Some Biochemical Differences between Juvenile and Young Olive Plant Material

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Abstract

The protein profiles of juvenile and young olive plant tissues have been compared by SDS-PAGE analysis. Differences were found between the juvenile and young leaves extracts corresponding to the expression of 3 bands of 29, 35 and 63 kD which were partial or totally absent in the sucker leaves extract. This suggests that suckers are not a juvenile material. On the other hand, the polypeptide bands associated to juvenility in the olive tree were also found in the flower tissue, indicating that the flowers may be at, or close to the juvenile phase.

INTRODUCTION

Researchers have tried to identify biochemical markers, which could help to characterize the adult or juvenile phase of the plants. To do this, juvenile and adult tissues have been compared and some differences in the protein expression between such materials reported. Nevertheless, in most occasions the juvenile material used for this kind of work is not really juvenile but rather rejuvenated tissues of adult origin as the shoots emerging from the base of the trunk of mature trees (“suckers”). Though rejuvenated plants can acquire some characteristics of juvenility, this does not mean in fact that a complete reversion of the adult phase has been attained.

In the case of the olive tree, work on biochemical juvenility markers is very scarce (Tazzari et al., 1995). The aim of this work was to compare the protein profiles of juvenile and young olive plant tissues by SDS-PAGE analysis.

MATERIAL AND METHODS

Plant Material

The plant material used included leaves of juvenile seedlings obtained through in vitro embryo germination and cultured in vitro or in pots, leaves of basal shoots (“suckers”), growing from the base of adult trees and flower tissue collected before anthesis. All the plant material was cv. Manzanillo.

Electrophoretic Analysis

Proteins were extracted as described elsewhere (García, 1999). Protein concentration of the extracts was determined according to Bensadoun and Weinstein (1976), with BSA as standard. Samples containing equal amounts of protein (60 µg of protein) were subjected to SDS-polyacrylamide gel electrophoresis as described by Laemmli (1970). Electrophoresis was performed at 35 mA until the marker dye reached the edge of the running gel. The gel slab was then stained with 0.25% Coomasie brilliant blue in 10% acetic acid and 50% methanol for 30 min. The gel was destained by repeated washing in 10% acetic acid and 40% methanol. The molecular weights of the protein bands were calculated by comparison with standards with molecular weights ranging from 14.4 kD to 116.3 kD.

RESULTS AND DISCUSSION

The protein profiles of the seedling leaves extracts (Fig. 1; lanes 1 and 2) were essentially identical despite of the different culture conditions of the plants (in vitro or in pot) but they showed differences when compared with the band pattern of the sucker.
leaves extract (Fig. 1; lane 3). These differences were related to three bands corresponding to molecular weights of 29, 35 and 63 kD which were partial or totally absent in the sucker leaves extract. In previous works we had reported that at least 2 of these bands (29 and 63 kD) were related to the juvenility of olive tissues since they were much less marked in the adult than in the seedling tissues (García, 1999).

Nevertheless, the protein pattern of the sucker leaves (Fig. 1; lane 3) show some characteristics that are different not only from the juvenile tissues but also from the adult ones (García, 1999). These features suggest that suckers are a material in a particular physiological situation and agree with the results of Tazzari et al. (1995). These authors compared the protein profiles of sucker and crown branch leaves of cv. Leccino trees and found that the sucker leaves lacked a 66 kD band.

Thus, olive suckers, although sharing some characteristics with the juvenile material, cannot be considered juvenile since it is an agamic tissue raised from ovuli existing in the trunk of adult trees. Then, they should rather be considered as a young or rejuvenated tissue and the fact of the differential electrophoretic profile in respect to the juvenile tissue seems to support this idea.

In most of the works where differential phase proteins have been reported, authors, rather than comparing adult and juvenile material, have compared adult tissues with tissue of adult origin that had been rejuvenated (Bon and Monteuuis, 1991; Huang et al., 1992) or even basal shoots with the same origin than the olive suckers (Amo-Marco et al., 1993; Bon et al., 1994). In this sense, it is particularly interesting the work of Bon (1988), who found a 16 kD membrane-associated protein (J16) in seedlings and rejuvenated mature tissue of *Sequoiaadendron giganteum*, which was absent in the mature tissues.

Our results show that the band patterns of the olive sucker leaves are different from those of real juvenile plants and indicate that the basal shoots are not in juvenile stage. In general it is quite difficult to distinguish between juvenile plants and rejuvenated or reinvigorized ones. Upon rejuvenation treatments, the plants can attain some characteristics of juvenility such as higher morphogenetic or rooting competence, but that does not mean a real and complete reversion of the adult phase (Bonga, 1982).

To date, complete reversion to juvenile phase is only achieved through sexual process or by somatic embryogenesis (Bonga, 1987), although this last is exceptional in woody species (Tulecke, 1987).

The flower tissue extract showed less quantity of a band corresponding to a molecular weight of 55 kD and lacked another band of 14 kD. These bands were identified, according to the literature, as the large and small subunits of RuBisCo, characteristic of photosynthetic tissues (Hubbs and Roy, 1992). It is worthwhile to point out that the 29 and 63 kD bands associated to juvenility were also found in the flower tissue extract with similar intensity than in the juvenile tissues. Flowers develop from adult vegetative meristems and through meiosis and fertilization result in whole juvenile embryos. Then, the epigenetic change from mature to juvenile can take place in floral tissues before the embryo is formed (Meyer, 1983). Thus, the fact of finding the juvenile-associated polypeptides in the flower tissue with a similar level of expression to that of the juvenile leaves suggests that flowers are at, or close to the juvenile phase.

**Literature Cited**


Fig. 1. Electrophoretic patterns of: (1) leaves of in vitro cultured juvenile plant; (2) leaves of juvenile plant grown in pot; (3) sucker leaves; (4) flower tissue before anthesis. Arrows indicate the 63, 35 and 29 kD peptides which show differential expression between juvenile plant and sucker leaves. An 8-15% polyacrylamide gradient running gel was used; 60 µg of protein were applied to the gel.