

The agronomic characteristics and combining ability of glutinous Thermo-sensitive Genic Male Sterile rice (*Oryza sativa*) lines

Nguyen Thanh Tung¹, Dang Thi Huong², Tran Thi Huyen¹, Le Thi Tuyet Cham², Chu Duc Ha³, Nguyen Van Loc², Tran Van Quang^{2*}

¹Crops Research and Development of Institute, Vietnam National University of Agriculture, Trau Quy, Gia Lam, Hanoi, 131000, Vietnam

²Faculty of Agronomy, Vietnam National University of Agriculture, Trau Quy, Gia Lam, Hanoi, 131000, Vietnam

³Faculty of Agricultural Technology, University of Engineering and Technology, Vietnam National University Hanoi, Xuan Thuy, Cau Giay, Hanoi, 122300, Vietnam

Corresponding author: tvquang@vnua.edu.vn

Submitted:
29/08/2023

Revised:
10/01/2024

Accepted:
02/04/2024

Abstract: Glutinous rice (*Oryza sativa*), despite its unique culinary qualities, typically yields less than other rice varieties, posing agricultural and economic challenges. The construction of a glutinous thermo-sensitive genic male sterile (TGMS) rice variety could indeed be a way to improve the yield and cultivation efficiency of glutinous rice. Among them, the two-line system operates by crossing a TGMS line, which is sterile at high temperatures but fertile at lower temperatures, with a normal fertile line. In this study, we conducted to access morphological characteristics and combining ability of some glutinous TGMS lines. The parental lines were characterized on 13 agronomic traits including growth duration, plant height, flag leaf width, flag leaf length, panicle length, rachis length, number of primary branches, number of secondary branches, number of panicles per plant, percentage of filled grains, P1000 seeds, grain yield, and amylose content. The obtained results revealed significant variations in growth traits, yield, and amylose content of studied rice lines. Hierarchical clustering based on principal component analysis of rice lines on all measured traits showed three major clusters with high genetic diversity. Furthermore, fifteen glutinous TGMS rice lines and three testers were used in a Line x Tester mating system to produce 45 hybrids. The 45 hybrids were evaluated on 6 traits related to grain yield and morphology in a randomized complete block design experiment with three replications. A line x tester analysis was conducted to estimate the combining ability, genetic variance, and the contribution of parental lines to genetic variation in hybrids. These findings were valuable for rice breeders to orient the strategy for breeding of hybrid glutinous rice varieties with high efficiency.

Keywords: Combining ability analysis, GCA, SCA, glutinous TGMS rice.

Introduction

Glutinous rice cultivated throughout in Asia and which is widely used in various staple foods (Zhang et al. 2021). As people's living standards improve, the market demand for glutinous rice, especially high-quality glutinous rice, continues to increase year by year (Han et al, 2019). Currently, conventional rice remains the primary glutinous rice in production, yet it faces significant issues with low yield and poor resistance (Deng et al. 1992). In recent years, some hybrid glutinous rice has been approved, which overcomes, to some extent, the problem of low yield, but the problem of poor quality is prominent. Breeding new glutinous rice varieties with good quality and high yield are effective ways to solve the market demand in the future.

In comparison to the three-line system, the two-line system has a significant advantage in terms of labor and time savings, grain quality, crop yields, breeding efficiency, and economic benefits due to its widespread use of restorers (Luo et al., 2013). Thermo-sensitive genic male sterility (TGMS) in rice represents a key advancement in hybrid rice breeding, providing a unique mechanism to facilitate hybrid seed production without the need for

manual emasculation (Ding et al, 2012; Luo et al, 2013). TGMS lines exhibit male sterility at certain elevated temperatures but regain fertility at lower temperatures (Ding et al., 2012; Luo et al., 2013). This system is characterized by the expression of specific genes that are sensitive to temperature changes, leading to the induction of male sterility under certain thermal conditions. Particularly, the *tms5* gene was found to exhibit a point mutation results in the production of a malfunctioning RNA, inducing male sterility in response to higher temperatures. The interaction between these genetic factors and environmental stimuli, particularly temperature, is crucial in determining the expression of the TGMS trait. Elevated temperatures can trigger male sterility in TGMS plants, while cooler conditions may reverse this effect, reinstating male fertility. The genetic underpinning of this phenomenon often involves the disruption of pollen or anther development, manifesting through various pathways such as compromised pollen wall formation, aberrant meiotic processes, or the cessation of microspore development. The development and utilization of TGMS

Table 1. Plant growth and morphological characteristics of glutinous TGMS rice lines.

Line	Growth duration (days)	Plant height (cm)	Flag leaf length (cm)	Flag leaf width (cm)	Panicle length (cm)	Rachis length (cm)	Number of primary branches	Number of secondary branches
TG-N1	139	76.3	34.9	1.8	23.3	2.1	10.6	35.4
TG-N2	141	75.4	37.9	1.7	25.4	3.0	10.8	34.8
TG-N5	140	80.9	30.3	2.0	26.9	-0.5	9.8	32.6
TG-N6	140	84.0	37.5	1.7	25.3	-1.4	9.9	36.2
TG-N9	140	80.2	35.1	1.9	23.6	1.8	10.3	35.4
TG-N10	140	79.2	34.2	2.1	25.1	1.2	9.7	33.2
TG-N12	141	77.4	34.1	1.8	23.6	2.7	10.7	34.2
TG-N15	138	83.7	36.8	1.9	23.9	1.6	10.5	36.0
TG-N18	140	78.6	32.3	1.7	25.2	-1.5	9.8	30.8
TG-N20	141	75.4	37.7	2.0	23.8	0.4	9.8	32.6
TG-N24	138	75.7	35.1	1.8	25.7	1.8	10.7	35.8
TG-N25	139	77.5	33.2	1.8	24.6	2.2	10.3	36.0
TG-N28	139	81.6	36.3	1.9	24.7	3.6	10.6	37.0
TG-N29	141	79.0	39.3	1.7	24.2	2.9	10.8	37.2
TG-N30	139	74.5	34.5	1.8	24.8	1.9	10.6	36.2
E15	140	86.7	36.5	1.6	23.3	2.7	9.7	31.4

Note: The negative value in rachis length indicates that it has not emerged from the flag leaf sheath.

lines have been instrumental in the large-scale production of hybrid rice, as they allow for natural outcrossing with selected lines during the sterile phase, followed by seed set in the fertile phase (Tran et al., 2021). Thus, the construction of new TGMS rice lines is required to ensure the long-term viability of rice production. Although rice hybrid breeding by using TGMS lines has been remarkably successful in past decades, however, a few hybrid glutinous rice varieties have been released. It could be explained that the glutinous attribute is predominantly controlled by a single gene, but its expression can be altered by other genetic factors, while the breeders also keep other desired traits like yield, disease resistance, and stress tolerance. In this study, we reported a comprehensive analysis of agronomic characteristics and assessed combining ability of glutinous of the glutinous TGMS rice lines.

Results

Plant growth and morphological characteristics of glutinous TGMS rice lines

The evaluation results for the agronomic traits of the thermo-sensitive genic male sterility (TGMS) rice lines in the spring cropping season of 2022 are presented in Table 1. The growth duration of these lines ranged from 138 to 141 days. Overall, the TGMS glutinous rice lines exhibited relatively consistent growth durations, comparable to the growth duration of the control variety E15.

The final plant height of the TGMS glutinous rice lines showed a wide range, varying from 74.5cm to 80.9cm, whereas the control variety E15 had a final plant height of 86.6cm.

Differences in flag leaf length were observed among the studied lines, ranging from 30.3 to 37.9 cm. Flag leaf width ranged from 1.6 to 2.1 cm. The TG-N10 line was investigated with the widest flag leaves at 2.1 cm, while the control variety E15 had the narrowest flag leaves at 1.6 cm. Panicle length in the glutinous TGMS rice lines varied from 23.3 to 26.9 cm. The TG-N1 line had a panicle length equivalent to the control variety E15 (23.3 cm) and also had the shortest panicle length. On the other hand, the TG-N5 line displayed the longest panicle length at 26.9 cm.

Most of the glutinous TGMS rice lines exhibited complete exertion, with only 3 lines showing non-exsertion with negative (-) panicle rachis lengths, which were the TG-N5,

TG-N6, and TG-N18 lines. The TG-N28 line had the most exertion, with a rachis length of 3.6 cm.

The lines exhibited a range of primary branches, varying from 9.7 to 10.8. The TG-N2 and TG-N29 lines had the highest number of primary branches at 10.8, whereas the TG-N10 line, along with the control variety E15, had the lowest number of primary branches at 9.7. The TG-N29 line also recorded the highest number of secondary branches at 37.2. In contrast, the TG-N18 line exhibited smaller panicles, evident from the lowest number of secondary branches at 30.8. The control variety E15 had a secondary branch count of 31.4.

Yield and yield components of glutinous TGMS rice lines

The number of panicles per plant in the glutinous TGMS rice lines ranged from 5.7 to 7.0 panicles. The number of grains per panicle among the lines varied from 143.4 to 169.7 grains. All TGMS rice lines had a higher number of grains per panicle compared to the E15 control variety (Table 2).

The TG-N9 line had the highest number of filled grains per panicle at 149.3. Following closely, the TG-N12 and TG-N28 lines also exhibited notable counts of filled grains (>140). In contrast, the control variety E15 had 124.6 filled grains per panicle (Table 2).

Almost all lines had filled grain percentages above 85%, with the TG-N9 line achieving the highest percentage at 91.7%, except for TG-N6, TG-N10, TG-N18, and TG-N24, which showed a lower percentages of filled grains (82-83%), falling below the E15 control variety (86.9%)(Table 2)..

Overall, the glutinous TGMS rice lines demonstrated larger grain sizes, with a 1000-grain weight ranging from 26.2 to 27.1g. The control variety E15 had a 1000-grain weight of 24.0g.

The individual grain yield of glutinous TGMS lines ranged from 16.1 to 23.1 g, the control variety E15 had an individual grain yield of 18.1 g. Notably, the TG-N9 line achieved the highest individual grain yield at 23.1 g (Table 2)..

The amylose content analysis results for the glutinous TGMS rice lines revealed low amylose content ranging from 0.7% to 3.2%. In contrast, the control variety E15 had an amylose content of 15.8%(Table 2).

Table 2. Yield and yield components of glutinous TGMS rice lines.

STT	Line	Number of panicles/plant	Number of grains/panicle	Number of filled grain	Proportion of filled grain (%)	1000-grain weight (g)	Individual grain yield (g)	Amylose content (%)
1	TG-N1	6.1	155.3	137.2	88.3	26.9	20.5	2.1
2	TG-N2	6.3	164.4	138.7	84.4	26.6	21.2	1.8
3	TG-N5	5.6	150.8	128.6	85.3	26.3	16.7	1.4
4	TG-N6	5.7	146.6	121.5	82.9	26.2	16.1	3.2
5	TG-N9	6.5	162.8	149.3	91.7	26.9	23.1	2.3
6	TG-N10	5.9	152.8	125.4	82.1	26.4	19.5	2.6
7	TG-N12	6.9	163.6	143.6	87.8	27.0	22.8	1.5
8	TG-N15	6.8	162.2	140.8	86.8	26.8	21.7	3.0
9	TG-N18	5.7	145.1	119.7	82.5	26.5	16.2	2.5
10	TG-N20	6.3	156.5	139.2	88.9	26.6	20.3	2.6
11	TG-N24	6.7	169.7	139.6	82.3	27.1	21.3	1.2
12	TG-N25	6.0	160.6	142.5	88.7	26.7	20.8	0.7
13	TG-N28	6.6	167.3	144.2	86.2	27.0	22.7	2.0
14	TG-N29	7.0	158.2	140.1	88.6	26.9	22.4	1.9
15	TG-N30	6.5	154.7	137.4	88.8	26.7	21.8	1.6
16	E15	6.4	143.4	124.6	86.9	24.0	18.1	15.8

Table 3. Analysis of general combining ability of parental lines for 6 investigated characteristics

Line	Plant height (cm)	Number of grains/panicle	Number of filled grain	Proportion of filled grain (%)	1000-grains weight (g)	Grain yield (g)
Female lines						
TG-N1	2.23*	0.41	5.44	1.24	0.07	0.07
TG-N2	1.73	0.30	4.46	1.61	-0.39*	0.34
TG-N5	3.69*	-0.95*	-16.27*	-0.17	-0.47*	-2.67*
TG-N6	-0.39	-0.75*	-16.10*	-1.45	-0.43*	-2.94**
TG-N9	3.35*	0.56	5.52	4.42*	0.36*	1.24
TG-N10	-1.23	-0.74*	-15.89*	-2.64*	-0.14	-0.98
TG-N12	-2.11	0.62*	3.74	1.09	0.45*	2.00*
TG-N15	5.00**	0.55	7.02	-0.90	0.16	0.46
TG-N18	-0.53	-0.85*	-4.96	-3.90*	-0.26	-1.38
TG-N20	-2.61*	-0.29	-0.48	2.82*	-0.47*	0.67
TG-N24	-4.13*	0.86*	11.25*	-4.95**	0.41*	1.31
TG-N25	0.55	-0.55	2.27	1.32	0.16	0.56
TG-N28	3.15*	0.41	13.86*	-0.11	0.56*	1.86*
TG-N29	-3.01*	0.58	3.32	2.53*	0.03	-0.07
TG-N30	-5.69**	-0.13	-3.18	-0.89	-0.03	-0.47
Male lines (Tester)						
N87	-0.16	-0.06*	7.72	-0.75	0.19	1.02
N97	-2.35*	0.03	6.79	-0.58	0.10	0.45
Nep A Sao	2.51*	0.04	-14.51*	1.33*	-0.30*	-1.48*
SE_{male line}	2.23	0.61	9.27	2.47	0.34	1.43
SE_{Tester}	1.99	0.05	10.27	0.94	0.21	1.07

SE_{L × T}: standard error; *, and **: significant at $P \leq 0.1$, $P \leq 0.05$, and $P \leq 0.01$, respectively.

Genetic diversity in glutinous TGMS rice lines

Hierarchical clustering based on principal component analysis of glutinous TGMS rice lines (Figure 1) on all measured traits showed three major clusters presented high genetic diversity of glutinous TGMS rice lines. The first cluster included TG-N24, TG-N29, TG-N28, TG-N2, TG-N15, TG-N20, TG-N9, TG-N12, TG-N1, TG-N25, and TG-N39. The second cluster had only E15. The third cluster included TG-N6, TG-N10, TG-N18, and TG-N5 (Figure 2).

Analysis of combining ability for glutinous TGMS rice lines

General combining ability

The evaluation results for the general combining ability of different lines (L) and tester varieties (T) are presented in table 3. For plant height, female lines TG-N20, TG-N24, TG-

N29, TG-N30, and male N97 line showed low combining ability. Number of grains/ panicle, female lines TG-N12 and TG-N24 exhibited high combining (with respective values of 0.62* and 0.86* in GGA). For number of filled grain, the obtained results indicates that female lines TG-N24 and TG-N28 have high combined ability (with GGA values of 11.25* and 13.86*). For proportion of filled grain (%), female lines including TG-N9, TG-20, TG-N29, and tester line Nep A Sao demonstrate high combined ability (with values of 4.42*, 2.82*, 2.53*, and 1.33*). In terms of 1000-grains weight (g), Table 3 reveals that female lines TG-N9 (0.36*), TG-N12 (0.45*), TG-N24 (0.41*), and TG-N28 (0.56*) have high values. For individual grain yield, the results suggested that female lines TG-N12 and TG-N28 possess high combined ability (with values of 2.00** and 1.86*).

Table 4. Specific combining ability for plant height.

Line	Tester		
	N87	N97	Nep A Sao
TG-N1	-0.82	0.14	0.68
TG-N2	-3.05**	1.77*	1.28
TG-N5	0.53	-0.42	-0.11
TG-N6	3.24**	2.46*	-5.70**
TG-N9	0.79	-0.78	-0.01
TG-N10	0.42	0.74	-1.15
TG-N12	-0.24	-0.25	0.49
TG-N15	-0.78	0.67	0.11
TG-N18	-1.12	0.11	1.01
TG-N20	1.36	-0.65	-0.71
TG-N24	0.45	-0.83	0.38
TG-N25	-0.91	-0.62	1.52*
TG-N28	0.46	-0.98	0.52
TG-N29	0.66	-1.25	0.59
TG-N30	-0.99	-0.11	1.10
SE_{L x T}	1.37		

SE_{L x T}: standard error for specific combining ability between female line and tester; *, and **: significant at $P \leq 0.1$, $P \leq 0.05$, and $P \leq 0.01$, respectively.

Specific combining ability.

In Table 4, it is shown that there are 24 positive SCA (Specific Combining Ability) values and 21 negative SCA values, indicating the role of non-additive genes in controlling plant height. There are 2 combinations with negative SCA values, TG-N2/N87 (-3.05**) and TG-N6/Nep A Sao (-5.70*), which are significant in the search for hybrid rice combinations with shorter plant height.

The results of the analysis of individual combining ability for yield of the hybrid combinations, as shown in Table 5, reveal that there are 22 positive SCA values, accounting for 48.89%, and 23 negative SCA values, demonstrating the role of additive genes in controlling individual yield. The effectiveness of additive genes in controlling this trait is evaluated. From Table 8, we can see that 7 combinations with positive SCA values are significant, with the highest being the TG-N30/Nep A Sao combination (1.39**), and the lowest being the TG-N28/N87 combination (0.59**). These initial assessments are important in the search for promising hybrid combinations in terms of yield.

Discussion

Rice varieties with different genetic structure are a good source of materials to guide in breeding and selecting new rice varieties. The evaluation of genetic diversity can be based on phenotype (agro-morphological trait) or genotype (using molecular markers), in which phenotypes are expressed through the interaction between genotypes and the environment. Agro-morphological study of collected rice landraces deals on the plant growth structure characterization (morphological) and yield component of an important work. Accordingly, the practice of agro-morphological characterization is the easiest method among the formal and standardized approaches of measuring crop genetic diversity (Watson and Eyzaguirre, 2002). Thermo-sensitive genic male sterile (TGMS) lines have been widely used for two-line hybrid rice. Diversity in TGMS rice lines is crucial for the success of rice breeding programs. The diversity among TGMS lines allows breeders to develop a wide range of hybrid rice varieties with different characteristics. In this study, Hierarchical clustering based on principal component analysis of glutinous TGMS rice lines on all measured traits showed three major clusters presented high genetic diversity of glutinous TGMS rice lines. The findings will serve

conservation, provide information on genetic resources and exploit glutinous TGMS rice varieties with high yield and good quality.

The general combining ability (GCA) of an inbred refers to its average performance across a series of hybrid combinations. The GCA effects of the parental lines are instrumental in identifying suitable parental candidates (Cheng et al., 2019). The most promising TGMS lines developed within high combining ability backgrounds could be used for further assessment and validation of their general combining ability. Combining ability serves as a valuable indirect criterion for parent selection. However, to our knowledge, no study on the combining ability of glutinous rice using TGMS lines has been reported. In this study, GCA for six important traits PH, NoG, NoFG, PoFG, P1000 seeds, and GY were investigated. The results suggested some glutinous TGMS lines with high combined ability were excellent parents with greater GCA.

Screening hybrid rice combinations with SCA is able to breed strong superiority combinations with practical values (Gaballah et al., 2022). In this study, the research results indicate the specific combining ability of Glutinous TGMS rice lines with different tester plants on PH and GY. Specifically, in terms of GY, there were 7 combinations with high SCA, with the highest being the hybrid combination TG-N30/Nep A Sao, followed by TG-N24/N97, TG-N9/N87, TG-N12/N87, TG-N18/Nep A Sao, TG-N29/N97, and TG-N28/N87. The plant height traits of the combinations TG-N2/N87 and TG-N6/Nep A Sao exhibited the lowest specific combining ability. Notably, lines TG-N12, TG-N24, and TG-N28 demonstrated both high GCA and SCA. These findings provided a good source of material for the glutinous rice breeding program.

Materials and methods

Plant materials and cultivation

The experimental materials in this study comprised of 16 rice lines including 15 female (Thermo-Sensitive Genic Sterile - TGMS) rice lines and one line E15 as control variety, and their 45 hybrids derived from a Line x Tester (15x3) mating system. Fifteen TGMS lines were selected and stored at Crops Research and Development Institute (CRDI) of the Vietnam National University of Agriculture, Hanoi, Vietnam. E15 was Selected from a hybrid 135S x "Hoa sũa"; in which, 135S is a TGMS lines and "Hoa sũa" is a pure line imported from USA. Testers were "N87", "N97" and "Nép A Sao". "N87" was selected from the combination of Yunshin//I.316/IR26. "N97" was derived from the combination of "N87" and "N415". The rice variety "Nép A sũa" was developed by ThaiBinh Seed Company. All experiments were conducted at CRDI. In the first experiment, 16 rice lines was arranged sequentially without repeating in the Spring Cropping Season of 2022. For the second experiment, the 18-day old seedlings of hybrids and parents were transplanted in a randomized complete block design (RCBD) with three replications in the Spring Cropping Season of 2023. Each experimental plot area was 15 m²; the growing density was 40 plants /m². The agricultural practices applied for rice on the open field, including field preparation, fertilization, irrigation, pest and diseases managements were followed in accordance with National Technical Regulation on Testing for Value of Cultivation and Use of rice varieties (QCVN 01-55: 2011/BNNPTNT) issued by Ministry of Agriculture and Rural Development of Vietnam (MARD, 2011).

Table 5. Specific combining ability for grain yield.

Male lines	Tester lines		
	N87	N97	Nep A Sao
TG-N1	-0.02	-0.22	0.24
TG-N2	0.04	0.05	-0.09
TG-N5	-0.15	0.06	0.09
TG-N6	-0.31	-0.14	0.45
TG-N9	1.24**	-0.42	-0.82*
TG-N10	0.56	-0.63*	0.06
TG-N12	0.89*	-0.24	-0.65
TG-N15	0.22	-0.27	0.05
TG-N18	-0.90*	0.04	0.86*
TG-N20	0.08	-0.05	-0.02
TG-N24	-0.29	1.35**	-1.06
TG-N25	-0.35	0.13	0.22
TG-N28	0.59*	0.06	-0.65*
TG-N29	-0.58*	0.66*	-0.08
TG-N30	-1.01*	-0.37	1.39**
SE_{L x T}	0.57		

SE_{L x T}: standard error for specific combining ability between female line and tester; *, and **: significant at $P \leq 0.1$, $P \leq 0.05$, and $P \leq 0.01$, respectively.

Data collection

Agro-morphological traits

The agro-morphological traits were assessed according to the rice genetic resource evaluation criteria of the International Rice Research Institute (2013). Assessment of agro-morphological traits including growth duration, plant height, flag leaf width, flag leaf length, panicle length, rachis length, number of primary braches, number of secondary braches, number of panicles per plant, percentage of filled grains, P1000 seeds, grain yield and amylose content were conducted in this study. Growth duration was number of days from sowing to grain ripening (85% of grain on panicle are mature) at growth stage 9. Plant height was measured from soil surface to tip of the tallest panicle at growth stage 7- 9. The flag leaf width was measured from margin to margin at the middle portion of the leaf blade. The flag leaf length (cm) was measured from base to tip of the fully expanded leaf blade. The panicle length was measured from the base of the rachis to the tip of the top panicle branch and calculating the average length for each plant. Rachis length was measured as the distance in cm between the top and the bottom node of the rachis. The number of primary braches, number of secondary braches was counted on all panicles of 10 random plants. The number of panicles per plant was counted by the average number of panicles containing filled grain on 10 random plants. The percentage of filled grains was measured by the average proportion of filled grain on all panicles of 10 random plants. P1000 seeds was measured in grams of 1000 well- developed whole grain at growth stage 9, dried to 13% moisture content, using precision balance, three samples per line/hybrid. The individual grain yield was measured by wieghting 13% moisture content-grains of 10 random plants. The amylose content (AC) is usually measured by an iodine colorimetric method using the starch as a sample.

Data analysis

The statistical description was conducted for the measured traits in the first experiment. The data analysis was conducted on the "Variance analysis LINE x TESTER Ver. 2.0" software following the Line x Tester analysis method for the second experiment.

Combining ability analysis: The contribution of combining ability effect on the morphological value was based on the report of Tran et al. (2021):

$$Y_{ijk} = \mu + g_i + g_j + S_{ij} + e_{ijk}$$

In which, Y_{ijk} : value of hybrid between i^{th} line and j^{th} tester in k^{th} replication; μ : the average value of all hybrids in all replication (the general mean value); g_i : general combining ability (GCA) of i^{th} line; g_j : general combining ability (GCA) of j^{th} tester; S_{ij} : specific combining ability (SCA) between i^{th} line and j^{th} tester; e_{ijk} : error (environmental and replication effects to the $(ijk)^{\text{th}}$ individual).

The combining ability data was estimated by the following formulas:

$$\text{GCA of } i^{\text{th}} \text{ line: } g_i = \frac{Y_{i.}}{tr} - \mu$$

$$\text{GCA of } j^{\text{th}} \text{ tester: } g_j = \frac{Y_{.j}}{tr} - \mu$$

$$\text{SCA between } i^{\text{th}} \text{ line and } j^{\text{th}} \text{ tester: } S_{ij} = \frac{Y_{ij}}{r} - g_i - g_j - \mu$$

Whereby $Y_{i.}$: sum value of hybrids between i^{th} line and all testers; $Y_{.j}$: sum value of hybrids between j^{th} tester and all lines; Y_{ij} : sum value of hybrids between i^{th} line and j^{th} tester in all replications; g_i : general combining ability (GCA) of i^{th} line; g_j : general combining ability (GCA) of j^{th} tester; μ : the average value of all hybrids in all replication (the general mean value); t : the number of testers; l : the number of lines; r : the number of replication.

Ethics approval: Not applicable.

Declaration of interests: The authors declare that they have no known competing financial interests of personal relationships that could have appeared to influence the work reported in this paper.

Funding: This work was supported by grants of Vietnam National University of Agriculture (T2022-01-11TĐ)

Declaration of interests: The authors declare that they have no known competing financial interests of personal relationships that could have appeared to influence the work reported in this paper.

References

- Chen J, Zhou H, Xie W, Xia D, Gao G, Zhang Q, Wang G, Lian X, Xiao J, He Y (2019) Genome-wide association analyses reveal the genetic basis of combining ability in rice. *Plant Biotechnology Journal*. 17(11): 2211-2222.
- Deng XL (1992) Selection of two-line hybrid glutinous rice. *Journal of Hybrid Rice*. 7: 33-34.
- Ding J, Lu Q, Ouyang Y, Mao H, Zhang P, Yao J, Xu C, Li X, Xiao J, Zhang Q (2012) A long noncoding RNA regulates photoperiod-sensitive male sterility, an essential component of hybrid rice. *Proceedings of the National Academy of Sciences of the United States of America*. 109: 2654-2659.
- Gaballah MM, Attia KA, Ghoneim AM, Khan N, El-Ezz AF, Yang B, Xiao L, Ibrahim EI, Al-Doss AA (2022) Assessment of genetic parameters and gene action associated with heterosis for enhancing yield characters in novel hybrid rice parental lines. *Plants*. 11:266. doi: 10.3390/plants11030266
- Han Y, Teng K, Nawaz G, Feng X, Usman B, Wang X, Luo L, Zhao N, Liu Y, Li R (2019) Generation of semi-dwarf rice (*Oryza sativa* L.) lines by CRISPR/Cas9-directed mutagenesis of OsGA20ox2 and proteomic analysis of unveiled changes caused by mutations. *3 Biotech*. 9:1-17.
- Luo D, Xu H, Liu Z, Guo J, Li H, Chen L, Fang C, Zhang Q, Bai M, Yao N, Wu H, Wu H, Ji C, Zheng H, Chen Y, Ye S, Li X, Zhao X, Li R, Liu YG (2013) A detrimental mitochondrial-nuclear interaction causes cytoplasmic male sterility in rice. *Nature Genetics*, 45, 573-577.
- MARD (2011) *QCVN 01-55: 2011/ BNNPTNT- National Regulation on Testing for Value of Cultivation and Use of rice varieties*.
- Tran QV, Tran LT, Nguyen DTK, Ta LH, Nguyen LV, Nguyen TT (2021) Dataset on the agronomic characteristics and combining ability of new parental lines in the two-line hybrid rice systems in Vietnam. *Data in Brief*. 36: 107069.
- Watson JW, Eyzaguirre PB (2002) *Proceedings of the second International Home Gardens Workshop*. 17-19th July, 2001. Witzhausen Fed. Rep Germany, IPGRI, Rome.
- Zhang O, Liang C, Yang B, You H, Xu L, Chen Y, Xiang X (2021) Effects of Starch Synthesis-Related Genes Polymorphism on Quality of Glutinous Rice. *Frontiers in Plant Science*. 12:707992.