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LINKAGES BETWEEN RESTRICTION FRAGMENT LENGTH, ISOZYME AND MORPHOLOGICAL MARKERS IN RICE

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ABSTRACT

A genetic linkage map of rice comprising 130 centimorgans (cM) was constructed from ten restriction fragment length, six isozyme and one morphological markers in F_2 population of a rice cross (IR 36 X Ma Hae). The DNA probes were labelled through nonradioactive dUTP. The relative distance between two markers varied from 5.7 cM to 24.1 cM. The morphological marker (Xa-4A : bacterial leaf blight resistance) could be placed between restriction fragment length polymorphic (RFLP) probes RG 303 and and RZ 536 at the distance of 18.9 cM and 16.1 cM, respectively. The RFLP marker RG 167 and isozyme marker Pgd-1 were located at the same position, i.e. 5.7 map units away from RG 1094. None of the isozyme marker could be detected to be closely linked to morphological marker under study.

Key words: RFLP, isozyme, morphological marker, rice, integration.

Knowledge of detailed genetic architecture is essential for applying new tools of biotechnology in crop improvement programmes. All the three kinds of markers viz., morphological, isozyme and DNA (RFLP) are extensively being used for mapping of rice genome. It is, however, often difficult to identify a large number of morphological and isozyme markers segregating in a cross of interest. Therefore, RFLP markers are being preferred because of their added advantages of developmental stability and availability in enumerable numbers. The linkage between RFLP markers and agronomic characters can increase the selection efficiency through indirect selection based on the markers. The RFLP, isozyme and morphological markers have already been used for mapping of quantitative trait loci and monitoring of selection responses in maize and tomato [1-4]. In this study attempt has been made for mapping of rice chromosome with the help of isozyme, morphological and RFLP markers.

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MATERIALS AND METHODS

The experimental material comprised F_2 population of a rice cross IR 36 X Ma Hae, six isozyme markers and ten RFLP probes. Observations based on 72 F_2 plants were used for linkage relationships between these markers. Bacterial leaf blight resistance was considered as morphological marker. IR36 was resistant to bacterial leaf blight while Ma Hae was susceptible. The F_2 population was grown under greenhouse condition to create artificial epiphytotic condition following the technique developed at the IRRI. Individual plants were scored for disease reaction. All the six isozyme loci (Adh-1, Pgd-1, Fdp-1, Tpi-1, Sud-3 and G6pd-1) and ten RFLP markers (CDO 127, CDO 520, CDO 534, RG 2, RG 118, RG 167, RG 303, RG 1094, RZ 536 and RZ 797) were showing polymorphism between two cultivars IR 36 and Ma Hae, hence, were used for linkage studies as per programme suggested [5]. Isozyme analysis was done as per procedure developed by Glazmann et al. [6]. DNA isolated from the fresh leaves of each individual F_2 plant was digested with six restriction enzymes (Bam H1, Dra 1, EcoR V, Hind III, Pst 1, Xba 1) as per standard procedure. The southern blots were hybridized using nonradioactive-digoxigenin-deoxyuridine triphosphate (dUTP) labelled DNA probes as per protocol suggested [7].

RESULTS AND DISCUSSIONS

Relative positions of different isozyme, RFLP and morphological markers is given in Fig. 1. Results indicated that all the isozyme markers except G6pd-1 were belonging to one linkage group. These markers were placed on chromosome 11 in earlier study [8]. Likewise all the RFLP markers could be placed on same linkage group suggesting that they too belong to chromosome 11. The results on RFLP markers are in conformity with the linkage map developed at Cornell University [9, 10]. Further, the morphological marker, Xa-4A was also located on same linkage group. Therefore, these markers can successfully be used for marker aided selection for the characters of agronomic importance located on this linkage group. Two RFLP probes viz., RG 303 and RZ 536 were found to be closely linked with Xa-4A as

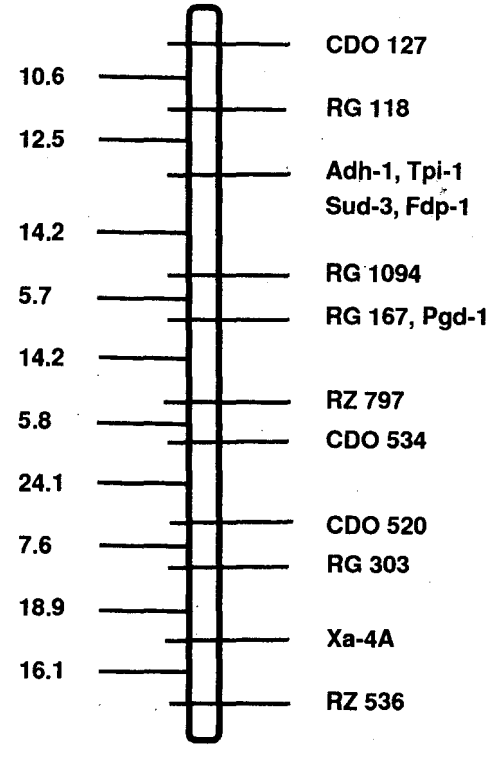

Fig. 1. Mapping of RFLP and isozyme markers in rice.

compared to other markers. They were located at 18.9 cM and 16.1 cM, respectively from Xa-4A. However, there are possibilities to investigate some more markers between RG 303 and RZ 536 so as to obtain more closely linked marker with Xa-4A. None of the isozyme marker was found to be closely linked with Xa-4A. These markers can be used for linkage analysis of other markers situated on chromosome 11. Regarding the relative distance of different markers, the least gap was found between RFLP markers RZ 797 and CDO 534 (5.8 cM) as compared to CDO 534 and CDO 520 where maximum distance (24.1 cM) was noticed. Four isozyme markers (Adh-1, Tpi- 3, Sud-3, Fdp-1) were located 12.5 cM apart from RFLP probe RG 1094 and 14.2 cM from RG 1094. The another isozyme loci Pgd-1 could be placed along with RFLP probe RG 167 which were showing distances of 5.7 cM and 14.2 cM from RG 1094 and RZ 797, respectively.

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