09	1.00**
RR-21-01 × GR 1	0	-0.23	-2.26	0.27	-1.48*	-0.19
RR-21-01 × RKR 1033	-0.02	0.43	-0.85	0.06	1.42*	-0.09
RR-21-01 × HUR	0.02	-0.20	3.11	-0.34	0.06	0.29
RR-21-12 × GR 1	0.31	-0.39	-0.11	-0.27	1.83*	0.50**
RR-21-12 × RKR 1033	0.20	-0.15	3.33	0.54	-0.15	0.32
RR-21-12 × HUR	-0.51	0.53	-3.22	-0.26	-1.68*	-0.81**

*and** indicate significant at 5 percent and 1 percent levels of significance, respectively

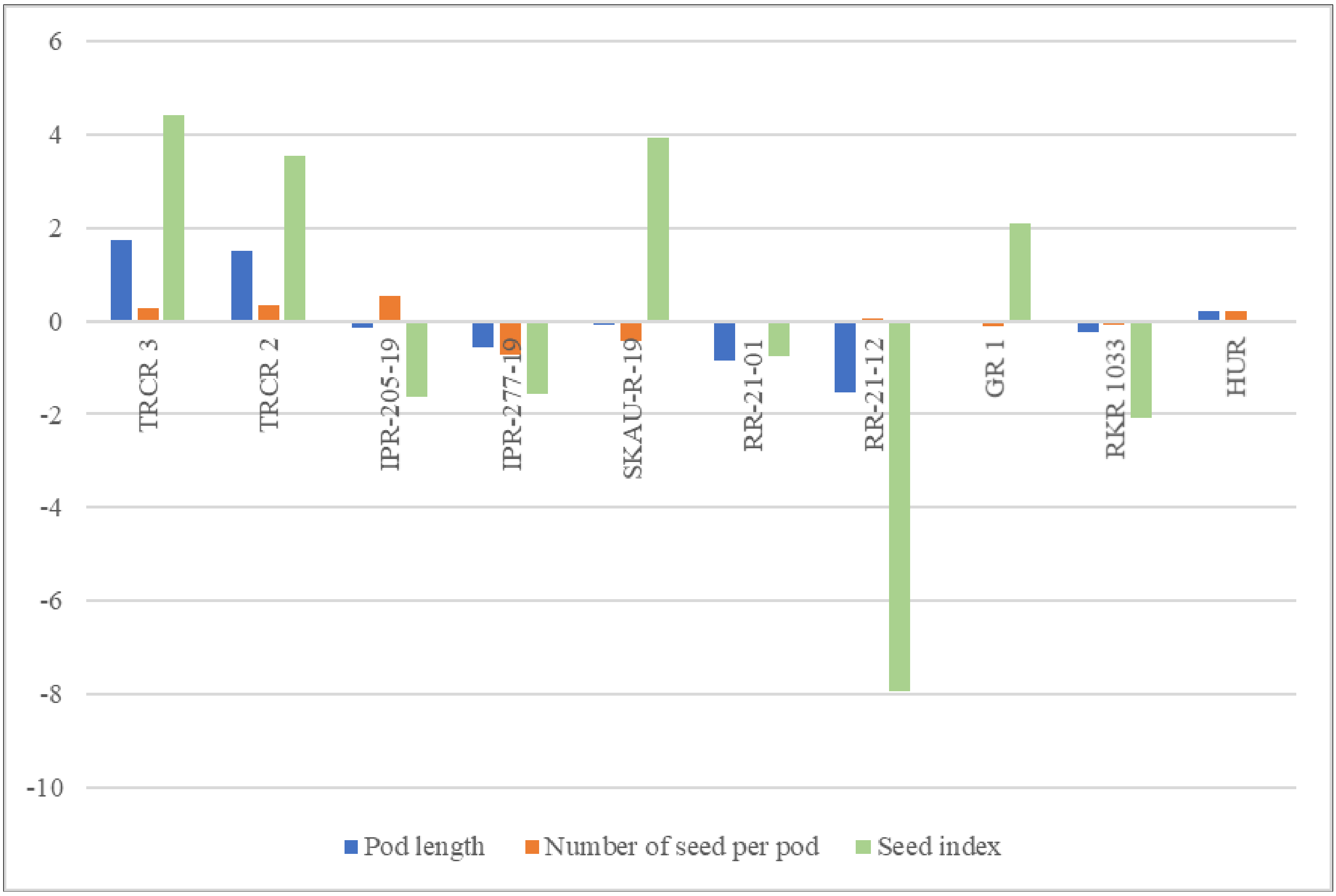

Fig 1: GCA effect of parents for physical quality traits

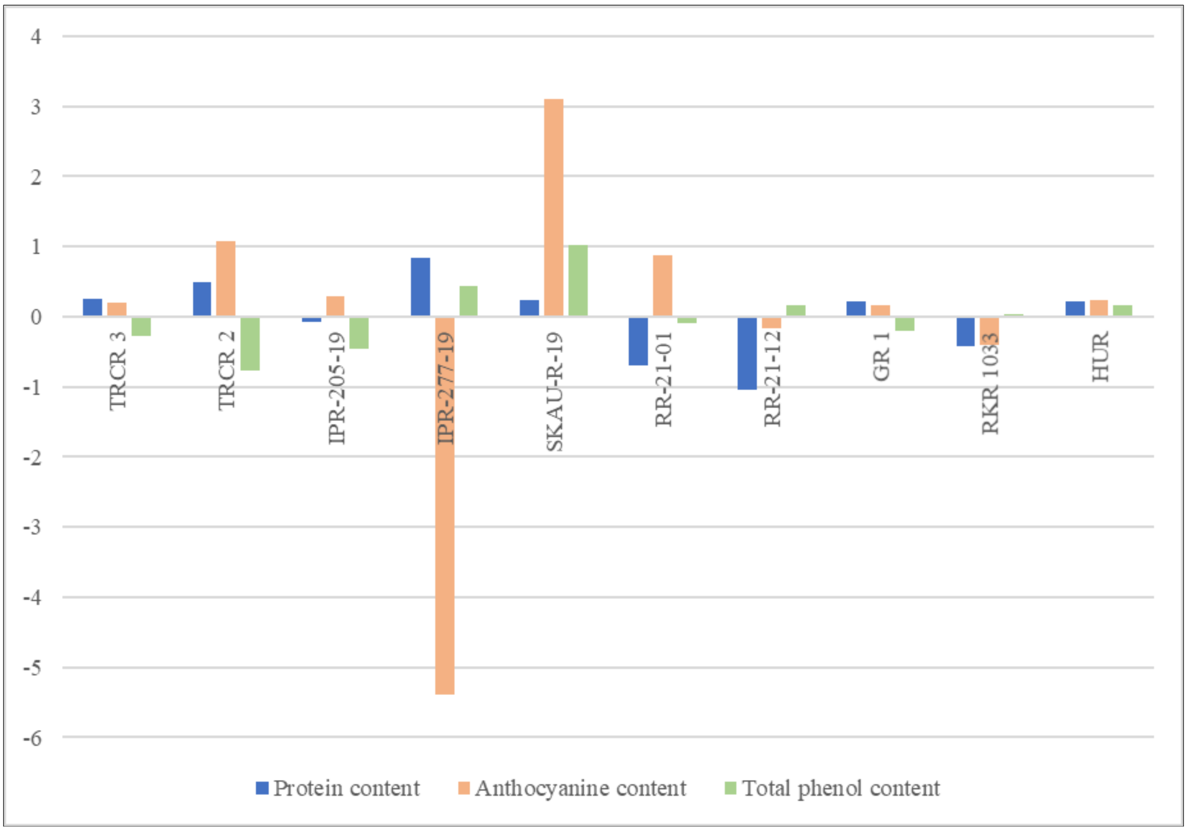

Fig 2: GCA effect of parents for biochemical quality traits

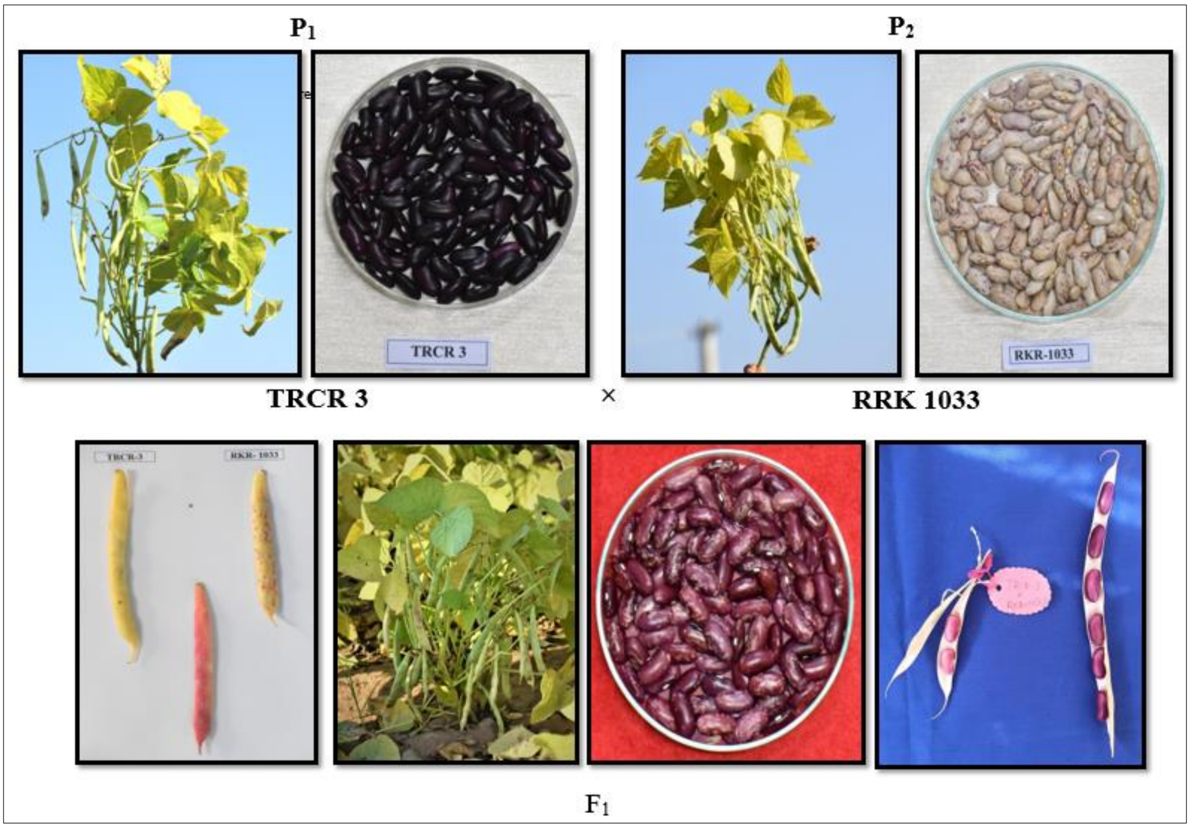

Fig 3: Parents and their F_1

Conclusion

Rajmash is sold as beans in markets, where consumer preference is for long pods with many large seeds per pod. Notably, lines such as TRCR 3 and TRCR 2 exhibited positive GCA effects for pod length, with implications for increased seed yield due to the correlation between pod length and seeds per pod. This holds significant promise for the development of genotypes with enhanced productivity. The importance of biochemical traits was also underscored in this study. Lines exhibiting positive GCA effects for protein content, such as TRCR 2 and IPR-277-19, hold the potential to contribute to enhanced protein levels in future generations, aligning with the nutritional demands of pulse crops. Phenol content in beans is associated with bitterness and astringency, which may affect the taste. Consumer preference for low phenol content aligns with a desire for a sweeter taste. specific hybrid combinations, including RR-21-12 × HUR, TRCR 2 × HUR, SKAU-R-19 × GR 1, SKAU-R-19 × RKR 1033, IPR-205-19 × RKR 1033, and PR-277-19 × GR 1, revealed significant negative SCA effects for phenol content. This finding suggests a potential avenue for reducing phenol content and subsequently improving taste in rajmash through controlled hybridization. Inheritance studies can help determine the genetic basis of phenol content. Combining ability studies can identify parental combinations that yield offspring with lower phenol content while maintaining other desirable traits. These characteristics are likely to be valued by consumers for both culinary and nutritional reasons. Long pods with many large seeds mean more beans to use in cooking, while a high anthocyanin content could imply potential health benefits associated with these pigments, such as antioxidant properties. The combination RR-21-12 × GR 1 demonstrated positive SCA effects in a desirable direction, positioning it as a potential specific combiner for anthocyanine content. This highlights the possibility of generating progeny with elevated anthocyanin levels through targeted hybridization efforts. These studies provide crucial information for developing improved varieties with enhanced agronomic and nutritional attributes, contributing to food security and human health.

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