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DETECTION OF PROTEIN MARKERS FOR IDENTIFICATION OF RICE GENOTYPES RESISTANT TO GREEN LEAFHOPPER

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ABSTRACT

SDS-PAGE of total seed proteins of 11 rice genotypes resistant to green leafhopper (GLH) and a susceptible variety, Taichung (Native) T(N) 1 facilitated scoring of 11 distinct polypeptide bands of heterogeneous molecular weights ranging from 84.1 to 24.0 kD. Seven bands exhibited polymorphism with regard to presence/absence or molecular weight. Two bands (46.8 and 42.7 kD) were present only in the susceptible variety and are potentially reliable markers in screening for GLH resistance. Five bands of 53.7, 38.0, 33.9, 28.2 and 24.0 kD were polymorphic for presence/absence or molecular weight even within the resistant genotypes. These polymorphic bands can be used as markers in breeding for GLH resistance as well as for detection of identification of unknown resistance genes and understanding the genetics of resistance.

Key words : Rice, Green Leafhopper, Resistance, Protein Markers, Marker Assisted Breeding

Green leafhopper (GLH), *Nephotettix virescens* (Distant) is a serious rice pest in South and South east Asia. It damages rice crop by direct feeding and acting as the vector of rice tungro virus that causes yield losses as high as 100% [1]. Eight genes conferring resistance to GLH have so far been identified [2-3]. Some of these donors have already been used for incorporation of resistance into high yielding genotypes [4]. Besides, five breeding lines identified as resistant to this insect pest at the Directorate of Rice Research (DRR), ICAR, Hyderabad, (India) can also be used as donors. Identity and genetics of these resistance sources are, however, yet to be established. Molecular markers can be effectively employed to facilitate investigations on the identity of these unknown genes, besides ensuring the introgression of resistance genes into high yielding susceptible cultivars. Protein markers have successfully been used for varietal identification in several crops [5-13]. Electrophoretic banding patterns have been used for characterization and identification of varieties

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in rice also [14-16]. We report here, for the first time, on the detection of seven protein markers to identify putative resistant genotypes for varietal screening and breeding for GLH resistance.

MATERIALS AND METHODS

Eleven GLH resistant rice genotypes studied included six varieties with known resistance genes: Pankhari 203 (*Glh1*), IR8 (*Glh3*) [17]; Ptb8 (*Glh4*), ASD8 (*Glh5*) [18]; TAPL 796 (*Glh6*) and Maddai Karuppan (*Glh7*) [2]; and five resistant breeding lines identified at DRR: IET 15120, IET 15359, IET 13341, IET 12175 and IET 13268. The high yielding variety T(N)1 was used as the susceptible test genotype.

For extraction of total protein, seed flour was suspended for 30 min. in an extraction buffer containing 0.5M NaCl (pH 2.4) followed by centrifugation at 12000 rpm at 10°C for 5 min. An equal volume of cracking buffer containing 0.125M Tris. HCl (pH 6.8), 4% SDS, 20% Glycerol, 10% 2-Mercaptoethanol and 0.01% Bromophenol Blue was added to the supernatant and the mixture was denatured by boiling at 100°C for 2 min. Denatured protein samples were electrophoresed in one-dimensional 12% SDS- polyacrylamide gel following Laemmli [19]. A molecular weight marker lane was also incorporated in the gel to determine the molecular weight of the polypeptide bands of the rice proteins.

RESULTS AND DISCUSSION

SDS-PAGE of total seed proteins of 12 rice genotypes resulted in scoring of a maximum number of 11 polypeptide bands of heterogeneous molecular weights and varying intensity (Fig. 1). Total protein profiles, therefore, belong to either albumin or globulin [20]. Sarkar and Bose [16] analyzed salt soluble seed proteins in eight rice varieties and reported two to four low molecular weight (17 to 24kD) bands, the 24kD band being prominent and universally present. Five most frequently occurring albumin bands in their study ranged between 41 and 69kD. However, Mawal *et al.* [21] reported the Con A purified rice albumin to consist of a single band of 60kD.

The four high molecular weight polypeptide bands of 84.1, 74.1, 66.1 and 60.4kD were universally present in the test genotypes. Two bands of 46.8kD and 42.7kD were present only in the susceptible variety, T(N) 1 which can be used as reliable markers in ascertaining the introgression of resistance genes. Similarly, a band of 33.9 kD was present in T(N) 1 and absent in all the resistant genotypes except IET 12175. This band also can be used in marker assisted introgression of the resistance genes from the 10 genotypes devoid of this protein expression. Three bands of 53.7,

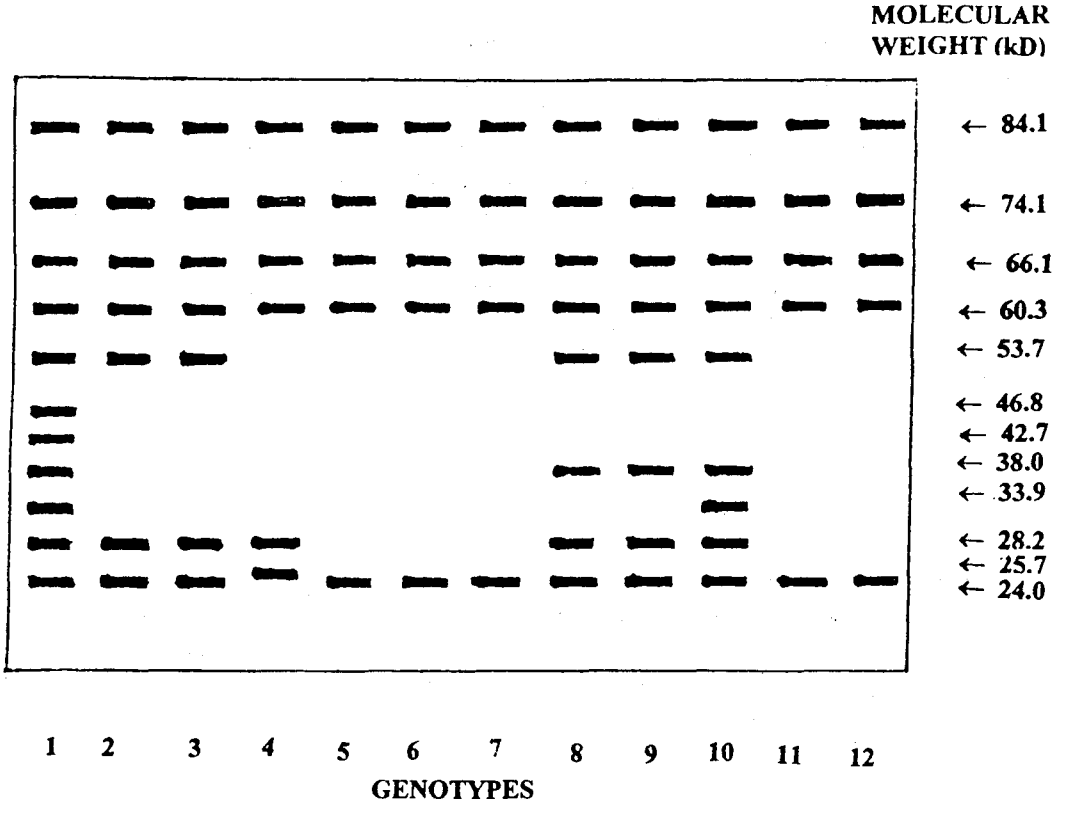

Fig. 1. Seed protein profiles of 12 rice genotypes: 1. T(N) 1, 2. Pankhari 203, 3. IR8, 4. Maddai Karuppan, 5. TAPL 796, 6. ASD8, 7. Ptb8, 8. IET 15120, 9. IET 15359, 10. IET 12175, 11. IET 13268, 12. IET 13341; Molecular weights (in kD) on the right

38.0 and 28.2kD present in the susceptible variety and absent in six (Maddai Karuppan, TAPL 796, ASD8, Ptb8, IET 13268 and IET 13341); eight (Pankhari 203, IR8, Maddai Karuppan, TAPL 796, ASD 8, Ptb8, IET 13268 and IET 13341); or five (TAPL 796, ASD8, Ptb8, IET 13268 and IET 13341) of the resistant genotypes, respectively can be used as markers for incorporation of resistance genes from the above eight resistant genotypes. Further, these can be used to monitor crosses of the resistant genotypes *inter se* designed to detect the identity of the unknown resistance genes and also the nature of inheritance of resistance in the five breeding lines. The band with the lowest molecular weight (24.0kD) was present in all the 12 rice genotypes with the exception of the variety Maddai karuppan, while had a high molecular weight variant (25.7kD). This band may again serve as a marker to introgress the resistance gene from this donor and also in monitoring crosses with other 10 resistant genotypes.

The genes controlling the expression of the polypeptides 84.1, 74.1, 66.1 and 60.3kD appear to behave as a single block. Similarly the genes controlling the polypeptides of 46.8 and 42.7kD might be linked. This assumption could, however, be verified through linkage analysis using segregating populations. Multigene inheritance of seed protein expression is well established in other crops also (for review please see de Lumen [22]). Seed protein expression is known to be under monogenic control with codominance of alleles for diverse molecular weight variants and dominance of presence over absence. Studies on inheritance of the seven polymorphic polypeptides are in progress.

Association of protein bands with agrobotanic characters has previously been reported. Whereas Larsen [5] observed genotypes with a particular banding pattern to have linkage with hilum colour in soybean, Osborn *et al.* [23] have reported association of arcelin polypeptides with resistance to bruchids in frenchbean. The present study provides evidence for association of polypeptides with susceptibility to the rice insect pest green leafhopper suggesting the possibility of using these polypeptides in marker assisted screening for resistance to this pest. Analysis of salt soluble proteins comprising mainly globulin and albumin has led to the detection of seven polymorphic polypeptides. Further studies on the major seed protein fractions of rice, such as glutelin and prolamin could possibly generate more polymorphism to pave way for obtaining protein profiles specific to individual resistant and susceptible genotypes.

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