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Electronic identification-based Web 2.0 application for plant pathology purposes

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ABSTRACT

In order to integrate Web-based tools in plant pathology for storing, updating and sharing information, an electronic identification system based on radiofrequency technology was used for linking plants or samples to associated data. Radiofrequency identification microchips working at low or ultra high frequency were associated to different items such as organism, matrix or container commonly involved in a plant pathology test. Moreover, the microchips were subjected to various environmental conditions, such as thermal and chemical stress. A collaborative Web 2.0-based workspace was used to support research data management and interaction between users. Our findings demonstrate that the microchips maintained their reliability following environmental treatments, while the selected Web 2.0 collaborative workspace allowed useful data interchange and communications between labs during long-term trials as sanitary selection of grapevine.

1. Introduction

Digitalization of data relative to plants has been used for health monitoring, sample collecting and retrieving sanitary information (Vai, 2005; Kumagai and Miller, 2006; Luchi et al., 2008; Thrane, 2008; Cunha et al., 2010). For these purposes, multimedia data were used, such as text linked to digital images, videos, global positioning system (GPS) coordinates or electronic maps that can be stored in off-line or on-line databases (Luvisi et al., 2011a). To establish a safe link between data and plant-associated samples, radiofrequency identification (RFID) microchips have been proposed (Bowman, 2005; Bollen et al., 2007; Bandinelli et al., 2009); their use in plant pathology has also been proposed (Kumagai and Miller, 2006; Luvisi et al., 2010a). Plant health monitoring represents a broad field of activity, involving plants with different structural tissues, samples and containers, sample preparations and stocking stresses. Therefore comprehensive tests of the associated electronic tools in their specific environments can be useful to define relationships within procedures for plant health monitoring.

The aim of RFID technology is to acquire information about objects, animals or people through microprocessors associated with them (Ngai et al., 2008). Generally, an RFID system is composed of an electronic label, generally called tag, a reader and a management system. The tag incorporates a unique code (ID code) that is readable. Using radio waves, the RFID reader identifies a single item, and the process of communication between tag and reader is without physical contact. Compared to traditional systems of identification, such as barcodes, RFID offers many advantages, for example, the possibility of reading a non-visible tag (i.e. when it is concealed inside plants or samples) or simultaneous reading of multiple tags. Moreover, it is possible to store data within the tag memory or associate information using a specific database for storing the history of the plant (Luvisi et al., 2010a).

The management of RFID-tagged plants or samples involves common elements to Web 2.0 (O'Reilly, 2005): a web-based interface is considered as platform where data are controlled by users, many services are provided and it is designed for user contribution. Network- or internet-friendly equipment such as RFID microchips associated with research samples represent an optimal link between laboratories, and their integration with a web-based system has been achieved in agriculture (Voulodimos et al., 2010; Luvisi et al., 2010a). Cunha et al. (2010) described a system that combines contextualization elements such as microchips placed in vines, with readers based on widely available mobile devices, integrating web-based applications. The term Web 2.0 has become a descriptor for the increased functionality of web sites; the 2.0 suffix is related to biological fields such as medicine (Giustini, 2006; Eysenbach, 2008) or clinical pathology (Schreiber and Giustini, 2009). Its features are being incorporated into medical web sites and some potential applications of Web 2.0 in pathology and laboratory medicine are proposed in order to achieve an increased interaction between labs and to facilitate information sharing (Boulos et al., 2006; McLean et al., 2007; Sandars et al., 2008; Schreiber and Giustini, 2009). With regard to farm management information systems, Sørensen et al. (2011) affirmed that communication and automated processing of data require a digital form and a machine-readable format which can be interpreted unambiguously by all entities involved in the information flows. Moreover a system network and communication platform has been

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applied for computerised agricultural management systems: different communication platform concepts, such as controller area network, wireless technologies, ethernet and internet tools and email tools, were used to achieve a network with low-cost, flexible, and functional characteristics (Serôdio et al., 2001).

This paper presents the testing of an RFID application supported by Web 2.0 tools, in particular considering a collaborative web-based workspace, for plant pathology purposes. A grapevine sanitary selection process was reported as case of study. Grapevine selection is based on sequential steps in which a number of grapevines are monitored in vineyards for years, as well as their relative propagated grapevines used for sanitarian selection, comparative studies or conservation in screenhouses (European Commission directive 2005/43/EC). This process may involve more than one conservative breeder, research lab, several experimental fields and a large number of plants variously distributed in a large territory linked to that of the originally selected grapevine. Traditional labels are characterized by decolouration, degradation and are subject to loss or removal: these issues represent a critical point in clonal selection, considering the long periods of time during which plants are monitored. The association of an identification microchip with a plant reduces the occurrence of these problems, in particular when a microchip is inserted into a grapevine, its removal or errors in data-to-plant association are impossible. Moreover, the different conservative breeders and respective research labs constantly have to update their knowledge of selected plants during the clonal selection process, and fast and safe communication between grapevine breeders can help to avoid errors and loss of information.

2. Materials and methods

2.1. Electronic material

Two types of microchips were used, operating at Low Frequencies (LF, 30–300 kHz) and Ultra High Frequencies (UHF, 300-3 GHz). Scanner systems of similar costs were used. An easy-to-handle and inexpensive tag was assembled using a commercial UHF tag (Higgs-3, Alien Technology, USA) that was rolled up around a bamboo stick (2.0 mm diameter and 40 mm length), and covered by a polyolefin film (3 M Italia Spa, Milan, Italy). A UHF reader using USB v2.0 (Kenetics Group Lmt, St. Helier, UK) operating at the frequency 840–960 MHz (EPC Gen2) was used to read tags. Data recovery was performed using a netbook PC (Eee PC, ASUSTek, Taiwan, ROC). Transponder glass tag RFIDs were employed (2.1 mm diameter and 12 mm length), working at a frequency of 125 kHz (InterMedia Sas, Forlì, Italy). Tags were electronically read using a Card Flash reader connected by SD slot to a palm-PC (Axim X51, Dell, Round Rock, TX). Data recovery was performed using a palm-PC containing a database specifically programmed using SprintDB Pro (KaioneSoft, Seoul, South Korea). For external tagging trials, microchips were attached to plastic labels, in the form of a RFID wristband.

2.2. Microchip associations

Tests of microchip reliability by Kumagai and Miller (2006) were relative to external LF tags in woody plants: tags were subjected to thermal or chemical stress to simulate their use during the laboratory test. In order to evaluate a tagging system able to adapt to the principal steps involved in plant pathology trials, it was necessary to include other factors relative to the organism, sample or matrix and sample container. Sources of variation for each factor were then defined in order to have a comprehensive test of LF or UHF microchips that can be utilized in plant pathology activities with the aim of determining microchip accuracy and reliability.

In order to consider microchip association to an organism, herbaceous or woody plants were considered. Tobacco plants used for virus assay were externally marked with a LF or UHF wristband, while LF tags were implanted in plum tree, olive tree and grapevine subjected to periodical health assays. For plum tree and grapevine, LF tags were inserted inside the pith of one-year-old rootstocks after direct drilling of the pith from the distal cut of the rootstock just before grafting, followed by microchip localization below the grafting point (Bandinelli et al., 2009; Luvisi et al., 2011b). For olive tree, transversal drilling of the trunk 5 cm below graft point was performed on four-year-old plants, implanting UHF tags. Various matrices were selected for LF and UHF microchip association. Sandy, loam and clay soils with different moisture-holding capacity (10%, 50%, 90%) used for physical and chemical assay were selected for microchip burial. Similarly, freshwater and saltwater (50 g l^{-1} NaCl), were used for microchip immersion. Media for culturing micro-organisms (potato dextrose agar, water–agar) and plant explants (Murashige and Skoog, 1962), or crude sap from leaf or wood of tagged grapevine were prepared to include the microchip in the medium. Microchips were covered by 1–5 or 6–10 cm of fresh matrix and read immediately. LF and UHF microchips were covered by paper, glass or polystyrene (10 × 10 cm, 1 or 2 mm thickness) to simulate tag response inside containers that are used for collecting organisms or matrix. To estimate the system accuracy in selected environmental conditions, the number of detected tags was divided by the total, with 15 tags for three replications. Replications were necessary because the accuracy is essentially a random variable and therefore mean values have to be estimated (Ampatzidis and Vougioukas, 2009).

The microchips were subjected to various thermal and chemical stresses. LF and UHF microchips were stored at −80 °C (ultrafreezer PBI International, Milan, Italy), −20 °C (freezer Bosh, Gerlingen, Germany), 100 °C (oven PBI International, Milan, Italy) or in liquid nitrogen for 30 days, alcohol (96%), phenol (99%) and chloroform (99%) for 60 min, exceeding treatment times reported for widely used biological protocols, such as Chomczynski and Sacchi (2006). Tags underwent two cycles of autoclaving (121 °C, 100 kPa, 40 min) to permit their insertion in aseptic environments such as media for culturing. To estimate tag reliability, the number of functional tags was divided by the total, with 15 tags for three replications. Functionality of tags was determined by a successful reading of the tag.

Differences in tagging system accuracy and reliability were determined using three-way or two-way analysis of variance (ANOVA) and pairwise multiple comparisons on significant effects and interactions using the Holm-Sidak method. Data expressed in percent were converted in arcsin values. $P < 0.05$ was considered to be significant.

2.3. Collaborative workspace

Collaborative web-based workspace software was selected in order to grant free-to-use application, web interface, support to proprietary formats (such as .doc, .docx .xls and .xlsx) and ISO standard OpenDocument format. The workspace had to allow the opening, sharing, and editing of documents by multiple users at the same time, notifying changes of documents (i.e. via e-mail, appointment manager or Really Simple Syndication, 'RSS feed'), providing an essential tool for rapid and effective communication between laboratories. The selected software was Google™ Docs (Google Inc., Mountain View, CA). The workspace for plant pathology trials was composed of query forms relative to plant or sample types and analysis techniques involved in trials. Microchip codes and assay results were recorded with workspace