Ilmu Pertanian Vol. 9 No. 2: 52-58

MOLECULAR MAPPING OF SEED SIZE IN MUNGBEAN

PEMETAAN MOLEKULAR UKURAN BENIH KACANG HIJAU

Sholihin[1)]

ABSTRACT

The objective of this study was to map the quantitative trait loci (QTL) for seed size in mungbean. Phenotypic evaluation of recombinant inbred lines (RILs) for seed size was conducted in screen-house. Results showed that seed size of the RILs ranged from 1.3 – 5.2 g per 100 seeds. For AFLP analysis, the parental materials ('TC 1966' and 'Pagasa 7') were used. Linkage analysis of AFLP markers was done using MAPMAKER™ ver. 2.0 (for Macintosh®) (Lander et al., *1987). One-way analysis of variance was performed for individual markers to be identified as putatively associated with seed size loci with each marker considered as treatment or independent variable with two levels and the phenotype of each trait as the dependent variable.*

*Among 103 AFLP markers, 70 AFLP markers were mapped to 9 linkage groups, which covered a total map length of 655.5 cM with an average distance of 10.7 cM between markers. There were four AFLP markers (*aflp24, aflp33, aflp34, *and* aflp49*) putatively associated with seed size and if combined they would explain 31.2 % of the trait's variation.* Aflp33, aflp34, *and* aflp49 *were in linkage groups E, C, and D, respectively. The linkage group containing* aflp24 *is still unidentified. This map may facilitate gene tagging, QTL mapping, and further useful gene transfer for mungbean breeding.*

Keywords: *mungbean, molecular mapping, QTL, AFLP.*

INTISARI

Penelitian ini bertujuan untuk memetakan lokus-lokus sifat kuantitatif (*QTL*) bagi ukuran biji kacang hijau. Evaluasi fenotipik galur-galur inbred untuk ukuran biji dilakukan di rumah kawat. Hasil penelitian menunjukkan bahwa ukuran biji dari galur-galur inbred berkisar antara 1,3 – 5,2 g per 100 biji. Kedua tetua ('TC 1966' dan 'Pagasa 7') dianalisis dengan teknik AFLP. Analisis keterpautan penanda AFLP dilakukan dengan menggunakan MAPMAKER™ ver. 2.0 (untuk Macintosh®) (Lander *et al.*, 1987).

Di antara 103 penanda AFLP, 70 penanda AFLP dipetakan menjadi 9 kelompok yang bertautan, yang meliputi panjang peta total 655,5 cM dengan jarak rata-rata antarpenanda 10,7 cM. Ada empat penanda AFLP (*aflp24*, *aflp33*, *aflp34*, dan *aflp49*) yang berkaitan dengan ukuran biji dan jika dikombinasikan akan mampu menjelaskan 31,2% variasi untuk sifat ini. Penanda *aflp33*, *aflp34*, dan *aflp49* masing-masing bertaut di kelompok tautan E, C, dan D. Kelompok tautan tempat penanda *aflp24* berada belum teridentifikasi. Peta ini dapat dipakai sebagai alat pelacak gen, pemetaan QTL, dan selanjutnya berman-faat dalam pemindahan gen dalam pemuliaan kacang hijau.

Kata kunci: kacang hijau, pemetaan molekular, QTL, AFLP.

[1)] Peneliti BALITKABI Malang

INTRODUCTION

Molecular markers have been employed in crop improvement primarily to make breeding more efficient, and thus reduce cost and time required for the production of new varieties. Markers, on a genome wide basis, have also been used to characterize germplasm collections, to identify new source of genetic variation for faster progress in breeding. Markers associated with traits of agronomic interest, have also been used to provide an accurate picture of the breeding value of genotypes, by eliminating the confounding influences on the phenotype of other deleterious loci and the environment. Markers have also been used to map resistant genes, for use in negative marker-assisted selection of diseases resistance in the absence of the pathogen (elimination of susceptible genotypes). Markers are also the start-off point for the cloning of these resistant genes. Finally, associations between molecular markers and traits of agronomic interests, which are mostly quantitatively inherited, are being employed to elucidated the genetics of these traits.

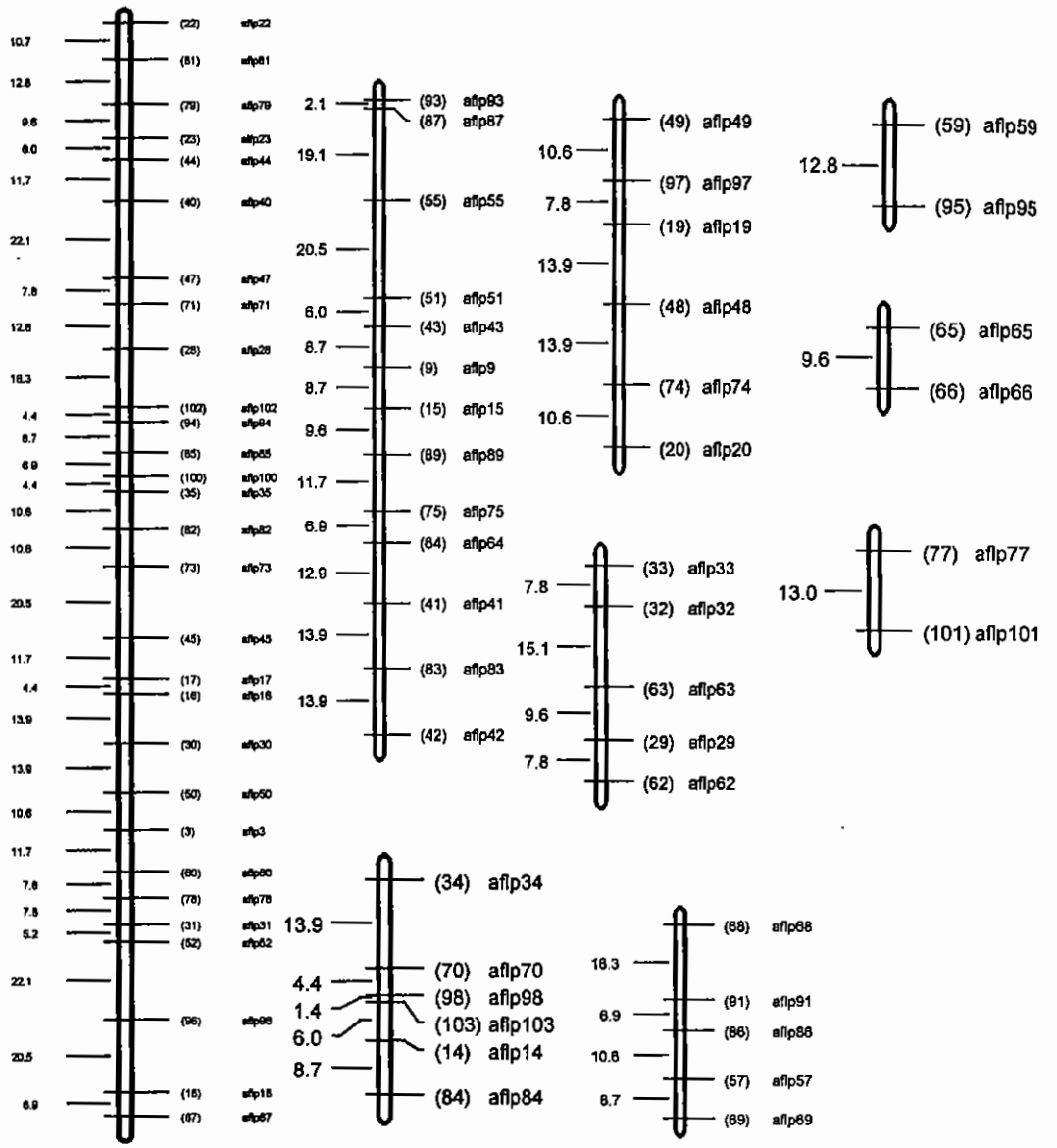
Recent development in molecular marker technologies, such as the Restriction Fragment Length Polymorphism (RFLP), the Randomly Amplified Polymorphic DNA (RAPD), and the Amplified Fragment Length Polymorphism (AFLP), provides opportunities for mapping the gene(s) governing the trait of interest. AFLP marker technology has several advantages compared with other molecular marker technologies as explained by Vos *et al.* (1995) and Thomas *et al.* (1995), namely: 1) An AFLP fingerprinting analysis yields on the average 6,000 to 8,000 bands per gel, with a resolution of up to 100 bands per reaction. By way of comparison, in a conventional RFLP analysis, only one restriction fragment, or perhaps a few at most, are visualized per single reaction; 2) The PCR-based AFLP reaction needs only small amounts of starting material and is less labor intensive, which translate into a much lower cost for a comparable amount of information; 3) AFLP requires no probe collection and can be used on any plant or animal species without prior knowledge of its DNA sequence or genome structure; 4) The AFLP technique is also distinctly superior to other PCR based fingerprinting techniques, such as RAPDs, in terms of resolution and reproducibility; 5) The advantage of AFLP over microsatellite lies in the high multiplex value of AFLP, i.e., a larger number of DNA markers can be diagnosed simultaneously, which in certain applications can be as high as 50 to 75 DNA markers per assay; 6) The large number of AFLP markers potentially available makes them an attractive choice for fine-scale mapping; and 7) AFLP is a powerful, reliable, stable, and rapid assay with potential application in genome mapping, DNA fingerprinting, and marker-assisted breeding. In addition, theory suggests that the entire genome (including both coding and non-coding regions) could be covered using AFLP markers because of the random amplification involved. The AFLP assay is sensitive to single base changes that cause the loss or gain of restriction sites, many kind of rearrangements, and changes in the DNA sequence recognized by the selective nucleotides. AFLP analysis is also less time-consuming than alternative strategies because large numbers of loci can be assessed in a single assay. AFLP has already been used for constructing linkage maps of various crops such as rice (Price *et al.*, 2000), maritime pine (*Pinus pinaster*) (Costa *et al.*, 2000), sugi (*Cryptomeria japonica*) (Nikaido *et al.*, 2000), *Allium* spp. (Van Heusden *et al.*, 2000), soybean (Hayes and Saghai Maroof, 2000), sugar beet (Setiawan *et al.*, 2000), carrot (Shim and Jorgensen, 2000), populus (Wu *et al.*, 2000), coconut (Herran *et al.*, 2000), and larch (*Larix sp.*) (Arcade *et al.*, 2000). The objective of this study was to map the quantitative trait loci (QTL) for seed size of mungbean.

MATERIALS AND METHODS

Molecular Marker Analysis

The $F_{9:6}$ recombinant inbred lines (RILs) were used in this study. The population was derived from the cross between 'Pagasa 7' and 'TC 1966'. The F_1 plants were selfed to produce the F_2 population. Individual F_2 plants were advanced by Del Rosario *et al.* (1995) through single seed descent (SSD) method.

DNA from the parental genotypes and seventy six of F_9 recombinant inbred lines were extracted using the procedure described by Tohme *et al.* (1996). AFLP analysis, which involved restriction



Unlinked markers

Aflp1	Aflp5	Aflp8	Aflp12	Aflp24	Aflp27	Aflp38	Aflp53	Aflp58	Aflp76
Aflp2	Aflp6	Aflp10	Aflp13	Aflp25	Aflp36	Aflp39	Aflp54	Aflp61	Aflp80
Aflp4	Aflp7	Aflp11	Aflp21	Aflp26	Aflp37	Aflp46	Aflp56	Aflp72	Aflp88
Aflp90	Aflp92	Aflp99							

Figure 1. Linkage map of mungbean composed of 103 AFLP markers based on LOD 5.5 and maximum recombination of 0.30

digestion of genomic DNA, ligation of adapter sequences, pre-amplification, and selective amplification, followed the procedure outlined in the User Manual (Life Technologies Catalogue No. 10717-015).

DNA fragments were detected by silver staining method. The detailed method was as follows. The gel was fixed for 20 min in solution containing 200 mℓ of glacial acetic acid and 1800 mℓ of ultra-pure water or double distilled water. The gel was rinsed 3 times (2 min. each) with ultra-pure water. The gel was put in staining solution (containing 2 g silver nitrate and 3 mℓ 37% formaldehyde and 2 ℓ ultra-pure water) for 30 min. The gel was dipped into the tray containing ultra-pure water (the time taken to do this should not exceed 10 sec.) The gel was agitated in solution containing 2 ℓ ultra-pure water, dissolved 60 g sodium carbonate, 400 µℓ 10mg/mℓ sodium thiosulfate, and 3 mℓ of 37% formaldehyde. The gel was fixed in solution (same solution which was used for fixing before) with shaking for 2–3 min. The gel was rinsed twice for 2 min each using ultra-pure water. The gel was dried by keeping it at room temperature.

Phenotypic Evaluation of seed size of Recombinant Inbred Lines

The total of seventy six recombinant inbred lines (RILs) were used in this study. The population was derived from the cross between 'Pagasa 7' (big seed size) and 'TC 1966' (small seed size). The F_1 plants were selfed to produce the F_2 population. Individual F_2 plants were advanced by Del Rosario *et al.* (1995) through single seed descent (SSD) method. The planting was conducted in June 2000 in the screen-house using a completely randomized design with two replications. Each replication was one pot with four plants.

RESULTS AND DISCUSSION

Construction of AFLP Map

The AFLP genetic map for each group was generated using MAPMAKER™ ver. 2.0 (for Macintosh®) (Lander *et al.*, 1987). The promising linkage groups were determined by using the Two Point/Group command using a minimum LOD score of 5.5 and a recombination fraction (θ value) maximum of 0.30. The order of the markers was determined using Multipoint/First order command. Linkage map was established using a Map command with Kosambi Mapping Function. Among 103 AFLP markers, 70 AFLP markers were mapped to 9 linkage groups, 2 large (A and B), 4 medium (C, D, E, and F), and 3 small (G, H, and I). The larger linkage group consisting of 13-29 markers had a total map length of 134.0-312.2 cM; the medium linkage group consisting of 5-6 markers, 34.3-56.9 cM; and the small linkage group consisting of 2 markers, 9.6-13.0 cM (Figure 1). The total map length covered by the 9 linkage groups was 655.5 cM with an average distance of 10.7 cM between markers which was around 42% of the total map length (1570 cM with an average distance of 9

Table 1. Putative markers associated with seed size using single marker analysis.

MARKER	Molecular Weight (bp)	R^2 (%)	ALLELIC MEAN (%)		PROBABILITY
			'TC 1966'	'PAGASA 7'	
aflp24	165	5.3	30.7	34.2	0.052*
aflp33	100	12.3	36.1	30.9	0.003*
aflp34	509	8.2	30.7	34.9	0.015
aflp49	103	5.4	31.3	34.6	0.049*

Note:* : Significant at 5% probability level.
** : Significant at 1% probability level.
R^2 = The proportion of the total phenotype variance among lines that could be explained by a marker.

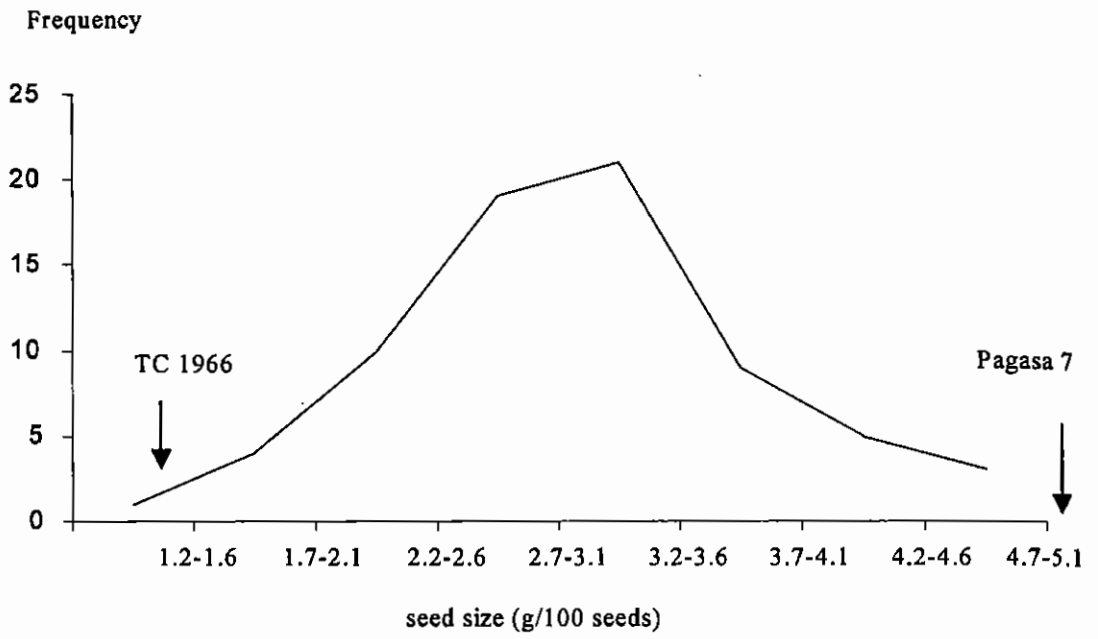


Figure 2. Frequency distribution of seed size of recombinant inbred lines of mungbean

cM between markers) for mungbean identified by RFLP-based markers (Menancio-Hautea *et al.*, 1992).

The map has still not coalesced into the 11 chromosomes of mungbean because of many minor linkage groups and unlinked markers, which suggests both that the AFLP markers identified are not randomly sampled throughout an entire chromosome (Panglia *et al.*, 1998) and the existence of hot spots of recombination (Kesseli *et al.*, 1994). To bridge these gaps, the segregation of terminal and unlinked markers in expanded backcross population can be analyzed and targeting by bulked segregant analysis markers to these regions can be done.

The current genetic map can be made denser by adding more markers genome wide. However, a more efficient approach is to develop bulked segregant analysis (BSA) to target markers to genomic region of particular interest. With the aid of BSA, we can identify and include additional molecular markers linked to the target genes. The BSA technique can be used to bridge the gaps existing in a map by linking co-segregated markers to form a larger genetic group.

Through the construction of a consensus map, this technique can also map genes that do not segregate in an original mapping population by combining information from multiple crosses.

AFLP markers associated with seed size

The phenotypic distribution of seed size is presented in Figure 2. The medium seed size (3.2-3.6g per 100 seeds) had the highest frequency, while the low and high seed size had low frequencies. These phenotypic variations were used for QTL analysis. One-way analysis of variance was performed for individual markers (103 AFLP markers) to be identified as putatively associated with seed size loci with each marker considered as treatment or independent variable with two levels and the phenotype of each trait as the dependent variable.

There were four AFLP markers (*aflp24*, *aflp33*, *aflp34*, and *aflp49* with respective

molecular weights of 165, 100, 509, and 103 bp) that were putatively associated with seed size loci and if combined could explain 31.2% of total variation in seed size (Table 1). *Aflp33*, *aflp34*, and *aflp49* were in linkage groups E, C, and D, respectively. The linkage group of *aflp24* is still unidentified (Figure 1). Each marker explained 5.4-12.3% of variation in seed size. Marker *aflp33* in linkage group E (Figure 1) explained the highest amount of variation in this trait (R^2 = 12.3%). 'TC1966' allelle at this locus was responsible for bigger seed size of the progeny. At three of the four marker loci, 'Pagasa7' alleles were associated with bigger seed size of the progeny, while the 'TC1966' allelles did so at the remaining locus (Table 1). A total 31.2% of genetic variation in the trait could be explained by the detected QTLs.

This ability of both parents to contribute towards the bigger seed size of the progeny explained the transgressive segregation observed among the progeny in this population.

Transgressive segregation in both directions was observed for seed size (Table 1), indicating that neither parent carried all positive or all negative allelles. Transgression also has been reported for several quantitative traits in other crops such as in soybean and rice (Ni *et al.*, 1998; Mian *et al.*, 1996; Tripathy *et al.*, 2000; Tang *et al.*, 2000, Price *et al.*, 2000).

A two-factors analysis of variance was used to detect epistatic interaction between all possible pairs of independent AFLP loci. The result showed that epistatic interactions were detected between two putative independent markers (*aflp24* and *aflp49*), while the other pairs (*aflp 24* and *aflp33*; *aflp24* and *aflp34*; *aflp33* and *aflp34*; *aflp34* and *aflp49*) showed no epistatic interactions.

CONCLUSION

Among 103 AFLP markers, 70 AFLP markers were mapped to 9 linkage groups, which covered a total map length of 655.5 cM with an average distance of 10.7 cM between markers. There was four AFLP markers (*aflp24*, *aflp33*, *aflp34*, and *aflp49*) putatively associated with seed size.

LITERATURE CITED

Arcade, A., F. Anselin, P. Faivre Rampant, M.C. Lesage, L.E. Paques, and D. Prat. 2000. Appication of AFLP, RAPD, and ISSR markers to genetic mapping of European and Japanese larch. *Theor. Appl. Genet.* 100: 299-307.

Costa, P., D. Pot, C. Dubos, J.M. Frigerio, C. Pionneau, C. Bodenes, E. Bertocchi, M.T. Cervera, D.L. Remington, and C. Plomion. 2000. A genetic map of maritime pine based on AFLP, RAPD, and protein markers. *Theor. Appl. Genet.* 100: 39-48.

Del Rosario, E. E., R.A. Hautea, C.B. Adalla, And M.L.J. Sison. 1995. Development of bruchid resistant mungbeans utilizing wild germplasm. (Abstr.) *Phil. J. Crop Sci.* 20:8.

Hayes, A.J. And M.A. Saghai Maroof. 2000. Targeted resistance gene mapping in soybean using modified AFLPs. *Theor Appl. Genet.* 100: 1279-1283.

Herran, A., L. Estioko, D. Becker, M.J.B. Rodriguez, W. Rohde, and E. Ritter. 2000. Linkage mapping and QTL analysis in coconut (*Cocos nucifera* L.). *Theor. Appl. Genet.* 101: 292-300.

Kesseli, R.V., I. Paran, and R.W. Michelmore. 1994. Analysis of a detailed genetic linkage map of *Lactuca sativa* (lettuce) constructed from RFLP and RAPD markers. *Genetics* 136: 1435-1446.

Lander, E. S., P. Green, J. Barahamson, A. Barlow, M. J. Daly, S.E. Lincoln, and L. Newbury. 1987. Mapmaker: An interactive computer package for constructing primary genetic linkage maps of experimental and natural population. *Genomics* 1:174-181.

Menancio-Hautea, D., L. Kumar, D. Danesh, and N. Young. 1992. A genome map for mungbean [*Vigna radiata* (L.) Wilczek] based on DNA genetic markers. *In*: S. O'Brien (ed). *Genetic Maps* 6th edition. Cold Spring Harbor Laboratory Press.

Mian, M. A. R., M. A. Bailey, D.A. Ashley, R. Wells, T. E. Carter, W. A. Parrott, and H. R. Boerma, 1996. Molecular marker associated with water use efficiency and leaf ash in soybean. *Crop Sci.* 36:1252-1257.

Nikaido, A.M., T. Ujino, H. Iwata, K. Yoshimura, H. Yoshimura, Y. Suyama, M. Murai, and K. Nagasaka. 2000. AFLP and caps linkage maps of *Cryptomeria japonica*. *Theor. Appl. Genet.* 100: 825-831.

Ni, J.J., P. Wu, D. Senadhira, and N. Huang. 1998. Mapping QTLs for phosphorous deficiency tolerance in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 97:1361-1369.

Panglia, G.P., A.M. Olivieri, and M. Morgante. 1998. Towards second-generation sts (sequence-tagged sites) linkage maps in conifers: a genetic map of norway spruce (*Picea abies* K). *Mol Gen Genet.* 258: 466-478.

Price, A.H., K.A. Steele, B.J. Moore, P.B. Barraclough, and L.J. Clarck. 2000. A combined RFLP and AFLP linkage map of upland rice (*Oryza sativa* L.) used to identify QTLs for root-penetration ability. *Theor. Appl. Genet.* 100: 49-56.

Qi, X., P. Stam, and P. Lindhout. 1998. Use of locus-specific AFLP markers to construct a high-density molecular map in barley. *Theor. Appl. Genet.* 96 : 376-384.

Shim, S.I. and R.B. Jorgensen. 2000. Genetic structure in cultivated and wild carrots (*Daucus carota* L.) revealed by AFLP analysis. *Theor. Appl. Genet.* 101:227-233.

Setiawan, A., G. Koch, S.R. Barnes, and C. Jung. 2000. Mapping quantitative trait loci (QTLs) for resistance to Cercospora leaf spot disease (*Cercospora beticola* Sacc.) in sugar beet (*Beta vulgaris* L.). *Theor. Appl. Genet.* 100: 1176-1182.

Tohme, J., D. O. Gonzales, S. Beebe, and M. C. Duque. 1996. AFLP analysis of gene pools of a wild bean core collection in plant genetic resources. *Crop Sci.* 36:1375-1384.

Thomas, C.M., P. Vos, M. Zabeau, D.A. Jones, K.A. Norcott, B.P. Chadwick, and J.D.G. Jones. 1995. Identification of amplified restriction fragment polymorphism (AFLP) markers tightly linked to tomato cf-9 gene for resistance to *Cladosporium fulvum*. *Plant J.* 8:785-794.

Tripathy, J.N., J. Zhang, S. Robin, Th. T. Nguyen, and H.T. Nguyen. 2000. QTLs for cell-membrane stability mapped in rice (*Oryza sativa* L.) under drought stress. *Theor. Appl. Genet.* 100: 1197-1202.

Tang, D., W. Wu, W. Li, H. Lu, and A.J. Worland. 2000. Mapping of QTLs conferring resistance to bacterial leaf streak in rice. *Theor. Appl. Genet.* 101: 286-291.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Lee, M, Hornes, A. Fritjers, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Res.* 23:4407-4414.

Van Heusden, A.W., J.W. van Ooujen, R. Vrielink-Van Ginkel, W.H.J. Verbeek, W.A. Wietsma, and C. Kik. 2000. A genetic map of an interspecific cross in *Allium* based on Amplified Fragment Length Polymorphism (AFLP) markers. *Theor. Appl. Genet.* 100: 118-126.

Wu, R.L., Y.F. Han, J.J. Hu, J.J. Fang, L.Li, M.L. Li, And B.Zeng. 2000. An integrated genetic map of Populus deltoids based on Amplified Fragment Length Polymorphism. *Theor. Appl. Genet.* 100: 1249-1256.