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Full Length Research Paper

Analysis of combining ability and heredity parameters of glucosinolates in Chinese kale

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The study was carried out with six Chinese kale lines as materials. Complete diallel crossing was designed with 6 × 6 to calculate the combining ability and the main genetic parameters. The results are as follows: The GCA effect of P_1, P_2 and P_5 was excellent. They were used as parants to get hybrids, the heterosis of anti-cancer glucosinolates of their hybrid was very high. By analyzing the SCA effects, 1 × 2 was an outstanding hybrid. The broad sense heritability of the main GSs were relatively higher, and the narrow sense heritability were lower, so the potential of heterosis was bigger.

Key words: Chinese kale, glucosinolate, combining ability, heritability, heterosis.

INTRODUCTION

Glucosinolate (GS) is a secondary metabolite widely contained in the crucifers, it has been found close to 120 species so far. Glucosinolates can be degraded by catalysis of myrosinase or bacterial enzyme in gastro-intestinal tract, and these degradation products perform active biological and biochemical characteristics, which make the product have a special flavor. It is also a chemical protector that can prevent the occurrence of many types of cancer. At present, sulforaphen which is the degradation product of glucoraphanin is the main subject of investigation (Fahey and Talalay, 1999; Nestle,1997). The *in vitro* test of human rectal cancer cells has proved that sulforaphane has the function of inhibiting the growth of tumor cells (Gamet-payrastre et al., 1998).

At present there are few researches on glucosinolates in Chinese kale, and its rule of inheritance has not been studied and reported. Six varieties originated in Guang- dong Province were used as experimental material, and the genetic laws and combining ability of RAA (glucoraphanin, anti-cancer) and PRO (progoitrin harmful to animal) were analysed to discover their medical value and also to find high-quality resources of Chinese kale.

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Abbreviations: GCA , General combining ability; **SCA,** specific combining ability; **GS,** glucosinolate; **PRO,** progoitrin; **RAA,** glucoraphanin; **SIN,** sinigrin; **NAP,** gluconapin; **4OH,** 4-hydroxyglucobrassicin; **GBN,** glucobrassicanpin; **GBC,** glucobrassicin; **4ME,** 4-methoxyglucobrassicin; **NEO,** neoglucobrassicin; **HPLC,** high performance liquid chromatography.

MATERIALS AND METHODS

Materials and production

Two varieties with high content of RAA (P1, P2), and two varieties with low content of RAA (P5, P6), and two varieties with middle content of RAA (P3, P4) were used as materials.

Complete diallel crossing was designed. The seeds were sowed in August 25, 2008, and transplanted in September 21. The test field was divided into 36 plots. The experiment was repeated three times. The field management was the same as general production. Plants were harvested when the main sprouts grew fully and the buds have not opened. Five plants were harvested in each plot, including the tender leaves (including petiole) and the main sprouts. After harvesting, the materials were freeze-dried and crushed, then the content of their glucosinolates were measured.

Sample handling and conditions of analysis

Five hundred milligrams of each sample were extracted in 4 ml 70% methanol at 75℃ for 20 min with vortex mixing every 5 min to facilitate the extraction and then 2 ml 0.4 mol/L barium acetate was

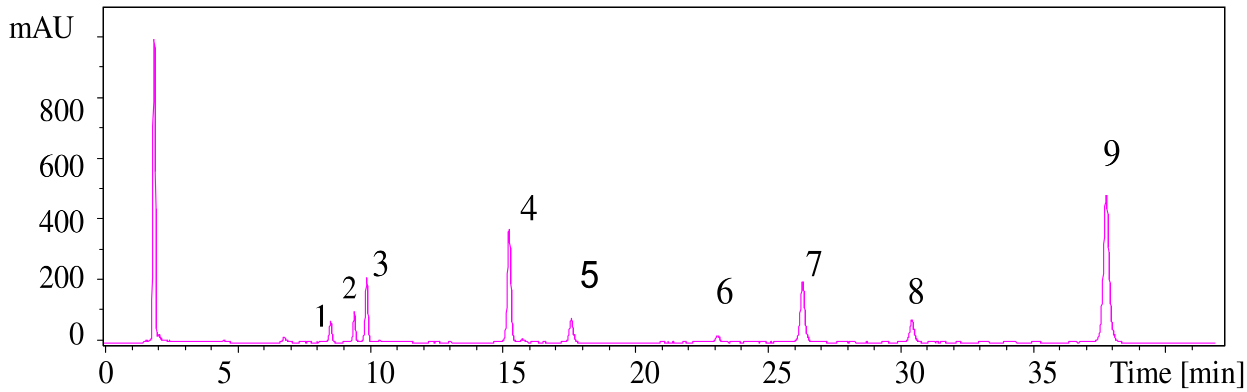

Figure 1. HPLC chromatogram of GSs in Wankuai Jielan. 1: Progoitrin (PRO); 2: Glucoraphanin (RAA); 3: Sinigrin (SIN); 4: Gluconapin (NAP); 5: 4-Hydroxyglucobrassicin (4OH); 6: Glucobrassicanpin(GBN); 7: Glucobrassicin (GBC); 8: 4-Methoxyglucobrassicin (4ME); 9: Neoglucobrassicin (NEO).

added. The samples were centrifuged (4000 rpm, 10 min, 4℃). The precipitates were extracted again as above. The supernatants were collected and combined. Five ml extract and 500 µl sulfatase solution (0.5 mg/ml) were poured into the chromatography column (filled with 500 µl DEAE Sephadex A25) slowly and incubate for 16 h at 29℃ and then was eluted with 3 ml ultrapure water. At the same time a parallel repetition was carried out, 100 µl 5 mg/mL glucotropaeolin was added into the sample as an internal standard, the other operations are thesame as above.

Twenty microliters (20 µl) eluate was used for HPLC analysis. Column is Prontosil ODS2 (250 mm × 4 mm, 5 µm; Dalian Jiangshen company). The mobile phase was a mixture of (A) ultrapure water and (B) acetonitrile. The flow rate was 1 ml min^{-1} in a linear gradient starting with 0% B at 0 - 32 min, reaching 20% B at 33 – 38 min, 20 - 100% B at 39 – 40 min. The flow rate was 1 mlmin-1, column temperature was 30℃. Chromatograms were recorded at 227 nm for glucosinolates.

Statistics and analysis

Analyses of variance were made according to a randomized complete block design. Combining ability was analyzed using Griffing I (model 1). The genetic variance component was estimated by MINQUE (1). The value of genetic effects was predicted by AUP. The standard error of estimated value and predicted values were calculated by Jackknife's method (Zhu, 1993).

RESULTS AND ANALYSIS

Analysis of glucosinolates composition in Chinese kale

In this study, some experimental conditions such as the ratio of mobile phase polarity and flow settings were explored and optimized based on the method of Krumbein et al., (2001) and Chen (2006). Finally, a total of nine glucosino- late compositions were detected. Figure 1 is HPLC chromatogram of GSs in Wankuai Jielan.

Variance analysis of the distribution of Chinese kale glucosinolates components

Firstly, analysis of variance was made among combinations according to the experimental design. The results showed that there were significant difference among the nine glucosinolate components. This indicated that there was a true difference among the nine characters, so combining ability analysis can be carried out further.

Table 1 showed that all of the ratio of mean square value between GCA and SCA were less than 5 in PRO, RAA, SIN, NAP, 4OH, GBN, 4ME, GBC, NEO, which indicated that these glucosinolates were controled by both additive effect and dominant effect. There was a significant difference at the level of 0.01 among the general combining ability and specific general combining ability of the nine glucosinolate components, therefore, it was necessary to do further analyse of combining ability .

Analysis of the general combining ability of different glucosinolate components in parents

The general combining ability (GCA) of parental character reflects it's average performance showed in a series of cross combinations. The effect of general combining ability is determined by the additive effect of the genotype of their parents, which is heritable. The GCA is proportional to the heredity of the characters. There is a higher heritability of a certain character if its general combining ability is high. This character is less affected by the environment, and play an important role in the future generations. Table 2 showed the effect of general combining ability of every inbred line.

Table 2 showed that there were great differences among different inbred lines in GCA of the same character.

Table 2. The value of general combining ability effect of parents.

Parents	PRO	Significant level of 5%	Significant level of 1%	Parents	RAA	Significant level of 5%	Significant level of 1%	Parents	SIN	Significant level of 5%	Significant level of 1%
5	0.7	a	A	1	0.48	a	A	1	0.45	a	A
3	0.12	b	B	2	0.19	b	B	3	0.36	b	B
6	0.03	c	B	3	-0.11	c	C	6	0.07	c	C
1	-0.09	d	C	5	-0.16	c	C	5	-0.21	d	D
4	-0.32	e	D	6	-0.18	c	C	2	-0.25	d	D
2	-0.44	f	E	4	-0.23	c	C	4	-0.43	e	E
Parents	**NAP**	**Significant level of 5%**	**Significant level of 1%**	**Parents**	**4OH**	**Significant level of 5%**	**Significant level of 1%**	**Parents**	**GBN**	**Significant level of 5%**	**Significant level of 1%**
3	1.89	a	A	1	0.08	a	A	1	0.23	a	A
6	1.54	a	A	2	0.06	a	A	2	0.19	b	A
1	0.79	b	B	6	0	b	B	3	0.04	c	B
2	-0.03	c	C	5	-0.04	bc	BC	5	-0.02	d	C
5	-1.59	d	D	3	-0.04	bc	BC	6	-0.19	e	D
4	-2.61	e	E	4	-0.06	c	C	4	-0.25	f	E
Parents	**GBC**	**Significant level of 5%**	**Significant level of 1%**	**Parents**	**4ME**	**Significant level of 5%**	**Significant level of 1%**	**Parents**	**NEO**	**Significant level of 5%**	**Significant level of 1%**
1	1.12	a	A	1	0.07	a	A	1	0.53	a	A
5	0.17	b	B	2	0.04	b	B	5	0.45	a	A
2	-0.01	c	C	6	0.02	c	BC	6	0.02	b	B
3	-0.35	d	D	5	0.00	c	C	3	-0.07	c	B
6	-0.36	d	D	3	-0.06	d	D	2	-0.35	d	C
4	-0.57	e	E	4	-0.07	d	D	4	-0.58	e	D

From GCA of PRO, there was low negative effect on parents of P_1, and high negative effect on P_4 and P_2. This indicated that it was rather difficult to get a hybrid combination with high content of PRO using these inbred lines as parents. RAA is an anti-cancer active substance studied widely. The higher the content, the better it is to human body. Table 2 showed that there were high positive effect on Parents P_1 and P_2, which indicated that it was possible to develop hybrid combinations with high RAA content using P_1 and P_2 as parents.

Similarly, it is easy to get a hybrid combination of higher content of SIN with P_1, P_3 and P_6 as parents, a hybrid combination of higher content of NAP with P_1, P_3 and P_6 as parents, a hybrid combination of higher content of 4OH with P_1, P_2 and P_6 as parents, a hybrid combination of higher content of GBC with P_1, P_2 and P_3 as parents, a hybrid combination of higher content of 4ME with P_1, P_2 and P_6 as parents, a hybrid combination of higher content of NEO with P5, P1 and P6 as parents.

Table 3. Specific combining ability of combinations.

Combination	PRO	RAA	SIN	NAP	4OH	GBN	GBC	4ME	NEO
1×2	-0.01	0.98**	0.00	-1.01	0.21**	0.48**	-0.02	0.02	-0.27
1×3	-0.30	-0.21	0.11*	2.41**	0.06	0.12**	-0.41	0.04*	0.04
1×4	1.13**	-0.15	0.76**	-1.30	0.02	-0.10	-0.65	0.05*	-0.16
1×5	-0.42	-0.73	-0.24	-1.69	-0.15	-0.32	-0.68	-0.22	0.26**
1×6	-0.07	-0.11	0.12*	1.46**	-0.02	-0.22	-0.87	-0.04	-0.32
2×3	-0.36	-0.55	-0.15	-3.15	-0.08	-0.55	-0.24	-0.05	-0.45
2×4	0.00	0.06	0.07	0.65	0.02	0.07*	-0.17	0.00	0.00
2×5	-0.75	-0.05	1.03**	3.82**	0.06	0.35**	0.30**	0.08**	-0.02
2×6	1.00**	-0.15	-0.88	2.60**	-0.06	0.15**	-0.20	0.02	0.60**
3×4	-0.47	-0.37	-0.89	2.20**	-0.11	-0.24	0.07	-0.12	-0.27
3×5	-0.93	0.74**	0.67**	6.85**	0.08	0.57**	0.77	0.08**	1.14**
3×6	-0.45	0.18	2.12**	1.65**	0.07	0.05	-0.03	0.07**	0.03
4×5	-0.67	0.06	-0.17	-2.85	-0.04	-0.11	-0.48	-0.05	-0.57
4×6	-0.01	0.10	0.23**	2.93**	0.05	0.16**	0.27**	0.04*	-0.06
5×6	0.34	0.27*	-0.58	-4.29	0.06	-0.04	-0.36	0.01	-0.34
LSD 0.05	0.15	0.25	0.11	0.90	0.09	0.07	0.17	0.04	0.18
LSD 0.01	0.20	0.33	0.15	1.20	0.12	0.10	0.22	0.06	0.24

* and ** indicate 5 and 1% level of significance, respectively.

Analysis of specific combining ability of every combination

Table 3 showed that there were big differences in the specific combining ability of different characters among different combinations, which indicated that the non-additive effect and variation of characters caused by non-additive effect were different in different hybridized. In addition, there were also differences in the specific combining ability of different characters among different combinations that have the same parent. This proved that there were significant differences in the effect of non-additive becuse of the different genetic background of different inbred lines.

Most parents with high effect of GCA were involved in the cross combinations with higher effect of SCA, indicating that SCA and GCA were consistent. But there was also a situation that parents with low GCA effects organized a combination with higher SCA effects, and this is special performance. Therefore, the breeder should select the inbred lines with high general combining ability as parents, but these combinations should be used with a lower general combining ability and with a higher specific combining ability.

One tmes two (1 × 2) was an excellent cross combination in the 15 combinations through a comprehensive evaluation. On one hand, the RAA concent of combinations and their parents was the highest, and the PRO content was the lowest. On the other hand, the SCA and GCA effects of combinations and their parents in RAA were the highest. So this combination was the best one.

Analysis of the major genetic parameters of glucosinolate in Chinese kale

Analysis of heritability of glucosinolate components

Table 4 showed that the narrow-sense heritability of all of the glucosinolate compositions were lower, but broad-sense heritability were high. Low narrow-sense heritability shows that the additive effect in the i gluco- sinolate content is small i, and the non-additive effects play a decisive role in their heredity, the proportion of phenotypic variation of the traits determine by genetic factors is large, and the phenotypic value is less affected by environmental conditions; these two kinds of genetic parameters are significative to the selection of parents and traits in hybrid offsprings.

The order of broad-sense heritability was GBC > NEO > PRO > RAA > SIN > 4ME > NAP > GBN > 4OH, the date was from 49 - 92%. The order of narrow sense heritability was GBC > NEO > RAA > 4ME > PRO > GBN > 4OH > SIN > NAP, the maximum was 54%. The order of the broad-sense heritability was not fully consistent with the narrow-sense heritability, which indicated that there was a difference among the genetic effects of the glucosinolates. For example, the broad-sense heritability of 4ME was 63%, while the narrow-sense heritability was only 27%, it indicates that the proportion of variance of dominant gene

Table 4. Heritability estimates of glucosinolate in Chinse kale.

Glucosinolates	Narrow sense heritability	Broad sense heritability
PRO	0.18 ± 0.03*	0.70 ± 0.02**
RAA	0.28 ± 0.07*	0.70 ± 0.07**
SIN	0.00	0.66 ± 0.00**
NAP	0.00	0.62 ± 0.03**
4OH	0.10 ± 0.05	0.49 ± 0.16*
GBN	0.17 ± 0.02**	0.58 ± 0.02**
GBC	0.54 ± 0.01**	0.92 ± 0.03**
4ME	0.27 ± 0.05*	0.63 ± 0.09*
NEO	0.31 ± 0.03**	0.82± 0.03**

* and ** indicate 5 and 1% level of significance, respectively. Values are expressed in mean ± SE..

Table 5. The average heterosis of 30 hybrid combinations.

Glucosinolates	G(F_1)	Hpm(F_1)	Hpb(F_1)
PRO	1.08 ± 0.01**	-0.49 ± 0.01**	-1.07 ± 0.01**
RAA	0.99 ± 0.01	0.03 ± 0.01	-0.03 ± 0.00*
SIN	1.48 ± 0.00**	0.73 ± 0.00**	0.54 ± 0.00**
NAP	44.83 ± 0.52**	14.74 ± 0.12**	10.29 ± 0.12**
4OH	0.30 ± 0.00	0.01 ± 0.00**	0.01 ± 0.00**
GBN	0.54 ± 0.01	0.04 ± 0.00**	0.01 ± 0.00**
GBC	1.28 ± 0.01**	-0.44 ± 0.01**	-1.10 ± 0.01**
4ME	0.33 ± 0.00	0.00	-0.01 ± 0.00**
NEO	1.42 ± 0.01	0.02 ± 0.00*	-0.11 ± 0.00**

* and ** indicate 5 and 1% level of significance, respectively. Values are expressed in mean ± SE; n = 3.

to the total genetic variance was larger than additive effect, and the characters of glucosinolates in the hybrids was unstable, so it was unsuitable to select in the early generations.

The prediction of genotypic value and heterosis of glucosinolates in Chinese kale

The genotypic value and heterosis (Table 5) of glucosinolates were predicated in the combinations of F_1 using the method proposed by Zhu (1993). Among the combinations, in addition to PRO, GBC, and 4ME, there were significant or very significant differences in average heterosis among the other six glucosinolates. The mean heterobeltiosis of PRO, RAA, 4ME, GBC, NEO and the total indole glucosinolates was significantly less than 0. Combining this two factors, the other six glucosinolates has obvious heterosis except PRO, GBC and 4ME.

DISCUSSION

After combining ability analysis, in this study, all of the glucosinolate components were both controlled by the additive effect and dominant effect, and this result was just opposite to the conclusion obtained by Hu (1987) in *Brassica napus*. He concluded that the geneticeffect of the total glucosinolates fit in with the additive-dominant model, the additive effect was more important than the dominant effect. This difference may be due to the different species.

The effect of Parental general combining ability is the basis in choosing parents, which is important in breeding but the hybrids derived from excellent parants may not be excellently hybridized, therefore, the breeder must consider both the general combining ability and the special combining ability in order to make a correct assessment of a combination. Among these 15 combinations obtained in this study, 1 × 2 was a perfect combination. On the one hand, the RAA (anti-cancer) content of the their parents was highest and with the lowest content of PRO (result in goiter); on the other hand, the RAA content of the hybrid was the highest, and their content were 3.18 μmol/g FW, which were higher than the anti-cancer vegetables broccoli (0.29 - 0.88 μmol/g) and purple cabbage (0.47 - 0.67 μmol/g) that was popular internationally (Rosa , 1997). At the same time, the SCA and GCA effect of the combinations and their parents in RAA were the highest. So this combination was a desirable combination.

The heritability affects the scale of the hybrid and offspring directiy, and also impact the selection time, methods, the environment in hybrids. The application of heritability has played a positive role in improving breeding efficiency. The broad sense heritability of the glucosinolates was relatively higher in Chinese Kale in this study, and the narrow sense heritability was lower, so the breeder can choose high- RAA hybrid varieties by heterosis breeding.

We concluded that, in addition to PRO, GBC, and 4ME, the other six glucosinolate components showed significant heterosis. The heterosis application is an effective and important measure to improve the yield and quality, and it is a widespread biological phenomenon. However, not all of the hybrid combinations have heterosis due to the different species and genetic differences among different characters and the interaction between these genes. So it needs to analyse specific species and specific traits in order to obtain the instructive conclusions in breeding.

With the development of nutrition and epidemiology, "diet and health" becomes more and more popular, it is particularly important to research and develop bioactive substances, and how to cultivate a vegetable variety with high content of bioactive substances is a collar job. In this paper, the rule of inheritance of glucosinolates in Chinese Kale was explored preliminarily, which provided a basis for developing high RAA varieties.

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