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Study of nature and magnitude of gene action in hybrid rice (*Oryza sativa* L.) through experiment of line x tester mating design

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Abstract

An experiment of line x tester mating design was conducted to study the nature and magnitude of gene action i.e. general combining ability (GCA) and specific combining ability (SCA) of 12 yield and yield contributing characters. The experiment was conducted with 10 rice genotypes and 25 F₁s at the experiment field of SAU during June 2013 to June 2014. Highly significant differences for genotypes and lines were observed which revealed the wide range of variability among the genotypes and lines studied. The SCA variances were found significant for most of the characters. Higher ratio of SCA and GCA variances indicating preponderance of non-additive gene actions in the inheritance of all the characters except filled spikelet per panicle. The contribution of either lines or testers was observed to be higher than that of the interactions of line x tester. Such results revealed the higher estimates of GCA variances i. e. additive gene action but in case of 1000 grain weight, days to flowering and grain yield per hill higher estimates of SCA variance i.e. non-additive gene action were found. Among the male parents, SAU509R and SAU525R were observed to be good general combiner for most of the character studied. Cross combinations, IR62829A x SAU509R, IR62829A x SAU523R, Jin23A x SAU507R, Dakshahi A x SAU525R and Luhagura A x SAU523R were observed to be good specific cross combinations for magnitude of gene action.

Keywords: Combining ability, gene action, Line × tester, male sterile line, hybrid rice.

1. Introduction

Success of any plant breeding program depends on the choice of appropriate genotypes as parents in the hybridization program. The combining ability studies of the parents provide information which helps in the selection of better parents for effective breeding.

Bangladesh is the fourth largest producer and consumer of rice in the world with an annual production ranging from 25 to 30 million tons. Rice occupies 77% of total cropped area. At present, rice alone constitutes about 92% of the total food grains produced annually in the country. It provides 75% of the calories and 55% of the proteins in the average daily diet of the people (Bhuiyan *et al.*, 2002) [2].

The increasing population pressure, the demand of cereal is increasing day by day. Rice is the most important staple food for about two-third of the world's population. It ranks second position by production in the world. More than 90% of the rice produced and consumed in Asia as a staple food, which provides 35-60% of the required calories. Rice is the most important cereal crop of different nation of the world including Bangladesh. Bangladesh ranks third among the rice producing countries of the world though yield is relatively low'. So, in Bangladesh, development of high yield potential variety is one of the ways to satisfy the future demand. Rice is considered as a major crop in Bangladesh as it constitutes 94.38% of the total food grain (rice & wheat) production of 26.7 million metric tons. Rice (*Oryza sativa* L.) is a self-pollinated cereal crop. It belongs to the family Gramineae (synonym-Poaceae) having chromosome number 2n=24 under the Order Cyperales and Class Monocotyledon. It is a perennial, bisexual cereal crop but cultivated as annual crop. The family Gramineae is rich in cereal crops like rice, wheat, maize, barley, sorghum, millet, etc. that are grown tropical and temperate countries over a wide range of soil and climatic condition.

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Hybrid rice offers an opportunity to boost the yield potential of rice. It has a yield advantage of 15-20% over conventional high yielding variety. The breeding of yield rich and quantitatively better rice varieties is not possible without prior knowledge of their genetic properties. The breeders therefore try, with the help of suitable quantitative genetic method to combine the desired properties of different varieties. Combining ability analysis is one of the powerful tools available to estimate the combining ability effects and aids in selecting the desirable parent and crosses for the exploration of heterosis. There is a bright scope to study the combining ability that is prerequisite for developing hybrid rice varieties with good quality in Bangladesh.

Combining ability is a powerful tool in identifying the best combiners that may be used in crosses either to exploit heterosis or to accumulate fixable genes and obtain desirable segregates. It will help to understand the genetic architecture of various characters that enable the breeder to design effective breeding plan for future up gradation of the existing materials. This information may also be useful to breeders for genetic improvement of the existing genotypes on the basis of the performance in various hybrid combinations. The line * tester analysis in rice is used principally to determine the general and specific combining ability of the quantitative properties.

Considering the above idea in mind the present investigation was undertaken with the following objectives:

1. To determine the general and specific combining ability of different male sterile and restorer lines.
2. To determine the specific combining ability of different male sterile and restorer lines.

2. Review of Literature

Rice (*Oryza sativa* L., $2n=2x=24$) is the world's most important staple food crop. It grows extensively in the humid tropical and subtropical regions of the world. China, India, Japan, Korea, South-Eastern Asia and adjacent islands of the Pacific account for about 90% of the world's rice production (Ram and Singh, 1998) [12]. Its importance as staple food emphasizes its improvement undoubtedly. It is grown all the year round in most of the areas of Bangladesh. Selection of appropriate rice genotype for hybridization is one of the important and prerequisite steps for the improvement of this crop. Hybridization program would be efficient and effective if the genotypes are selected on the basis of their genetic value i.e. additive and non-additive value. In rice, many workers studied the nature of combining ability of parents and hybrids for different growth character and panicle traits. Which are reviewed below-

Singh and Singh (1993) [22] reported the information on combining ability in rice derived from data on yield and 6 of its components in 8 genotypes and their F₁ hybrids from a dial lei cross without reciprocals grown during kharif 1989-90. ARC 10550 was a good general combiner for most traits and was recommended for breeding purposes.

Singh *et al.* (1993) [22] reported that days to heading, studied in an 8-parent half-diallel, appeared to be controlled by both additive and dominance gene effects in rice. Parents carried an abundance of dominant alleles operating towards lateness and displaying over dominance. Heritability in the narrow sense reflected preponderance of additive genetic variance. Per se performance was closely associated with general combining ability effects of parents and specific combining ability effects of crosses.

Kumar and Chandrapa (1994) [10] studied combining ability for yield and its component traits in rice. Variance due to GCA was higher than variance due to SCA for all the characters except productive tiller number, grain yield per panicle and grain yield per plant.

Singh *et al.* (1996) [23] studied a rice line x tester set obtained by crossing 3 CMS lines with 13 elite cultivars in rice. The 39 F₁s and their parents were evaluated for 7 quantitative characters. Additive genetic variance predominantly governed the expression of plant height and days to 50% flowering. Non-additive genetic variance mainly controlled yield, number of panicles/plant, number of grains/panicle and 1000-grain weight.

Ganesan and Rangaswamy (1997) [4] reported highly significant heterotic rice genotypes in 5 line x 8 tester analysis of combining ability for days to flowering, number of productive tillers, panicle length, spikelet fertility and yield/plant.

Geetha *et al.* (1998) [5] studied combining ability analysis for quantitative traits in rice. Information on combining ability was derived from data on 12 yield-related traits in parents and F₁ progeny of a dial lei cross involving six rice cultivars. Data indicated a predominance of additive gene action for all the characters studied. The lines IR50 and ADT4I were rated the best parents based on per se performance and GCA effects for all characters. The cross IR50/ADT4I was recommended for improvement of both grain yield and grain length/breadth ratio by recombination breeding.

Anand *et al.* (1999) [1] reported combining ability for 8 quantitative characters in rice under cold conditions using line * tester analysis involving 4 CMS lines and 10 cold tolerant testers. Parents and their 40 F₁ hybrids were grown at Keelagudalore in the Cumbum Valley, a cold-prone area of Tamil Nadu, during August-December 1994. Combining ability analysis indicated non-additive gene action governing all 8 characters. Pragathi A, BR203-70-B-2, IR31406-MS and IR50400-64-I-2-2-2 were good general combiners for grain yield. Four crosses were identified as the best, having high heterosis and high specific combining ability effects, and could be used in breeding for cold tolerance.

Yadav *et al.* (1999) [26] studied thirty hybrids generated from crossing three lines with 10 testers along with the parents for combining ability for days to 50% flowering, days to maturity, plant height, flag leaf length, panicle length, number of ear bearing tillers plant⁻¹, per cent pollen sterility, number of spikelet panicle⁻¹, number of filled spikelet panicle⁻¹, grain yield plant⁻¹ and harvest index.

The existence of both additive and non-additive type of gene action for various yield traits has also been reported by Montazeri *et al.* (2014) [11]. Predominance of non-additive gene action for grain yield and its components has been reported by Satyanarayana *et al.* (2000) [19], Rita and Motiramani (2005) [13], Singh *et al.* (2005) [20], Venkatesan *et al.* (2007) [25], Dalvi and Patel (2009) [3], Saidaiah *et al.* (2010) [21] and Hasan *et al.* (2013) [7]. However, Saravanan *et al.* (2006), Kumar *et al.* (2004) [8] and Thakare *et al.* (2013) [24] while working in the rice reported lower value of σ^2A than σ^2D for all the characters studied indicating the predominance of non-additive gene action.

3. Materials and Methods

The study was conducted at the experimental farm of Sher-e-Bangla Agricultural University (SAU), Dhaka, during June 2013 to December 2013 and December 2013 to June 2014.

The climate of the experimental site is subtropical characterized by heavy rainfall during May to September and scanty during the rest of the year. The record of air temperature, humidity and rainfall during the period of experiment were noted from the meteorological station of Sher-e-Bangla Agricultural University (SAU). The soil of the experimental plot belongs to the Madhupur tract, but modified to a great extent through addition of fine alluvial sand to make the soil texture loamy. The soil pH ranges from 6.45-6.55. Ten high yielding inbred lines were used as parents in crossing program among them five were cytoplasmic-genetic male sterile (CMS) lines IR75595A, IR62829A, Jin23A, Dakshahi A and Luhagura A, which were used as female parents. Rests five were restorer lines, SAU507R, SAU509R, SAU521R, SAU523R and SAU525R those were used as male parents.

Five cytoplasmic-genetic male sterile (CMS) rice genotypes (female) were crossed with five restorer genotypes (male) in one direction during October 2013 to November 2013. Thus 25 experimental hybrids were produced.

During flowering several rice hills of five CMS genotypes were picked up along with soil with Khurpi and was placed on pot previously filled with soil for facilitating emasculation in laboratory. Emasculation of panicle of CMS rice genotypes was done in evening and hand pollination was carried out in the following morning by dusting pollen from the panicle of restorer genotypes to the panicle of emasculated CMS genotypes. The emasculated and crossed panicles were tagged and bagged properly with white and brown paper respectively. Bagging after pollination continued for 3-4 days to avoid unwanted pollination. After maturity spikelet were collected from panicles, dried and kept in cool seed store for the study in following year. The seeds of five cytoplasmic male sterile rice genotype, five restorer parents and their 25 F₁s obtained from previous year were used as plant materials. Seeds of 25 F₁s were first decoded carefully by fingernails and unwounded seeds were soaked in Petri dishes on December 27, 2013. Germination was completed in a germinator. Two third portions of the 35 pots were filled up with mud. Upper portion of the pots was leveled by hand. Sprouted seeds were carefully placed on mud on January 01, 2014. Irrigation with 1-3 cm water levels was maintained until uprooting the seedling. Weeding and other intercultural operation and special care were taken during raising of seedling. The experimental plot was at a lower elevation with high water holding capacity, the land was prepared thoroughly by 3-4 times ploughing and cross ploughing followed by laddering to attain a good puddle.

The seedlings of 25 F₁s (hybrids) and 10 parents (5 lines and 5 testers) were grown in Randomized Completely Block (RCB) Design with 2 replications. Single row of 3 m each constituted the experimental unit. Treatment was distributed in the experimental unit through randomization by suitable computer software. Adequate soil fertility was ensured by applying additional quantities of Urea, TSP, MP, Gypsum, Zinc sulphate and one fourth of the total urea were applied in final land preparation. Rest of the Urea was applied in three installments, at 20 days after transplanting (DAT), 35 DAT and during heading stage (Julfikar, 1999) [6]. Healthy seedlings of 40 days old were transplanted in separate lines in the experimental field. The row to low spacing was 20 cm having plant spacing of 20 cm within the row. Single seedling per hill was transplanted.

3.1 Data collection

Data were collected from 10 hills of each genotype on individual plant basis.

3.1.1 Plant height: The height was measured in cm on the tallest panicle from ground level to the tip of the panicle.

3.1.2 Tillers per hill: The tiller number was measured during heading stage of rice.

3.1.3 Days to flowering: The data were recorded when first flowering occurs in panicle

3.1.4 Days to maturity: The data were recorded from soaking date of seed to maturity of 80% spikelet.

3.1.5 Panicles per hill: The panicle number was recorded during maturity stage of rice. Panicle weight: The weight was recorded in gram, of the whole sun dried panicle. Primary branches per panicle: the total number of primary branches of 10 panicles were measured and averaged by dividing by 10.

3.1.6 Filled spikelet per panicle: The total number of tilled spikelet of 10 panicles were measured and averaged by dividing 10.

3.1.7 Panicle length: The length was recorded in cm from first node of the rachis to tip of the top most spikelet.

3.1.8 Flag leaf length: The Hag leaf length was measured in cm after harvesting of rice from collar to nodal end of the leaf.

4. Results and Discussion

In the present investigation, ten diverse genotypes (5 CMS and 5 restorer lines) and their 25 crosses were studied. The results presented here provide some information on general combining ability (GCA) and specific combining (SCA) for yield and yield attributes. Mean square from the analysis of variances for genotypes, general combining ability and specific combining, ratio of SCA and GCA variances, proportional contribution of lines, tester, and interactions of line and tester to the total sum of square due to crosses, general combining ability and specific combining ability effects and mean values of different characters of parents and crosses are shown in Table 1 to 5. The results obtained are presented and discussed under the following headings.

The results are presented in Table 1 and 2. Character-wise results on above heads are presented and discussed below:

4.1 Plant height (cm)

The analysis of variances due to genotypes and lines showed highly significant differences indicating wide range of variability for this character. Highly significant specific combining ability variances were observed. Analysis of variance due to combining ability and ratio of SCA and GCA (3.44) indicating the preponderance of non-additive gene action (Table 1). Rogbell and Subbaraman (1997a) [15], Sreedhar *et al.* (1997) [18], Ramalingam *et al.* (1995) in rice also found the similar preponderance of non-additive gene action for this character.

The contribution of lines to the total sum of square due to crosses were higher than the interaction of line x tester that

indicated higher estimates for variances due to general combining ability (Table 2).

4.2 Tillers per hill

The analysis of variances due to genotypes, lines, testers and interactions of line and tester were highly significant indicating wide range of variability for this character. Yadav *et al.* (1999) [26] also found similar results for lines, testers and interactions of line x tester in rice. Highly significant specific combining ability variances were observed. Ganesen and Rangaswamy (1997) [4] found similar significant variances in rice for specific combining ability. Analysis of variance due to specific combining ability and ratio of SCA and GCA (9.16) indicating the preponderance of non-additive gene action (Table 1). These results were in agreement with the findings of Anand *et al.* (1999) [1] in rice.

The contribution of line and tester to the total sum of square due to crosses were higher than the interactions of line x tester that indicated higher estimates for variances due to general combining ability (Table 2).

4.3 Panicles per hill

The highly significant estimates of variances due to genotypes, line, tester and interaction of line and tester indicated wide range of variability for this character. Ganesen and Rangaswamy (1997) [4] also found significant variances due to genotypes in rice. Specific combining ability variances were highly significant and the ratio of SCA and GCA (6.63) was very high. These facts indicated the preponderance of non-additive gene action over additive gene action (Table 1). Singh *et al.* (1996) [23], Ramalingam *et al.* (1993) [16], Sreedhar and Kulkarni (1997) [18] in rice also observed similar results.

Table 1: Analysis of variance for combining ability for different characters in rice

Source of variation	Degrees Of freedom	Plant Height (cm)	Tillers per hill	Panicles per hill	Panicle Length (cm)	Primary branches per panicle	Filled spikelet per panicle
Replication	1	0.67	0.616	0.93	0.90	7.14	7.72
Genotype	34	279.89**	86.20**	73.80**	15.50**	3.02**	1437.14**
Line	4	740.68**	354.93**	339.56**	21.77**	13.82**	1603.65**
Tester	4	106.10	69.419**	53.60**	3.56*	1.63	577.86
Line x tester	16	82.11	46.041**	31.17**	0.82	0.47	337.60
Error	34	52.69	7.904	3.71	1.34	0.79	321.25
σ^2 gca	9	4.27	2.08	2.07	0.15	0.09	37.54
σ^2 sca	24	14.71**	19.07**	13.73**	0.26	0.16	8.15
σ^2 sca/ σ^2 gca		3.44	9.26	6.63	1.73	1.78	0.008

*Significant at 5% level of significance and ** Significant at 1% level of significance.

σ^2 gca = variance of general combining ability and

σ^2 sca = variance of specific combining ability

Table 1: (Cont'd)

Source of variation	Degrees Of freedom	Plant Height (cm)	Tillers per hill	Panicles per hill	Panicle Length (cm)	Primary branches per panicle	Filled spikelet per panicle
Replication	1	0.00	0.01	37.30	1.73	0.129	1.25
Genotype	34	0.71**	1.15**	22.61**	158.37**	105.67**	59.16**
Line	4	3.97**	1.03**	117.37**	188.47**	399.21**	109.52**
Tester	4	0.15*	0.43**	4.04	102.78**	42.81	55.91**
Line x tester	16	0.18**	0.31**	10.02	109.83**	97.64**	28.14**
Error	34	0.06	0.10	8.85	17.2	18.1	1.93
σ^2 gca	9	0.02	0.01	0.63	0.45	1.54	0.68
σ^2 sca	24	0.06**	0.17**	0.58	42.79**	12.36**	13.11**
σ^2 sca/ σ^2 gca		3.00	17.00	0.92	95.09	8.03	19.28

*Significant at 5% level of significance and ** Significant at 1% level of significance.

σ^2 gca = variance of general combining ability and

σ^2 sca = variance of specific combining ability

The contribution of lines to the total sum of square due to crosses were higher than that of the interactions of line x tester which indicated higher estimates for variances due to general combining ability (Table 2).

4.4 Panicle length (cm)

Highly significant estimates of variances of genotypes, lines and testers revealed wide range of variability for this character. Ganesen and Rangaswamy (1997) [4] in rice found similar results for this character. Specific and general combining ability variances were insignificant but the ratio of SCA and GCA (1.75) was high. Non-additive gene actions were predominating over additive gene action due to higher ratio of SCA and GCA variances (Table 1).

The contribution of lines to the total sum of square due to crosses were higher than that of the interactions of line x

tester which indicated higher estimates for variances due to general combining ability (Table 2).

4.5 Primary branches per panicle

The analysis of variances due to genotypes and line showed highly significant estimates indicating wide range of variability for this character concerned.

Specific and general combining ability variances were insignificant but the ratio of SCA and GCA (1.7) was high. Predominate non-additive gene actions over additive gene action due to higher ratio of SCA and GCA variances (Table 1) were observed.

Higher estimates of variances for general combining ability were observed due to the higher contribution of lines to the total sum of square due to crosses than that of the interaction of line x tester (Table 2).

Table 2: Proportional contribution (%) of lines, testers and their interactions to total variance in rice

Line/Tester/ LxT*	Panicle weight	1000 grain weight	Flag leaf length	Days to flowering	Days to maturity	Grain yield per hill
Due to line	63.02	71.68	65.57	63.02	79.84	75.99
Due to tester	9.03	19.07	10.35	9.03	9.42	7.19
Due to line x tester	27.95	0.27	24.08	27.95	10.75	16.81

* = Line x Tester

Table 2: (Cont'd)

Line/Tester/ LxT*	Panicle weight	1000 grain weight	Flag leaf length	Days to flowering	Days to maturity	Grain yield per hill
Due to line	82.10	38.20	72.68	25.80	47.95	39.40
Due to tester	18.72	15.87	2.50	14.07	5.14	20.11
Due to line x tester	14.70	45.93	24.82	60.14	46.91	40.49

* = Line x Tester

Filled spikelet per panicle

Highly significant analysis of variances due to genotypes and line indicated wide range of variability for this character. Specific and general combining ability variances were insignificant but the ratio of SCA and GCA (0.008) was lower than unity indicating additive gene action over non-additive gene action (Table 1).

The contribution of line to the total sum of square due to crosses were higher than the interactions of line x tester that indicated higher estimates for variances due to general combining ability (Table 2).

4.6 Panicle weight (g)

Wide range of variability among genotypes, lines, testers and interactions of line and tester were observed for this character due to highly significant and significant values. Specific combining ability variances were highly significant and the ratio of SCA and GCA (3) was very high. Non-additive gene action was predominant due to higher ratio of SCA and GCA variances (Table 1).

The contribution of either lines or tester to the total sum of square due to crosses were higher than that of the interactions of line x tester that indicated higher estimates for variances due to general combining ability (Table 2).

4.7 1000 grain weight (g)

Highly significant variances for genotypes, lines, testers and interactions of line and tester revealed wide range of variability for this character. Specific combining ability variance was highly significant and the ratio of SCA and GCA (17) was very high. These facts indicated the preponderance of non-additive gene action over additive gene action (Table 1). Similar results were observed by Sreedhar *et al.* (1997)^[18], Singh *et al.* (1996)^[23] in rice.

The contribution of either lines or tester to the total sum of square due to crosses were lower than that of the interactions of line x tester that indicated lower estimates for variances due to general combining ability (Table 2).

4.8 Flag leaf length (cm)

The analysis of variances due to genotypes and lines showed highly significant indicating wide range of variability among the genotypes for this character.

Insignificant general and specific combining ability was observed and the ratio of SCA and GCA (0.92) was lower than unity indicating preponderance of additive gene action over non-additive gene action (Table 1)

The contribution of lines to the total sum of square due to crosses were much higher than the interactions of line x tester indicating higher estimates of variances due to general combining ability (Table 2).

4.9 Days to flowering

Highly significant estimates of variances due to genotypes, lines, testers and interactions of line and tester revealed wide range of variability among the genotypes for this character concerned. Highly significant specific combining ability variances and the higher ratio of SCA and GCA (95.09) were observed. Such results indicated the preponderance of non-additive gene action over additive gene action (Table 1).

The contribution of either lines or tester to the total sum of square due to crosses were lower than that of the interactions of line x tester that indicated lower estimates for variances due to general combining ability (Table 2).

4.10 Days to maturity

Significant estimates of variances due to genotypes, lines and interactions of line and tester revealed wide range of variability among the genotypes for this character. Highly significant specific combining ability variances and the higher ratio of SCA and GCA (8.03) were also observed. Such results indicated the preponderance of non-additive gene action over additive gene action (Table 1). Similar preponderance of non-additive gene action was reported by Ramalingam *et al.* (1993)^[16] and Rogbell and Subbaraman in rice.

The contribution of lines to the total sum of square due to crosses were higher than that of the interactions of line x tester that indicated higher estimates for variances due to general combining ability (Table 2).

4.11 Grain yield per hill (g)

Highly significant estimates of variances due to genotypes, lines, testers and interaction of line and tester revealed wide range of variability among the genotypes for this character. Highly significant specific combining ability variance was observed for this trait. The higher ratio of SCA and GCA (19.28) indicated the preponderance of non-additive gene action (Table 1). Rogbell *et al.* (1998), Salam *et al.* (1996)^[17], Singh *et al.* (1996)^[23] in rice observed similar results.

The contribution of either lines or tester to the total sum of square due to crosses were lower than that of the interactions of line x tester that indicated lower estimates for variances due to general combining ability (Table 2).

5. Conclusion

The mean square from the combining ability analysis of the variances revealed that there was non-significant difference in general combining ability among the parents for all the characters studied. But highly significant difference due to specific combining ability was observed for plant height, tillers per hill, panicles per hill, panicle weight, 1000 grain weight, days to flowering, days to maturity and grain yield per hill and insignificant due to specific combining ability variance were observed for panicle length, primary branches per panicle, filled spikelet per panicle and flag leaf length. Good specific crosses were obtained from high x high, high x low, low x high and low x low general combiner indicating predominance of non-additive gene action. In few cases high x high general combiner parents produced inferior cross combinations. The ratio of SCA and GCA variances was very high for all the characters except filled spikelet per panicle and flag leaf length studied revealed the preponderance of non-additive gene action over the additive gene action. The contribution of either lines or testers to the total sum square due to crosses was higher than that of interaction of line x tester for all characters except 1000 grain weight, days to flowering and grain yield per hill. The smaller contribution of interaction of the line x tester than either lines or testers indicating higher estimates of variances due to general combining ability and higher contribution of interaction of line x tester than either lines or testers indicating higher estimates of variances due to specific combining ability. On the basis of the results of the present experiment the following conclusions could be made:

Selection of the male and female parents on the basis of GCA effects would be effective for most of the 12 characters. The CMS line IR75595A and IR62829A could be used in production of diversified CMS lines as well as hybrid seed production with SAU509R and SAU525R good general combiner male parents.

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