dominant seed which starves the rest of the seeds leading to their death. It is interesting to note that a similar mechanism operates in the widely-separated genera, *Dalbergia sissoo*[26,27] suggesting a possible common mechanism of seed abortion across plant systems.

1. Arathi, H. S., M Sc thesis, University of Agricultural Sciences, Bangalore, 1990.
2. Arathi, H. S., Ganeshaiah, K. N., Uma Shaanker, R. and Hegde, S. G., *Int. J. Plant Sci*., 1996, **157**, 49–52.
3. Uma Shaanker, R. and Ganeshaiah, K. N., *Evol. Trends Plants*, 1988, **2**, 91–98.
4. Ganeshaiah, K. N. and Uma Shaanker, R., *Oecologia*, 1988, **77**, 135–139.
5. Uma Shaanker, R., Ganeshaiah, K. N. and Bawa, K. S., *Annu. Rev. Ecol. Syst*., 1988, **19**, 177–205.
6. Uma Shaanker, R. and Ganeshaiah, K. N., *Evol. Trends Plants*, 1989, **3**, 59–64.
7. Ganeshaiah, K. N. and Uma Shaanker, R., *Oikos*, 1991, **60**, 3–6.
8. Hegde, S. G., Uma Shaanker, R. and Ganeshaiah, K. N., *Oikos*, 1991, **62**, 100–101.
9. Ganeshaiah, K. N., Uma Shaanker, R. and Hegde, S. G., *J. Genet*., 1991, **70**, 103–127.
10. Hegde, S. G., Uma Shaanker, R. and Ganeshaiah, K. N., *Evol. Trends Plants*, 1991, **5**, 131–135.
11. Lokesha, R., Hegde, S. G., Uma Shaanker, R. and Ganeshaiah, K. N., *Am. Nat*., 1992, **140**, 520–525.
12. Ganeshaiah, K. N. and Uma Shaanker, R., *Physiol. Plant*, 1994, **91**, 81–89.
13. Bawa, K. S., Hegde, S. G., Ganeshaiah, K. N. and Uma Shaanker, R., *Nature*, 1989, **342**, 625–626.
14. Bawa, K. S. and Webb, C. J., *Am. J. Bot*., 1984, **71**, 736–751.
15. Wiens, D., *Oecologia*, 1984, **64**, 47–53.
16. Lee, T. D. and Bazzazz, F. A., *Oecologia*, 1986, **68**, 439–465.
17. Stephenson, A. G., *Annu. Rev. Ecol. Syst*., 1981, **12**, 253–279.
18. Willson, M. F. and Schemske, D. W., *Bull. Torrey Bot. Club*, 1980, **107**, 401–408.
19. Zimmerman, M. and Pyke, G. H., *Am. Nat*., 1988, **131**, 723–738.
20. Bierzychudek, P., *Am. Nat*., 1981, **117**, 837–840.
21. Gross, R. S. and Werner, P. A., *Ecol. Monogr*., 1983, **53**, 95–117.
22. Schemske, D. W. and Paulter, L. P., *Oecologia*, 1984, **62**, 31–36.
23. Snow, A. A., *Oecologia*, 1982, **55**, 231–237.
24. Wiens, D., Calvin, C. I., Wilson, C. A., Davern, C. I., Frank, D. and Seavey, S. R., *Oecologia*, 1987, **71**, 501–509.
25. Krishnamurthy, K. S., Ph D thesis, University of Agricultural Sciences, Bangalore, 1995.
26. Mohan Raju, B., M Sc (Agriculture) thesis, University of Agricultural Sciences, Bangalore, 1993.
27. Mohan Raju, B., Uma Shaanker, R. and Ganeshaiah, K. N., *Sexual Plant Reprod*., 1996, **9**, 273–278.
28. Mohan Raju, B., Uma Shaanker, R. and Ganeshaiah, K. N., *Malaysian For*., 1995, **58**, 97–100.
29. Mohan Raju, B., Uma Shaanker, R. and Ganeshaiah, K. N., *Curr. Sci*., 1995, **68**, 1114–1118.
30. Normanly, J., Cohen, J. D. and Fink, G. R., *Proc. Natl. Acad. Sci. USA*, 1993, **90**, 10355–10359.
31. Naren, A., Prasad, T. G., Udaya Kumar, M. and Sashidhar, V. R., *Indian J. Exp. Biol*., 1996, **34**, 257–261.
32. Ganeshaiah, K. N. and Uma Shaanker, R., *Curr. Sci*., 1994, **62**, 359–365.
33. Ganeshaiah, K. N., Uma Shaanker, R. and Krishnamurthy, K. S., *Int. J. Plant Sci*., 1996, **156**, 650–657.
34. Ganeshaiah, K. N., Vasudeva, R. and Uma Shaanker, R., *Ann. Bot*., 1996, **76**, 71–77.
35. Bangerth, F., *Physiol. Plant*., 1989, **76**, 608–614.
36. Jacobs, M. and Gilbert, S. F., *Science*, 1983, **220**, 1297–1300.
37. Burg, S. P. and Burg, E. A., *Plant Physiol*., 1968, **43**, 1069–1074.
38. Russel, W. and Thimann, K. V., International Conference on Plant Growth Substances, Calgary, Canada, 1988, Abstr No. 227.
39. Kondo, S., *Bull. Akita Fruit Tree Expt. Station*, 1990, **21**, 56.
40. Engvild, K. C., *Physiol. Plant*, 1989, **77**, 282–285.

ACKNOWLEDGEMENT. This work was supported by grants from the Department of Science and Technology, Government of India, New Delhi.

Received 22 April 1997; revised accepted 3 October 1997

Application of marker-assisted selection in rice for bacterial blight resistance gene, *Xa21*

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Polymerase chain reaction (PCR)-based marker-assisted selection for a disease resistance gene was employed in a rice improvement programme. PCR amplification using sequence-tagged site (STS) primers specific to bacterial blight resistance gene *Xa21*-linked marker resulted in amplified products of the expected molecular weights for each of the three genotypic classes, homozygous resistant, homozygous susceptible and heterozygous and facilitated selection of plants carrying the desired gene for further advancing the breeding material.

BACTERIAL blight, caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is the most important bacterial disease of rice and is a serious constraint for rice production in the irrigated and rainfed lowland environments. No chemical control measure is presently available for controlling this disease. Growing resistant varieties, the only option available for minimizing the losses caused by this disease, is not only economical, but is also an environment-friendly method. However, resistant varieties, particularly those carrying single gene for resistance, often break down (become susceptible). Pyramiding resistance

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genes in a cultivar offer promise for broad spectrum and long-lasting resistance, presumably due to quantitative complementation[1]. However, in such breeding efforts, phenotypic selection of individual plants with resistance is difficult to do with specific pathogen isolates by artificial inoculations. Under these circumstances, identification of plants with multiple genes can be carried out using DNA markers. Knowledge on polymorphism among the parents used is a pre-requisite for employing marker-assisted selection in a breeding programme.

The gene, *Xa21* introgressed from *Oryza longistaminata* into *O. sativa* (IR24 background) located in chromosome 11 confers broad spectrum resistance to most isolates of *Xoo*, making it a valuable gene for plant breeding[2,3]. Ronald *et al.*[4] identified a random primer (RAPD248) specific to *Xa21*-linked marker which amplifies two polymorphic bands, one from the susceptible (IR24) and one from the resistant isoline 1188 (IRBB21), indicating an insertion/deletion type polymorphism, cloned the amplified product and demonstrated its use as RFLP marker. From the sequence of this genomic clone (RAPD248), two primers, PB7 (5′-AGA CGC GGA AGG GTG GTT CCC GGA-3′) and PB8 (5′-AGA CGC GGT AAT CGA AAG ATG AAA-3′) were designed [sequence-tagged site (STS) markers][5]. This provides an effective, fast and easy tool for undertaking PCR-based marker-assisted selection of bacterial blight resistant plants in a segregating population. In this study, we focus on the use of these sequence-tagged site (STS) markers for analysis of parental polymorphism and selection of individual plants carrying homozygous resistance allele (*Xa21*) among a segregating F_2 population in a marker-assisted breeding programme aimed at improving one of the most popular rice varieties in the favourable lowlands of eastern India, Swarna for bacterial blight resistance.

The segregating population was derived from a double cross between Swarna (susceptible to bacterial blight used as recurrent parent)/IRBB21, (carrying *Xa21* allele and is a near isogenic line of IR24 which is susceptible) and Swarna/*O. minuta* derivative line WHD IS 78-1-5 (resistant plant carrying an undefined gene). Phenotyping of the plants was performed at the Central Rice Research Institute in India and the genotyping was undertaken at the International Rice Research Institute in the Philippines. Its usage for marker-assisted selection was confirmed at the Central Rice Research Institute in India.

DNA from Swarna, IRBB 21, IR 24 and 114 F_2 plants was extracted as per the rapid micro-level extraction procedure described by Zheng *et al.*[6]. Polymerase chain reaction (PCR) amplification was performed in a 20-μl reaction mixture containing 0.5 μM each of the two opposing primers, 50 ng of genomic DNA, 185 μM each of four dNTPs, approximately 2.5 units of *Taq* polymerase (Boehringer Mannheim) in PCR Buffer no. 9 (10 mM Tris, pH 9.2, 25 mM KCl; 1.5 mM $MgCl_2$; 15 mM $(NH_4)_2SO_4$). The reaction mixture was overlaid with one drop of mineral oil, initially denatured for 5 min at 94°C, and then subjected to 30 cycles of 30 s denaturation at 94°C, 30 s annealing at 55°C and 60 s extension at 72°C and a final extension of 5 min at 72°C using a Thermal Cycler (PTC-100) manufactured by MJ Research (Watertown, MA, USA).

Figure 1. PCR amplification pattern of DNA from parents and F_2 population derived from cross, Swarna/IRBB21\\Swarna/*O. minuta* derivative line WHD IS 78-1-5. Primers PB7 and PB8 were used in the reactions. Lanes 1 and 16 contain molecular weight marker (1-kb ladder, BRL Life Technologies, Inc.), lanes 2–15 contain amplification products from susceptible parent, Swarna (lane 2); IR24 (susceptible, lane 3); resistant parent, IRBB21 (lane 4) and F_2 population: homozygous susceptible individuals (lanes 5–7), heterozygous individuals (lanes 8–13) and homozygous resistant individuals (lanes 14, 15).

PCR products were resolved on gels composed of 0.5 × TBE and 1.5% agarose at 100 V for 4 h. The gels were stained with ethidium bromide, and then photographed using Polaroid Type 665 film.

PCR amplified products of the expected molecular weights for both bacterial blight-resistant and susceptible lines for each of the three genotype classes generated in this study (Figure 1). Clear polymorphism between susceptible and resistant parents used in this study was evident. The PCR products amplified from bacterial blight resistant (IRBB21) and susceptible (IR24) lines were approximately 900 and 700 bp, respectively[5]. However, in case of the susceptible parent, Swarna used in this study, the PCR product amplified was around 800 bp which is clearly distinct from that of the resistant parent (IRBB21). The PCR product amplified in other susceptible lowland rices, Tulasi and Sabita was also around 800 bp (data not presented), suggesting that there are multiple alleles governing susceptibility. In the segregating population, based on the banding patterns, plants were classified into homozygous susceptible, homozygous resistant and heterozygous. However, among the heterozygotes, those with Swarna

(recurrent parent used in this study) background and those with IR24 background could also be distinguished. Nevertheless, of the 114 F_2 plants screened, 27 were found to be homozygous resistant (RR), 55 heterozygous (Rr) and 32 homozygous susceptible (rr) following the normal Mendelian ratio of 1:2:1. The selected homozygous resistant plants will be used for further advancing the breeding material.

1. Yoshimura, S., Yoshimura, A., Iwata, N., McCouch, S. R., Abenes, M. L., Baraoidan, M. R., Mew, T. W. and Nelson, R., *Mol. Breed.*, 1995. **1**, 375–387.
2. Khush, G. S., Mackill, D. J. and Sidhu, G. S., in Proceedings of the International Workshop on Bacterial Blight of Rice, International Rice Research Institute, Los Banos, Philippines, 1989, pp. 207–217.
3. Ikeda, R., Khush, G. S. and Tabien, R. E., *Jpn. J. Breed.*, 1990, **40**, 280–281.
4. Ronald, P. C., Albano, B., Tabien, R., Abenes, L., Wu, K-S., McCouch, S. and, Tanksley, S. D., *Mol. Genet.*, 1992, **236**, 113–120.
5. Chunwongse, J., Martin, G. B. and Tanksley, S. D., *Theor. Appl. Genet.*, 1993, **86**, 694–698.
6. Zheng, K., Huang, N., Bennett, J. and Khush, G. S., *IRRI Discussion Paper Series No. 12*, 1995, p. 24.

ACKNOWLEDGEMENTS. The study was funded by a collaborative project between IRRI and CRRI (ICAR) by a grant from Asian Development Bank to IRRI (RETA 5667) for the Asian Rice Biotechnology Network. We thank Dr. K. C. Mathur, Director, CRRI for his encouragement and interest.

Received 4 June 1997; revised accepted 30 September 1997

Cheliped laterality in freshwater prawn, *Macrobrachium nobilii* (Henderson and Matthai, 1910)

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A field study was undertaken to find out the cheliped laterality in the freshwater prawn *Macrobrachium nobilii* which inhabit the river Cauvery. Among 664 *M. nobilii* collected from three sites over a period of three months, 553 possess differentiated chelipeds with major and minor chela distributed on right or left side. In overall population and in site two, male-handedness deviated from the expected 1:1 ratio, when compared with female.

CHELIPED, the multi-functional organ in decapod crustaceans has drawn much attention due to its versatile use in foraging[1], agonistic and aggressive interactions at inter- and intra-species level[2–4], and mate selection[5,6], handling and guarding[7]. Simultaneously the morphometric features of cheliped also have gained equal importance. Based on their morphology, the chelipeds are classified into major (crusher with molariform teeth) and minor (cutter with pointed teeth laden with abundant setae)[8]. Sexual dimorphism in cheliped size is also recorded in some species[9].

In decapods, handedness or laterality is the possession of dimorphic claws with one larger than the other[10]. The animal is called right or left-handed, based on the location of major chela on the corresponding side. Among the right and left-handed animals, the former wields more functional advantages like breaking dextral gastropod shell and resources utilization[10,11]. Even distribution of handedness and/or its deviation from right or left has been reported between and within species. For example in *Ocypode quadrata*, half of the sampled population are right-handed[12] but in *Ocypode gudichaudii*, the occurrence of major chela is essentially skewed to the left[13]. When the major chela is automized, minor chela becomes the major and the regenerated one becomes the minor leading to reversal of handedness[14]. This had led to a controversy over the use of chela as a dependable taxonomic character.

Studies on other decapods like lobster, *Homarus americanus*[15,16], stomatopod, *Gonodactylus falcatus*[17], crayfish, *Orconectes rusticus*[3], and freshwater prawn. *Macrobrachium rosenbergii*[2] have focused on the functional advantages of chelipeds in aggressive interactions and displays. In *Macrobrachium* spp, there are a few studies, which report only on the size of the cheliped and not on handedness[18,19]. *Macrobrachium nobilii*, an endemic prawn, inhabiting river Cauvery is called as stone prawn due its affinity to seek shelter under boulders. It is aggressive and at a given time 16.4% of the population suffers autotomy[20]. This paper reports on the handedness in *Macrobrachium nobilii*.

M. nobilii was sampled in three different sites – Jedarpalayam (a minor check dam), Upper Anicut (a major reservoir) in river Cauvery and Check Post point in Colleron river (a tributary of Cauvery) near Tiruchirapalli (10.50"N; 78.43"E) during May to July 1997. Each time about 75 ± 5 (Mean ± SD) prawns were collected at random by using hand net with equal amount of fishing effect from 0600 to 0800 hours and sorted out into juveniles (30 ± 5 mm), males (45 ± 5 mm) and females (40 ± 5 mm). Juveniles were not considered for the study since handedness between chelae is discernible only on attaining sexual maturity[13]. The occlusive surface of major chela contains abundant 'molariform' teeth but minor chela possesses pointed teeth laden with numerous setae (Figure 1). Based on this, the data on handedness recorded separately for males and females were tested through χ^2 test[21].