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Mapping QTL for popping expansion volume in popcorn with simple sequence repeat markers

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Abstract Popping expansion volume is the most important quality trait in popcorn (*Zea mays* L.), but its genetics is not well understood. The objectives of this study were to map quantitative trait loci (QTLs) responsible for popping expansion volume in a popcorn × dent corn cross, and to compare the predicted efficiencies of phenotypic selection, marker-based selection, and marker-assisted selection for popping expansion volume. Of 259 simple sequence repeat (SSR) primer pairs screened, 83 pairs were polymorphic between the H123 (dent corn) and AG19 (popcorn) parental inbreds. Popping test data were obtained for 160 S_1 families developed from the [AG19(H123 × AG19)] BC_1 population. The heritability (h^2) for popping expansion volume on an S_1 family mean basis was 0.73. The presence of the gametophyte factor *Ga1*^{s} in popcorn complicates the analysis of popcorn × dent corn crosses. But, from a practical perspective, the linkage between a favorable QTL allele and *Ga1*^{s} in popcorn will lead to selection for the favorable QTL allele. Four QTLs, on chromosomes 1S, 3S, 5S and 5L, jointly explained 45% of the phenotypic variation. Marker-based selection for popping expansion volume would require less time and work than phenotypic selection. But due to the high h^2 of popping expansion volume, marker-based selection was predicted to be only 92% as efficient as phenotypic selection. Marker-assisted selection, which comprises index selection on phenotypic and marker scores, was predicted to be 106% as efficient as phenotypic selection. Overall, our results suggest that phenotypic selection will remain the preferred method for selection in popcorn × dent corn crosses.

Keywords Popcorn · Popping expansion volume · Quantitative trait loci (QTL) · Marker-assisted selection

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Introduction

Popping expansion volume, defined as the volume of popped corn per gram of unpopped corn, is the most important quality trait in popcorn. Lyerly (1942) reported that crosses of high expansion popcorn inbreds tended to have high expansion volumes, whereas hybrids between low expansion inbreds tended to have low expansion volumes. Crosses of high × low expansion inbreds tended to be intermediate in expansion. In crosses between popcorn and dent corn, Crumbaker et al. (1949) found that low popping volume and dent-type kernels were partially dominant over high popping volume and flint-type kernels. Their data showed that two backcrosses to the popcorn recurrent parent are sufficient to recover popping expansion volume equal to that of the popcorn parent. Dofing et al. (1991) found additive genetic effects for popping expansion in two popcorn × dent corn crosses and dominant effects for reduced expansion volume in one popcorn × dent corn cross. Information on the number of genes affecting popping expansion volume is limited. In a preliminary study with 60 BC_1 families evaluated in one location, Kantety (1997) identified three putative QTLs for popping expansion.

Popcorn generally is inferior to dent corn in yield and other agronomic traits (Robbins and Ashman 1984; Dofing et al. 1991). Johnson and Eldredge (1953) reported that some inbreds developed from one or two backcrosses of popcorn × dent corn progeny to popcorn had good popping expansion and improved stalk strength. It is thus desirable to introduce dent corn germplasm into popcorn to improve traits such as yield and resistance to stalk and root lodging in popcorn. However, the expan-

sion volume of the popcorn parent must be recovered during the introgression of dent corn germplasm into popcorn.

Molecular markers are powerful tools for mapping genes (including QTLs) in maize (Dudley 1993). Because of their simplicity, abundance, and distribution throughout the genome, SSRs have become a major marker system utilized in maize (Smith et al. 1997; Senior et al. 1998). Marker-assisted selection or marker-based selection could be useful to facilitate the improvement of popcorn when backcrosses are made to recover popping expansion. Our objectives in this study were: (1) to identify putative QTLs for popping expansion volume in a popcorn × dent corn cross, and (2) to compare the predicted efficiencies of phenotypic selection, marker-based selection and marker-assisted selection in the improvement of popping expansion volume.

Materials and methods

Mapping population

AG19 is a Supergold popcorn inbred developed by Ag Alumni Seed Improvement Association, Incorporated (Romney, Indiana, USA), whereas H123 is a dent corn inbred developed at Purdue University from the [B73(B73 × H84)] BC_1 cross. The H123 × AG19 cross was made at the Ag Alumni nursery, Romney, Indiana, in 1998. The F_1 was backcrossed to AG19 in a Puerto Rico nursery in winter 1998. The BC_1 plants were selfed to generate BC_1S_1 lines at the Purdue Agronomy Farm, West Lafayette, Indiana, in 1999. Selfed ears from 160 random BC_1 plants were harvested.

SSR analysis

Twenty kernels of each of the 160 BC_1S_1 lines, the two parents and the F_1 hybrid were planted in a greenhouse. The leaf tissues (<2 weeks old) were harvested and bulked for each entry and were stored in a –70 °C freezer before lyophilization. The lyophilized samples were ground to fine powder with a grinder, and stored at –20 °C until DNA extraction. Genomic DNA was extracted using the CTAB method (Saghai-Maroof et al. 1984). Concentrations of the genomic DNA preparations were determined by spectrophotometry at 260 nm using a Gene Quant (Pharmacia Biotech) spectrophotometer and diluted to 25 ng μl^{-1} with TE buffer (pH 8.0). DNA was then stored in a –20 °C refrigerator until it was used in polymerase chain reaction (PCR) amplification.

A total of 259 SSR primers pairs, chosen from Maize DB (http://www.agron.missouri.edu) for their distribution throughout all ten maize chromosomes, were initially screened for their polymorphism between parents. The primers were synthesized by Research Genetics (Huntsville, Ala.). Primer codes assigned by Research Genetics (i.e., Maize MapPairs) are used herein. The primer pairs that amplified polymorphic bands between parents and generated codominant bands in their F_1 were subsequently used to genotype each BC_1S_1 family (i.e., each equivalent to a BC_1 individual plant).

PCR amplifications were performed in a PTC-100 Programmable Thermocycler (MJ Research, Watertown, Mass.). The 25-µl PCR reaction mixture contained 2.5 µl of 10 × buffer [500 mM of KCl, 100 mM of Tris (pH 9.0 at 25 °C, 1% Triton X-100, and 20 mM of $MgCl_2$], 5 µl of 5 × buffer [60% Sucrose and 1 mM of Cresol-red (Sigma C 9877)], 1.25 mM each of dATP, dGTP, dCTP and dTTP, 0.8 mM of SSR primer, 0.5 of U *Taq* polymerase, and 25 ng of genomic DNA as a template. The thermal cycler was programmed for a first denaturation step of 30 s at 92 °C, followed by 40 cycles of 92 °C for 30 s, 55 °C for 1 min, and 72 °C for 2 min. After the completion of 40 cycles, a final extension at 75 °C was carried out for 10 min. The completed reactions were kept at 4 °C. The SSR products were resolved in 4% Nusieve GTG agarose gels (BioWhittaker Molecular Applications, Rockland, Me.) that were subjected to electrophoresis at 80–120 V for 4–6 h in 0.5 × TBE buffer. The gels were stained with ethidium bromide and photographed under ultraviolet light. All names and nucleotide sequences of the SSR primer pairs used for genotypic analysis are available on request.

Measurement of popping expansion volume

Field experiments were conducted at three locations in 2000 with a single replication in each location. Given that our interest was in the average performance across locations, the use of one replication per location leads to the most efficient allocation of resources (Nyquist 1991). The 160 BC_1S_1 lines were randomly assigned to one-row plots, each row 6.0 to 7.0 m long and spaced 0.76 m apart. The plant population density was 43,900 plants ha^{-1} at the Purdue Agronomy Farm, West Lafayette, Indiana (i.e., West Lafayette) and at Throckmorton, Lafayette, Indiana, and 65,800 plants ha^{-1} at the Ag Alumni nursery (i.e., Romney). Standard cultural management practices were used at each location. The [AG19(H123 × AG19)] BC_1S_1 families yielded well at all three locations, and we harvested the plots by hand at Romney and at West Lafayette for popping tests. The ears were conditioned (i.e., dried slowly) until the kernels reached the optimum moisture for popping (13.5% ± 0.5%). The conditioned ears for each plot were shelled and bulked for popping expansion tests. Popping expansion tests were conducted as described by Robbins and Ashman (1984).

Data analysis

Mean, variation and distribution for values of popping expansion volume were analyzed by PROC UNIVARIATE in SAS (SAS Institute Inc 1989). The heritability (h^2), on an entry mean basis was estimated by Nyquist (1991) as: $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_e^2/l)$, where σ_g^2 denotes the genetic variance, σ_e^2 is experimental error, and l is number of locations. The estimates of σ_g^2 and σ_e^2 were obtained from an analysis of variance assuming the locations were random.

Segregation ratios for SSR marker classes were tested by a chi-square test for conformation to the expected 1:1 ratios at the $\alpha = 0.05$ significance level. A linkage map was constructed with MAPMAKER (Lander et al. 1987). Interval mapping, using MAPMAKER/QTL (Lincoln and Lander 1990) was conducted for those markers that were in linkage groups containing two or more markers. The presence of a QTL was declared at a LOD score threshold of 3.0. Single-factor analyses of variance were performed for the other markers that were not linked to any group. A significant F-test ($P \leq 0.05$) indicated an association of the marker locus with the quantitative trait.

We predicted the efficiencies of three selection strategies for improving popping expansion volume: (1) selection based on the popping test only, i.e., phenotypic selection; (2) selection based only on markers linked to QTLs, i.e., marker-based selection; and (3) selection based on both the phenotype and markers linked to QTLs, i.e., marker-assisted selection. The method for such comparisons was described by Lande and Thompson (1990). The predicted efficiency of marker-based selection relative to phenotypic selection was $(p/h^2)^{1/2}$ where p was the proportion of the additive genetic variance explained by the marker loci. We assumed that dominance and epistasis were absent and that all genetic variance was additive. The predicted relative efficiency of marker-assisted selection to phenotypic selection was calculated as $[p/h^2 + (1 - p^2/(1 - h^2p)]^{1/2}$.

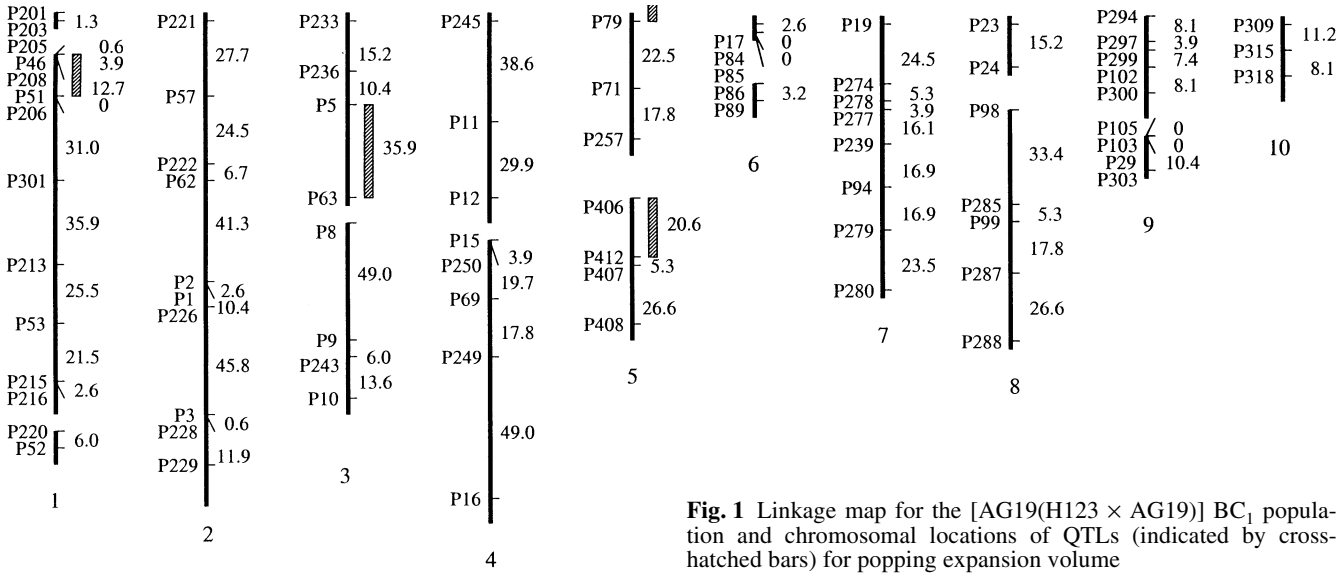

Fig. 1 Linkage map for the [AG19(H123 × AG19)] BC_1 population and chromosomal locations of QTLs (indicated by cross-hatched bars) for popping expansion volume

Results and discussion

Linkage map and effects of *Ga1*^{s}

Of 259 SSR primer pairs screened, 83 (32%) pairs amplified clear, unambiguous polymorphic DNA fragments between parents AG19 and H123. Eighty markers were assigned to 18 linkage groups that comprised 963 cM (Fig. 1), whereas three markers (P33, P272 and P291) were unlinked. Primer pair P239 (bnlg1022) was chosen from chromosome 3 in MaizeDB, but our results indicated that it was on chromosome 7.

Chi-square tests ($\alpha < 0.05$) showed that markers P11, P12 and P245 did not segregate in the expected 1:1 ratios. These three markers were located on the short arm of chromosome 4. The *Ga1*^{s} gametophyte factor, present on the short arm chromosome 4 (Neuffer et al. 1997, p 41) in popcorn, complicates the construction of linkage maps and the subsequent linkage mapping of QTLs in popcorn × dent corn crosses. *Ga1*^{s} and *ga1*^{s} pollen are equally competitive on *ga1*^{s}/*ga1*^{s} silks. On *Ga1*^{s}/*Ga1*^{s} or *Ga1*^{s}/*ga1*^{s} silks, however, *Ga1*^{s} pollen is much more competitive in pollination, to the point where the frequency of pollination by *ga1*^{s} pollen is close to zero (Schwartz 1950). The presence of *Ga1*^{s} in popcorn complicates the analysis of popcorn × dent corn crosses, regardless of how the crosses are made. If the F_1 (*Ga1*^{s}/*ga1*^{s}) was used as the male and AG19 (*Ga1*^{s}/*Ga1*^{s}) as the female, as we did in this study, then all the resulting BC_1 plants have the *Ga1*^{s}/*Ga1*^{s} genotype. The BC_1 S_1 families will uniformly have the *Ga1*^{s}/*Ga1*^{s} genotype. But if the F_1 (*Ga1*^{s}/*ga1*^{s}) was used as the female and AG19 (*Ga1*^{s}/*Ga1*^{s}) was used as the male, then the resulting BC_1 population is 50% *Ga1*^{s}/*Ga1*^{s} and 50% *Ga1*^{s}/*ga1*^{s}. Selfing the BC_1 will lead to segregation distortion in the S_1 families of BC_1 plants with the *Ga1*^{s}/*ga1*^{s} genotype.

The presence of *Ga1*^{s} on chromosome 4 in popcorn therefore prevents the detection of QTLs in the region around it, i.e., within 50 cM of *Ga1*^{s}. From a practical perspective, however, *Ga1*^{s} actually enhances the retention of QTL alleles for popping expansion volume: the linkage between a favorable QTL allele and *Ga1*^{s} in popcorn will lead to selection for that particular QTL allele in popcorn × dent corn crosses.

Table 1 QTLs for popping expansion volume detected in the [AG19 (H123 × AG19)] BC_1 population

Chromosome	Flanking markers (cM)[a]	Estimated QTL effect (cm^3 g^{-1})	Percentage of variance explained by QTLs
1S	P51 (0), P208 (12)	2.92	16.2
3S	P5 (14), P63 (22)	2.84	15.5
5S	P79 (0)	2.60	12.9
5L	P412 (14), P406 (7)	2.77	14.9
			Total = 45.1[b]

[a] Estimated map distance between the QTL and each flanking marker is in parentheses
[b] From multiple linear regression analysis in MAPMAKER/QTL

Phenotypic data and QTL detected

Among the [AG19(H123 × AG19)] BC_1S_1 families, popping expansion volume ranged from 27.3 and 48 $(cm)^3$ g^{-1}, a mean of 38.4 $(cm)^3$ g^{-1}. The data were normally distributed as shown by the Wilks statistic ($P = 0.56$). The h^2 of popping expansion volume was 0.73.

Four QTLs were identified in the [AG19(H123 × AG19)] BC_1 population. These QTLs were mapped on the short arm of chromosome 1 (1S), the short arm of chromosome 3 (3S), the short arm of chromosome 5 (5S) and the long arm of chromosome 5 (5L) (Table 1, Fig. 1). The three unlinked markers were not associated with popping expansion volume. The total amount of

phenotypic variation explained by these QTLs was 45.1%. The QTL flanked by markers P51 and P208 on chromosome 1S had the largest effect (2.92 $cm^3\ g^{-1}$) and explained 16.2% of the total phenotypic variation, whereas the QTL on 5S had the smallest effect (2.60 $cm^3\ g^{-1}$).

The estimated distance between a QTL and its nearest flanking marker ranged from 0 cM for the QTL on 1S, to 14 cM for the QTL on 3S. Marker P79 was at the end of chromosome 5S. For the QTL on chromosome 5S, the LOD scores decreased as the distance from marker P79 increased, indicating that for our linkage map the marker itself provided the best estimate of QTL position. We consider these linkages between flanking markers and the putative QTLs to be tight enough for QTL introgression. For simplicity, suppose the map distances are equal to the recombination frequencies. The probability that the P5–P63 chromosomal segment will not carry the QTL allele (i.e., due to a double crossover) is only $0.14 \times 0.22 = 3\%$. These probabilities are lower for the other QTL.

It has been postulated that three to four genes may be involved in the inheritance of popping expansion volume in popcorn (W.A. Robbins, personal communication 2001). A comparison between the results from this study and those in two preliminary studies suggests some common QTLs for popping expansion volume. In the first preliminary study, which involved 60 earliest flowering [AG19(AG19 × A662)] BC_1 plants (A662 is a dent corn) evaluated in one location, Kantety (1997) found three QTLs for popping expansion volume, two of them being on chromosome 5L and the third being on chromosome 6. In the second preliminary study, which involved 119 [AG03(C103 × AG03)] BC_1 lines (C103 is a dent corn) evaluated at one location, one QTL was detected near the centromere on chromosome 1 and a second QTL was detected on chromosome 5L (H.-J. Lu and R. Bernardo, unpublished). The QTL on 1L in [AG19(H123 × AG19)] BC_1 in the present study seems to be the same QTL detected in [AG03(C103 × AG03)] BC_1 (i.e., the unpublished preliminary study); primer P208 was a flanking marker for the QTL detected in both populations. Likewise, the QTL on 5L in [AG19(H123 × AG19)] BC_1 might be the same as that in [AG03(C103 × AG03)] BC_1 because the primer P406 flanked the QTL in both populations. Our results for [AG19(H123 × AG19)] BC_1 and those of Kantety (1997) for [AG19(AG19 × A662)] BC_1 are not directly comparable because of the different marker systems utilized in the each study, but some general information can still be obtained. Both studies showed two QTLs on chromosome 5, but our study indicated that two QTLs were located on different arms, other than on the same arm (long arm) as reported by Kantety (1997). The QTL near marker UMC126A reported by Kantety (1997) might be the same QTL flanked by markers P406 and P412 in the [AG19(H123 × AG19)] BC_1 and flanked by markers P406 and P403 in the [AG03(C103 × AG03)] BC_1, because the chromosomal positions of markers UMC126A and P406 are close. The results from the present study, from the Kantety (1997) study, and from our unpublished preliminary study indicate that the QTL on chromosome 5L was perhaps the most important QTL because it was consistently detected in different populations and in different environments.

Predicted efficiencies of different selection strategies

Phenotypic selection for popping expansion volume is laborious and time-consuming. Thus, molecular markers associated with QTLs governing the trait can enhance the improvement of the trait. Lande and Thompson (1990) indicated that for a trait with a low h^2 the relative efficiency of marker-assisted selection can be high, and the relative efficiency of marker-based selection increases as p (i.e., the proportion of the additive genetic variance explained by the marker loci) increases. In the [AG19 (H123 × AG19)] BC_1, the value of p was $R^2/h^2 = 0.62$, which was less than the h^2 (0.73) of the trait. When all of the four QTLs detected used in selection for popping expansion volume, marker-based selection is predicted to be 92% as efficient as phenotypic selection, whereas marker-assisted selection is predicted to be 106% as efficient as phenotypic selection.

The conditioning of ears for popping expansion tests requires more time and work than marker-based selection. A cost-benefit analysis is therefore needed to determine whether the savings in time and effort with marker-based selection would offset its lower efficiency compared with phenotypic selection. In addition, we are aware that QTL effects are overestimated if the mapping population is small and if many QTLs control the trait (Beavis 1994). The results of Beavis suggest that if a total of ten QTLs control popping expansion volume, our use of 160 BC_1S_1 families reduces, but does not eliminate, the upwards bias in the estimates of QTL effects. If the value of p is overestimated, then the efficiency of marker-based selection and marker-assisted selection will be lower than that predicted from our results. All in all, our results seem to suggest that phenotypic selection will remain the preferred method for selection in popcorn × dent corn crosses.

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