ARTICLE

REML/BLUP-based dissection of combining ability and maternal influence on soybean grain protein

Crop Breeding and Applied Biotechnology
26(2): e554926210, 2026
Brazilian Society of Plant Breeding.
Printed in Brazil
http://dx.doi.org/10.1590/1984-70332026v26n2a25

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Abstract: *This study aimed to analyze the additive and non-additive genetic effects governing protein content in soybean grains and to identify superior parents and maternal contributions in advanced generations. A total of 170 F_8 soybean lines and 95 commercial cultivars were evaluated during the 2024/2025 growing season. The total protein content in the grains (%) was determined. Deviance analysis confirmed significant effects of general combining ability (GCA) and specific combining ability (SCA). Narrow-sense heritability was low (2%), while broad-sense heritability reached 52%, demonstrating total genetic variability, attributable to dominance and epistatic effects. The average overall crude protein content was 36.15%. The parents NS5958RR, Maradona, and DM5.8BmxApoloRR showed the highest positive GCA estimates, indicating the presence of favorable additive alleles. The most promising specific combination was L77, with a 1.16% increase in protein, followed by L87. Maternal contributions were evident, particularly when NS5958RR and Maradona were used as female parents.*

Keywords: *Maternal effects, circulating diallel, heritability*

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is one of the world's leading crops, essential for food security due to its vegetable oil production and high-quality protein for human and animal consumption, contributing to global market competitiveness and socioeconomic development (Singh et al. 2008, Vasconcelos et al. 2020). Thus, breeding programs prioritize the development of high-protein cultivars with strong agronomic performance under biotic and abiotic stresses (Anderson et al. 2019, Liu et al. 2023). In this context, multi-trait selection in multi-environment trials has become a key strategy to manage environmental heterogeneity and genotype × environment interaction, enabling the identification of stable, high-performing genotypes (Kehl et al. 2022).

The genetic control of protein content in soybean is quantitative in nature, characterized by the action of multiple genes, strong environmental influence and management (Bosaz et al. 2019), which poses challenges to direct selection, especially in early breeding stages, thus requiring improved techniques for identifying these quantitative loci (Van and McHale 2017). In this context, biometric tools that enable the identification of superior

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Scientific Editor:
Luiz Antônio dos Santos Dias

Received: 18 February 2026
Accepted: 3 April 2026
Published: 11 April 2026

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parents and promising hybrid combinations are fundamental for increasing the efficiency of breeding programs (Borém et al. 2021).

Combining ability, widely used in quantitative genetics studies, is a robust method for evaluating the genetic potential of lines, parents, and combinations in crosses. By estimating the genetic effects of general combining ability (GCA) and specific combining ability (SCA), it is possible to infer the predominance of additive and non-additive effects in controlling the variable of interest. GCA corresponds to additive gene action and reflects the mean performance of a parent across different crosses, while SCA is associated with dominance and epistatic interactions (Cruz et al. 2012, Carvalho et al. 2017).

Studies of combining analysis have been widely applied in grain legumes to dissect the genetic architecture of yield and nutritional traits. In pea, dominance gene effects were reported to be predominant for yield components (Ceyhan et al. 2008, Ceyhan and Kahraman 2013, Ceyhan et al. 2014a, Şimsek and Ceyhan 2017). In common bean, significant GCA and SCA effects were identified for protein and mineral accumulation, demonstrating the relevance of additive gene action for nutritional improvement (Ceyhan et al. 2014b, Ceyhan et al. 2025). Similarly, diallel analyses in cowpea revealed substantial genetic variability and heritability for protein and mineral composition (Jou-Nteufa and Ceyhan 2024, Dalgıç et al. 2025). These findings emphasize that protein-related traits in legumes are governed by both additive and non-additive genetic effects, depending on species and population structure. However, such analyses remain limited in soybean advanced-generation lines, particularly regarding maternal influence and residual heterozygosity effects in F_8 materials.

Studies of combining ability have been widely used for agronomic traits of soybean, such as grain yield, plant height, and cycle (Bagatelli et al. 2020, Sangiovo et al. 2025). However, there are still few analyses focused on grain protein content, especially those involving lines in advanced generations that exhibit greater homozygosity and genetic stability. Evaluating combining ability in these lines allows the identification of parents with a higher frequency of alleles favorable to protein production, as well as crosses capable of generating superior progeny, contributing to the development of more efficient cultivars from a nutritional and industrial perspective (Meena et al. 2024).

Given this scenario, studying the combining ability of soybean as a function of grain protein content is essential to understanding the genetic basis of this trait and to guiding precise selection strategies, without confounding from heterozygous genes. This study aimed to evaluate the general and specific combining ability of elite soybean lines (F_8 generation: 0.78% heterozygous genes), seeking to identify promising parents and their maternal effects on increasing soybean grain protein.

MATERIAL AND METHODS

The experiment was conducted at the Experimental Farm School of the Regional University of Northwest Rio Grande do Sul (UNIJUÍ), located in the municipality of Augusto Pestana, Rio Grande do Sul, during the 2024/2025 growing season. The experimental design was conducted using an augmented block design with interspersed controls. The regular treatments consisted of 170 soybean lines from the F_8 generation, originating from crosses in a circulating diallel (Figure 1 and Table 1), and 95 soybean cultivars, included as interspersed controls, all arranged in four blocks. The experimental units consisted of a sowing row fifteen meters long, spaced 0.5 meters apart. For all genotypes, a sowing density of 16 seeds per linear meter was used, with a base fertilization of 300 kg ha^{-1} of 04-30-20 (N-P-K).

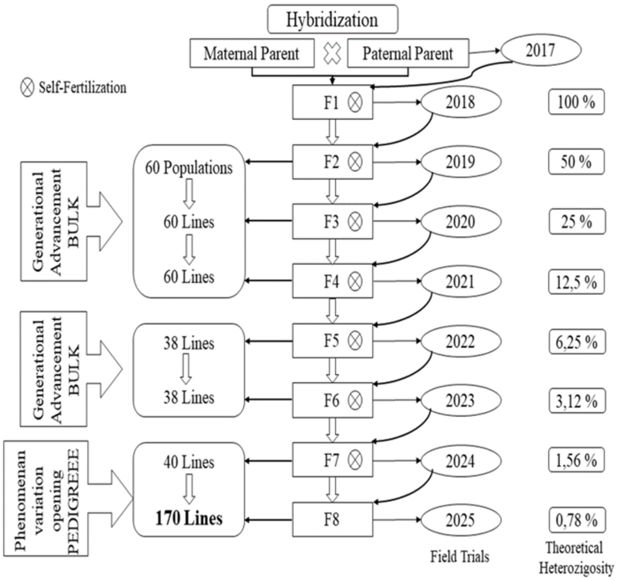

Figure 1. Chronological diagram of the genetic improvement process through hybridizations up to the F8 stage, to obtain the 170 inbred lines.

The control cultivars were: M6410IPRO, DM5958IPRO, 95Y52RR, FPS1859RR, TMGManacáXTD, C2570RR, FPSJúpiterRR, BMXTorqueI2x, FPS2565IPRO, TMG6123IPRO, SoyTech570I2x, GH2361IPRO, DM59IX61I2x, IPRBasalto, M6210IPRO, FPSSolimõesRR, SoyTech580I2x, IPRPé-Vermelho, NEO531I2x, 95R40IPRO, BRS525, NEO590I2x, NEO510IPRO, BMXLótusIPRO, C2560CE, NEO620IPRO, BMXCromoTFIPRO, SYN1059RR, M6130I2x, NS6601IPRO, NS4823RR, TMG7363RR, BRS511, TMG7061RR, DM64I63IPRO, NS6446I2x, BR2606IPRO, GH2459I2x, NS5252I2x, 95Y02IPRO, DM54IX57I2x, BRS5804RR, NS5922IPRO, M5939I2x, M5947IPRO, SoyTech541I2x, IPRPetrovita, BMXNexusI2x, BMXTitaniumI2x, DM60I64I2x, BMXTrovãoI2x, BRS546, BMXRaçaE, 95R51IPRO, AS3590IPRO, M5705IPRO, BMXVênusCE, SoyTech616I2x, BMXImuneTFI2x, TMGGuanandiI2x, BRS6013, NEO600I2x, C2550E, NS6006IPRO, FPSParanapanemaRR, NEO581E, BMXCompactaIPRO, 95Y95IPRO, URNRS23, BRS10001IPRO, M5917IPRO, GH5933IPRO, NEO610IPRO, NEO560IPRO, BRS537, DM65IX67I2x, BMXLançaIPRO, TMG7362IPRO, BMXFibraIPRO, M5710I2x, NS5959IPRO, DM56I59IPRO, NS5624I2x, M5834XTD, BRS539, DM57I52IPRO, BMXZeusIPRO, NA5909RGRR, TMG7262RR, SoyTech535I2x, M5838IPRO, BMXFúriaCE, and BMXRaioIPRO.

Table 1. Genealogy of the F_8 lines with their respective parents and the population of origin

Population	Maternal parent	No. PP	Paternal parent	No. MP	No. of lines
IRC001	DM7.0BmxMagnaRR	1	Fundacep66RR	20	13
IRC002	DM5.8BmxApoloRR	3	Fundacep66RR	20	8
IRC003	DM7.0BmxMagnaRR	1	MonascaRR	21	4
IRC005	RoosCaminoRR	2	FPSParanapanemaRR	22	3
IRC007	DM5.8BmxApoloRR	3	Maradona M4	8	2
IRC011	BmxPotênciaRR	4	Maradona M4	8	2
IRC012	FPSNetunoRR	5	DM5.8BmxApoloRR	3	15
IRC013	DM7.0BmxMagnaRR	1	Fundacep66RR	20	4
IRC016	RoosCaminoRR	2	FPSUranoRR	23	1
IRC017	DM7.0BmxMagnaRR	1	Fundacep66RR	20	4
IRC021	TMG7062IPRO	6	6700RR	7	7
IRC022	6700RR	7	BmxAtivaRR	24	3
IRC023	6700RR	7	5601IPRO	25	1
IRC025	Maradona (MP)	8	Maradona (PP pd)	8	11
IRC028	Maradona (MP)	8	Maradona (PP pt)	8	2
IRC030	4823RR	9	Rota54	26	1
IRC031	NS5958RR	10	Maradona M2	8	6
IRC032	DM7.0BmxMagnaRR	1	NS5909RR	12	2
IRC033	Maradona M4	8	NS5909RR	12	6
IRC034	NS5958RR	10	6700RR	7	14
IRC035	BmxForçaRR	11	Maradona M4	8	3
IRC037	NS5909RR	12	Maradona M4	8	3
IRC038	6700RR	7	Maradona M3	8	1
IRC039	TMG7166RR	13	NS5909RR	12	6
IRC040	BmxTurboRR	11	TMG7166RR	13	2
IRC042	DM7.0BmxMagnaRR	1	DM5.9BmxAlvoRR	27	2
IRC043	DM6563IPRO	14	NS5958RR	10	3
IRC044	M5710	15	TMG2757	28	7
IRC045	FPSJúpiterRR	16	DM7.0BmxMagnaRR	1	2
IRC046	DM7.0BmxMagnaRR	1	BmxAtivaRR	24	2
IRC047	TMG7062IPRO	6	NS5958RR	10	1
IRC048	DM7.0BmxMagnaRR	1	FPSParanapanemaRR	22	2
IRC049	6700RR	7	DM6160IPRO	30	4
IRC050	BmxPotênciaRR	4	NS5909RR	12	9
IRC056	HOPuricaIPRO	17	HOAmaboyIPRO	31	2
IRC058	15b70IPRO	18	DM6563IPRO	14	7
IRC060	HOJacuiIPRO	19	HOPuricaIPRO	17	4

PP number: paternal parent number; MP number: maternal parent number.

After maturation, 50 representative plants per experimental unit were harvested, from which the grains were grouped, homogenized, dried until they reached 13% moisture, and formed a 100 g sample from each experimental unit. For each sample, the percentage of total crude protein (%) was determined by the acid-catalytic digestion method, followed by distillation and titration using the Kjeldahl method, employing a conversion factor of 6.25, indicated for soybean cultivation (Kjeldahl 1883).

The grain protein data obtained from the control lines and cultivars were subjected to analysis of model assumptions, including normality, homogeneity of residual variances, additivity, and independence of errors. The Restricted Maximum Likelihood (REML) method was applied to estimate variance components and genetic parameters, based on the linear mixed model $y = X\beta + Za + Wf + e$, where *y* is the vector of individual phenotypic observations; *X* is the incidence matrix relating observations to fixed effects; *β* is the vector of fixed effects in the model; *Z* is the incidence matrix relating observations to additive genetic effects; *a* is the vector of random additive genetic effects of individuals, with $a \sim N(0, A\sigma_a^2)$, where *A* is the additive relationship matrix and σ_a^2 represents the additive genetic variance (Va); *W* is the incidence matrix relating observations to family effects; *f* is the vector of random family effects, with $f \sim N(0, I\sigma_{fam}^2)$, where *I* is the identity matrix and σ_{fam}^2 represents the variance among families (Vfam); and *e* is the vector of residual random errors, with $e \sim N(0, I\sigma_e^2)$, where σ_e^2 represents the residual variance (Ve).

Significance determined by a *Deviance* analysis at a 5% significance level using the Chi-square test (χ^2). The significant model allowed the estimation of the following parameters: additive variance (*Va*); family variance (Vfam); residual variance (*Ve*); phenotypic variance (*Vf*); narrow-sense heritability based on additive effects (*h2a*); broad-sense heritability (*h2g*); and mean family heritability (*h2fam*). From these parameters, the Best Linear Unbiased Predictor (BLUP) was extracted to estimate the genetic deviations from the general combining ability of the parents and the specific combining ability, using the *metan* package (Olivoto and Lúcio 2020). The analyses were performed using the R software (R Core Team 2026) with the *plant breeding*, *lmDiallel*, and *sommer* packages.

RESULTS AND DISCUSSION

Table 2. Estimates of variance components and genetic parameters (*REML*) for crude soybean grain protein

Variance componentes	**Grain protein** (%)
Va	0.22
Vfam	1.16
Ve	7,96
Vf	9.37
h2a	0.02
h2g	0.52
h2fam	0.24
Overall mean	36.15
Deviance	6,91*

Va: additive variance; *Vfam*: family variance; *Ve*: residual variance; *Vf*: phenotypic variance; *h2a*: narrow-sense heritability; *h2g*: broad-sense heritability; *h2fam*: general family heritability; * significance at 5% probability by chi-square test.

Deviance analysis revealed the significance of the general and specific combining abilities of the genotypes for the soybean grain protein content at a 5% probability level using the chi-square test (χ^2), validating the estimation of variance components and genetic parameters (Table 2). The significance of both GCA and SCA components indicates that soybean grain protein is controlled by a mixed genetic model involving both additive and non-additive gene effects. The relative contribution of these components suggests that although additive variance is limited, dominance and possible epistatic interactions substantially influence phenotypic expression (Ceyhan et al. 2008, Ceyhan et al. 2014a). The parameters related to the heritability of genotypes for grain protein reveal that the narrow-sense heritability (*h2a*) is only 2%, which is low but essential for selection strategies. The narrow-sense heritability (2%) observed in this study is considerably lower than values reported in cowpea (0.64–0.99 for protein; Dalgıç et al. 2025) and common bean populations (Ceyhan et al. 2014b). This discrepancy may be attributed to the advanced generation (F_8), environmental variance, and potential genotype × environment interactions affecting protein accumulation in soybean. Low additive variance suggests that direct phenotypic selection may be inefficient, and parent-based selection strategies should be emphasized.

In contrast, considering overall variability through broad-sense heritability (*h2g*) shows 52% genetic variance, allowing the selection of promising lines for this attribute. The moderate broad-sense heritability indicates that total genetic variance, including dominance and epistatic components, remains substantial. This reinforces the importance of exploiting non-additive gene action through strategic crossing schemes rather than relying exclusively on within-line selection (Tekin and Ceyhan 2023, Tamüksek and Ceyhan 2024, Jou-Nteufa and Ceyhan 2024). The overall family heritability (*h2fam*) indicates that selection based on this parameter accounts for 24% of the genetic variation. The overall mean for crude protein was 36.15%, considering 95 control cultivars, 30 parent cultivars, and 170 elite lines. When considering these genetic gains via heritability for increasing grain protein, breeding programs need adequate, readily available analytical approaches, that primarily accounts for additive effects.

Diallel analysis for recombinant soybean genotypes in early generations provides an estimate of general combining ability (GCA), revealing additive genetic effects on the mean parental performance relative to the overall mean, and specific combining ability (SCA), indicating the deviation of the cross from parental performance, based on dominance and epistasis effects (Cruz et al. 2012, Oliveira et al. 2014). However, in F_8 generation lines considered elite in the breeding program, which exhibit high homozygosity, this approach helps identify the superior parents, position future crosses, and assess contributions, inheritances, and maternal effects responsible for cytoplasmic, mitochondrial, metabolic, and photosynthetic traits (Singh and Hadley 1972). In advanced generations such as F8, reduced segregating variance and increased allele fixation may limit detectable additive variance relative to early-generation diallel studies. Therefore, the interpretation of combining ability parameters in highly homozygous populations must consider the genetic background stabilization inherent to elite breeding materials. The great potential of these estimates in future generations lies in minimizing errors caused by heterozygote bias.

Estimates of the means (*BLUP*) for general combining ability were made for the 30 parent cultivars and for specific combining ability for 170 lines. To increase protein content in new soybean genotypes, the performance of control cultivars for grain protein content was evaluated (Figure 2), and these results can be incorporated into genetic improvement programs to obtain progenies with high protein content. The cultivars that presented protein contents higher than the mean specific combining ability (SCA) of 37% were M6410IPRO (44%), DM5958IPRO (43%), 95Y52RR (41%), FPS1859RR (40%), TMGManacáXTD (40%), C2570RR (40%), FPSJúpiterRR (38%), BMXTorqueI2x (38%), FPS2565IPRO (38%), TMG6123IPRO (38%) and SoyTech570I2x (38%). For general combining ability (GCA), the controls that exceeded 36% crude protein were GH2361IPRO, DM59IX61I2x, IPRBasalto, M6210IPRO, FPSSolimõesRR, SoyTech580I2x, IPRPé-Vermelho, NEO531I2x, 95R40IPRO, BRS525, NEO590I2x, NEO510IPRO, BMXLótusIPRO, C2560CE, and NEO620IPRO.

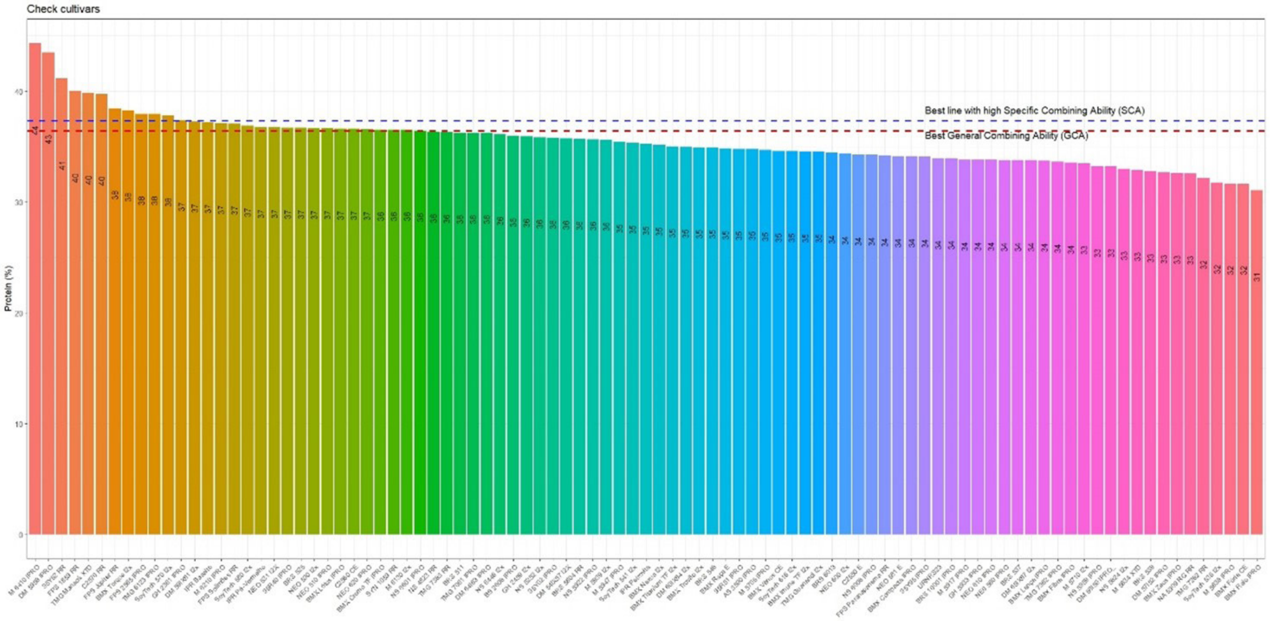

Figure 2. Ranked performance of 95 control cultivars for grain protein (%), and rows of mean performances of general (GCA) and specific (SCA) combining abilities.

The selection of superior parents for a given trait can be aided by analyzing general combining ability, in which the highest estimates (positive for an increase or negative for a decrease in the trait) indicate which parents are superior or inferior to the overall mean of the combinations (Miranda et al. 1996). From the classification (Table 3) and the graph (Figure 3), it can be observed that the parents NS5958RR, Maradona, and DM5.8BmxApoloRR showed the highest estimates and increases in grain protein, at 0.27%, 0.22%, and 0.20%, respectively. Despite low narrow-sense heritability, significant GCA effects for NS5958RR, Maradona, and DM5.8BmxApoloRR indicate the presence of favorable additive alleles. Similar findings were reported in common bean, where cultivars with positive GCA values significantly contributed to protein enhancement in hybrid populations (Ceyhan et al. 2025). This confirms that combining ability analysis remains a powerful tool for selecting superior parents, even under conditions of limited additive variance. Furthermore, the parents TMG7166RR, MonascarRR, FPSNetunoRR, NS5909RR, BmxPotênciaRR, BmxForçaRR, 4823RR, Rota54, TMG2757, M5710, 5601IPRO, DM6563IPRO, and 15B70IPRO showed positive genetic deviations for increased protein, but less than 0.1%.

GCA demonstrated estimates of reduced grain protein content for the parents FPSParanapanemaRR, Fundacep66RR, HOJacuiIPRO, FPSJúpiterRR, DM5.9BmxAlvoRR, HOPuricaIPRO, HOAmaboyIPRO, DM7.0BmxMagnaRR, TMG7062IPRO, FPSUranoRR, BmxAtivaRR, RoosCaminoRR, with DM6160IPRO and 6700RR standing out with genetic deviations of -0.19% and -0.20%, respectively. Through general combining ability, it was observed that some parents carry alleles that favor increases or decreases in protein content, which be fixed in the transgressive progenies of the program (Pimentel et al. 2013).

Table 3. Ranking of estimates of the genetic deviation of the general combining ability (GCA) of the parents for grain protein (%)

Rank	Parent	No. of parent	GCA	BLUP
1	NS5958RR	10	0.2725	36.43
2	Maradona	8	0.2209	36.40
3	DM5.8BmxApoloRR	3	0.1954	36.38
4	TMG7166RR	13	0.0989	36.35
5	MonascaRR	21	0.0905	36.33
6	FPSNetunoRR	5	0.0828	36.31
7	NS5909RR	12	0.0586	36.30
8	BmxPotênciaRR	4	0.0542	36.29
9	BmxForçaRR	11	0.0471	36.28
10	4823RR	9	0.0398	36.27
11	Rota54	26	0.0398	36.26
12	TMG2757	28	0.0255	36.26
13	M5710	15	0.0255	36.25
14	5601IPRO	25	0.0252	36.24
15	DM6563IPRO	14	0.0073	36.24
16	15B70IPRO	18	0.0006	36.23
17	FPSParanapanemaRR	22	-0.0194	36.22
18	Fundacep66RR	20	-0.0217	36.22
19	HOJacuiIPRO	19	-0.0243	36.21
20	FPSJúpiterRR	16	-0.0278	36.20
21	DM5.9BmxAlvoRR	27	-0.029	36.20
22	HOPuricaIPRO	17	-0.0764	36.20
23	HOAmaboyIPRO	31	-0.0764	36.19
24	DM7.0BmxMagnaRR	1	-0.0825	36.19
25	TMG7062IPRO	6	-0.1099	36.18
26	FPSUranoRR	23	-0.1121	36.18
27	BmxAtivaRR	24	-0.1251	36.17
28	RoosCaminoRR	2	-0.1315	36.17
29	DM6160IPRO	30	-0.1911	36.16
30	6700RR	7	-0.2075	36.15

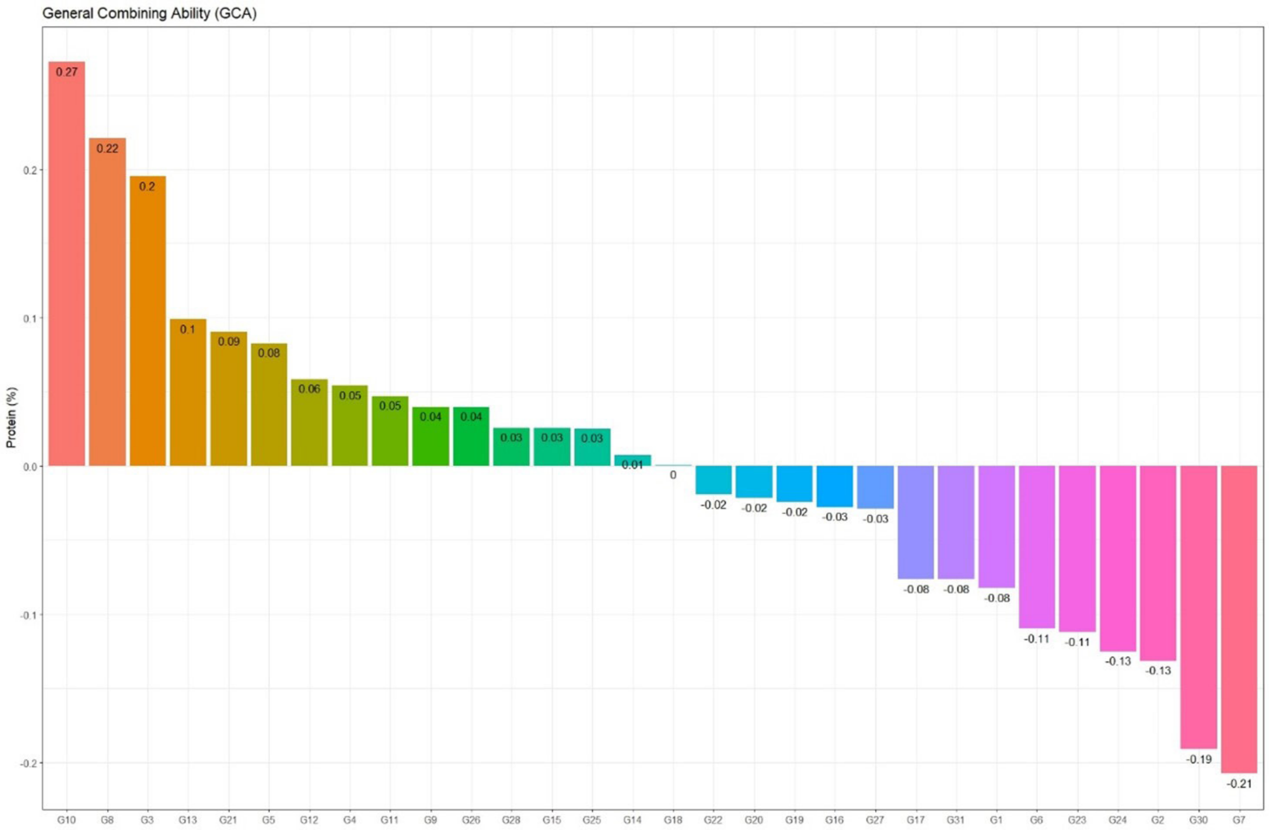

Figure 3. Estimates of genetic deviation from ordered general combining ability (GCA) of parents for grain protein.

Significant estimates of general combining ability were also obtained for grain weight per plant in a partial diallel with three parents (Zorzetto et al. 2008), and in the selection of soybean parents to enhance grain yield in the Brazilian Cerrado (Daronch et al. 2014). In the study by Teodoro et al. (2019), the general combining ability analysis using 11 parent cultivars and 28 hybrid combinations revealed strong additive effects for net photosynthesis, stomatal conductance, internal CO_2 concentration, and transpiration, with high estimates for TMG7062IPRO. Greater additive effects were reported in estimates of general combining ability by Bagatelli et al. (2020), Santos et al. (2025), and Sangiovo et al. (2025). These works aimed to select soybean genotypes based on main yield components and cycle reduction.

Estimates of specific combining ability (Figure 4) show that, out of 170 lines, 96 combinations presented an increase in soybean grain protein content compared to the overall mean of 37%. The best combinations for increasing protein were L77 (Maradona x Maradona) with 1.16%, followed by 0.71% through L87 (Maradona x NS5958RR) and 0.67% through L30 (Maradona x DM5.8BmxApoloRR). It is noteworthy that the genotypes NS5958RR, Maradona, and DM5.8BmxApoloRR, which showed the best combinations, also exhibited the highest general combining ability values, a desired result in diallel analyses for identifying the superior parents, indicating the relevant additive effects on protein content in soybean grains (Cruz et al. 2012).

Conversely, lower estimates of specific combining ability were obtained from L14 and L4, both from the cross Fundacep66RR x DM7.0 BmxMagnaRR, with a reduction of -1.16% and -1.15% in grain protein, as well as from L102 (6700RR x NS5958RR), which reduced -0.99%. It is noteworthy that these parents 6700RR, Fundacep66RR, and DM7.0BmxMagnaRR showed low general combining ability, indicating the effectiveness of diallel analysis. Low additive effects for the oil and soybean grain protein content are attributed to SCA and higher GCA in generations of high heterozygosity (Meena et al.

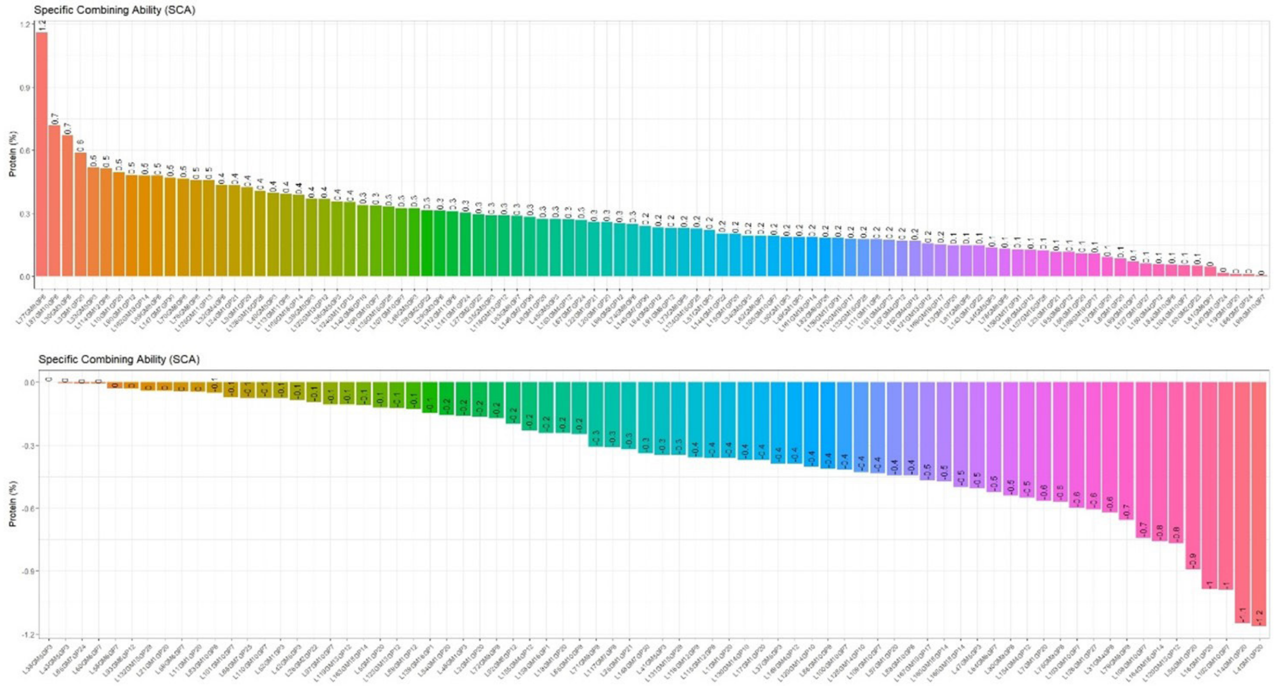

Figure 4. Barplot graph showing estimates of the genetic deviation from specific combining ability (SCA) of genotypes ordered for grain protein (%).

2024). In this scenario, it becomes evident that the choice of parents based on this approach for grain quality attributes should be prioritized in later breeding generations, in line with the premises presented in this work.

Specific combining ability (SCA) in advanced generations (F_8) of soybean consolidates the results due to the additive effects fixed in each line, complementarity of alleles and genes, as well as the correct positioning of the pollen-receiving and pollen-donating parents, due to the large maternal contribution to the genetic potential of the line (Pimentel et al. 2013, Carvalho et al. 2017). Relating the superior parent expressed by general combining ability, NS5958RR (0.27%), with the best combination via specific combining ability, this maternal parent was L87 (Maradona x NS5958RR), contributing 0.44% to the protein magnitude. The superiority of certain parent lines suggests potential effects of maternal inheritance. Maternal contributions have also been highlighted in reciprocal diallel analyses in pea and cowpea (Ceyhan and Kahraman 2013, Jou-Nteufa and Ceyhan 2024). Such effects may arise from interactions between the cytoplasmic genome or from maternal regulation of seed storage protein synthesis. In soybean, major seed storage proteins such as glycinin and β-conglycinin are synthesized under large maternal tissue influence during seed development, which may partially explain the maternal contribution detected in advanced-generation lines. Therefore, cytoplasmic–nuclear interactions should be considered in protein-oriented breeding strategies. The Maradona parent revealed better specific combining ability and maternal effect for the trait in question.

Studies by Singh and Hadley (1972) showed that maternal and cytoplasmic effects are crucial for the protein composition in soybean grains during early breeding generations. Similar trends were reported by Pucinelli (1992), who postulated that the soybean grain protein results from the successful combination of favorable genes with large additive effects. Maternal genetic effects in the F_1, F_2, and F_3 generations of soybean are confirmed for isoflavone content (Liang et al. 2007), fatty acids (Gilsinger et al. 2010), and tolerance to Asian soybean rust (Cruz et al. 2011). The results suggest that improving soybean grain protein in advanced generations should prioritize parental selection based on GCA estimates, complemented by evaluating superior SCA combinations and maternal contributions. Integrating combining ability analysis with high-throughput protein phenotyping platforms may accelerate genetic gain in soybean breeding programs.

CONCLUSION

Soybean grain protein in advanced generation (F_8) material is governed by a mixed genetic architecture, involving additive and non-additive gene effects. Although narrow-sense heritability was low (2%), indicating limited exploitable additive variance at this stage, moderate broad-sense heritability (52%) confirmed the presence of substantial total genetic variability.

General combining ability (GCA) analysis identified parents such as NS5958RR, Maradona, and DM5.8BmxApoloRR as superior for increasing grain protein. Superior specific combining ability (SCA), such as Maradona × NS5958RR, showed significant protein gains, demonstrating that strategic crossing schemes remains essential to maximize genetic progress. Maternal effects were evident in several superior combinations, suggesting the influence of cytoplasmic-nuclear interactions and maternal regulation of seed storage protein synthesis.

ACKNOWLEDGMENTS

Authors are thankful to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Nº: 306334/2024-0, and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the financial support.

CREDIT STATEMENT

GMW Bruinsma: Conceptualization; IR Carvalho: Data curation and methodology; CF Colet and ADC Lúcio: Formal analysis; JP Sangiovo: Investigation; JPD Roza: Project management.

DATA AVAILABILITY

The datasets generated and/or analyzed during the current research are available from the corresponding author upon reasonable request.

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