THE PHYSIOLOGICAL STATUS OF MICROPROPAGATED PLANTS - A REVIEW

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ABSTRACT

Tissue cultured shoots or plantlets share certain characteristic features that are inconsistent with the development under greenhouse or field conditions. The greenhouse and field have substantially lower relative humidities, higher light levels and septic environments that are stressful to micropropagated plants compared to in vitro conditions. The benefits of any micropropagation system can, however, only be fully realised by the successful transfer of plantlets from tissue culture vessels to the ambient conditions found ex vitro. The tissue culture plants are often characterised by abnormal leaf morphology and anatomy, poor photosynthetic efficiency, malfunctioning of stomata and a marked decrease in epicuticular waxes. This review will examine the anatomical, morphological and physiological factors that account for the fragility of cultured plants when removed from culture.

Plants tissue culture is an increasingly important aspect of plant biotechnology and this laboratory based technology has introduced exciting possibilities for the conservation of genetic resources by in vitro methods. The advantages of this methodology is that, as and when required, germplasm can be coiinally multiplied by routine micropropagation methods. Such a system, therefore, offers considerable potential for the conservation of important germplasm of crop and ornamental plants. The success of this approach, however, can only be effectively measured by the number of plants which are successfully transferred from the tissue culture laboratory to field conditions. The tissue culture conditions that promote rapid growth and multiplication of shoots often result in the formation of structurally and physiologically abnormal plants. Plantlets or shoots that have grown in vitro have been continuously exposed to a unique minimal stress and optimum conditions for plants multiplication. Plantlets develop within the culture vessels under low level of light, aseptic conditions, on a medium containing ample sugar and nutrients to allow for heterotrophic growth or in an atmosphere with high relative humidity. These contribute a culture induced phenotype that cannot survive the environmental conditions when directly placed in a greenhouse or field. In this review, an attempt has been made to discuss the major abnormalities in tissue culture plants that require attention at the time of transplantation to field.

Photosynthetic efficiency

\textit{In vitro} plants rely principally on sugar as a carbon source (Conner and Thomas, 1982) and \textit{CO}_2 uptake capability is low (Donnelly and Vidaver 1984a, Donnelly \textit{et al}, 1984). High source and salt containing media, often employed for raising cultures and poor light conditions seem to restrict photosynthetic efficiency of the leafy shoots. For \textit{in vitro} growth, a continuous supply of exogenous sucrose is required. Although, such plantlets may appear normal, they are unlikely to be active photosynthetically. This is because of exogenous supply of sucrose, which does not necessitate the normal development of photosynthetic apparatus. Using cauliflower plantlets growing \textit{in vitro} Grout and Aston (1978) measured \textit{CO}_2 uptake using radiolabelled carbon and gas ex-
change using an infrared gas analyzer. They found negligible carbon dioxide uptake under light by the plantlets grown in vitro. The regenerating plantlets also had lower chlorophyll content than 4 week old seedlings in greenhouse (Grout and Aston, 1977b). Similar results were obtained with strawberry plantlets grown in vitro. (Grout and Millam, 1985). Birch plantlets regenerated in vitro had approximately one third the photosynthetic rate compared to greenhouse grown seedlings (Smith et al., 1986). In Leucaena leucocephala, the leaves from cultured shoots and plantlets exhibited total lack of starch grains normally found in mesophyll cells of fields grown plants, thereby suggesting the photosynthetic inefficiency of the plants in cultures (Dhawan and Bhojwani, 1987). The low rate of photosynthesis in plantlets grown in vitro have been attributed to low ribulose biphosphate carboxylase activity (Grout 1988, Grout and Price 1987), low light and inadequate gas exchange (Kozai et al., 1987a, 1987b, Fujiwara et al., 1987, Desjardins et al., 1987). The presence of sugar in the medium enhances the level of photosynthetic component and photosynthetic performance (Kovtun and Daie, 1995). Koch (1996) observed that sugar feeding results in down regulation of photosynthesis and loss of proteins of photosynthetic apparatus. Ticha et al. (1998) found that high light acclimation potential of plants grown in vitro are especially prone to photoinhibition. Although some of the photosynthetic rate reduction under low light may be attributed to a temporary increase in respiration resulting from higher concentration of available carbohydrates (Sims and Pearcy, 1991), it has been found that the CO₂ concentration in the air tight vessel containing chlorophyllus plantlets is often as low as CO₂ compensation point during most of the photoperiod (Fujiwara et al., 1987), much lower than normal atmospheric CO₂ concentration of 345 μ mol mol⁻¹. Even in loosely capped vessels or vessels capped with gas permeable film, the concentration is often lower than 200 μ mol mol⁻¹ (Kozai et al., 1988; Kozai et al., 1991). It increase with time during the dark period up to 3000 to 9000 μ mol mol⁻¹ but decrease sharply with time to 100 to 200 μ mol mol⁻¹ within a few hours after the onset of the photoperiod and remains the same until the dark period begins. This reduction in CO₂ concentration during the photoperiod has been observed for different plant species (Fujiwara et al., 1988). The presence of sugar in the medium itself inhibits the photosynthetic activity of plantlets in vitro (Kozai, 1990). The literature showed that the net photosynthetic rates of rose (Capellades, 1990), potato (Dejardins et al., 1988) and Spathiphyllum (Watanabe et al., 1990) plantlets in vitro decreases with increasing sugar concentration in the medium.

The cuticle

The cuticle is a membrane composed of a cutin matrix together with embedded and surface waxes that covers above ground tissues of plants. The primary function of the cuticle is to limit transpirational water loss, water permeability through the cuticle is influenced primarily by the structure and amount of cuticular and epicuticular waxes (Martin and Juniper, 1970). Epicuticular wax on leaves of cauliflower (Grout, 1975), carnation (Sutter and Langhans, 1979) and cabbage (Sutter and Langhans, 1982) plants grown in vitro lacked the characteristic structure of wax on greenhouse grown plants. Low deposition of protective epicuticular wax on the surface of the leaves of the in vitro grown plants, has been regarded as one of the most important factor responsible for excessive loss of water, leading to poor transplantation success (Grout and Aston 1977a, Sutter and Langhans, 1982, Fuchigami et al., 1981; Hazarika, 1999, Brainerd and Fuchigami, 1981; Wetzstein and Sommer, 1982). Differences in the rate of water loss by leaves at different stages of micropropagation have also been reported in
Malus domestica (Brainerd et al., 1981), Solanum laciniatum (Conner and Conner, 1984). Sutter (1984) reported that wax on leaves formed in vitro had higher proportion of esters and polar compounds and significantly less long chain hydrocarbons than that on greenhouse grown leaves. Since polar compounds afford greater water permeability than long chain hydrocarbons, it was probable that the chemical composition of wax also contributed to water loss. Storey and Treeby (1994) observed depositon of crystalline epicuticular wax in the early stage of fruit development when albedo cells were beginning enlargement and differentiation in orange.

Stomata

Stomatal structure and functioning have been implicated in the water imbalance exhibited by micropropagated plants upon removal from culture. Wetzstein and Sommer (1993) observed that leaves of sweetgum plants cultured in vitro had significantly more stomata per unit area than the acclimatized or field grown plants. Fewer stomata were observed in the cultured plants of Prunus instititia (Brainerd et al., 1981), Malus domestica (Brainerd and Fuchigami 1981), Rubus ideaus (Donnelly and Vidaver, 1984b) and Leucaena leucocephalla (Dhawan and Bhojwani, 1987). Stomatal density of in vitro plants, measured per mm² was greater in apple (Blanke and Belcher, 1989) and rose (Capellades et al., 1990) when expressed as an index per number of epidermal cells. There was no significant differences in stomatal frequency among in vitro, acclimatized and greenhouse grown plants (Conner and Conner, 1984). Many researchers have reported that stomata of cultured plants shows a characteristic inability to close when first removed from culture. Stomata on excised leaves of micropropagated apple plants did not close when treated with ABA, CO₂ or mannitol immediately after removal from culture whereas stomata on plants that had been acclimatized responded as expected by closing immediately when treated (Brainerd and Fuchigami, 1982). Stomata on epidermal strips of chrysanthemum also did not respond to applications of ABA, whereas stomata on leaves of greenhouse grown and acclimatized plants closed in response to this treatment (Wetzstein and Sommer, 1983). Martin et al. (1988) reported that upto 80% of stomata of excised leaves of Prunus cerasus closed when removed from culture and placed in 45% relative humidity. Stomata of apple shoots remained open after removal from culture, but upto 78% of stomata of cherry and sweetgum plants closed after one hour of exposure to ambient condition on a laboratory bench (Sutter, 1988). One cannot generalized that stomata of all micropropagated plants are unable to close in response to chemical treatment, darkness or an increased water vapour pressure gradient. Sutter and Langhans (1982) stated that stomata on excised leaves of cultured cabbage plants close after the leaves were allowed to wilt for 5 minutes. Shackel et al. (1990) reported that stomata of intact apple shoots do have the ability to close in an atmosphere of 90% relative humidity. They showed that water loss of micropropagated plants immediately after removal from culture, measured in a specially adapted cuvette attached to a steady-state porometer decreased over a period of 24 hours to steady continuous rate. The size of root xylem vessel and leaf stomatal density showed direct relationship with plant vigour while the size of vascular bundle in roots and leaves, size of leaf xylem vessels and leaf epidermal cells did not show any appreciable trend for predicting plant vigour (Saroj et al., 1997). There was a predominance of normal stomata in leaves from normal plantlets and of abnormal stomata in leaves from vitrified plantlets (M'guens et al., 1993).

Anatomy

The poor mesophyll differentiation and weak vasculature of the leaves formed in vitro render the plants highly susceptible to
transplantation shock. The cauliflower leaves formed in vitro lacked or had poorly developed palisade cells as compared to transplanted plants (Grout and Aston, 1978). Similarly in 'Pixy' plum, the layer of palisade cells were shallow and the mesophyll air space were greater in cultured plantlet than in green-house plants (Brainerd et al., 1981). Such dissimilarities in leaf anatomy of in vivo and in vitro grown plants were also observed in Liquidambar styraciflua (Wetzstein and Sommer, 1982) and Rubus idaeus (Donnelly and Vidaver, 1984a). The changes in leaf anatomy that occurred during acclimatization were most in leaves that developed after the plants were removed from culture. Tissue culture plants of Rubus idaeus showed very poor development of mechanical tissues (collenchyma and sclerenchyma) in petiole, stem and root (Donnelly et al., 1985). Persistant leaves of strawberry plants became thicker due to enlargement of the palisade cells (Fabbri et al., 1986) but there was no change in numbers of layers of palisade cells or in amount of mesophyll air space, the connection between roots and shoots was shown to be incomplete in cauliflower plants, resulting in insufficient water transfer between the roots and shoots (Grout and Aston, 1977a). However, xylem appeared continuous and was functional between the roots and shoot in vitro rooted Prunus cerasus (Martin et al., 1988). During acclimatization leaves present as primordial leaves in vitro assumed intermediate characteristics between leaves grown in vitro and green-house or field leaves. Only new leaves that formed completely after removal from culture resembled greenhouse grown leaves (Wetzstein and Sommer, 1982; Donnelly et al., 1985).

Transplant phenotypes ex vitro: The persistant leaves

Leaves that developed in culture were retained after transplantation for a week to several months (Grout and Millam, 1985). Persistence depend on the plant species and the degree of environmental stress ex vitro. These persistant leaves increased in size slightly mainly due to cell elongation (Fabbri et al., 1986). In most cases stomatal function has been equated with closure (Brainerd et al., 1981, Sutter, 1988, Wetzstein and Sommer, 1982). The role of persistant leaves is very important issue. Photosynthetic capacity appears to very with plant species in culture and may determine the ex vitro contribution of persistant leaves. Cultured plants are divisible in photosynthetically non competent and competent species. In the non competent group cultured cauliflower and strawberry leaves that develop in culture deteriorated rapidly after transplantation (Grout and Aston 1978; Grout and Millan 1985). Such leaves have been referred to as storage organ or pseudo-cotyledonary tissues (Wardle et al., 1983). Non competence in strawberry has been attributed to irreversibly reduced levels of ribulose biphosphate carboxylase activity in leaves developed in the presence of sucrose. Leaves of competent species did not deteriorate rapidly after transplantation (Grout and Donkin, 1987). Persistant leaves of asian white birch (Smith et al., 1986) and red raspberry (Donnelly and Vidaver, 1984a) seem to fall in to the competent group. Red raspberry plantlets photosynthesizes at a low level after transplantation. The new leaves

The phenotypes of new leaves formed ex vitro varies with the species, the culture and transplant environment and the age of the transplant. New leaves of cauliflower (a non-competent species) that formed the second week after transplantation apparently exhibited green house control levels of CO₂ uptake (Grout and Aston, 1977). However, new leaves of red raspberry (a competent species) were transitional in the sense that weekly flushes of new leaves became progressively larger eventually with control type anatomy, functional stomata and improved CO₂ uptake capability (Donnelly et al., 1985). The number of transitional leaves produced by a transplant may
depend on the number of immature leaf buds formed in culture. The degree of transition of these leaves and how closely they resemble those of control plants is probably a reflection of the stage of development of leaf primordia when the plantlets was transferred from culture and the conflicting stresses imposed on leaf development by both the culture environment and the new ambient environment (Donnelly and Vidaver 1984a). Studies carried out with micropropogated strawberry plantlets showed that they did not fix enough carbon to sustain independent growth in the absence of added sucrose in the culture medium. (Grout and Price, 1987). Capellades et al. (1991) observed that the size and number of starch granules increased with the level of sucrose in the culture medium in Rosa multiflora. Strach content in leaves of shootlets grown with 5% sucrose was higher than those grown with 3% and 1% sucrose. Grout and Aston (1978) reported that formation of chlorophyll in culture was depended on an external supply sucrose whereas Pamplin and Chapman (1975) found that sucrose was often inhibitory to chlorophyll synthesis. We thank anonymous reviewer for this vice comments on the manuscript.

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REFERENCES


