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Article

Agric. Rev., 22 (1) : 65 - 67, 2001

BIOCHEMICAL BASIS OF ACCLIMATIZATION OF MICROPROPAGATED PLANTLETS - A REVIEW

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ABSTRACT

Exhaustive work done on *in vitro* propagation concerns mostly with providing chemical supplement to growth medium and amiable conditions to culture environment. Not much has been done on vital tissues which are cultured, in respect to their biochemical status. Hence, there is need to work on biochemical aspects to get an insight into *in vitro* differenciation and acclimatization phenomenon in order to make suitable amendments in the medium to increase efficiency of the *in vitro* propagation in economic terms. This review attempts to bring together the current informations on biochemical changes during *in vitro* differenciation and hardening of tissue cultured plant.

The ultimate success of micropropagation on commercial scale depends on the ability to transfer plants out of culture on a large scale, at a low cost and with high survival rate. The tissue cultured plants are often characterised by abnormal leaf morphology and anatomy, poor photosynthetic efficiency, mulfunctioning of stomata and marked deposition of epicuticular waxes. The heteroprophic mode of nutrition and poor mechanism to control water loss render micropropagated plants vulnerable to the transplantation shocks. Although considerable efforts have been directed to optimise the conditions for the *in vitro* stage of micropropagation, scant attention has been paid to understand the biochemical changes taking place during acclimatization. Consequently, the transplantation stage continues to be a major bottleneck in the micropropagation of many plants. In this review, an attempt has been made to assimilate the current knowledge on biochemical changes during differenciation and acclimatization of micropropagated plants.

Epicuticular Wax : Scant deposition of the proptective epicuticular wax on the surface of the leaves of the *in vitro* grown plants has been regarded as one of the most important factors responsible for excessive loss of water, leading to poor transplantation success (Sutter and Langhans, 1982). The chemical nature of the wax deposited on the surface of the leaves under *in vitro* conditions is also known to differ from that form under natural conditions, allowing excessive diffusion of water from *in vitro* formed leaves (Sutter, 1984). Measurement of the amount of epicuticular wax in the leaves of cultured plants revealed that the lack of crystalline structure was correlated with significantly less epicuticular wax compared to that on green house grown plants (Preece and Sutter, 1991). Epicuticular wax on cauliflower and cabbage leaves *in vitro* was only 25% of that plants grown in green house (Grout and Aston, 1977). The micropropagated *Leucaena leucocephala* have attained wax density comparble to that of field grown plants within 6-7 weeks of transplantation (Dhawan and Bhojwani, 1987). Newly formed leaves developed increasingly greater amount of wax and more complex crystalline structure over time (Sutter and Langhans, 1982). Similar results were noted in carnation (Sutter, 1979) and cabbage (Sutter, 1983). Differences in the rate of water loss by leaves at different stages of micropropagation have also been reported in *Malus domestica* (Brainered and Fuchigami, 1981) and *Prunus insititia* (Brainerd *et al.*, 1981).

In most of the studies discussed so far, there was a positive correlation between the amount of wax and survival of the plants when

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removed from culture. Sutter (1985) noted, however, that no such relationship existed in the foliage plants she studied and concluded that the quantity of epicuticular wax alone was not a good predictor of survival of micropropagated plantlets in the green house during acclimatization. Based on earlier studies reporting, the relationship between environment and formation of epicuticular wax, Grout and Aston (1977) theorized that, the lack of epicuticular wax on plants *in vitro* was due to high humidity within the culture vessel. Data supporting this theory were reported by Sutter and Langhans (1982) who produced glaucous cabbage plants with structured wax by reducing their relative humidity *in vitro* to 35%. Wardle *et al.* (1983) produced glaucous cauliflower plants with clearly greater amount of epicuticular wax by reducing the relative humidity *in vitro* with the used of a desiccant. Ziv *et al.* (1982) found that factors resulting in glaucous appearance of carnation shoot grown *in vitro* included increased concentration of agar and sucrose in addition to relative humidity was reduced. Other environmental conditions including light and temperature might also interact with relative humidity and affects the deposition of epicuticular wax. Transplantation rates were significantly higher in leaves of cultured plants lacking epicuticular wax compared with rates in green house plants (Wardle *et al.* 1983). Smith *et al.* (1990b) reported that paclobutrazol (0.5-4 mgl^{-1}) in the rooting medium resulted in increased epicuticular wax and reduction in wilting after transfer to compost. Ritchie *et al.* (1991) found that plants cultured on growth media supplemented with 10.0mM paclobutrazol displayed increased epicuticular wax, improved stomatal functioning and reduced leaf dehydration and reduction in plant height resulting higher *ex vitro* survival. Inclusion of paclobutrazol (0.25-4.0 mgl^{-1}) in the rooting medium progressively increased epicuticular wax in cultured citrus plantlets and increased *ex vitro* survival (Hazarika, 1999).

Carbohydrates Protein and Phenol : The process of hardening has been found to be associated with an altered balance in carbohydrate metabolism in white clover (Koster and Lynch, 1992). The concentration of sucrose was higher in hardy plants than in less hardy plants at the maximum level of hardiness (Svenning *et al.*, 1997). During dehardening, level of soluble sugar and sucrose decreased in all temperature treatments and there was a highly significant positive correlation between sucrose level and LT_{50} values during dehardening (Svenning *et al.*, 1997). A decrease in sucrose content during dehardening has also been reported for cabbage (Sasaki *et al.*, 1996). Sucrose content is positively correlated with the degree of hardiness in contrasting cultivars/ecotypes both during hardiness and dehardening. Estimation of total carbohydrates and proteins at various stages of culture showed interesting magnitude of variation (Sarma *et al.*, 1997). The level of total carbohydrates were highest in the explant and reduced at subsequent stages. Conversely, protein content was lowest in the explant and showed an increase of 2.5 times in poliferation stage. However, after completing rooting, the total carbohydrates and proteins have shown slight increase over multiplication stage in banana. Tissue type had significant effect on total sugar, aminoacid, protein and tennis (Upadhyay *et al.*, 1999). Antioxidants did not significantly influence the level of metabolities in the explants as well as their leaching in the medium. The content of phenols and OD phenols start increasing 120 hrs. after inoculation. Total sugars were higher in tissues cultured in liquid medium whereas amino-acid was higher in solid medium (Upadhyay *et al.*, 1999). Paclobutrazol treated plants contained higher total phenol in apple (Wang and Steffens, 1987a). Higher level of total phenol in paclobutrazol treated plants may be derived from the higher organic acid content

(Siegleman, 1964). Inclusion of paclobutrazol in the rooting medium increased starch and total phenol content in cultured plantlets (Hazarika, 1999) of citrus. Starch content in the leaves of shootlets grown at 5% sucrose was higher than growth with 3% and 1% sucrose. Plants grown in lower level of sucrose recorded lower reducing sugar and starch (Capellades *et al.*., 1991). The accumulation of starch was posttively affected by sugar feeding (Galzy and Compan, 1992, Paul and Stiff, 1993, Ticha *et al.*, 1998).

Proline : Proline is also known to confer crypotective effects (Guy, 1991, Kruuv and Glofcheski, 1999) and has been shown to be correlated with hardiness during hardening in wheat (Dorffling *et al.*, 1993) and to some degree in white clover (Sandli *et al.*, 1993). Accumulation of proline acts as a indicator for drought resistance. Induced proline accumulation due to water stress plays a protective role (Tyankova, 1969) during recovery process upon rehydration and performs a function of storing carbon and nitrogen without damaging the cells (Palfi *et al.*, 1974). On the otherhand, Hanson (1979) suggested that accumulation of proline is a mere symptom of deleterious response of the plant to the stress and does not have any adaptive value.

CONCLUSION

Understanding the physiological and morphological characteristics of tissues cultured plants and biochemical changes they undergo during hardening process should facilitate the development of efficient transplantation protools. Therefore, additional data must be gathered on these aspects by extending the studies to more species.

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