Construction of a High-density Linkage Map in *Brassica juncea*

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ABSTRACT

Brassica juncea (Indian mustard) is a major oilseed crop of India and is grown in around six million hectares of land during the winter season. The conventional breeding methods through utilization of adapted pool of elite germplasm have contributed only marginally towards increase in productivity. To enhance productivity in a significant manner, it is necessary to utilize new sources of desirable genes present in exotic *B. juncea* lines from Canada, eastern Europe and Australia as these constitute a rich source of agronomically important traits such as quality, seed coat colour, resistance to *Albugo* and many yield component traits. In order to transfer these traits in a precise manner through marker assisted breeding, a high-density genetic linkage map was constructed using a cross between most extensively grown Indian cultivar Varuna and a canola quality exotic line Heera. More than 1000 AFLP and RFLP markers have been mapped using a F1-derived doubled haploid (DH) population of 123 individuals. The segregation of each marker and linkage analysis was performed using the program JoinMap version 2.0. The mapped-markers were aligned in 18 linkage groups, which is the haploid chromosome number of the species, at LOD values ranging from 5-8. Further work is being conducted to put more RFLP and microsatellite markers on the map.

Keyword: AFLP – RFLP – *Brassica juncea* – Linkage map

Introduction

Productivity in Indian mustard (*Brassica juncea*) could be substantially increased by use of new sources of variations that exist in exotic germplasm. The exotic *B. juncea* lines from eastern Europe and Australia are the rich source of agronomically important traits such as quality, disease resistance and many yield components but are ill adapted to Indian agro-climatic conditions. Due to the poor adaptability, these germplasm have not been successfully utilized in conventional recombination breeding programmes in India. Recent advances in molecular techniques particularly the DNA markers have provided new tools that can increase the efficiency of plant breeding methods through precise marker assisted transfer of qualitative and quantitative traits from unadapted to adapted cultivars (Khush, 2002). In order to transfer the desirable traits from exotic germplasm to cultivated Indian cultivars, a high-density linkage map in *B. juncea* was made containing 996 AFLP and 33 RFLP markers using a F1-derived doubled haploid (DH) population of 123 individuals from a cross between a widely adapted Indian cultivar, Varuna and a canola quality exotic line, Heera (Pradhan et al., 2003). We also identified a set of 26 AFLP primers covering 96% of the mapped *B. juncea* genome that could be used for whole genome selection in backcross breeding programmes in *B. juncea*. We report here the distribution of these selected AFLP markers on the mapped *B. juncea* genome.

Materials and Methods

The two parental lines used for construction of linkage map are highly divergent and belong to two distinct gene pools – Varuna to Indian and Heera to eastern European pool (Srivastava et al.

2001). DNA extraction, AFLP and RFLP analysis from parents and 123 DH lines were carried out as described previously by Pradhan et al. (2003). Segregation and linkage analyses were performed using the program JoinMap version 2.0 (Stam and Van Ooijen, 1996). Markers were grouped at a LOD value of 6.0 and were mapped at maximum recombination fraction of 0.45.

Results and Discussion

A total of 1029 markers consisting of 996 AFLPs and 33 RFLPs were mapped of which 16.5% showed distortion in segregation. The total dataset consisted of 51% Varuna and 49% Heera specific alleles. When markers were grouped at LOD values ranging from 4 – 8, it was observed that all the markers were grouped into 18 linkage groups (LG) at LOD values 5 – 8. Hence, the markers were mapped to 18 LGs covering a total length of 1629 cM with an average interval size of 3.5 cM. AFLP markers generated by Eco RI were more clustered, whereas Pst I markers showed more extensive distribution. A set of 26 AFLP primers consisting of 9 Eco RI/ Mse I, 6 Eco RI/Taq I, 6 Pst I/Mse I and 5 Pst I/Mse I selected on the basis of their extensive coverage of the genome were identified as core AFLP primer set that could be used for whole genome selection. These primers generated 385 markers covering 96% of the mapped *B. juncea* genome (Fig 1). These primer pairs were e31m47, e31m49, e31m58, e31m60, e34m49, e39m32, e39m50, e44m39, e45m34, e35t63, e47t70, e47t71, e50t66, e51t78, e60t77, p33m32, p53m33, p33m38, p53m47, p58m35, p62m59, p32t77, p33t65, p48t67, p48t94 and p62t78.

The *B. juncea* map developed in the present study will be used for tagging agronomically important genes and identification of superior QTLs from east European germplasm. This tagging information will subsequently be utilized for precise transfer of these desirable genes from exotic germplasm to Indian varieties through marker assisted breeding. The set of 26 AFLP primers identified for whole genome selection will be used for rapid recovery of the recipient genome in backcross breeding programme.

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