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ScienceDirect
Plant Science 174 (2008) 502–509
PLANT SCIENCE
www.elsevier.com/locate/plantsci

# Influence of graft incompatibility on gene expression and enzymatic activity of UDP-glucose pyrophosphorylase

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Received 4 October 2007; received in revised form 10 December 2007; accepted 21 January 2008
Available online 9 February 2008

**Abstract**

Graft compatibility response is a complex mechanism with a wide range of different physiological, biochemical, and anatomical interactions. However, little is known about the biochemical basis for incompatibility and the molecular mechanism involved in this response. In the present paper, the influence of grafting on the protein pattern in the incompatible heterograft combination of apricot (*Prunus armeniaca* L. cv. Moniqui) on plum (*Prunus cerasifera* × *Prunus munsoniana* cv. Marianna 2624) and their homograft combinations using callus fusion *in vitro* was investigated. A decrease in a 55 kDa protein band at the stock of the incompatible union was observed during the 2 weeks after grafting. Using SDS-PAGE combined with MALDI-TOF, this protein was identified as an UDP-glucose pyrophosphorylase (UGPase) with a high homology to other higher plants. In addition, the UGPase mRNA transcript and enzyme activity were also diminished in the stock from the incompatible combination at 10 DAG. Furthermore, genotypic differences in UGPase activity were observed between the apricot cultivar 'Moniqui' and the plum rootstock 'Marianna 2624'. These results suggested that this protein could be related to the graft compatibility/incompatibility responses.


*Keywords:* Graft compatibility; *In vitro* callus unions; Protein profile; *Prunus* spp.; UGPase

## 1. Introduction

Plant grafting is a widely used means of fruit tree propagation and growth control that is of considerable importance in the adaptation of interesting cultivars in appropriated areas. The formation of a successful graft is a complex biochemical and structural process that includes an immediate wound response, callus formation, establishment of new vascular tissue, and formation of functional vascular system between both partners. However, when divergent genotypes are grafted, they do not always constitute a successful union and show their disagreement in the form of incompatibility. In the case of apricot combinations grafted on some *Prunus* rootstocks, this incompatibility is associated with mechanical weakness and breakage of the union due to morphological and physiological alterations in the process of grafting [1,2].

Whereas an increasing number of studies have led to the observation of these morphological and physiological changes between compatible and incompatible unions in herbaceous [3,4] and woody plants [5,6], there is limited information on the biochemical basis for incompatibility and the molecular mechanism involved [7,8]. Different compatibility/incompatibility mechanisms have been proposed based on the interaction of cells of different herbaceous species with special reference to the possibility that a phenomenon of cellular recognition must occur as part of adhesion and the events that follow in successful graft union formation [9–11]. Convincing experimental evidences suggest that in the graft *Cucumis*/*Cucurbita*, changes in protein banding may be due to polypeptides migrating symplastically across the graft union via the connecting phloem [12] in such a way that translocation of signalling molecules, such as polypeptides in the phloem, could be significant in cell recognition and compatibility between the graft partners. Likewise, it has been reported that the adhesion between cells of the scion and rootstock is performed by beadlike projections in the cell walls composed of pectins,

*Abbreviations:* DAG, days after grafting; DAW, days after wounding; MALDI-TOF, matrix-assisted laser desorption ionization time of flight; RT-PCR, reverse transcriptase-PCR; UGPase, UDP-glucose pyrophosphorylase.

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doi:10.1016/j.plantsci.2008.01.015

proteins, carbohydrates and fatty acids [13], which may act as binding or cementing cells with an active role in cell recognition and the successful merging of tissues of the graft partners.

Despite the fact that the callus formation occurs as a wound reaction and it is found in compatible and incompatible grafts, the development of future vascular connections depends on this cell to cell contact when the callus first touch. Here, the possibility of detecting differences in compatible and incompatible unions at an early stage of the graft response is explored by callus fusion *in vitro*. The use of tissue culture techniques may be an interesting approach to study compatibility–incompatibility phenomena provided there occurs a good correlation between *in vivo* and *in vitro* systems. In this sense, previous studies have shown that *in vitro* graft systems match very closely to *in vivo* graft systems [2,6,14] and that the strong interdigitation of callus cells observed *in vitro* is similar to that recorded *in vivo*.

The aim of the present study was to determine whether there are differences in the protein profile between compatible/incompatible apricot combinations due to the influence of grafting. Furthermore, a protein band differently expressed on homografts/heterografts was identified by means SDS-PAGE and MALDI-TOF combined technique. In order to gain more insight into the regulation of the graft development, analyses of expression and protein activity were performed. The involvement of this protein in the complex process of union formation is discussed.

## 2. Materials and methods

### 2.1. Plant material and grafting (obtaining callus tissue of plum and apricot)

Callus tissue of the apricot (*Prunus armeniaca* L.) cv. Moniqui ('MO') and from the interspecific plum rootstock (*P. cerasifera* × *P. munsoniana*) cv. Marianna 2624 ('MN') were used to establish either homografts (MN/MN and MO/MO) or heterografts (MO/MN). For callus obtention, stem fragments of each cultivar were cut into 0.5 cm segments under sterile conditions and placed *in vitro* with a modified Murashige and Skoog medium [15] supplemented with indole-3-butyric acid (IBA, 0.5 μM), 6-benzylaminopurine (BA, 0.5 μM), 3% sucrose and 0.7% Difco-Bacto agar. Cultures were maintained in a growth chamber at 22 ± 2 °C and cool white fluorescent tubes provided a 16-h photoperiod of 17-μmol $m^{-2}$ $s^{-1}$. Subcultures were made in monthly intervals.

The graft combinations were established by placing two callus pieces upright on the agar layer under sterile conditions in the same culture medium and conditions as used for callus initiation. The graft combinations were sampled 5 and 10 DAG. Ungrafted and wounded calli served as control.

### 2.2. Protein extraction

The contacting surfaces of the two callus masses were prepared separately for protein extraction (Fig. 1). Callus tissues were frozen in liquid nitrogen and the frozen tissue was ground to a fine powder with a precooled mortar and pestle. Total protein was extracted with 100 mM Tris–HCl pH 8.2, 5 mM EDTA, 2 mM PMSF, 1% SDS, and 2-β mercaptoethanol. The cellular debris was pelleted at 15,000 rpm for 10 min, at 4 °C, and the supernatant was collected. To ensure equal loading of samples, an aliquot of this crude protein extract was used for protein determination using the Bio-Rad protein assay based on the method of Bradford [16] with bovine serum album (BSA) as standard.

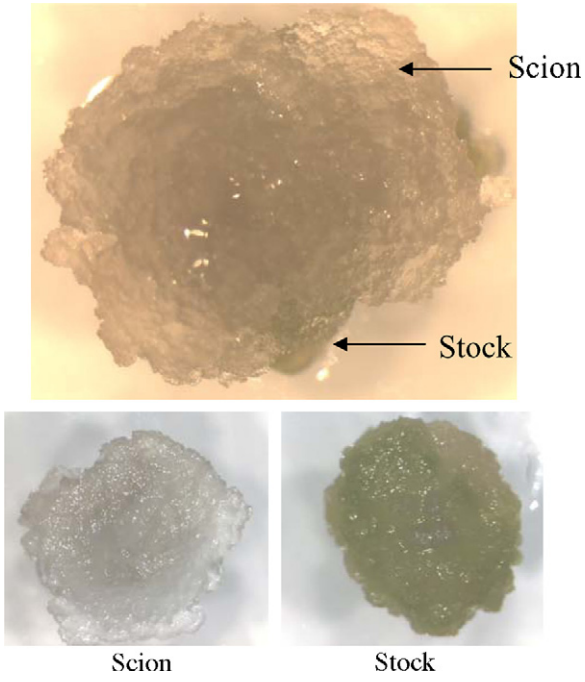


Fig. 1. Callus union showing an apricot/plum heterograft (MO/MN) established *in vitro* where the two components are prepared separately for protein extraction.

### 2.3. SDS-PAGE

Total protein was separated by SDS-PAGE in 4% stacking gel and 15% resolving gel according to the procedure of Laemmli [17]. Experiments used 0.75-mm gels with dimensions of 8.3 cm × 7.3 cm (miniprotean II-System Bio-Rad). Each well of the gel was loaded with 24 μg total protein per lane and after electrophoresis, the gels were stained with Comassie brilliant blue R-250 overnight. The SDS-PAGE analysis was performed three times with similar results.

### 2.4. Mass spectrometry

The callus proteins were collected from ungrafted calli 'Marianna 2624', fractionated by SDS-PAGE in 15% polyacrylamide gels, and stained with Coomassie blue. The putative UGPase band (approximately 55 kDa) was excised from the gel and washed several times with sterilized water. Peptide mass mapping by MALDI-TOF mass spectrometry was performed by the CNB proteomic service (Centro Nacional de Biotecnología, CSIC, Madrid) and peptide mass fingerprints were