RESEARCH ARTICLE

# QTL identification of grain protein concentration and its genetic correlation with starch concentration and grain weight using two populations in maize (*Zea mays* L.)

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## Abstract

Protein is one of the three main storage chemical components in maize grains, and is negatively correlated with starch concentration (SC). Our objective was to analyse the influence of genetic backgrounds on QTL detection for protein concentration (PC) and to reveal the molecular genetic associations between PC and both SC and grain weight (GWP). Two hundred and eighty-four (Pop1) and 265 (Pop2) $F_{2:3}$ families were developed from two crosses between one high-oil maize inbred GY220 and two normal maize inbreds 8984 and 8622 respectively, and were genotyped with 185 and 173 pairs of SSR markers. PC, SC and GWP were evaluated under two environments. Composite interval mapping (CIM) and multiple interval mapping (MIM) methods were used to detect single-trait QTL for PC, and multiple-trait QTL for PC with both SC and GWP. No common QTL were shared between the two populations for their four and one PC QTL. Common QTL with opposite signs of effects for PC and SC/GWP were detected on three marker intervals at bins 6.07–6.08, 8.03 and 8.03–8.04. Multiple-traits QTL mapping showed that tightly-linked QTL, pleiotropic QTL and QTL having effects with opposite directions for PC and SC/GWP were all observed in Pop1, while all QTL reflected opposite effects in Pop2.

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## Introduction

Protein is one of the three most important storage chemical components in maize grains. Generally, protein concentration (PC) is negatively and positively correlated with the other two grain components, starch and oil concentration, respectively (Dudley and Lambert 2004; Dudley *et al*. 2004, 2007; Clark *et al*. 2006; Willmot *et al*. 2006; Liu 2007; Liu *et al*. 2008; Zhang *et al*. 2008). Starch concentration (SC) plays an important role in grain-yield potential (Boyer and Hannah 2001). For example, the mean protein and oil concentrations of the Illinois high-protein (IHP) strains were increased 0.12% and 0.01% per generation from generations 67 to 99, while the mean SC was decreased 0.26% per generation. For the Illinois high-oil (IHO) strains from generations 65 to 99, the mean protein and oil concentrations were increased 0.17% and 0.03% per generation, while the mean SC was decreased 0.28% per generation (Dudley and Lambert 2004). IHP, IHO and other long-term selected high-oil germplasms, such as Beijing high-oil (BHO) (Zhang *et al*. 2008), tropical (Mangolin *et al*. 2004) and Alexo single-kernel (ASK) (Zheng *et al*. 2008), provide unique resources to identify QTL controlling major chemical composition in maize grain.

Previous studies on QTL detection for PC have been done using IHP/Illinois low protein (ILP) (Goldman *et al*. 1993; Dudley *et al*. 2004, 2007), IHO/Illinois low oil (ILO) (Berke and Rocheford 1995; Clark *et al*. 2006; Willmot *et al*. 2006), BHO (Zhang *et al*. 2008), European flint maize inbreds (Melchinger *et al*. 1998) and popcorn inbreds (Liu *et al*. 2008). Although some consistency in QTL detection has been reported, considerable differences in numbers of detected QTL and their locations were observed. Therefore, it is desirable to conduct further studies using more relevant germplasm to reveal the genetic nature of PC. QTL mapping using several connected multi-parental crosses could in-

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crease the probability of QTL detection, and consequently reveal the effects of genetic background on the expression of QTL and phenotypic traits (Chardon *et al*. 2004; Mihaljevic *et al*. 2004; Blanc *et al*. 2006; Meyer *et al*. 2007). In addition, to use the results of detected QTL, the concerned germplasms should be incorporated in the current breeding programme (Dudley *et al*. 2007). On the other hand, some common QTL with opposite effects for grain protein and SC have been detected (Berke and Rocheford 1995; Melchinger *et al*. 1998; Clark *et al*. 2006; Willmot *et al*. 2006; Liu *et al*. 2008; Zhang *et al*. 2008). It remains to be determined whether these QTLs were tightly-linked trait-specific QTL or single QTL with pleiotropic effects. The multiple-trait joint QTL analysis developed by Jiang and Zeng (1995) could be used to evaluate the genetic associations among different correlated traits.

The first objective of present work was to analyse the influence of genetic backgrounds on QTL detection for PC, using two connected populations derived from one elite high-oil inbred and two normal inbred lines, under the same environments. The second objective was to reveal the genetic associations between PC and both SC and grain weight using multiple-trait QTL mapping method. These results were expected to provide useful information in marker-assisted selection (MAS) for PC in quality maize breeding. According to our knowledge, this is the first report of the use of multiple-trait QTL mapping method on two related populations, to reveal the genetic mechanism of grain PC and its genetic association with both SC and grain weight in maize.

## Materials and methods

### *Genetic materials*

The high-oil maize inbred line GY220 (as the paternal parent) was crossed with two dent corn inbred lines 8984 and 8622 (as maternal parents) to generate two related populations, 8984 × GY220 (Pop1) and 8622 × GY220 (Pop2). GY220 was selected and provided by Agricultural University, China. The two dent corn inbred lines 8984 and 8622 were developed in our laboratory, and belonged to the Chinese Reid heterotic group. Each $F_1$ plant from the two crosses was selfpollinated and unselected $F_2$ plants were selfpollinated to produce 284 and 265 $F_{2:3}$ family lines in Pop1 and Pop2, respectively.

### *Field trails and trait evaluation*

The two sets of $F_{2:3}$ family lines, including their $F_1$ and parental inbreds, were evaluated in two adjacent field plot trials in one row plots with two replications. The trials employed an α-design under the same conditions and were conducted in 2006, during the spring in Luoyang, and summer in Xuchang, Henan, China. Each row was 4-m long with distance of 0.67 m between rows. Plots were planted by hand at a density of 60,000 plants $ha^{-1}$. Standard cultivation management practices were used.

All plants were sib-pollinated within each plot by hand to avoid xenia effect. After maturity, 10 consecutive plants starting from a third of each row were harvested. Ears were naturally dried and grain weight per plant (GWP, in g) was evaluated. Grain protein and SC were measured on grain samples mixed within each plot with a MATRIX-1 NIR Spectroscope (Bruker, Greenleaf, Germany).

### *Phenotypic data analysis*

The correlation coefficients among the three traits were calculated using the statistical software package SPSS 12.0 (SPSS, Chicago, USA). Broad-sense heritability ($H^2$) for all traits in the $F_{2:3}$ families on an entry mean basis were estimated by dividing the genotypic by the phenotypic variances (Hallauer and Mirando 1981).

### *SSR analysis and map construction*

Leaf samples were collected at seedling stage from each $F_2$ plant, two $F_1$ and the three parental lines 8984, 8622 and GY220, and stored at −80°C. DNA extraction and SSR analysis were conducted as in Li *et al*. (2006).

A total of 665 SSR primer pairs were chosen from Maize Genome Database (http://www.maizegdb.org) according to their uniform distribution throughout all 10 maize chromosomes. The primer pairs were initially screened for polymorphisms between the two pairs of parents, 8984/GY220 and 8622/GY220. Ultimately, 212 and 205 polymorphic markers were selected that clearly showed co-dominant segregation in the two populations, respectively. Markers showing serious segregation distortion or failing to be assigned to any linkage group in the two populations were excluded, and finally the two genetic linkage maps were constructed with 185 and 173 SSR markers using Mapmaker 3.0 (Lincolin *et al*. 1992) at an LOD threshold > 3.0. The recombination frequency between linked loci was transformed into centimorgan (cM) distances by applying Kosambi's (1944) mapping function.

### *QTL analysis*

QTL mapping and the estimation of QTL effects for PC was done using composite interval mapping (CIM) (Zeng 1994) in Model 6 of the Zmapqtl procedure in QTL Cartographer version 2.5 (Wang *et al*. 2006). To identify an accurate significance threshold for each trait, an empirical threshold was determined for CIM using 1000 per mutations (Churchill and Doerge 1994). QTL positions were assigned to relevant regions at the point of a maximum LOD score. Two peaks for the same trait on the same chromosome were accepted as two different QTL (Groh *et al*. 1998) if they were separated by at least two markers and with a minimum distance of 20 cM. QTL confidence/support intervals were calculated as the point along the significance peak where the LOD score was 1.0 unit less than the peak LOD scores. Gene action was determined based on the average level of dominance following the criteria of Stuber *et al*. (1987) as follows: additive (A),

0 – 0.2; partial dominance (PD), 0.21 – 0.80; dominance (D), 0.81 – 1.20; and overdominance (OD) > 1.20.

Joint QTL analysis for PC and GWP/SC was carried out according to the multiple interval mapping (MIM) method in WinQTLCart (Jiang and Zeng 1995) with Cartographer version 2.5 (Wang *et al*. 2006). A significance threshold was identified by the quick method for computing approximate thresholds for QTL detection (Piepho 2001).

## Results

### *Performance for PC in the two relevant $F_{2:3}$ populations*

The PC of the three parent lines, two $F_1$ and $F_{2:3}$ family lines are shown in table 1. Among the three parent lines, the value of the high-oil maize inbred GY220 was slightly higher than the normal inbred line 8984 and much higher than another normal inbred line 8622. The values of the two $F_1$ crosses were all lower than the low parent. There was considerable variation among $F_{2:3}$ family lines in both populations, showing a continuous distribution pattern around the means, and transgressive segregations exceeding the high and low parent values. This reflected that the two pairs of parents had different favourable and unfavourable alleles for PC.

### *Correlation analysis among PC, SC and GWP*

Highly significant negative correlations were observed between PC and SC, and PC and GWP in the two populations, while highly significant positive but low correlation was observed between SC and GWP (table 2). The $H^2$ estimates for grain protein and SC was moderate, but it was low for GWP, reflecting less influence of environmental conditions on variation in grain protein and SC than on GWP.

### *QTL detected for PC in the two relevant $F_{2:3}$ populations*

A total of five QTL significantly associated with PC were detected in the two $F_{2:3}$ populations. The four QTL detected in Pop1 were located on chromosomes three, six and eight (two) (table 3). The contributions to phenotypic variation for a single QTL varied between 6.7% and 13.4%, with total contribution to phenotypic variation 36.3% and *qPRO1-8-1* the highest. The positive alleles of the two QTL on chromosomes three and six were contributed by the normal maize

**Table 1.** Phenotypic performance of protein concentration for the parents. $F_1$ and $F_{2:3}$ family lines of the two populations based on conbined data across two environments.

| | Parents | | | $F_{2:3}$ family lines | | | | |
|---|---|---|---|---|---|---|---|---|
| Population | GY220 | 8784/8622 | $F_1$ | mean ± s.e. | Range | CV (%) | Skewness | Kurtosis |
| Pop1 | 14.71 | 14.56 | 12.14 | 13.38 ± 0.87 | 10.26–17.46 | 6.52 | 0.80 | 2.38 |
| Pop2 | 14.71 | 12.09 | 11.70 | 12.23 ± 0.84 | 9.92–14.95 | 6.84 | 0.25 | 0.44 |

**Table 2.** Phenotypic correlation coefficients among protein concentration (PC), starch concentration (SC) and grain weight per plant (GWP), and their broad sense heritability in the two populations[a].

| Trait | PC | SC | GWP | $H^2$ | 90% CI on $H^2$[b] |
|---|---|---|---|---|---|
| PC | | −0.67** | −0.37** | 0.52 | 0.39–0.62 |
| SC | −0.75** | | 0.27** | 0.46 | 0.31–0.57 |
| GWP | −0.36** | 0.30** | | 0.33 | 0.15–0.47 |
| $H^2$ | 0.46 | 0.54 | 0.27 | | |
| 90% CI on $H^2$ | 0.31–0.57 | 0.41-0.63 | 0.17–0.42 | | |

[a]Pop1 below diagonal, Pop2 above diagonal.

**Significant at $P < 0.01$.

**Table 3.** Putative QTL detected for grain protein concentration in the two connected $F_{2:3}$ populations.

| Population | QTL | Marker interval | Bin locus | Position | LOD | *A* | *D* | $R^2$% | Effect mode[a] |
|---|---|---|---|---|---|---|---|---|---|
| Pop1 | *qPRO1-3-1* | *umc*1320 ~ *bnlg*1754 | 3.08–3.09 | 248.7 | 4.1 | 0.38 | −0.51 | 8.9 | OD |
| | *qPRO1-6-1* | *umc*1653 ~ *umc*1127 | 6.07–6.08 | 4.0 | 4.5 | 0.41 | −0.16 | 7.3 | PD |
| | *qPRO1-8-1* | *bnlg*1067 ~ *bnlg*2082 | 8.03 | 139.9 | 5.2 | −0.24 | −0.31 | 13.4 | OD |
| | *qPRO1-8-2* | *bnlg*1863 ~ *bnlg*2046 | 8.03–8.04 | 178.5 | 3.9 | −0.28 | −0.16 | 6.7 | D |
| Pop2 | *qPRO2-10-1* | *phi*96342 ~ *umc*19384 | 10.02–10.03 | 53.2 | 3.5 | 0.02 | −0.37 | 6.2 | OD |

[a]OD, overdominance; PD, partial dominance; D, dominance.

parent 8984, while the positive alleles of the two QTL on chromosome eight were contributed by high-oil maize parent GY220. In Pop2, only one QTL for PC was detected, with contribution to phenotypic variation 6.2%. The positive allele was contributed by the normal maize parent 8622. OD, D and PD effects were observed in Pop1, while the QTL in Pop2 expressed OD effect.

### *Genetic correlations between PC and both SC and GWP in the two populations*

To further analyse the genetic correlations between PC and SC, and PC and GWP, joint analyses for PC and SC and for PC and GWP were conducted in the two populations (table 4; figure 1). In Pop1, joint analysis for grain protein and SC detected six QTL, which were located in six marker intervals on chromosomes five, eight and ten. Comparing with single-trait QTL detected for PC and SC (Wang 2007), three QTL on chromosomes five (*phi*008~*umc*2115), eight (*umc*1562~*bnlg*162) and ten (*bnlg*2190~*bnlg*1360) were additional QTL, suggesting the higher statistical power of the joint-analysis method. One protein QTL on chromosome eight (*bnlg*1067~*bnlg*2082), and three starch QTL on chromosomes five (*bnlg*1879~*umc*1162), eight (*bnlg*1067~*bnlg*2082) and ten (*phi*050~*umc*2163) were detected after single-trait mapping. The graph peaks of LOD curves for protein and starch concentrations between *umc*1162 and *bnlg*2323 on chromosome five changed simultaneously and in the same direction, suggesting the existence of pleiotropic QTL controlling protein and starch concentrations simultaneously (figure 1). The protein QTL on chromosomes three and six failed to show significant effects in joint-trait analysis. This suggested that these QTL had effects with opposite directions for protein and starch concentrations. Joint analysis for PC and GWP detected one additional QTL on chromosome three. Both protein and GWP QTL on chromosome six were detected after single-trait mapping for PC and GWP. The graph peaks of LOD curves for PC and GWP between *umc*1653 and *umc*1127 changed in the same close direction, suggesting QTL controlled PC and GWP with a tight link in this marker interval. Three PC QTLs on chromosomes three and eight failed to show significant effects in joint-trait analysis. This suggested that these QTL had effects with opposite directions for PC and GWP.

No joint-trait QTL were detected in Pop2. The protein QTL on chromosome ten, the three starch QTL on chromosomes two and six (two), and the two GWP QTL on chromosome six all failed to show significant effects in joint-trait analysis. This suggested that these QTL had effects with opposite directions for PC and SC, and for PC and GWP.

## Discussion

### *Comparison of QTL detected in the two populations and with other researches*

In our present study, no common QTL for PC was found between the two populations, although the same high-oil corn parent inbred GY220 was used and the trial conditions were same. Obviously, the main influence was from the two dent maize inbred lines. In our previous analysis in combining ability between nine high-oil maize inbreds and 21 normal inbreds, great difference in combining ability was observed between 8984 and 8622. The general combining ability of 8984 and 8622 for PC were −0.05 and 0.50 ($P < 0.01$), respectively. And the special combining ability between 8984 and GY220, and between 8622 and GY220 for PC were −0.33 and 0.52 (Liu 2007).

Although consistency in QTL detection for PC has been reported in previous researches, different influence from genetic background have always been observed (Goldman *et al*. 1993; Berke and Rocheford 1995; Melchinger *et al*. 1998; Dudley *et al*. 2004, 2007; Clark *et al*. 2006; Willmot *et al*. 2006; Liu *et al*. 2008; Zhang *et al*. 2008). Therefore, to reveal the global molecular genetic mechanism of QTL for PC in maize, it was needed to make intensive studies using quite a large number of populations derived from different parents representing different germplasms. QTL mapping using several relevant multi-parental crosses will increase the probability of QTL detection, and consequently reveal the effects of genetic background on QTL and phenotypic traits (Chardon *et al*. 2004; Mihaljevic *et al*. 2004; Blanc *et al*. 2006; Meyer *et al*. 2007). According to our knowledge, this was the first study to conduct QTL analysis for PC simultaneously using two relevant $F_{2:3}$ populations in high-oil maize.

**Table 4.** Joint QTL analysis of protein concentration with starch concentration, and protein concentration with grain weight per plant (GWP) in Pop1.

| Trait | Chromosome | Marker interval | Bin locus | Position | LOD |
|---|---|---|---|---|---|
| Protein–starch | 5 | *phi*008 ~ *umc*2115 | 5.02 | 12 | 4.8 |
| | 5 | *umc*1162 ~ *bnlg*2323 | 5.04 | 45.9 | 5.1 |
| | 8 | *bnlg*1067 ~ *bnlg*2082 | 8.03 | 139.9 | 6.2 |
| | 8 | *umc*1562 ~ *bnlg*162 | 8.05 | 199.2 | 5.1 |
| | 10 | *phi*050 ~ *umc*2163 | 10.03–10.04 | 119.1 | 5.7 |
| | 10 | *bnlg*2190 ~ *bnlg*1360 | 10.06–10.07 | 167.7 | 6.6 |
| Protein–GWP | 3 | *umc*2118 ~ *umc*1746 | 3.0–3.01 | 27.3 | 4.8 |
| | 6 | *umc*1653 ~ *umc*1127 | 6.07–6.08 | 6.0 | 5.9 |

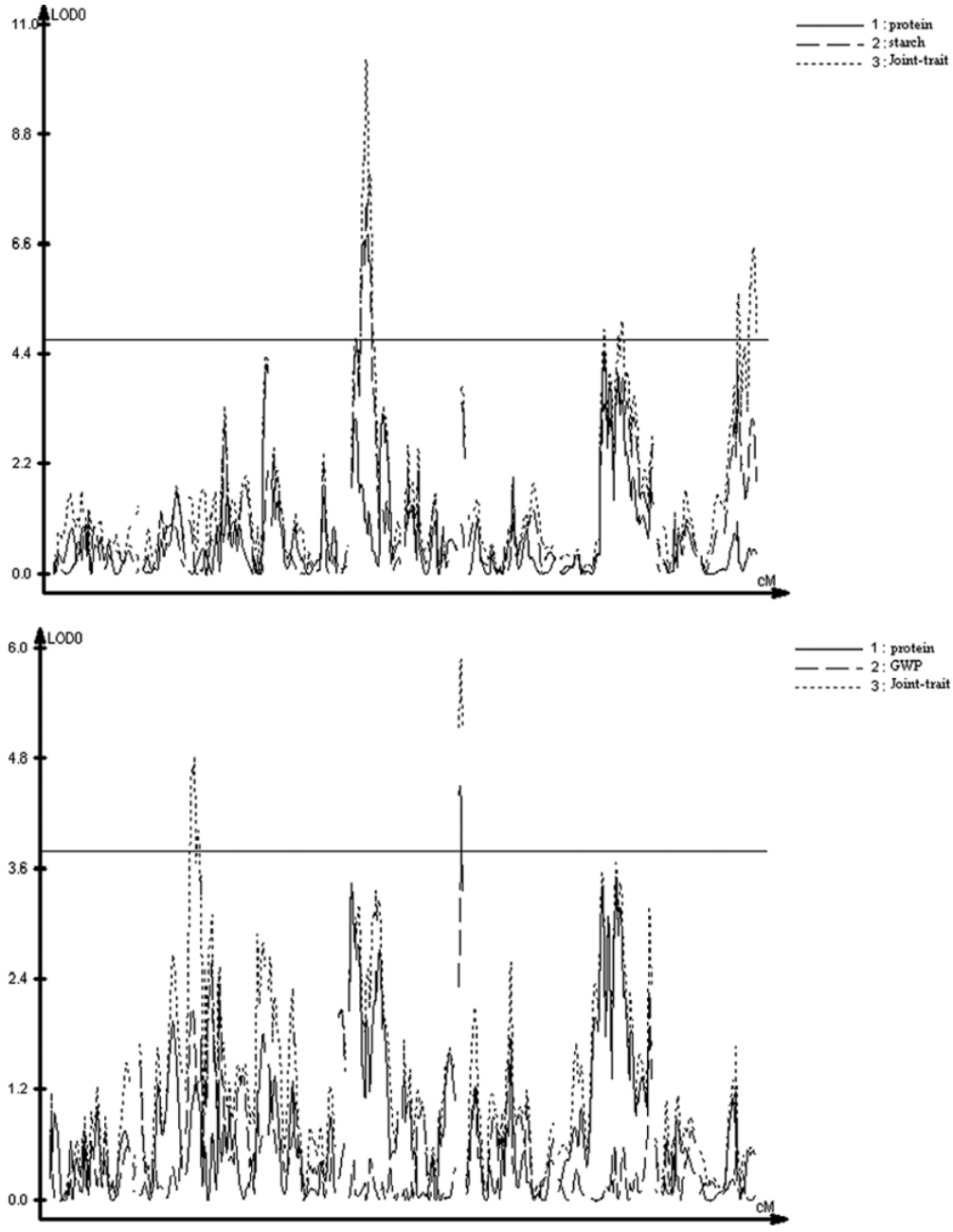


**Figure 1.** Joint QTL analysis of protein concentration with starch concentration, and protein concentration with grain weight per plant in Pop1.

The existence of great influence from dent maize inbred lines on protein QTL mapping could provide an useful references in revealing the genetic mechanism of PC and also in high-oil maize breeding.

Comparing with previous researches, several regions associated with QTL for PC in our study have been reported. For example, the QTL at bin 8.03 with the largest effects for PC in Pop1 have also been detected by Berke and Rocheford (1995), Dudley *et al*. (2004), Melchinger *et al*. (1998) and Willmot *et al*. (2006) using IHO/ILO, IHP/ILP, and European flint normal maize germplasms, respectively. Similarly, the QTL found in this study at bin 3.08 ~ 3.09 in Pop1 has also been detected by Dudley *et al*. (2004), Goldman *et al*. (1993) and Willmot *et al*. (2006); QTL at bin 6.07 ~ 6.08 in Pop1 has been detected by Melchinger *et al*. (1998), Liu *et al*. (2008) and Willmot *et al*. (2006). Mean while, in Pop2, QTL at bin 10.02 ~ 10.03 has also been detected by Berke and Rocheford (1995), Dudley *et al*. (2004) and Willmot *et al*. (2006). This shows that QTL for PC at all the four regions had high stability across different maize germplasms, genetic

backgrounds, and environments. It will be worth conducting further research on these QTL in near-isogenic lines (NILs), fine mapping, MAS and even cloning.

***Genetic correlations between grain protein and starch concentration***

Relationships among different traits could be inferred from correlations among them, common single-trait QTL and their signs of effects, and the results of joint QTL analysis. Significant negative correlations between protein and starch concentrations in maize grain have generally been observed in previous researches (Dudley *et al*. 2004, 2007; Clark *et al*. 2006; Willmot *et al*. 2006; Liu 2007; Liu *et al*. 2008; Zhang *et al*. 2008). In the present study, PC was negatively correlated with SC in both populations at $P < 0.01$. Comparing the single-trait QTL detected for PC in this study and for SC by Wang (2007), QTL for both traits were detected in the same two marker intervals (*bnlg*1067~*bnlg*2082 and *bnlg*1863~*bnlg*2046) on chromosome eight in Pop1. However, the favourable alleles for PC were contributed by the high-oil maize parent GY220, while the favourable alleles for SC were contributed by the normal parent 8622. Such results indicated that pleiotropic or tightly linked QTL might exist in these marker intervals. Similar results have been observed by Clark *et al*. (2006), Dudley *et al*. (2004, 2007), Willmot *et al*. (2006) and Zhang *et al*. (2008).

Joint QTL analysis for multiple traits was first used in this study to reveal the genetic associations between grain protein and starch concentrations. The results revealed that tightly linked QTL, pleiotropic QTL and QTL having effects with opposite directions existed for the two traits in Pop1. In Pop2, all single-trait QTL showed effects with opposite directions for both pairs of traits. Therefore, to break-up the undesirable associations between grain protein and SC, more effort should be put into selection. With MAS, this objective could be realized easily and fast. Although such correlations should be proven in further research using QTL-NILs, our results in this study and in previous studies (Flint-Garcia *et al*. 2003; Li *et al*. 2007) demonstrate that multiple-trait joint analysis could reveal the genetic correlations among correlated traits at the molecular level.

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