

SUMMARY
AND CONCLUSION

During the present investigation on mulberry variety S₃₆, protocols were developed for direct regeneration from nodal explant, shoot apex and *in vitro* derived shoots (with 1 -3 nodes). Different concentrations of BAP, Kn, GA₃, IBA and 4 pu were used, each separately and also in combinations of two of them for micropropagation studies. BAP at 1.0 mg/l and 4 pu at 0.5 mg/l were found to be optimal concentrations required to promote direct shoot induction from nodal explant and also from shoot apex on semisolid as well as liquid media, respectively. High concentration of BAP (2.0 -10.0 mg/l) and 4 pu (1.0 - 1.5 mg/l) were noted to suppress the shoot formation in the said explants, however, high concentration of BAP (8.0 mg/l) was required to induce multiple shoots from shoot apex cultures. Combination of high concentration of GA₃ (5.0 mg/l) with low BAP (1.0 mg/l) yielded the best result in the case of *in vitro* derived shoot explants for further multiplication. Among the tested explants, the shoot apex culture yielded maximum number of multiple shoots per explant on MS medium supplemented with BAP (8.0 mg/l) and 4pu (0.5 mg/l) on semisolid and liquid-shake culture conditions, respectively. The regenerated shoots were rooted on 1/4 MS basal medium (hormone free) and hardened in a mixture of soil, sand and compost (2:1:1).

A somaclonal variant has been successfully isolated directly (in contrast to indirect organogenesis) in long term shoot apex explant culture. The said culture was initially raised at high concentration of BAP (8.0 mg/l) supplemented MS medium and maintained by series of subcultures on the same medium with low concentration of BAP (1.0 mg/l). The somaclonal variant isolated from this culture work exhibited improved morphological characters such as high sprouting, better growth with more number of branches and high rooting ability. The somaclonal variant also showed variations in biochemical parameters like total sugar, phenol and chlorophyll content,

compared to the control plant. The present studies showed for the first time that a somaclonal variant can be obtained through meristem (shoot apex) cultures in mulberry.

Internodal segments and leaf explants cultured on both MS and B5 media supplemented with different concentrations of 2,4-D, BAP, IAA, NAA and IBA resulted in callus formation of various degree. It was noted that MS medium was better for the growth of callus than B5 medium. 2,4-D at 1.0 mg/l was recorded as the most potent auxin for inducing callus from both internodal segment and leaf explants. Similarly, BAP at low concentration (0.5 mg/l) along with 2,4-D (1.0 mg/l) also enhanced the callus formation in both the explants stated already. A combination of BAP (1.0 mg/l) and IAA (0.5 mg/l) was also found to be equally effective for induction of nodular callus and regeneration of adventitious shoot buds especially from leaf callus. Thus, in the present work, the leaf explant was proved to be better for regeneration by indirect organogenesis compared to the internodal segment, however, a low frequency (20%) of adventitious shoot formation was achieved following subculture of the leaf callus on MS medium with BAP (1.0 mg/l) + CH (100 mg/l). The reason for callus differentiation only in a low frequency of adventitious shoots could not however be ascertained but it could be due to recalcitrant nature of callus in the presently worked out mulberry var. S₃₆.

In vitro mutagenesis in mulberry var. S₃₆ was investigated by treating explants and also calli derived from both internodal segment and leaf explants with the physical and chemical mutagens and finally, five solid mutants with improved agronomical traits were isolated (from nodal explant cultures) in the present work.

Two explants viz., nodal explant and shoot apex were treated with different doses / concentrations of gamma irradiation and EMS. LD₅₀ dose at 3 kR

and 2 kR of gamma treatment was ascertained, respectively as effective doses of mutagen for nodal explant and shoot apex explants cultures. Similarly, LC₅₀ at 0.2 % for 4 hours treatment was determined as effective dose for nodal explants. In the present study, chemical mutagen (EMS) of the two mutagens employed was found to be efficient in inducing high number of mutants compared to gamma rays. A combination treatment of gamma and EMS, however, failed to induce mutagenesis.

Sprouting percentage and growth of variant plants following mutagenic treatments were severely affected with the increased dosages / concentrations of gamma rays and EMS, respectively in the tested explants. Shoot apex explant was found to be more sensitive to mutagenic treatment than nodal explant. Stimulatory effect on growth of the shoots for both the explants was noticed in lower doses of 1kR of gamma and also in 0.1% of EMS treatments.

Mutants that were isolated at M₂ generation exhibited differences as to their morphological parameters. Detailed morphological data collected from M₁ and M₂ generations in somaclonal variant and mutant plants were analysed statistically regarding sprouting ability, plant height, number of branches, internodal distance, leaf area and rooting ability. The shift in mean values and analysis of variance were worked out on morphological characters to record the degree of induced variability.

The encapsulation technique employed, was found to be more effective to recover more mutant plants especially from axenic nodal explants. M₂ to M₄ generations were probed following mutagenic treatments of axenic nodal explants. The regeneration capacity of the isolated mutants remained same even after M₄ generation.

Differential requirement of auxins viz., IBA and NAA (0.5 - 1.0 mg/l each) for inducing roots in mutants indicated possible genotypic variations in mulberry var. S₃₆ compared to the control (*i.e.*, on MS basal medium).

An attempt was also been made to obtain regeneration from the calli derived from internodal segment and leaf explants, following treatment with different doses / concentrations of gamma rays and EMS. The mutagenic treatments at low doses / concentration resulted only in green calli, however, at higher doses/ concentrations, a steady decline in the growth of callus was noted.

Besides the morphological traits, the ultra structural variations of leaves in somaclonal variant and mutants at M₂ generation were also studied. It was noted that there was a wide range of variation among the isolated variants (*i.e.*, somaclonal variant and mutants) compared to control as to the shape and size of idioblasts and trichomes.

The cytological investigation in somaclonal variant and mutant plants at M₂ generation revealed no variation in chromosome number. In all the isolated/ induced variants, chromosome number is noted to be diploid ($2n = 28$).

Variation in biochemical parameters was recorded at M₁ and M₂ generations in somaclonal variant and mutants of mulberry var. S₃₆. The biochemical contents such as total nitrogen, chlorophyll, phenol and sugar increased in the somaclonal variant. There was a marginal increase in chlorophyll content in all the mutants isolated indicating possible enhancement of photosynthetic rate. Both somaclonal variant and mutant - 4 showed increased phenol content compared to control.

The SDS-PAGE protein profile was investigated at M_2 generation in somaclonal variant and mutant plants of mulberry var. S_{36} . It exhibited difference in presence or absence of total protein bands.

Conclusion:

1. Protocols for rapid *in vitro* micropropagation of *M.indica* var. S_{36} were developed using nodal explant, shoot apex and *in vitro* derived shoot segment on MS medium supplemented with BAP 1.0 mg/l; 8.0 mg/l, 4 pu 0.5 mg/l followed by subsequent subculture on BAP 1.0 mg/l + GA₃ 5.0 mg/l for further multiplication. Protocol for callus formation from internodal segment and young leaf explants was developed. Protocol for adventitious shoot bud formation from leaf explant was also developed.
2. Somaclonal variation in mulberry variety S_{36} was achieved, from long term shoot apex culture. Based on morphological and biochemical characters at M_1 and M_2 generations, it was conformed that somaclonal variant was superior to control plant (parent plant).
3. For *in vitro* mutagenesis, semilethal doses (LD₅₀) and semilethal concentrations (LC₅₀) using gamma and EMS, respectively were identified for nodal explants and also shoot apex in *M. indica* var. S_{36} M_1 and M_2 generations were studied for stabilization of mutant traits.
4. Encapsulation method has been effectively used in mulberry var. S_{36} to recover more number of mutants from axenic nodal explants of M_1 plants.
5. Differential requirement of auxins viz., IBA and NAA (0.5 - 1.0 mg/l each) for inducing roots in somaclonal variant and mutants indicates possible genotypic variations compared to the control plant (i.e., on MS basal medium)

6. Five mutants were isolated at M_2 generation in mulberry var. S_{36} . Mutation affecting morphological characters at M_1 and M_2 generations were identified and confirmed in comparison with the control.
7. Ultra structural and cytological variations were also investigated at M_2 generation in somaclonal variant and mutants in mulberry var. S_{36} .
8. Bio-chemical parameters studied at M_1 and M_2 generations conform the variations in bio-chemical constituents of somaclonal variant and mutants compared to the control.