

Genetic analysis of carbon metabolism-related traits in maize using mixed major and polygene models

Yang Wang^{†1}, Shuang Yang^{†2}, Muhammad Irfan^{1*}, Chunyu Zhang¹, Quan Sun¹, Suwen Wu³, Feng Lin^{1*}

¹Biotechnology and Bioscience College, Shenyang Agricultural University, Shenyang 110866, China

²Biotechnology Research Center, Shenyang Academy of Agricultural Sciences, Shenyang 110034, China

³College of Science Institute, Shenyang Agricultural University, Shenyang 110866, China

*Corresponding author: fenglinsn@126.com; mirfanashraf@yahoo.com

† Considered as first author

Abstract

Carbon metabolism is the foundation for crop growth, which has direct impact on the formation and transforming crop photosynthetic products, and also its yield and quality. In this study, the inheritance of the carbon metabolism-related traits including net-photosynthesis rate, chlorophyll contents, Phosphoenol pyruvate carboxylase activity and Sucrose Phosphate Synthase activity was analyzed by the joint segregation analysis of multiple generations P₁, P₂, F₁, and F_{2:3} from the cross Shen3336 × Shen3265 using the mixed major genes and polygenes inheritance models. Seeds of these generations were grown in three replications of randomized complete block design from year 2010-2012 and the data obtained was statistically analyzed for the selection of best fitting genetic model. The results showed that net-photosynthesis rate, chlorophyll contents, and SPS activity were all controlled by two major genes. The model E-1 and B-1 was best fit for net-photosynthesis rate, chlorophyll contents and SPS activity, respectively. The heritability for net-photosynthesis rate, chlorophyll contents, and SPS activity were 41%, 56.2% and 75.7% respectively. Major genes and polygenes were jointly controlled the net-photosynthesis rate. Both chlorophyll content and SPS activity was mainly controlled by two major genes. PEPC activity was controlled by polygenes with best-fitting model C-0 showing heritability of 46.3%. The present results were very meaningful to understand the inheritance characteristics of carbon metabolism in maize, and to guide the practical breeding.

Keywords: maize; mixed major genes and polygenes inheritance model; net-photosynthesis rate; chlorophyll content; SPS activity; PEPC activity.

Abbreviation: PEPC_Phosphoenol pyruvate carboxylase, SPS_Sucrose phosphate synthase, QTL_Quantitative trait locus, IECM_Iterated expectation and conditional maximization, AIC_Akaike's information criterion.

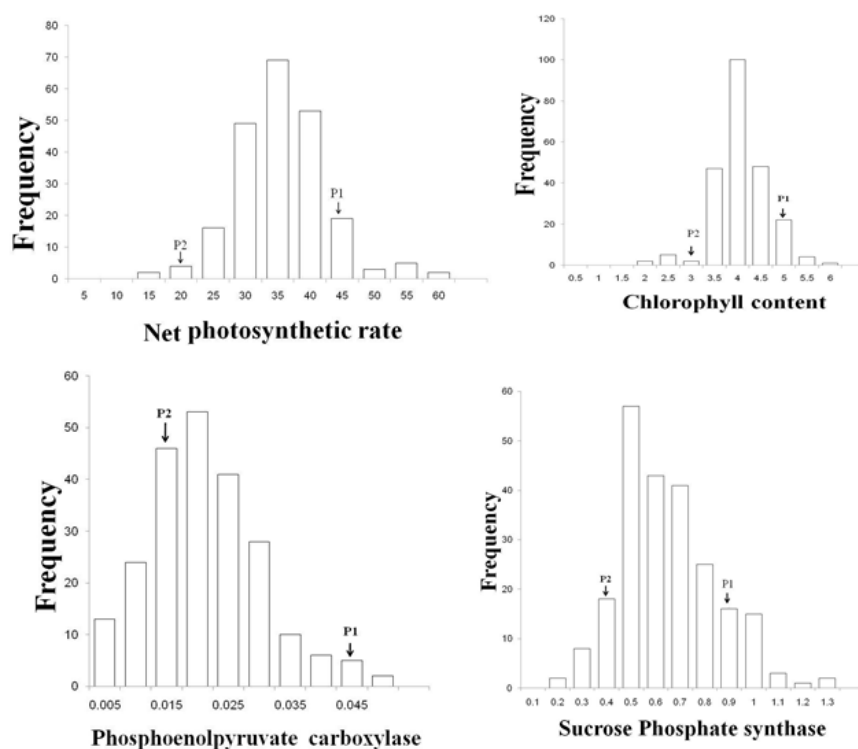
Introduction

Carbon is the basic essential nutrient for metabolic growth and development which affects the photosynthetic process in plants. Carbon metabolism directly influences the formation and transformation of photosynthate and also its yield and quality in plants. Photosynthesis, as an important part of carbon metabolism, is not only affected by quantity and quality of light, temperature, concentration of CO₂, water, mineral nutrients, and also chlorophyll contents, photosynthetic rates and enzymes. By using appropriate techniques, Zelith et al. (1982) found tightly relationship between net photosynthesis rate and yield. Photosynthetic rate showed a significant positive correlation with growth rate and grain yield during the filling stage in different wheat genotypes (Fischer et al., 1981). In maize, yield improvement might be partly due to the higher net photosynthesis rate (Dwyer et al., 1988). The photosynthetic performance and yield were affected by chlorophyll contents, a pigment which allows plants to absorb and transmit light energy (Ma et al., 1995). Chlorophyll contents and photosynthetic rate were closely related under high light intensity and the former had positively effect the later (Thomas et al., 2005; Nakazawa et al., 1990; Eihide and Shigesaburo, 1979). Bansal et al. (1999) reported that high chlorophyll contents, blade erection and slow aging had significantly

positive effects on rice yield. In addition, among all the photosynthesis enzymes, phosphoenolpyruvate carboxylase (PEPC) is one of the most important enzymes which played a vital role in enrichment of CO₂ for improving the photosynthetic efficiency in C₄ plants (Chollet et al., 1996). At present, research for maize PEPC is mainly focused on PEPC gene cloning and breeding. Using agrobacterium-mediated transformation, Maurice et al. (1999) transferred a PEPC gene from maize (C₄) to rice (C₃) and got equal or higher expression levels than that of in maize. In higher plants, sucrose is the main product of photosynthesis, the main form of carbon transport, and also the major metabolic substrate (Farrar et al., 2000). Sucrose phosphate synthase is one of the key enzymes required for the production of sucrose in various metabolic pathways. Harbron et al. (1981) suggested that sucrose phosphate synthase might be one of the important control points of sucrose biosynthetic pathway and its activity reflects the ability of biosynthetic pathway. Huber and Huber (1996) reported that SPS activity had negative correlation with starch accumulation, but had positive correlation with sucrose formation. Hence, SPS activity influences photosynthate distribution between starch and sucrose. The higher SPS activity, the more accumulation of sucrose and vice versa.

Table 1. Statistical analysis of carbon metabolism-related traits among 4 generations derived from shen3336 x shen3265.

Trait	Generation	Maximum	Minimum	Range	Mean	SD	CV%
Net photosynthesis rate	P ₁	47.0	35.7	11.3	42	3.22	7.6%
	P ₂	25.9	15.3	10.6	20.0	3.11	15.5%
	F ₁	38.5	25.3	13.2	30.8	4.03	13.1%
	F _{2:3}	56.2	13.6	42.6	33.1	6.91	28.8%
Chlorophyll content	P ₁	5.92	3.98	1.94	4.55	0.48	11%
	P ₂	3.77	2.75	1.02	3.0	0.29	9.7%
	F ₁	5.00	3.8	1.20	4.50	0.33	7%
	F _{2:3}	5.52	1.61	3.91	3.82	0.57	15%
PEPC activity	P ₁	0.05	0.03	0.02	0.045	0.008	17.8%
	P ₂	0.021	0.011	0.01	0.013	0.004	30.8%
	F ₁	0.06	0.03	0.03	0.05	0.009	18%
	F _{2:3}	0.05	0.001	0.049	0.019	0.01	52.9%
SPS activity	P ₁	0.998	0.696	0.303	0.854	0.097	11.4%
	P ₂	0.521	0.261	0.26	0.36	0.075	20.8%
	F ₁	0.799	0.531	0.268	0.675	0.076	11.2%
	F _{2:3}	1.30	0.166	1.13	0.6	0.2	32.8%

**Fig1.** Frequency distribution of Carbon metabolism-related traits in F_{2:3}

Various crops and vegetables such as maize (Zhan et al., 2001), wheat (Ge et al., 2004; Hou et al., 2006), rice (Wang et al., 2000; Xue et al., 2007; Zhu et al., 2008), cotton (Xin et al 2012; Hao et al., 2008; Yuan et al., 2002), soybean (Wang and Gai, 1997, 2001), rapeseed (Qi et al., 2001; Tian et al., 2009; Xu et al., 2010; Zhang et al., 2008), broccoli (Liu et al., 2009), chickpea (Anbessa et al., 2006), tomato (Li et al., 2006), cucumber (Zhang et al., 2007), egg plant (Pang et al., 2008), Chinese cabbage (Zuo et al., 2009), melon (Cheng, 2006; Zhang et al., 2009) and pepper (Chen et al., 2006; Chen and Chen, 2006) has been studied by joint segregation analysis to check the inheritance of various traits. The formation of carbon metabolites is the result of synergistic effects of a large number of enzymes in the entire metabolic pathways. Therefore, most traits related to carbon metabolism perform quantitative

inheritance. In this study, we described the polygenic inheritance of carbon metabolism-related traits like photosynthetic rate, chlorophyll contents, PEPC activity and SPS activity in order to make clear the genetic models and gene interaction modes, to assess the genetic effects and heritability, and to provide a valuable information for maize genetic breeding and the QTL mapping of the above mentioned 4 traits related to carbon metabolism.

Results

Frequency distribution of carbon metabolism-related traits in parents, F₁ and F₂ populations

The phenotypic values of C4 metabolism-related traits among 4

generations derived from the cross shen3336 x shen3265 were listed in Table 1. The chlorophyll contents, net photosynthesis rate and SPS activity of F₁ plants were between two parents, and close to the average means of two parents. PEPC activity of F₁ plants was higher than two parents, suggested that genes controlling PEPC activity were dominant, while the F₁ plants for PEPC activity had heterosis. All the coefficients of variation (CV %) of C4 metabolism-related traits among F_{2:3} generation were higher than those of P₁, P₂ and F₁ generations, indicating the existence of real variations of heredity. As shown in Fig.1, chlorophyll contents, net photosynthesis rate, SPS activity and PEPC activity showed a continuous distribution, suggesting quantitative inheritance. The distribution curves of chlorophyll contents, net photosynthesis rate and SPS activity displayed two major peaks, indicating that chlorophyll contents, net photosynthesis rate and SPS activity were controlled by one major gene while modified by several minor genes. The PEPC activity revealed approximately normal distribution, which indicated that PEPC activity was only controlled by many minor genes.

Inheritance analysis of carbon metabolism-related traits in maize

Based on mixed major gene plus polygene inheritance model, 24 genetic models, coded as A-E, were tested with the data of C4 metabolism-related traits from P₁, P₂, F₁ and F_{2:3} from the cross shen3336 x shen3265 (Table 2). Models C-0 and E-1 for net photosynthesis rate, models C-0 and E-1 for chlorophyll contents, models C-0 and D-0 for PEPC activity, and models B-1 and E-1 for SPS activity were chosen as the candidate models for the inheritance analysis due to the lower Akaike's information criterion (AIC) values of these models than the others. According to the fitness, evaluation was done by using 5 statistical parameters such as U₁², U₂², U₃², nW², and Dn as shown in Table 3. The model E-1 (two additive-dominance-epistasis major genes plus additive-dominance polygenes) was the best-fitting model for both net-photosynthesis rate and chlorophyll contents while model B-1 (two additive-dominance-epistasis major genes) and model C-0 (polygenes without major genes) was fit for SPS and PEPC activity respectively.

Genetic parameter estimation

Using the IECM method, max-likelihood values of parameter distribution means of the best-fitting models were obtained, and the first order and the second order parameters were estimated (Table 4). Two major genes controlling net-photosynthesis rate had close additive-effects and dominance effects. The additive×additive interaction effect (*i*) was 2.628039, whereas the dominance×dominance interaction effect (*l*) was -19.049299. Both additive×dominance (*j_{ab}*) and dominance×additive (*j_{ba}*) were -8.979408. Heritability of major gene was 41.0 % (h^2_{mg}), whereas heritability of polygenes was 34.9 % (h^2_{pg}). There were no additive effects between two major genes controlling chlorophyll contents. The dominance effects of the two major genes were 0.481714 and -0.182698, respectively, larger effect by the first gene. The additive ($[d]=0.685533$) and dominance ($[h]=-1.122663$) effects of polygenes can be either positive or negative among crosses, which shows differences in their polygenic background. The additive×additive interaction effect (*i*) was -0.034057, whereas the dominance×dominance interaction effect (*l*) was 1.457208. Both additive×dominance (*j_{ab}*) and dominance×additive (*j_{ba}*) were 1.251403 and 0.219850 respectively. Heritability of major gene was greater ($h^2_{mg}=56.2\%$) than heritability of polygenes ($h^2_{pg}=2.5\%$). The

additive effects and dominance effects of two major genes controlling SPS activity were all small. The additive effect *d_a* (0.062605) was larger than *d_b* (0.062571), and dominance effect *h_a* (-0.075795) was larger than *h_b* (0.067836), suggested larger effects of both additive and dominance by the first gene. The interaction effects between two major genes were not obvious. Heritability of major gene was 75.7 % (h^2_{mg}). The major genes controlling PEPC activity were not detected yet, whereas its heritability of polygenes was 46.3 %.

Discussion

The classic Mendelian method usually views the quantitative traits as multigenes and overall estimates gene effect sizes and modes of gene action. By this way, it cannot tell the genetic behavior of individual genes in the system. Gai and Wang (1998) proposed a genetic model called a mixed major gene and polygene inheritance model. By imposing the mixed genetic model, the existence of major genes and polygenes can be identified. Furthermore, the genetic parameters related to both major genes and minor genes can be estimated. So far, there were many reports about the application of the mixed genetic model (Wang and Gai, 2001; Zhang et al., 2012; Hiroshi et al., 2012; Xin et al., 2012). Net-photosynthesis rate, chlorophyll contents, PEPC activity and SPS activity are all complex quantitative traits and also the most important traits in the carbon metabolism. In the present study, the joint segregation analysis of multiple generations P₁, P₂, F₁, and F_{2:3} were used to reanalyze the genetic data. The results showed that net-photosynthesis rate, chlorophyll contents, and SPS activity were all controlled by two major genes with additive, dominance effects and interaction effects. SPS was controlled only by major genes without polygenes. The joint segregation analysis method of mixed major gene plus polygene genetic model was used to study the inheritance of erucic acid in *Brassica napus* L. from five populations which indicated that inheritance of erucic acid was followed by two major genes with additive- dominance-epistatic effects plus polygenes with additive-dominance effects (Zhang et al., 2008). The two major genes controlling net-photosynthesis rate with close additive and dominance effects, suggesting that there was little difference between the two major genes. In contrast, the interaction effects were remarkable. Net photosynthetic rate was also conditioned by polygenes. The dominance effect of polygenes was 28.216787 ([h]), leading to the increase of net photosynthetic rate. Heritability was higher for both the major gene (41%) and the minor genes (34.9%), indicating that major genes and minor genes jointly controlled net photosynthetic rate. In addition, net photosynthetic rate was also affected by environment with environmental variance accounting for 24.1% of the phenotypic variance. In a recent study, Xin et al. (2012) reported the heritabilities of major genes in B₁, B₂ and F₂ were 83.62, 76.84 and 90.56% respectively while the heritability of polygene in B₂ was 7.76% from cross of cotton varieties H077 x H113. There was no additive effect between two major genes controlling chlorophyll contents. The dominance effects of the two major genes were larger by the first gene. The interaction effects were obvious. Meanwhile, chlorophyll contents had also polygene modification effect. The dominance effect of polygenes was -1.122663 ([h]), leading to the increase of chlorophyll contents. Heritability was higher for the major gene (56.2%) than that of the minor genes (2.5%). Environmental variance accounted for 41.3% of the phenotypic variance which greatly influenced the genetic of chlorophyll contents. The fluorescence of chlorophyll was controlled by additive type of gene action (Farshadfar et al., 2011). Total chlorophyll content in plants (cucumber, rice) was controlled

Table 2. Max-likelihood values and Akaike's information criterion (AIC) values under different genetic models.

Trait	Model	Max log likelihood value	AIC value	Model	Max log likelihood value	AIC value
Net photosynthetic rate	A-1	-955.929749	1919.859497	D-0	-913.036194	1842.072388
	A-2	-955.941162	1917.882324	D-1	-915.768616	1845.537231
	A-3	-958.006653	1922.013306	D-2	-915.767883	1843.535767
	A-4	-957.903076	1921.806152	D-3	-915.774536	1843.549072
	B-1	-943.960999	1907.921997	D-4	-915.766174	1843.532349
	B-2	-925.309082	1862.618164	E-0	-912.997192	1853.994385
	B-3	-953.543640	1915.087280	E-1	-906.357910	1838.715820
	B-4	-974.285828	1956.571655	E-2	-913.841980	1845.683960
	B-5	-955.887939	1921.775879	E-3	-915.763855	1845.527710
	B-6	-950.745911	1909.491821	E-4	-915.763855	1843.527710
	C-0	-912.997192	1837.994385	E-5	-915.763733	1845.527466
	C-1	-915.763855	1841.527710	E-6	-915.763855	1843.527710
	Chlorophyll content	A-1	-251.835220	511.670410	D-0	-220.692993
A-2		-257.457886	520.915771	D-1	-226.644119	467.288239
A-3		-252.575760	511.151489	D-2	-226.613892	465.227783
A-4		-264.913361	535.826721	D-3	-226.615326	465.230652
B-1		-233.664856	487.329712	D-4	-226.612122	465.224243
B-2		-241.446838	494.893677	E-0	-220.635803	469.271606
B-3		-252.363266	512.726562	E-1	-210.133057	446.266113
B-4		-274.194641	556.389282	E-2	-220.604538	459.209076
B-5		-252.123398	514.246826	E-3	-226.609344	467.218689
B-6		-256.017059	520.034119	E-4	-226.609344	465.218689
C-0		-220.635803	453.271606	E-5	-226.609375	467.218750
C-1		-226.609344	463.218689	E-6	-226.609329	465.218658
PEPC activity		A-1	771.038330	-1534.076660	D-0	909.501099
	A-2	769.787781	-1533.575562	D-1	774.793579	-1535.587158
	A-3	769.958435	-1533.916870	D-2	774.793579	-1537.587158
	A-4	770.060486	-1534.120972	D-3	774.793579	-1537.587158
	B-1	846.130676	-1672.261353	D-4	774.793579	-1537.587158
	B-2	771.850403	-1531.700806	E-0	910.464478	-1792.928955
	B-3	769.790283	-1531.580566	E-1	890.960632	-1755.921265
	B-4	754.971985	-1501.943970	E-2	785.012512	-1552.025024
	B-5	485.036987	-960.073975	E-3	774.793579	-1535.587158
	B-6	799.361145	-1590.722290	E-4	774.793579	-1537.587158
	C-0	910.464478	-1808.928955	E-5	774.793640	-1535.587280
	C-1	774.793579	-1539.587158	E-6	774.793579	-1537.587158
	SPS activity	A-1	76.074509	-144.149017	D-0	117.448647
A-2		70.041809	-134.083618	D-1	99.146912	-184.293823
A-3		58.288078	-110.576157	D-2	92.761108	-173.522217
A-4		74.884071	-143.768143	D-3	90.603050	-169.206100
B-1		124.420189	-228.840378	D-4	99.318909	-186.637817
B-2		100.198586	-188.397171	E-0	113.298904	-198.597809
B-3		80.456726	-152.913452	E-1	125.103134	-224.206268
B-4		41.299690	-74.599380	E-2	108.368355	-198.736710
B-5		40.962845	-91.925690	E-3	90.605675	-167.211349
B-6		57.090553	-106.181107	E-4	90.605682	-169.211365
C-0		113.298897	-214.597794	E-5	90.604813	-167.209625
C-1		90.605675	-171.211349	E-6	90.605652	-169.211304

by mini-polygene which was affected by environmental factors (Li et al., 2009; Yang et al., 2006). Zhang et al. (2009) reported that the heritability of chlorophyll *a* and *b* was high in wheat. Chlorophyll content and photosynthetic rate is correlated with each other, thus an effective way to increase the biomass production and grain yield in crops is to increase the chlorophyll content in crops (Wang et al. 2008). The first gene effects were larger for the additive effects and dominance effects of two major genes controlling SPS activity. There were no polygene effects. Heritability was higher for the major gene (75.7%). Environmental variance accounted for 18.3% of the phenotypic variance. No major genes controlling PEPC activity had been detected. Polygenes mainly conditioned PEPC activity and the heritability of minor gene was 46.3%. These results indicated that chlorophyll contents and SPS activity were mainly controlled by 2 major genes, which can be

selected in the early generations during breeding procedure through single or simple recombinant backcross method. PEPC activity was controlled by polygenes. In order to improve breeding efficiency, the trait of PEPC activity can be selected in the higher generations during breeding procedure through recurrent selection and pyramiding backcross method. The inheritance of anthracnose leaf blight in maize was studied by mixed model using six generations. Results of this study indicated mixed model major genes and polygenes were very helpful in revealing the inheritance of anthracnose leaf blight in maize (Rezende et al 2004). In another study (Xingtao et al., 2011) tolerance of low level of potassium in maize was investigated by mixed model of major gene plus polygene. The findings of this study indicated that tolerance to low-potassium stress in maize was dominated by major genes which were very helpful in future maize breeding programs. The present study

Table 3. Tests for goodness-of-fit of candidate models.

Trait	Model	Generation	U ₁ ²	U ₂ ²	U ₃ ²	nW ²	Dn
Net photosynthetic rate	C-0	P ₁	0.001(0.9773)	0.000(0.9998)	0.012(0.9116)	0.0333	0.1199(CD(0.05)=0.3206)
		P ₂	0.012(0.9120)	0.019(0.8906)	0.015(0.9030)	0.0325	0.1053(CD(0.05)=0.3206)
		F ₁	0.020(0.8865)	0.104(0.7476)	3.385(0.0658)	0.1131	0.1779(CD(0.05)=0.3206)
		F _{2:3}	0.043(0.8353)	0.155(0.6941)	5.657(0.0174)*	0.1722	0.0517(CD(0.05)=0.0917)
	E-1	P ₁	0.002(0.9677)	0.004(0.9469)	0.012(0.9126)	0.0335	0.1255(CD(0.05)=0.3206)
		P ₂	0.032(0.8573)	0.041(0.8399)	0.013(0.9109)	0.0349	0.1113(CD(0.05)=0.3206)
		F ₁	0.033(0.8564)	0.082(0.7751)	3.400(0.0652)	0.1143	0.1813(CD(0.05)=0.3206)
		F _{2:3}	0.062(0.8035)	0.001(0.9770)	0.719(0.3963)	0.0440	0.0366(CD(0.05)=0.0917)
Chlorophyll content	C-0	P ₁	0.795(0.3726)	0.551(0.4578)	0.234(0.6288)	0.2014	0.2189(CD(0.05)=0.3206)
		P ₂	0.010(0.9213)	0.132(0.7160)	1.151(0.2834)	0.0479	0.1185(CD(0.05)=0.3206)
		F ₁	0.057(0.8107)	0.030(0.8630)	0.057(0.8121)	0.1169	0.1789(CD(0.05)=0.3206)
		F _{2:3}	0.037(0.8481)	0.192(0.6613)	6.222(0.0126)*	0.2633	0.0673(CD(0.05)=0.0899)
	E-1	P ₁	0.867(0.3519)	0.597(0.4399)	0.266(0.6060)	0.2106	0.2226(CD(0.05)=0.3206)
		P ₂	0.021(0.8854)	0.165(0.6848)	1.135(0.2868)	0.0494	0.1166(CD(0.05)=0.3206)
		F ₁	0.045(0.8327)	0.020(0.8865)	0.061(0.8046)	0.1149	0.1764(CD(0.05)=0.3206)
		F _{2:3}	0.048(0.8261)	0.008(0.9288)	0.243(0.6217)	0.0494	0.0439(CD(0.05)=0.0899)
PEPC activity	C-0	P ₁	0.009(0.9229)	0.513(0.4736)	6.207(0.0127)*	0.1861	0.1980(CD(0.05)=0.3206)
		P ₂	0.005(0.9459)	0.192(0.6612)	2.222(0.1361)	0.0967	0.1606(CD(0.05)=0.3206)
		F ₁	1.071(0.3008)	0.632(0.4267)	0.686(0.4076)	0.2233	0.2292(CD(0.05)=0.3206)
		F _{2:3}	1.083(0.2981)	1.904(0.1677)	2.218(0.1364)	0.3235	0.0745(CD(0.05)=0.0901)
	D-0	P ₁	0.009(0.9227)	0.513(0.4740)	6.189(0.0129)*	0.1856	0.1978(CD(0.05)=0.3206)
		P ₂	0.005(0.9458)	0.191(0.6620)	2.207(0.1374)	0.0965	0.1604(CD(0.05)=0.3206)
		F ₁	1.072(0.3004)	0.635(0.4257)	0.680(0.4097)	0.2233	0.2291(CD(0.05)=0.3206)
		F _{2:3}	0.292(0.5890)	1.193(0.2747)	5.182(0.0228)*	0.2189	0.0665(CD(0.05)=0.0901)
SPS The activity of SPS	B-1	P ₁	0.149(0.6995)	0.002(0.9625)	2.833(0.0923)	0.1235	0.1816(CD(0.05)=0.3206)
		P ₂	0.113(0.7365)	0.129(0.7194)	0.018(0.8934)	0.0950	0.1854(CD(0.05)=0.3206)
		F ₁	0.001(0.9809)	0.013(0.9099)	0.130(0.7189)	0.0214	0.0817(CD(0.05)=0.3206)
		F _{2:3}	0.000(0.9974)	0.000(0.9943)	0.002(0.9672)	0.0175	0.0234(CD(0.05)=0.0899)
	E-1	P ₁	0.001(0.9709)	0.172(0.6784)	3.240(0.0719)	0.1184	0.1640(CD(0.05)=0.3206)
		P ₂	0.115(0.7350)	0.111(0.7394)	0.000(0.9846)	0.0955	0.1860(CD(0.05)=0.3206)
		F ₁	0.001(0.9729)	0.009(0.9248)	0.061(0.8056)	0.0205	0.0859(CD(0.05)=0.3206)
		F _{2:3}	0.004(0.9506)	0.001(0.9733)	0.011(0.9156)	0.0187	0.0284(CD(0.05)=0.0899)

Table 4. The estimates of genetic parameters of 4 carbon metabolism-related traits of F_{2:3} population.

Trait	Model	1st order parameter	Estimate	1st order parameter	Estimate	2nd order parameter	Estimate
Net photosynthetic rate	E-1	m	28.572163	j _{ab}	-8.979408	σ _e ²	11.48354
		d _a	4.215199	j _{ba}	-8.979408	σ _p ²	47.74215
		d _b	4.215200	l	-19.049299	σ _{mg} ²	19.58745
		h _a	-3.474782	[d]	-2.595898	σ _{pg} ²	16.67116
		h _b	-3.474761	[h]	28.216787	h _{mg} ²	41.0 %
		i	2.628039			h _{pg} ²	34.9 %
Chlorophyll content	E-1	m	3.903294	j _{ab}	1.251403	σ _e ²	0.132753
		d _a	0.000000	j _{ba}	0.219850	σ _p ²	0.32114
		d _b	0.000000	l	1.457208	σ _{mg} ²	0.180364
		h _a	0.481714	[d]	0.685533	σ _{pg} ²	0.008023
		h _b	-0.182698	[h]	-1.122663	h _{mg} ²	56.2%
		i	-0.034057			h _{pg} ²	2.5%
SPS activity	B-1	m	0.598359	j _{ab}	0.397015	σ _e ²	0.007051
		d _a	0.062605	j _{ba}	0.030464	σ _p ²	0.038599
		d _b	0.062571	l	0.084753	σ _{mg} ²	0.029236
		h _a	-0.075795	[d]		σ _{pg} ²	0.002312
		h _b	0.067836	[h]		h _{mg} ²	75.7%
		i	0.138393			h _{pg} ²	6%

Note: d, Additive effect; h, Dominance effect; l, Additive×Additive effect; j, Dominance×Additive effect; l, Dominance×Dominance effect; [d], Additive effects of the polygenes; [h], Dominance effects of the polygenes; σ_{mg}², Major gene variance; σ_{pg}², Polygene variance; h_{mg}², Major gene heritability; h_{pg}², Polygene heritability. Subscript a and b refer to two major genes.

revealed the preliminary genetic mechanism of carbon metabolism-related traits in maize, which provided a new theory basis for the future maize breeding.

Materials and methods

Plant materials

To study genetic of carbon metabolism-related traits, maize hybrid Shenyu 21 (F₁), bred by the Breeding Institute, Shenyang Academy of Agricultural Sciences, was used to construct a segregation population. The female parent Shen3336 has high photosynthetic efficiency and tall while the male parent Shen3265 had weak photosynthetic efficiency and dwarf. Shenyu21 seeds were grown in San Taizi experiment field of Shenyang Academy of Agricultural Sciences for F₂ seed production in May 1, 2010. In the same year, F₃ lines were advanced from individual F₂ plants in Hainan Province, China. During the 2011 crop season, the parents, Shenyu21 (F₁) and F₃ population were planted at San Taizi experiment field of Shenyang Academy of Agricultural Sciences on April 28. 60 Shenyu 21 (F₁) seeds were planted along 4 rows with 15 seeds in one row. For the 235 F₃ lines, about 15 seeds from each line were planted in a 4 m row with 0.6 m apart between rows. Because of limited seed quantities, only one experiment field (site) was conducted and replications were not possible in 2011. In 2012, the parents, Shenyu21 (F₁) and F₄ population were sown on 2nd May 2012 at San Taizi experiment field and 4th May 2012 at New Chengzi experiment field of Shenyang Leiao Seed Company. The experimental plots were completely randomized with three replications at each site. F₃ and F₄ lines were advanced from individual F₂ plants through single seed-descent. The ear leaves from 5 normal plants per line were sampled at filling stage with 3 replications.

Measurement of traits

The net photosynthesis rate was measured during cloudless days at 9:30-11:30AM, the net photosynthesis rate in ear leaves during the filling stage was measured with a portable TPS-2 photosynthesis System (TPS-2, PP System, USA) at 2000 $\mu\text{mol m}^{-2}\text{s}^{-1}$ PPFD (i.e., photosynthetic photon flux density) using the natural light source. 3-5 plants were selected for measuring twice. The leaf temperature was maintained at 25°C. Chlorophyll content in the leaves was measured by the method as described by Arnon et al. (1949). Phosphoenolpyruvate Carboxylase (PEPC) activity was estimated by following method of Blanke et al. (1986) and Sucrose phosphate synthase (SPS) activity was determined by the method of Miron and Schaffer (1991)

Statistical analysis

All the data was analyzed statistically according to Gai et al. (2003) using joint analysis of 4 generations (P₁, P₂, F₁ and F₂₋₃) from the cross shen3336 x shen3265 was carried out to investigate the inheritance of 4 carbon metabolism-related traits using the model of major gene plus polygene for multiple correlated quantitative traits. By maximum log likelihood method and IECM (Iterated expectation and conditional maximization), component distribution parameters of the above four generations were calculated and candidate genetic models were selected referring to Akaike's information criterion (AIC) value. Further, the best-fitting model was determined by 5 statistical parameters (U₁², U₂², U₃², nW² and Dn). The gene effects and genetic variances were estimated by the component distribution parameters of best-fitting models using least

squares estimation (LSE). The genetic variances of major gene (σ_{mg}^2) and polygene (σ_{pg}^2) were computed as $\sigma_{mg}^2 = \sigma_p^2 - \sigma_f^2$ and $\sigma_{pg}^2 = \sigma_f^2 - \sigma_e^2$, respectively, with σ_p^2 , the phenotypic variance of population; σ_f^2 , the distribution variance of population; σ_e^2 , the error variance. The heritability of major gene (h_{mg}^2) and polygene (h_{pg}^2) were obtained according to formula $h_{mg}^2 = \sigma_{mg}^2 / \sigma_p^2 \times 100\%$ and $h_{pg}^2 = \sigma_{pg}^2 / \sigma_p^2 \times 100\%$.

Acknowledgements

This research was supported by Shenyang basic research for application project (F10-205-1-24) and sponsored by the "Planned Science and Technology Project of Shenyang, China" (NO.F12-277-1-24)

References

- Anbessa Y, Warkentin T, Vandenberg A, Ball R (2006) Inheritance of time to flowering in chickpea in a short-season temperate environment. *J Hered* 97: 55-61.
- Arnon DL (1949) A copper enzyme is isolated chloroplast polyphenol oxidase in *Beta vulgaris*. *Plant Physiol* 24:1-15.
- Bansal UK, Saini RG, Kaur A (1999) Genetic variability in leaf area and chlorophyll content of aromatic rice. *Int Rice Res Notes* 24:21.
- Blanke M, Notton B, Hucklesby D (1986) Physical and kinetic properties of photosynthetic PEP carboxylase in developing apple fruit. *Phytochem* 25: 601-606.
- Chen XJ, Chen JF, Fang R, Cheng ZF, Wang SB (2006) Inheritance of the node for first flower in pepper (*Capsicum annuum* L.). *Acta Horti Sin* 33: 152-154.
- Chen XJ, Chen JF (2006) Genetic analysis of plant height in pepper (*Capsicum annuum* L.). *Acta Bot Boreal-Occident Sin* 26: 1342-1345.
- Cheng ZJ (2006) The genetic mechanisms of resistance to powdery mildews and ALFP marker linked to the disease resistance in melon [M.S. Dissertation]. Nanjing: Nanjing Agricultural University.
- Chollet R, Vidal J, O'Leary MH. (1996) Phosphoenolpyruvate carboxylase: a ubiquitous, highly regulated enzyme in plants. *Annu Rev Plant Physiol Plant Mol Biol* 47: 273-298.
- Dwyer LM, Tollenaar M. (1989) Genetic improvement in photosynthetic response of hybrid maize cultivars 1959 to 1988. *Can J Plant Sci* 69: 81-91.
- Eihide M, Shigesaburo J. (1979) Photosynthetic heterosis in maize. *Japan J Breed* 29: 159-165.
- Farrar J, Pollock C, Gallagher J (2000) Sucrose and the integration of metabolism in vascular plants. *Plant Sci* 154:1-11.
- Farshadfar E, Valiollah R, Jaime Teixeira D, Farshadfar M (2011) Inheritance of drought tolerance indicators in bread wheat (*Triticum aestivum* L.) using a diallel technique. *Aust J Crop Sci* 5: 308-317.
- Fischer RA, Bidinger F, Syme JR, Wall PC (1981) Leaf photosynthesis, leaf permeability, crop growth, and yield of short spring wheat genotypes under irrigation. *Crop Sci* 21-367.
- Gai JY, Wang JK (1998) Identification and estimation of a QTL model and its effects. *Theor Appl Genet* 97:1162-1168.
- Gai JY, Zhang YM, Wang JK (2003) Genetic system of quantitative traits in plants. Science Press, Beijing. Pp. 224-260. (in Chinese)
- Ge XX, Zhang LP, HE ZH, Zhang YM (2004) The mixed inheritance analysis of polyphenol oxidase activities in winter wheat. *Acta Agvonomica Sin* 30: 18-20.

- Harbron S, Foyer C, Walker D (1981) The purification and properties of sucrose-phosphate synthetase from spinach leaves: the involvement of this enzyme and fructose biphosphatase in the regulation of sucrose biosynthesis. *Arch Biochem Biophys* 212:237-246.
- Hao JJ, Yu SX, Ma QX, Fan SL, Song MZ (2008) Inheritance of time of flowering in upland cotton under natural conditions. *Plant Breed* 127: 383-390.
- Hiroshi I, Shigeki M, Nobuhiro K, Naozumi M, Sae TS, Kazuyuki A (2012) Mode of inheritance in fruit acidity in apple analysed with a mixed model of a major gene and polygenes using large complex pedigree. *Plant Breed* 131:322-328.
- Hou BW, Dou BD, Zhang YM, Li SHQ, Yang JB, Liu FX, Du JK, Sun QX (2006) The Mixed Major Gene Plus Polygenes Inheritance for Female Fertility in Wheat (*Triticum aestivum* L.). *Hereditas* 28:1567-1572.
- Huber SC, Huber JL (1996) Role and regulation sucrose phosphate synthase in higher plants. *Ann Rev Plant Physiol Plant Mol Biol* 47:431-445.
- Li JS, Shen HL, Shi ZQ (2006) Analysis on the major gene and polygene mixed in heritance of lycopene content in fresh consumptive tomato fruit. *Hereditas (Beijing)* 28: 458-462.
- Li DD, Si LT, Li J, Jin ZA, Niu HH (2009) Genetic analysis on chlorophyll contents of cucumber seedling under low light. *Acta Agr Boreali-Sinica* 24: 1-4.
- Liu EY, Liu YM, Fang ZY, Yang LM, Zhuang M, Zhang YY, Yuan SX, Sun JF, Li ZS, Sun PT (2009) Genetic analysis of head-leaf traits using mixed major gene plus polygene inheritance model in *Brassica oleracea* L. var. *italica* Planch. *Acta Horti Sin* 36: 1611-1618.
- Ma BL, Morrison MJ, Voldeng HD (1995) Leaf greenness and photo-synthetic rates in soybean. *Crop Sci* 35: 1411-1414.
- Maurice SBK, Sakae A, Mika N, Hiroko Tsuchi d, Kazuko O, Sakiko H, Seiichi T, Mitsue M, Makoto M (1999) High level expression of maize phospho enolpyruvate carboxylase in transgenic rice plants. *Nature Biotechnol* 17: 76-80.
- Miron D, Schaffer AA (1991) Sucrose phosphate synthase sucrose synthase, and invertase activities in developing fruit of *lycopersicon esculentum* mill and the sucrose accumulating *lycopersicon hirsutum* humb. and bonpl. *Plant Physiol* 95: 623-627.
- Nakazawa F, Tunoda K, Torikura H (1990) On the photosynthetic characteristics of high yielding rice varieties I. Leaf photosynthetic rate. *Japan Crop Sci* 59: 72-79.
- Pang WL, Liu FZ, Chen YH, Lian Y (2008) Genetic study on fruit color traits of eggplant. *Acta Horti Sin* 35: 979-986.
- Qi CK, Gai JY, Zhang YM (2001) Major gene plus polygene inheritance of erucic acid content in *Brassicu napus* L.. *Acta Genetica Sin* 28:182-187.
- Rezende VF, Vencovsky R, Cárdenas FEN, da Silva HP, Bearzoti E, Camargo LEA (2004) Mixed inheritance model for resistance to anthracnose leaf blight in maize. *Crop Breed Appl Biotechnol* 4:115-122.
- Thomas JA, Jeffrey AC, Atsuko K, David MK (2005) Regulating the proton budget of higher plant photosynthesis. *Proc Natl Acad Sci USA* 102: 9709-9713.
- Tian LS, Niu YZ, Yu QQ, Guo SX, Liu L (2009) Genetic Analysis of White Flower Color with Mixed Model of Major Gene Plus Polygene in *Brassica napus* L.. *Sci Agric Sin* 42: 3987-3995.
- Wang JK, Gai JY (2001) Mixed inheritance model for resistance to agromyzid beanfly (*Melanagromyza sojae* Zehntner) in soybean. *Euphytica* 122: 9-18.
- Wang JS, Wang JK, Zhu LH, Gai JY (2000) Major-polygene effect analysis of resistance to Bacterial Blight (*Xanthomonas campestris* pv. *oryzae*) in rice. *Acta Genet Sin* 27: 34-38.
- Wang FH, Wang GX, Li XY, Huang JL, Zheng JK (2008) Heredity, physiology and mapping of a chlorophyll content gene of rice (*Oryza sativa* L.). *J Plant Physiol* 165: 324-330.
- Wang JK, Gai JY (1997) Identification of major gene and polygene mixed inheritance model and estimation of genetic parameters of a quantitative trait from F2 progeny. *Acta Genet Sin* 24: 398-404.
- Xin Z, Chang-qi L, Xi-yuan W, Guo-ping C, Jin-bao Z, Rui-yang Z (2012) Genetic analysis of cryotolerance in cotton during the over wintering period using mixed model of major gene and polygene. *J Integ Agr* 11:537-544.
- Xingtiao L, Minjian C, Haiqiu Y (2011) Genetic analysis of tolerance to low-potassium stress in maize using mixed model of major gene plus polygene. *J Maize Sci* 19:93-97 (in Chinese).
- Xue Y, Jiang L, Su N, Wang JK, Deng P, Ma JF, Zhai HQ, Wan JM (2007). The genetic basic and fine-mapping of a stable quantitative trait loci for aluminium tolerance in rice. *Planta* 227: 255-262.
- Xu AX, Huang Z, Li JH, Lu RW, Xiao ES, Zhang XS (2010) Inheritance of erucic acid content of *Brassica juncea* in Northern Shaanxi with mixed model of major gene plus polygene. *Acta Bot Boreal-Occident Sin* 30: 0933-0938.
- Yang GH, Li SQ, Feng LL, Kong J, Li H, Li YH (2006) Analysis of QTL underlying the traits relative to the chlorophyll contents of the flag leaf in Rice. *Acta Wuhan Univ* 52: 751-756.
- Yuan YL, Zhang TZ, Guo WB, Yu J, Kohel RJ (2002) Major polygene effect analysis of super quality fiber properties in upland cotton. *Acta Genet Sin* 29: 827-834.
- Zelitch L (1982) The close relationship between net photosynthesis and crop yield. *Biol Sci* 32:796-802.
- Zhang CJ, Wang Y, Wang YF, Li FL, Li FG (2012) Mixed major gene and polygene inheritance analysis of embryogenesis callus induction ratio in upland cotton mature leaf petioles as explants. *Cott Sci* 24: 3-9.
- Zhan QW, Gai JY, Zhang YM, Sun ZD (2001) Development and expression process of inheritance of resistance to cotton worm (*Prodenia litura*) in soybeans. *Acta Genet Sin* 28: 956-963.
- Zhang H, Wang HS, He CX, Zhang ZB, Zhang X, Yi HP, Wu MZ (2009) Genetic study on sugar and sour traits of melon (*Cucumis melo* L.). *Acta Horti Sin* 36: 989-996.
- Zhang SF, Fu TD, Zhu JC, Wang JP, Wen YC, Ma CZ (2008) Genetic analysis of erucic acid in *Brassica napus* L. using mixed major gene and polygene inheritance model. *Sci Agric Sin* 41: 3343-3349.
- Zhang SQ, GU XF, Zhang SP, Zou ZR (2007) Inheritance of downy mildew resistance in cucumber (*Cucumis sativus* L.). *Acta Bot Boreal.-Occident Sin* 27: 1342-1345.
- Zhang K, Zhang Y, Chen G, Tian J (2009) Genetic analysis of grain yield and leaf chlorophyll content in common wheat. *Cereal Res Comm* 37:499-511.
- Zhu SY, Guo Y, Hong DL (2008) Genetic analysis on aging-resistant in rice seed. *Hereditas* 30: 217-224.
- Zhuo ZC, Wan EM, Zhang LG, Zhang MK, Hui MX (2009) Major gene plus polygene inheritance analysis of bolting trait in heading Chinese cabbage. *Acta Bot. Boreal.-Occident Sin* 29: 0867-0873.