Dynamic Genetic Effects on Threonine Content in Rapeseed (*Brassica napus* L.) Meal at Different Developmental Stages

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Abstract: Dynamic genetic effects on threonine content (TC) of rapeseed (*Brassica napus* L.) meal were analysed at 5 developmental times/stages using the genetic models for diploid plant seeds. Results indicated that the expression of diploid embryo, cytoplasmic and diploid maternal plant genes were all important for the performance of TC at various developmental times/stages of rapeseed, especially at the early and middle developmental stages. Among different genetic systems, TC was mainly controlled by the cumulative or net maternal main effects and the genotype × environment (GE) interaction effects, followed by the embryo main effects and GE interaction effects. The expression of genes was more easily influenced by the environmental factors at the first three developmental stages. The total narrow-sense heritabilities for TC on 15, 22, 29, 36 and 43 days post anthesis were 46.50, 62.60, 57.10, 84.70 and 59.50%, respectively, of which the interaction heritabilities were more important at the first three developmental stages of rapeseed. The improvement in TC of rapeseed meal could be expected by selection based on the higher narrow-sense heritabilities near maturity. The predicted genetic effects of parents showed that TC of progeny could be improved by using the parent Gaoyou 605.

Keywords: developmental genetics; genetic variances and conditional genetic variances; heritability; rapeseed meal; threonine content

Oilseed rape, *Brassica napus* L., is one of the most important oilseed crops around the world, which is mainly planted for edible oil and as potential biofuel for industrial applications. Rapeseed is a rich source of oil and protein. On average, rapeseeds contain 22.5% of crude protein in addition to 43.5% of crude fat (Becker *et al*. 1999). Rapeseed meal has a high protein content and is an excellent feed for animals (Goding *et al*. 1972; Huisman & Tolman 1990). The overall nutritive value of rapeseed is not lower than that of soybean (Simbaya *et al*. 1995).

Threonine is an essential amino acid. It plays important physiological roles in animal growth and immune functions. A preliminary study has suggested that human adults need about 15 mg of this amino acid per day per kilogram of body weight (Borgonha *et al*. 2002). It is well known that dietary threonine imbalance could decrease the growth of the small intestine, liver, and skeletal muscle in young animals. Wang *et al.* (2007) found out that either excess or deficiency of dietary threonine reduces protein synthesis in rapidly growing tissues of young pigs. In recent years, lysine, methionine and threonine additives have been widely applied in animal feeds. For centuries, *Brassica napus* L. species have been exploited, domesticated and refined by man. Although its economic value mainly depends on the seed oil content, the increase of seed protein content is also an important breeding objective. Development of cultivars with high protein or essential

amino acid content has therefore been proposed for achieving the full potential benefits of *Brassica* crops (Röbbelen 1981). Further research on TC in rapeseed meal is useful for livestock and poultry production.

Most of the quality traits of rapeseed are quantitative traits. Genetic analysis of quality traits in rapeseed using traditional statistical method has been reported (Shi *et al*. 2003; Zhang *et al*. 2004a, b). The protein content of rapeseed may be controlled by various genetic systems, such as embryo nuclear genes (Brandle & McVetty 1988), cytoplasmic genes (McVetty & Pinnisch 1994), diploid maternal plant nuclear genes (Grami & Steffansson 1977). Wu *et al*. (2005) found that the protein content of rapeseed meal was governed by the genetic main effects from embryo (cotyledon), cytoplasmic, diploid maternal plant genetic system and their genotype × environment (GE) interaction effects. The contents of amino acids, such as glutamic acid (Glu), glycine (Gly) and arginine (Arg), were confirmed to be subjected to different genetic effects (Ren *et al*. 2005). However, in these studies the genetic effects were inferred from the analysis based on the phenotypic values of traits in mature seeds. The protein and oil contents in seeds were the results of the expression of genes through a series of developmental stages after pollination (Variath *et al*. 2009; 2010a, b). So variation in genetic effects and expression of genes for the amino acid content of rapeseed might exist at different developmental stages.

The theory of developmental quantitative genetics proposes that the performances for complex quantitative traits are controlled by the different temporal and spatial patterns of gene expression during the developmental process across environments (Stewart & Hunt 1982; Atchley 1984; Atchley & Zhu 1997). One of the major goals of developmental quantitative genetics is to understand the dynamic variations of gene expression under different environments. The genetic models and corresponding statistical analysis methods proposed by Zhu could be effectively applied to analyse the genetic effects at different developmental times or at any one specific period (t–1→t) (Zhu 1995). These models have been successfully used to study the developmental genetics and net genetic effects on the quantitative quality traits in rice and cotton (Shi *et al*. 2002a, b, 2005, 2006; Ye *et al*. 2003; Zhang *et al*. 2004c). But, the dynamic expression of genes affecting the nutrition quality traits of rapeseed just began to be recognized. Threonine is one of the essential amino acids and its content is one of the major determinants of the nutrition quality of rapeseed meal. However, little has been known about the developmental behaviour of TC of rapeseed up to date.

In this study, dynamic genetic behaviour of TC in rapeseed meal was investigated using unconditional and conditional genetic models and the related statistical methods. Unconditional analysis was used to estimate the accumulated effects of genes (0→ t) expressed from flowering (0) to a particular time (t) in the whole developmental period of rapeseed; conditional analysis was aimed at the net effects of new expression of genes in the special period t–1 to time t (t–1→t) during different seed development stages. The accumulative or net genetic effects of gene expression on TC of rapeseed meal at different developmental times/stages were analysed; the difference was discussed in gene expression. In addition, the general and interaction heritability at different developmental times was estimated among different developmental times.

MATERIAL AND METHODS

Field experiments

The field experiments were carried out in the years 2006–07 and 2007–08 based on F_1 seed of 2005–06 on the experimental farm of Zhejiang University at Hangzhou (120°11'27''E, 30°16'28''N). In a complete diallel design (not including reciprocal crosses), nine parental lines of *B. napus* including Youcai 601 (P1), Shuang 20-4 (P2), Huashuang 3 (P3), Gaoyou 605 (P4), Zhongyou 821(P5), Eyouchangjia (P6), Zhong R-888 (P7), Tower (P8) and Zheshang 72 (P9) were used. The 36 combinations were made by P1 × Pi (P2 toP9), P2 × Pi (P3 to P9), P3 × Pi (P4 to P9), P4 × Pi (P5 to P9), P5 × Pi (P6 to P9), P6 × Pi (P7 to P9), P7 × Pi (P8 to P9), and P8 × P9. The field experiments used a randomized block design with two replications. The seeds of parental and F_1 lines were sown on October 9, 2006 and October 13, 2007, respectively. 38-days-old seedlings were transplanted to plots at a spacing of 35 cm × 30 cm (32 plants/plot). Flowers were labelled and recorded on the day of anthesis. Seed samples of parents and F_2 on F_1 plants were derived from 12 plants in the middle part of each

plot, whose several inflorescences were randomly set in the photic bag before anthesis. The seed developmental period was divided into 5 stages, namely the initial (1–15 days post-anthesis – dpa), early (16–22 dpa), middle (23–29 dpa), late (30–36 dpa) and mature (37–43 dpa) stages. The F_1 seed samples at each developmental time/stage were obtained from the 36 crosses made using hand emasculation among the 9 parental lines during the same growing seasons. Regarding the sample size, the following strategy was followed: 15d seed sample comprised about 50 inflorescences/plot followed by 45 for 22 days, 30 for 29 days, 20 for 36 days and 15 for 43 days. Each inflorescence contained about 2~7 freshly crossed florets which were recorded and bagged after removing the unopened florets for each day of the flowering period of rapeseed.

Developing calibration equation for TC of rapeseed meal

A total of 621 primary rapeseed samples were used to develop the calibration equation of amino acid content. Some samples selected from breeding programmes and developmental genetic experiments were also included. The remaining rapeseed samples were chosen from rapeseed cultivars for oil production. Therefore, the seed samples had a wide range of variation in amino acid content. At least 3 g of intact seeds from each sample were scanned in a 36mm inner-diameter ring cup (Shenk & Westerhaus 1993) using a NIRSystems model 5000 near-infrared reflectance spectroscope (NIRSystems, Inc., Silver Spring, MD, USA) according to the instructions of WinISI II manual for routine analysis (FOSS NIRSystems/TECTOR, Infrasoft International, LLC.). The calibration set comprising 226 rapeseed samples was selected from the 621 primary samples by using CENTER and SELECT algorithm based on the spectra variation. TC in the rapeseed meal was estimated using an amino acid auto-analyser (model L-8900; Hitachi High-Technologies Corporation, Japan) after oil extraction and analysis which were done using the Soxhelt Extractive Method (GB2906-82) on a crude fat analyser (model SZF-06; Shanhai Xinjia Electronic Co Ltd., China). The calibration equation was developed using a standard normal variant + de-trending scatter correction and a 2, 4, 4, 1 mathematical treatment and a modified partial least squares (MPLS) regression method. The equation was developed for TC with an r^2 or the RSQ of 0.964, and the corresponding standard error of cross-validation (SEC) of 0.061, respectively (CHEN *et al.* 2011).

Determination of TC in rapeseed meal

TC (%) of rapeseed meal was calculated using the above equations and NIRSystems model 5000 near-infrared reflectance spectroscope. At least 3 g of seeds from each sample were scanned in a 36 mm inner-diameter ring cup (Shenk & Westerhaus 1993). All samples from each parental, F_1, and F_2 lines were measured with 2 replications.

Statistical methods

The genetic models and statistical procedures for quantitative traits of diploid plant seeds (Zhu & Weir 1994, 1996; Zhu 1995) were applied to estimate the unconditional and conditional genetic main effects and GE interaction effects. For a mating design from a set of inbred lines, the generation mean (y_{hijk}) of mating type k from maternal line i and paternal line j in block l of environment h can be expressed as follows:

$$y_{hijkl} = \mu + E_h + G_{ijk} + GE_{hijk} + B_{l(h)} + e_{hijkl}$$

where:

μ – population mean, fixed
E_h – environmental main effect, fixed
G_{ijk} – genotypic main effect, random
GE_{hijk} – genotype × environment interaction effect, random
$B_{l(h)}$ – block effect, random
e_{hijkl} – residual effect, random

The unconditional genetic effects from different genetic systems were defined as the accumulative effects of gene expression from anthesis (0 dpa) to a particular time (t) during the developmental period ($0 \rightarrow t$) by the above equation. The unconditional genetic main effect variance ($V_{G(t)}$) consists of unconditional embryo additive variance ($V_{A(t)}$), unconditional embryo dominance variance ($V_{D(t)}$), unconditional cytoplasmic variance ($V_{C(t)}$), unconditional maternal additive variance ($V_{Am(t)}$) and unconditional maternal dominance variance ($V_{Dm(t)}$). The unconditional GE interaction variance ($V_{GE(t)}$) included unconditional embryo additive

interaction variance ($V_{AE(t)}$), unconditional embryo dominance interaction variance ($V_{DE(t)}$), unconditional cytoplasm interaction variance ($V_{CE(t)}$), unconditional maternal additive interaction variance ($V_{AmE(t)}$) and unconditional maternal dominance interaction variance ($V_{DmE(t)}$) at the rapeseed developmental time t (Zhu & Weir 1994; Zhu 1996). The unconditional embryo and maternal additive covariance ($C_{A.Am(t)}$) or dominance covariance ($C_{D.Dm(t)}$), and unconditional embryo and maternal additive interaction covariance ($C_{AE.AmE(t)}$) or dominance interaction covariance ($C_{DE.DmE(t)}$) were estimated, because partial embryo genes derived from maternal plants and the covariance might exist between embryo and maternal genetic effects. The partitioning for the unconditional phenotypic variance ($V_{P(t)}$) is as follows:

$$V_{P(t)} = V_{A(t)} + V_{D(t)} + V_{C(t)} + V_{Am(t)} + V_{Dm(t)} + V_{AE(t)} + V_{DE(t)} + V_{CE(t)} + V_{AmE(t)} + V_{DmE(t)} + 2(C_{A.Am(t)} + C_{D.Dm(t)}) + 2(C_{AE.AmE(t)} + C_{DE.DmE(t)}) + V_{e(t)}$$

For the conditional analysis, the developmental genetic models and statistical methods developed by Zhu (1995) were used to evaluate the conditional genetic variance ($V_{G(t|t-1)}$) components including conditional embryo additive variance ($V_{A(t|t-1)}$), conditional embryo dominance variance ($V_{D(t|t-1)}$), conditional cytoplasmic variance ($V_{C(t|t-1)}$), conditional maternal additive variance ($C_{A.Am(t|t-1)}$) and conditional maternal dominance variance ($V_{Dm(t|t-1)}$), and the conditional GE interaction variance ($V_{GE(t|t-1)}$) divided into conditional embryo additive interaction variance ($V_{AE(t|t-1)}$), conditional embryo dominance interaction variance ($V_{DE(t|t-1)}$), conditional cytoplasm interaction variance ($V_{CE(t|t-1)}$), conditional maternal additive interaction variance ($V_{AmE(t|t-1)}$) and conditional maternal dominance interaction variance ($V_{DmE(t|t-1)}$) at the special period ($t-1 \rightarrow t$) with seed developing. The conditional covariances between embryo and maternal effects including $C_{A.Am(t|t-1)}$, $C_{D.Dm(t|t-1)}$, $C_{AE.AmE(t|t-1)}$ and $C_{DE.DmE(t|t-1)}$ were also estimated. The partitioning for the conditional phenotypic variance ($V_{P(t|t-1)}$) is as follows:

$$V_{P(t|t-1)} = V_{A(t|t-1)} + V_{D(t|t-1)} + V_{C(t|t-1)} + V_{Am(t|t-1)} + V_{Dm(t|t-1)} + V_{AE(t|t-1)} + V_{DE(t|t-1)} + V_{CE(t|t-1)} + V_{AmE(t|t-1)} + V_{DmE(t|t-1)} + 2(C_{A.Am(t|t-1)} + C_{D.Dm(t|t-1)}) + 2(C_{AE.AmE(t|t-1)} + C_{DE.DmE(t|t-1)}) + V_{e(t|t-1)}$$

Net genetic effects or conditional genetic effects, which were defined as the effects of new expression of genes activated in the period $t-1$ to time t ($t-1 \rightarrow t$), were detected using conditional analysis through the conditional variable ($Y(*) = Y(t|t-1)$ derived from the equation ($Cov(Y(*), Y(t-1)) = 0$). In the present experiments, 15d|0d indicated the cumulative effects of gene expression from 0 to 15 dpa, while 22d|15d represents the measures on the 22nd day given the phenotypic value estimated on the 15th day for conditional analysis, and so on.

The estimated total narrow-sense heritability (h^2) at different developmental times was further differentiated into general heritability (h^2_G) controlled by genetic main effects and interaction heritability (h^2_{GE}) controlled by GE interaction effects. General heritability has the components of embryo general heritability (h^2_{Go}), cytoplasmic general heritability (h^2_{Gc}) and maternal general heritability (h^2_{Gm}). Interaction heritability has the components of embryo interaction heritability (h^2_{GoE}), cytoplasmic interaction heritability (h^2_{GcE}) and maternal interaction heritability (h^2_{GmE}). The AUP method was used to predict the genetic main effects including embryo additive main effect (A), cytoplasmic main effect (C), maternal additive main effect (Am) as well as their GE interaction effects (AE, CE and AmE) (Zhu 1993; Zhu & Weir 1996).

The Jackknife resampling methods were applied by sampling generation means of entries to derive the standard errors of estimated unconditional or conditional variance, heritability and correlation coefficient components (Miller 1974; Zhu & Weir 1996).

RESULTS

Estimation of TC in rapeseed meal of parental and descendant lines

The means and standard deviation of TC in rapeseed meal of the parental (P1 to P9), F_1 (36 combinations mentioned above) and F_2 samples (from F_1 plants of 36 combinations) at different developmental times are shown in Table 1. The mean values at 5 developmental times were 0.748, 1.008, 1.269, 1.485 and 1.561%, respectively, for the parental lines in 2007. The respective standard deviations were 0.125, 0.203, 0.216, 0.165 and 0.182%. For F_1, the mean values and the standard deviations were 0.906, 1.026, 1.240, 1.537, 1.660 and

Table 1. The means and range of threonine content (TC) in rapeseed meal

Generations	Time (dpa)	2007				2008			
		mean	min	max	SD	mean	min	max	SD
Parent	15	0.748	0.570	0.932	0.125	1.123	0.681	1.557	0.210
	22	1.008	0.781	1.378	0.203	0.942	0.551	1.246	0.222
	29	1.269	0.934	1.579	0.216	1.132	0.758	1.427	0.194
	36	1.485	1.147	1.708	0.165	1.386	1.069	1.653	0.180
	43	1.561	1.188	1.794	0.182	1.515	1.321	1.699	0.117
F_1	15	0.906	0.604	1.338	0.185	0.985	0.697	1.442	0.176
	22	1.026	0.753	1.471	0.219	0.727	0.530	1.044	0.131
	29	1.240	0.838	1.615	0.212	1.169	0.658	1.506	0.224
	36	1.537	1.064	1.739	0.148	1.465	1.066	1.688	0.125
	43	1.660	1.395	1.860	0.105	1.585	1.267	1.800	0.098
F_2	15	0.750	0.563	1.032	0.113	0.980	0.759	1.368	0.116
	22	0.994	0.728	1.433	0.154	0.981	0.554	1.406	0.207
	29	1.258	0.909	1.560	0.144	1.006	0.710	1.403	0.145
	36	1.459	1.189	1.667	0.125	1.354	1.155	1.603	0.084
	43	1.566	1.301	1.772	0.105	1.471	1.256	1.671	0.071

SD – standard deviation; dpa – days post anthesis

0.185, 0.219, 0.212, 0.148 and 0.105%, respectively. The mean values of F_2 were 0.750~1.566%, with a standard deviation of 0.105~0.113%. In all cases, TC increased as the seeds develop after flowering, reaching the plateau upon maturation. There were visible differences in the means of TC of rapeseed meal between 2007 and 2008. The results of the performance for TC among 3 generations at 5 different developmental times were found to be easily influenced by environmental factors.

Unconditional variance analysis for TC at different developmental times

Unconditional variance components for TC of rapeseed meal at the 5 developmental times (15, 22, 29, 36 and 43 dpa) are listed in Table 2. TC of rapeseed meal appeared to be controlled by both the genetic main effects and GE interaction effects, since most of the unconditional genetic variances from various genetic systems were significant at different developmental times. At the first three developmental times (15, 22, 29 dpa), variation in the TC was mainly dependent on the GE interaction effects, since $V_{GE(t)}$ ($V_{AE(t)} + V_{DE(t)} + V_{CE(t)} + V_{AmE(t)} + V_{DmE(t)}$) accounted for 74.51, 86.81 and 81.23% of the total genetic variance ($V_{G(t)} + V_{GE(t)}$, $V_{G(t)} = V_{A(t)} + V_{D(t)} + V_{C(t)} + V_{Am(t)} + V_{Dm(t)}$), respectively. At the last 2 developmental times (36 and 43 dpa), TC were mainly controlled by the genetic main effects, and $V_{G(t)}$ accounted for 51.47 and 74.38% of the total genetic variance, respectively. This suggested that the gene expression for TC at the first three developmental stages was much more easily influenced by the environmental conditions.

Among the genetic effects from embryo, cytoplasmic and maternal plant genetic systems for TC, it was observed that the maternal effects ($V_{Am(t)} + V_{Dm(t)} + V_{AmE(t)} + V_{DmE(t)}$) were more prominent at different developmental times which accounted for 59.91, 53.89, 58.69, 59.34 and 72.53% of the total genetic variance, respectively, followed by the embryo effects ($V_{A(t)} + V_{D(t)} + V_{AE(t)} + V_{DE(t)}$) which accounted for 23.29~41.31%. The cytoplasmic main effects were not significant at all developmental times, while the cytoplasmic interaction effects were significant at 3 developmental times (15, 22, and 36 dpa). The expression

Table 2. The estimated unconditional variance components for threonine content (TC) in rapeseed meal at different developmental times

Parameter	Developmental times of rapeseed (dpa)				
	15	22	29	36	43
$V_{A(t)}$	0.000	0.000	0.000	0.011**	0.003**
$V_{D(t)}$	0.013**	0.010**	0.012**	0.003**	0.002**
$V_{C(t)}$	0.000	0.000	0.000	0.000	0.000
$V_{Am(t)}$	0.000	0.000	0.000	0.009**	0.009**
$V_{Dm(t)}$	0.021**	0.026**	0.025**	0.006**	0.004**
$V_{AE(t)}$	0.003**	0.071**	0.049**	0.000	0.000
$V_{DE(t)}$	0.015**	0.009**	0.019**	0.004**	0.002**
$V_{CE(t)}$	0.022**	0.036**	0.000	0.005**	0.000
$V_{AmE(t)}$	0.039**	0.097**	0.061**	0.013**	0.000
$V_{DmE(t)}$	0.019**	0.025**	0.028**	0.005**	0.004**
$C_{A\cdot Am(t)}$	0.000	0.000	0.000	0.034**	0.004*
$C_{D\cdot Dm(t)}$	–0.004	–0.005	–0.004	–0.001	0.000
$C_{AE\cdot AmE(t)}$	–0.175	–0.030	–0.006	0.000	0.000
$C_{DE\cdot DmE(t)}$	–0.002	0.003	–0.005	–0.001	0.000
$V_{e(t)}$	0.008**	0.019**	0.008**	0.003**	0.003**

*$P < 0.05$; **$P < 0.01$; dpa – days post anthesis; $V_{A(t)}$ – embryo additive variance; $V_{D(t)}$ – embryo dominance variance; $V_{C(t)}$ – cytoplasmic variance; $V_{Am(t)}$ – maternal additive variance; $V_{Dm(t)}$ – maternal dominance variance; $V_{AE(t)}$ – embryo additive interaction variance; $V_{DE(t)}$ – embryo dominance interaction variance; $V_{CE(t)}$ – cytoplasm interaction variance; $V_{AmE(t)}$ – maternal additive interaction variance; $V_{DmE(t)}$ – maternal dominance interaction variance; $C_{A\cdot Am(t)}$ – embryo and maternal additive covariance; $C_{D\cdot Dm(t)}$ – embryo and maternal dominance covariance; $C_{AE\cdot AmE(t)}$ – embryo and maternal additive interaction covariance; $C_{DE\cdot DmE(t)}$ – embryo and maternal dominance interaction covariance

of cytoplasmic genes should not be ignored for TC of rapeseed at some developmental times.

Given the observations that the additive variances accounted for much of the total genetic variances for TC at the developmental times of 22, 29, 36 and 43 dpa ($(V_{A(t)} + V_{Am(t)} + V_{AE(t)} + V_{AmE(t)})/(V_{G(t)} + V_{GE(t)})$ = 60.99, 56.65, 58.98 and 50.26%, respectively), while the dominance variance was important at the developmental time of 15 dpa ($(V_{D(t)} + V_{Dm(t)} + V_{DE(t)} + V_{DmE(t)})/(V_{G(t)} + V_{GE(t)})$ = 50.95%). Therefore, the additive effects of the gene expression from different genetic systems at most of the developmental times were more crucial in controlling the performance of TC.

The results in Table 2 show that the unconditional covariances ($C_{A.Am(t)}$, $C_{D.Dm(t)}$, $C_{AE.AmE(t)}$ and $C_{DE.DmE(t)}$) were not significant for TC except for the significant positive additive covariances ($C_{A.Am(t)}$) at 36 and 43 dpa, which indicated that there were visible relationships between embryo and maternal additive main effects at these two developmental times. The residual variances at various developmental times were all significant, so TC of rapeseed meal could be significantly influenced by sampling errors. Nevertheless, it is speculated that TC was mainly affected by genetic effects because of the small values for the estimated $V_{e(t)}$.

Conditional analysis for TC at different developmental stages

Genes affecting the TC might be expressed in different ways during the developmental stages of rapeseed. The genetic variances estimated by the unconditional analysis at the developmental time t only can reveal the variations of accumulated genetic effects expressed from flowering

(0) to time t (0→t). Therefore, the results could not clearly elucidate the gene expression at one specific developmental stage (t–1→t), such as the developmental stage from 30 to 36 dpa. Accordingly, conditional analysis approaches are needed to decipher the dynamic gene expression at each developmental stage. The results in Table 3 show the variance components estimated by the conditional method.

The results showed that the activation of quantitative genes for TC gradually occurred through the different developmental stages and there existed diversity of the magnitude or type of conditional genetic effects among the various developmental stages. The new expression of genes at certain developmental stages was much higher, especially for the periods of 23~29 and 16~22 dpa, indicating that the embryo, cytoplasmic and/or maternal effects from the new expression of genes were visible at the specific stage(s). Furthermore, since the conditional GE interaction variances ($V_{G(t|t-1)}$) for TC at 5 developmental stages of rapeseed accounted for 74.51, 83.62, 85.42, 62.97 and 90.68% of total genetic variances ($V_{G(t|t-1)} + V_{GE(t|t-1)}$), respectively, the net GE interaction effects from different genetic systems were more important for this nutrient quality trait of rapeseed meal at all specific developmental stages. Hence, the new expression of genes during the whole developmental period of rapeseed was sensitive to environmental conditions.

For TC, the new expression of maternal nuclear genes at the first three developmental stages was assumed to be larger than in the others, because the net maternal effects accounted for 59.91, 49.59 and 59.75% (($V_{Am(t|t-1)} + V_{Dm(t|t-1)} + V_{AmE(t|t-1)} +$

Table 3. The estimated conditional variance components for threonine content (TC) at different developmental stages in rapeseed

Parameter	Developmental stages of rapeseed (dpa)				
	15|0	22|15	29|22	36|29	43|36
$V_{A(t\|t-1)}$	0.000	0.000	0.000	0.008**	0.000
$V_{D(t\|t-1)}$	0.013**	0.010**	0.008**	0.003**	0.002**
$V_{C(t\|t-1)}$	0.000	0.000	0.000	0.003**	0.003**
$V_{Am(t\|t-1)}$	0.000	0.000	0.000	0.000	0.000
$V_{Dm(t\|t-1)}$	0.021**	0.024**	0.025**	0.004**	0.004**
$V_{AE(t\|t-1)}$	0.003**	0.045**	0.046**	0.009**	0.050**
$V_{DE(t\|t-1)}$	0.015**	0.009**	0.022**	0.003**	0.002**
$V_{CE(t\|t-1)}$	0.022**	0.039**	0.013**	0.000	0.000
$V_{AmE(t\|t-1)}$	0.039**	0.052**	0.079**	0.013**	0.022**
$V_{DmE(t\|t-1)}$	0.019**	0.026**	0.030**	0.005**	0.005**
$C_{A\cdot Am(t\|t-1)}$	0.000	0.000	0.000	0.000	0.000
$C_{D\cdot Dm(t\|t-1)}$	–0.004	–0.004	–0.042	0.000	0.000
$C_{AE\cdot AmE(t\|t-1)}$	–0.175	–0.012	–0.019	–0.002	–0.016
$C_{DE\cdot DmE(t\|t-1)}$	–0.002	0.002	–0.007	–0.001	0.000
$V_{e(t\|t-1)}$	0.008**	0.019**	0.008**	0.003**	0.003**

**$P < 0.01$; dpa – days post anthesis; $V_{A(t|t-1)}$ – conditional embryo additive variance; $V_{D(t|t-1)}$ – conditional embryo dominance variance; $V_{C(t|t-1)}$ – conditional cytoplasmic variance; $V_{Am(t|t-1)}$ – conditional maternal additive variance; $V_{Dm(t|t-1)}$ – conditional maternal dominance variance; $V_{AE(t|t-1)}$ – conditional embryo additive interaction variance; $V_{DE(t|t-1)}$ – conditional embryo dominance interaction variance; $V_{CE(t|t-1)}$ – conditional cytoplasm interaction variance; $V_{AmE(t|t-1)}$ – conditional maternal additive interaction variance; $V_{DmE(t|t-1)}$ – conditional maternal dominance interaction variance; $C_{A\cdot Am(t|t-1)}$ – conditional embryo and maternal additive covariance; $C_{D\cdot Dm(t|t-1)}$ – conditional embryo and maternal dominance covariance; $C_{AE\cdot AmE(t|t-1)}$ – conditional embryo and maternal additive interaction covariance; $C_{DE\cdot DmE(t|t-1)}$ – conditional embryo and maternal dominance interaction covariance

$V_{DmE(t|t-1)}$)/($V_{G(t|t-1)}$ + $V_{GE(t|t-1)}$)), while the net embryo effects were larger at the next two developmental stages [arriving at 47.69 and 61.72% (($V_{A(t|t-1)}$ + $V_{D(t|t-1)}$ + $V_{AE(t|t-1)}$ + $V_{DE(t|t-1)}$)/($V_{G(t|t-1)}$ + $V_{GE(t|t-1)}$))], respectively.

Significant conditional cytoplasmic interaction effects were observed at the first three developmental stages, while there were significant conditional cytoplasmic main effects at the next two developmental stages. These results revealed that the expression of cytoplasmic genes at the earlier developmental period was more easily influenced by environmental conditions than that at the later developmental period. Compared to the different types of conditional variance components, the total net additive and cytoplasmic effects appeared to be very important for TC at the whole developmental period of rapeseed, which were closely associated with the selective effect of genetic improvement in the breeding for rapeseed quality.

The theory of developmental quantitative genetics considers that the quantitative polygene might be orderly and selectively expressed during the development of quality traits. In the present study, the results of conditional genetic analysis revealed that $V_{A(15d|0d)}$, $V_{A(22d|15d)}$, $V_{A(29d|22d)}$, $V_{A(43d|36d)}$, $V_{C(15d|0d)}$, $V_{C(22d|15d)}$, $V_{C(29d|22d)}$, $V_{Am(t|t\text{-}1)}$, $V_{CE(29d|22d)}$, $V_{CE(36d|29d)}$ and $V_{CE(43d|36d)}$ were not found. Hence, it could be suggested that there were no new expressions of genes for the above genetic effects at these specific developmental stages, and the corresponding significant unconditional genetic effects detected in Table 2 at certain developmental times might be attributable to the extended expression of activated genes at the previous developmental stages. It was also indicated that the expression of genes might be discontinued at certain stages by developmental regulatory mechanisms. For example, no $V_{A(43d|36d)}$ was found for TC but this was not the case in unconditional variance analysis.

The results shown in Table 3 revealed that the conditional covariances ($C_{A.Am(t|t-1)}$, $C_{D.Dm(t|t-1)}$, $C_{AE.AmE(t|t-1)}$, and $C_{DE.DmE(t|t-1)}$) were not all significant. In addition, a small but significant conditional residual variance ($V_{e(t|t-1)}$) was observed, indicating that the new expression of genes for TC could be influenced by sampling errors.

Estimation of general and interaction heritabilities at different developmental times

The narrow-sense heritability (h^2) can be used to evaluate the performance of TC for progeny selection. The results in Table 4 show that the total narrow-sense heritabilities for TC at 15, 22, 29, 36 and 43 dpa were 46.5, 62.6, 57.1, 84.7 and 59.5%, respectively. For the components of heritability, general heritability related to the embryo and maternal plant systems was detected only at the two later developmental times (36 and 43 dpa). Cytoplasmic general heritability was not found at all developmental times. With respect to interaction heritability arising from interaction effect, maternal interaction heritabilities were more important except for 43 dpa, while embryo interaction heritabilities were relatively vital at 22 and 29 dpa. The cytoplasmic interaction heritabilities were also visible at 15, 22 and 36 dpa. In general, the interaction heritabilities

Table 4. The estimated heritabilities of rapeseed threonine content (TC) at different developmental times

Parameter	Developmental times of rapeseed (dpa)				
	15	22	29	36	43
h^2_{Go}	0.000	0.000	0.000	0.357**	0.207**
h^2_{Gc}	0.000	0.000	0.000	0.000	0.000
h^2_{Gm}	0.000	0.000	0.000	0.342**	0.387**
h^2_{GoE}	0.000	0.178**	0.251**	0.000	0.000
h^2_{GcE}	0.168**	0.156**	0.000	0.040**	0.000
h^2_{GmE}	0.297**	0.291**	0.319**	0.108**	0.000

**P < 0.01; dpa – days post anthesis; h^2_{Go} – embryo general heritability, h^2_{Gc} – cytoplasmic general heritability, h^2_{Gm} – maternal general heritability, h^2_{GoE} – embryo interaction heritability, h^2_{GcE} – cytoplasmic interaction heritability, h^2_{GmE} – maternal interaction heritability

including embryo, cytoplasm and maternal interaction heritabilities were dominant at the first three developmental times, while general heritabilities were higher at the two later developmental times. Hence maternal and cytoplasm general heritabilities and their interaction heritabilities were relatively higher at all the developmental stages ($(h^2_{Gc} + h^2_{Gm} + h^2_{GcE} + h^2_{GmE})/h^2$ = 100.00, 71.55, 55.96, 57.88 and 65.14%, respectively). These results suggest that an improvement in TC would be more efficient when selection is based on maternal plants in the early generations.

Prediction of embryo, cytoplasmic and maternal effects and their GE interaction effects

In the breeding for rapeseed quality, the breeders can predict the genetic merit of the parental lines besides understanding the inheritance patterns. Evaluating the breeding value which includes embryo additive effect (*A*), cytoplasmic effect (*C*), maternal additive effect (*Am*), embryo additive interaction effect (*AE*), cytoplasmic interaction effect (*CE*), and maternal additive interaction effect (*AmE*) of parents across different environments (years), the breeders could select better parent(s) for a breeding programme. The results showed that genetic main effects as well as GE interaction effects of parents could influence the performance of TC in the progeny (Table 5), since there were marked differences in the genetic effect components among parents. The total genetic effects (Gt1 =*A* + *AE*1 + *C* + *CE*1 + *Am* + *AmE*1; Gt2 =*A* + *AE*2 + *C* + *CE*2 + *Am* + *AmE*2) were significantly positive for some parental lines, such as Gaoyou 605 (P4), Huanshuang 3 (P3), Youcai 601 (P1) and Eyouchangjia (P6). Predicted genetic effects of these parents could increase TC of the offspring, but not for most parents used in

Table 5. Predicted parental genetic effects on threonine content (TC) (%) in rapeseed meal

Parent	Time (dpa)	Embryo additive effect			Cytoplasmic effect			Maternal additive effect			Gt1	Gt 2
		Ao	*AoE1*	*AoE2*	*C*	*CE1*	*CE2*	*Am*	*AmE1*	*AmE2*		
P1	15	–	0.06	–0.14	–	0.06	–0.19*	–	0.07	–0.10	0.19	–0.43
	22	–	–0.01	0.08*	–	–0.16**	0.17**	–	0.08	–0.11**	–0.10	0.14
	29	–	0.14$^+$	–0.13$^+$	–	–	–	–	–0.06	0.11$^+$	0.08	–0.02
	36	–	–	–	–	–0.03	0.02	0.09	–0.02	0.06**	0.05	0.18
	43	–0.02	–	–	–	–	–	0.06**	–	–	0.04	0.04
P2	15	–	0.35	–0.30	–	0.27*	–0.27*	–	0.07	–0.03	0.69	–0.60
	22	–	–0.18*	0.14	–	0.23*	–0.34*	–	0.07	–	0.12	–0.21
	29	–	–0.01	–0.01	–	–	–	–	–0.08	0.02	–0.09	0.01
	36	–0.09	–	–	–	–	–0.01	–0.01	–0.03	0.04	–0.12	–0.06
	43	–0.01	–	–	–	–	–	–0.01	–	–	–0.02	–0.02
P3	15	–	0.03	0.04	–	0.16*	–0.13*	–	0.02	–0.02	0.22	–0.11
	22	–	–0.15*	0.11	–	–0.02	0.07	–	0.12	–0.09	–0.04	0.08
	29	–	0.16	–0.12	–	–	–	–	–0.19*	0.17*	–0.03	0.04
	36	0.04	–	–	–	0.08$^+$	–0.05	0.07	0.07*	–0.03	0.26	0.03
	43	0.02	–	–	–	–	–	0.02	–	–	0.04	0.04
P4	15	–	0.04$^+$	–0.01	–	–0.16*	0.04	–	0.10	–0.07	–0.02	–0.04
	22	–	–0.12	0.12	–	–0.11	0.11	–	0.19*	–0.23**	–0.04	0.01
	29	–	–0.02	0.10	–	–	–	–	0.12	–0.12	0.10	–0.02
	36	0.11*	–	–	–	0.07$^+$	–0.09$^+$	0.02	–0.02	0.03	0.18	0.07
	43	0.03	–	–	–	–	–	0.04*	–	–	0.07	0.07

Table 5 to be continued

Parent	Time (dpa)	Embryo additive effect			Cytoplasmic effect			Maternal additive effect			Gt1	Gt 2
		Ao	*AoE1*	*AoE2*	*C*	*CE1*	*CE2*	*Am*	*AmE1*	*AmE2*		
	15	–	0.02	0.13	–	–0.07	–0.03	–	0.08	–	0.03	0.10
	22	–	–0.04	0.03	–	–0.26*	0.36*	–	0.16^{+}	–0.14	–0.14	0.25
P5	29	–	0.07	–0.06	–	–	–	–	–0.08	0.12	–0.01	0.07
	36	–0.01	–	–	–	–0.01	0.10	0.06^{+}	0.12^{+}	–0.09*	0.15	0.06
	43	–0.03	–	–	–	–	–	0.02	–	–	–0.01	–0.01
	15	–	–0.22	–0.06	–	–0.22*	0.25*	–	–0.09	0.01	–0.53	0.20
	22	–	0.25**	–0.20**	–	0.13^{+}	0.01	–	–0.27**	0.25**	0.11	0.06
P6	29	–	–0.10	0.09	–	–	–	–	0.08	–0.01	–0.02	0.08
	36	0.04^{+}	–	–	–	–0.06	0.08	0.02	–0.04	0.03	–0.04	0.17
	43	–	–	–	–	–	–	0.02	–	–	0.02	0.02
	15	–	–0.13	0.19	–	0.15*	0.02	–	–0.06	0.11	–0.04	0.33
	22	–	0.23^{+}	–0.20*	–	0.19^{+}	–0.18*	–	–0.30*	0.29**	0.12	–0.09
P7	29	–	–0.14	0.10	–	–	–	–	0.15	–0.16	0.00	–0.06
	36	–0.08*	–	–	–	–0.14^{+}	0.01	–	0.07*	–0.05^{+}	–0.15	–0.13
	43	–0.02	–	–	–	–	–	–0.03	–	–	–0.05	–0.05
	15	–	–0.16	0.17	–	0.14^{+}	–0.12	–	–0.18	0.11	–0.19	0.16
	22	–	0.01	–0.03	–	–0.01	–	–	–0.05	0.02	–0.05	0.00
P8	29	–	–0.03	0.02	–	–	–	–	–0.01	–0.05	–0.04	–0.02
	36	–0.03	–	–	–	0.05	–0.04	–0.13	–0.07	–	–0.18	–0.20
	43	–0.02	–	–	–	–	–	–0.08**	–	–	–0.10	–0.10
	15	–	–	–0.02	–	–0.34*	0.43*	–	–0.02	–0.02	–0.35	0.39
	22	–	0.01	–0.06	–	0.01	–0.19*	–	–	0.01	0.02	–0.24
P9	29	–	–0.06	0.01	–	–	–	–	0.07	–0.08	0.00	–0.07
	36	0.02	–	–	–	0.04	–0.02	–0.12	–0.08^{+}	0.01	–0.15	–0.11
	43	0.01	–	–	–	*	–	–0.04*	–	–	–0.04	–0.04

$^{+}P < 0.10$; $^{*}P < 0.05$; $^{**}P < 0.01$; "–"indicates the genetic effect was not detected; dpa – days post anthesis; the parental lines used were: Youcai 601 (P1), Double 20–4 (P2), Huanshuang 3 (P3), Gaoyou 605 (P4), Zhongyou 821 (P5), Eyouchangjia (P6), Zhong R–888 (P7), Tower (P8) and Zheshang 72 (P9); *Ao* – embryo additive main effect, *AoE* – embryo additive interaction effect, *C* – cytoplasm main effect, *CE* – cytoplasmic interaction effect, *Am* – maternal additive main effect, *AmE* – maternal additive interaction effect. *Gt* – total genetic effect, with 1 and 2 referring to the 2007 and 2008 seasons, respectively

the present experiment because their predicted breeding values were negative. Among genetic main effects, *Am* was relatively more important than *A* for most parents. In GE interaction effects, *AE*, *CE* or *AmE* of most parents were variable in direction between two different growing environments. The results suggested that Gaoyou 605 (P4), Huanshuang 3 (P3), and Youcai 601 (P1) were better parental lines for an improvement in TC because of the larger total predicted genetic effects (Gt1 in 2007 and Gt2 in 2008); both Gt1 and Gt2 were 0.07, 0.04 and 0.04%, respectively.

DISCUSSION

Most of the nutrition quality traits in crops are controlled by complex genetic systems. Genes affecting nutrition quality traits are expressed in a developmental stage- and tissue-specific manner. The genetic control of quality traits would change along with developmental stages and environments. Therefore, the study on variation of the genetic mechanism of quantitative traits has been the highlight of developmental genetics. Many nutrition traits in rapeseed were simultaneously controlled by genetic systems including diploid embryo nuclear genes, cytoplasm genes, and diploid maternal nuclear genes (Shi *et al*. 2003; Variath *et al*. 2009). The present results revealed that TC of rapeseed meal at different developmental times/stages was controlled by different genetic components from embryo, cytoplasm, and diploid maternal genetic systems. There existed more maternal effects at different developmental stages, followed by embryo effects. Additive effects, including embryo and maternal additive effects, became more important at later developmental stages. Conditional variance analyses in the present study further proved that there existed differences in net genetic effects at most developmental stages.

The developmental genetic models and corresponding statistical analysis methods proposed by Zhu could be effectively used to analyse the dynamic variation in the expression of genes at specific stages from $t-1$ to t for the quality traits (Zhu 1995). Net genetic effects from different genetic systems were detected at most of the specific stages. The results of conditional analysis in the present experiment revealed that the activation of quantitative genes on TC of rapeseed was gradually carried out throughout developmental stages. There also existed an interrupted phenomenon of the gene expression of embryo, cytoplasm, and maternal genetic effects among rapeseed developmental stages. For example, the embryo additive main effect occurred at 30–36 dpa, cytoplasm main effects appeared at the two later developmental stages, but cytoplasm interaction effects were found at the first three developmental stages. The expression of genes was more active at middle (23~29 dpa) and early developmental stages (16~22 dpa). Furthermore, the results of conditional analysis revealed that the new expression of genes from the embryo, cytoplasm and maternal plant genetic systems was essential for the final phenotypic performance of TC. If the seed traits were simultaneously controlled by the genes of the three genetic systems at different developmental stages, the genetic estimations for seed traits obtained only based on the final phenotypic value without considering the activation of various genetic systems at the whole developmental period from flowering would not be complete. The results documented the time sequence of gene expression and relative contribution of different genetic effects to the performance of the quantitative trait studied. Hence, our results provide further information on how the TC of rapeseed meal could be simultaneously affected by the genetic main effects and GE interaction effects from the different genetic systems during the seed development process. These results might be helpful for the analysis of quantitative trait loci (QTL) of TC.

Rapeseeds growing in the field are affected by environmental conditions. The environmental factors and cultivation practices are among the main factors that could affect the gene expression of the nutrition quality traits. Besides the genetic main effects, this study showed that GE interaction effects were important for TC of rapeseed meal. GE interaction effects, including embryo additive and dominant interaction effects, cytoplasmic interaction effects, and maternal additive and dominant interaction effects, were found at most developmental stages by conditional analysis. Significant GE interaction effects also indicated that the sequential expression of genes from embryo, cytoplasm and maternal genetic systems was influenced by the environmental factors. For example, the accumulated temperature during the growing season of 2007 was lower than that of 2008. In addition, there was much rainfall in 2007. These environmental differences could cause the change of gene expression for TC between the two growing seasons. Understanding the magnitude and direction of the parental genetic effects is very useful for improving the efficiency of selection in breeding. A breeder is well advised to pay careful attention to the GE interaction effects in the breeding for rapeseed quality. Based on this study, certain parental lines such as Gaoyou 605 may have a higher breeding value for an improvement in TC due to its positive predicted value and stable genetic effects across environments.

The genetic basis of the nutrition quality traits of rapeseed is more complex than in other agro-

nomic traits. It is labour-consuming to obtain large numbers of F_1 seeds by hand emasculation because of the small seed size. In order to understand the contribution of different genetic effects to TC in rapeseed, the genetic models proposed by Zhu and Weir (Zhu & Weir 1994; Zhu 1995) were applied in the present study. This method only requires the means of three generations including parents, F_1, and F_2 with two or three replications in the diallel design to analyse the unconditional and conditional genetic main effects and GE interaction effects from embryo, cytoplasm and diploid maternal plant genetic systems. The results from such analyses could help understand the dynamic genetic mechanisms governing the nutrition quality traits in rapeseed. The method used in this experiment might be more suitable and practicable for the genetic analysis of other crops to study developmental genetics of seed nutrition quality traits.

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