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Developmental Analysis on Genetic Behavior of Quality Traits of Flue-cured Tobacco (*Nicotiana tabacum*) in Multiple Environments

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ABSTRACT

Analysis of genetic main effects and genotype × environment interaction effects for total sugar and reducing sugar of flue-cured tobacco were conducted at two locations and diallel cross by using the additive, dominance and additive × additive model. Unconditional and conditional analyses were employed to investigate the developing behavior of quantitative traits and uncover the genetic mechanisms of flue-cured tobacco. Total and reducing sugars were greatly affected by genotype × environment interaction effect rather than genetic main effect, and gene intermittently expressed and exhibited different genetic mode in different growth stages. It was suggested that genetic improvement for total sugars of flue-cured tobacco could be achieved by selection of pure lines in the period of Gs1118 to Gs1121 in early generations and by selection of heterosis in the period before Gs1109 in advanced generations under multiple environments. As for RS, it would be better to select hybrid in the period of Gs1112 to Gs1121 in advanced generations under specific environments.

Key Words: Flue-cured tobacco; Developmental stages; Conditional analysis; Quality Traits; G×E Interaction

INTRODUCTION

The flue-cured tobacco (*Nicotiana tabacum* L.) is one of the most important economically valuable crops in the world. The leaf quality of flue-cured tobacco is very important for tobacco industry and the main objective of tobacco breeding is to select cultivars with high flue-cured-leaf quality. Chemical characteristics, such as total sugar (TS) and reducing sugar (RS) are indicative of taste and flavor of cigarettes (Pandeya *et al.*, 1985). Understanding genetic mechanism is prerequisite for breeders to improve such quality traits.

Epistasis is considered as an important genetic feature of quantitative traits in many recent studies (Cockerham & Zeng, 1996; Yu *et al.*, 1997; Wang *et al.*, 1999) and the complex epistatic effects might be the resource of heterosis rather than dominance (Minvielle, 1987; Birchler *et al.*, 2010). Epistatic effects have been detected in some genetic studies on quality traits of the flue-cured tobacco and were indicated the complexity of the expression of gene system (Gopinath *et al.*, 1967; Lewis *et al.*, 2007). Researchers have recognized that phenotypic variations of many valuable traits of flue-cured tobacco are controlled not only by genetic main effects, but also by the interaction of the genotype with environment so that genetic variance and heterosis would be affected by genotype-year interaction (Matzinger *et al.*, 1971). This necessitates that epistasis and *GE* interaction effects should be considered when we study the genetic behavior of flue-cured tobacco quality traits.

In light of the principles of the general genetic model (Cockerham, 1980), Zhu (1989) proposed an additive, dominance and additive × additive (*ADAA*) model that has been widely used in recent genetic studies of quantitative traits (Quijada *et al.*, 2006; Zhou *et al.*, 2009; Saha *et al.*, 2011). The *ADAA* model can give out unbiased estimates of variance components even if the additive × additive epistasis does not exist (Xu & Zhu, 2000). Additive × dominance epistasis and dominance × dominance epistasis are more complicated compared to additive-additive epistasis, but they are generally negligible and will decline quickly as generations increases, and hence the later are not included in the *ADAA* model (Xu & Zhu, 1999).

The conventional analysis methods (correlation analysis, multiple linear regression analysis, path analysis, etc.) can not reveal the mechanism of the genetic variation for developmental traits (Cramer & Wehner, 2000; Ball *et al.*, 2001; Wu *et al.*, 2002). It is very important to clarify the gene expression in developmental genetics (Zhu, 1995; Cao *et al.*, 2001). The conditional analysis method (Zhu, 1995) can estimate net genetic variance and broad-sense/narrow-sense heritability, and predict the gene expression in specific growth duration. So far, this method has been used to study

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the developmental traits in mice (Atchley & Zhu, 1997; Liang *et al.*, 2009), rice (Shi *et al.*, 2002b; Zhang *et al.*, 2004; Shi *et al.*, 2006) and cotton (Ye *et al.*, 2003; Wu *et al.*, 2004; McCarty *et al.*, 2008). The conditional analysis method was also used to estimate genetic contribution of agronomic traits to TS in flue-cured tobacco (Xiao *et al.*, 2007). But few reports are available about conditional analysis on developmental behavior of flue-cured tobacco quality traits in different growth periods. Present studies uncover the developmental behavior of gene expression of flue-cured tobacco quality traits, such as TS and RS, in different developmental stages under various environments. The unconditional and conditional analyses were used to evaluate genetic effects, as well as their interactions with environments.

MATERIALS AND METHODS

Plant material: Eight cultivars, Yunnan tobacco No. 87 (YT87), Guizhou tobacco No. 11 (GT11), G28, NC32, K346, Ti245, Dixie Bright 101 (DB101) and K326 were used as the parents for constructing a diallel matting design with F_1s and nine F_2s.

Field experiment: An 8×8 diallel cross of eight cultivars was conducted in 2006 at two locations (Fuquan & Jinsha) in Guizhou, southwest of China. The experiment was arranged in a randomized complete block design with two replications at each location. Flue-cured tobacco seeds were sown on 20 February and transplanted on 10 May at both locations. Each block consisted of 80 parent plants, 80 F_1 plants and 120 F_2 plants. Normal field management was carried out in the process of field evaluation, curing and grading.

TS and RS were measured at five developmental stages, which were growth stage 1109, growth stage 1112, growth stage 1115, growth stage 1118 and growth stage 1121 (CORESTA, 2009) and denoted subsequently as Gs1109, Gs1112, Gs1115, Gs1118 and Gs1121, respectively. The initial stage of transplanting was named for Gs1100. Two leaves of the flue-cured tobacco were sampled per plant every time randomly from each block. All harvest leaves were killed out at 105°C for 30 min and dried at 40°C to constant weight, then ground into powder and sieved with 40-mesh sieve, and kept in the drying oven.

TS (RS) was calculated in the formula:

$$TS(RS)(\%) = \frac{c \times V}{m \times (1-W)} \times 100$$

Where *c* was the sample solution of TS (RS), *V* was the volume of extract liquor, *m* was the mass of sample and *W* was the moisture content of sample material. The TS and RS in generation mean were calculated using five competitive plants for parent and F_1 offspring and twenty competitive plants for F_2 offspring in each block.

Statistical method: In this study, developmental genetic analysis was conducted to estimate the unconditional and conditional genetic main effects and *GE* interaction effect of flue-cured tobacco (Zhu, 1996). The data were analyzed using a genetic model including additive (*A*), dominance (*D*) and additive × additive epistatic (*AA*) effects and their interaction effects with environments (Xu & Zhu, 1999). The phenotypic mean value y_{hijkl} of the k^{th} mating type ($k = 0$ for parent, $k = 1$ for F_1, $k = 2$ for F_2) from lines i *and* j in the l^{th} block under h^{th} environment can be partitioned as:

$$y_{hijkl} = \mu + E_h + A_{ik} + A_{jk} + D_{ijk} + AA_{ijk} + AE_{hik} + AE_{hjk} + DE_{hijk} + AAE_{hijk} + B_{l(h)} + e_{hijkl}$$

Where μ is the population mean and the E_h is the environmental effect; A_{ik} or A_{jk} is the additive effect and AE_{hik} or AE_{hjk} is the additive interaction effect with environment; D_{ijk} is the dominance effect and DE_{hijk} is the dominance interaction effect with environment; AA_{ijk} is the epistatic effect and AAE_{hijk} is the additive-additive epistasis interaction effect with environment; $B_{l(h)}$ and e_{hijkl} are the block effect and residual effect, respectively.

The MINQUE (1) method (Zhu & Weir, 1996; 1998; Sabaghnia *et al.*, 2010) was employed in data analysis and the genetic effects were predicted by the adjusted unbiased prediction (AUP) method (Zhu, 1995; Zhu & Weir, 1996; Jiang *et al.*, 2010). Unconditional genetic effects at time *t* were the accumulated effects of gene expression from the initial time to time *t*; the conditional variance analysis detected the variation of net genetic effects in a specific period of growth. Jackknifing was employed to estimate standard errors of genetic variances and correlation coefficients (Miller, 1974; Zhu, 1996). All statistical analysis was carried out by QGAStation software (http://ibi.zju.edu.cn/software/qga/index.htm).

RESULTS AND DISCUSSION

Unconditional variance analysis: The estimated variance components for TS, RS are presented in Table I. For TS, additive variance (V_A) at Gs1109 and Gs1112, dominance variance (V_D) at Gs1109, Gs1112 and Gs1115, additive×additive epistatic variance (V_{AA}) at Gs1115, Gs1118 and Gs1121 were significant; and additive×environment interaction variance (V_{AE}) in whole growth stages except Gs1109, dominance × environment interaction variance (V_{DE}) at Gs1112, Gs1118 and Gs1121, additive-additive epistasis by environment interaction variance (V_{AAE}) at Gs1109 and Gs1115 were also

significant (Table I). TS was mainly affected by *GE* interaction effects at Gs1109, Gs1112, Gs1118 and Gs1121, the proportions of variance due to *GE* interaction effects reached as high as 44.0%, 50.4%, 71.3% and 68.1% in these developmental stages, respectively. We also found the *GE* interaction was mainly constituted by the dominance × environment interaction effects, which accounted for 41.5%, 65.8% and 64.5% of genotype by environment interaction variance for Gs1112, Gs1118 and Gs1121. Xiao *et al.* (2005, 2007) also observed lager genotype × environment interaction effects (35.9%) and lager dominance × environment interaction effects (22.3%) compared with V_G (20.5%) and V_D (8.9%) for TS based on a two generations diallel cross experiment. Therefore, dominance × environment interaction was the main factor of genetic effects, indicating breeder should take different selection strategy of dominance or heterosis under different environments.

For RS, the additive × environment interaction variance (V_{AE}) at all developmental stages (Table I), the dominance × environment interaction variance (V_{DE}) in all growth stages except Gs1115, the additive × additive epistatic variance (V_{AA}) at Gs1112, Gs1118 and Gs1121, the additive variance (V_A) at Gs1115 and dominance variance (V_D) at Gs1109 and Gs1115 all reached significant. However, the additive-additive epistasis × environment interaction variance (V_{AAE}) was not detected in five developmental stages. The performance of RS at Gs1109, Gs1112, Gs1118 and Gs1121 were mainly affected by *GE* interaction, with the *GE* interaction variance ($V_{GE} = V_{AE} + V_{DE} + V_{AAE}$) about 30.7%, 83.1%, 70.4% and 61.3% of the total genetic variance ($V_G + V_{GE}$), respectively. Xiao *et al.* (2005) also detected relatively lager *GE* variance (29.6%) compared with the V_G (26.5%). The RS of flue-cured tobacco varieties could be improved by selection of dominance effect in early stage or in later stages under specific environments.

The genetic behavior of flue-cured tobacco quantitative traits was controlled by genetic main effects and *GE* interaction effects, and the *GE* interaction effects accounted for a large proportion of total phenotypic variation of TS and RS. Former research indicated that additive gene action is the major component in determining the expression of TS and RS and dominance effect plays a minor role (Pandeya *et al.*, 1985; Chen *et al.*, 2004), but they rarely considered epistatic and *GE* interaction effect of flue-cured tobacco. Our research showed that the expression of genes involved in the genetic performance of flue-cured tobacco quality traits are greatly affected by environment factors; the interaction effects are the main cause of genetic difference across different environments. There were some climatic differences between two locations; the temperature in Fuquan of 2006 was lower than that in Jinsha, and the annual precipitation in Jinsha was more than that in Fuquan. These climatic factors might be the cause of the difference in gene expression for TS and RS and make it imperative to conduct experiments in multiple environments and include environment effect and *GE* interaction effect in analysis for quality traits of flue-cured tobacco. Xiao *et al.* (2008; 2005) reported that TS and RS were affected largely by environment factor, which was in accord with our research. The residual variance (V_e) of TS and RS were all significant, only at Gs1115 for TS and Gs1109 for RS and the proportion of residual variances in total phenotypic variance reached 41.8% and 39.6%, all others are less than 30%; in contrast with our results, larger residual variance proportions occurred in that of Xiao *et al.* (2005). It also should be noted that additive-additive epistatic effects and additive-additive × environment interaction effects are included in the model for our analysis, while these effects could not be analyzed in the study of Xiao *et al.* (2005; 2008) because of only two generations materials (Parents & F_1) planted in their experiment.

Conditional variance analysis: One of the main objectives of developmental quantitative genetics study is to understand the dynamic behavior of gene expression in process of trait development (Shi *et al.*, 2002a). Shi *et al.* (2002b & c; 2006) and Ye *et al.* (2003) used conditional analysis approach to evaluate developmental behavior of quantitative traits on rice and cotton, respectively. Until now, no genetic study is reported about the use of conditional analysis method to uncover the genetic mechanisms of quality traits on flue-cured tobacco. In this study, we used conditional analysis method to analyze the developmental behavior of traits, clarify the performance of net genetic effects in different developmental stages and their relationships. Conditional genetic variance of gene expression for TS and RS in specific growth stages were estimated by using the conditional variance analysis method (Table II).

For TS, the conditional additive variance ($V_{A(t|t-1)}$) from Gs1109 to Gs1112 and from Gs1115 to Gs1121, the conditional dominance variance ($V_{D(t|t-1)}$) from Gs1100 to Gs1109, from Gs1112 to Gs1115 and from Gs1118 to Gs1121, the conditional additive × additive epistatic variance ($V_{AA(t|t-1)}$) from Gs1112 to Gs1121, the conditional additive × environment interaction variance ($V_{AE(t|t-1)}$) from Gs1112 to Gs1115 and from Gs1118 to Gs1121, the conditional dominance × environment interaction variance ($V_{DE(t|t-1)}$) from Gs1118 to Gs1121 and the conditional additive-additive epistasis × environment interaction variance ($V_{AAE(t|t-1)}$) in whole developmental stages except the period of Gs1112 to Gs1115 were all

significantly detected. The conditional *GE* interaction variances ($V_{GE(t|t-1)} = V_{AE(t|t-1)} + V_{DE(t|t-1)} + V_{AAE(t|t-1)}$) in the periods of Gs1100 to Gs1109, Gs1109 to Gs1112 and Gs1115 to Gs1118, accounted for about 44.0%, 38.8% and 57.3% of the total conditional genetic variance ($V_{G(t|t-1)} + V_{GE(t|t-1)}$), respectively, which were larger than that of genetic main variance ($V_{G(t|t-1)} = V_{A(t|t-1)} + V_{D(t|t-1)} + V_{AA(t|t-1)}$); therefore, the conditional *GE* interaction effects for TS were more important than genetic main effects in the periods of Gs1100 to Gs1109, Gs1109 to Gs1112 and Gs1115 to Gs1118, environmental effects should be considered when breeders selected flue-cured tobacco with high quality traits.

Dominance variance at Gs1112, the additive-additive epistasis × environment interaction variance at Gs1115 and the additive × environment interaction variance at Gs1118 were significantly detected (Table I); whereas, the significance of the conditional variances for dominance, additive-additive epistasis × environment interaction and additive × environment interaction effects were not detected in the periods of Gs1109 to Gs1112, Gs1112 to Gs1115 and Gs1115 to Gs1118, respectively (Table II). These significant unconditional variances were derived from the expression of activated genes in earlier developmental stages. The significant conditional additive × additive epistatic variance ($V_{AA(t)}$) at Gs1115 (Table I) resulted from the expression of the activated genes in the period of Gs1112 to Gs1115 or earlier periods, indicating genes expressed in early stages were closed in the period of Gs1109 to Gs1112 and then re-expressed at Gs1115. The results also revealed that gene expression exhibited interruptive mode in the process of development of flue-cured tobacco quality traits.

Gene expression of TS was mainly controlled by additive effect which conditional variance accounted for 37% of the total conditional genetic variance in the periods of Gs1118 to Gs1121 and additive-additive epistasis × environment interaction effects accounted for about 44.0% in the period of Gs1100 to Gs1109 and 38.8% in the period of Gs1109 to Gs1112, respectively. It also showed that net additive effect of gene expression of TS occurred in early and later developmental stages (the periods of Gs1100 to Gs1112 & Gs1115 to Gs1121), the net dominance × environment interaction effects were mostly detected in later developmental stages (the periods of Gs1115 to Gs1118 & Gs1118 to Gs1121) and the net additive-additive epistasis × environment interaction effect was found at all growth stages except the period from Gs1112 to Gs1115. The additive variance and additive-additive epistasis × environment interaction variances of TS were higher than other genetic variance components in the periods of Gs1118 to Gs1121 and Gs1100 to Gs1109, respectively. Therefore, gene expression of TS was mainly controlled by additive effect in the period of Gs1118 to Gs1121 and by additive-additive epistasis × environment interaction effect in the period of Gs1100 to Gs1109.

The additive effect of TS in the period of Gs1115 to Gs1121, dominance effect in the period of Gs1118 to Gs1121 and the additive-additive epistasis × environment interaction effect in the periods of Gs1109 to Gs1112 and Gs1115 to Gs1121 could be significantly detected (Table II) by the conditional analysis method, however, such effects could not be detected by the unconditional method.

For RS, the conditional dominance × environment interaction variance ($V_{DE(t|t-1)}$) in whole growth stage, conditional additive × additive epistatic variance ($V_{AA(t|t-1)}$) in five developmental stages except the period of Gs1100 to Gs1109, conditional additive × environment interaction variance ($V_{AE(t|t-1)}$) in whole growth stages except the period of Gs1115 to Gs1118 all reached significant (Table II). The conditional *GE* interaction variances in five developmental stages accounted for about 30.7%, 77.7%, 80.0%, 68.2% and 70.9% of total conditional genetic variance, respectively it showed that the environment had a huge effect on genotype. The conditional dominance × environment interaction variance ($V_{DE(t|t-1)}$) took a large majority of total conditional genetic variances in the periods of Gs1112 to Gs1115 and Gs1118 to Gs1121, accounted for about 59.6% and 49.1%, respectively; thus, it could be concluded that RS was largely affected by dominance × environment interaction effects in these periods. During the period of Gs1115 to Gs1118, RS was mainly controlled by additive-additive epistasis × environment interaction effects ($V_{AAE(t|t-1)}$), which could be selected in advanced generations under different environment.

Conditional genetic variance analyses for TS and RS showed that gene intermittently expressed in the process of trait development (Table II). For example, dominance effect of TS in the period of Gs1109 to Gs1112 and additive × environment interaction effect of RS in the period of Gs1115 to Gs1118 could not be detected, but the dominance variance of TS at Gs1112 and additive × environment interaction variance of RS at Gs1118 were significant, which accounted for about 9.5% and 19.8% of the total genetic variances, respectively. Those effects might be the rest effects of gene expression in early developmental stages and indicated that gene expression would be interrupted in specific growth stages.

The conditional residual variances ($V_{e(t|t-1)}$) for TS and RS were all detected significantly during whole growth periods, and the proportions of total variation were mostly less than 30% for most periods, except that the periods of Gs1109 to Gs1112 (32.4%) and Gs1112 to Gs1115 (37.2%) for TS and the period of Gs1100 to Gs1109 (39.6%) for RS. Since residual effects in different periods were independent of each other, residual effect always existed in whole

Table I: Genetic variance components for total sugar and reducing sugar of flue-cured tobacco in five developmental stages

Parameter	TS [c]					RS				
	Gs1109[b]	Gs1112	Gs1115	Gs1118	Gs1121	Gs1109	Gs1112	Gs1115	Gs1118	Gs1121
$V_{A(t)}$	6.466**	0.787**	0.000	0.000	0.000	0.000	0.000	1.690**	0.000	0.000
$V_{D(t)}$	3.600**	0.655**	1.899**	0.000	0.000	4.048**	0.000	6.281**	0.000	0.000
$V_{AA(t)}$	0.000	0.000	0.585**	2.142**	2.460**	0.000	1.583**	0.000	8.742**	9.977**
$V_{AE(t)}$	0.000	0.619**	1.277**	0.810**	0.637**	3.137**	6.809**	2.741**	7.653**	8.100**
$V_{DE(t)}$	0.000	2.871**	0.000	9.697**	11.425**	1.039**	8.937**	0.000	19.505**	13.178**
$V_{AAE(t)}$	11.145*	0.000	0.730**	0.000	0.000	0.000	0.000	0.000	0.000	0.000
$V_{e(t)}$	4.141**	1.989**	3.223**	2.079**	3.195**	5.388**	1.620**	3.908**	2.690**	3.475*

[a] $V_{A(t)}$, $V_{D(t)}$, $V_{AA(t)}$, $V_{AE(t)}$, $V_{DE(t)}$, $V_{AAE(t)}$, $V_{e(t)}$, are genetic variance components due to additive, dominance, additive × additive epistasis, additive × environment interaction, dominance × environment interaction, additive-additive epistasis × environment interaction and residual effects, respectively;

[b] Gs1109, Gs1112, Gs1115, Gs1118, Gs1121 denote the growth stage1109, growth stage1112, growth stage1115, growth stage1118, growth stage1121, respectively;

[c] TS= total sugar; RS= reducing sugar;

*, ** significance at 0.05 and 0.01 probabilities, respectively

Table II: Conditional variance components for total sugar and reducing sugar of flue-cured tobacco in different developmental stages

Parameter [a]	TS [c]					RS				
	Gs1109| Gs1100 [b]	Gs1112| Gs1109	Gs1115| Gs1112	Gs1118| Gs1115	Gs1121| Gs1118	Gs1109| Gs1100	Gs1112| Gs1109	Gs1115| Gs1112	Gs1118| Gs1115	Gs1121| Gs1118
$V_{A(t\|t-1)}$	6.466**	1.442**	0.000	1.132**	7.960**	0.000	0.000	0.000	0.000	0.000
$V_{D(t\|t-1)}$	3.600**	0.000	1.297**	0.000	1.925**	4.048**	0.000	0.000	0.000	0.000
$V_{AA(t\|t-1)}$	0.000	0.000	1.366**	0.065**	2.610**	0.000	2.451**	11.981**	0.427**	22.253**
$V_{AE(t\|t-1)}$	0.000	0.000	2.115**	0.000	2.226**	3.137**	8.216**	16.376**	0.000	19.699**
$V_{DE(t\|t-1)}$	0.000	0.000	0.000	3.669**	3.165**	1.039**	5.420**	47.990**	0.734**	44.246**
$V_{AAE(t\|t-1)}$	11.145*	1.944**	0.000	0.092**	0.635**	0.000	0.000	0.000	2.915**	0.000
$V_{e(t\|t-1)}$	4.141**	1.624**	2.830**	1.608**	3.006**	5.388**	1.464**	4.128**	1.271**	3.940**

[a] $V_{A(t|t-1)}, V_{D(t|t-1)}, V_{AA(t|t-1)}, V_{AE(t|t-1)}, V_{DE(t|t-1)}, V_{AAE(t|t-1)}, V_{e(t|t-1)}$, are conditional variances for additive, dominance, additive-additive epistatic effects and their interaction effects with environment for TS and RS in the period of time (*t*-1) to time *t*, respectively;

[b] Gs1109|Gs1100 denote the stage from growth stage1100 to growth stage1109; Gs1112|Gs1109, Gs1115|Gs1112, Gs1118|Gs1115 and Gs1121|Gs1118, are the stages from growth stage1109 to growth stage1112, from growth stage1112 to growth stage1115, from growth stage1115 to growth stage1118, from growth stage1118 to growth stage1121, respectively;

[c] TS= the total sugar; RS= the reducing sugar;

*, ** significance at 0.05 and 0.01 probabilities, respectively

process of trait development, and its significance can be easily detected.

Broad-sense and narrow-sense heritabilities based on unconditional variances: The general broad-sense and narrow-sense heritabilities and interaction heritabilities were estimated for different developmental stages (Table III). For TS, It was noted that general broad-sense heritabilities (H_G^2) were higher than general narrow-sense heritabilities (h_G^2) at Gs1109, Gs1112 and Gs1115, but they were equal at Gs1118 and Gs1121; in other words, dominance effects could be detected at Gs1109, Gs1112 and Gs1115 but not at Gs1118 and Gs1121. By comparison of h_{GE}^2 and H_{GE}^2, we could observed that dominance × environment interaction effects were not detected at Gs1109 and Gs1115, but exhibited maximum at Gs1121; it indicated that selection of dominance in early growth stages were valid for all environments, but would exhibit larger different in later stages among environments.

For RS, it was observed that narrow-sense general

Table III: Broad-sense and narrow-sense heritabilities for total sugar and reducing sugar traits of flue-cured tobacco

Parameter [a]	TS [c]					RS				
	Gs1109 [b]	Gs1112	Gs1115	Gs1118	Gs1121	Gs1109	Gs1112	Gs1115	Gs1118	Gs1121
h_G^2	0.255**	0.114**	0.076**	0.145**	0.139**	0.000	0.084**	0.116**	0.227**	0.287**
H_G^2	0.397**	0.208**	0.322**	0.145**	0.139**	0.297**	0.084*	0.545**	0.227**	0.287**
h_{GE}^2	0.440**	0.089**	0.260	0.055*	0.036	0.230**	0.359**	0.187**	0.198**	0.233**
H_{GE}^2	0.440**	0.504**	0.260**	0.713**	0.681**	0.307**	0.831**	0.187**	0.704**	0.613**

[a] h_G^2 and h_{GE}^2 are the general narrow- sense heritability and interaction heritability; H_G^2 and H_{GE}^2 are the general broad-sense heritability and interaction heritability;
[b]Gs1109, Gs1112, Gs1115, Gs1118, Gs1121, denote the growth stage1109, growth stage1112, growth stage1115, growth stage1118, growth stage1121, respectively;
[c]TS= total sugar; RS= reducing sugar;
*, ** significance at 0.05 and 0.01 probability, respectively

Table IV: Broad-sense and narrow-sense heritabilities estimated by conditional analysis method for total sugar and reducing sugar traits in flue-cured tobacco

Parameter [a]	TS [c]					RS				
	Gs1109| Gs1100 [b]	Gs1112| Gs1109	Gs1115| Gs1112	Gs1118| Gs1115	Gs1121| Gs1118	Gs1109| Gs1100	Gs1112| Gs1109	Gs1115| Gs1112	Gs1118| Gs1115	Gs1121| Gs1118
$h_{G(t\|t-1)}^2$	0.255**	0.288**	0.180**	0.182**	0.491**	0.000	0.140**	0.149**	0.080**	0.247**
$H_{G(t\|t-1)}^2$	0.397**	0.288**	0.350**	0.182**	0.580**	0.297**	0.140**	0.149**	0.080**	0.247**
$h_{GE(t\|t-1)}^2$	0.440**	0.388**	0.278**	0.014	0.133**	0.230**	0.468**	0.203**	0.545**	0.219**
$H_{GE(t\|t-1)}^2$	0.440**	0.388**	0.278**	0.573**	0.280**	0.307**	0.777**	0.800**	0.682**	0.709**

[a] $h_{G(t|t-1)}^2$ and $h_{GE(t|t-1)}^2$ are the general conditional narrow-sense heritability and interaction heritability predicted by conditional analysis method; $H_{G(t|t-1)}^2$ and $H_{GE(t|t-1)}^2$ are the general conditional broad-sense heritability and interaction heritability predicted by conditional analysis method;
[b] Gs1109|Gs1100, denote the stage from growth stage1100 to growth stage1109; Gs1112|Gs1109, Gs1115|Gs1112, Gs1118|Gs1115 and Gs1121|Gs1118, are the stages from growth stage1109 to growth stage1112, from growth stage1112 to growth stage1115, from growth stage1115 to growth stage1118, from growth stage1118 to growth stage1121, respectively;
[c] TS= total sugar; RS= reducing sugar;
*, ** significance at 0.05 and 0 .01 probabilities, respectively

heritability was zero at Gs1109 and equivalent to broad-sense general heritabilities at Gs1112, Gs1118 and Gs1121, indicating dominance effect occurred only at Gs1109 and Gs1115. The broad-sense interaction heritabilities (H_{GE}^2) were larger than the narrow-sense interaction heritabilities (h_{GE}^2) in all developmental stages except Gs1115, but equivalent at Gs1115.

Broad-sense and narrow-sense heritabilities based on conditional variances: The broad-sense and narrow-sense heritabilities calculated by conditional variance components (Table IV) revealed that for TS, the conditional general broad-sense heritability ($H_{G(t|t-1)}^2$) was equal to the conditional general narrow-sense heritability ($h_{G(t|t-1)}^2$) in the periods of Gs1109 to Gs1112 and Gs1115 to Gs1118, indicating that there were no net dominance effects during these periods. The conditional broad-sense interaction heritabilities ($H_{GE(t|t-1)}^2$) were equivalent to the conditional narrow-sense interaction heritabilities ($h_{GE(t|t-1)}^2$) in the periods of Gs1109 to Gs1112 and Gs1112 to Gs1115, then the conditional narrow-sense interaction heritability decreased to minimum and the conditional broad-sense interaction heritability reached maximum in the period of Gs1115 to Gs1118. It revealed that there were no net dominance × environment interaction effect in the period of Gs1109 to Gs1115 and larger significant dominance × environment interaction effect was detected in the period of Gs1115 to Gs1118. It came to a conclusion that we could select hybrid to improve TS in the period of Gs1112 to Gs1115 in advanced generations under multiple environments, or we could achieve that from Gs1115 to Gs1118 under specific environments.

As for the broad-sense and narrow-sense heritabilities of RS, there was no net dominance effect in the period of Gs1109 to Gs1121 and net dominance effect reached maximum in the period of Gs1100 to Gs1109. In addition, the net dominance × environment interaction effects could be detected in whole developmental stages, but those effects were relatively smaller in the period of Gs1100 to Gs1109 and Gs1115 to Gs1118, compared to that in other growth stages. The genetic improvement for RS of flue-cured tobacco could be achieved by selection of hybrid varieties in early developmental stage under multiple environments or in the period from Gs1112 to Gs1115 under specific environments.

The genetic variation of quantitative traits is usually controlled by some minor-effect genes and environments, while, epistatic effects between different genes and interaction between gene and environment are also involved (Mackay *et al.*, 2009). Previous genetic studies on agronomic or chemical component traits of flue-cured tobacco usually ignored either genotype × environment interaction effect or epistatic effect (Pandeya *et al.*, 1985; Chen *et al.*, 2004; Xiao *et al.*, 2005, 2007, 2008), which might introduce deviation of analysis results. Our research is the first time to employ the additive, dominance and additive × additive model and condition analysis method to investigate the developing behavior of quantitative traits and uncover the genetic mechanisms of flue-cured tobacco with a diallel cross experiment of three generations materials (Parents, F_1 & F_2). Our study showed that the genetic behavior of flue-cured tobacco for quantitative traits was controlled by genetic main effects and *GE* interaction effects, and the *GE* interaction effects accounted for a large proportion of total phenotypic variation of TS and RS and the genes intermittently expressed in the process of trait development. TS could be improved by selecting pure lines in the period of Gs1118 to Gs1121 in early generations or hybrids in the period of Gs1100 to Gs1109 in advanced generations under specific environments. Heterosis could be used to improve RS of flue-cured tobacco varieties in middle or later periods (the periods of Gs1112 to Gs1115, Gs1115 to Gs1118 & Gs1118 to Gs1121) in advanced generations under specific environments.

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