Scientia Horticulturae 124 (2010) 349–353

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

RFID microchip internal implants: Effects on grapevine histology

A. Luvisi [a,*], A. Panattoni [a], R. Bandinelli [b], E. Rinaldelli [b], M. Pagano [b], B. Gini [c], E. Triolo [a]

[a] Department of Tree Science, Entomology, and Plant Pathology "G. Scaramuzzi", University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy
[b] Department of Crop, Soil and Environmental Science, University of Florence, Viale delle Idee 30, 50019 Sesto Fiorentino (FI), Italy
[c] Vivai New Plants di Barbara Gini, Via Togliatti 41, 56040 Cenaia (PI), Italy

ARTICLE INFO

Article history:
Received 28 September 2009
Received in revised form 17 December 2009
Accepted 12 January 2010

Keywords:
Nursery
Radiofrequency identification
Traceability
Pith
Grapevines

ABSTRACT

Interesting applications for traceability in agriculture have recently been developed using radio-frequency identification (RFID) technology. A preliminary report of survival and growth in grapevine suggested pith not only as optimal microchip localization within the plant, but only continuous monitoring of performances, supported by histological observation of tissues around microchips, can validate this approach as a long-term strategy for grapevine identification. In this study, histological assays of grapevine plants are reported, considering different strategies in RFID marking. Microchip insertion after direct drilling of pith from a distal cut on rootstocks did not show any differences in tissue status compared to control, and this can be adequately correlated to an absence of effect in plant growth. Conversely, a "U" cut performed laterally on the rootstock to insert the microchip, which involved tissues from bark to pith, caused development of callus tissues, restoring transversal continuity, but with a partial loss of functionality in terms of open vessels. This phenomenon can be considered permanent damage to plant vascular function, but with limited extension.

1. Introduction

Plant traceability in grapevine nurseries is closely linked to health and qualitative characteristics, and is currently reported on labels as required by European Union regulations (68/193/CEE). These traditional labels represent the only tool available for commercial grapevine identification as they contain the datasheet for the material. These labels can pose some limitations, for example their colour can fade and written information can be lost over time, labels can fall off or be torn, and often the space available is not sufficient to contain the information. Moreover, labels do not refer to every single plant, but rather to groups of grapevines joined by laces.

These limitations suggest that development of new, more plant-specific tools for identification, capable of containing much more data could be useful. There has been increased interest regarding traceability in agriculture, thus implementation of supporting technology for this purpose, linking all aspects of the production line, could have positive effects for food safety and for protection of the breeder's right. Interesting applications have been widely developed using radiofrequency identification (RFID) technology in animal identification systems (Artmann, 1999; Jansen and Eradus, 1999), following a reduction of technology costs (CNIPA, 2007) and worldwide spread (Das, 2005). Recently, application of this technology was studied in plants, considering *Prunus* spp. (Bowman, 2005), *Citrus* spp. (Grieco et al., 2006) and *Vitis* spp. (Luvisi, 2007; Triolo et al., 2007; Bandinelli et al., 2009), with different techniques and microchip allocations. However, compared to the widely described effects of RFID application in animals, little information about the impact of RFID on plant development, growth and health status is available, especially with regard to microchip insertion in plants.

A preliminary report in grapevine (Triolo et al., 2007) suggests pith as optimal microchip localization within the plant. Microchip insertion in grapevine pith can be obtained by two different procedures, and each method has its own advantages, i.e. it can be performed during grafting phase without additional wound to the plant (defined as procedure A), or with a more easily mechanized procedure (defined as procedure B). Data about the impact of microchip implantation on growth in grapevine were reported on nursery stage (Luvisi, 2007; Triolo et al., 2007) and during two years of vineyard farming (Bandinelli et al., 2009). In these reports no decrement in growth was recorded for procedure A, and limited effects were noted following procedure B. However, the microchip represents an alien item inside living tissues, and potential effects due to space occupation, materials, radiofrequency waves and

Abbreviation: RFID, radiofrequency identification.

* Corresponding author at: Department of Tree Science, Entomology and Plant Pathology "G. Scaramuzzi", University of Pisa, via del Borghetto 80, 56124 Pisa, Italy.
E-mail address: aluvisi@agr.unipi.it (A. Luvisi).

0304-4238/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.scienta.2010.01.015

wounds relative to insertion need to be evaluated before long-term predictions can be made. Thus, a histological approach could support the validation of these procedures, taking into account the differences between the methods and their impact on plant morphology.

In this paper, histological assays on grapevine plants are reported, considering different strategies in RFID marking.

2. Materials and methods

2.1. Experimental design

The experimental trials reported were conducted at a nursery specialized in grafted-cutting productions. 4 replicates of 25 grapevine plants per treatment were established following a random layout (300 plants in total). 5 plants from each replicate were random collected for histological and growth assay.

2.2. Plant material

Trials involved grafted cuttings of *Vitis vinifera* cv. Sangiovese (clone I-SS-F9-A5-48) grafted in 2007 on rootstock 1103 Paulsen (*Vitis berlandieri* × *Vitis rupestris*), supplied by the Associazione Toscana Costitutori Viticoli (TOS.CO.VIT., San Piero a Grado, Pisa, Italy, http://www.toscovit.it).

2.3. Electronic material

Transponder glass TAG RFID were used, 2.1 mm diameter and 12 mm length, working at a frequency of 125 kHz (InterMedia Sas, Forlì, Italy, http://www.RFID360.net). TAGs were electronically read by way of a 14-length identification number using a Card Flash reader connected by SD slot to palm-PC (Dell Axim X51) able to identify the microchips from 20 cm distance. Data recovery was performed using a palm-PC containing a database software specifically programmed using SprintDB Pro (http://www.kaio-ne.com) for storing the data of each plant.

2.4. Methods of microchip implantation

TAGs were inserted inside pith of rootstock (average cutting length 30 cm, average diameter 1.5 cm) in April 2007, following two different procedures (Fig. 1), that are deposited for patent registration (RM2009A000271). The first one (A) involves microchip insertion after 4-cm depth direct drilling of pith from distal cut of rootstock just before omega grafting. Then, the microchip was located 3 cm below grafting point. The second procedure (B) were performed after omega grafting, and it consists in a "U" cut performed laterally on the rootstock 3 cm below the grafting point, by a designed machine by Authors (Bandinelli et al., 2009), involving tissues from bark to pith. After each procedure, the microchip was located inside the pith, and cut tissues were manually reassembled. Unmarked omega grafted plants were used as control.

2.5. Image analysis and histological observation

Measurements of vascular tissue area by image analysis were made on samples collected in January 2008 (two years old plants) and January 2009 (three years old plants), on fresh trunk sections in proximity of the microchip location (starting from 3 cm below graft point), at approximately mid-length of the microchip ("height 0"), 3 mm higher ("height 3") and 3 mm lower ("height −3"). In unmarked plants, sections were taken at the same height of marked ones. Vascular tissue area was calculated using software for image analysis (Cerri et al., 1993), measuring total vascular tissue area and non-necrotic vascular tissue area.

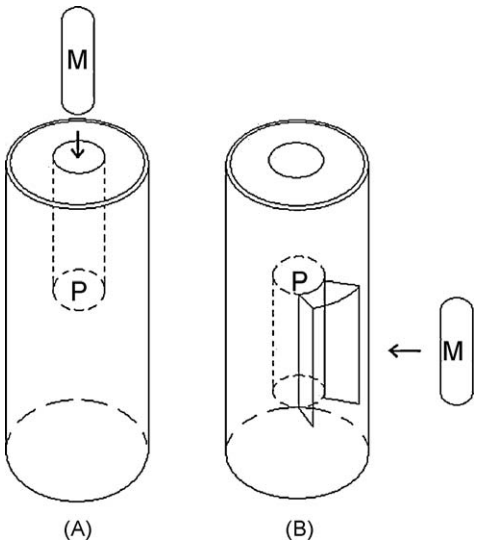

Fig. 1. Schematization of microchip insertion: (A) procedure A; (B) procedure B; (M) microchip; (P) pith.

For histological observation, fresh transversal sections (20-μm thick) were made with a rotary microtome (Reichert-Jung, Autocut 2040, Osterreich) and stained with Toluidine Blue O (Sigma–Aldrich Corporation, USA); sections were immediately observed with a light microscope (Leica, Wetzlar, Germany).

2.6. Growth assay and microchip test

No pruning was performed during 2007, while two branches were allowed to develop during 2008. For plant growth, mean relative growth rate (MRGR) was calculate using equation reported by Kolb and Steiner (1990), with one sampling period of 90 days, calculated since shoots start growing. The dry weight was performed drying the shoots in an oven set at 100 °C overnight, and cooling shoots in a closed plastic bag.

The RFID system was tested evaluating TAGs reliability, performing a microchip reading before sampling for histological observation.

2.7. Data analysis

The effects of treatments were compared, even considering differences between years of sampling, by analysis of variance in a random design. The Duncan's multiple range test at 5% level (Duncan, 1955) was calculated in order to compare treatments for functional vascular area, characterized by undamaged vessels and in which xylem rays are developed as control, and for growth parameters. Effects of treatments were expressed as functional vascular tissue area out of total (%) and MRGR (mg day^{-1}). Data in percentage were normalized by arc sin square root transformation (Camussi et al., 1995).

3. Results

3.1. Image analysis

In Table 1, mean functional vascular tissue areas (%) are reported at 3 heights in 2008 and in 2009, considering each treatment. Since 2008, no effects due to procedure A were registered compared to control considering each height. Conversely, procedure B causes a reduction in functional vascular tissue area in proximity to microchip and above it, whereas below microchip allocation any alteration was not observed. Anyway, non-necrotic