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Full Length Research Paper

QTL analysis of kernel soluble sugar content in super-sweet corn

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A genetic linkage map was constructed with SSR markers based on a super-sweet corn population consisting of 208 F_2 individuals from the cross Ji557 (sh2) × Ji165 (sh2). Density of the linkage groups varied from 2.2 cM to 65.3 cM, with an average of 13.0 2 cM between two adjacent markers. The map covered 1470.9 cM of the total recombination length of the super-sweet corn. Using Mapmaker/QTL, 11 quantitative trait loci (QTLs) were identified for kernel soluble sugar content, explaining 3.5 - 20.3% of soluble sugar content variance and collectively accounting for 63.7% of the trait variance. Of the 11 QTLs associated with kernel soluble sugar content, 2 (18.2%) showed additive effects, 3 (27.3%) showed partially dominant effects, 3 (27.3%) showed dominant effects and 3 (27.3%) showed over-dominant effects. All four types of genetic effects appeared to play important roles in determining the kernel soluble sugar content in super-sweet corn cultivars.

Key words: Super-sweet corn, genetic map, SSR, QTLs, kernel soluble sugar content.

INTRODUCTION

Sweet corn originally occurred as a spontaneous mutation in the corn field and was grown by several native American tribes. The Iroquois gave the first recorded sweet corn (also called Papoon) to European settlers in 1779. There are three known genetic mutations responsible for the various types of sweet-corn lines. Early varieties, such as those used by the native Americans, were the result of the mutant *su* ("sugary") allele. The second gene mutation discovered is the *se* or "sugary enhanced" allele, responsible for so-called "Everlasting Heritage" varieties (Gonzales et al., 1976; Douglas and Tsung, 1994). Super-sweet corns are varieties of sweet corn which produce higher levels of sugar, firstly developed by Laughnan (1953). Super-sweet corn is characterized by sweet, waxy, tender and dulcet, had higher nutrition and economic values, also known as a fruit as well as a vegetable, so called "king of fruit" (Larson, 2003). With the ever-increasingly appetite-driven needs of people, ever-better eating quality is required.

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These requirements in the quality of soluble sugar content, tenderness and pericarp thickness have attracted many crop scientists to work on super-sweet corns. Conventional breeding strategies have been employed to enhance eating quality of super-sweet corn; however, it had limited efficiency to improve super-sweet corn because of its lower efficiency and long period. Recent advances in plant biotechnology in general and DNA markers in particular offer crop breeders a rapid and precise alternative approach in addition to conventional breeding schemes to improve quantitative traits including quality (Lubberstedt et al., 2005).

Grain yield, plant- and ear-height, anti-adversity, resistance to disease and insect, kernel protein-, oil-, starch- and sugar-content and many other traits are all important for maize production (Hallauer and Carena, 2009). Much time and efforts have been endeavored to identify markers associated with these phenotypic traits in maize (Ajmone-Marsan et al., 1995; Berke and Rocheford, 1995; Dudley et al., 2004; Goldman et al., 1993; Lebreton et al., 1995; Schon et al., 1993; Berke and Rocheford, 1995). However, hitherto QTL analysis for quality traits in sweet corn has been reported in only a few studies (Azanza et al., 1996; Chandler and Tracy, 2007). For

Table 1. Soluble sugar content (%) of parents, F_1 and $F_{2:3}$ families.

Parental inbred lines		F_1	$F_{2:3}$ families		
Ji557	Ji165		Means	Range	CV (%)
6.75	17.81	14.82	11.37±1.75	4.46-17.81	32.96

example, only Azanza et al. (1995, 1996) investigated QTLs of edible quality and the influencing chemical and sensory characteristics of eating quality in sweet corn.

Shrunken2 (Sh2) encodes the large regulatory subunits of the heterotetrameric ADP-glucose pyrophosphorylase (AGPase) present in maize kernel endosperm and affects the starch content (Thevenot et al., 2005). Because maize lines containing gene have high soluble sugar content, these lines have been widely used for super-sweet corn breeding.

By using parental inbred lines, Ji557 (sh2) and Ji165 (sh2), developed by us, we have constructed a genetic linkage map for super-sweet corn by employing 450 mapped SSR markers based on a segregating population consisting of 208 F2 individuals. The objectives of the present study were to identify QTLs for soluble sugar content and evaluate their genetic effects. We believe that the results obtained in this study will help facilitate further fine-mapping for the trait of kernel soluble sugar content, map-based cloning of the underlying gene(s), as well as marker-assisted selection in super-sweet corn breeding.

MATERIALS AND METHODS

Plant materials and DNA isolation

The inbred parental lines Ji557 (sh_2) and Ji165 (sh_2) chosen to create a mapping population because they differed markedly in the content of soluble sugar, with Ji557 (sh_2) being lower (6.75%) and Ji165 (sh_2) being higher (17.81%) in soluble sugar contents. A F_2 population containing 208 individuals derived from Ji557 (sh_2) x Ji165 (sh_2) was used to construct the genetic map and the F_2 plants were then self-pollinated to create a mapping population consisting of 208 $F_{2:3}$ families. Leaf samples were collected from each F_2 plants, the parental lines and F_1 at the seedling stage. The tissues were then ground to fine powder under liquid nitrogen and stored at -80°C until to be used for DNA isolation. DNA was isolated following the procedure described previously (Saghai-Maroof, 1984).

Growing condition and soluble sugar measurements

The 208 $F_{2:3}$ families, the parental lines and the F_1 plants were evaluated in a randomized design of single-row plots (5 x 0.65 m) with two replications at the Experimental Station of Jilin Agricultural University, Changchun, China, in 2005. The plots were thinned to 20 plants per row. The plants were hand-pollinated with the pollen mixture of all plants in the row. At maturity, the plots were harvested manually and a random bulk sample of grain was used for measuring content of kernel soluble sugar using the anthrone method (Yemm and Willis, 1954).

Genotypic analysis and construction of the genetic linkage map

SSR analysis including PCR reaction, gel electrophoresis and silver staining was performed following the protocol described by Senior and Manfred (1993). The SSR markers were verified to fit Mendel segregation ratios by the Chi-square test. The genetic map was constructed with MapMaker version 3.0b (Lincoln et al., 1992). Linkage groups were created with a LOD score of 3.0 and a recombination fraction of 0.5 by the "group" command. The commands "compare", "try" and "ripple" were used sequentially to generate the linkage groups which were assigned to chromosomes based on the microsatellite consensus map of B73 × Mo17 (http://www.agron.missouri.edu). The Kosambi mapping function was applied to transform recombination frequencies into centiMorgans (cM) as map distances. The genetic map was drawn by the software Mapdraw (Liu and Meng, 2003). Loci were named according to primer combinations with multiple markers being generated by a given primer combination ordered by decreasing molecular sizes.

QTL mapping

QTLs were detected by the segregation data of all markers and soluble sugar content in 208 F_2 plants using the software package MAPMAKER/QTL version 1.1b (Lincoln et al., 1993) with a 2.0 LOD threshold. Mapping of QTLs and estimation of their effects were performed using the interval mapping method (Lander et al., 1987). Gene action was determined on the basis of the average level of dominance by using the criteria described by Stuber et al. (1987).

RESULTS

Measurement of the targeted trait - kernel soluble sugar content

The two parental inbred lines, Ji557 and Ji165, showed contrasting phenotypes for the specific trait of kernel soluble sugar content, which are averaged 6.75% and 17.81%, respectively. The soluble sugar content of the F_1 pooled individuals was intermediate between the two parental lines (averaged 14.82%) and that of the $F_{2:3}$ families showed a continuous distribution, as expected for a typical quantitative trait (Table 1).

Segregation analysis and linkage map construction

A total of 450 mapped SSR primer pairs (based on http://www.agron.missouri.edu/ssr.html) were assessed for polymorphism between the two parental lines. Of the 450 loci, 121 (26.9%) were found to be polymorphic and 113

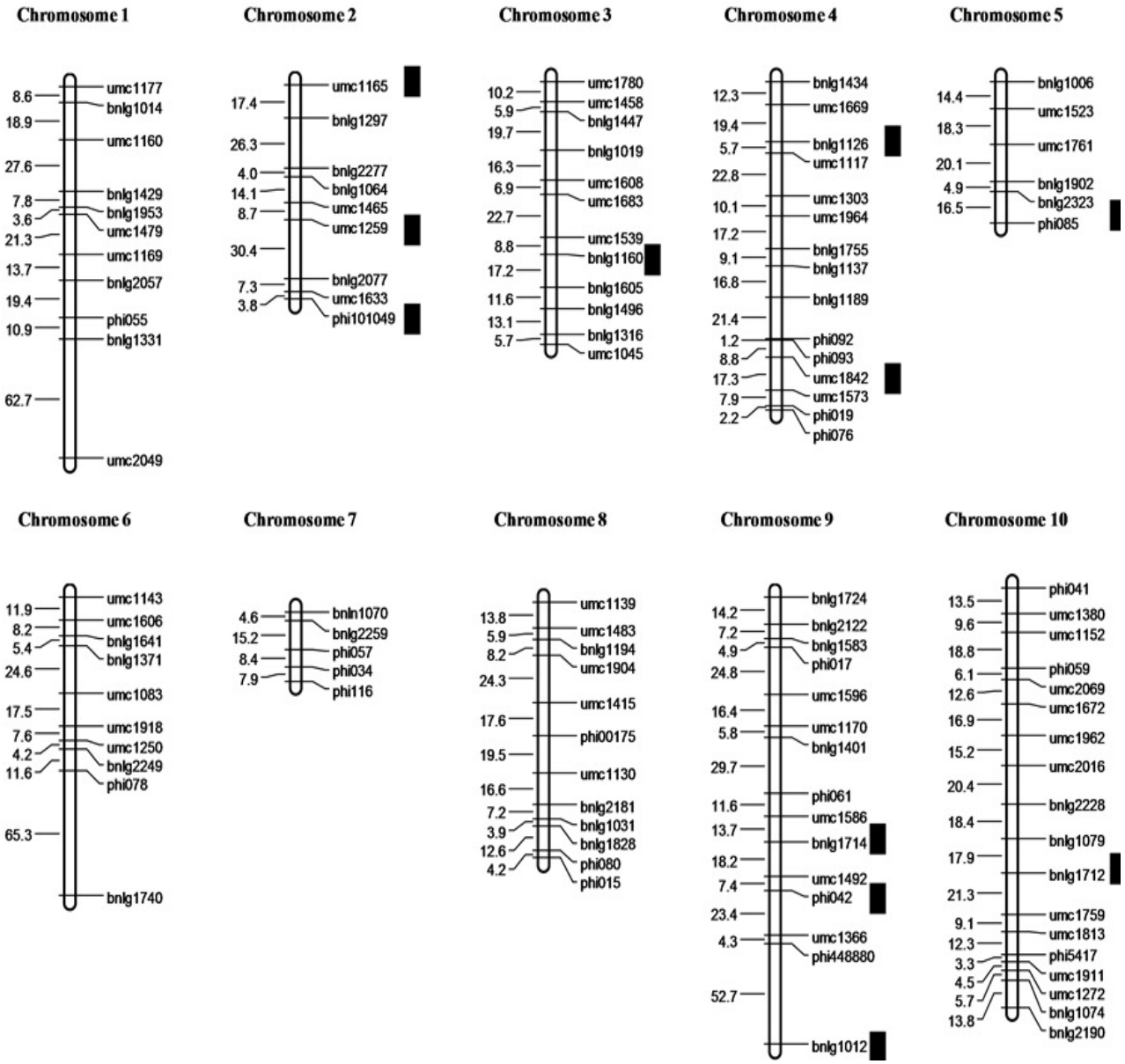

Figure 1. Linkage map with SSR markers and QTLs for soluble sugar content. ▌: QTL region of soluble sugar content.

markers with good amplification profile and even coverage of the genome, were selected and used for genotyping the 208 F_2 individuals derived corn the cross of Ji557 (sh_2) x Ji165 (sh_2). Chi-square test revealed that 96 markers (accounting for 79.4%) accorded with the expected ratio of 1:2:1 and 25 markers (accounting for 20.6%) showed distortion from the expected ratio. The linkage map of the Ji557 (sh_2) x Ji165 (sh_2) cross (Figure 1) largely agreed with previously published maize maps (MaizeGDB, 2004) except for seven loci (*umc1045, bn1g2228, phi5417, umc1813, umc1759, umc1672* and *bnlg1316*) which mapped to different positions. Those markers covered 1470.9 cM of the 10 maize chromosomes, with an average distance of 13.02 cM between adjacent markers.

QTL analysis

Eleven QTLs influencing soluble sugar content were detected on chromosomes 2 - 5, 9 and 10, with three on chromosomes 2 and 9, two on chromosome 4 and one on all others (Figure 1), explaining in total 63.7% of the phenotypic variance (Table 2). Individual QTLs accounted for 3.5 - 20.3% of the phenotypic variance and displayed dominant, partial dominant, over dominance and additive gene actions. SSC1 (14.3%), SSC3 (20.3%), SSC4 (13.6 %), SSC9 (10.7%) and SSC11 (13.9%) among the QTLs

Table 2. Results of QTLs analysis on soluble sugar content.

QTLs	Chr.	Nearest marker	Position[a] (cM)	LOD	Phenotypic variation[b] (%)	Genetic effects[c]			Model gene action[d]
						A	D	D/A	
SSC1	2	umc1259	- 1.6	3.81	14.3	0.1139	0.2654	2.33	OD
SSC2	2	umc1165	13.9	2.39	7.2	0.3564	-0.4157	- 1.17	D
SSC3	2	phi01049	5.5	4.12	20.3	0.1176	-0.0154	- 0.13	A
SSC4	3	bnlg1160	8.4	2.07	13.6	- 0.2544	- 0.1127	0.44	PD
SSC5	4	bnlg1126	0	3.73	3.5	0.4871	0.3198	0.66	PD
SSC6	4	umc1842	9.4	2.24	4.7	0.6234	0.5246	0.84	D
SSC7	5	phi085	- 4.8	2.60	9.4	- 0.3542	- 0.1548	0.44	PD
SSC8	9	bnlg1012	- 7.7	3.05	6.8	0.1087	0.1179	1.08	D
SSC9	9	bnlg1714	- 3.9	2.11	10.7	- 0.4994	0.1021	- 0.20	A
SSC10	9	phi042	0.6	4.66	4.1	- 0.4281	- 0.8235	1.92	OD
SSC11	10	bnlg1712	5.8	2.33	13.9	0.2845	0.5138	1.81	OD
Total					63.7				

[a] The distance was measured from the nearest marker to the maximum LOD peak of a QTL. A positive distance was given for QTLs located downward the marker and a negative distance was given for QTLs located upward the marker; [b] percentage of phenotypic variance explained by the given QTL; [c] model of gene action was determined from the ratio d/a. DR = d/a., DR = 0 - 0.20 additive, DR = 0.21 - 0.80 partial dominance, DR = 0.81 - 1.20 dominance, DR = 1.21 over dominance; [d] total is the percent of phenotypic variation accounted for in a multiple model of all QTLs.

explained more than 10% of the phenotypic variation for kernel soluble sugar content. These QTLs could thus be considered as major QTLs.

DISCUSSION

The power of QTL detection is only moderate for QTLs with weak effects (Melchinger et al., 2004). Consequently QTLs were only considered for LOD values higher than 2.5, thereby producing reliable results. In this study, we detected 11 QTLs for kernel soluble sugar content in super-sweet corn; these QTLs, which individually explained 3.5 - 20.3% of the phenotypic variation for the targeted trait (kernel soluble sugar content) can be considered as major effect QTLs (accounting for over 10% of the phenotypic variation). The possible reasons leading to lower total contribution are conceivably several, but predominantly might include an influence exerted by genetic background of the mapping population. For example, Bubeck et al. (1993) obtained discrepant results when they analyzed QTLs controlling resistance to gray leaf spot in maize using different mapping population. Additionally, interactive effect and epistasis may also play an important role apart from additive, partially dominant, dominant and over-dominant effects (Senea et al., 2000; Yamamoto et al., 2000; Wei et al., 2009; Liu et al., 2009). Finally, because QTL detection depends on phenotypic variation among the $F_{2:3}$ families, estimates of QTL positions might vary because of progeny type (Ji557 or Ji165) and genotype X environment interactions. Therefore, it is likely that some QTLs contributing to kernel soluble sugar content determination remain to be identified.

We noted that of the QTLs detected in this study, none was mapped to chromosome 6, which is incongruent with some previous reports (Goldman et al., 1994; Berke and Rocheford, 1995; Sene et al., 2001; Wassom et al., 2008) showing that this chromosome harbors important loci for kernel traits, e.g., oil, starch and protein contents. This discrepancy warrants further investigations.

Super-sweet corn is an endosperm mutant which was controlled by the sh2 gene, but kernel soluble sugar content, being a quantitative trait, is likely affected by both genetic loci and non-genetic factors like epigenetic modifiers. In the present study, only SSC3 among five major QTLs had a contribution larger than 15%, but several QTLs each had a contribution over 10%; these major QTLs should be amenable to be used for marker-assisted selections (MAS) in future breeding efforts (Yousef and Juvik, 2002). An integrated breeding strategy of phenotypic selection and MAS should be more effective for enhancing soluble sugar content in super-sweet corn. On the other hand the closely linked DNA markers to the QTLs detected in this study could be used for the future positional cloning of these QTLs.

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