Genetic analysis of leaf expansion trait in *Brassica* rapa L. ssp. chinensis (L.) Hanelt

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Abstract—Brassica rapa L. ssp. chinensis (L.) Hanelt is an important vegetable in eastern Asia. Its leaf area is one of main factor of influencing on the yield of individual plant. To reveal the mechanism of the leaf expansion, the joint segregation analysis of multiple generations $(P_1, P_2, F_1, B_1, B_2 \text{ and } F_2)$ was used to analyze the genetics of leaf expansion trait in combination SW-13×L-118 of Brassica rapa L. ssp. chinensis (L.) Hanelt. In this paper, the results showed that the leaf expansion trait was controlled by one pair of additive-dominant major genes plus additive-dominant-epistatic polygene (D model). The additive effect of the major gene was -2.3061, the dominant effect was -1.4525. The heritability of major gene of leaf expansion was 2.56% in B₁ generation, 1.55% in B₂ generation and 12.12% in F₂ generation. The heritabilites of the polygene were 54.04% in B₁ generation, 25.11% in B_2 generation, and 62.60% in F_2 generation, respectively. It appeared that the polygene effects should be given prior to the improvement of leaf expansion trait of combination SW-13×L-118, and the selection was effective in later generation, and the negative additive effect and negative dominant effect of major gene were noticed.

Keywords—Brassica rapa L. ssp. chinensis (L.) Hanelt; leaf expansion; major gene plus polygene; genetic model

I. INTRODUCTION

Brassica rapa L. ssp. *chinensis* (L.) Hanelt, known as nonheading Chinese cabbage in China, is an important vegetable in eastern Asia [1]. The leaf expansion of *Brassica rapa* L. ssp. *chinensis* (L.) Hanelt, belonging to quantitative trait, is one of important agronomy trait closely related to yield since its rosette leaves are major edible position. It can provide instruction for the genetic improvement of yield of *Brassica rapa* L. ssp. *chinensis* (L.) to analyze genetic system of the leaf expansion.

The quantitative traits were controlled by a stack of minipolygene according to traditional quantitative genetics [2]. Although traditional quantitative genetics can estimate integrated gene effects as a whole, it can not discriminate the individual gene effects and the differences among different gene effects [3]. Moreover, the results of QTL mappings Xue-bin Li³, Chao-jun He¹ 3. Henan Institute of Science and Technology, Xinxiang 453003, P. R. China

showed there also existed many differences in gene effect size of controlling quantitative traits [4]. In fact, many quantitative traits existed both grouping trend and illegible sideline phenomena in separate generation, which indicated that the genes of quantitative traits are controlled by some major genes with larger genetic effects and some minor polygenes with smaller effect [5]. But the mixed major gene and polygene inheritance model can be used to evaluate the genetic effects of individual major genes and the collective genetic effects of polygenes [6-8]. The general idea of this method is: 5 types, 24 kinds of genetic models, included one pair of gene (additive-dominant, additive, complete dominant and negative complete dominant), two pair of major genes (additivedominant-epistasis, additive-dominant, equal additive, complete dominant and equal dominant), polygenes (complete model, additive-dominant), one pair of major gene plus plolygene and two pairs of major genes plus polygenes, coordinate phenotypical distributions of quantitative traits derived from various populations, from which the optimum model is selected, then the genetic parameters of major gene and polygenes are estimated.

At present, the procedure was widely applied to the genetic analysis of soybean [9-10], rice [11-12], cotton [13-14], wheat [15], adzuki bean [16], rape [17-19] *et al*, whereas it is not reported in the leaf expansion of *Brassica rapa* L. ssp. *chinensis* (L.) Hanelt. In this paper, the genetic system of leaf expansion of *Brassica rapa* L. ssp. *chinensis* (L.) Hanelt was analyzed using the mixed major gene plus polygene joint segregation analysis of multiple generations to identify the gene effect controlled the leaf area genetic, and to provide theoretical reference for the improvement of leaf area.

II. MATERIALS AND RESULTS

1. Materials and genetic design

2 inbred-lines SW-13 (P₁) and L-118 (P₂) came from Nanjing Agriculture University (NAU). Parents P₁ and P₂ were plant in Experimental Farm of NAU in autumn of 2004, bagged P₁ and P₂, inbreded and crossbreeded on P₁ and P₂ at early

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blooming in spring of 2004, and harvested F_1 hybrids after the seeds were matured. $P_1 \, \times \, P_2$ and F_1 were inbreded, backcrosseded P_1 to F_1 and P_2 to F_1 in spring of 2005, respectively. 20 $P_1 \times 20 P_2$, 20 F_1 , 98 B_1 , 120 B_2 and 91 F_2 were transplanted in spring of 2006. Plant and row spacing was 18 cm \times 20 cm. The leaf-expansion of the largest leaf was investigated when after transplanted 60 days.

2. Date analysis

The mixed major gene plus polygene joint segregation analysis of six generations was used to analyze the leaf expansion of combination SW-13×L-118. The parameters of generations and component distributions were estimated by maximum likelihood method and IECM (Iterated expectation and conditional maximization). The relative optimum model was selected by AIC value (Akaike's information criterion), and tested a set of goodness of fits (including homogeneity $U_1^2 \\limbda U_2^2$ and U_3^2 tests, Smirnov test ($_nW^2$) and Kolmogorov test (D^n)), hereby, selected the most optimum model. The effects of genes were estimated by parameters of component distributions using least squares method according to the optimum genetic model, then estimated genetic variances.

Heritability of major gene:

$$h_{mg}^2 = \frac{\sigma_{mg}^2}{\sigma_{p}^2} \times \%$$
(1)

Heritability of polygene:

$$h_{pg}^{2} = \frac{\sigma_{pg}^{2}}{\sigma_{p}^{2}} \times \%$$
⁽²⁾

 σ_{mg}^2 : Major gene variance. σ_{pg}^2 : Polygene variance. σ_p^2 : Phenotypic variance.

III. RESULTS AND ANALYSIS

A. The frequency distributions of the leaf expansion of six generations

According to the frequency distributions of the leaf expansion of P_1 , P_2 , F_1 , B_1 , B_2 and F_2 of six generations derived from combination SW-13×L-118, the leaf expansion of B_1 , B_2 and F_2 generations appeared skewness of unimodal distribution, which indicated that there might be mixture of multi-distributions (Table 1, Figure 1).

B. Genetic model of leaf expansion

The maximum likelihood estimators and AIC values of 5 types, 24 kinds of genetic models, included one major gene (A), two major genes (B), polygenes (C), one major gene plus

plolygene (D) and two major genes plus polygenes (E), were acquired by IECM arithmetic to jointly analyse the leaf expansion of six generations from combination SW-13×L-118 using the mixed major gene plus polygene inheritance model (Table 2). According to AIC criterion, model C and model D became alternative genetic model with smaller AIC value.

The model C and model D were further tested by a set of tests of goodness of fit $(U_1^2, U_2^2, U_3^2, {}_nW^2 \text{ and } D^n)$ (Table 3). The results showed that 5 statistics in model C and model D reached significance level among 30 statistics. The AIC value of Model D was smaller compared with model C, which indicated that the data types of leaf expansion from combination SW-13×L-118 most approached the theoretic distribution of model D with one additive-dominant major gene plus additive-dominant-epistatic polygene. Hereby the mixed major gene plus polygenes inheritance model was the optimum genetic model of leaf expansion trait.

C. Estimation of genetic parameters

The genetic parameters of leaf expansion of combination SW-13×L-118 estimated by component distribution of model D showed that the additive effect of major gene of leaf expansion was -2.3061, the dominant effect was -1.4525. The heritability of major gene of leaf expansion was 2.56% in B₁ generation, 1.55% in B₂ generation and 12.12% in F₂ generation. The heritability of polygene of leaf expansion was 54.04% in B₁ generation, 25.11% in B₂ generation and 62.60% in F₂ generation (Table 4). The results indicated the leaf expansion traits of combination SW-13×L-118 was controlled by major gene and polygene. 74.73% of the phenotypic variance was determined by the effect of major gene plus polygene in F₂ generation, whereas 25.27% of the phenotypic variance was determined by environment factor.

IV. DISCUSSION

The leaf weight is one of main factor of influencing on the yield of individual plant of *Brassica rapa* L. ssp. *chinensis* (L.) Hanelt. Most varieties of *Brassica rapa* L. ssp. *chinensis* (L.) Hanelt is belonging to leaf-weight-type, and the expansion of leaf area is the important factor except for the petiole weight gain. Therefore, clarifying the genetic of the leaf area seems prominent in yield breeding of *Brassica rapa* L. ssp. *chinensis* (L.) Hanelt.

In this paper, six generation joint segregation analysis of genetic system of quantitative traits method was used to analyze the leaf expansion traits of combination SW-13×L-118. The results showed that the leaf expansion traits genetic was controlled by one major gene plus polygene, of which there were both the negative additive effect (-2.3061) and the negative dominant effect (-1.4525). The negative additive effect was almost twice as much as the negative dominant effect. The heritability of major gene of leaf expansion was 2.56% in B₁ generation, 1.55% in B₂ generation and 12.12% in F₂ generation. The heritability of the polygene was 62.60% in F2 generation.

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controlled by polygenes and the weaker major gene on the basis of genetic effects information. There was similar in the genetic of the largest leaf length and plant expansion of cabbage [20]. The mappings of QTLs also indicated that some QTLs were observed in the plant expansion of *Brassica rapa* L [21-23]. Hence, if the leaf expansion trait of *Brassica rapa* L. ssp. *chinensis* (L.) Hanelt. was improved, the higher heritability of polygene could be used. According to the results, it appeared that the polygene effects should be given prior to the improvement of leaf expansion trait of combination SW-13×L-118, and the selection was effective in later generation, and the negative additive effect and negative dominant effect of major gene were noticed.

There were three statistics in model C and model D reached significance level among 30 statistics, which was the reason why the experiment was lack of replication and resulted in error, the precision and credibility should be enhanced by six generations families, recombinant lines or DH population to reduce experimental errors.

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REFERENCES

- G. Eason, Jian-Feng Geng, Cheng-Song Zhu, Xiao-Wei Zhang, Yan Cheng, Yuan-Ming Zhang, and Xi-Lin Hou, "A Genetic Linkage Map of Nonheading Chinese Cabbage," J. Amer. Soc. Hort, vol 132, Jun. 2007, pp. 816–823.
- [2] Mather K, and Jink S J K, Biometrical genetics, 3rd ed., London: Chapman and Hall, 1982, pp. 65-103.
- [3] Guo-Ping Zeng, and Shou-Chun CAO, "Genetic studies of some important characters in non-heading Chinese cabbage II. Analysis of genetic effects and heritability for 15 agronomic characters," Journal of Nanjing Agricultural University, vol 21, Jan. 1998, pp. 31-35.
- [4] Kearsy M J, and Farquhar A G L, "QTL analysis in plants: where are we now?," Heredity, vol 80, 1998, pp. 137-142.
- [5] Jian-Kang Wang, and Jun-Yi Gai, "Identification of major gene and polygene mixed inheritance model and estimation of genetic parameters of a quantitative trait from F₂ progeny," Acta Genetica Sinica, vol 24, May 1997, pp. 432-440.
- [6] Jun-Yi Gai, Yuan-Ming Zhang, and Jian-Kang Wang, Genetic system of quantitative traits in plants, Beijing: Science Press, 2003.
- [7] Yuan-Ming Zhang, Jun-Yi Gai, and Jian-Kang Wang, "Identification of major gene and polygene mixed inheritance model and estimation of genetic parameters of a quantitative trait from B₁ and B₂ or F₂ progeny," Journal of Biomathematics, vol 15, Mar. 2000, pp. 358-366.

- [8] Wang J, Podlic D W, Cooper M, and Delacy I H, "Power of the joint segregation analysis method for testing mixed major-gene and polygene inheritance models of quantitative traits," Theor Appl Genet, vol 103, May 2001, pp. 804-816.
- [9] Zu-Dong Sun, and Jun-Yi Gai, "Studies on the inheritance of resistance to cotton worm *Prodenia litura(Fabricius)*," Acta Agronomica Sinica, vol 26, May 2000, pp. 342-346.
- [10] Hua Liu, Hui Wang, Qun Li, Peng Xu, Jun-Yi Gai, and De-Yue Yu, "Inheritance analysis and mapping QTLs related to cotton worm resistance in soybeans," Scientia Agricultura Sinica, vol 38, Jul. 2005, pp. 1369-1372.
- [11] Jian-She Wang, Jian-Kang Wang, Li-Hong Zhu, and Jun-Yi Gai, "Major-polygene effect analysis of resistance to bacterial blight (*Xanthomonas campestris* pv. oryzae) in rice," Acta Genetica Sinica, vol 27, Jan. 2000, pp. 34-38.
- [12] Qing-Yu Wang, Li-Hong Zhu, Jun-Yi Gai, and Jian-Kang Wang, "Analysis on the major gene and multigene mixed inheritance of wide compatibility gene in rice," Hereditas(Beijing), vol 26, Jun. 2004, pp. 898-902.
- [13] Jian-Mei Yin, Yao-Ting, Wu Xie-Fei Zhu, and Tian-Zhen Zhang, "Genetic analysis of yield traits and fiber qualities by using major gene plus polygene mixed inheritance model in upland cotton (*G. hirsutum* L.)," Cotton Science, vol 15, Feb. 2003, 1pp. 67-72.
- [14] Pei-Tong Zhang, Xie-Fei Zhu, Wang-Zhen Guo, Jing-Zhong Yu, and Tian-Zhen Zhang, "Genetic analysis of yield and its components for high yield cultivar Simian 3 in *G. hirsutum* L.," Acta Agronomica Sinica, vol 32, 2006, pp. 1011-1017.
- [15] Xiu-Xiu Ge, Li-Ping Zhang, Zhong-Hu He, and Yuan-Ming Zhang, "The mixed inheritance analysis of polyphenol oxidase activities in winter wheat," Acta Agronomica Sinica, vol 30, Jan. 2004, pp. 18-20.
- [16] Wen-Lin Jjn, Lu Bai, Zi-Xiang Wen, Shao-Jing Pu, and Bo Zhao, "Analysis on genetic system of 100-seed weight in adzuki bean," Acta Agronomica Sinica, vol 32, Sep. 2006, pp. 1410-1412.
- [17] Cun-Kou Qi, Jun-Yi Gai, Shou-Zhong Fu, Hui-Ming Pu, Jie-Fu Zhang, Xin-Jun Chen, and Jian-Qin Gao, "Analysis of genetic system of 1000seed weight in *Brassica napus* L.," Acta Agronomica Sinica, vol 30, Dec. 2004, pp. 1274-1277.
- [18] Hui Gu, and Cun-Kou Qi, "Genetic Analysis of Lodging Resistance with Mixed Model of Major Gene Plus Polygene in *Brassica napus* L.," Acta Agronomica Sinica, vol 34, Mar. 2008, pp. 376–381.
- [19] Ye Cong, Yong Cheng, Chong-Shun Zou, Xue-Kun Zhang, and Han-Zhong Wang, "Genetic analysis of waterlogging tolerance for germinated seeds of rapeseed(*Brassica napus* L.) with Mixed Model of Major Gene Plus Polygene," Acta Agronomica Sinica, vol 35, Aug. 2009, pp. 1462–1467.
- [20] Tiyun Miao, Yumei Liu, Zhiyuan Fang, Limei Yang, Mu Zhuang, Yangyong Zhang, Suxia Yuan, and Peitian Sun, "Genetic analysis of the main agronomic traits of DH population in *Brassica oleracea* var. *capitata*," Acta Horticuhurae Sinica, vol 35, Jan. 2008, pp. 59-64.
- [21] Song K M, Slocum M K, and Osborn T C, "Molecular marker analysis of genes controlling morphological variation in *Brassica campestris*(syn. *Rapa*)," Theoretical and Applied Genetics, vol 90, 1995, pp. 1-10.
- [22] Gang Lu, Jia-Shu Cao, Hang Cheng, and Xun Xiang, "QTLs mapping of some horticultural traits of chinese cabbage," Scientia Agricultura Sinica, vol 35 Aug. pp. 969-974.
- [23] Shuang-Cang Yu, Yong-Jian Wang, and Xiao-Ying Zheng, "Mapping and analysis of QTL controlling some morphological traits in Chinese Cabbage(*Brassica campestris* L. ssp. *Pekinensis*)," Acta Genetica Sinica, vol 30, Dec. 2003, pp. 1153-1160.



Figure 1 Frequency distribution of the leaf expansion of B1, B2 and F2 generations derived from SW-13×L-118

TABLE 1 THE FRENQUENCY DISTRIBUTIONS OF THE LEAF EXPANSION OF SIX GENERATIONS

Generation	Leaf expansion/cm ²								N	SD				
	9.5	13	16	19	22	25	28	31	34	37	40	43	IN SL	5D
P_1				2	6	7	5						20	3.10
P_2						6	7	3	3	1			20	3.25
\mathbf{F}_1									3	8	7	3	20	3.06
\mathbf{B}_1			9	12	26	20	19	7	4				98	4.46
B_2			3	7	48	32	22	8					120	3.43
F_2	8	11	19	21	16	6	5	2	2	1			91	5.85

N is the number of sample, and SD is the standard deviation.

 TABLE 2
 MAXINUM LIKEHOOD FUNCTION VALUES (MLV) AND AIC VALUES OF DIFFERENT GENETIC MODEL OBTAINED FROM IECM

 ALGORITHM

Model	MLV	AIC	Model	MLV	AIC
A-1	-1061.0856	2130.1711	D	-936.1020	1896.2040
A-2	-1071.3113	2148.6226	D-1	-970.1522	1958.3044
A-3	-1074.3636	2154.7273	D-2	-969.4483	1954.8966
A-4	-1004.3172	2014.6344	D-3	-970.3008	1956.6017
B-1	-949.7438	1919.4877	D-4	-972.9123	1961.8246
B-2	-1062.1445	2136.2891	Е	-934.6459	1905.2917
B-3	-1074.9996	2157.9993	E-1	-937.9789	1905.9578
B-4	-1071.7029	2149.4058	E-2	-1053.3987	2128.7974
B-5	-1074.3566	2156.7131	E-3	-1031.6104	2081.2207
B-6	-1074.3566	2154.7131	E-4	-1053.3988	2122.7976
С	-936.6601	1893.3202	E-5	-1053.3989	2124.7979
C-1	-1038.3398	2090.6797	E-6	-1053.4337	2122.8674

AIC: Akaike's information criterion

Model	Generation	U_1^2	$U_2^{\ 2}$	U_3^2	$_{n}W^{2}$	D _n
	P_1	0.047(0.8285)	0.123(0.7257)	0.318(0.5726)	0.0919	0.1774(p<0.05)
	P_2	0.009(0.9229)	0.080(0.7770)	0.574(0.4485)	0.0820	0.1492(p<0.05)
C	F_1	0.068(0.7944)	0.020(0.8863)	0.191(0.6618)	0.0748	0.1384(p<0.05)
C	B_1	0.004(0.9496)	0.032(0.8579)	0.222(0.6373)	0.0481	0.0882(p<0.05)
	B_2	0.120(0.7288)	0.052(0.8199)	0.187(0.6658)	0.3395	0.1278(p>0.05)
	F_2	0.394(0.5302)	0.620(0.4311)	0.516(0.4726)	0.1432	0.1108(p<0.05)
D	P ₁	0.055(0.8150)	0.188(0.6642)	0.690(0.4063)	0.1028	0.1854(p<0.05)
	P_2	0.011(0.9160)	0.131(0.7175)	1.079(0.2989)	0.0945	0.1585(p<0.05)
	F_1	0.077(0.7808)	0.010(0.9200)	0.457(0.4991)	0.0822	0.1400(p<0.05)
	B_1	0.004(0.9496)	0.032(0.8579)	0.222(0.6373)	0.0481	0.0882(p<0.05)
	B_2	0.186(0.6660)	0.184(0.6678)	0.002(0.9644)	0.3357	0.1312(p>0.05)
	F_2	0.394(0.5300)	0.583(0.4452)	0.386(0.5342)	0.1366	0.1087(p<0.05)

TABLE 3 TESTS FOR GOODNESS OF FIT OF MODEL D AND MODEL C OF LEAF EXPANSION

The value in brackets in U_1^2 , U_2^2 , U_3^2 column is the distribution value in theory; the critical value of $_nW^2$ is 0.461 at 5% significant level.

TABLE 4 ESTIMATES OF GENETIC PARAMETERS OF LEAF EXPANSION IN D MODEL

1 st order peremeter	Estimata	2 nd order perometer	Estimate				
i order parameter	Estimate	2 order parameter	B_1	\mathbf{B}_2	F_2		
ml	24.4561	σ_p^2	19.9205	11.7898	34.2120		
m2	37.3175	${\sigma_{ m mg}}^2$	0.5092	0.1822	4.1475		
m3	19.8439	$\sigma_{pg}{}^2$	10.7645	2.9608	21.4177		
m4	23.6105	σ_e^2	8.6468	8.6468	8.6468		
m5	21.8555	h _{mg} %	2.56	1.55	12.12		
m6	18.0111	h _{pg} %	54.04	25.11	62.60		
d	-2.3061	h_{mg} + h_{pg} %	56.59	26.66	74.73		
h	-1.4525						

d: Additive effect of major gene. h: Dominant effect of major gene.