SCREENING AND SELECTION OF SALT TOLERANT GENOTYPES OF *MORUS ALBA* L. (LOCAL AND SUJANPURI) UNDER ALKALINITY STRESS

By

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DEDICATED

TO MY

MUMMY AND DADDY

CERTIFICATE

This is to certify that the thesis entitled "Screening and selection of salt tolerant genotypes of *Morus alba* L. (Local and Sujanpuri) under alkalinity stress" submitted by Mr. Parvaiz Ahmad Ganaie has been prepared under our guidance with the rules and regulations of Indian Institute of Technology Delhi, India. The research report and results presented in this thesis have not been submitted for any degree or diploma in any other institute or university.

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In the name of Allah, the most Gracious and the most Merciful
He is Allah, the Creator, the Inventor of all things, the
Bestower of forms. To Him belongs the best names.
All that is in the heavens and the earth glorify Him.
And He is the All-Mighty, the All-Wise
(Al-Quran, 59: 24)

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ABSTRACT

The extent of degraded land in India is 173.6 mha that is about 53% of the entire landmass in the country. Out of total degraded land, the problem of salt affected soil is one of the oldest and most serious limiting the land capacity of optimum plant growth. Due to the increasing demand of the expanding population for food and energy from the limited resources it is important to bring the degraded land under cultivation by applying better cultivation and management techniques. Accumulation of salts in soil occurs through natural and human-induced activities resulting in the problem of soil salinity and alkalinity and this is expected to boost in the future with desertification and greenhouse effect. The salt affected land area is increasing with time throughout the world. Production of altered plants to suit the environment is one of the biological approaches for mitigating the problem. The challenge is to maintain higher levels of productivity while using biological means of giving inputs.

One of the important applications of modern biotechnology in agriculture is its use in tissue culture. *In vitro* micropropagation techniques are increasingly being applied to large scale production of quality planting materials especially in fruit crops and woody timber trees. Unlike the other field crops mulberry is being cultivated for its leaf which is sole food for silkworm *Bombyx mori* L. and its foliage forms the basis of commercial sericulture. Mulberry is a multipurpose tree; all parts of the plant (root, stem, bark, leaf and fruits) are useful in many ways. The conventional methods of mulberry propagation are through stem cuttings and grafting but only 30-40% of them survive. Propagation through seeds is undesirable owing to cross pollination and the resultant heterozygosity; the true-to-type is not conserved in the propagated plants. The dioecious nature of the genus is another serious barrier to genetic improvement by conventional techniques. Keeping all these facts into

consideration the present work was focused to screen and select the $NaHCO_3$ and Na_2CO_3 tolerant genotype of *Morus alba* L. The study was performed in two parts i.e. *in vitro* and *in vivo*.

The different phytohormones and explants were standardized for shoot and root differentiation. The standardized hormone combinations were used for the *in vitro* screening of mulberry cultivars for Na_2CO_3 and $NaHCO_3$ tolerance.

The salt gradient was developed by using Na_2CO_3 (0.41, 1.20, 2.49 mM) and $NaHCO_3$ (3.57, 20.0, 59.0 mM) which raised the pH of the medium to 6.2, 7.2 and 8.2 respectively. The well developed salt tolerant plantlets were shifted for hardening and further to waste land for field evaluation. The biochemical parameters viz, (chlorophyll, proline, protein, sugar), antioxidant enzymes, (superoxide dismutase, catalase, peroxidase, glutathione reductase) and lipid peroxidation (MDA content) of the *in vitro* grown plantlets were analyzed. The induction or disappearance of proteins due to salt stress was also studied using SDS-PAGE.

In case of *in vivo* experiment the alkalinity was developed by applying Na_2CO_3 (0, 10.4, 20.5 and 30.2 gm of Na_2CO_3/kg soil) and $NaHCO_3$ (0, 30.4, 40 and 50 gm of $NaHCO_3$/kg soil), which raised the pH of the soil to 7.8, 9.1, 9.8 and 10.3 respectively. The data pertaining to different parameters i.e. sprouting and survival percentage, biomass yield, biochemical parameters, antioxidant enzymes, SDS-PAGE and amino acid composition were obtained and analyzed statistically.

The best results were obtained with nodal explant with axillary bud using phytohormone combination 2.5 mgl^{-1} BAP + 0.3 mgl^{-1} GA_3. The rooting was easily achieved with IBA 1 mgl^{-1}.

The results of *in vitro* and *in vivo* screening and selection of Na_2CO_3 and $NaHCO_3$ tolerant mulberry plants revealed that the morphological parameters i.e. sprouting/survival percentage, shoot length, rooting percentage, number of roots/shoot, and length of roots/shoot were greatly affected with increasing concentration of salts. The biochemical parameters and antioxidant enzymes were also altered. The proline content and antioxidant enzymes were greatly enhanced in Local cultivar. Protein and sugar content however were initially increased with increasing concentration of salt but at higher concentration it showed decrease. Same trend was found during *in vivo* studies. The protein profile of salt tolerant saplings (*in vitro*) showed some unknown proteins of the molecular weight 22.5 kDa, 32.1 kDa, 25 kDa, , 10 kDa and the expression of some proteins (14.5 kDa, 17.5 kDa) got increased. The screened salt tolerant (*in vitro*), well rooted saplings were hardened in jam bottles having cocopeat and solirite (2:1 w/w). The well acclimatized and developed saplings were then shifted to pots for further evaluation. The *in vitro* and *in vivo* screened salt tolerant saplings were evaluated for salt tolerance under field conditions and they showed better performance than the control plants.

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