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RFLP analysis of genomic regions associated with cooked-kernel elongation in rice

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Abstract. As a result of earlier breeding efforts, portions of the genome of "Basmati 370" have been introgressed into a rice breeding line, B8462T3-710. Cooked-kernel elongation was increased in this breeding line to a level equal to that of "Basmati 370". The objective of this study was to identify and locate quantitative trait loci (QTLs) associated with cooked-kernel elongation in an F3 population derived from a cross between B8462T3-710 and the reduced-elongation recurrent parent variety, Dellmont. DNA from the parental lines and "Basmati 370" as a control, were screened for RFLPs using 170 clones chosen to cover the rice genome at intervals of 8 cM on average. Eighteen markers identified RFLPs common to Basmati 370 and B8462T3-710, but different from Dellmont, suggesting possible associations with kernel elongation. The B8462T3-710/Dellmont F3 population was analyzed for segregation of those RFLPs and for kernel elongation. Analysis of variance of the kernel elongation ratio revealed that two markers, 14.6 cM apart on chromosome 8, are significantly associated with this trait (RZ323 $P \leq 0.005$, RZ562 $P \leq 0.05$). Interval mapping suggests a single QTL with a close proximity to RZ323. This QTL was tested in F6 lines derived from the same cross and the presence of the B8462T3-710 segment detected by RZ323 caused a highly significant increase of the kernel elongation ratio ($P \leq 0.04$). In addition, the QTL for kernel elongation and a gene for aroma, which are major components of the grain quality characteristics of Basmati-type rices, showed linkage. The availability of linked markers to the QTL may facilitate early selection for kernel elongation in rice breeding programs.

Key words: Cooked-kernel elongation – Rice (*Oryza sativa* L.) – RFLP markers – QTL – Grain quality

Introduction

Kernel elongation without significant increase in breadth after cooking is, in addition to soft texture, a desired property for some fine-grained and scented rices such as Basmati. This type of rice is an important commercial commodity for the economy of countries like India, Pakistan and Thailand (Khush et al. 1979). 'Bahra' of Afghanistan, 'Domsia' of Iran and 'D25-4' from Burma all show this elongation (Khush et al. 1979), and *Japonica* rices also elongate appreciably during cooking (Juliano and Perez 1984). Such elongating rices tend to have a low starch-gelatinization temperature (<70 °C), a low to intermediate amylose content (<25%), and medium gel consistency (41–60 mm) (Juliano 1979).

In view of their commercial importance, many countries have initiated breeding programmes to develop suitable Basmati varieties with enhanced grain elongation. Attempts to improve the yield potential of poor-yielding Basmati varieties by combining the high-yielding ability of semidwarfs with the grain quality characteristics of Basmati types have been largely unsuccessful (Khush et al. 1979). The lack of desirable recombinants in the breeding programmes from crosses involving semidwarf parents and Basmati varieties, and the reversion to parental types in the backcross programmes, seemed to suggest that probably *indica* and Basmati types are phylogenetically divergent (Khush et al. 1979). A study by Glaszmann (1987) on Asian rice varieties using isozyme markers indicates that Basmati types are clustered in the group V gene pool and separated from the *indica* and *japonica* types. This group was the most polymorphic, and further subgrouping was suggested. Wang and Tanksley (1989), using RFLP markers, demonstrated that although accessions that belong to isozyme Group V

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cluster together, they form two distinct groups linking to the *indica* cluster at two different nodes.

Kernel elongation, like scent, is a major component of the grain quality characteristics of Basmati types (Sood et al. 1979); however, no genetic studies have been carried out on this characteristic. This might be due to the fact that this is an endosperm character which is expressed in a quantitative manner and affected by the environment, and is therefore difficult to measure (Khush et al. 1979).

Molecular markers have been utilized to identify QTLs for fruit size, soluble solids, and pH (Paterson et al. 1988, 1991; Tanksley and Hewitt 1988) in tomato; plant height (Beavis et al. 1991) and yield (Stuber et al. 1987) in maize; seed protein and oil content (Diers et al. 1992) and several reproductive and morphological traits (Keim et al. 1990) in soybean. These molecular markers can be used to select desired genotypes based on their RFLP alleles at QTLs, substituting for multiple evaluations over years and locations. The objective of this study was to use RFLPs to identify genomic regions associated with cooked-kernel elongation in rice.

Materials and methods

Plant material

An F3 population, segregating for kernel elongation and derived from the cross B8462T3-710 × Dellmont, was used in this study. The B8462T3-710 line (hereafter referred to as *B*), which was derived from a single plant, was developed through anther culture of the cross B7836A1-44-5/Rexmont. The female parent, B7836A1-44-5, was an F4 selection from the cross Basmati 370/RU7703075. RU7703075 was an F6 selection from the cross CI9881/PI331581. Since RU7703075 and Rexmont were non-elongating types, the superior kernel elongation character of the *B* line most likely comes from Basmati 370 which also has genes for aroma (Reddy and Sathyanarayanaiah 1980). Dellmont (hereafter referred to as *D*) was derived from the cross Della/Lemont*5 followed by several generations of selfing and pedigree selection. Lemont was derived from the cross Lebonnet//CI9881/PI33158. Dellmont is a non-elongating type and has a gene for aroma derived from Della.

Evaluation of kernel elongation

For each family, ten random milled F3 kernels harvested from F2 plants were measured (mm) prior to cooking. These ten kernels were cooked with about 20 other kernels from the same family. After cooking, ten kernels were taken randomly from the cooked sample and measured for length (mm). The remnant F3 kernels were grown in a greenhouse at Cornell University, Ithaca, N.Y., for fresh-tissue harvest. Measurements of kernel elongation of the parentals, *D* and *B*, were repeated several times through the analysis. The 'Basmati' check was from a sample of rice originally grown in India. F6 kernels from F5 plants were measured in the same way. The kernel elongation ratio was calculated as the mean length of the cooked kernel divided by the mean length of the raw kernel. Measurements of the kernel elongation ratio were done in Beaumont, Texas.

RFLP analysis

An RFLP map of the rice genome constructed by McCouch et al. (1988), augmented with additional DNA markers to a total of more than 500 loci (Causse et al., in preparation), was the basis for the marker analysis. A total of 170 RFLP clones from rice genomic (RG), cDNA (RZ), and oat cDNA (CDO) libraries were used to identify DNA polymorphisms between parents in the survey filters (see Fig. 1). Polymorphism survey filters consisted of three lanes per enzyme including the parents (*D* and *B*) and Basmati 370. DNA from Basmati 370 was included to eliminate from consideration those polymorphic clones that were less likely to be associated with the kernel elongation of Basmati 370. Seven restriction enzymes (*Dra*I, *Xba*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Bgl*II, and *Bam*HI) were used to digest DNA for survey filters. Procedures for DNA extraction, restriction enzyme digests, and Southern blotting were according to McCouch et al. (1988). Labeling of probes was via primer extension (Feinberg and Vogelstein 1983). Twenty-two polymorphic markers were used to construct the linkage map, which served as the basis for QTL analysis (see Fig. 1). The map based on 85 F3 families was constructed with MAPMAKER computer software using the Kosambi map unit function (Kosambi 1944; Lander et al. 1987).

QTL mapping

The association between markers and QTLs for kernel elongation was tested using two different procedures. First, a one-way analysis of variance was conducted to identify significant associations between individual RFLP markers and kernel elongation ratio using the computer program StatView II (Abacus Concepts, Inc., Berkeley, Calif.). This analysis included contrasts to compare the genotypic means of the homozygous and heterozygous classes for each RFLP marker. The second method was interval mapping which searches for the effects of a QTL using sets of linked markers (Lander and Botstein 1989). A LOD score of 2.0 was chosen as the threshold for detecting QTL locations. LOD peaks were used to position QTLs on the RFLP linkage map. The additive effect (a), the dominance deviation (d), and the degree of dominance (d/a), were calculated for each QTL using MAPMAKER QTL software. Environmental variance for kernel elongation ratio was estimated by averaging the variance of the control plants (*B* and *D*). Data on the F1 could not be obtained due to the limited numbers of F1 kernels available to measure elongation ratio and the unusual shape of the kernels (banana shaped). The phenotypic variation accounted for by significant QTLs in the F3 was estimated using MAPMAKER-QTL software. The proportion of genetic variance was estimated by multiplying the total variance of the F3 by the proportion of phenotypic variation and dividing this by the genetic variance of the F3 (total F3 variance minus environmental error). Tests for two-way interactions were made between significant markers and all other marker loci segregating in the population using the computer program StatView II.

Results and discussion

Parental screening

Of the 170 RFLP clones that were surveyed for polymorphism, 22 (13%) gave a polymorphism between the parents (Fig. 1). Eighteen of the twenty-two clones produced the same RFLP in Basmati 370 and *B* but a different RFLP than *D*, indicating a possible association of those markers with kernel elongation. Four of the 22 clones (*RZ566, RZ599, RG28* and *CDO99*) produced the same RFLP in Basmati 370 and *D* but a different RFLP than *B*. The low level of polymorphism is probably attributable to the fact that *D* and *B* share CI 9881 and PI

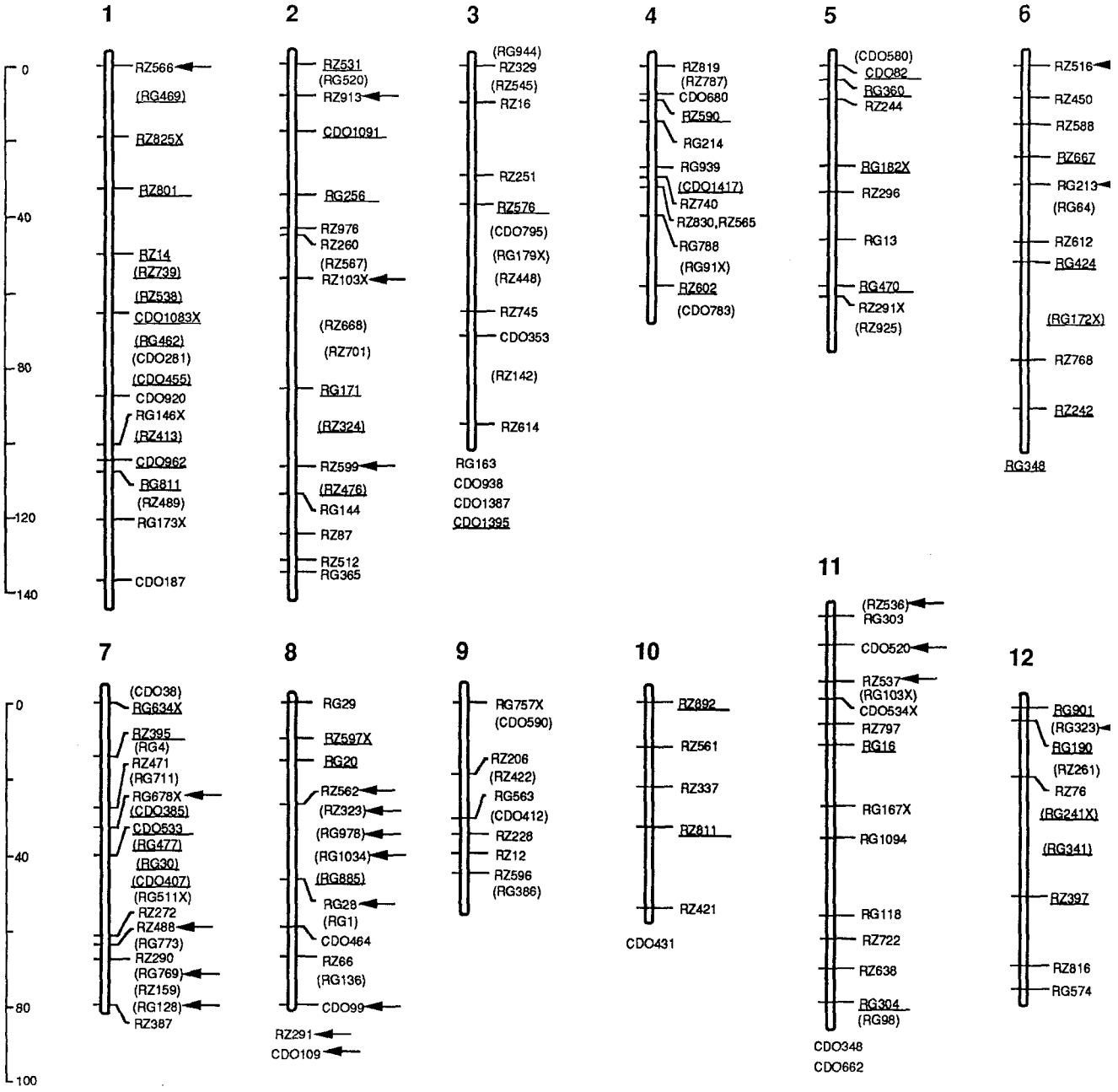

Fig. 1. Distribution of markers evaluated on kernel elongation based on the molecular map of rice developed by Dr. Tanksley's lab at Cornell University. Scale in Kosambi cM is shown on left. Markers *in parenthesis* have been approximated from a previously published map (McCouch et al. 1988) or located to intervals with LOD < 2. Markers followed by "*X*" are multiple-copy clones (see the rice map for details). *Arrows* point to markers which detected polymorphisms between the parents (22 markers). Four of twenty-two markers produced the same RFLP in Basmati 370 and Dellmont and a different RFLP than B8462T3-710 (*RZ566, RZ599, RG28* and *CDO99*). The other eighteen markers were used to construct the linkage map for interval analysis. Two markers on chromosome 2 (*RZ913* and *RZ103X*) did not form a linkage group. Linkage groups on chromosomes 6, 7, 8, and 11 comprise 188 cM in total explaining 18% of the rice genome examined. Markers *underlined* produced the same RFLP in Dellmont and B8462T3-710 and a different RFLP than Basmati 370. Markers under a chromosome were linked to that chromsome but not placed to intervals

331581 as their ancestors so they have similar genetic backgrounds.

Fifty-one (30%) out of the one-hundred and seventy clones which were located randomly throughout the genome produced the same RFLP in *D* and *B*, but a different RFLP than Basmati 370; therefore these clones were thought not to be associated with the kernel elongation of Basmati 370. By utilizing previously developed genetic materials to identify a few RFLP loci with potential linkage to the kernel elongation gene(s), we were able to

avoid analyzing a large population for segregation of RFLPs at many loci scattered randomly throughout the genome.

F3 population

ANOVA. The distribution of kernel elongation ratio for the segregating F3 population is shown in Fig. 2, which includes means for the parents and Basmati 370. The potential association of kernel elongation with markers was tested with analysis of variance procedures using each marker as a treatment. Two linked markers on chromosome 8, *RZ323* and *RZ562*, were found to be significantly associated with kernel elongation (Fig. 1). *RZ323* showed the most significant association ($P \leq 0.005$) in the F3, and the b/b (B homozygotes) and b/d (heterozygotes) genotypes were approximately the same with respect to kernel elongation ratio. Both were significantly higher than the d/d genotype (*D* homozygotes) indicating that, in this genetic background, the segment containing *RZ323* is behaving in a dominant manner with respect to kernel elongation (Table 1). The segment from Basmati 370 on chromosome 8 is also marked by three other loci; *RZ291, RG1034*, and *RG978* (Fig. 1); however, none of them showed a significant association based on ANOVA (Table 1).

The Basmati 370 segments marked by *RZ913, RZ103, RZ516*, and *RG323* on chromosomes 2, 2, 6, and 12, respectively, had no detectable effects on kernel elongation in the F3 population. Several intervening markers between *RG678* and *RZ488* and between *RZ488* and *RG769* were tested and found not to derive from Basmati 370. This indicated that three separate fragments of chromosome 7 had been introgressed from Basmati 370. Three introgressed loci were detected on chromosome 11 and comprise about an 18 cM section at the terminal position on the short arm of that chromosome. The segments on chromosomes 7 and 11 had no detectable effects on kernel elongation and may be residual segments that, by chance, were maintained during the breeding program or else were selected for characters other than grain elongation.

RZ323 and *RZ562* were tested for possible two-way interactions with all other segregating markers. None of the interactions were significant ($P \leq 0.05$), suggesting

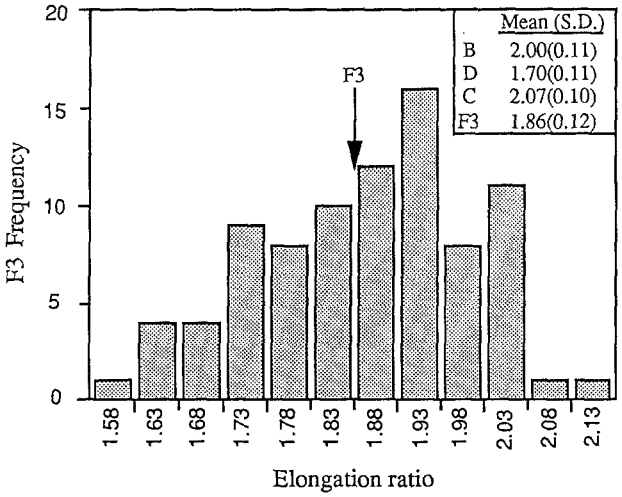

Fig. 2. Frequency distribution for kernel elongation ratio in the parents B8462T3-710 (denoted *B*) and Dellmont (denoted *D*) and in Basmati 370 (denoted *C*). Means and standard deviations for the distributions of the parents, Basmati 370 and F3 populations appear in the upper right of histogram. The distribution for kernel elongation ratio is approximately normal

Table 1. Means and contrasts between means for kernel elongation ratio of different genotypic classes in F3 and F6 populations. Numbers in ( ) = no. of individuals examined; b/b(1) = homozygous B8462T3-710; b/d(2) = heterozygous; d/d(3) = homozygous Dellmont

Population	Marker	Genotype			F	Significance	Differences in means		
		b/b	b/d	d/d			1/2	1/3	2/3
F3	RZ323	1.91 (20)	1.89 (37)	1.80 (25)	5.66	0.005	0.02 ns[a]	0.11**	0.09**
	RZ562	1.90 (22)	1.89 (32)	1.82 (28)	3.55	0.03	0.01 ns	0.08*	0.07 ns
	RG1034	1.90 (19)	1.87 (32)	1.83 (25)	2.18	0.12	0.03 ns	0.07*	0.04 ns
	RG978	1.89 (20)	1.85 (27)	1.84 (32)	1.03	0.36	0.04 ns	0.05 ns	0.01 ns
	RZ291	1.89 (24)	1.89 (16)	1.83 (28)	2.29	0.11	0.00 ns	0.06 ns	0.06 ns
F6	RZ323	2.17 (10)	2.07 (7)	1.98 (6)	3.95	0.04	0.1 ns	0.19*	0.09 ns
	RZ562	2.15 (10)	2.04 (7)	2.06 (6)	1.36	0.28	0.11 ns	0.09 ns	0.02 ns

[a] ns = nonsignificant at $P > 0.05$, * = significant at $P < 0.05$ and ** = significant at $P < 0.01$ as determined by Fisher PLSD

that there is little or no interaction between loci with regards to kernel elongation. This is supported by the fact that *B* with portions of the genome of 'Basmati 370' showed an elongation ratio equal to that of 'Basmati 370' and the segment on chromosome 8 continued to exert the same effects in the F6 generation as seen in *B* (see next section). If the effects of this segment had been dependent on the presence of 'Basmati 370' alleles elsewhere in the genome, it is expected that the loss of such 'Basmati 370' alleles during the selection process would have modified the effects of this segment.

Interval analysis. When the kernel elongation ratio was subjected to interval analysis in the F3 population, one QTL (LOD > 2) could be detected. The *b* allele increases the kernel elongation ratio by 0.055 with the dominance effect (d) being 0.029. The LOD score plot was then drawn to localize the gene(s) for kernel elongation more precisely (Fig. 3). *RZ323* is the most likely location. The percentage of phenotypic variance explained by this QTL was 13.6%, which is equivalent to 45% of the genetic variance. The large amount of variation explained by this QTL seems to suggest that the inheritance of this trait was controlled partly by a gene(s) with large effects and possibly a few major genes (C. Bollich, personal communication). *RG28*, which is linked to a scent gene (*fgr*, Ahn et al. 1992), produced the same RFLP in Basmati 370 and *D* but a different RFLP than *B*. This seems to indicate that the genetic origin of this scent gene in the two varieties may be the same. It is interesting to note that although analysis of variance with *RG28* and *fgr* did not produce differences in kernel elongation ratios among the three genotypes, the QTL for kernel elongation appears to show loose linkage to *fgr* (Fig. 3).

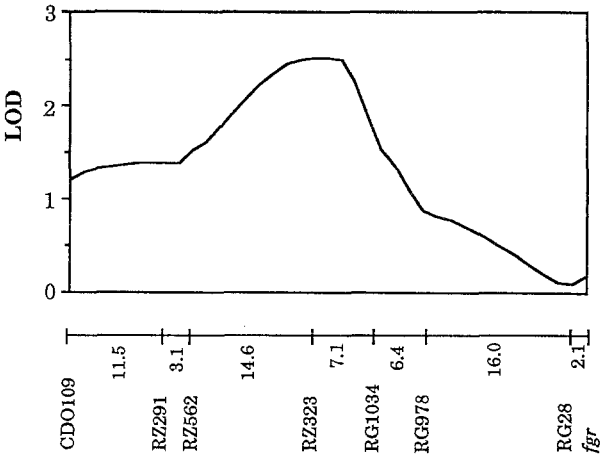

Fig. 3. QTL plot for kernel elongation ratio monitored in the F3 population (chromosome 8). The plot is for LOD scores based on MAPMAKER-QTL. A gene for aroma (*fgr*) segregated in this population and was included in the map of chromosome 8. The number between markers indicates map distance in Kosambi map function

F6 study

To further test the effects of the QTL detected in the F3 population, 23 F6 lines, derived from the F2 population described above, were screened for segregation of *RZ323* and *RZ562* and kernel elongation. The effect of the QTL was estimated using ANOVA. The results confirm the effect of the QTL on kernel elongation ($P \leq 0.04$, Table 1) and the presence of a *B* chromosomal segment detected by *RZ323* caused a significant increase in kernel elongation ($P \leq 0.05$). For *RZ323* in the F6, the b/b genotype had a mean kernel elongation ratio value 0.19 higher than that of the d/d genotype. In the F3, the difference between these genotypes was 0.11. For *RZ562*, the b/b genotype was higher than the d/d genotype but the effects were statistically non-significant.

The variability seen in the data of kernel elongation ratio between the F3 and the F6 (Table 1) could be attributed to various factors (genotype × environment interaction, environment effects, and alterations in genotype, due to inbreeding and selection.)

Limitations to detection of quantitative trait loci

The goal of this research was to identify associations between QTLs for kernel elongation in rice with RFLP markers so that such markers may be used as indirect criteria for selection. The QTL identified in this study should be considered as the minimum number of those segregating in this population. The following factors might explain why more QTLs were not identified: the population used in the study, the action of minor QTLs, environmental effects, and error associated with measurement of the kernel elongation ratio. The effects of the latter three could be reduced by evaluating larger populations in more environments.

Although the 170 clones surveyed were chosen to cover the rice genome at intervals ranging from 2 to 23 cM, there is still a possibility that small Basmati 370 fragments containing QTLs went undetected. Monomorphic regions of the genome would be less likely to have differences in DNA sequence and consequently less likely to be segregating for QTLs for kernel elongation.

The ability to detect a QTL appears to be affected by the population used in the QTL mapping. By using an interspecific cross of tomato between *L. esculentum* and *L. chmielewskii*, a wild species with a high level of soluble solids, Paterson et al. (1988) was able to identify four QTLs for the concentration of soluble solids. Only one of these QTLs was detected in LA1653, a strain with increased soluble solids produced through backcrossing a different strain of *L. esculentum* to *L. chmielewskii*, and this QTL was detected in a region which failed to show effects on the concentration of soluble solids in a single-environment test by Tanksley and Hewitt (1988). The other three QTLs identified by Paterson et al. (1988) were

not introgressed into LA1653. Although different *L. esculentum* strains were used in these two experiements, they seem to suggest that the QTLs detected by interval mapping may differ from those that would be fixed by repeated selection for a trait in the breeding programme. Although *B* showed a kernel elongation equal to that of 'Basmati 370', it is not clear whether all the genes involved in kernel elongation were introgressed from 'Basmati 370' during the breeding program. In this regard, it would be interesting to use other mapping populations derived from crosses involving 'Basmati 370' or other lines showing kernel elongation. Because a relatively small population (85 F3 families) was used in this study, it is likely that only loci with a relatively large effect on kernel elongation were detected. It is possible that more loci with smaller effects on kernel elongation segregate in this population and larger population sizes would be needed to identify such minor loci.

As was discussed by Tanksley and Hewitt (1988), in order to have confidence in the effects of QTLs they should be tested in a variety of genetic backgrounds. Caution should be taken in asserting that rice breeding lines could be bred by selection of the markers linked to this QTL. The effect of this QTL should be tested in different genetic backgrounds, and the segment containing it should also be tested for associated effects with other characters of agronomic importance.

Conclusions

In this study we have utilized previously developed genetic materials to identify RFLP loci that potentially were associated with loci affecting kernel elongation in rice. It is apparent from the data presented in this report that a segment of chromosome 8 introgressed from Basmati 370, contains a gene(s) affecting kernel elongation. Having mapped a QTL with a relatively large effect, flanking markers could be used to facilitate early selection for this character and to transfer the chromosome segment containing the QTL into breeding lines or varieties with a high degree of certainty. Improvements in selection for kernel elongation could be expected by the identification of markers with tighter linkages to the QTL reported here and by identifying additional QTLs. Having mapped a QTL for grain elongation along with *fgr* to chromosome 8 allows a point of comparison for other studies of the inheritance of kernel elongation and may lead to a better understanding of the genetic basis of polymorphism for Basmati-type rices throughout the world. Finally precise localization of the QTL for grain elongation could ultimately lead to the isolation of a gene(s) controlling this character through map-based cloning.

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