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Mapping a new source of resistance to powdery mildew in mungbean

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With 2 figures and 1 table

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Abstract

Both restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphism (AFLP) analyses were employed to map a new source of resistance to powdery mildew in mungbean. Disease scores of an F_2 population derived from the cross between a moderately resistant breeding line VC1210A and a susceptible wild relative (*Vigna radiata* var. *sublobata*, accession TC1966) showed a continuous distribution and was treated as a quantitative trait. Although no significant quantitative trait loci (QTL) that can explain the variation was detected by QTL analysis based on the reconstructed RFLP linkage map, new marker loci associated with resistance were discovered by AFLP analysis. The RFLP loci detected by two of the cloned AFLP bands are associated with resistance and constitute a new linkage group. A major resistance quantitative trait locus was found on this linkage group that accounted for 64.9% of the variation in resistance to powdery mildew. One of the probes developed in this study has the potential to assist in breeding for powdery mildew resistance in mungbean.

Key words: *Erysiphe polygoni* — *Vigna radiata* — AFLP — molecular markers — quantitative trait loci — RFLP

Mungbean, a diploid (2n = 22), primarily inbreeding species, is cultivated throughout tropical Asia where it is a major source of protein for humans (Shanmugasundaram and Kim 1996). India accounts for about 45% of the world production of mungbean, but Thailand and China are also important producers. Mungbean production is adversely affected by many factors such as low genetic potential of current varieties, environmental stresses, diseases and insect pests, and poor cultural practices (Poehlman 1991).

The main foliar diseases that affect production of mungbean are powdery mildew and *Cercospora* leafspot. Powdery mildew has a wider geographical range than *Cercospora* leafspot (Poehlman 1991) and also has a wide host range (Moseman 1966). Severe infection by powdery mildew occurs in cool, dry months when it can reduce the yield of mungbean by between 20% and 40% (Soria and Quebral 1973, AVRDC 1984). Mungbean incurs maximum damage when powdery mildew infects plants just before the flowering stage (Poehlman 1991). Inheritance of resistance to powdery mildew in mungbean has been reported as a single dominant gene in two breeding lines from India, Mung Ludhiana ML-3 and ML-5 (AVRDC 1979), two dominant genes in RUM breeding lines from India (Reddy et al. 1994) and quantitative genes (Yohe and Poehlman 1972, AVRDC 1981a, b, Young et al. 1993).

Powdery mildew is an obligate parasite, therefore, disease resistance can be identified when the disease is present in the field or greenhouse. Inoculation can be performed using infected plants and dusting spores onto the target plants, but environmental factors can influence the severity of the infection (Thakur and Agrawal 1995). Backcrossing is the main breeding method used to transfer resistance to the disease. Progress in breeding is hampered because direct selection after inoculation is difficult to assay on individual plants if infection is weak. In such circumstances, indirect selection using molecular markers linked to resistant genes should be an effective approach.

Many molecular markers have been developed and widely used in plant genetics and breeding. They can be used to study both qualitative traits (Fazio et al. 1999), and quantitative traits (Veldboom and Lee 1994). In addition, markers tightly linked to genes of interest are useful in breeding programmes since they can enable marker-assisted selection to overcome inaccuracy in field evaluation caused by environmental factors (Tanksley et al. 1989).

The present study was undertaken to determine whether the breeding line, VC1210A, resistant to races of powdery mildew in north east Thailand represents a new gene source for mungbean breeding. The potential of using this breeding line in mungbean improvement is discussed.

Materials and Methods

Plant materials: During field screening, four lines of mungbean, *Vigna radiata* (L.) Wilczek, from the Asian Vegetable Research and Development Centre (AVRDC), VC1210A, VC1482A, VC2273, and VC3528A, were found to be resistant to powdery mildew in northeast Thailand. These four lines were re-evaluated in two trials on October 20, 1999 and on December 20, 1999, respectively, at Surananee University of Technology (SUT), Nakhon Ratchasima, Thailand 15°N, 102°E. An F_2 mapping population of 96 individuals was developed from the cross between VC1210A (*V. radiata* var. *radiata*), the most resistant line to powdery mildew, and TC1966, *V. radiata* var. *sublobata* (Roxb.) Verdc., a wild relative of mungbean that is susceptible to powdery mildew.

Disease assay: The parents, F_1, and F_2 progenies were planted at SUT, Nakhon Ratchasima, Thailand on October 30, 2000. Susceptible varieties, CN36, M5-5, and TC1966, were planted around and between the plots as a source of powdery mildew inoculum. Individual plants of parents, F_1 and F_2 were scored for powdery mildew response at 55 days after germination using the scoring system described by

Young et al. (1993) as follows: 1, no visible mycelial growth; 2, 0–25% foliage area covered by fungus; 3, 26–50% foliage covered; 4, 51–75% foliage covered; and 5, 76–100% foliage covered.

Restriction fragment length polymorphism (RFLP) analysis: DNA was isolated from young leaves using a procedure based on the cetyltrimethylammonium bromide (CTAB) method (Draper and Scott 1988). DNA from P_1, P_2 and F_2 individuals was digested with *Bgl*II, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, and *Xba*I restriction enzymes under conditions recommended by the manufacturer (New England BioLabs, Bishops Stortford, UK). The digested DNA was separated on 1% agarose gel and transferred onto Hybond N+ membrane (Amersham Pharmacia Biotech, Cambridge, UK) by alkaline solution (0.4 M NaOH, 1.5 M NaCl) and fixed by UV Crosslinker at 60 mJ/cm^2 (Amersham Pharmacia Biotech). An RFLP linkage map for mungbean has been developed using the F_2 population of a cross between a cultivar VC3890 and the wild relative of mungbean TC1966 (Menancio-Hautea et al. 1993). Markers were chosen based on a revision of this linkage map available from Beangenes Database (http://beangenes.cws.ndsu.nodak.edu) for RFLP analysis. The mungbean, soybean and common bean probes were kindly supplied by the University of Minnesota, St. Paul, USA, Iowa State University, Ames, USA and CIAT, Cali, Colombia, respectively. DNA hybridization was carried out by ECL® direct nucleic acid labelling and detection systems according to the manufacturer's instructions (Amersham Pharmacia Biotech).

Amplified fragment length polymorphism (AFLP) analysis: AFLP analysis was performed following Vos et al. (1995). Total genomic DNA from P_1, P_2, bulked resistant (six plants with the lowest disease score) and bulked susceptible F_2 plants (six plants with the highest disease score) were digested with *Eco*RI and *Mse*I (New England BioLabs). Digested DNA fragments were ligated to *Eco*RI and *Mse*I adaptors with T4 DNA ligase (Roche Molecular Biochemicals, Mannheim, Germany). The restriction–ligation products were used as primary template DNA for the first polymerase chain reaction (PCR) step (preamplification) with E_{00} and M_{00} primers, which have no selective nucleotides at the 3′ end. The initial PCR products were used in the second PCR (selective PCR) with 100 primer combinations (10 *Eco*RI primers and 10 *Mse*I primers) with two and three selective nucleotides at the 3′ end. Denatured second PCR products were run on 6% denaturing polyacrylamide gel (19 : 1) and stained according to the Silver Sequence DNA sequencing system (Promega, Madison, WI, USA).

The bands of interest were excised from gels and squashed in microcentrifuge tubes containing distilled H_2O. The solution was used as the template DNA for PCR to re-amplify the fragments using the same PCR conditions and primer combinations as for producing polymorphic bands. The PCR products were cloned into a pGEM-T Easy plasmid vector according to the protocol of the manufacturer (Promega). The plasmid DNA was extracted from *Escherichia coli* by the small-scale preparation method described by Maniatis et al. (1989) and used as template DNA for probe preparation. The PCR products were checked for the correct insert size by electrophoresis.

Linkage and quantitative trait loci (QTL) analysis: The genotypic data from RFLP analysis were analysed with MAPMAKER/EXP version 3.0 (Lander et al. 1987, Lincoln et al. 1992b) to reconstruct the RFLP linkage map of mungbean. An LOD score of 3.0 and Haldane functions (Haldane and Waddington 1931) were used. The positions of cloned fragments from AFLP analysis were determined by pairwise command against all RFLP markers at a threshold of LOD 3.0. The position was then determined using the compare command.

The mapping of QTL was performed by the method of interval mapping (Lander and Botstein 1989) using MAPMAKER/QTL version 1.1 (Lincoln et al. 1992a) based on the phenotypic and linkage map data. Scan command at threshold of LOD 2.0 was used to identify putative QTL in the linkage map. By fixing the strongest QTL, others were searched. The Try command was used to evaluate the genetic models. One-way ANOVA was also used to determine the presence of QTL at different marker positions.

Results

Of the four lines evaluated for resistance to powdery mildew, VC1210A exhibited superior resistance. This line exhibited rapid necrosis around a focus of powdery mildew infection and was selected as the resistant parent in this study. One individual of accession TC1966 (P_2) was crossed onto one individual of VC1210A (P_1 — female parent). P_1, P_2, F_1 and F_2 individuals were planted together and evaluated for disease response. The average scores for powdery mildew resistance of P_1 (31 plants), P_2 (34), F_1 (7) and F_2 (96) were 1.45 ± 0.09, 3.91 ± 0.09, 2.00 ± 0.00 and 2.67 ± 0.10, respectively. Broad-sense heritability of this population was 85.5%. The P_1 and P_2 differed significantly in the disease score with P_1 showing a lower score. The score of the F_1 was not intermediate between P_1 and P_2, and was almost as low as P_1. Disease scores of F_2 plants from the VC1210A × TC1966 cross showed a continuous distribution (Fig. 1).

A QTL analysis based on a reconstructed linkage map with 42 mungbean, 29 soybean, and 27 common bean probes revealed only one marker, Bng065, located on linkage group 2 (Menancio-Hautea et al. 1993), which appeared to have a significant association with powdery mildew resistance ($P = 0.009$) by ANOVA (Table 1). However, in MAPMAKER/QTL analysis it fell below the LOD threshold of 2 (LOD = 1.79). This quantitative trait locus was tentatively named as *PMR2* (*p*owdery *m*ildew *r*esistance). The average score for powdery mildew resistance of the homologous allele from VC1210A and TC1966 was 2.1 and 2.88, respectively. The score of the heterozygous plants was 2.79, nearly as susceptible to powdery mildew as TC1966. The phenotypic variation explained by *PMR2* was 10.0%. Thus, the direction of gene action and extent of genetic variation explained by *PMR2* did not agree with the variation of the F_2 population. No other genomic region showed significant associations based on interval mapping. Since RFLP analysis did not provide markers closely linked to the resistance gene, a search for additional markers was undertaken using AFLP analysis.

The initial screening DNA from P_1, P_2, and bulked DNA of six resistant and six susceptible F_2 plants with 100 primer

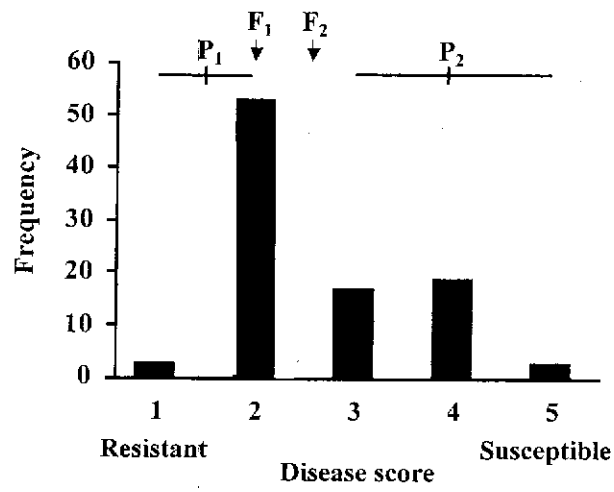

Fig. 1: Frequency distribution of the F_2 population from the VC1210A × TC1966 cross for powdery mildew disease scores. Parents, the F_1 and F_2 population were designated as P_1 (VC1210A), P_2 (TC1966), F_1 and F_2, respectively. Ranges for parents are indicated with bars

Table 1: Restriction fragment length polymorphism (RFLP) markers showing significant association with powder mildew (*Erysiphe polygoni*) resistance based on analysis of variance

RFLP markers	Marker mean[1]					
	P	R^2 (%)	A/A	A/B	B/B	Name of QTL[2]
Mac71a, Mac114	$P = 1.35 \times e^{-19}$	63.16	1.84	2.35	3.80	*PMR1*
Bng065	$P = 0.009$	10.00	2.10	2.79	2.88	*PMR2*

[1] A, VC1210A; B, TC1966.
[2] QTL, quantitative trait loci.

combinations showed 5725 and 5723 bands from bulked resistant and bulked susceptible F_2 DNA, respectively. Among them only four polymorphic bands revealed by four primer combinations, 71 (E-AGT/M-AGG), 86 (E-CAC/M-AGC), 95 (E-CGT/M-AGG) and 114 (E-CGT/M-AAC), corresponded to powdery mildew resistance. The approximate sizes of each band were 200 bp, 100 bp, 150 bp, and 180 bp, respectively. Three primer combinations, 71, 86, and 95, revealed bands in the resistant parent and resistant F_2 bulk but not in the susceptible parent and susceptible F_2 bulk. One primer combination, 114, showed bands in the susceptible parent and susceptible bulk and not the resistant parent or resistant bulk. Prior to cloning, polymorphism of these bands was reconfirmed using the four primer combinations to amplify the DNA from P_1, P_2, six individual resistant plants and six individual susceptible plants. The results revealed cosegregating patterns of the four bands with the bulked DNA from resistant and susceptible plants. The three bands in the resistant parent and one in the susceptible parent were cloned and used as probes in RFLP analysis. These probes were named after their primer combinations as Mac71, Mac86, Mac95 and Mac114 (Mac = *m*ungbean *A*FLP *c*lone). In the screening of parental polymorphism, Mac95 showed no polymorphism and no band was detected with Mac86. These probes possibly derived from byproducts that occurred during the cloning re-amplification step and have a similar size to the original polymorphic AFLP bands. Mac71 and Mac114 revealed multiple and single polymorphic bands, respectively. Four loci of Mac71 were designated as Mac71a, Mac71b, Mac71c and Mac71d. Two of these, Mac71a and Mac71b, were codominant loci and the other two, Mac71c (P_1 dominant) and Mac71d (P_2 dominant), were dominant loci. There was no association of these loci with RFLP marker loci on the current mungbean linkage map. However, linkage analysis revealed that these loci were tightly linked to each other. The best order of the Mac probes was determined using the compare command of MAPMAKER (Fig. 2). No recombination was found between Mac71a, Mac71d and Mac114. The sequences for Mac71 and Mac114 appear as accession numbers AB085909 and AB085910 in DDBJ, EMBR and GenBank.

The QTL interval mapping revealed a LOD score peak at this new linkage group. A major resistance quantitative trait locus was detected at marker Mac71a and Mac114. This was tentatively named as *PMR1*. It had a LOD score of 20.22, additive value (a) of 0.98, dominant value (d) of −0.50 and dominant to additive ratio of −0.51. This quantitative trait locus accounted for 64.9% of the variance for plant reaction to the disease. The main effect of *PMR1* on the mean of the disease score per plant was 1.854 (A/A, VC1210A), 2.334 (A/B) and 3.814 (B/B, TC1966). The score of the heterozygous plants was not intermediate between VC1210A and TC1966, and almost as low as VC1210A. Results from analysis of variance were similar to results found by MAPMAKER/QTL analysis (Table 1). No other quantitative trait locus was identified from rescanning by fixing this quantitative trait locus to the whole linkage map.

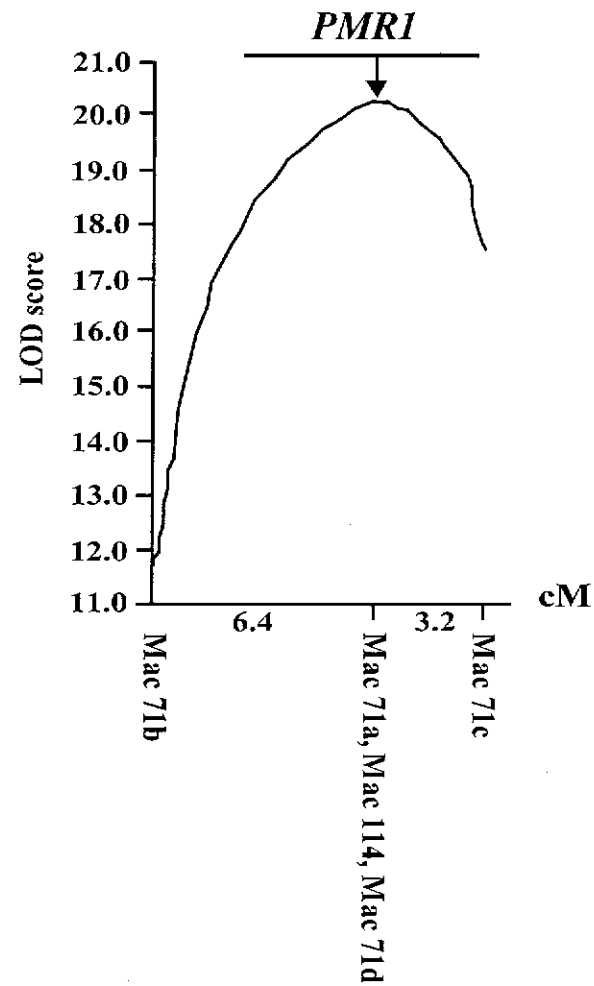

Fig. 2: A quantitative trait loci likelihood map for powdery mildew resistance in the new linkage group. The vertical axis indicates the LOD score based on MAPMAKER/QTL. The most likely position of quantitative trait locus peak is indicated with an arrow. The bar on the top of the quantitative trait locus map corresponds to a 2 LOD support interval from the peak LOD

Discussion

Resistance gene

Although several researchers have confirmed resistance to powdery mildew in mungbean genetic resources based on field studies, only a few resistance genes have been reported. Under controlled environmental conditions using field isolates of powdery mildew, two independent dominant genes *Pm*1 and

Pm2 have been identified in RUM lines in India (Reddy et al. 1994). Environmental factors have a large effect on the extent and severity of powdery mildew in the field (Thakur and Agrawal 1995). The disease score of F_2 progenies in the cross, VC1210A × TC1966, showed a continuous distribution but seemed somewhat skewed towards the resistant parent, suggesting resistance is conferred by a dominant gene with modifiers (Fig. 1). Since the field resistance in this cross appeared to be controlled by several genes it was treated as a quantitative trait.

The QTL analysis based on molecular markers can provide information on the inheritance of quantitative traits. Previously, three putative field resistance QTL for powdery mildew were identified in a cross VC3890 × TC1966 by QTL mapping (Young et al. 1993). These three markers, sgK472, mgM208 and mgQ039 are located on linkage groups 3, 7 and 8, respectively. In this study, RFLP markers located across all 13 linkage groups of the current mungbean linkage map were checked to identify the resistant QTL in VC1210A × TC1966. Although the involvement of a gene with a large effect was anticipated because of the high heritability, distribution pattern of the disease score of F_2 population and the low disease score of the F_1, only one quantitative trait locus with a small effect was detected on linkage group 2. The quantitative trait locus, named *PMR2*, showed significant association with powdery mildew resistance by ANOVA (Table 1). The effect of *PMR2* appears to enhance susceptibility to powdery mildew since the heterozygous condition has a similar value to the homozygous susceptible. Thus, *PMR2* may be one of the modifiers that reduce powdery mildew resistance. This contrasts with *PMR1*, which is discussed below (Table 1).

The haploid chromosome number of mungbean is 11, but the number of linkage groups is currently 13 and many markers are unlinked. The mungbean linkage map construction is incomplete, with many large gaps in the current 13 linkage groups. Consequently, the major locus associated with powdery mildew resistance in this cross is believed to be in a genomic region not yet covered by the current linkage map.

DNA pooling strategies based on phenotypic information can be reliably used to tag QTL with large effects (Wang and Paterson 1994). Using this strategy combined with AFLP analysis an attempt was made to find more markers linked to powdery mildew resistance. Among approximately 5700 fragments visualized by AFLP analysis, four polymorphic fragments were confirmed to cosegregate with powdery mildew resistance among individuals that constituted the pooled DNA. These four polymorphic fragments were cloned to develop probes named Mac71, Mac85, Mac95 and Mac114. Five loci identified by Mac71 and Mac114 were not associated with any linkage group on the mungbean linkage map and constitute a new linkage group (Fig. 2). Both MAPMAKER/QTL analysis and ANOVA indicated that the small genomic region contained a quantitative trait locus (*PMR1*) with large effect for the plant reaction to powdery mildew. The resistant parent allele at this position enhances powdery mildew resistance with a partially dominant effect, supporting the low disease score of the F_1. Since environmental effects and experimental error from field evaluation of the disease affected the phenotypic data, whether or not this putative quantitative trait locus is a single partial dominant gene requires additional analysis in their progeny. The results of this study strongly suggest that a major gene controls the resistance to powdery mildew in VC1210A.

Pathogen

VC1210A exhibits a clear resistance response to powdery mildew infection not seen in other resistant lines in north east Thailand. This resistance was characterized by localized necrosis in the host plant around the site of infection. Currently, there are a lack of systematic studies of *E. polygoni* in relation to mungbean. Research suggests the presence of different physiological races of powdery mildew that affect mungbean in India, Taiwan and the USA. Resistant parent VC1210A used in this study was found to be a useful genetic resource for resistance to both powdery mildew and *Cercospora* leafspot during tests in Taiwan (S. Shanmugasundaram, AVRDC, 2001 pers. comm.). The ancestral line, ML-3, was resistant to powdery mildew in Taiwan but susceptible in India (Reddy et al. 1987), suggesting that powdery mildew isolates at each location are different and probably represent races. The relationship between the putative QTL identified here and physiological races of powdery mildew in Thailand will be crucial in determining the value of this resistance source.

Marker-assisted selection

Marker-assisted selection may be used to shorten the time needed to fix resistance to powdery mildew in segregating populations. Markers tightly linked to genes of interest increase the efficiency and accuracy of selection (Sanchez et al. 2000). The putative quantitative trait locus for powdery mildew resistance reported here was positioned in the centre of a new, small linkage group. It remains to be determined whether the markers linked to the new putative quantitative trait locus will reveal polymorphism among breeding lines. However, the probe Mac71 has the potential to develop more PCR-based markers that can assist in breeding for powdery mildew resistance in mungbean because it is possible to detect recombination at three positions surrounding the quantitative trait locus at the same time. This study has shown the need for new markers to fill gaps in the current mungbean linkage map since no flanking markers around this linkage group are currently available. An improved high-density mungbean linkage map will enable substantial progress to be made in the application of marker-assisted selection in mungbean breeding.

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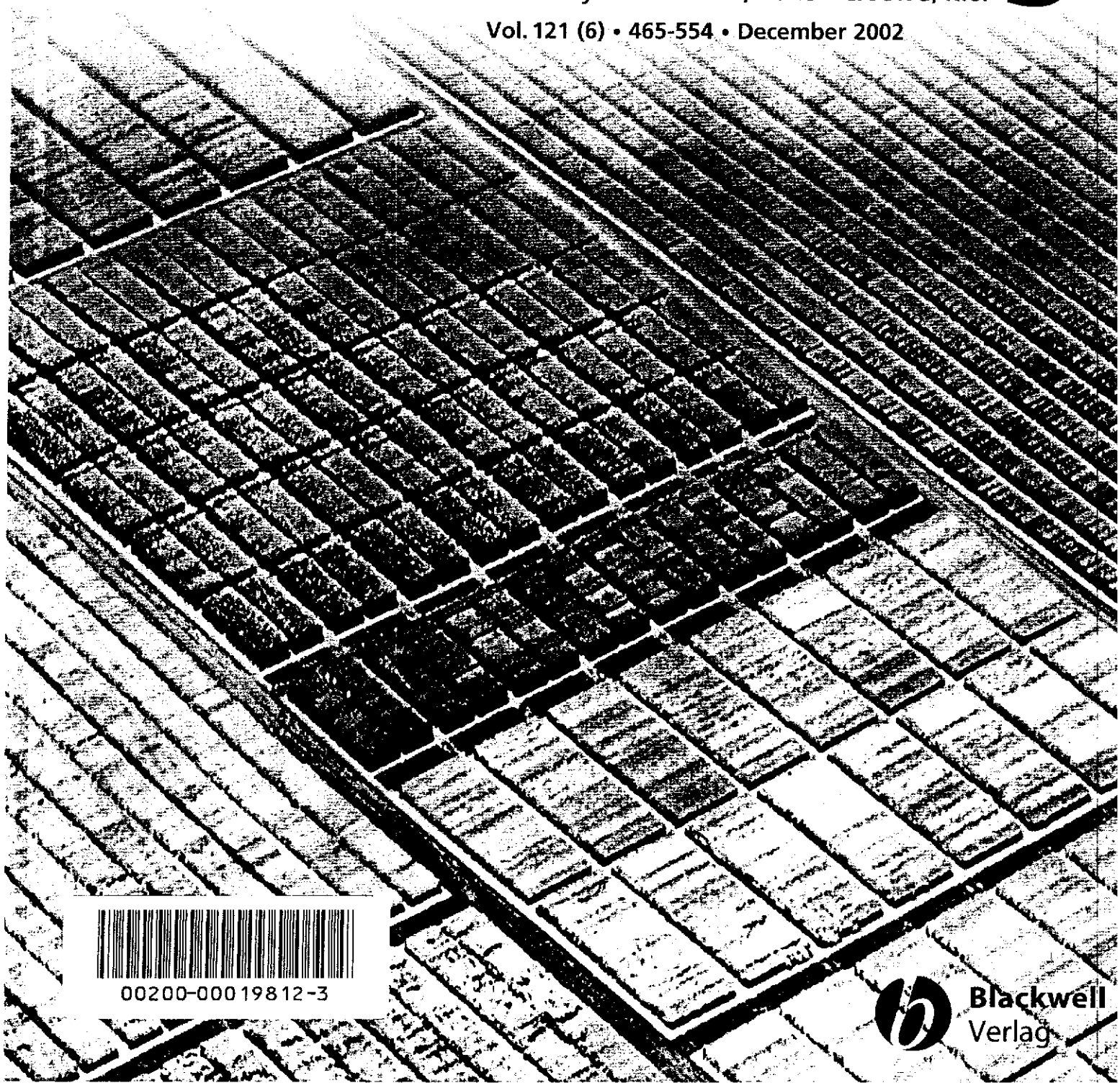

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