EFFECT OF ABIOTIC AND BIOTIC FACTORS ON ACCLIMATIZATION OF TISSUE CULTURED PLANTS

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Abstract: The ultimate success of micropropagation on a commercial scale depends on the ability to transfer plants out of culture on a large scale at low cost and with high survival rate. Tissue culture raised plants possess certain characteristic features i.e Culture induced phenotype due to their acclimatization to special environment in vitro. The in vitro culture conditions result in the plantlets with altered morphology, anatomy and physiology. During field transfer the in vitro grown plantlets are unable to compete with soil microbes and to cope with environmental conditions. Except humidity all other physical factors like temperature, light intensity, water potential, water loss, hydraulic conductivity are low at in vitro conditions where as in ex vitro conditions they are high. The anatomical factors like stomata density, number, wax formation, cuticle formation, calcium amount in guard cells, chloroplast number are low at in vitro conditions where as in ex vitro conditions they are high. This review is focused on the effect of both abiotic (physical & chemical environment) and biotic (biotization) during acclimatization of plantlets to ex vitro conditions.

Keywords: In vitro and ex vitro growth, Biotization, Acclimatization, Biotic and abiotic factors, Micropropagation.

INTRODUCTION:

Micropropagated plantlets suffer high mortality when transferred from in vitro to ex vitro conditions. Plantlets should be slowly acclimatized to ex vitro conditions with high light intensity & low humidity conditions. In the hardening technique, attempts has to be made to economize the production process and simplify the technique with low sophistication, which could be adapted at village bio centers in order to extend the scientific technology from lab to land. The use of biofertilizer and biocontrol agents during acclimatization reduces the loss due to microbial infection of plants and this avoids the cost of maintaining strict and vigorous sterile conditions in shade house during primary hardening involving ex vitro rooting. Thus Biotization of micropropagated plants results in enhanced growth and survival during lab to land transfer. Bacterial inoculants containing Bacillus species was found to be effective in improving the survival of tissue culture raised tea plants against fungal attack during acclimatization. The micro cloned plantlets of Chlorophytum borivilianum [1] registered more than 95% establishment in soil following treatments with bioinoculants like Glomus aggregatum, Trichoderma harzianum and Piriformospora indica. Sahay and Verma [2] used P. indica as a potential agent for use in the acclimatization of micropropagated tobacco and brinjal. The plant endophytic bacteria and VAM fungi promote plant growth, resistance to pests and increased productivity. Hence research
must be focused on the control of both biotic and abiotic factors in order to increase growth, reduce cost & mortality in plantlets at the acclimatization stage.

**Effect of various abiotic factors on acclimatization:**

**Humidity**

During in vitro conditions plantlets were grown under relatively air-tight culture vessels where humidity is higher and irradiance lower than conventional cultures. These conditions result in the formation of plantlets of abnormal morphology and anatomy which causes high stomatal and cuticular transpiration rates when taken out of the culture vessels [3]. This typical in vitro anatomy can be prevented by increasing the vapour pressure gradient between the leaf and the atmosphere [4]. The plants that develop under lower relative humidity have fewer transpiration and translocation problems *ex vitro* and persistent leaves that look like normal leaves [5]. Leaves of Chrysanthemum and Sugar beet which were initiated and developed at relative humidity below 100%, displayed increased epicuticular wax, stomatal functioning and reduced leaf dehydration [6]. Humidity of the culture vessel can also be reduced by the use of dessicans, by coating the medium with oily materials, by using large culture vessels, by using special closures that facilitate water loss there by improving the internal structure of plantlets [7,8].

**Temperature & Light intensity**

The plantlets grown under in vitro conditions at low light intensity (1,200–3,000 Lux) and temperature (25 ± 2°C), when directly transferred to broad spectrum sunlight (4,000–12,000 lux) and temperature (26–36°C) caused charring of leaves and wilting of plantlets due to chlorophyll photo-bleaching and photo-inhibition [9]. To avoid this, the culture containers can be kept at room temperature for few days and later in the greenhouse with loose lids for 1-2 weeks. Micropropagated plantlets can be left in shade for 3–6 days under diffused natural light to make them adjust to the conditions of new environment. This helps in semi-hardening of plants and leads to shoot elongation. So this approach might decrease photo-inhibition which was the cause for the transient decrease in photosynthesis after transplantation. When *Nicotiana tobaccum* plantlets were acclimatized in two phases, first in green house (30-90µmolm⁻²s⁻¹) and then in open air (200-1400 µmolm⁻²s⁻¹) no photo-inhibition was found and photosynthetic capacity increased 46 days after transfer [10].

**Sucrose concentration**

Reports suggest that carbohydrate concentration influences the acclimatization process because plantlets switch from heterotrophic to autotrophic growth and any treatment before and after transfer increases the photosynthetic capacity of plants, which may improve plant establishment [11]. Alternatively, the sugar was reduced or completely eliminated from the medium, while the photosynthetic photon flux and the carbon dioxide concentration were increased. Kozai reported that the growth of plantlets *in vitro*
is often greater under photoautotrophic conditions than under heterotrophic conditions, provided that the in vitro environment is properly controlled for promoting photosynthesis [12]. Deng and Donnelly reported that sucrose in the medium promoted plantlet growth but depressed photosynthesis and reduced in vitro hardening [13]. Photoautotrophic growth of plantlets on medium without saccharides enables the development of fully functional photosynthetic apparatus at high CO2 concentration and irradiance [14]. Bhatt and Dhar reported that rooted microshoots of Bauhinia vahlii, preconditioned in different sucrose solutions before transferring did not enhance percent survival but resulted in better quality of shoots [15].

**Use of antitranspirants & growth retardants**

Acclimatization can be enhanced by hardening of plantlets in vitro (or) after transplantation by decreasing the transpiration rate using antitranspirants and growth retardants. Amaregouda et al. [16] found that stomatal resistance was more in plants treated with 1,500 ppm of phenylmercuric acetate. Smith et al. [17] reported that several growth retardants can be used in micropropagation to reduce damage due to wilting without deleterious side effects. Use of paclobutrazol (0.5–4 mg/l) in the rooting medium is reported to result in reduced stomatal apertures, increased epicuticular wax, shortened stems and thickened roots, reduction in wilting after transfer to compost, and also increased chlorophyll concentration per unit area of leaf. Mc Kinless et al [18] reported the formation of rhizome buds from aerial shoot auxiliary buds in L. rosea by adding paclobutrazol in the culture medium. Abscisic acid (ABA), plays an important role in plant water balance and in the adaptation of plants to stress environments. It is transported via xylem to the shoot, where it regulates transpiration water loss and leaf growth. Wardle et al., [19] were able to substantially decrease stomatal transpiration of micropropagated cauliflower plantlets with a leaf spray of 10 mM ABA on persistent leaves. Adie et al. showed that ABA is an essential signal for plant resistance to pathogens effecting jasmonic acid (JA) biosynthesis and the activation of defenses in Arabidopsis [20]. Role of abscisic acid on tolerance to abiotic stresses has also been studied by Aguilar et al., in Tagetes erecta in controlling leaf water loss and plant survival [21]. Hemavathi found that accumulation of ascorbic acid in transgenic potato plantlets increased tolerance to abiotic stresses [22]. Other leaf surface covering agents such as glycerol,paraffin and grease promoted ex vitro survival of several herbaceous species, but have not been evaluated over a long term or examined on woody species [23].

**Effect of various biotic factors on acclimatization**

Another major cause of high mortality of microshoots is their sudden exposure particularly the root system to microbial communities present in the soil as they do not possess sufficient resistance against the soil microflora. Biotization is an emerging dimension of micropropagation technique where the young in vitro raised plantlets are exposed to useful endophytes (both fungi& bacteria) which invade the tissues of plants there by promoting growth of the host plant & the formation of secondary metabolites related to plant defense[24].
Mycorrhization of tissue cultured plants provides advantage in terms of nutrient availability, soil pH, aeration and protection from water stress. Strawberry plantlets inoculated in vitro and ex vitro outperform non mycorrhizal plantlets in both fresh & dry weight gain [25]. The VAM- Glomus aggregatum reduces the osmotic potential and pre adapts the in vitro developed plantlets during transfer to Greenhouse . Estrada- Luna et al reported that mycorrhizal fungi enhances growth and gas exchange of micropropagated Guava plants [26] . Hao et al., reported that endophytic fungi promote growth of Ginkgo biloba by the formation of secondary metabolites related to plant defense [27]. Arbuscular mycorrhizal fungi form an abundant mycelium in the soil, absorb relatively immobile mineral nutrients such as phosphorus and transfer them to their host plant in exchange of carbon compounds. This results in modification of root system morphology and plant physiology which is in favour of the development of beneficial microbes in the rhizosphere and limits pathogenic microorganisms development [28]. 100 % ex vitro survival was reported from biotization of tissue culture raised tea plants with B.subtilis and P. corrugata [29]. Trivedi and Pandey also reported inoculation of microshoots of Picrorhiza kurrooa with B.megaterium recorded maximum survival (95%) of plants after transfer to soil [30]. Mathur et al. reported that microcloned plantlets of Chlorophytum borivilianum registered more than 95% establishment in soil after treatment with various bioinoculants. Senthil kumar et al., reported that endophytic colonization in rice tissue culture raised plants treated with Azorhizobium caulinodans strains ORS 571 & AA – SK-5 showed significant increase I protein content, total nitrogen and nitrogenase activity [31]. Vyas and his group reported root colonization and enhanced acclimatization of micropropagated Feronia limonia by P. indica [32]. Lavanya et al., reported successful acclimatization of neem microshoots using bacterial and fungal isolates [33]. Biotization of young raspberry plants during the acclimatization phase has proved to be useful in improving plant survival and increased tolerance to pathogen Phytophthora fragariae.var. Rubi [34]. Biological hardening of micropropagated Chlorophytum spps with P.indica and P.flourescens improved survival rate, nutrient acquisition and field performance. Suthar et al., [35,36] reported improved growth performance of micropropagated Terminalia bellarica when inoculated with Piriformospora indica. Biopriming of micropropagated Boswellia serrata Roxb plantlets with Piriformospora indica enhanced 75% ex vitro survival of plantlets. Sandal et al., [37] reported the use of liquid medium allows the close contact with the tissue which stimulates and facilitates the uptake of nutrients and phytohormones, leading to better shoot and root growth. Continuous shaking promotes lesser expression of apical dominance which generally leads to induction and proliferation of numerous axillary buds. From this it was understood that research must be focused on understanding the plant microbe interactions to optimize the beneficial plant-endophyte bacterial relationships.

**Summary and Conclusion:**

Plantlets should slowly be acclimatized to ex vitro conditions with high light intensity and low humidity. Acclimatization can be enhanced by hardening of plantlets in vitro (or) after transplantation by decreasing the transpiration rate using anti-transpirants and growth retardants or by increasing photosynthetic rate by elevated CO₂
concentration. Switching plantlets from heterotrophic to autotrophic mode by regulating physical and chemical environment, using liquid medium, bioinoculants reduces production cost and survival rate. Biotization helps to economize the production process and simplify the technique with low sophistication which could be adapted at Village Bio-centers in order to extend the scientific technology from laboratory to land. So, further research should be focused on the study of abiotic and biotic factors on acclimatization as the benefit of any micropropagation system can, however, only be fully realized by the successful transfer of plantlets from tissue-culture vessels to the ambient conditions found \textit{ex vitro}.

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\textbf{References :}


