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Aroma in rice: genetic analysis of a quantitative trait

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to be determined. In the study presented here, the genetic mapping of aroma was improved by combining for the first time the two following approaches: (1) a precise and objective aromatic analysis by gas chromatography, permitted by the utilisation of doubled haploid lines, which gave sufficient quantities of grains (Petrov et al. 1995), and (2) by saturation of the map of the chromosome 8 with several molecular markers, allowing the placement of flanking markers around the AcPy gene.

Materials and methods

Strategy

A core genetic map consists of markers covering all the genome at middle density, i.e. with no gap larger than 20–25 cM. Therefore, there will be at least 1 marker sufficiently linked at 10 cM or less to the genes(s) underlying a trait. A core rice map was developed at IRRI, Philippines (Huang et al. 1994) on the basis of 145 RFLP markers, for the most part ones that had already been placed on the interspecific saturated map (Causse et al. 1994). For this purpose, a population of 135 doubled haploid (DH) lines was used. After evaluation for aromatic compounds of the DH lines by gas chromatography at ENSIAA, France, chromosome segments of interest were identified by segregation/QTL (quantitative trait locus) analysis. Saturation of the major segment with different types of molecular markers was then done at ORSTOM-LRGAPT, France and at IRRI.

Genetic material and DNA extraction

The mapping population of (DH) lines was derived from the F_1 hybrid 'IR 64'×'Azucena' through anther culture at IRRI (Guiderdoni et al. 1992). Azucena is a scented *japonica* landrace from Philippines, and IR 64 is a non-scented *indica* variety obtained from IRRI. DNA was isolated from lyophilised leaves using the CTAB method (Murray and Thompson 1980).

Restriction fragment length polymorphism markers

In this study, we used probes from the saturated RFLP rice map developed by Causse et al. (1994). Probes were kindly provided by Dr. S. McCouch (Cornell University, USA). Southern transfers, hybridisations and non-radioactive DNA labelling used for revelation of hybridisations were done according to IRRI or CIMMYT protocols

Sequence-tagged sites (STSs)

STSs are PCR markers obtained by the amplification of DNA between two primers corresponding to the two bounds of a genomic probe. STSs have the advantages of RAPD markers (they are based on PCR) and those of RFLP markers (they are locus-specific and codominant). Such 20-mer primers were published by Inoue et al. (1994). These primers correspond to probes of the saturated map of the Rice Genome Research Program – Japan (Kurata et al. 1994). PCR conditions were the same as in Inoue et al. (1994). As polymorphism between parents was not detected directly, digestion of amplification products was done with 4 bp restriction enzymes.

Isozymes

Isozyme analyses were performed following the protocol of Guiderdoni et al. (1989).

Evaluation of aroma

The evaluation of rice aroma is not easy, and classical smelling or chewing methods are not supposed to be totally reliable because of their subjective nature. The method used here, detailed in Petrov et al. (1995), is based on a gas chromatography quantification of volatile compounds contained by the cooking water of 100 g. of grains. By this technique, the presence of 2-acetyl-1-pyrroline at the ppb (1 ppb equals to 1 ng/g) level can be detected with good repeatability (Petrov et al. 1995). This compound is known to be the major agent of aroma in rice (Buttery et al. 1983, 1988). As several DH lines were partially sterile, we could not obtain sufficient amounts of grains for the analysis of these lines. Consequently, the analysis was performed on only 84 lines of the mapping population. Measurements on parents were replicated 12 times.

The method of scent revelation with KOH (Sood and Siddiq 1978) using leaves and seeds was also applied on a replication of the population grown in the glasshouse. For the test using leaves, one or two leaves per line were cut into small pieces and put into petri boxes with 10 ml of 1.7% KOH. After 30 min, the boxes were opened and immediately smelled. DH lines were then scored as aromatic or non aromatic. For the test using seeds, ten seeds per line were ground and aroma revelation and scoring were identical to that used for leaves. Each line was evaluated by a minimum of four persons chosen for their capacity to easily distinguish between the two parents. Since these tests need only ten seeds or one or two leaves from each line, the entire population (135 lines) was evaluated for both leaves and seeds. These two tests permitted us to compare the results of sensitive tests using leaves or seeds to those of gas chromatography.

where a, b and c are the frequencies of AB, Ab and aB classes, respectively. The variance of this estimate is

$$V_{\hat{r}_c} = \frac{r(1+r)(r-1)(ru-r-u-1)}{2n},$$

where u is the selection coefficient of class ab and r is the corrected estimate. This variance is slightly smaller than that of the classical estimate under the conditions of selection observed on chromosome 8 ($u<1$).

The standard deviation of the Kosambi map distance $\hat{d}_c$ associated to the estimate $\hat{r}_c$ is:

$$s_{\hat{d}_c} = \frac{1}{(1-4r^2)} \sqrt{\frac{r(1+r)(r-1)(ru-r-u-1)}{2n}}.$$

Cosegregation analysis between markers and characters

Two approaches were used:

– A QTL detection approach based on quantitative evaluation of AcPy by gas chromatography. The interval mapping method (MAPMAKER/QTL for Unix v. 1.1; Lander and Botstein 1989) was used. As AcPy was not normally distributed, ANOVA 1 (SAS-IML®) and the Kruskall & Wallis test (MapQTL for Unix v. 2.4 ; van Ooijen 1992) were used to confirm results of interval mapping. In order to detect putative minor QTLs, we repeated the interval mapping after putting the loci detected by the first analysis as cofactors.

– A Mendelian approach based on coding AcPy in the presence/absence and on sensitive tests. The data were included in the marker data matrix and analysed using MAPMAKER v. 2.0 for a Macintosh computer.

Results

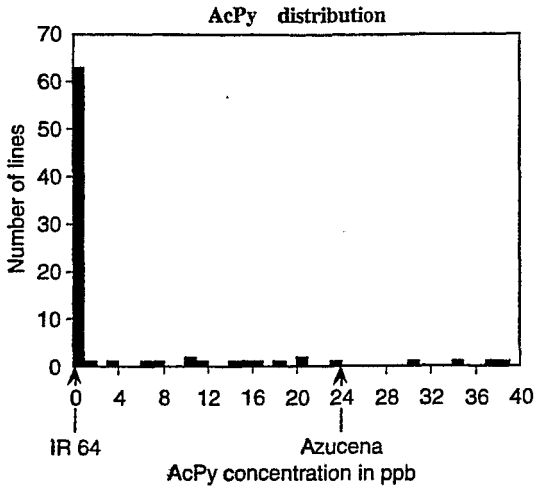


Fig. 1 Distribution of 2-acetyl-1-pyrroline concentration in the DH lines coming from the F_1 hybrid 'IR 64'×Azucena. The deviation in favor of non scented lines (AcPy concentration=0 ppb) is due to strong segregation distortion on chromosome 8 (see text for details). Among the scented lines (AcPy concentration > 0 ppb), some lines are intermediate between 'IR 64' and Azucena, some contain as much AcPy as Azucena and others contain more AcPy than Azucena

ed LOD=1.6. The associated probabilities to the LODs of these two QTLs were 0.004 and 0.008, respectively.

As the major gene was located on chromosome 8, the mapping effort was concentrated on this linkage group. Thus, sixteen markers were mapped. The minimum two-

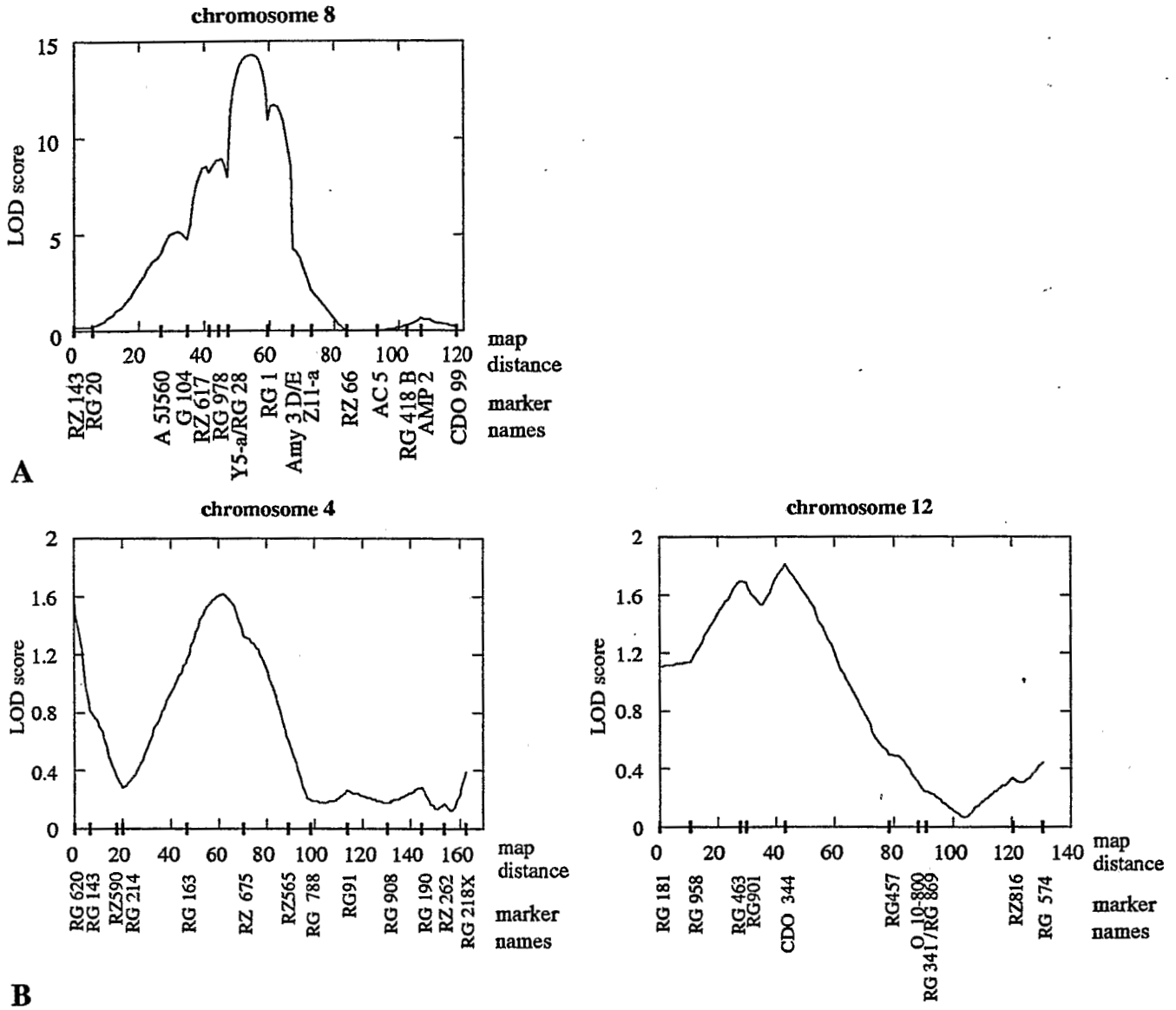


Fig. 2A, B A QTL found for AcPy on chromosome 8, corresponding to a major gene controlling aroma in rice. **B** QTLs on chromosomes 4 and 12 obtained after putting QTL on chromosome 8 as a cofactor in the analysis

the two-point map distance estimate corrected for segregation distortion, $\hat{d}_c$, gave the total length of the group as 117.5 cM, leading to a reduction of map distances of about 27%, compared with those obtained by MAPMAKER. Three-point models corrected for distortion revealed that the order was not modified by segregation distortion.

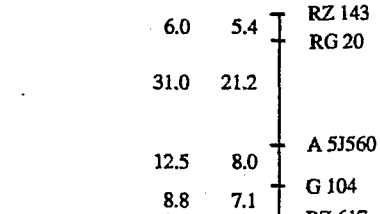
Discussion

This study permitted us to tag a major gene for aroma between close flanking markers. Moreover, two QTLs were identified that may affect the strength of aroma in rice varieties. Although these QTLs were not highly significant, it is very probable that we would have obtained more significant tests if the numbers of scented and non-scented DHs had been roughly the same (it can be shown that segregation distortion affects the power of QTL detection but does not generate false positive detection). It was also confirmed that 2-acetyl-1-pyrroline (AcPy) is the major component of aroma in rice, since *AcPy* and *Aroma* were perfectly correlated and mapped at the same locus.

The map length of chromosome 8 corrected for segregation distortion (117.5 cM) is in good accordance with that found in the intraspecific saturated map (124.8 cM; Kurata et al. 1994), indicating that the proposed estimate of recombination fractions was appropriate for this linkage group. The corrected length is logically larger than chromosome 8 of the interspecific map (Causse et al. 1994), which shows a reduction of recombination due to the genetic distance of the parentals involved in the cross.

segregation distortion is observed on all markers except for RZ143 and RG20. Markers are listed in map order

Marker name	IR 64	Azucena	Total	χ^2	Probability
RZ 143	73	55	128	2.53	0.11161
RG 20	62	58	120	0.13	0.71500
A 5J560	39	18	57	7.74	0.00541
G 104	84	49	133	9.21	0.00241
RZ 617	84	34	118	21.19	$< 1 \cdot 10^{-5}$
RG 978	85	40	125	16.20	0.00006
Y 5-a	89	44	133	15.23	0.00010
RG 28	87	43	130	14.89	0.00011



located at the same locus by Yano et al. (1992) who used an F_2 population and RFLP markers. Moreover, aroma was found to be linked at 7 cM to a RAPD marker that mapped close to aroma in our DH progeny using a cross involving 'Basmati 370' (data not shown). Pinson (1994) found that all scented varieties, including 'Basmati', 'Jasmine 85' and a mutant of 'Della' share the same gene (which is that of chromosome 8). According to several authors, the contradictory conclusions are due to problems in the handling of the endospermic nature of aroma. In F_2 progenies, this confusion may lead, for instance, to the conclusion that the gene is dominant instead of recessive. Segregation distortion can also strongly modify the segregation pattern of a gene (e.g. a 1:1 segregation may be interpreted as a 3:1 segregation in a DH or backcross population). In our study, without molecular marker information we would have to conclude the existence of two complementary aromatic genes coming from Azucena. It is not impossible that the two-gene segregations observed by Pinson (1994) were due to this phenomena, which could not be detected since no marker data were available.

An immediate application of our results is the introgression of the major gene for aroma in a high yielding variety using successive backcrosses, with the aid of the flanking markers. RG 28 and RG 1 are RFLP markers and may be converted into STSs in order to perform a rapid succession of gene introgression. Y5 is a RAPD marker and could be used as it is or after conversion into STS. The advantages of a such approach are: (1) a twofold decrease in the number of necessary generations by choosing in the BC1 progeny the individual with the higher percentage of recurrent alleles, (2) the complete elimination of linkage drag (Ragot et al. 1994) and (3) the direct following of the allele for aroma in successive generations by markers, without the need of the self-pollination steps that are necessary in classical breeding schemes involving recessive characters. Another promising application is marker-assisted selection (MAS), which can integrate major gene and QTL information in selection indexes. It can not be excluded that other QTLs for aroma may be revealed by using a larger prog-

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