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Electrophoretic studies in induced mutants of diploid mulberry genotype S_{13}

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Electrophoretic leaf protein profiles of *Morus alba* variety S_{13}, irradiated with different dosages of gamma radiation, revealed proteins (polypeptides) of both high and low molecular weights; 44 kDa was the major protein component. Gamma irradiation influenced the quantitative differences in the minor components and low molecular weight proteins present in trace amounts. Significant decrease in the quantity of 55 kDa protein was observed with the increase in dosage from 7 kR to 10 kR. thus, necessitating to limit the dosage of gamma irradiation to 6 kR.

Keywords: leaf protein profiles, mulberry, silver staining
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Mulberry has immense economic importance in silk industry due to its foliage, which constitutes the chief food for the silkworm, *Bombyx mori* L. Improvement in food value, which has been a breeding objective in mulberry, is mainly determined by the protein quantity and quality[1,2]. Mutation breeding employing radiation is in use to explore the feasibility of developing new varieties and it has been proved useful in creating new variability in the existing gene pool. Of late, genetically effective radiations have been widely used for induction of mutation in mulberry[3-11]. Gamma-ray induced diploids have resulted in many beneficial traits, such as larger leaf-size, bigger leaf area, dark green coloured leaves, etc. However, the genetic variations of the leaf protein profile have not been properly investigated. Hence, in the present study, the leaf protein profile in the induced variants has been investigated using SDS-PAGE electrophoresis and silver staining with variety S_{13} of *Morus alba*. Also, the standard molecular weights of the protein profiles were evaluated.

Hardwood stem cuttings of S_{13} mulberry variety were irradiated with 4, 5, 6, 7, 8, 9 and 10 kR dosages of gamma rays from a Co 60 gamma ray source. The irradiated cuttings were raised with all the necessary agricultural inputs. The fresh leaves of gamma irradiated diploid plants along with the leaves of the control plant were used as the source material. SDS-PAGE analysis of total proteins was performed according to the method of Laemmli[12]. Silver stained gels (slab) were scored for the presence or absence of protein bands for gamma irradiated plants and control plants in comparison to standard marker (PMW-M 97.400-14.300 kDa).

Leaf protein profile of variety S_{13} revealed five major proteins (polypeptides). Of which, two high molecular weight proteins (HMW_1 and HMW_2) were common in all the samples, while 64, 55 and 44 kDa proteins were present with quantitative variations. The 44 kDa protein was the major component of the leaf proteins, whereas 42 kDa protein was present only in the control sample. The quantity of 64 and 55 kDa proteins decreased with the increase in dosage of gamma irradiation. There were also minor low molecular weight proteins present in trace quantities. In general, the influence of gamma irradiation on synthesis of the different proteins was not significant, except for 55 kDa protein as their quantity decreased with the increase in dosage above 6 kR (Fig. 1).

Rubisco (ribulose-1, 5-biphosphate carboxylase-/oxygenase) is a major soluble chloroplast protein, which can account for upto 50% of total protein in mulberry leaf[1]. The large subunit of the native enzyme has a molecular weight of 55 kDa[13]. Rubisco is the most abundant protein on the earth, which captures CO_2 in the first reaction of photosynthesis. It is a large molecule having a molecular mass of about 560 kDa, consisting of eight copies of two types of subunits within this holoenzyme. The *rbcL* gene of chloroplast encodes the 55 kDa large subunits, each of which contain an α/β barrel active site for photosynthetic CO_2 fixation. The 15 kDa small subunits are coded by family of nuclear *rbcS* genes. Within the chloroplast, the rubisco holoenzyme catalyses the reaction between CO_2 and RuBP (ribulose-1, 5-bisphosphate) and is directly responsible for plant productivity[14]. Morphological, physiological and biochemical gradients which occur

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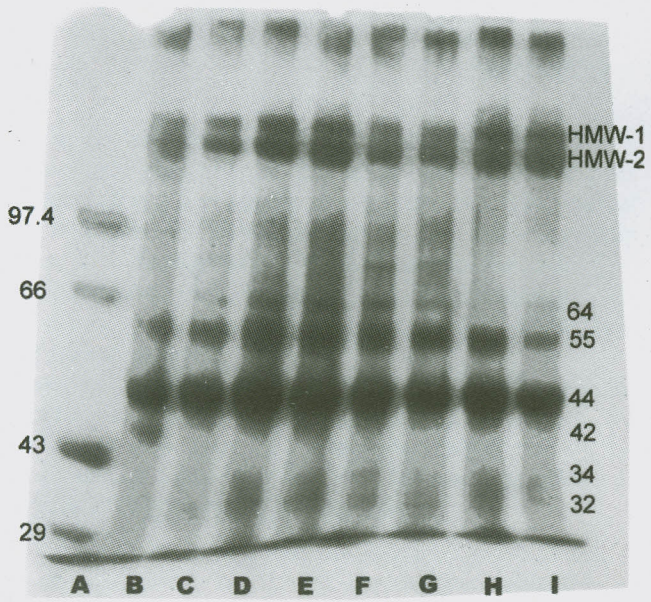

Fig. 1—Electrophoretic leaf protein profiles of induced diploid plant of S_{13} mulberry variety studied using standard protein marker PMW-M and silver staining; A-Marker, B-Control, C to I-irradiated with 4 to 10 kR gamma rays, respectively (at the interval of 1 kR).

in plant systems seem to be related to a gradient in the ploidy level as suggested by Kraus *et al*[15].

The gradual decrease in the concentration with the increase in dosage of irradiation above 6 kR indicates that the *rbcL* genes responsible for coding 55 kDa subunit of rubisco enzyme are inhibited. The dosages above 6 kR also resulted in stunted growth of mulberry variety S_{13}[16]. Therefore, the variety S_{13} seems to be sensitive for gamma ray irradiation above 6 kR and the decline in quantitative parameters of productivity can be directly correlated to the photosynthetic productivity.

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