SHORT COMMUNICATION

Medicago scutellata – A Possible Source of Weevil Resistance for Lucerne Improvement

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Alfalfa or lucerne *(Medicago sativa* L.) is a herbaceous, perennial legume that has often been called the "Queen of forages." It is the third most important forage crop in India and occupies more than one million hectare area and provides 60 to 130 tones green forage per hectare (Hazra, 1995). Lucerne is well-adapted to a wide range of climatic and soil conditions. It is relatively drought tolerant but also responds very well to irrigation. It tolerates some alkalinity, but does not perform well on highly-alkaline or salty soils. It is a perennial, out-crossing, autotetraploid crop domesticated presumably in the Near East (Iran, plain of Mesopotamia) and/ or in central Asia around 5000 BC.

The lucerne weevil (*Hypera postica*) is one of the primary insect defoliators of lucerne. This is the most damaging pest of lucerne, occurring in all the lucerne growing areas of the country and is particularly severe in north-western part, western Himalaya, Gangetic and central plains. Besides lucerne, it also damages sweet clover, peas, species of *Medicago* and *Mains* spp. The damage is mostly caused by the larvae that feed within the plant tips, on the young leaves and under heavy infestation the lower foliage is also consumed. The adults also feed on the foliage. Though difficult to estimate, the insects is estimated to reduce yields by 10 to 15% annually (Fig. 1a) where forage quality not taken into account (Pandey and Faruqui, 1990). A number of insecticides are available for control of this weevil, but due to inherent property of these chemicals to have residual toxicity, to non-target species and causing ecological disruptions, their use is restrictive. Integrated Pest Management (IPM) is a commonsense approach to crop protection. This concept relies on combinations of insect suppression and crop production techniques to optimize yield and quality. Developing resistant variety is an important aspect of IPM. However, earlier research conducted at IGFRI, Jhansi, has not yielded any lines of *M. sativa* showing reasonable level of weevil resistance. In order to search resistant material, germplasm of exotic species were procured and evaluated under hot-spot condition at Indian Grassland and Fodder Research Institute, Jhansi to identify and characterize the suitable genetic materials. Six accessions of *M. scutellata* (L.) Mill. along with 43 different species belonging to *Medicago* genus (data not given) were evaluated during 2004-05 and 2005-06. Genomic DNA from six accessions of *M. scutellata* was isolated from young, fresh leaves and PCR amplification was carried out as described earlier. The SSR amplification was performed by following the procedure of Diwan *et al.* (1997). Similarity matrices of both RAPD and SSR data sets were calculated using NTSYS computer software. SAHN clustering was used to construct the dendrogram employing UPGMA module.

Germination of all six accessions of *M. scutellata* was more than 90 % with intense flowering and seed setting. The level of infestation caused by weevil insect among the lines varied from 5-75 %. Accessions EC541686 and EC541685 showed 5 to 10 % infestations (Fig. 1b). The results indicate the presence of reasonable level of weevil resistance in these lines (Table 1). The identified weevil resistant lines of *M. scutellata* can be exploited in breeding with cultivated *M. sativa* for transferring the gene responsible for weevil resistance. It is generally agreed that the basic chromosome numbers for the genus *Medicago* are x = 7 and x = 8. Most species are 2n = 2x = 16, 2n = 4x = 32 or 2n = 6x = 48. The cultivated and perennial *M. sativa* is 2n = 4x = 32. Before

Table 1. Level of infestation and other details of different accessions of *M. scutellata* lines

Accession number	Per cent infestation
EC541685	75
EC541686	5
EC547738	35
EC543739	30
EC547740	10
EC547741	30

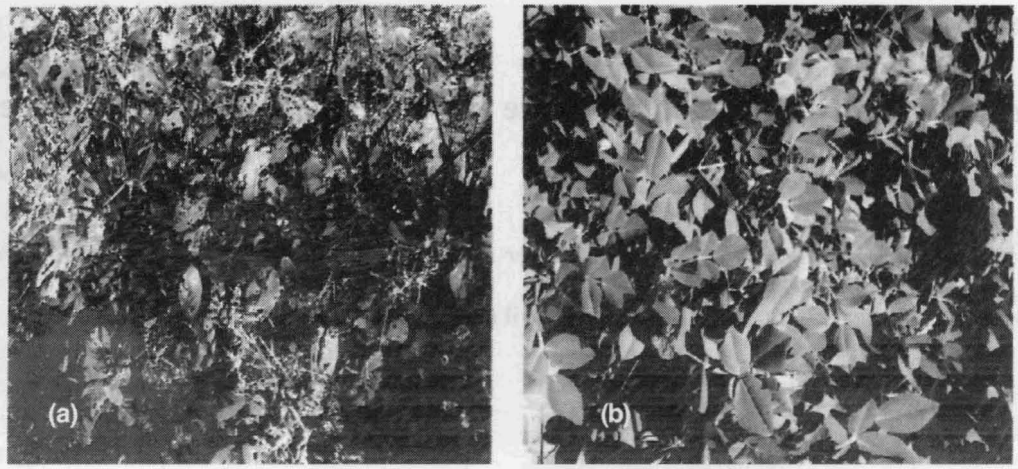

Fig. 1: *Highly weevil infested M. sativa plant (a) and No infestation observed on M. scutellata (b)*

1984, *M. scutellata*, was considered as annual and tetraploid with 2n = 32 (Simon and Simon, 1965; Lesins and Lesins, 1979). Thus, hybridization between this annual species and *M. sativa* may be possible because of their common ploidy level. The only successful hybridization between *M. scutellata* and *M. sativa* was reported by Sangdeun *et al.* (1982), who recovered one plant from the cross. However, this plant was chimeric and sterile. Contrary to the earlier report, Bauchan and Elgin Jr (1984) cytologically observed counts of 2n = 30 in *M. scutellata* at both mitotic and meiotic stages. Diploid accessions were not found and meiosis was normal with 15 bivalents forming at metaphase I suggested *M. scutellata* is an allo-tetraploid species. Further authors have suggested the presence of two pairs of SAT-chromosome in *M. scutellata* and probably this led to the erroneous counts of 2n - 32, recorded previously. Two hypothesis has been proposed for the development of a somatic number of 2n = 30, one is the hybridization of a 2n = 14 and a 2n = 16 species followed by the polyploidization of the hybrid. Another possibility would be the loss of a pair of chromosomes from a 2n = 32 polyploid. The gain of a pair of chromosomes is unlikely because a 2n - 28 species has not been found in the genus *Medicago.*

The next logical step in improving the *M. sativa* was to identify the two progenitors of *M. scutellata* and study the relationship among the genotypes of two progenitors of *M. scutellata* to pinpoint the most closely associated accessions of progenitors. Based on phenolic-taxometric studies *M. rigidula* (L.) All., *M. murex* Willd., *M. doliata* Carmian., *M. muricoleptis* Willd. and *M. rotata* Boiss. species showed a close relationship to *M. scutellata* (Classen *et al.,* 1982). Two of the species are 2n = 14 *(M. murex* and *M. rigidula)* and the others are 2n = 16. The presence of these species and close relationships *M. scutellala* suggest that 2n = 30 species may have arisen through hybridization followed by polyploidization (Bauchan and Elgin Jr., 1984).

Molecular analysis of six species of *M. scutellala* indicated variability among the lines. Four hundred ninety-nine RAPDs and 150 SSR markers when employed for analysis through unweighted pair group method with arithmetic mean (UPGMA) algorithm and dendrogram formed by Sequential Agglomerative Hierachical and Nested (SAHN) clustering showed two clusters by both marker systems (Figs. 2a, 2b). The

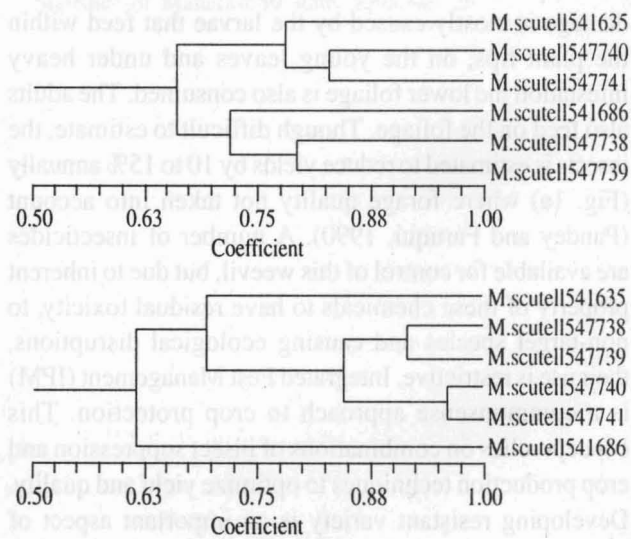

Fig. 2: *Dendrogram showing genetic ^elatedness among six accessions of M. scutellata based on RAPD (top) and SSR (bottom) markers*

genetic distance ranged from 0.58 to 0.96 with RAPD and 0.59 to 0.83 with SSR markers indicated large variability among the lines could be exploited in breeding programme.

Since the crosses between species with unequal ploidy levels have been successful due to the production of unreduced gametes (Bingham and Saunders, 1974) and the identification of weevil resistant *M. scutellata* having different ploidy to that of *M. sativa* can be used in attempting the crosses for introgression of weevil resistant genes. The results also indicated a reasonable level of genetic variability among the *M. sculellata* lines, better for crossing. If success rate of getting fertile plant is less, the biotechnological tools like embryo rescue can be attempted. Another possibility of getting resistant *M. sativa* (2n = 4x = 32) would be to cross the diploid *M. sativa* lines with the progenitors of *M. scutellata* especially those possessing 2n = 16 chromosome.

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