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Full Length Article

Combining Ability of Mineral Element Contents in Hybrid Rice

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Abstract

Understanding the genetic control of mineral element contents in the last three leaves of hybrid rice will shed light on breeding hybrid rice with ideal mineral element contents. In this study, 25 hybrid rice combinations were created using five elite male sterile lines and five restorer lines. The combining ability of potassium (K), calcium (Ca), iron (Fe), copper (Cu), magnesium (Mg), manganese (Mn), phosphorus (P), sulfur (S) and zinc (Zn) in the last three leaves of these crosses were analyzed at both active tillering stage and milky stage. The results showed that the variance of the general combining ability (GCA) of these parents was larger than the variance of the specific combining ability (SCA) of all crosses. Moreover, larger variation range of heterosis was found in the lines crossed from the parents with the larger variance of SCA. The restorer lines exhibited stronger heritability than sterile lines for Ca, Cu, Mn, S, Zn at active tillering stage and for K, Ca, S, Zn at milky stage. The further phenotypic evaluation for tested crosses revealed that the content of some mineral elements in the last three leaves showed positively significant correlation with yield-related traits. Selecting parents with higher GCA and larger variance of SCA will help us develop the hybrid combination with ideal content of mineral elements and good yield performance for the future hybrid rice breeding program.

Keywords: Mineral elements; Combining ability; Rice; Correlation analysis; Heterosis

Introduction

Rice (*Oryza sativa* L.) is one of the most important crops that feed about half of the world's population (Izawa and Shimamoto, 1996; Khush, 2005). It is also considered to be a good supplier for mineral element because of its high level of consumption in people daily diet, especially those poor in Asia, Africa and Latin American (Gregorio *et al*., 2000). The mineral element is proven to be of vital important in rice growth and development, yield formation and grain quality (Shannon *et al*., 2014). Hence, investigating the heterosis and heredity for the content of mineral element in rice is helpful in modern rice breeding program, particularly in hybrid breeding program.

Previous studies have mainly focused on the heterosis and heredity of mineral elements in rice grain. The contents of Ca, Fe, Mn, Mg, P, S and Zn in hybrid rice were investigated to be higher than that in their parents, suggesting a positive heterosis (Yang and TzerKaun, 2010). The variance of both general combining ability (GCA) and specific combining ability (SCA) for the contents of Fe, Mn and Zn were also analyzed (Lai *et al*., 1994; Zhang *et al*., 1996; Gregorio *et al*., 2000). The heredity for the content of various mineral elements, such as Fe, Zn, Mn, Cu, Ca, Mg, P and K, was studied as well. It was found that a wide range of heritability was presented, while the additive effect, non-additive effect, over-dominance effect, partial dominance effect, epispastic effect, genotype × environment interaction effects and environmental effect have their respective effect on determining the content of mineral elements in rice grains (Zhang *et al*., 1993, 1996; Lai *et al*., 1994; Gregorio *et al*., 1999, 2000; Tin, 2000; Jiang *et al*., 2007; Garcia-Oliveira *et al*., 2009; Yang and TzerKaun, 2010; Du *et al.*, 2013; Huang *et al.*, 2016). In addition, some significant correlation between mineral elements content and agronomic characters were detected. For example, it was found that the yield of rice was closely related with K, Mg, Zn, Se and other elements (Chen *et al*., 2015).

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Although a number of reports are available on the heredity of mineral elements in grains, none of these studies focused on the heredity of mineral elements in leaves, which is reported to be the main source of mineral elements in grains. The last three leaves in rice, which contain the flag leaf, the second leaf and the third leaf from the top, are the main supplier of photoassimilates, rice panicle development and spikelet establishment (Grusak and DellaPenna, 1999; Narayanan *et al*., 2007), and are critical for and determine the final grain production (Khush, 1995). The mineral element in the last three leaves was regarded as the main source of some mineral element in rice grains, such as Zn and Fe (Sperottoa *et al*., 2010). In addition, the proper content of mineral in the last three leaves was reported to retard the senescence in leaves, and prompt rice grain production (Duan *et al*., 1997). Therefore, it is of great importance to explore the content of mineral elements in the last three leaves and their genetic regularity for hybrid rice breeding program.

In this study, in order to investigate the combing ability and heredity of mineral element in the last three leaves of rice, 25 combinations were created according to NC II design method using five male sterile lines and five restorer lines which was widely utilized in the rice production. The content of 9 kinds of mineral element, including K, Ca, Fe, Cu, Mn, Mg, P, S and Zn, were measured in all tested sterile lines, restorer lines and all their crosses at two growing stages. The combing ability, including the variance of the GCA and SCA was analyzed. Meanwhile, we also evaluated the heredity of them. Furthermore, the content of mineral elements and their correlation with important agronomic traits were investigated. These results would be helpful to uncover the theoretical basis for selection of hybrid rice combinations with optimal mineral element contents and learn more about the genetic rules of nine mineral elements (K, Ca, Fe, Cu, Mg, Mn, P, S and Zn) in the last three leaves.

Materials and Methods

Materials and Field Experiment

Five CMS lines (A) and five restorer lines (R) (Table 1) were used to make 25 hybrids. All materials were provided by Prof. Qinquan Yan, from Hunan Agricultural University.

Twenty-five combinations were made in incomplete diallel design (Shanyou 63 acts as a control) at the breeding base of Hunan Agricultural University (Hai Tangwan Town, Sanya City, Hainan Province, China). All 25 combinations and their parents (5 sterile lines and 5 restorer lines) were planted at the didactical farm of Hunan Agricultural University. Thirty-day-old seedlings were transplanted at a spacing of 20 cm×20 cm with single seedling per hill. The plots were arranged in the field in a randomized block design with three replications following standard field management practice.

Sampling

The last three leaves from 5 typical plants in the central part of each plot were collected at active tillering and milky stage. For each sample, we had 3 biological replications. All leaf samples were rinsed with tap water and dried at 60°C in oven until a constant weight was recorded. Nine mineral elements were measured including K, Ca, Fe, Cu, Mn, Mg, P, S and Zn.

Sample Pretreatment and Mineral Element Measurement

Extraction of mineral elements was modified from previous study (Du, 2003). Briefly, 0.5 g dried sample was placed in conical flask, 12 mL of HNO_3-$HClO_4$ (4:1, v/v) was added into the flask and covered with watch glass overnight in fume hood. The sample was further digested on electric stove until the organics decompose completely into colorless or faint yellow digestive juice 20 mL of water was added after cooling (four times, 5 mL each time), the sample was dried by heating and evaporation approach to 0.5 mL^{-1} mL. Dilute the solution to 25 mL with 0.02% HCl after cooling. The diluted solution were used to measure the content of potassium (K), calcium (Ca), iron (Fe), copper (Cu), manganese (Mn), magnesium (Mg), phosphorus (P), sulfur (S) and zinc (Zn) by IRIS intrepid II XPS (Thermo, USA).

Statistical Analyses

General combining ability and specific combining ability were calculated by the formulae: $g_i = \bar{y}_{i.} - \bar{y}$; $g_j = \bar{y}_{.j} - \bar{y}$, $s_{ij} = \bar{y}_{ij} - \bar{y}_{i.} - \bar{y}_{.j} + \bar{y}$, where g_i and g_j stand for GCA of parent, s_{ij} stands for SCA of cross, $\bar{y}_{i.}$, $\bar{y}_{.j}$, $\bar{y}_{ij}$ and $\bar{y}$ stand for the mean of crosses with same parent Pi, the mean of crosses with same parent Pj, the mean of crosses Pi/Pj, the mean of all crosses, respectively (Mo, 1982). Descriptive statistics, ANOVA and correlation analysis were implemented using software DPS (v12.01).

Results

Heterosis for the Content of Mineral Elements

In the last three leaves of tested rice hybrid, there was a wide variation range of the heterosis in various mineral elements content at both the active tillering stage and milky stage (Table 2). At the active tillering stage, the content of Mn showed a wider range of variation for mid-parent heterosis than other tested mineral elements. Its range of variation for mid-parent heterosis varied from 12.54~74.86%, with an extreme deviation of 62.32, and 25 combinations exhibited positive mid-parent heterosis. The minimum variation is the content of P, with a variation of mid-parent heterosis ranged from -6.65~10.19% and an extreme deviation of 19.27. There were 11 combinations

Table 1: The hybrid rice parents used in this experiment

No.	Male sterile line	No.	Restorer line
A1	K17A	R1	R617
A2	You IA	R2	R612
A3	D62A	R3	R63
A4	Gang46A	R4	R654
A5	Zhenshan97A	R5	R665

Table 2: Performance of heterosis for contents of different mineral elements in the last three leaves of hybrid rice at active tillering stage and milky stage

Growth period	Element	Mid-parents heterosis						Over-better-parent heterosis					
		Mean	Max	Min	Range	CV	Number of combinations with positive heterosis	Mean	Max	Min	Range	CV	Number of combinations with positive heterosis
Active tillering stage	K	-3.90	6.11	-17.71	23.82	-1.52	6	-8.70	6.05	-20.17	26.22	-0.73	2
	Ca	3.63	24.65	-22.98	47.63	3.16	15	-6.78	18.39	-30.38	48.77	-1.89	7
	Fe	-18.09	5.71	-33.96	39.67	-0.55	1	-40.42	-24.55	-53.19	28.64	-0.18	0
	Cu	7.02	39.01	-20.81	59.82	2.60	15	1.34	37.60	-29.65	67.25	13.87	13
	Mg	-3.62	16.92	-18.92	35.84	-3.21	10	-18.94	7.77	-41.47	49.24	-0.99	10
	Mn	34.30	74.86	12.54	62.32	0.47	25	22.81	59.19	-2.42	61.61	0.68	24
	P	8.83	14.66	-0.42	15.08	0.42	24	0.40	10.19	-6.65	16.84	11.00	11
	S	-4.72	6.93	-12.34	19.27	-0.95	3	-12.30	-4.32	-24.38	20.06	-0.43	0
	Zn	-1.85	14.81	-16.07	30.88	-4.44	12	-1.85	14.81	-16.07	30.88	-4.44	12
Milky stage	K	-0.29	20.25	-30.41	50.66	-44.35	15	-8.54	18.05	-36.11	54.16	-1.69	6
	Ca	-3.82	17.48	-41.49	58.97	-3.76	10	-10.37	26.50	-44.82	71.32	-1.59	6
	Fe	12.44	56.12	-4.15	60.27	1.01	22	-1.51	36.38	-29.87	66.25	-10.97	11
	Cu	-1.74	18.05	-17.62	35.67	-6.00	11	-7.11	16.85	-25.89	42.74	-1.54	6
	Mg	-9.57	16.61	-33.66	50.27	-1.45	7	-23.58	1.61	-47.73	49.34	-0.48	1
	Mn	13.04	35.17	-19.42	54.59	1.01	22	11.67	66.59	-22.38	88.97	1.90	16
	P	-0.17	31.31	-21.66	52.97	-91.29	10	-10.83	22.40	-26.77	49.17	-1.29	5
	S	-13.08	5.53	-26.17	31.70	-0.58	1	-20.94	2.10	-38.25	40.35	-0.45	1
	Zn	16.76	34.47	-1.79	36.26	0.62	24	9.40	34.03	-11.81	45.84	1.37	17

exhibited positive mid-parent heterosis in the content of P.

When it came to the last three leaves of milky stage, the content of Fe showed the widest range of variation for mid-parent heterosis in all tested mineral elements. Its range of variation for mid-parent heterosis varied from -4.15~56.12%, with an extreme deviation of 60.27. The minimum variation is the content of S, with a variation of mid-parent heterosis ranged from -26.13~5.33% and an extreme deviation of 31.7. However, only one combination exhibited positive mid-parent heterosis in the content of S. The results also revealed that the content of Mn showed the widest range of variation for heterobeltiosis in all tested mineral elements.

ANOVA of Combining Ability

Analysis of combing ability variance demonstrated that the GCA between the tested restoring lines showed significant ($p<0.05$) or very significant ($p<0.01$) difference in the content of all tested mineral elements in the last three leaves at two tested stages, except the content of Mg, Mn and P at the active tillering stage, and Mg at the milking stage (Table 3). The GCA between the tested sterile lines showed significant ($p<0.05$) or very significant ($p<0.01$) difference in the content of all tested mineral elements in the last three leaves at two tested stages, except the content of K and Mg at the milking stage. The SCA of the content about tested mineral element in the last three leaves all exhibited very significant ($p<0.01$) difference. The ratio of GCA/SCA ranged from 1.15 in K to 3.58 in Fe at the active tillering stage, while ranged from 1.32 in Cu to 3.17 in Mn at the milky stage. It suggested that the additive gene effect was larger than that of no-additive gene effect.

The General Combining Ability

It was found that the general combining ability of the tested traits all reached the extremely significant level (Table 4). Both the general combining ability for the same traits between different parents and the general combining ability for different traits of the same parents showed the wide variation according to our result. It was also found that the general combining ability for the same trait was distinct in different growing stages. For example, all the tested parents exhibited a higher level of general combining ability for the content of K and Ca in the active tillering stage than in the milky stage. However, when it came to the content of Fe, Mg and Mn, the changing trend showed in the opposite direction. In addition, we can also find that some parents, such as D62A, Gang46A, R617 and R665, showed a higher GCA in almost tested traits than other parents at both two growing stages, suggesting that they had a higher general combing ability and maybe more suitable for breeding program.

Table 3: ANOVA of combining ability for contents of different mineral elements in the last three leaves (F value)

Growth period		K	Ca	Fe	Cu	Mg	Mn	P	S	Zn
Active tillering stage	RGCA	0.29*	0.21**	19.77*	1.21**	0.00	0.01**	0.00	0.02**	1.77**
	AGCA	0.41*	0.09*	103.13**	0.68*	0.02**	0.01**	0.00	0.01**	0.92*
	SCA	0.61**	0.18**	34.33**	1.31**	0.01**	0.01**	0.00	0.01**	2.09**
	GCA/SCA	1.15	1.67	3.58	1.44	3.37	2.57	3.04	3.17	1.29
Milky stage	RGCA	1.36**	0.99**	165.76**	0.38**	0.02	0.00	0.01	0.02**	3.67**
	AGCA	0.27	0.44*	239.73**	0.86**	0.15**	0.05**	0.05**	0.01*	1.18
	SCA	1.09**	0.84**	179.48**	0.39**	0.06**	0.04**	0.00**	0.01**	3.57**
	GCA/SCA	1.49	1.72	2.26	3.17	3.01	1.32	1.34	2.43	1.36

* $p<0.05$，** $p<0.01$

RGCA is short for "the variance of the general combining ability for restorer line", AGCA is short for "the variance of the general combining ability for sterility line"

Table 4: General combining ability for the contents of different mineral elements

Growth period	Parents		K		Ca		Fe		Cu		Mg		Mn		P		S		Zn	
Active tillering stage	Sterility line	A1	14.78**	Bb	29.30**	Aa	142.44**	Cc	8.78**	Bb	1.44**	D d	0.89**	B b	2.30**	Cc	2.32**	Dd	21.91**	Aa
		A2	13.79	Cc	27.00	Cd	144.48**	Bb	7.78	Dd	1.42	E e	0.60	D d	2.22**	Aa	2.19	Cc	24.33**	Cc
		A3	15.70**	Aa	28.60**	Bb	133.18	Dd	9.97**	Aa	2.25**	C c	0.95**	Aa	2.49**	Ee	2.47**	Ee	20.95	Ee
		A4	15.60**	Aa	28.33**	Bc	142.70**	Cc	8.03**	Cc	2.77**	Aa	0.60	Dd	2.21**	Dd	2.26**	Bb	22.43**	Dd
		A5	13.72	Cc	28.52**	Bbc	145.85**	Aa	8.68**	Bb	2.63**	B b	0.62**	Cc	2.19	Bb	2.29**	Aa	21.88**	Bb
	Restorer	R1	13.53**	Aa	29.30**	Aa	75.00 **	aA	8.20**	Aa	1.34**	B b	0.63	Bb	1.93**	Bb	1.90**	Cc	20.73**	Aa
		R2	13.11**	Bb	27.00	Cd	62.33 **	bB	7.77**	BCbc	1.24**	D d	0.71**	Cc	2.16**	Ee	1.92**	Aa	17.90**	Cc
		R3	12.66	Cc	28.60**	Bb	62.41 **	bB	7.02	Dd	1.28**	C c	0.68**	Aa	1.61	Aa	1.52	Ee	17.37	Ee
		R4	12.97*	BCb	28.33**	Bc	60.33	cC	7.68**	Cc	1.12	E e	0.69**	Dd	1.89**	Dd	2.39**	Bb	17.65**	Dd
		R5	13.78**	Aa	28.52**	Bbc	62.33**	bB	7.87**	Bb	1.48**	Aa	0.73**	Ee	2.06**	Cc	2.43**	Dd	20.06**	Bb
Milky stage	Sterility line	A1	8.04**	dD	8.31**	bB	171.89**	Bb	7.12	Ee	2.36	Ee	1.04	Cc	1.45**	Cc	1.99**	Dd	20.78**	Cc
		A2	9.08**	cC	8.67**	aA	117.35**	Dd	7.34**	Dd	2.48**	Dd	1.02	Cc	1.42	Dd	1.65	Ee	17.33	Ee
		A3	7.42	eE	7.52**	cC	226.21**	Aa	7.78**	Cc	3.28**	Aa	1.28**	Bb	2.10**	Bb	2.24**	Bb	20.49**	Dd
		A4	10.25**	bB	7.17	eE	169.20**	Cc	9.97**	Aa	3.01**	Cc	1.67**	Aa	2.23**	Aa	2.44**	Aa	22.14**	Bb
		A5	10.80**	aA	7.44**	dD	108.46	Ee	8.68**	Bb	3.11**	Bb	1.55**	Aa	1.45**	Cc	2.10**	Cc	23.35**	Aa
	Restorer	R1	6.27	eE	9.08**	bB	111.12**	Dd	7.94**	Aa	2.12**	Bb	1.60	Bb	1.26**	Dd	1.77**	Cc	18.04**	Cc
		R2	7.61**	dD	8.54**	cC	122.85**	Cc	7.87**	Bb	1.95**	Cc	1.57	Bb	1.23	Ee	1.57	Ee	16.99	Ee
		R3	8.57**	bB	9.76**	aA	104.83	Ee	6.88	Ee	1.89**	Dd	1.55	Bb	1.53**	Cc	1.73**	Dd	17.63**	Dd
		R4	8.50**	cC	6.68**	dD	129.72**	Bb	7.18**	Dd	1.55	Ee	1.54	Bb	1.95**	Aa	2.08**	Bb	19.15**	Bb
		R5	10.55**	aA	6.59	eE	145.17**	Aa	7.76**	Cc	2.23**	Aa	2.12**	Aa	1.94**	Bb	2.78**	Aa	20.63**	Aa

Note: * $p<0.05$，** $p<0.01$

The different capital indicate both of parents have highly significant difference, the different lowercase indicate both of parents have significant difference

A1 represents K17A, A2 represents You ⅠA, A3 represents D62A, A4 represents Gong 46A, A5 represents Zhenshan 97A, R1 represents R617, R2 represents R612, R3 represents Minghui 63, R4 represents R654, R5 represents R665

The Specific Combining Ability

The specific combining ability of content of various mineral elements in the last three leaves of rice hybrid showed a large variation (Table 5). At the active tillering stage, the number of cross which had a positive specific heterosis in the content of K, Ca, Fe, Cu, Mg, Mn, P, S and Zn was 14, 13, 12, 14, 13, 14, 12, 8 and 11, respectively, while the corresponding number at the milky stage ranged from 10 to 14. In a specific combination, the SCA about content of different mineral elements in the last three leaves was also presented great discrepancy. For example, K17A×R617, one of the total 25 tested combinations, showed the positive SCA in seven kinds of mineral elements content in both two tested stages, while nine kinds of mineral elements content were tested. You IA×R617 showed the positive SCA in Ca, S, Zn, K, Cu and Mn contents at the active tillering stage, while K, P, Fe, S and Zn contents at the milky stage. The similar phenomenon was detected in other combinations, such as K17A×R63, You IA×R63, K17A×R654, and so on.

The Correlation between Combining Ability and Heterosis

The correlation between combining ability and heterosis about the content of mineral elements in the last three leaves of rice hybrid has been studied (Table 6).

At the active tillering stage, the mid-parent and heterobeltiosis of the contents of all tested mineral elements showed significant or very significant positive correlation with its SCA, except the content of Mg. The similar results were also presented about the correlation between heterobeltiosis of mineral element contents and the RGCA. However, the mid-parent heterosis of the content of P exhibited the significant negative correlation with the RGCA, while the mid-parent heterosis of the content of Mg, and the heterobeltiosis of the content of Mg and P showed significant or very significant negative correlation with the AGCA. The mid-parent heterosis of the content of Mg, Mn, Fe and S, and the heterobeltiosis of the content of Mg, Mn, P and Zn showed significant

Table 5: Crosses with beneficial specific combining ability for the nine elements at active tillering stage and milky stage

Active tillering stage									Milky stage								
K	Ca	Fe	Cu	Mg	Mn	P	S	Zn	K	Ca	Fe	Cu	Mg	Mn	P	S	Zn
A1/R1	A2/R1	A1/R1	A1/R1	A4/R1	A1/R1	A3/R1	A2/R1	A2/R1	A2/R1	A4/R1	A1/R1	A3/R1	A3/R1	A4/R1	A2/R1	A1/R1	A1/R1
A2/R1	A4/R1	A3/R1	A2/R1	A5/R1	A2/R1	A4/R1	A3/R2	A2/R2	A5/R1	A5/R1	A2/R1	A4/R1	A4/R1	A5/R1	A3/R1	A2/R1	A2/R1
A5/R1	A1/R2	A2/R2	A4/R1	A3/R2	A4/R1	A1/R2	A2/R3	A3/R2	A1/R2	A3/R2	A1/R2	A5/R1	A3/R2	A1/R2	A5/R1	A3/R1	A3/R1
A3/R2	A2/R2	A4/R2	A2/R2	A4/R2	A3/R2	A2/R2	A5/R3	A4/R2	A4/R2	A2/R3	A3/R2	A1/R2	A4/R2	A2/R2	A3/R2	A4/R2	A4/R1
A4/R2	A2/R3	A2/R3	A3/R2	A5/R2	A5/R2	A3/R2	A1/R4	A1/R3	A5/R2	A3/R3	A4/R2	A2/R2	A1/R3	A4/R2	A4/R2	A5/R2	A2/R2
A1/R3	A3/R3	A3/R3	A5/R2	A1/R3	A1/R3	A5/R2	A4/R4	A3/R3	A1/R3	A4/R3	A1/R3	A1/R3	A2/R3	A2/R3	A1/R3	A1/R3	A5/R2
A5/R3	A1/R4	A3/R4	A1/R3	A2/R3	A2/R3	A1/R3	A4/R5	A3/R4	A2/R3	A1/R4	A2/R3	A2/R3	A4/R3	A3/R3	A3/R3	A5/R3	A1/R3
A1/R4	A3/R4	A4/R4	A2/R3	A3/R3	A5/R3	A3/R3	A5/R5	A4/R4	A3/R3	A4/R4	A5/R3	A1/R4	A5/R3	A5/R3	A1/R4	A2/R4	A2/R3
A3/R4	A4/R4	A5/R4	A5/R3	A1/R4	A1/R4	A1/R4		A5/R4	A4/R4	A5/R4	A1/R4	A2/R4	A1/R4	A1/R4	A2/R4	A5/R4	A3/R3
A4/R4	A5/R4	A1/R5	A3/R4	A2/R4	A3/R4	A2/R4		A4/R5	A5/R4	A1/R5	A2/R4	A3/R4	A2/R4	A3/R4	A3/R4	A1/R5	A1/R4
A5/R4	A2/R5	A2/R5	A4/R4	A3/R4	A4/R4	A4/R5		A5/R5	A2/R5	A2/R5	A3/R4	A4/R5	A4/R5	A5/R4	A4/R5		A3/R4
A2/R5	A3/R5	A5/R5	A2/R5	A4/R5	A3/R5	A5/R5			A4/R5	A3/R5	A4/R4	A5/R5	A5/R5	A1/R5	A5/R5		A4/R4
A3/R5	A5/R5		A4/R5	A5/R5	A4/R5				A5/R5		A4/R5			A2/R5			A3/R5
A4/R5			A5/R5		A5/R5						A5/R5						A4/R5
																	A5/R5

Note: A1 represents K17A, A2 represents You ⅠA, A3 represents D62A, A4 represents Gong 46A, A5 represents Zhenshan 97A, R1 represents R617, R2 represents R612, R3 represents Minghui 63, R4 represents R654, R5 represents R665

Table 6: The correlation between combining ability and heterosis

Growth period	Combining ability	Mid-parents heterosis									Over-better-parent heterosis								
		K	Ca	Fe	Cu	Mg	Mn	P	S	Zn	K	Ca	Fe	Cu	Mg	Mn	P	S	Zn
Active tillering stage	SCA	0.77**	0.70**	0.49*	0.61**	0.37	0.62**	0.61**	0.80**	0.77**	0.70**	0.53**	0.49*	0.55**	0.17	0.59**	0.50**	0.62**	0.62**
	RGCA	0.49*	0.39*	0.33	0.63**	-0.04	0.69**	-0.41*	-0.33	0.44*	0.63**	0.71**	0.47*	0.68**	0.08	0.61**	0.63**	0.67**	0.72**
	AGCA	0.33	0.54**	0.78**	0.42*	-0.68**	-0.03	0.07	0.44*	0.35	-0.07	0.42*	0.72**	0.28	-0.79**	-0.30	-0.41*	0.25	0.06
	RGCA+AGCA	0.58**	0.63**	0.85**	0.75**	-0.64**	0.48*	-0.24	-0.03	0.56**	0.36	0.82**	0.86**	0.71**	-0.71**	0.23	0.15	0.70**	0.60**
	RGCA-AGCA	0.08	0.01	-0.56**	0.23	0.62**	0.52**	-0.34	-0.52**	0.13	0.47*	0.34	-0.44*	0.36	0.77**	0.65**	0.74**	0.42*	0.54**
	ASCA	-0.09	0.42*	0.00	0.29	-0.46*	0.13	-0.23	0.00	0.15	0.14	0.29	0.00	0.10	-0.56**	-0.07	-0.24	-0.02	-0.13
	RSCA	0.10	-0.29	-0.11	-0.03	-0.06	0.59**	-0.33	0.07	-0.01	0.22	-0.18	-0.2	-0.01	0.00	0.53**	0.48*	0.02	0.26
Milky stage	SCA	0.72**	0.71**	0.69**	0.64**	0.60**	0.10	0.66**	0.59**	0.75**	0.70**	0.46*	0.47*	0.66**	0.60**	0.28	0.56**	0.40*	0.68**
	RGCA	0.39*	0.45*	0.31	0.49*	0.14	0.01	0.15	-0.11	0.55**	0.43*	0.58**	0.27	0.51**	0.42*	0.62**	0.16	-0.02	0.57**
	AGCA	0.19	0.41*	-0.40*	0.51**	0.71**	0.37	0.27	-0.15	0.12	0.05	0.44*	-0.64**	0.21	0.47*	0.34	0.09	-0.26	-0.04
	RGCA+AGCA	0.44*	0.61**	-0.10	0.70**	0.71**	0.34	0.31	-0.18	0.53**	0.40*	0.73**	-0.31	0.46*	0.59**	0.58**	0.15	-0.16	0.46*
	RGCA-AGCA	-0.39*	-0.45*	-0.31	-0.49*	-0.14	-0.01	-0.15	0.11	-0.55**	-0.43*	-0.58**	-0.27	-0.51**	-0.42*	-0.62**	-0.16	0.02	-0.57**
	ASCA	0.29	-0.05	0.46*	-0.27	0.12	0.14	0.27	-0.24	-0.27	0.17	-0.19	0.53**	-0.26	0.11	0.12	0.22	-0.26	-0.26
	RSCA	-0.12	-0.12	0.34	0.24	-0.01	-0.16	-0.15	0.10	0.12	0.03	-0.65**	0.27	0.24	-0.25	-0.34	-0.18	-0.06	0.17

* $p<0.05$，** $p<0.01$

Note: SCA represents the specific combining ability, RGCA represents the general combining ability of restorer line, AGCA represents the general combining ability of sterility line, RSCA represents the specific combining ability of restorer line, ASCA represents the specific combining ability of sterility line

or very significant positive correlation with (RGCA-AGCA). The mid-parent heterosis of the content of Mg and the heterobeltiosis of the content of Fe showed significant negative correlation with RGCA and AGCA.

At the milky stage, the mid-parent and heterobeltiosis of the contents of all tested mineral elements showed significant or very significant positive correlation with its SCA, except the content of Mn. Both the mid-parent and the heterobeltiosis of the content of K, Ca, Cu and Zn showed significant or very significant positive correlation with SCA, whereas the mid-parent and the heterobeltiosis of the content of Fe showed significant or very significant negative correlation. The mid-parent heterosis of the content of K, Ca, Cu, Mg, Mn and Zn, and the heterobeltiosis of the content of K, Ca, Cu, Mg, and Zn showed significant or very significant positive correlation with (RGCA+AGCA). The mid-parent heterosis of the content of K, Ca, Cu, and Zn, and the heterobeltiosis of the content of K, Ca, Cu, Mg, Mn and Zn showed significant or very significant negative correlation with (RGCA-AGCA). The mid-parent and the heterobeltiosis of the content of Fe showed significant or very significant positive correlation with ASCA. The heterobeltiosis of the content of Ca showed very significant positive correlation with RSCA.

All the results above indicated that the mid-parent and heterobeltiosis of the contents of all tested mineral elements in the last three leaves of different hybrid combinations showed significant or very significant correlation with the parents' GCA, (RGCA+AGCA), (RGCA-AGCA) and SCA, and exhibited no significant correlation with the variance of SCA about two parents at both the two tested stages.

The Correlation between the Content of Mineral Elements and Combining Ability

The performance of mineral content of hybrids had a significant correlation with heterosis and combining ability

Table 7: The correlation between the performance of hybrids and combining ability

Growth period		K	Ca	Fe	Cu	Mg	Mn	Na	P	S	Zn
Active Tillering stage	SCA	0.58**	0.53**	0.42*	0.55**	0.45*	0.46*	0.37	0.49*	0.43*	0.57**
	RGCA	0.54**	0.69**	0.40*	0.65**	0.31	0.63**	0.65**	0.61**	0.75**	0.65**
	AGCA	0.61**	0.49*	0.81**	0.52**	0.83**	0.62**	0.66**	0.63**	0.51*	0.51**
	RGCA+AGCA	0.81**	0.85**	0.90**	0.83**	0.89**	0.89**	0.93**	0.87**	0.90**	0.82**
Milky stage	SCA	0.54**	0.53**	0.49*	0.45*	0.45*	0.06	0.49*	0.58**	0.49*	0.56**
	RGCA	0.73**	0.69**	0.57**	0.51**	0.34	0.36	0.42*	0.38	0.72**	0.69**
	AGCA	0.41**	0.49*	0.66**	0.73**	0.83**	0.74**	0.76**	0.72**	0.49*	0.45*
	RGCA+AGCA	0.84**	0.85**	0.87**	0.89**	0.89**	0.82**	0.87**	0.82**	0.87**	0.83**

Note: SCA represents the specific combining ability, RGCA represents the general combining ability of restorer line, AGCA represents the general combining ability of sterility line

Table 8: The correlation between the content of mineral elements and important agronomic traits

Growth period	Traits	K	Ca	Fe	Cu	Mg	Mn	P	S	Zn
Active Tillering stage	Plant height	0.44*	-0.18	-0.36	0.45*	0.47*	0.35	0.26	0.21	-0.13
	Panicle number	0.17	0.04	-0.32	-0.09	-0.08	0.44*	0.24	0.16	-0.09
	Panicle length	-0.21	0.08	0.1	0.08	0.25	-0.36	-0.17	-0.09	-0.06
	Spikelets number	0.39	-0.41*	0.19	0.53**	0.47*	0.19	0.27	0.36	0.47*
	Grain Number	0.31	-0.44*	0.31	0.38	0.48*	0.18	0.22	0.35	0.36
	Seed Set Rate	-0.05	-0.19	0.37	-0.21	0.18	0.05	-0.04	0.08	-0.12
	Grain Weight	0.05	0.11	0.2	0.17	0.48*	0.08	0.24	0.08	-0.06
	Yield per plant	0.48*	-0.22	0.32	0.32	0.45*	0.40*	0.43*	0.37	0.28
Milky stage	Plant height	0.20	-0.33	0.50**	0.46*	0.42*	0.22	0.45*	0.19	0.31
	Panicle number	0.09	0.13	0.25	-0.32	-0.21	-0.27	0.01	-0.21	-0.10
	Panicle length	-0.03	-0.05	-0.23	0.30	0.20	0.26	0.03	0.03	0.04
	Spikelets number	0.43*	-0.53**	0.36	0.48*	0.46*	0.39*	0.43*	0.36	0.28
	Grain Number	0.66**	-0.56**	0.33	0.37	0.38	0.49*	0.32	0.32	0.32
	Seed Set Rate	0.71**	-0.23	0.06	-0.11	-0.05	0.37	-0.13	-0.01	0.17
	Grain Weight	0.33	-0.29	0.06	0.53**	0.45*	0.41*	0.08	-0.14	0.10
	Yield per plant	0.64**	-0.59**	0.41*	0.46*	0.53**	0.45*	0.46*	0.32	0.32

Note: a=0.05, r^2=0.39; a=0.01, r^2=0.50

(Table 7). For example, the contents of all tested mineral elements in the last three leaves of hybrid rice showed significant or very significant positive correlation with SCA at both active tillering and milky stage, except the content of Mn at milky stage. Moreover, the contents of all tested mineral elements in the last three leaves of hybrid rice showed significant or very significant positive correlation with AGCA except the content of Mg at the active tilling stage. The significant or very significant positive correlation between content of mineral elements and AGCA and RGCA demonstrated that it was co-controlled by both additive and non-additive gene effect.

The Correlation between the Content of Mineral Elements and Important Agronomic Traits

To detect the possible correlation between the content of mineral elements and important agronomic traits, we evaluated several key agronomic traits, including plant height, panicle number, grain number, seed setting rate, grain weight and yield per plant in all the 25 tested crosses (Table 8). It demonstrated that there was a significant positive correlation between the single plant yield and the content of Cu and Fe in the last three leaves of tested crosses at milky stage, and K, Mg, Mn and P at both active tillering stage and milky stage. Meanwhile, grain number was significantly and positively correlated with the content of Zn at active tillering stage, K and Mn at milky stage, and Cu and Mg at both stages described above In addition, the plant height of tested crosses positively related with content of K at active milling stage, Fe and P at milky stage, and Cu and Mg at the both stages. Interestingly, the content of Ca in the last three leaves showed significant negative correlation with single plant yield and grain number of the tested crosses. These results suggested that there are phenotypic and genetic correlationships between the content of mineral elements and the phenotypes of important agronomic traits, which should be utilized to rice breeding.

Discussion

In this study, we employed 5 sterile lines and 5 restorer lines to make 25 crosses by NCII design and analyzed heterosis and combining ability of 9 mineral element contents in the last three leaves of hybrid rice.

Previous studies revealed that the contents of Ca, Fe, Mn, Mg, P, S and Zn in the grain of hybrid were found to be higher than that in their parents, indicating the heterosis with positive effect (Yang and TzerKaun, 2010). However, our result did not consistent with the previous study in hybrid grains. Although the content of Mn in the last three leaves at two tested growing stages showed a positive heterosis, the content of S at two stages, the content of Fe at active tillering stage, and the content of Mg at milky stage exhibited the negative heterosis. These results suggested that we can increase the content of Mn in the last three leaves by hybrid breeding strategy, while the regulation of the content of S was not suitable.

In previous studies, a wide range of heritability was discovered, while the content of mineral elements in rice grains was determined by the additive effect, non-additive effect, over-dominance effect, partial dominance effect, epispastic effect, genotype × environment interaction effects and environmental effect (Zhang *et al*., 1993, 1996; Lai *et al*., 1994; Gregorio *et al*., 1999, 2000; Tin, 2000; Garcia-Oliveira *et al*., 2009; Yang and TzerKaun, 2010; Du *et al*., 2013; Huang *et al.*, 2016). The GCA variance for the content of Fe and Zn contents was larger than its SCA variance (Lai *et al*., 1994; Zhang *et al*., 1996; Gregorio *et al*., 2000). The results of this paper showed that both the variance of GCA and SCA for parents were significant or remarkably significant except the variance of GCA for Mg content in restorer lines at active tillering stage. Moreover, the variance of GCA was larger than that of SCA which showed that the contents of nine elements in the last three leaves were controlled by both additive gene effect and no-additive gene effect, whereas the role of additive gene effect was larger than that of no-additive gene effect.

The mineral element in the last three leaves was regarded as the main source of some mineral element in rice grains, such as Zn and Fe (Sperottoa *et al*., 2010). In addition, the proper content of mineral in the last three leaves was reported to retard the senescence in leaves, and prompt rice grain production (Duan *et al*., 1997). Therefore, it is of great importance to study the content of mineral elements and their genetic regularity for hybrid rice breeding with ideal content of mineral elements. Previous studies have mainly focused on the combing ability of mineral element in grains. Our results revealed that the content of mineral elements in the last three leaves at active tillering and milky stages have been investigated, and we found that some of the tested traits were closely related to the final yield production. It was known that the mineral elements from various rice tissues all play a key role in rice growth and development. Hence, we may further investigate the combing ability of mineral element in other important tissues, such as root, stem, and uncover their relationship with mineral element content in grains.

Conclusion

In this study, we not only evaluate the combing ability of mineral elements in the last three leaves of hybrid rice at active tilling stage and milky stage, but also find the significant correlation between the content of mineral elements and the key agronomic traits, such as grain yield, plant height, in the tested crosses. It suggested that the content of mineral elements in the last three leaves is of vital important in the formation of rice key agronomic traits. In fact, the crosses with good performance in the content of mineral elements of the last three leaves indeed exhibited higher grain production in our study. Therefore, selecting parents with higher GCA and larger variance of SCA will help us develop the hybrid combination with ideal content of mineral elements and good yield performance for the future hybrid rice breeding program.

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