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DIALLEL ANALYSIS OF EIGHT NUTRIENT QUALITY RELATED CHARACTERS IN THREE ECOTYPES OF CHINESE CABBAGE

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Key words: *Brassica pekinensis*, Nutrient quality, Genetic effect, Heritability, Diallel cross

Abstract

A four-year experiment with advanced inbred lines of different ecotypes of Chinese cabbage revealed that the levels of Ca, Fe, K, dry matter and crude fiber in the cabbage head fit to additive dominant model, with the narrow-sense heritability being 4.7, 21.0, 41.8, 16.9 and 13.3%, respectively. The concentrations of Zn, ascorbic acid and amino acid fit to additive dominant model with maternal effects, their narrow-sense heritability accounted for 31.8, 58.4 and 59.2%, respectively. Zinc, potassium, ascorbic acid and amino acid possessed relatively high narrow-sense heritability, they can be selected in early generations; the other nutritional characters had low heritability and seriously suffered from environmental effects, so they suited to be selected in late generations. Calcium level was significantly correlated with Fe, Zn and amino acid, with the related coefficients being 0.362, 0.176 and 0.180, respectively. Moreover, the dry matter in the cabbage head has got a negative significant correlation with crude fiber (–0.207), but the ascorbic acid had a positive significant correlation with amino acid. The results can serve as an important basis for parent selection and combination pairing in quality breeding of Chinese cabbage.

Introduction

Chinese cabbage (*Brassica pekinensis*) which contains vitamins and more mineral nutrients is a good source of Fe, Ca, K and other elements needed for human health (Ensminger *et al.* 1983). Improvement of nutrient quality of Chinese cabbage has been a concern of breeders worldwide. An FAO survey stated that the Ca, Fe, Mn, Zn and other microelements in people's food and drink of most developing countries were seriously deficient. Therefore, finding mineral-rich germplasm and breeding new varieties containing high levels of minerals become an effective remedy for the nutrition crisis. So it is of great importance to carry nutritional quality breeding of Chinese cabbage, mainly aiming at increase of mineral concentration.

Minerals (such as K, Ca and Mg) are not only the key factors in physiological and biochemical reaction of plants but also the important portion of plant genes and enzymes (such as P, Fe, Mn, Zn and Cu) (Yu and Tang 1998). Hayman (1954, 1960) advanced some diallel cross models in his articles. According to Griffging (1956), some authors including Yu *et al.* (2005), Zhao *et al.* (2006) and Yang *et al.* (2008) analysed the genetic correlations among crude fiber, protein, soluble sugar and other important nutrients in Chinese cabbage. On account of Cockerham's (1980) broad-sense genetic modeling principles, Zhu (1993) and Mo (1993) proposed ADM (additive dominant model) and ADMME (additive dominant model with maternal effects) which were successfully used in rice (Shen 1997, Lin *et al.* 2011, Zhu 2012), cotton (Xu 1999) and Maize (Zhou 2011). To date, little has been published concerning genetic effects of minerals in Chinese cabbage. In breeding practice, unclear knowledge of genetic laws of minerals in Chinese cabbage made a blind choice of parents and hybrid offspring, resulting in a

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low breeding efficiency. In addition, the objectives of dominant breeding have been done to date in Chinese cabbage did not yet touch upon minerals'levels. Consequently, six advanced selfed lines of three ecotypes of Chinese cabbage were chosen. Diallal crosses were made for analysis of genetic effects and genetic correlations among Ca, Fe, Zn, K, ascorbic acid, dry matter, crude fiber and amino acid, so as to find out the genetic laws of these nutrient quality related characters and the effects of genotypes in interaction with environments (years) on the production of eight characters, providing a theoretical basis for quality breeding of Chinese cabbage.

Materials and Methods

Six advanced inbred lines from three ecotypes were selected. Experiments were conducted at the Institute of Vegetable Crops, Shandong Academy of Agricultural Sciences, Jinan. On November 10, 2005, the standard maternal plants from six parents were chosen and transplanted in a greenhouse. In spring 2006, a single-plant inbreeding was carried out, with pollination and seed collecting being done in accordance with complete diallel cross, so the seeds of 30 combinations from six parents were obtained. The materials got in spring were seeded on August 15 the same year and on August 15, 2008, respectively. Plots were arranged in a randomized complete-block design, with three replications, 14 plants in each plot, 40 cm apart within rows and 60 cm between rows. As field managements, precautions were taken against flood and drought at the seedling stage, heavily fertilize nitrogen and potassium and in-time irrigate at the early-heading stage, pay attention to timely disease and insect pests control at the growing stage. At the post-heading stage, three plants were harvested from each plot and the samples were gained by quartering. After being dried, the samples were mixed thoroughly and determined for Ca, Fe, Zn and K by atomic absorption spectrophotometry, for ascorbic acid by 2.6-dichloro-indophenolsodiummetry, for dry matter by oven-drying and weighing, for crude fiber by acid-base washing, for amino acid by an amino acid analyzer Hitachi 83550, respectively. Sampling and determination were duplicated three times.

Table 1. Head shape, original material and ecotype of six advanced inbred lines of Chinese cabbage.

Inbred line	Code	Head shape	Original material	Ecotype
Xinfu474	P1	Oval	Fushanbaotou	Marine climatic
Xinfu1042	P2	Oval	Fushanerbaotou	"
200-95	P3	Flat	Shite	"
Wei214	P4	Flat	Weigu	"
99-682	P5	Cylindric	Tianjinqingmaye	Interlaced climatic
99-683	P6	Cylindric	Tianjinlu	"

Determination of minerals in soil of the plots in 2006 and 2008 indicated that the concentrations had no significant difference in different years (Table 2), suggesting the mineral contents unchanged with years under the conditions of uniform field managements.

Genetic effects of eight nutritional characters in Chinese cabbage were analysed with the help of ADM or ADMME provided by Zhu (1993) and Mo (1993), respectively. If we intend randomly draw p number of homozygote samples from a certain genetic group and use them as parents for diallel cross to produce a group of cross combinations, so this genetic model can be shown by the following linear formula: $Yijk = u+Eh+Ai+Aj+Dij+Mi+AEhi+AEhj+DEhij+MEhi+Bk(h)+ehijk$

Here the yijk is the mean phenotypic value in k block number of the cross combination with i as femaleparent and j as male parent; u stands for the mean value of a group, Ai or Aj for the accumulative additive effect, Dij for the accumulative dominant effect and Mij for the accumulative maternal effect; AEhi or AEhj is a mutual effect of additivity × environment, with the DEhij being the mutual effect of dominance × environment, and MEhi being the mutual effect of female parent × environment; Bk(h) represents the block effect in a certain environment and eij serves as the residual effect.

Table 2. Mineral concentrations in soil of the plots for planting Chinese cabbage in 2006 and 2008.

Year	Ca (mg/100mg DW)	Fe (mg/kg DW)	Zn (mg/kg DW)	K (mg/100 mg DW)
2006	0.498 a*	19.2 a	16.60 a	0.0354 a
2008	0.494 a	22.1 a	20.62 a	0.0362 a

*Figures followed by the same letters within columns mean significant at p = 0.05 level.

The phenotypic variance (yhijk) is : $\sigma y^2 = 2\sigma^2_A+\sigma^2_D+\sigma^2_M+2\sigma^2_{AE}+2\sigma^2_{DE}+2\sigma^2_{ME}+\sigma^2_B+\sigma^2_e$

With help of MINQUE (minimum norm quadratic unbiased estimation), the ADM or ADMME which includes mutual effects of female parent × environment can be used for any nutritional character to unbiasedly estimate its genetic variance component ($V_P = V_A+V_D+V_{AE}+V_{ED}+V_e$), heritability (=VA/Vp) and covariance component of genetic effects between paired characters. At the same time the LUP (linear unbiased prediction) or AUP (adjusted unbiased prediction) can be applied to predict various genetic effect values including additive effect value (V_A), dominant effect value (V_D), maternal effect value (V_M) and others, and predict interaction of these genetic effects × environment effect. The above mentioned predictive results were used to assess the breeding value of parents and their combinations, and evaluate the vigor of hybrids. All the data were treated by use of Zhu Jun's software.

Results and Discussion

Mean values of the eight characters in Chinese cabbage of six parents and the results of ANOVA are listed in Table 3. ANOVA suggested that there existed a significant or extremely significant difference in levels of the eight characters among parents.

Results (Table 4) showed that the genetic maternal effects of Zn, ascorbic acid and amino acid analysed by ADMME reached significant or very significant levels, but that of Ca, Fe, K, crude fiber and dry matter analysed by ADM had no significance in levels.

It can be seen from Table 4 that the estimated variance of the additive and dominant effects of Ca, Fe, Zn, ascorbic acid, dry matter and crude fiber reach significant or very significant levels ($p > 0.01$) , that of additive, dominant and maternal effects of Zn, ascorbic acid and amino acid reach very significant levels, suggesting that the heredity of the latter three is controlled by genes of both the cell nucleus and cytoplasm, that of the other five nutritional characters is controlled only by the nucleus. The residual variance values of all the eight nutritional characters have attained very significant levels of P at $p > 0.01$), demonstrating all these characters are affected not only by inheritance but also by environment conditions.

It also can be seen from Table 4 that for the eight characters, both the narrow heritability (h^2_N) acted by additive effect and the broad heritability (h^2_B) acted by both additive and dominant effects have got significant or very significant levels, manifesting their productivity is inherited to the progeny of Chinese cabbage mainly via cell nuclei. A further comparison of the eight characters revealed that the narrow heritability of Zn, K, ascorbic acid and amino acid was relatively high, suitable for early generation selection; the other four characters which got relatively low direct heritability can be selected in advanced lines, moreover, single plant selection may exert a certain effect on their improvement.

Table 3. Mean values of eight nutrient quality related characters in Chinese cabbage of six parents.

Parent	Ca (mg/100 mg DW)	Fe (mg/100 mg DW)	Zn (mg/kg DW)	K (mg/100 mg DW)	Ascorbic acid (mg/ 100g FW)	Dry matter (g/100 g FW)	Crude fiber (mg/100 mg DW)	Amino acid (mg/100 mg DW)
P1	0.776^{B}*	0.045^{cD}	50.96^{aA}	1.62^{bB}	13.82dC	6.34bB	12.51aA	10.77eD
P2	0.833^{B}	0.059^{bBC}	43.81^{cC}	1.53^{cC}	19.92bcB	5.40dC	12.58aA	13.50cdBC
P3	0.966^{A}	0.060^{bB}	45.35^{cC}	1.41dD	20.93bB	4.93eD	11.14bb	13.34cdBC
P4	0.826^{B}	$0.052b^{cBCD}$	34.91^{dD}	1.64bAB	19.90cB	6.06cB	10.10bB	14.48bcBC
P5	0.818^{B}	0.047^{cCD}	47.78^{bB}	1.45dD	23.68aA	6.92aA	10.38bB	16.15aA
P6	0.831^{B}	0.077^{aA}	32.20^{eE}	1.69aA	18.44cB	7.05aA	10.27bB	14.87bAB

*Each value is the average of six measurements taken during two years. The followed small and capital letters stand for significance at $p \leq 0.05$ and 0.01, respectively.

Table 4. The estimated genetic variances and heritability components of eight nutrient quality related characters in Chinese cabbage.

Component of variance	Ca	Fe	Zn	K	Ascorbic acid	Dry matter	Crude fiber	Amino acid
V_A/V_P	0.047*	0.210**	0.147**	0.418**	0.460**	0.169**	0.133*	0.389**
V_D/V_P	0.141**	0.169**	0.139**	0.005	0.042**	0.277**	0.259**	0.011+
V_M/V_P	0.000	0.000	0.171**	0.000	0.124**	0.000	0.000	0.202**
V_{AE}/V_P	0.000	0.048*	0.000	0.000	0.021+	0.000	0.038	0.046**
V_{DE}/V_P	0.362**	0.188*	0.418**	0.301**	0.180**	0.330**	0.219	0.148**
V_{ME}/V_P	0.000	0.000	0.034+	0.000	0.000	0.000	0.000	0.169**
V_e/V_P	0.449**	0.382**	0.088**	0.274**	0.031**	0.222**	0.348**	0.032**

Significance of ANOVA is at $p \leq 0.1$(+) or 0.05(*) or 0.01(**) levels. The abbreviations in left column stand for : V_A = additive variance, Vp = phenotypic variance, V_D= dominant variance, V_M = maternal variance, V_e = error variance, h^2_N = heritability in narrow sense, h^2_B = heritability in broad sense.

The potential breeding values of varieties can be achieved after analysis of the genetic effect values of their parents. The additive effects of eight characters in six parents and the predicted values of dominant effects in their combinations are listed in Table 5 from which we can obtain following information.

Parent P4 has got a very significant additive predicted value for Ca, proving P4 is an ideal parent for improvement of Ca level. Combinations P2×P4, P2×P5, P4×P5, P4×P6 and P5×P6 had significant or extremely significant significant dominant effects, illustrating they are good for increase of Ca level. Parents P2, P3 and P6 reached very significant levels in Fe, and their combinations P1×P2, P1×P6, P2×P3, P2×P4, P2×P5, P3×P4, P4×P5 and P5×P6 also came up to a significance at 5% or 1% level of predicted random dominant effect. For Zn, the P1, P2 and P3

Table 5. Predicted genetic effects of eight nutrient quality related characters in parents and their F_1 of Chinese cabbage.

Parent and combination	Ca	Fe	Zn	K	Ascorbic acid	Dry matter	Crude fiber	Amino acid
P1	–0.035**	-0.005**	1.233*	0.0094	–2.791**	0.068*	0.322**	–1.231**
P2	–0.008*	0.003**	1.271**	–0.0113 *	0.357**	–0.152**	0.225*	–0.362*
P3	0.001	0.002*	1.616 **	–0.0644 **	1.052**	–0.288**	0.204**	–0.358**
P4	0.049**	–0.001*	–3.346**	0.0248 **	0.698**	0.049*	–0.333**	–0.361**
P5	0.007	–0.004**	0.567+	–0.0340 **	1.466**	0.071**	–0.245**	1.078**
P6	–0.013 *	0.006**	–1.341*	0.0754 **	–0.782**	0.253**	–0.174*	1.234**
D12	0.049	0.003*	5.891**	–0.0002	–1.024**	0.066*	0.560**	0.223
D13	–0.025 *	–0.0003	–0.416	–0.0075	1.010**	0.107**	–0.311+	0.144
D14	0.023	–0.002*	–2.061**	–0.0014	–0.061	0.259**	–0.197+	0.052
D15	–0.047 +	–0.0003	–2.789**	0.0045	0.730**	–0.213**	–0.536**	0.428
D16	0.039	0.005*	2.104**	0.0077	0.631**	0.047	1.548**	0.011
D23	–0.041 *	0.011**	–2.176**	0.0063	0.250	0.458**	0.211 +	–0.231
D24	0.107 **	0.004**	2.912**	-0.0044	0.745**	0.016	0.448**	0.201
D25	0.034 **	0.003*	–0.889 +	0.0105	–0.848**	–0.129*	–0.077	–0.142
D26	–0.063 *	–0.0004	–0.419	0.0056	0.047	0.435**	–0.901**	–0.061
D34	0.022	0.008**	3.393*	0.0097	0.799**	0.204**	0.721 *	–0.036 +
D35	0.028	–0.001	1.009	-0.0001	–0.377**	0.382**	1.078**	0.063
D36	–0.043**	–0.005*	2.660**	0.0078	–0.242*	–0.142*	1.204**	0.248
D45	0.127**	0.003*	–0.738**	0.0024	–0.456**	–0.095	0.375**	–0.088
D46	0.090**	–0.001	2.534**	0.0015	–0.497*	0.285**	–0.271	0.173
D56	0.042 *	0.004*	1.396*	0.0093	0.361*	–0.724**	0.313*	0.004

+, *, ** Significant at $p > 0.1$, $p > 0.05$ or $p > 0.01\%$, respectively.

achieved significant or very significant levels of predicted dominant value; meanwhile, for ascorbic acid, the P2, P3, P4 and P5 got the same. The related combinations P1 × P3, P1 × P5, P1 × P6, P2 × P4, P3 × P4 and P5 × P6 had 5% or 1% significant level of genetic dominant effect value for ascorbic acid, indicating there existed a marked heterosis between crossed different parent lines. Concerning dry matter, parents P1, P4, P5 and P6 achieved significant or very significant level of predicted additive effect, and the related cross combinations P1 × P2, P1 × P3, P1 × P4, P2 × P3, P2 × P6, P3 × P4, P3 × P5 and P4 × P6 appeared dominant in heterozygosity. For crude fiber, parents P4, P5 and P6 had significant or very significant levels of negative additive effect, the combinations P1 × P5 and P2 × P5 got negative dominant effect, so they are ideal parents for decrease of fiber level. There existed a significant additive effect for P4 and P6 in K, and for P5 and P6 in amino acid, but all the combinations did not get a significant level of random dominant effect, illustrating they have no apparent hybrid vigor in K and amino acid.

Generally, the contents in progeny can be increased by cross with P1 for zinc and dry matter, with P2 or P3 for both Fe and ascorbic acid, with P4 for Ca and K, with P5 or P6 for crude fiber and amino acid.

Table 6. Covariance and correlation between paired nutritional characters in Chinese cabbage.

Paired character	Covariance of phenotype (Cp)	Covariance of genotype (Cg)	Phenotypic correlation (rP)	Genotypic correlation (rG)
Ca and Fe	0.00025**	0.00051**	0.10651**	0.36154**
Ca and Zn	0.09239+	0.15333**	0.07138+	0.17599**
Ca and K	0.00033	–0.00041	0.01714	–0.03376
Ca and ascorbic acid	0.00301	0.06376**	0.00592	0.20006
Ca and dry matter	–0.00135	0.002018	–0.01129	0.02562
Ca and crude fiber	0.00895	0.00370	0.03538	0.02445
Ca and amino acid	0.03964**	0.03082*	0.1404 **	0.18004**
Fe and Zn	–0.00551	–0.01244**	–0.05908	–0.18715*
Fe and K	0.00026**	0.00028**	0.19138**	0.30026**
Fe and ascorbic acid	0.00230*	0.00136	0.06280*	0.05611
Fe and dry matter	0.00082**	0.00059*	0.09534**	0.09866*
Fe and crude fiber	0.00047	0.00192**	0.02578	0.16640*
Fe and amino acid	0.00119+	0.00169*	0.05884+	0.12982*
Zn and K	–0.06854*	–0.06677+	–0.09134*	–0.11518*
Zn and ascorbic acid	–1.54314*	–1.08326	–0.07887*	–0.07232+
Zn and dry matter	–0.40060*	–0.40827*	–0.08651*	–0.11025*
Zn and crude fiber	4.4621**	4.7797**	0.45848**	0.67100**
Zn and amino acid	0.23072	–0.96807**	0.02124	–0.12031+
K and ascorbic acid	–0.07783**	–0.0660**	–0.26392**	–0.31133**
K and dry matter	0.01865**	0.01766**	0.26720**	0.33697**
K and crude fiber	0.01450+	0.00232	0.09888+	0.02309
K and amino acid	0.02697*	0.02105	0.16481*	0.18477+
Vc and dry matter	–0.30906**	–0.27593**	–0.16982**	–0.20369**
Vc and crude fiber	0.01582	–0.14411**	0.00413	–0.05530
Vc and amino acid	1.59736**	1.77856**	0.37426**	0.60421**
Dry matter and crude fiber	–0.14099**	–0.13348**	–0.15574**	–0.20719**
Dry matter and amino acid	0.19143**	0.10731	0.18952**	0.14745+
Crude fiber and amino acid	–0.38054*	–0.26447	–0.17924*	–0.18892

+ = $p \leq 0.1$, *= $p \leq 0.05$, ** = $p \leq 0.0$.

The MINQUE could be adopted for unbiased estimation of covariances and related coefficients of genetic effects in paired characters. Analysis of genetic correlation (Table 6) made clear that Ca has a significant positive correlation with Fe, Zn and amino acid; Fe has the same correlation with K, dry matter, crude fiber and amino acid; but there exists a significant negative correlation between Zn : K, K: ascorbic acid, dry matter: ascorbic acid, dry matter: crude fiber. Moreover, Zn, K and ascorbic acid are very significantly correlated with crude fiber, dry matter and amino acid, respectively. The results suggested that with increase of Ca, Zn, ascorbic acid and crude fiber, the dry matter and amino acid could be increased, but the Fe and K might fall down in concentration. The fact that there exists a very significant negative correlation would bring unfavourable effects on simultaneous improvement of the two characters in Chinese cabbage breeding.

Griffing's combining ability model and his analytic method are based on ANOVA. For a given diallel cross material, Griffing's analytic method fails to estimate combining ability of parents after which further estimate combining variance components and heritability. Researchers usually assess heritability using Griffing'sdiallel analytic method then calculate genetic variance components and heritability adopting the diallel analytic method created by Hayman (1954). Since the two genetic models and the two analytic methods are different, so the analytic results got by them may be less than uniform. For this reason, we employed ADM and ADMME along with MINQUE to estimate genetic variance and heritability, and predict genetic effect values, thereby to evaluate the genetic performance of parents and combinations. The results demonstrated that the additive and maternal effects of Zn, ascorbic acid and amino acid in Chinese cabbage were significant, with the narrow heritabilities being 31.8, 58.4 and 59.2%, respectively. For the three characters, a marked hybrid vigor has been got in progeny from cross between different ecotypes. For ascorbic acid, the result gained by us was inconsistent to that got by Zhang et al. (1998) who stated the ascorbic acid in Chinese cabbage fit the ADM. Judging from this, each character in different plant materials might perform inconsistent under different environment conditions. The genetic effects of Ca, Fe, K, crude fiber and dry matter were not significant, with the narrow heritability being 4.7, 21.0, 41.8, 16.9 and 13.3%, respectively. The ascorbic acid in progeny crossed from different ecotypes got a relative strong hybrid vigor.

Concerning selection of parent lines, there exist yet some problems such as high prime costs and time-consuming in measurement of nutrient levels of Chinese cabbage. In breeding practice, estimation of genetic variance components and heritability could provide a basis for knowledge of the genetic effect and relative contribution of each nutritional character, which favors to work out a scheme for effective selection of parents and for selective breeding of cross combinations. Our treatments made clear that the Ca, Fe, crude fiber and dry matter in Chinese cabbage had a lower heritability, they could be greatly affected by environment thus unreliable to be selected in early generations; while the Zn, K, ascorbic acid and amino acid got a higher heritability, possessed a marked additive effect thus some fine single plants with high levels of these nutrients could be selected in early generations. In practice, one can use the parent line especially rich in some nutritional characters to be combined with that which owns fine comprehensive characters, so as to get some ideal cross combinations. If we can find out the correlations between these characters, their heritabilities can be used as indirect indices of selection in order to increase selective effects of characters with low heritability. Our research proved that Ca had a significant positive correlation with Fe, Zn and amino acid; meanwhile, Fe had a significant negative correlation with Zn but had a significant positive correlation with K, dry matter and amino acid. Furthermore, there appeared a significant negative correlation between dry matter and crude fiber, but a significant positive correlation between ascorbic acid and amino acid. The results just mentioned can serve as an important basis for selection and combination of parents. It is an important goal of quality breeding to properly heighten levels of ascorbic acid, amino acid and dry matter, and to reduce crude fiber level.

Present work disclosed again that besides genetic control, the environment also greatly affected the contents of nutritional characters in Chinese cabbage, which fit the results reported by Zhang (2000) in black rice. It is one target of quality breeding for Chinese cabbage to create heritability-stable and adaptability-broad varieties which should adapt harmful environment variation and maintain relatively high contents of nutrients in different areas and years. For this reason, one can employ the nutrient-richparents in advanced lines to screen environment-adaptable materials and carry again a directional selection for several consecutive year in succetion thus obtain excellent progeny F_1.

For selection of materials, we paid attention to hire those that greatly differ in nutritional quality among ecotypes to make combinations, then evaluate the genetic effects of main nutritional characters in the gained progeny along with the genetic correlation between characters and the genetic effects of various parents and their combinations, as a result to provide a theoretic basis for quality breeding of Chinese cabbage. Although the used materials covered all the three ecotypes, they were yet slightly less in quantity, so the results obtained by us need a further study to be proved.

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