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Assessment of genetic diversity among leaf blight resistant and susceptible cultivars of barley by RAPD

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Molecular diversity was assessed using two leaf blight resistant and two susceptible genotypes in barley. Out of 25 RAPD primers used, 20 primers produced polymorphism. Similarity coefficients ranged from 0.65 (PL-760 and RD-2508) to 0.78 (PL-760 and DWR-28) among genotypes. The primers OPA-05, OPM-06, and OPB-11 produced 100 per cent polymorphism. Clustering pattern clearly differentiated resistant (DWR-28 and PL-760) and susceptible genotypes (RD-2508 and RD-2653).

Key words : Genetic Diversity, Leaf Blight Resistance, Barley

INTRODUCTION

BARLEY is cultivated over 0.658 mha in India and in Karnataka over 3000 ha (Anon, 1995). This crop is ex posed to various types of foliar diseases. Among them leaf blight (*Helminthosporium sativum* Pam., King and Bakke) of barley is an important production constraint and is capable of causing considerable yield loss. Genetic diversity in barley has been well documented based on molecular characterization. Molecular marker such as random amplified polymorphic DNA (RAPD), which is simple and fast, is widely used for the identification of genotypes, gene mapping and QTL analysis. In this study molecular diversity was assessed with leaf blight resistant and susceptible genotypes utilizing RAPD tolerance.

MATERIAL AND METHODS

Previous investigations have shown that barley genotypes DWR-28 and PL-760 were found to be moderately resistant to leaf blight pathogen and RD-2653 and RD-2508 were found to be susceptible to leaf blight pathogen (Anon, 1995). In the present study, we have utilized these four genotypes for RAPD analysis. DNA extraction was done by CTAB extraction method with few modifications (Dellaporta *et al.,* 1983). Five grams of leaves of 10-12 days old barley seedlings was grinded to fine Powder in liquid N_2 with pre cooled Pestle and Mortar. The ground tissue was transferred to polypropylene tube containing 15 ml extraction buffer (2-3 % w/v CTAB, 1mM Nacl, 20 mM EDTA, 100mM Tris Hcl pH 8, 0.03% â – mercapta ethanol) preheated to 65°C. Samples were incubated for 30 min with intermittent shaking at every 15 min. Equal volume of chloroform: iso amyl alcohol (24:1v/v) was added and gently agitated for 10 min to form an emulsion. Then tubes were centrifuged for 10 min at 6000 rpm at room temperature. Then supernatant was transferred to sterile tubes and 10 ml of chilled isoproponol was added to each tube, mixed by inverting and incubated at -20°C for 10min. The contents were centrifuged again for 20 min with 5000 rpm at 4°C and the pellet was retained by discarding the supernatant. The DNA pellet obtained was washed with 70 per cent ethanol and tubes were inverted on blotting paper to dry the pellet. Later DNA was suspended in 50 µl TE (10 mM Tris Hcl, 1 mM EDTA) buffer and stored at -20°C. DNA concentration of samples and purity was determined by taking ultraviolet absorbance at 260 nm and 280 nm in a spectrophotometer followed by agarose gel electrophoresis. Protocol for PCR was optimized by varying the following parameters. RAPD conditions for barley in the present investigation were standardized and the amplification assay contained template DNA 30 ng, Taq DNA polymerase (Genei) 0.3 units, $Mgcl_2$-1.5mM, dNTP (Genei) – 2.5 mM each of dATP, dGTP, dCTP, dTTP (Operon Technology) and 0.6 µM buffer (Genei) in a reaction volume of 25 ml.

The polymerase chain reaction was performed by using primer 96 plus supplied by MWG AG Biotech, Auzinger strasse TA, Eberiberg, Germany. The following PCR conditions were tried: 94°C for 4 min followed by 45 cycles of 94°C for 1 min, 38°C for 1 min, 72°C for 2 min with final extension of 72°C for 5 min. 25 RAPD primers from OPA, OPB and OPM series (Operon Technologies , Inc. USA) were screened using DNA from four barley genotypes. Amplification products obtained after the PCR reaction were loaded into individual channels of 1.2 per cent agarose horizontal gel in 1 x TAE (pH 8.0) buffer. Electrophoresis was carried out at 70 volts for 2hrs 30 min. Gels were stained with ethidium bromide (1 µg/ ml) and gels were photographed using gel documentation system (Hero lab EASY, Germany).

DATA ANALYSIS

The amplified fragments were scored as '1' for the presence and '0' for the absence of a band generating the 0 and 1 matrix and per cent polymorphism was calculated. Pair wise genetic similarities (Sij) between genotypes were estimated by DICE similarity coefficient, clustering was done using the symmetric matrix of similarity coefficient and clusters obtained based on unweighted pair group arithmetic mean (UPGMA) using SHAN module of NTSYSPC version 2.0 given by Rohif (1998).

RESULTS AND DISCUSSION

DNA fingerprinting of leaf blight resistant and susceptible genotypes of barley RAPD analysis revealed that 22 primers showed clear reproducible and scorable amplicons in each DNA sample (Table 1). RAPD banding pattern generated by primers OPA-04, OPB-08, OPM-11 and OPB-04 is shown in Fig 1. Among 22 primers which produced scorable amplicons2 primers produced monomorphism and remaining 20 primers produced polymorphism (Table 1). A total of 180 amplicon levels were resulted from 22 primers. Out of these, 119 bands were polymorphic. The primers OPA – 05, OPM-06 and OPM-07 produced maximum of 100 per cent polymorphism, followed by OPM-14 (90.90%), OPA-01(90%) (Table 1). The highest number of 13 amplicons was produced in the primer, OPM-11 followed by 12 amplicons in OPA-02. The lowest of 4 marker level amplicon was observed in OPB-02 and 5 marker level amplicons were observed in OPB-01 (Polymorphism). On an average there were 7.2 amplicon levels per primer of which 4.24 were polymorphic, indicating molecular diversity existing among the four genotypes of barley.

Based on simple matching co-efficients a genetic similarity

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