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BIOTECHNOLOGICAL INTERVENTIONS FOR CROP IMPROVEMENT IN *HEVEA BRASILIENSIS*: PRESENT STATUS AT RUBBER RESEARCH INSTITUTE OF INDIA

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Abstract

The recent developments in agricultural biotechnology offer tremendous potential for the genetic improvement of the perennial tree species, *Hevea brasiliensis*. The *in vitro* approaches initiated for the genetic improvement of *Hevea* at Rubber Research Institute of India (RRII) are 1) development of efficient protocols for micropropagation of elite *Hevea* clones through somatic embryogenesis, 2) development of haploid/dihaploids, triploids and polyploids in *Hevea*, 3) development of techniques for embryo rescue and induction of polyembryony and 4) development of transgenic plants for better adaptation to environmental stresses, tapping panel dryness, latex yield and disease tolerance. Being a perennial tree species crop improvement in this crop is limited by the long juvenile period, poor seed set and highly heterozygous nature of the seed propagated plants. Protocols for plant regeneration through somatic embryogenesis have been successfully established using different explants such as immature anther, immature inflorescence and leaf explants for the elite Indian clone, RRII 105. Field trials have been established with the somatic plants developed from immature inflorescence and are being evaluated for their field performance. Further, extensive optimization experiments were carried out to improve the somatic embryogenesis and plant regeneration efficiency. Modification of the media components such as replacement of ammonium nitrate with calcium/potassium nitrate as well as changing the hormonal composition by the addition of picloram along with BA, 2,4-D and the cultural conditions improved callus induction and plant regeneration frequency.

Embryo rescue experiments were attempted to improve the seedling recovery during hand pollinations and methods were successfully established to rescue embryos through *half ovulo* culture from five week old seeds. By manipulating the hormonal combinations multiple embryos of up to 40 numbers could be induced from a single ovule and plants were regenerated and field established. The single zygotic origin of these multiple embryos was confirmed through molecular analysis.

For the development of haploid plants different approaches such as direct culture of pollen grains, culturing of pollen protoplasts, isolated embryo sac culture, *in vitro* and *ex vitro* pollination with irradiated pollen grains and culturing of mature unfertilized

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ovule were attempted. Pollen protoplast culture could induce cell division leading to the formation of micro colonies and micro calli, which have further proliferated and turned out embryogenic. Culturing of isolated embryo sacs also resulted in embryogenic calli as well as embryos. Cytological analyses have proved the development of androgenic haploids from pollen protoplasts and gynogenic haploids from the embryo sac. Embryos rescued from fruits developed after pollination with irradiated pollen grains were cultured for germination and further analysis. Experiments were conducted for *in vitro* induction of polyploidy in diploid callus of *Hevea* through chemical treatment using colchicine. Colchicine treated cultures first turned white in colour and later gave rise to friable yellow calli which were further proliferated and subcultured for embryogenesis. Several embryos could be induced from these cultures and cytological studies are in progress to determine the ploidy levels. Although, triploid plantlets were developed earlier through endosperm culture, they could not be hardened. Endosperm tissue from *Hevea* seeds at different developmental stages were cultured for callus induction in MS basal medium fortified with different levels of 2,4-D, NAA, BA and Kin. Callus induction at a low frequency was obtained in the presence of 2.0 mg/l 2,4-D and 3.0 mg/l kinetin and a few embryos were developed.

Transgenic *Hevea* plants integrated with the genes coding for MnSOD, osmotin protein and hmgr1 were developed. The MnSOD transgenic plants are expected to have increased tolerance to drought and other abiotic stresses as well as tapping panel dryness. The drought tolerant traits of these plants growing in polybags under containment conditions were evaluated by withholding water and the physiological performance of the transgenic plants are found to be better compared with the non transgenic control plants. Experiments were initiated to analyse the drought tolerant traits of the transgenic plants integrated with the osmotin protein gene and the preliminary study indicated a higher level of proline accumulation during stress induction. The hmgr1 transgenic plants showed a higher level of hmgr1 activity in comparison with the non transgenic control plants. RRII has also initiated a programme to develop antibiotic marker free transgenic plants using Cre/LoxP recombination techniques. A binary vector carrying the Cre recombinase under the control of a heat shock promoter was developed. For the functional validation of the binary vector developed, transgenic tobacco plantlets were developed using the same binary vector.