Anatomical Aspects of IBA-treated Microcuttings of *Gomphrena macrocephala* St.-Hil.

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ABSTRACT

Gomphrena macrocephala *St.-Hil. (Amaranthaceae) is a perennial herb from the cerrado with medicinal properties and ornamental interest. Plants can be micropropagated through nodal segments, but acclimatization is difficult. The aim of this study was to establish a relationship between anatomical aspects of the roots and the acclimatization process. When cultures were supplemented with IBA, callus and thick and frangible roots appeared at the base of the microcuttings. A fragile vascular connection between roots and shoots was observed. Abnormal adventitious roots showing alteration in the vascular cylinder and hypertrophy of the cortical cells were also noted. These roots interfere in the transfer to extra vitrum conditions. When no growth regulator was used, no callus was formed, the adventitious roots were similar to those found in seedlings, and acclimatization could proceed. The results show that the origin and the structure of roots formed in the microcuttings play an important role in the acclimatization process and thus in the establishment of the micropropagated plants.*

Key words: *Gomphrena*, root anatomy, *in vitro* rooting, indol butiric acid, vascular connection.

INTRODUCTION

Gomphrena macrocephala St.-Hil. is a perennial herb of the Brazilian cerrado (Vieira, 1991). Medicinal properties of its tuberous root and of leaf infusions have been reported (e.g. Siqueira, 1987; Vieira et al., 1994). Figueiredo-Ribeiro et al. (1986) mentioned that besides the economic importance to food and pharmaceutical industries, the carbohydrates of the underground reserve organs may also play an important role in the physiology of these plants and in their adaptation to the cerrado environment. This is probably due to the availability of these compounds for organogenetic process (Appezzato-da-Glória & Estelita, 1995). The pink inflorescences of the plants provide an additional interest for this species as an ornamental plant. These features led to an increased interest in the popular use of this species and has caused an incorrect and overstressed harvesting in the cerrado.

In natural conditions plants of *G. macrocephala* require a long period to develop the underground organ. Sexual and vegetative propagation are difficult and impose limitation to plant multiplication in large scale (Mercier et al., 1992). Vegetative propagation through bud formation in root fragments can be achieved in many cerrado species (Rizzini & Heringer, 1966). Nevertheless, there are few anatomical studies showing how and where the adventitious organs are formed. Such information could be useful to understand the adaptive mechanisms of many plants to the cerrado environment (Appezzato-da-Glória & Estelita, 1995).

Mercier et al. (1992) observed that microcuttings of *G. macrocephala* formed roots only when auxin-like substances, such as naftalene-acetic acid (NAA) or indol-butyric acid (IBA) were added to the culture medium, as already reported for other species (Hartmann et al., 1990). Mercier et al. (1992) have not succeeded in the rooting response of microcuttings when no growth regulator was added to the medium culture or when indoleacetic acid (IAA) was supplemented. Using Mercier et al. (1992) protocol, we were able to propagate and root microcuttings of *G. macrocephala* in MS (Murashige & Skoog, 1962) medium but the only

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plants to survive more than three months were those rooted in the absence of growth regulators.

Adventitious root formation can be considered as a sequence of several biochemical (Gaspar, 1981) and histological (Lovell & White, 1986) events that can be identified by physiological changes caused by growth regulators. The changes occurring during adventitious root formation are well known. Auxin is required in the initial phases of the root formation, but it inhibits the subsequent growth and development of the root primordium; root-inhibiting responses caused by cytokinins occur specially during the first stage of the rooting process (De Klerk et al., 1990).

Adventitious root formation is a very complex multicellular event, often involving the reactivation of cell division in cells not directly involved in the formation of root meristemoids (Altamura, 1996). Two patterns of adventitious root formation on cuttings have been recognized in both herbaceous and woody plants. One consists of the direct development of the adventitious root primordia from cells associated with or in close proximity to the vascular system. The other is an indirect process in which adventitious root formation is preceeded by the proliferation of undifferentiated tissue (callus). Meristemoids with direct and indirect origins can form root primordia and roots (Altamura, 1996). In general, the direct pattern is found in most herbaceous plants (Hartmann et al., 1990, San-José et al., 1992).

The aim of this study was to analyse the origin of the root primordia and the anatomy of the adventitious roots formed in *G. macrocephala* microcuttings in the presence and in the absence of auxin-like substances in the culture medium, and to relate the data obtained to the ability of these microcuttings to acclimatize.

MATERIALS AND METHODS

Following the procedures of Mercier et al. (1992), seeds (achenes) of *Gomphrena macrocephala* St.-Hil. (SP167468, referred plant material according to Young et al., 1997) were collected from plants growing in a cerrado area (Itirapina, SP, 22°14'S and 47°49'W). The seeds were surface sterilized by washing with 70% aqueous ethanol for one minute and commercial chlorine bleach 20% containing a few drops of "Tween 20" for 20 minutes. Seeds were then washed three times in sterile distilled water and transferred to MS culture medium containing 30 $g.L^{-1}$ sucrose and 2.3 $g.L^{-1}$ phytagel (Sigma) for germination. The pH medium was adjusted to 5.8. Seedlings thus obtained and showing three nodes produced axillary shoots. The microcuttings obtained from these nodal segments were rooted in MS medium containing 10 $mg.L^{-1}$ IBA (Mercier et al., 1992) and in MS medium without growth regulator (control treatment). Each glass flask (350 ml) received 50 ml of culture medium and was sealed with a plastic lid. The *in vitro* cultures were maintained at 25 ± 2°C, under fluorescent and incandescent lamps (50.8 ± 6.6 $\mu mol.m^{-2}.s^{-1}$), and 12 h photoperiod.

Three samples from the base of the microcuttings were collected every five days from day 0 to day 45, from both rooting treatments. The samples were fixed in Karnovsky solution (Karnovsky, 1965), dehydrated in a graded ethanol series (10 to 100%), and then embedded in glycol methacrylate resin (Reichert-Jung). Transverse serial sections (5 μm), obtained in rotary microtome, were stained with toluidine blue (Sakai, 1973). Adventitious root samples were also cut by hand. The sections were stained with Iodin green and Congo Red according to Dop & Gautié (1928). Photomicrographs were taken from the sections prepared in histological slides.

RESULTS AND DISCUSSION

The anatomical structure of the base of the microcuttings on day 0 (Fig.1) showed a uniseriate epidermis with non-glandular hairs and stomata. The cortical parenchyma consisted of six layers of isodiametric cells, and the endodermis was not morphologically distinctive.

The central cylinder showed a uni or biseriate pericycle with smaller parenchyma cells when compared to the cortical cells (Fig.1, arrow). The vascular system showed a continuous procambium, already evidencing differentiated elements of the protophloem and protoxylem in some regions. The pith was well developed showing isodiametric parenchyma cells.

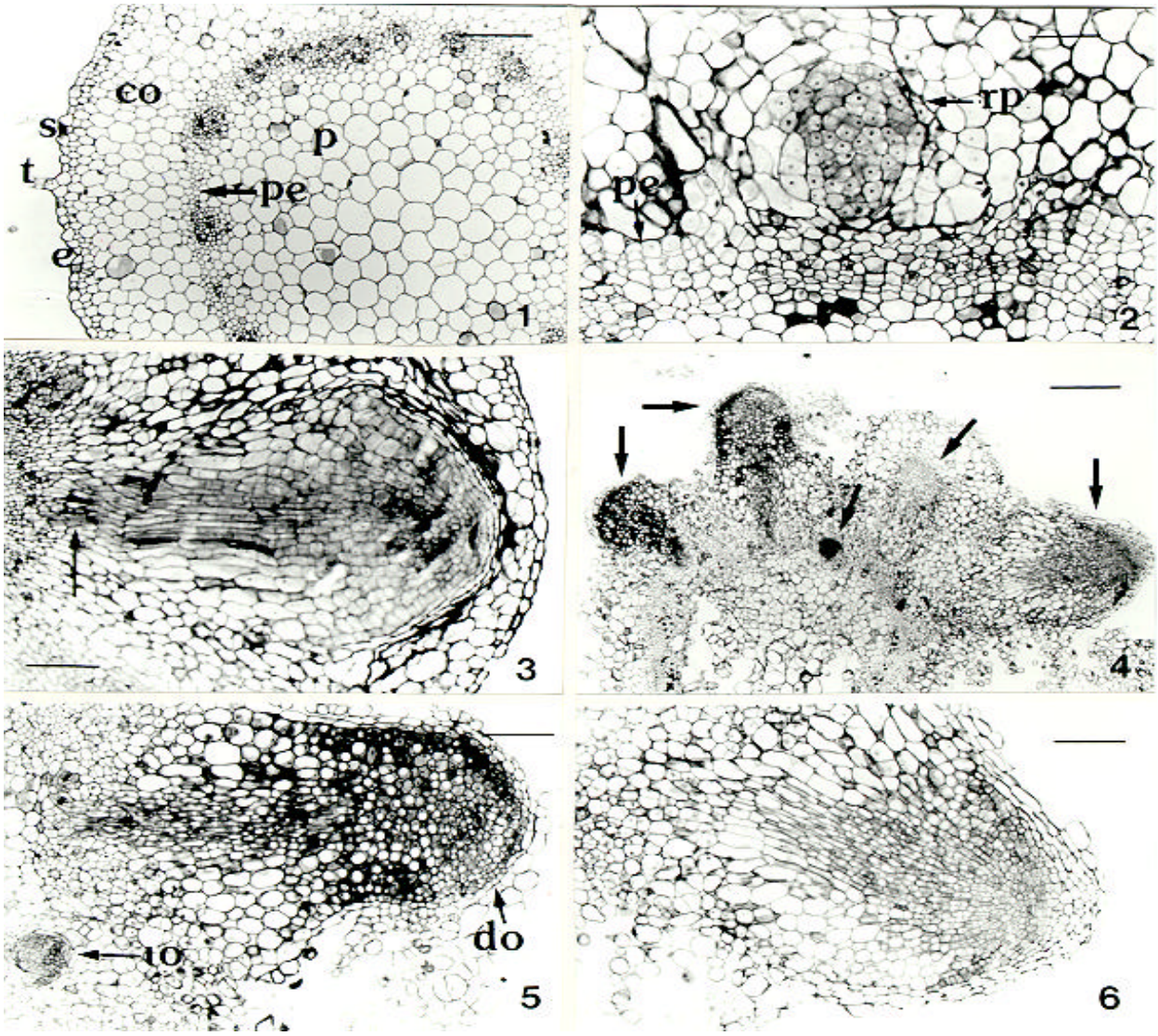

Figure 1-6 - Transverse sections at the base of *Gomphrena macrocephala* microcuttings during the rooting process. **1**: day 0. The arrow points to the pericycle (pe); epidermis (e); trichome (t); stomata (s); cortex (co); pith (p); (bar=204.6µm); **2**: day 10, development of a root primordia (rp) with direct origin, from the proliferating pericycle (pe) in the control microcuttings; (bar=53.2µm); **3**: day 10, development of the root primordia in control microcutting; the arrow points to the vascular connection (bar=204.6µm); **4**: day 10, adventitious roots of IBA-treated microcuttings in different developmental phases (arrows) (bar=552.4µm); **5**: detail from the same section showing the root primordia from direct (do) and indirect (io) origin, in the same microcutting (bar=204.6µm); **6**: another detail of fig 4, showing a root with indirect origin in the outer region of the IBA treated microcutting base (bar=204.6µm).

Ten days after the initiation of the rooting process, it was possible to observe morphological differences among the explants: some developed roots, while others showed only a swelling base. On day 10, microcuttings placed in the control medium presented root primordia formation generated from pericycle divisions (Fig.2, arrow) and thus in close association to the vascular cylinder. The development process of these root primordia was similar to those described by Esau (1977). They had an endogenous origin and were formed closely related to the vascular tissue, growing through tissues located around their point of origin and establishing a vascular connection with the microcutting (Fig.3).

The IBA-treated microcuttings presented (day 10) a modified anatomical structure compared to the control (Fig.4). In the various plans of sections, the epidermis and the cortex could not be

distinguished due to an intensive mitotic activity of the cortical parenchyma and pith cells (arrow), leading to callus formation. The vascular cylinder, although altered, could be visualised. The root primordia originated in two different ways: directly from pericycle divisions, similar to that observed in the control treatment, or indirectly, from meristemoids formed in the callus periphery (Fig.5). This modification in the origin of the adventitious roots has been already reported by Hartmann et al. (1990) and De Klerk et al. (1990). These authors have mentioned that root primordia could be formed directly from the original tissues of the cuttings, or indirectly, via newly formed callus. Altamura (1996) observed that both types of meristemoids originated directly or indirectly could form root primordia and roots. In microcuttings of *G. macrocephala*, the adventitious roots formed indirectly generally did not establish vascular connection with the microcuttings (Fig.6).

Friedman et al. (1979), studying rooting of *Phaseolus vulgaris* hypocotyl explants and Pluss & Schmid (1988), in *Populus tremula*, have verified that treatment with auxin-like substances did not modify the development of adventitious roots from the histological point of view. In the present study, however, it was verified that the origin of the root primordia could change in relation to the presence of an auxin-like substance. In fact, IBA could induce changes in some species, as reported by Hilaire et al. (1996). They observed that increasing concentrations of IBA may induce an increase in the number of roots in *Mussaenda erythrophylla*. These roots formed in auxin-treated microcuttings showed clear vascular connections with the main vascular tissue. Non-treated microcuttings, however, presented callus formation which, according to the authors, could delay the appearance of the few roots formed in these microcuttings.

M. erythrophylla microcuttings presented different behaviour than that of *G. macrocephala.* In this latter species, callus was formed only in the IBA-treated microcuttings (Fig.7-A). The roots, in this case, were thick and frangible (Fig. 7-A). Nevertheless, the adventitious roots formed in the control microcuttings (no IBA in the culture medium), were originated always in the pericycle layer, and were similar to the roots of seedlings (Fig.7-B).

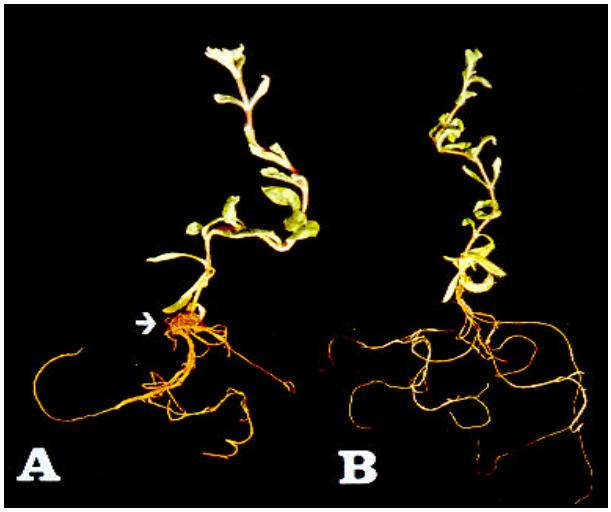

Figure 7 - *Gomphrena macrocephala* plants rooted *in vitro*. A. Plant derived from a microcutting rooted in MS medium supplemented with 10 mg.L^{-1} IBA; the arrow shows the presence of a callus. B. Plant derived from microcutting rooted in the absence of iBA, showing the development of thin roots and no callus formation.

The excellence of the root system, as mentioned by Gonçalves et al. (1998), is a key factor for the success of the acclimatization process. The incomplete vascular connection between shoot and roots of cauliflower rooted *in vitro* resulted in insufficient water translocation to the shoot, endangering the acclimatization of the new plants (Grout & Aston, 1977). The morphological differences observed in adventitious roots formed in the two culture conditions here used suggest that the roots have different anatomical structure that could change the performance of the rooted cuttings during acclimatization.

In fact, the anatomical structure of the adventitious roots developed in the microcuttings in the absence of IBA (Fig. 8 and 9) was similar to that found in seedlings *in vivo*, such as: uniseriated epidermis; cortical parenchyma constituted by four layers of isodiametric cells with wide intercellular spaces; endodermis presenting evident Casparian strips (Fig.9, arrow); central cylinder constituted by uniseriate pericycle and primary phloem strands alternated with primary xylem strands; protostelic roots. On the other hand, the anatomical structure of the adventitious roots formed in the IBA-treated microcuttings (Fig. 10 and 11) showed hypertrophied parenchyma cells and a malformed vascular cylinder, with primary xylem poles poorly defined.

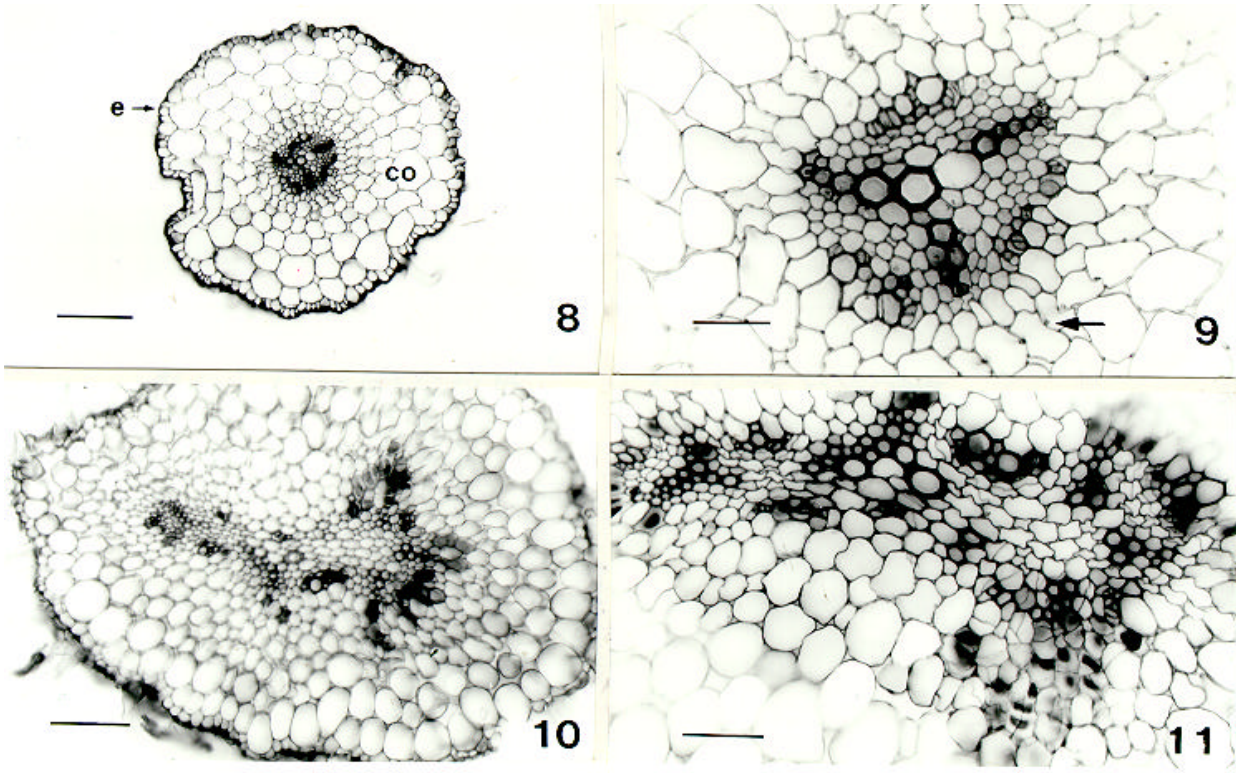

Figure 8-11 - Transverse section of adventitious roots formed in microcuttings with and without IBA 10 mg.L^{-1}. **8**: control treatment on day 20 (e=epidermis, co=cortex). Note that the root is protostelic (bar=204.6µm); 9: detail from fig. 8, showing the endodermis with Casparian strips (arrow) and the vascular cylinder (bar=53.2µm); **10**: roots of microcutting treated with 10 mg.L^{-1} IBA on day 20; it is possible to observe the root with hypertrophied cortical cells (bar= 102.3µm); **11**: detail of fig. 10, showing alteration of the root vascular cylinder due to the presence of IBA. The root, in this case, does not show the characteristic protostele organisation (bar=53.2µm).

The roots do not show the protostele characteristic (Fig.11). These data confirm the observations of Davies et al. (1982) about differentiated root primordia formed indirectly (from the callus tissue) and their poor further development.

The present study confirmed that *G. macrocephala* microcuttings could be propagated *in vitro*, as first observed by Mercier et al. (1992); but it also showed why the microcuttings rooted in culture medium supplemented with IBA, in the concentration used, did not survive for periods longer than three months after being transferred to *extra vitrum* conditions in the greenhouse. This occurred due to the fragile vascular connection and to the hypertrophy of the cortical cells found in this treatment that were probably jeopardising the performance of the roots during the acclimatization process. On the other hand, the microcuttings rooted in the absence of IBA presented roots similar to those found in seedlings and the rate of survival during the acclimatization process was superior to 90 %.

It can be concluded that the success of acclimatization in *G. macrocephala* microexplants lied on the origin and structure of the adventitious roots formed during micropropagation and thus on the formation of a normal anatomical structure of the roots with adequate vascular connections.

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RESUMO

G. macrocephala St.-Hil. (Amaranthaceae) é uma espécie herbácea perene do cerrado com propriedades farmacológicas e ornamentais. Pode ser micropropagada através de segmentos nodais, mas as novas plantas sobrevivem por pouco tempo. O presente estudo visou relacionar os efeitos de IBA na anatomia das raízes e a resposta de aclimatização das plantas micropropagadas. Na presença de IBA, a base das microestacas forma calo e raízes adventícias espessas e quebradiças, dificultando a transferência para as condições *extra vitrum*. Ocorre uma frágil conexão vascular entre o sistema radicular e a microestaca, além de raízes adventícias mal formadas, com alterações no cilindro vascular e hipertrofia das células corticais. Na ausência de IBA, as microestacas não formam calo, as raízes adventícias são morfologicamente similares às de plantas obtidas de sementes e o processo de aclimatização é bem sucedido. Os resultados indicam que as características das raízes formadas podem vir a comprometer a aclimatização e o estabelecimento das novas plantas.

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