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Review

Morpho-physiological disorders in in vitro culture of plants

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Abstract

The special conditions during in vitro culture results in the formation of plantlets of abnormal morphology, anatomy and physiology. Tissue culture conditions that promote rapid growth and multiplication of shoots often results in the formation of structurally and physiologically abnormal plants. They are often characterized by poor photosynthetic efficiency, malfunctioning of stomata and a marked decrease in epicuticular wax. Qualitatively also, the waxes present on the surface of the leaves of in vitro cultured plants may vary. The conditions under which most laboratories done tissue culture is high relative humidity and low light, no supplemental CO_2, high sucrose and nutrient containing medium may contribute to a phenotype that cannot survive the environmental conditions when directly placed in a greenhouse or field. Understanding these abnormalities is a prerequisite to develop efficient transplantation protocols. The present review summaries the major abnormalities in in vitro culture of plants and also highlight the current and developing methods that are satisfactory for acclimatization of in vitro cultured plantlets.

Keywords: Photosynthetic efficiency; Tissue culture; Stomatal function; Epicuticular wax; Anatomy; Culture-induced phenotype; Acclimatization

Contents

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1. Introduction

In vitro propagation has been extensively used for the rapid multiplication of many plant species. But the ultimate success of in vitro propagation 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 rates. In vitro cultured plants are generally susceptible to transplantation shocks leading to high mortality during final stage of micropropagation. Plantlets or shoots that have grown in vitro have been continuously exposed to a unique microenvironment that has been selected to provide minimal stress and optimum conditions for plant multiplication. Plantlets developed within the culture vessels under low level of light, aseptic conditions, on a medium containing ample sugar and nutrients to allow for heterotrophic growth and in an atmosphere with high relative humidity. Due to these conditions, in vitro plantlets can develop certain characteristic features that are inconsistent with the development under greenhouse or field conditions. The heterotrophic mode of nutrition and poor mechanism to control water loss render micropropagated plants vulnerable to the transplantation shocks when directly placed in a greenhouse of field. Although some aspects of culture-induced phenotypes are known (Brainerd et al., 1981; Debergh and Maene, 1984; Donnelly et al., 1984; Donnelly and Vidaver, 1984c; Sutter, 1985; Fabbri et al., 1986; Dhawan and Bhojwani, 1987; Grout, 1988; Ziv and Ariel, 1992; Hazarika et al., 2001b, 2002b; Lamhanedi et al., 2003) but the transplantation stage continues to be a major bottleneck in the micropropagation of many plants. Understanding the physiological and morphological characteristics of tissue culture plants and the changes they undergo during the hardening process should facilitate the development of efficient transplantation protocols. This article discuss the major abnormalities of in vitro cultured plants that accounts for the fragility of cultured plants and reviews the methods used to harden the plants for transplantation to soil.

2. Photosynthetic efficiency

High sucrose and salt containing media, low light level and the carbon dioxide concentration in the air in the culture vessel are some of the important limiting factors among various physical microenvironmental factors which influence photosynthesis of in vitro cultured plants (Fujiwara and Kozai, 1995; Jeong et al., 1995). For in vitro growth, a continuous supply of exogenous sucrose is required (2–3%) as a carbon source (Hazarika et al., 2000b, 2004; Hazarika, 2003a, 2003b; Wainwright and Scrace, 1989). High sucrose and salt containing media often employed for raising cultures and poor light conditions seems to restrict photosynthetic efficiency of leafy shoots. Although such plantlets may appear normal, they are unlikely to be actively photosynthesizing. This is because of the exogenous supply of sucrose, which does not necessitate the normal development of photosynthetic apparatus. Therefore in vitro cultured plants are either poor in chlorophyll content or the enzymes responsible for photosynthesis i.e. ribulose bisphosphate carboxylase (RubPcase) are inactive or absence altogether The low RubPcase activity may be due to presence of sucrose during the development of leaves (Grout and Aston, 1977a; Wetzstein and Sommer, 1982; Donnelly and Vidaver, 1984a).

The photosynthetic apparatus of regenerating cauliflower meristem culture is not sufficiently active to produce a net positive carbon balance in vitro. The chloroplasts have light stimulated electron transport comparable to control material but lower level of chlorophyll and RubPcase activity as shown in Fig. 1 resulting in correspondingly low carbon assimilation. This photosynthetic system does not develop further at transplanting as the in vitro foliage deteriorates rapidly, contributing little to net carbon uptake (Grout and Donkin, 1987). Studying cauliflower plantlets growing in vitro, Grout and Aston (1978) measured CO_2 uptake using radiolabelled carbon and gas exchange using an infrared gas analyzer. They found negligible carbon dioxide uptake in the light while the plantlets were in vitro. A negative CO_2 balance persisted up to 2 weeks after the plantlets had been transferred to soil in the greenhouse. The regenerated plantlets also had lower chlorophyll content than 4-week-old seedlings in a greenhouse (Grout and Aston, 1977b). Similar results were obtained with strawberry plantlets grown in vitro (Grout and Millam, 1985). After transplanting the strawberry to the greenhouse, most of the persistent leaves deteriorated rapidly and those that remained on the plants showed no increase in carbon fixation, indicating lack of development of photosynthetic competency. Birch plantlets regenerated in vitro had approximately one-

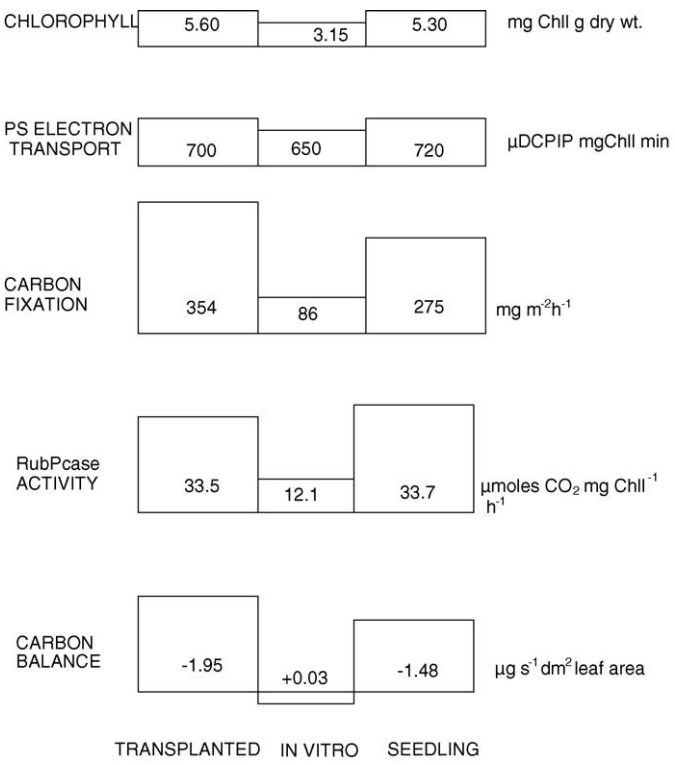

Fig. 1. Aspects of the photosynthetic system of cauliflower meristem cultures in vitro, compared to seedlings and plantlets established in soil (cultures 4 weeks after initiation, seedlings 2 weeks post-germination, transplants 4 weeks after transplanting—de novo foliage only) (Grout and Donkin, 1987).