ISSN: 0394-6169
ISNN: 1592-1573

ADVANCES IN HORTICULTURAL SCIENCE

n. 3 **2013**

formerly
«Rivista dell'Ortoflorofrutticoltura Italiana»
founded in 1876

FIRENZE UNIVERSITY PRESS

Advances in Horticultural Science
Published by **Firenze University Press** - University of Florence, Italy
Via Cittadella, 7 - 50144 Florence - Italy
http://www.fupress.com/ahs

Direttore Responsabile: **Franco Scaramuzzi**, University of Florence, Italy.

ADVANCES IN HORTICULTURAL SCIENCE

Formerly Rivista dell'Ortoflorofrutticoltura Italiana
founded in 1876 and issued by University of Florence, Italy

supported by ENTE CASSA DI RISPARMIO DI FIRENZE

Advances in Horticultural Science is covered in the following indexing and abstracting services: BIOBASE - Biological Abstracts - BIOSIS Previews - Horticultural Abstracts - Ornamental Horticulture - Plant Breeding Abstract

Advances in Horticultural Science is published by the Department of Agri-Food Production and Environmental Sciences, University of Florence, Viale delle Idee, 30, 50019 Sesto Fiorentino (FI), Italy.
Phone +39-055-4574021-22, Fax +39-055-4574078-17, E-mail: advances@dipsa.unifi.it, Homepage: http://www.dipsa.unifi.it.

SUBSCRIPTIONS - The subscription price of volume 27, 2013 is € 60. 00 in Italy and € 70.00 in other countries. Mailing costs: € 3 for Italy, € 6.50 for Europe and € 10.00 for the rest of the world. The subscription price of an issue is €17.00 in Italy and € 20.00 in other countries.

CONTENTS

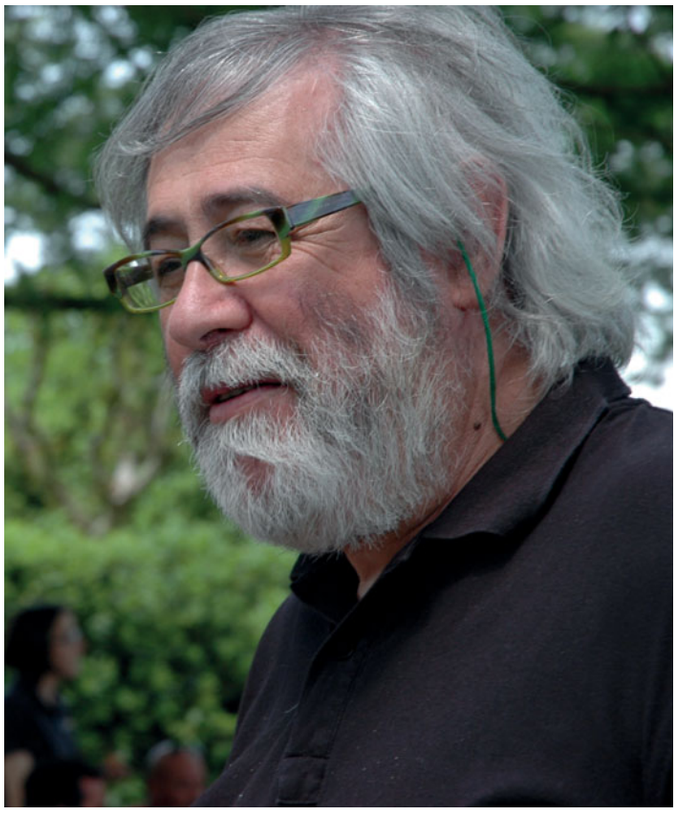
Both selection and preservation of the genetic patrimony of grapevine are fundamental for the establishment of sustainable and high quality viticulture. In fact, starting back in 1968 Europe regulated the production and marketing of grapevine propagation material (68/93EEC). This legislation required that adequate structures be set up able to manage "basic" material to be transferred into production as "certified" material through nurseries. In Italy this sort of structure exists with the centers for premultiplication of grapevine material and one of their primary roles is to preserve, spread and guarantee the germplasm for this important plant.

At a distance of 45 years from that first European legislation and 10 years from the founding of the Associazione Toscana Costitutori Viticoli (TOS.CO.VIT.), the premultiplication center in Tuscany, a study day has been organized entitled *I Nuclei di premoltiplicazione e altri interventi per la qualificazione del materiale di propagazione viticolo in Italia* (Premultiplication centers and other approaches to qualify grapevine propagation material in Italy). This occasion has made it possible to define the framework of some important realities in this sector through consideration of Tuscan experiences and others taking place in Italy to select and preserve grapevine germplasm, as well as discuss recent solutions to identify and protect it.

The event, held in Crespina (Pisa, Italy) on 15 November 2013, was attended by both private and public scholars and operators in the field and their contributions are collected in the following section.

Prof. Enrico Triolo

A BRIEF TRIBUTE TO PROFESSOR ENRICO TRIOLO (1946-2013)



Enrico Triolo graduated in 1972 in agriculture from the University of Pisa. His experimental thesis, supervised by Prof. Giovanni Scaramuzzi, investigated plant virology and dealt with the selection of grapevine based on health status, a topic which was avant-garde at the time and which later, thanks to his continued study, led to national legislation.

The 1970s were important strategic times for the school of phytopathology at Pisa: the scientific campus at San Piero a Grado was forming (essentially from nothing) and the newly graduated Dr. Triolo applied his organizational, and human- and equipment-resource management skills to its creation. Laboratories, greenhouses and experimental fields were built and established and technical personnel was trained. His academic career is equally worthy of note: he began as a young "precarious" staff member, then rose to permanent assistant professor, associate professor of plant virology and then in 1996 became a full professor of plant pathology. Prof. Triolo held numerous courses at the Faculty of Agriculture (where he was president of the degree course in agricultural science for a number of years) and Pharmacy at the University of Pisa. He was an enthusiastic teacher who encouraged his students to actively participate in his lectures and paid close attention to innovation. He was almost theatrical in his gestures and presentation - in fact his changes in vocal tone were famous - and these qualities left an indelible mark on his students and held their attention. It was impossible to remain untouched. His "theatrical" presence was not accidental: Prof. Triolo loved the arts, all of them, and was a talented amateur painter, respected critic, and lover of classical music. His enthusiasm was also directed toward other, perhaps less refined aspects of daily life such as football (a die-hard fan of Pisa, of course) and the historic annual Pisan game of strength, "Gioco del Ponte".

Prof. Triolo was active in various fields of research, in particular with regard to plant viruses, as pathogenetic and epidemiological facts, and with regard to therapies. As mentioned above, he was greatly involved in problems relative to health selection of grapevine, olive and other fruiting species. Another field in which he played an important role in research was in the fight against soil pathogens using ecocompatible techniques (for example, he was the first Italian to use solar energy to partially sterilize soil).

Prof. Triolo was the head of research groups, a speaker and organizer of conventions, involved in international study groups, and a member of important Accademies (Georgofili, Viti e Vino, Olivo). He also played a critical role in the formation of the Associazione Toscana Costitutori Viticoli (TOS.CO.VIT), and served as President as well.

Indeed, through this organization he brought together his research and the needs of the operative world, which is the true mission of a scientist: to support and carry forward the civil, social and economic progress of his country.

Adv. Hort. Sci., 2013 27(3): 95

System application and the availability of vine germplasm

G. Tempesta
Consorzio Italiano Vivaisti Viticoli Ampelos, Via Tebano, 45 Faenza (RA) 48018. info@ampelositalia.com gftempesta@vivaioenotria.it.

Viticulture systems are based, obviously, on the vine, man, his history, his knowledge and the environment, which combine to form the various "terroir" or Denomination of Origin (DO). Grapevine, by its nature, has a long lifespan and generational rhythms. Knowledge about the area, the variety and the characteristics of clones come from everyday life and an accumulation of understanding.

Different vine-growing areas claim historical and synergic structures for processing and marketing systems. While they are linked to tradition, innovation is necessary to respond to new lifestyles and preferences that reward typologies that are often based on new varietal ranges and clones.

This innovation, which has led to "Vigneto Italia", has been favored by public projects such as FEOGA 1970-1975 and incentives for renovation in 2000-2006.

Today, 15,000 to 18,000 hectares of the estimated 600,000 hectares of total cultivation area undergo renewal or replacement annually. This translates into a nursery demand for an estimated 50-60 million rootlings that must follow the rapid changes and stimuli of the market. A striking example of this are the varieties Prosecco and Pinot Grigio: the former has gone from two million grafts to nearly 18 million, and the latter from one million to the current 12 million. Similar significant variations have taken place for low-productivity Sangiovese clones or others that are utilized for the production of "commodity" wines.

While modern viticulture is based on tradition, at the same time, it requires nurseries to respond rapidly to changes in a hard-to-predict market. Nurseries attempt to face this challenge thanks to vast varietal and clonal offerings but to fully succeed in satisfying the variations in the market, a genetic patrimony that is ten times greater than what is currently available would be necessary. Greater availability could also compensate for declassifications resulting from material that is no longer suitable in sanitary or genetic terms. However, the economic commitment necessary to sustain public research bodies in their quest for new genetic material is hard to come by and the spread of already available initial and basic material is not always optimal.

Adv. Hort. Sci., 2013 27(3): 96-97

The evolution of clonal heritage registry available at TOS.CO.VIT.

R. Bandinelli*, M. Pagano**

* *Associazione Toscana Costitutori Viticoli (TOS.CO.VIT.), Via Vecchia di Marina, 6, 56010 San Piero a Grado, Pisa, Italy, bandinelli.roberto@libero.it.*

** *Ph.D. in Agrobiotechnology for Tropical Production.*

The first clonal selection works in Italy were carried out by Breviglieri in 1943, when he noticed an intravarietal variability in the varieties cultivated in Tuscany. Since then, the study of clones has stimulated research for new genetic materials characterized by high performance.

The Council Directive 68/193/EEC of 9 April 1968 (concerning the marketing of material for vegetative propagation of vines) and subsequently the Presidential Decree of 24 December 1969 (n.1164) entitled "production and marketing of vine pre-multiplication material" gave a positive impulse to promote clonal selection activity. These decrees forced a gradual change from standard (of massal origin) to certified material (obtained through clonal selection activity).

The Institutes of Pomology of the Universities of Florence and Pisa, during the period 1960-1975, promoted research to establish and reorganize Tuscany's ampelographic platform. These studies focused on identifying suitable varieties for cultivation in different Tuscan provinces. The clonal materials, identified by both universities, were preserved at the Monna Giovannella farm (Andrenelli, 1984). In 1976 clonal selection activity continued with the National Research Council project entitled "Improvement of crops for food and industry by genetic interventions - sub project - grapevines to wine grapes". Upon conclusion of the project, a new project was sponsored by the Ministry of Agriculture and Forestry entitled "Viticulture: Production of vegetative propagation material of grapevine through by clonal selection" (Triolo, 1976; Pisani and Bandinelli, 1990). In Tuscany, research is still in progress in this sector, as carried out at public Institutions and privately, and through collaborations.

Early clonal selection activities focused on finding plants with high productivity and vigor. In recent years, the trend has been to select individuals phenotypically characterized by a reduced vegetative growth, moderate fertility and higher tolerance to the most common plant diseases. Due to desirable enological and technological aspects, other preferred features include looser clusters, smaller berries, good rate of polyphenols and anthocyanins, early ripening and high sugar content. The health status of selected material is guaranteed thanks to MiPAAF (Ministry of Agriculture, Food and Forestry) protocols.

Public interest in the genetic material obtained from clonal selection activities stimulated the creation (on 24th March 1977) of a center for premultiplication of grapevine material, thanks to an agreement signed between the Region of Tuscany and the University of Pisa. The agreement confirms the public interest for the multiplication and spread of plant material obtained through genetic selection of varieties and rootstocks deemed important for our national production chain (Triolo, 2010). The research institutions involved provided the first registered clones to this center.

Nowadays, TOS.CO.VIT. (Association of Tuscan wine-makers, founded on 29 January 2003) carries on the natural evolution of the activities that began with the Center for premultiplication of grapevine. This institution continues pre-multiplication activities and distribution of the genetic material – selected in Tuscany – with regard to the basic category. A total of 63 clones of European grapevine, selected in Tuscany, from different projects (both public research institutions and private entities) are managed, such as: 32 clones of Sangiovese, three of Prugnolo gentile, four of Ansonica, three of Canaiolo nero, one of Ciliegiolo, two of Colorino, two of Trebbiano toscano, two of Vernaccia di San Gimignano, two of Malvasia bianca lunga, seven of Vermentino, one of Mammolo, one of Aleatico, one of Barsaglina, two of Moscato bianco. In addition, two clones of hybrid rootstocks are also present.

References

ANDRENELLI L., 1984 - *Riordinamento ampelografico e selezione clonale delle uve bianche in Toscana*. - Università degli Studi di Firenze, Tesi di laurea A.A. 1983-1984, pp. 76-83.

BREVIGLIERI N., 1943 - *I principali vitigni relativi ai più diffusi tipi di vino italiano*. - Estratto dal "Trattato di Enologia" del prof. Garoglio.

PISANI P.L., BANDINELLI R., 1990 - *Stato attuale della selezione clonale della vite in Toscana*. - Atti X Incontro su "Contributo ed influenza della chimica nella produzione e commercializzazione del vino". Siena, 7 Giugno, Istituto di Chimica Organica, Università degli Studi di Siena, pp. 41-50.

TRIOLO E., 1976 - *Selezione sanitaria e termoterapia della vite da vino in Toscana*. - L'Italia Agricola, 3:103.

TRIOLO E., 2010 - *L'Associazione Toscana Costitutori viticoli (TO.SCO.VIT.) e l'impiego dei sistemi a radiofrequenze RFID (Radio Frequency IDentification*). - Vivaismo viticolo: nuove performance di tracciabilità e sviluppi per il mercato vivaistico, Eds. Debatte, Italy, pp. 8-13.

Adv. Hort. Sci., 2013 27(3): 98

Sanitary and agronomic selection of Tuscan germplasm to improve wine production

P. Storchi*, F. Giannetti*, P. Valentini*, I. Bazzo, E. Angelini****

* *Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Unità di Ricerca per la Viticoltura, Arezzo, paolo.storchi@entecra.it.*

** *Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Centro di Ricerca per la Viticoltura, Conegliano (TV).*

Clonal selection of grape was launched in 1985 at the operative section in Arezzo of the former of Experimental Institute for Viticulture [currently the Research Unit for Viticulture of the Council for Research in Agriculture (C.R.A.)].

Studies focused on the search for putative clones of the most popular varieties in central Italy, carrying out detailed investigations in old vineyards in different wine-producing districts of Tuscany, initially in Maremma and later in Montepulciano, Montalcino, San Gimignano and Chianti (Armanni *et al*., 2010). Beginning in 1990, some experimental vineyards were established with the progenies of the clones, which were tested for their health and evaluated for their vegetative and productive characteristics.

The work was carried out in particular on the Sangiovese cultivar (Calò *et al*., 1995), but over the years many other varieties have been included, with particular attention to indigenous germplasm for which availability of propagation material with good sanitary status is still lacking (Borgo *et al*., 2009).

Particular attention was given to the results from experimental vinifications using virus-free clones, allowing expression of their genetic properties without interferences, especially those relating to the phenolic richness and aromatic complexity of the wines.

Through the research it was possible to evaluate the incidence of viruses in the ranges of selection. The leafroll agents, were very common, even considering that a careful visual preselection control of symptoms was carried out. Ampelovirus type 1 was identified in particular in materials from Pitigliano and Chianti Classico areas, while ampelovirus type 3 affected, almost exclusively, selections from Maremma. This virus, along with Fleck, was found in significant proportions in clones under selection in the vineyards of Tuscany. Among virus-like diseases of minor importance, the incidence of vein necrosis was very high.

The selection activity led to the registration in 2002 in the National Catalogue of Grape Varieties of clones ISV-RC1 and ISV2 of Sangiovese (Storchi *et al*., 2004); in 2011 other clones of Sangiovese, Aleatico, Vermentino, Trebbiano toscano and Canaiolo were registered. Still other clones will be ready soon for application to the Ministry of Agriculture once agronomic observations and sanitary checks are complete.

Currently, there are clone comparison vineyards in Arezzo and in the district of Chianti Classico and collaborations are in progress regarding the clonal selection with the "Consorzio Vivaisti Viticoli Italiani" and with the main wine cellars of Tuscany.

Since the protocol for the selection of a clone demands a long period of time, one of the next objectives will be to reduce the time needed to secure clone registration in the National Catalogue. In this sense, early selection of mother plants with stable and better features, compared to the standard variety, is crucial, as is subsequent rapid propagation of plant material to establish the fields for clone assessment and comparison in different environments.

References

ARMANNI A.B., RANDELLINI L., VALENTINI P., GIANNETTI F., LEPRINI M., STORCHI P., 2010 - *Recupero, tutela e valorizzazione delle identità viticole dell'Italia centrale*. - Rivista di Viticoltura e di Enologia, 1(4): 31-41.

BORGO M., COSTACURTA A., EGGER E., STORCHI P., SCALBRELLI G., LORETI F., 2009 - *La selezione clonale del vitigno Aleatico*. - Atti AIVV, Tornata Isola d'Elba, pp. 99-112.

CALÒ A., COSTACURTA A., PALUDETTI G., CRESPAN M., GIUST M., EGGER E., GRASSELLI A., STORCHI P., BORSA D., DI STEFANO R., 1995 - *Characterization of biotypes of Sangiovese as basis of clonal selection*. - Proc. Int. Symp. Clonal Selection, Portland, pp. 99-104.

STORCHI P., EGGER E., BORGO M., VALENTINI P., PIERI M., 2004 - *Contributo al miglioramento qualitativo del Sangiovese in Toscana: le selezioni ISV*. - II Simp. Sangiovese, Firenze, pp. 347-353.

Adv. Hort. Sci., 2013 27(3): 99

Innovative techniques for pre-multiplication of grapevine clones: the CE.PRE.MA.VI. experience

F. Mannini*, L. Nervo**

* *Istituto di Virologia Vegetale, CNR Unità di Grugliasco, Via Leonardo da Vinci 44, 10095, Grugliasco (TO), f.mannini@ivv.cnr.it.*

** *Vivaio Cooperativo Vivalb, Fraz. Vaccheria, 12051 Alba (CN).*

The IVV-CNR Unit of Grugliasco (TO) has selected and registered in the National Grapevine Cultivar Catalogue more than 100 clones belonging to 25 cultivars of north-west Italy. The primary sources of these clones are preserved and pre-multiplied by the Grapevine Premultiplication Centre of Piedmont (CEPREMAVI), based in Alba (CN) and managed by Vivalb on behalf of the Region of Piemonte. Premultiplication supplies the commercial nurseries with propagation material of the "basic" category, i.e. selected and virus-free. In the 2013 campaign CEPREMAVI produced more than 37,000 "basic" grafted rootlings.

Healthy primary sources, in accordance with official regulations, are kept individually potted inside a screen-house, where each clone is represented by three plants (on their own roots). Isolation from the soil and from the outside, thanks to a double insect-proof net, safeguards the original sanitary status of the clones from both virus and phytoplasma. The "initial material" collected from the primary sources is then used to establish mother plant vineyards (MPV) suitable for the production of 'basic' material. In recent times, due to the spread of phytoplasma diseases (Flavescence dorée and Bois noir), CEPREMAVI has undertaken a new approach in the production of "basic" clonal material, introducing complete isolation from the ground (except for roots) and the outside, also for MPV. The aim is to prevent, at the highest level, the propagated material from any possible virus and phytoplasma infection. The new technique consists of planting the MPV inside insect-proof tunnels provided with a double room entrance and with complete plastic mulching of the ground. Each plant row is isolated from the others. Along the rows a metallic structure is designed to support the net coverage as well as the wire sets to uphold the plant canopy. A plastic pipe runs through the upper part of the tunnels with sprinklers for fungicide and insecticide sprays. Vineyard management (i.e. the entry of workers inside the tunnels) is limited to green pruning and to routine sanitary inspections. During winter the nets are removed from the supports and rolled up on the ground near the rows.

In order to maintain the optimal sanitary status throughout the pre-multiplication steps, the woody propagation materials collected from the covered MPV are processed by hot water treatment at 50°C for 45 min before grafting. In addition, beginning with the 2013-14 campaign, the traditional open-field nursery will be eliminated. After the callusing period, the grafted cuttings will be potted (biodegradable pot) and maintained for about 20 days in a greenhouse, then transferred under a wide tunnel, ground-mulched and covered by an insect-proof net, where they will complete seasonal growth until fall. Thanks to the new technique, CEPREMAVI will be able to produce "basic" propagation material with the maximum degree of protection from virus and phytoplasma infection.

Adv. Hort. Sci., 2013 27(3): 100

Preservation and premultiplication of selected grape material in Trentino: collaboration between FEM- S. Michele all'Adige and Trentino Grape-Nurseries AVIT-Consortium

U. Malossini*, L. Gretter**

* *Edmund Mach Foundtion, Istituto Agrario di S. Michele all'Adige, Via Edmondo Mach, 1, 38010 San Michele all'Adige (TN), umberto.malossini@fmach.it.*

** *AVIT, Consorzio Vivaisti Viticoli Trentini, Via R. Guardini, 73, 38121 Trento.*

In Trentino, the Edmund Mach Foundation-San Michele all'Adige (FEM) is the institution responsible for the selection of grape propagation material for the wine industry, serving not only the local area. The premultiplication material, selected and maintained by FEM, is released commercially in the vine-nursery chain in collaboration with the Trentino Grape Nurseries Consortium (AVIT). FEM has made significant investments for the qualification of sanitary selection. In particular, the already existing structures (screen-house of approx. 500 m^2) have been totally renovated and new open-field greenhouses/tunnels (with anti-insect nets) have been realized. Special attention was paid to scientific training and updating of the personnel responsible for the control and treatment of clonal material primary sources. The personnel involved in the selection programs is also qualified thanks to participation in activities and projects carried out at national level, in collaboration with other premultiplication centres and scientific institutions, for the study and validation of official protocols of selection and/or premultiplication. Active participation in these initiatives has enabled a constant updating of procedures for internal audit on selected materials.

The preservation of "primary sources" of registered clones takes place both under screen house and in a micropropagation laboratory (tissue culture), allowing the possible "sanitation" of special accessions. This service is carried out also for other breeders. Annually FEM manages at their farm more than 3.5 hectares of surface area for "initial", "basic" and "certified" categories of *V. vinifera* and rootstock propagation material An additional approx. 1.5 hectares of vineyards, obtained with FEM selections but commercially classified in the "standard" category, is also under FEM control. The "initial" materials of 84 accessions of *V. vinifera* and *Vitis* hybrid rootstock clones are preserved under screen-house. These materials belong to clones registered by FEM, some of them in association with other institutions, or preserved on behalf of third parties. Each accession or clone is present with an average of five or more plants (grown in 70-L containers), depending on the "commercial" interest. The yield of buds or cuttings to supply grafting activity is significant. The two tunnel-like structures in the field (one for the production of rootstock cuttings and the other of *V. vinifera* scions) cover a total area of approx. 2,000 m^2 with a defined mapping of every single vine. Most of the materials under the tunnels belong to the "basic" category. In addition to vineyard management, a strict sanitary control is carried out by means of field surveys and visual monitoring of the main disease symptoms. Plants are routinely sampled for diagnostic virus assays, particularly for the official verifications required by certification services. All the operations are carefully recorded as required for genuine traceability of nursery materials and as a guarantee in the subsequent stages of propagation. Since 2008, AVIT acts as the only authorized nursery for management and marketing of "basic" category grafted rootlings of the FEM clones. AVIT, along with its administrative office in collaboration with Nucleo di Premoltiplicazione Viticola delle Venezie, arranges reservations, production and distribution of selected rootlings.

AVIT has recently been recognized as a co-breeder proponent, along with FEM, of seven new clones (registered in the National Catalogue). In 2012, a consortium was formed by FEM and CIVIT AVIT with the aim of pursuing a path of mutual interest, in the wake of innovation in the field of viticulture and wine making.

Adv. Hort. Sci., 2013 27(3): 101

Propagation of endangered grapevine cultivars: some reasons to recover and protect this patrimony

E. Dallavalle*, E. Triolo**

* *Agronomo Libero professionista, Bologna, elisabettadallavalle@libero.it*

** *Presidente TOS.CO.VIT., S. Piero a Grado (PI).*

Endangered grapevine plants of uncertain origin may represent a target for safeguarding. Current regulations guarantee the propagation of selected plant material following clonal procedures with the aim of excluding the most dangerous plant viruses. Thus, rare grapevine plants that survived the nineteenth-century plagues may be characterized by interesting epigenetic features. Their progeny could take on an important role with regard to today's viticulture: consider how these individuals could represent an important source of genetic material that not only possesses biological, historical, scientific and educational value, but also the qualitative potential to attract interest from the most demanding markets.

Moreover, in recent years, the recovery of ancient grapevines has been reassessed with the use of plants more than a hundred years old to produce new wines, and for which novel characteristics may be described on the label. In this context, in the last few years, intense research has been undertaken to evaluate forgotten plants and as a way to utilize old plantings. The 'Associazione Partriarchi' of Forli was the first to apply this approach to save a group of ancient vines that had been abandoned for decades. 'Uva Caveccia' and 'Uva Morta' were the vines involved: these very old vines have close ties to the Romagna plain in Italy where they were cultivated in connection with maple or elm, and often with almond, pear or plum. In another case, the trunk of a century-old, white grape vine has surfaced recently near Aosta (Moriondo *et al*., 2010). Genetic analysis was undertaken to determine its parentage: a descendant of Prié. The historical data, morphological measurements and parentage with Prié lead to the conclusion that the surviving plant belongs to the Blanc Comun variety. These are not singular examples of the use of old vines from the pre-phylloxera period. For instance, there has been interest in Valdobbiadene toward 100-150 year old plants located on steeper slopes between Farra di Soligo and Valdobbiadene. The Senarum Vinea project has made it possible to discover, also in Tuscany, ancient examples of century-old autochthonous/minor vines that have survived, essentially forgotten, up to the current day. Among the identified vines, we can mention 'Gorgottesco', 'Tenerone', 'Salamanna', 'Occhio di Pernice' and 'Rossone'.

In addition, another interesting application for old grapevines may derive from their use as ornamental plants, as suggested by some examples within the monastery of S. Maria Maddalena in Bologna or in the Olivetani cloister of the church of S. Maria in Regola in Imola.

References

MORIONDO G., SANDI R., VOUILLAMOZ J., 2010 - *Identificazione del Neret di Saint-Vincent, antico vitigno valdostano*. - L'Informatore Agricolo, 5.

Adv. Hort. Sci., 2013 27(3): 102-103

Characteristics of recent released clones selected by DiSAAA-A in Tuscan Coast line premultiplied by TOS.CO.VIT

G. Ferroni*, G. Scalabrelli*, G. Di Collalto**

* *Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto, 80, 56124 Pisa, giuseppe.ferroni@unipi.it*

** *Department of Crop, Soil and Environmental Science, University of Florence, Viale delle Idee, 30, 50019 Sesto Fiorentino (FI).*

At the conclusion of the research project "enhancement and renewal of viticulture in the province of Grosseto", "Sangiovese selection in the area of Morellino di Scansano" and "enhancement of candidate clones of Tuscan grapevines" have been homologated in 2007 four clones of Ansonica and five clones of Vermentino and in 2011 three clones of Sangiovese (Scalabrelli and Di Collalto, 1999; Scalabrelli *et al*., 2004, Scalabrelli *et al*., 2005, Scalabrelli *et al*, 2012).

Main traits of Ansonica clones

I-Cosa 1. Large conical bunches of medium firmness, elliptical-short berry Medium size, and greenish-yellow, slightly amber. Bud fertility below the average (1.18); bud break medium earliness, slightly more precocious ripening. Medium-high vigour. Pale straw yellow wine, with notes of tropical and fruity floral, light citrus, hazelnuts and honey, sapid and structured, the most appreciated by the panel. Quality Clone requires control of production. Suitable to produce structured white wine, even with aging.

I-Settefinestre 1. Conical bunches, sometimes winged, less than average size, medium sparse and separated, berry green-yellow, amber, slightly elliptical-short, slightly coarser than Cosa1 clone. Medium bud fertility (1.34). Time of bud break and ripening are slightly later than the average of the population. Higher vigour of clone Cosa 1, production lower than the average. Pale straw yellow wine, with notes of almond, slightly fruity and floral, with a good structure, flavor, freshness and agreeableness. Clone less productive, suggested for the production of assembled wines, especially with the previous clone.

I-Settefinestre 2. Bunches large, conical, mildly sparse, elliptical-short, medium size, green-yellow, amber. Bud fertility (1.46) and yield above the average. Average earliness of bud break and ripening. Medium-high vigour; pale straw yellow wine tropical fruity, note of floral and slightly spicy, soft nutty and honeyed notes, medium-bodied, balanced and pleasant. Clone productive of good quality, able to bring aromatic complexity, suitable to produce also varietal wines.

I-Settefinestre 3. Bunches of medium size, broad conical shaped, of medium size, largest of the clone Settefinestre 1 and smaller of clone Cosa 1; berry elliptical-short, medium size , yellow, amber. Production and fertility of the buds below average (1.20). The earliness of bud break and time of ripening are like the average of the population; medium-high vigour. Pale straw yellow wine, with Mediterranean spiced and nutty, with hints of fruity and honey, balanced structure and good pleasantness. Clone of intermediate level of production and quality, suitable to produce white wines ready to drink and for assemblage.

Main traits of Vermentino clones

I-Sirena 1. Time of bud break like the population mean, average vigour and low productivity, mean bud fertility (1.26), medium-small cluster, short conic shape and medium tightness, medium berry size, spherical shape and, firm skin. Earlier ripening compared to the population average (7-10 days), sugar accumulation influenced by territorial environment. Straw yellow wine, good structure, floral and fruity, palatable, sapid, with slightly bitter final, suitable for short aging.

I-Marem 1. Bud break timing similar to the population mean. Earlier ripening compared to the population average (7 days). Medium high plant vigour and fertility (1.40) yield lower than the population average. Loose bunch with a wing more evident, the berry is slightly smaller than the average, the skin is firm. It exhibits low sensitivity to the bunch rot. Sugar accumulation higher than the reference population. Straw yellow wine with notes of ripe fruit, citrus and spicy Mediterranean (very pronounced). It maintains high qualities of freshness, good body, suitable for the production of wines with a good structure which can be subjected to a medium aging period.

I- Marem 3. Bud break contemporary to the population. Earlier ripening than the population average (7days). Medium-high vigour and bud fertility (1.46) lower yield to the average. Bunch medium-small of truncated cylindrical shape, with a short wing, medium loosen. Berry short- medium ellipsoidal, good tolerance against botrytis. Accumulation of sugars compared to the reference population. Wine with aromatic complexity, good freshness, texture and excellent balance, suitable for producing structured wines and wines ready to drink.

I-Sileno 1. Bud break slightly earlier than the average of the population. Later ripening as compared to the average (about 10 days). Low vigorous and very productive, Medium-low bud fertility (1.31). Very large, composed and tight cluster, large berries. Sugar accumulation potential slightly below the average of the population. Straw yellow wine, perfume with notes of fresh fruitiness and marked by spicy Mediterranean, fresh, light structure, suitable for the grapes and the production of wines ready to drink and sparkling wines.

I-Sileno 3. Bud break slightly earlier than the average of the population. Later ripening (about 10 days after the avg.). Low vigour and fertility (1.25). Very large, composed and tight cluster, large berry, higher yield. Accumulation potential of sugars is below the average of the population. Straw yellow wine, aroma with notes of fresh fruitiness and marked by spicy Mediterranean, fresh, light structure, suitable for the assemblage with other clones for the production of wines ready to drink and sparkling wines.

Main traits of Sangiovese clones (selected in the DOCG area "Morellino di Scansano").

I-CHI 8. Production is on the average, plant vigour slightly below the average, bud fertility above the average. The bunch is medium-small, conical-winged and tightness on the average. Berry of spherical shape, uniform black blue color, waxy and thick skin, very rich in polyphenols and anthocyanins. The wine has an intense ruby red color, with intense fruity notes with evidence of red fruits and floral notes, well-structured and balanced suitable to aging.

I-CHI 10. Production and vigour on the average, bunches short, conical, rather compact. Berry black blue uniform, ellipsoidal, skin resistant, thick and waxy. It ripens on II-III decade of September. Ruby red wine with floral notes, spicy and fruity, with a good structure, balanced, suitable to aging. Clone suitable to the assemblage with other clones.

I-CHI 13. Bunch of size below the average, conical shape moderate compactness, sometimes winged. Berry medium-small size, ellipsoidal shape, blue black color, waxy and thick skin, rich in color. Ripening time in the second half of September. Intense ruby red wine with distinct notes of red fruit, good structure and balance, suitable to the production of assembled red wines to be aged.

These new clones of *Ansonica*, *Vermentino* and *Sangiovese*, possess different productive, qualitative and sensorial characteristics, some of which are complementary, therefore, they can respond to several growing and oenological requirements, allowing the establishment of polyclonal vineyards to achieve the wine target using the best combinations.

References

SCALABRELLI G., DI COLLALTO G., 1999 - *Rinnovamento e Valorizzazione della viticoltura della provincia di Grosseto*. - Provincia di Grosseto. ARSIA. Regione Toscana, pp. 5-24.

SCALABRELLI G., FERRONI G., BORGO M., PORRO D., DI COLLALTO G., 2005 - *La selezione clonale del "Vermentino" vitigno bianco di grande interesse*. - L'Informatore Agrario, 35: 67-74.

SCALABRELLI G., FERRONI, F., D'ONOFRIO C., BORGO M., PORRO D., STEFANINI M., 2012 - *La selezione clonale del vitigno Ansonica in Toscana*. - Italus Hortus, 3-5: 451-455.

SCALABRELLI G., LORETI F., FERRONI G., 2004 - *Aspetti agronomici e ampelografici della selezione clonale di Sangiovese nelle zone DOC Val di Cornia e Morellino di Scansano*. - Atti Convegno "Miglioramento qualitativo delle produzioni vitivinicole e del materiale di produzione, Quaderno ARSIA, 1: 27-46.

Adv. Hort. Sci., 2013 27(3): 104

Updated knowledge of the vine rootstocks

M. Bornice, G. Ferroni, G. Scalabrelli
Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto, 80, 56124 Pisa, marcello.bornice@for.unipi.it

For some time, the use of rootstocks in viticulture is no longer considered only as a means of agronomic method to avoid the phylloxera damages (*Daktulosphaira vitifoliae*, Fitch, 1856), but as a tool to be able to influence the physiology of the cultivars and to adapt the vine's behavior to the different soil conditions, the climatic characteristics and the cultivation techniques (Ferroni and Scalabrelli, 1995; Intrieri *et al.*, 1999; Di Collalto *et al*., 2001; Scalabrelli *et al.*, 2001). In particular, it is increased the need to use the rootstock as a means to control the physiological processes of the plant, being the root activity closely related to that of the canopy, by exchanging signals and metabolites.

The selection of the vine rootstocks started at the end of 1800, originated the main part of the rootstocks currently used in viticulture, using *V. riparia* and *V. berlandieri*. Subsequently, through the use of other genotypes were obtained new hybrids even more complex. Currently, in Italy there are 39 varieties of vine rootstock recognized on the National Register of Grapevine Varieties (agg. DM 23/03/2012, rev. 24/07/2012, http://catalogoviti.politicheagricole.it/catalogo.php) but are essentially five, those used on large surfaces: Kober 5BB, SO4, 140 Ru, 1103 P, 110 R (Bavaresco, 1998).

In the past, for the diffusion has had an important role the need for nurserymen, who tended to favor rootstocks with excellent performance in propagation. While today there is a greater interest to satisfy the needs of viticulture's techniques also as a consequence of the changed cultivations' conditions. In fact, we need of new rootstocks able to adapt to environmental stresses caused by climate change in progress (mainly droughts and changes in the distribution of rainfall) and/or linked to new growing environments which have restricting factors for the vines. Moreover, the need to obtain specific qualitative characteristics (polyphenols content, grapes' acidity, etc..), suggest to deepen even more the aspects that can influence the wine quality. At the same time, it is necessary to reduce the period of selection (a new rootstock from crossing takes between 20 to 25 years), which through the use of molecular markers could also be reduced by half.

In this paper we provided an overview about the behavior of some rootstocks tested in Tuscany during the last years. In particular, the survey covered the vigor, the productivity, and the characteristics of grapes at maturity, including the phenolic content. The rootstocks considered were: 420A, Kober 5BB, SO4, 161-49, 140 Ru, 1103P, 110R, 779P, 775P, 3309C, 101.14, Fercal, Gravesac and 41B (Scalabrelli *et al.,* 2003).

References

BAVARESCO L., 1998 - *Scelta del portinnesto in viticoltura*. - L'Informatore Agrario, 38: 53-59.

DI COLLALTO G., SCALABRELLI G., FERRONI G., 2001 - *Osservazioni preliminari sull'attività vegeto-produttiva del "Sangiovese" innestato su tredici portinnesti nella zona di produzione del "Morellino di Scansano"*. - In: ARSIA. *Il Sangiovese,* Atti del Simposio internazionale, Firenze, 15-17 February, 2000, ARSIA, Regione Toscana, pp. 259-264.

FERRONI G., SCALABRELLI G., 1995 - *Effect of rootstock on vegetative activity and yield in grapevine*. - Acta Horticulturae, 388: 37-42.

FREGONI C., 2012 - *Il portinnesto ideale*. - VQ in vigneto, 5: 24-26.

INTRIERI C., SILVESTRONI O., VESPIGNANI G., FILIPPETTI I., 1999 - *Indagini poliennali sull'impiego dei portinnesti diversi per il vitigno Lambrusco Grasparossa*. - Vignevini, 3: 49-54.

SCALABRELLI G., FERRONI G., D'ONOFRIO C. DI COLLALTO G., VENERINI F., 2003 - *Trials with 'Sangiovese' grafted on several grapevine rootstocks in two different areas of Tuscany*. - Acta Horticulturae, 617: 73-83.

SCALABRELLI G., LORETI F., PISANI P.L., BANDINELLI R., PORCINAI S., 2001 - *Comportamento bio-agronomico del "Sangiovese" su diversi portainnesti nel Chianti Classico*. - In: ARSIA. *Il Sangiovese,* Atti del Simposio internazionale, Firenze, 15-17 Februart, 2000, ARSIA, Regione Toscana, pp. 289-294.

Adv. Hort. Sci., 2013 27(3): 105

Micropropagation in viticulture: twenty years of experience

O. Navacchi, G. Zuccherelli
Vitroplant Italia, via Loreto 170, 47521 Cesena (FC), orianonavacchi@vitroplant.it.

Vitroplant began as a micropropagation laboratory in the early 1980's to respond to a growing need for hybrid peach-almond GF677 rootstocks that were particularly suited to excessively dry or soils inducing chlorosis and for replanting. This rootstock, selected in France, is relatively simple to propagate *in vitro* while it is more difficult to carry out multiplication by cuttings. In subsequent years the range of *in vitro* propagated species grew and, in addition to other peach rootstocks, self-rooting peach, apricot, kiwi, artichoke and pear rootstocks started being multiplied. Today, micropropagation is the most advanced agamic multiplication technique allowing an elevated number of genetically identical plants to be obtained in a short time due to an exponential growth rate of the number of individuals with each subculture step. *In vitro* culture offers the advantage of high quality and health safety of the plant material as it not subject to an accumulation of pathogens or viruses over the years, as can occur with other agamic *in vivo* propagation techniques. Moreover, *in vitro* procedures pass on to plants a physiologic equilibrium that is comparable to that of seed-grown individuals, demonstrating good vigor of the aerial structures and notable rhizogenic exploratory ability in roots. These behaviors can be associated with a "rejuvenation" of the plant material. For example, the majority of grapevine cultivars have been, over the millennia, vegetatively propagated and for this reason they present important problems from a health standpoint. Toward the end of the 1980's Prof. Carmine Liuni, director of the Viticulture Institute of Turi (Bari), proposed a collaboration with Vitroplant for *in vitro* propagation with the aim of resolving some of the numerous problems found in viticulture nurseries. Initially the idea was to use this propagation technique to multiply some known rootstocks and new seedless table grape varieties. The results obtained from field tests were very satisfactory. The micropropagated plants were very vigorous and demonstrated excellent development of rooting structures, highlighting the potential of this propagation technique also in grapevine. This type of development, comparable to seed-grown plants, is not due to genetic mutation but rather to phenotypic manifestations that come from the rejuvenation of *in vitro*-produced plants. Juvenile phases are recognizable in some arboreal species thanks to specific morphological, cellular or physiological traits such as a particular leaf size, shape or phyllotaxis, the presence of thorns or ability to root. Plants do not however undergo genetic variations as they pass from the juvenile to the adult phase, instead these morpho-physiologic differences are expressions of the genotype. Juvenile as well as adult tissues can be present on the same plant at the same time, a phenomenon called heteroblasty, and leaves on a single plant can have different forms. Juvenile tissues are generally found in the lower portion of the trunk and branches. To lower the point of transition from juvenile to adult tissues, it is possible to make a cut near ground-height, avoiding the risk of juvenile tissues in the shoots intended for production. Micropropagation has shown to be an essential technique for the production of healthy material in quantity and rapidly, two aspects that are very useful for clonal selection or genetic improvement programs, providing nurseries with sufficient starting material in a timely manner. Micropropagated plants, as self-rooted plants not needing rootstock, can be subject to phylloxera attack. With careful attention to the selection of soil for mother plant fields and suitable preventative measures, it is possible to keep this pathogen under control. In any case, after five or six years micropropagated mother plants have fulfilled their purpose: they have provided quickly healthy buds for propagation. Today it is increasingly difficult to keep mother plants healthy in open fields and a reduction of their useful period with frequent renewal can be predicted for the future. A new frontier for nurseries in coming years may be the use of rootstocks amenable to genic silencing to induce resistance to viruses or other pathogens of the varieties onto which they are grafted. Unfortunately considerable difficulty can be expected with regard to the spread of this technique in Europe where there is deep aversion toward biotechnology, while in other emerging, future-competitor grape-growing countries the technique has a better chance of taking hold.

Adv. Hort. Sci., 2013 27(3): 106

State of the art in grapevine variety and clone identification through polymorphism in DNA molecular markers

C. D'Onofrio
Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto, 80, 56124 Pisa, Italy, c.donofrio@agr.unipi.it.

Rapid and certain identification of grapevine clones is of topical interest in modern viticulture, especially with regard to the genetic control of propagation material.

In the beginning of the twentieth century, a specific branch of research called "ampelography" was established to identify grapevine varieties, biotypes and clones. The term derives from the Greek *ampelos*, for vine, and *graphon*, for description, as ampelography is based on visual observation of morphological and phenological characters (Viala and Vermorel, 1909). Currently ampelography is employed by international organizations such as the *Office International de la Vigne et du Vin* (OIV), the *International Board for Plant Genetic Resources* (IBPGR) and the *International Union for the Protection of New Varieties of Plants* (UPOV).

This type of genotypic identification of grapevines presents, however, some limitations. First and foremost, as the method is based on visual observations it is dependent on the subjectivity of experienced operators (ampelographers). Ampelographic description - based primarily on observation of the morphology of sprouts, adult leaves and clusters - is limited to adult plants during vegetative periods, and for this reason varietal and clonal impurities can only be detected some years after the planting of a vineyard. Furthermore, phenotypical changes induced by the cultivation environment, nutritional shortages or possible viral infections often make varietal identification difficult, and sometimes impossible. In fact, for correct varietal identification accessions must grow in the same vineyard and be virus free. However, given the great number of vine varieties (5,000-15,000), even when plants are grouped in a single vineyard, it is extremely difficult to differentiate them all simply on the basis of their morphological and phenological characteristics.

Thus, the availability of a rapid, practical, objective system to identify vine varieties is desirable to satisfy the need for quality control in nursery production, for legal protection of new selections, and as a fundamental tool to recover autochthonous vines with the aim of preserving biodiversity. In this context, as a support for the classic methods of varietal identification through morphological characters, DNA molecular markers are of fundamental importance. Among these, microsatellites are the most frequently used due to their high repeatability and reliability, high level of polymorphism and thus the degree of information they can provide, and their co-dominant nature that permits analysis of parentage. In addition, microsatellites have made it possible to develop numerous vine-related databases, including the "Database Viticolo Italiano" (www.vitisdb.it) which is managed by the author of the present abstract.

Although microsatellites allow rapid and certain identification of vines, they generally do not present sufficient polymorphism to distinguish biotypes and clones, even if some researchers have stated that analyzing an elevated number of microsatellite loci they were able to distinguish clones of Pinot and Carmenère.

Amplified Fragment Length Polymorphisms (AFLPs), a type of molecular marker that uses digestion of DNA with restriction enzymes, are much more polymorphic than microsatellites. Numerous publications have evidenced the ability of these markers to distinguish clones of various species, however they have not found application for legal purposes in the genotyping of clones due to problems of instability related to the use of restriction enzymes.

DNA molecular markers seem more stable, making it possible to combine AFLP with other markers such as SAMPL (Selective Amplification of Microsatellite Polymorfic Loci), M-AFLP (microsatellites amplified fragment length polymorphism) and S-SAP (Sequenze-Specific Amplification Polymorfism). The first two combine AFLP with microsatellites, while the latter combines AFLP technique with molecular markers based on transposons insertion polymorphism. In particular, S-SAP seems to be more stable than AFLP, and recently it was demonstrated that they are efficient in discriminating clones.

Another recently introduced DNA molecular marker for grapevine is Single Nucleotide Polymorphisms (SNPs). This type of marker has developed rapidly following sequencing of the entire grapevine genome. The resequencing of a large number of vines within the context of a specific European project has made it possible to plan an array which allows rapid, simultaneous analysis of more than 18,000 SNPs at a reasonable cost. Currently, many research groups are testing the utility of this type of marker in clonal discrimination and it is plausible that initial publications may appear by the time the present convention is held.

Adv. Hort. Sci., 2013 27(3): 107-108

Harmonization and validation of diagnostic protocols for the detection of grapevine viruses covered by phytosanitary rules

F. Faggioli[1], F. Anaclerio[2], E. Angelini[3], M. G. Antonelli[1], N. Bertazzon[3], G. Bianchi[4], P. Bianchedi[5], P.A. Bianco[6], S. Botti[7], P. Bragagna[5], M. Cardoni[7], P. Casati[6], R. Credi[8], E. De Luca[2], G. Durante[9], C. Gianinazzi[9], G. Gambino[10], V. Gualandri[5], D. Luison[1], A. Luvisi[11], U. Malossini[5], F. Mannini[10], P. Saldarelli[12], F. Terlizzi[8], E. Triolo[11], N. Trisciuzzi[13], M. Barba[1]

[1] *Consiglio per la Ricerca e sperimentazione in Agricoltura, Centro di Ricerca per la Patologia Vegetale, Via C.G. Bertero, 22, 00156 Rome, Italy. francesco.faggioli@entecra.it*

[2] *Vivai Cooperativi Rauscedo, Rauscedo, Italy.*

[3] *CRA, Centro di Ricerca per la Viticoltura, Conegliano Veneto, Italy.*

[4] *ERSA, Friuli Venezia Giulia, Pozzuolo del Friuli, Italy.*

[5] *Fondazione Edmund Mach, Istituto Agrario San Michele all'Adige, Italy.*

[6] *DISA, Dipartimento di Scienze agrarie e ambientali, Produzione, Territorio, Agroenergia, Università degli Studi di Milano, Italy.*

[7] *Centro Attività Vivaistiche, Tebano, Italy.*

[8] *Dipartimento di Scienze e Tecnologie Agroambientali, Patologia Vegetale, Alma Mater Studiorum, Università di Bologna, Italy.*

[9] *IPAD Lab, Lodi, Italy.*

[10] *CNR, Istituto di Virologia Vegetale, UOS di Grugliasco, Italy.*

[11] *Department of Agriculture, Food and Environment, Università di Pisa, Italy.*

[12] *CNR, Istituto di Virologia Vegetale, UOS di Bari, Italy.*

[13] *CRSA, Basile Caramia, Locorotondo, Italy.*

The Italian Ministry of Agriculture funded in 2009 the "ARNADIA" Project, aimed at producing validated reference diagnostic protocols for the control and monitoring of plant pathogens of phytosanitary interest and, among them, grapevine viruses. In this framework, the "Working group ARNADIA – grapevine viruses (WG)", composed of eight universities and research bodies, three accredited private laboratories, one plant health service and one association of grapevine nurseries, was established.

The aim of the WG was to produce referenced and validated serological and molecular protocols allowing for the harmonization of diagnosis of eight grapevine viruses: *Grapevine leafroll-associated virus* 1, 2, 3, (GLRaV 1, 2, 3) *Grapevine virus* A (GVA), *Grapevine virus* B (GVB), *Arabis mosaic virus* (ArMV), *Grapevine fanleaf virus* (GFLV) and *Grapevine fleck virus* (GFkV).

The validation of a protocol consists of the evaluation of specific parameters designed to determine their suitability to identify the presence of a specific "target". The parameters that influence the capability of the test to accurately predict the sample's infection status are the diagnostic sensitivity (ability of the utilized method to detect the presence of the pathogen in the samples truly infected by the pathogen in question - true positive) and diagnostic specificity (ability of the utilized method NOT to detect the presence of the pathogen in samples not infected by the pathogen in question - true negative). Other parameters that must be considered and which determine the efficiency of a protocol are the analytical sensitivity (the smallest amount of infectious entities that can be identified by the diagnostic method), repeatability or accordance (degree of conformity of the results obtained in replications of the process, made at short time intervals, using the same reference sample and in the same working conditions i.e. equipment, operator, laboratory) and reproducibility or concordance (degree of conformity of the results obtained using the same method with the same reference samples in different laboratories). The latter parameter was defined in collaboration with five laboratories of the Regional Phytosanitary Services.

Specifically, 122 grapevine samples (varieties, rootstocks and "pools" of five plants, of which only one infected) were analyzed by ELISA, using 25 antisera from three commercial companies (Agritest, Bioreba, Sediag) and multiplex RT-PCR protocols. For ELISA, the tests were conducted carefully following instructions provided by the companies; multiplex RT-PCR was performed using the protocol described by Gambino and Gribaudo (2006). The tests were performed in 18 laboratories using the same samples (analyzed in blind conditions) and reagents. In each laboratory, results were obtained using the same threshold value calculated on the basis of the spectrophotometer readings for ELISA and by analyzing the

electrophoretic gels for the multiplex RT-PCR. Processing of the obtained results (about 24,000 data points) led to definition of the validation parameters according to UNI/EN/ISO 16140 and 17025 and EPPO standards PM7/76 and PM7/98.

As reported in Table 1, ELISA proved to be a highly effective technique, comparable to the molecular method, although the latter turned out, as expected, to be more efficient for some viruses and on some specific samples (rootstocks and "pool").

Table 1 - Summary of validation parameters obtained by the ELISA test for each virus and antiserum and comparison with those obtained with the molecular protocol

Virus	Diagnostic protocol	Sensitivity	Specificity	Accuracy	Analytical sensitivity	Repeatability	Reproducibility
A rMV	Multiplex	92 %	99 %	98 %	10^{-2}	100%	100 %
	ELISA – A/B/S	64/48/50%	85/95/96%	74/72/72%	10^{-2}	100%	95%
GFLV	Multiplex	68 %	100%	90 %	10^{-3}	100%	76%
	ELISA – A/B/S	75/82/77%	96/92/92%	80/84/81%	10^{-2}	100%	90%
GFkV	Multiplex	95%	95%	95%	10^{-2}	100%	95%
	ELISA – A/B/S	90/90/30%	100%	92/92/46%	10^{-1}	98%	88%
GVA	Multiplex	96 %	99 %	98 %	10^{-2}	100%	94 %
	ELISA – A/B/S	77/45/87%	100/100/96%	83/58/89%	10^{-1}	98%	82%
GVB	Multiplex	100%	100%	100%	10^{-2}	100%	100%
	ELISA – A/B/S	86/nt/nt%	100%	92%	10^{0} (2^{-2})	100%	85%
GLRaV 1	Multiplex	74 %	100 %	94 %	10^{-2}	100%	70 %
	ELISA – A/B/S	89/94/96%	100%	93/96/98%	10^{-2}	100%	92%
GLRaV 2	Multiplex	84%	98%	85%	10^{-2}	95%	83%
	ELISA – A/B/S	86/67/87%	100%	93/96/98%	10^{0} (2^{-2})	93%	84%
GLRaV 3	Multiplex	100 %	93 %	95 %	10^{-3}	100%	100 %
	ELISA – A/B/S	81/90/97%	100%	84/92/97%	10^{-3}	100%	94%

A= Agritest; B= Bioreba; S= Sediag.

In conclusion, harmonized and validated reference diagnostic protocols for grapevine viruses subjected to phytosanitary rules are, for the first time, available. The efficiency and robustness of the protocols have been proven using a large number of samples in a variety of laboratories. On this basis, both serological and molecular protocols resulted valid, and their use could be as a function of different specific applications.

Acknowledgements

This study was funded by the MiPAAF project "ARON-ARNADIA" (2009 -11).

References

GAMBINO G., GRIBAUDO I., 2006 - *Simultaneous detection of nine grapevine viruses by Multiplex Reverse Transcription-Polymerase Chain Reaction with Co-amplification of a plant RNA as internal control*. - Phytopathology, 96(11): 1223-1229.

Adv. Hort. Sci., 2013 27(3): 109

Prevention and control strategies against *Agrobacterium* vitis in grapevine multiplication material

G. Lucchetta[1], G. Bevilacqua[2], C. Colla[3], S. Di Marco[4], R. Falconi[2], C. Frausin[5], R. Mirandola[2], G. Mordenti[4], E. Sartori[6], E. Angelini[1]

[1] *CRA-VIT, Centro di ricerca per la Viticoltura, Conegliano (TV), elisa.angelini@entecra.it.*
[2] *VITIVER Consorzio per la Valorizzazione dei Prodotti Ortoflorovivaistici Veronesi, Verona.*
[3] *MIVA Moltiplicatori Italiani Viticoli Associati, Firenze.*
[4] *CNR IBIMET, Bologna.*
[5] *ERSA Agenzia Regionale per lo Sviluppo Rurale, Pozzuolo del Friuli (UD).*
[6] *VCR Vivai Cooperativi Rauscedo, Rauscedo (PN).*

Agrobacterium vitis is the etiological agent of grapevine crown gall disease, an abnormal tissue growth occurring mostly in the basal part of the trunk. The infection starts at wound sites and it is often caused by freezing temperature. The pathogen does immediately not cause the tumour, but it can stay latent in the plant for a long time, without clear damage. Infections occurring in the first years of planting lead to debilitation of infected grapevines, together with poor quality and quantity of grape production.

In Italy in the last three years crown gall, a disease already known previously, has shown an unexpected increase, especially due to the cold temperatures in the 2009-2010 winters. This spread has caused serious damage for nurserymen, and the consequences continue now: grape growers are complaining about crown galls in one-year-old vineyards, and foreigner importers are asking for *A. vitis*-free rootlings.

Unfortunately, control strategies applied at present are successful in decreasing the damage but not in eliminating the pathogen. For these reasons, CRA-VIT, together with ERSA and several nurserymen (VITIVER, VCR, MIVA), is studying this disease in order to identify critical points in the grapevine production process and possible solutions to control the infection in mother plants and in nurseries.

The research activities include: i) monitoring of soils and mother plants in the Verona area; ii) monitoring and optimization of procedures in the grapevine production chain; iii) experimental trials to verify the effectiveness of *Trichoderma* spp. treatments of grafts and rootlings; iv) cleaning of multiplication material by chemical or alternative agents; v) hot water treatments; and vi) molecular characterization of *A. vitis* strains in order to establish molecular markers for traceability of infection sources.

Hot water treatments, unfortunately, are completely ineffective in obtaining bacterium-free rootlings (Lucchetta *et al*., 2013), while initial analyses of soils and mother plants in the Verona area have been encouraging; cleaning trials with acidic water and Virkon need further study. Other experimental trials are ongoing and initial results will be outlined in the presentation.

Acknowledgements

This work is financed by AGRO.FREE project (PSR Regione Veneto 2012-14), ERSA, MIVA and VCR. We are grateful to the CRA-VIT staff, the nurserymen involved and CBC Europe for the collaboration.

References

LUCCHETTA G., MIOTTI L., FORTE V., SARTORI E., ANGELINI E., 2013 - *Per il tumore della vite la termoterapia non funziona*. - L'Informatore Agrario, 17: 55-58.

Adv. Hort. Sci., 2013 27(3): 110-111

How information technology can support regulations and best practices for the management of health status of grapevine and product safety

A. Luvisi
Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto, 80, 56124 Pisa, Italy, aluvisi@agr.unipi.it

The need for knowledge about the origins and qualitative characteristics of food products or plants that are commercialized worldwide has increased due to consumer demands. This fact is due in part to recent negative events related to food production and has, in turn, led to stricter regulations to safeguard public health and ecosystems from the spread of pathogens. In an essential step to guarantee quality, beginning in the 1960s the former European Economic Community was involved in defining legal regulations regarding the health status of grapes and their production (68/93/EEC). The regulation of grapevine identity, health and production continued to develop until the last decade in Europe (2005/43/CE) and Italy (DM 13/12/2011), revealing its importance over the past 50 years. Regulations have followed the general trends in agriculture during the past century: not only with regard to new farming approaches and consequential environmental impact, but also the globalized trade of products which poses new challenges to import/export regulations. Food safety, market protection, property rights and ecological conservation are common themes of "global" consumers and governmental agencies. These concepts have been reinforced by the EU through ''The European White Paper on Food Safety".

Nowadays, many foods and agricultural products have to carry identifying labels or documents, as required by legal regulations (e.g. 2000/13/EC), to establish a safe traceability system. In the EU, grapevines in the certified category must be in line with the most recent directive (2005/43/CE), and associated labels have to report essential data such as the nursery where they were produced. Plant traceability, as in foods, can be supported by Information Technology (IT) and can be considered a best practice in agriculture, as is the case for livestock. The IT revolution, exemplified by the Internet, has made traceability and monitoring economically feasible and enabled traceability of food products through the labyrinth of the agricultural product supply chain. With regards to food plants, the implementation of IT solutions to trace the plant-to-food chain seems to be possible only in fruit trees, including grapevine, due to the difficulties in labeling and/or tracking herbaceous plants. The wine production line is characterized by many - effective or potential - IT innovations as technology has been able to permeate nearly every production step. With regard to the first step (i.e. selection and registration of a grapevine clone), an online database gives breeders, researchers and stakeholders an easy and lasting consulting system to share information regarding available clones. Several databases are available in Europe, such as the Italian "Catalogo Istituzionale del Registro Nazionale delle Varietà di Vite" or "Italian Vitis Data Base", the French "Base de données du Réseau Français des Conservatoires de Vignes", the German "The European Vitis Database" or "Vitis International Variety Catalogue". As for specific aims, some of these databases are more genetic-oriented than exhaustive digital archives, while others monitor production phases of premultiplication material. In any case, if the knowledge about varieties and clones is well supported by IT, the situation changes on farms or in vineyards where few IT solutions are available for health and quality management as most of them are in the prototype phase. In contrast to the situation with livestock, where technology plays an important role with electronically labeled and checked animals, farms generally have a low level of computerization, due to both the costs involved and the lack of urgency to shift to a more in-depth traceability system (Luvisi *et al*., 2012). However, available technology can satisfy various needs.

Radio-frequency identification (RFID) microchips can represent a safe tool to identify plants and foods that are protected by rights or subjected to specific regulations. The initial tests in grapevine by the Associazione Toscana Costitutori Viticoli (TOS.CO.VIT.) were carried out in 2006 on clones and involved the use of microchips implanted within the pith of rootstock (Bandinelli *et al*., 2009). This technology, if appropriately supported by information management systems, can support health controls and be a useful tool for managing risks related to environmental impacts of production systems, chemical residues and the worldwide spread of plant pathogens. In certified plant propagation and breeding programs, risk management may be a sufficient reason to change to RFID systems. Similar technology can be implemented in order to tracking the application of agrochemicals (Peets *et al*., 2009), the virtualization of vineyards by combining GPS technology (Luvisi *et al*., 2011), and the management of widespread monitoring stations using mobile devices

(Cunha *et al*., 2010). Finally, collaborative Web 2.0-based workspaces can be used to support sampling for health checks and the exchange of information between users and laboratories (Luvisi *et al*., 2012). IT can also offer real options for wine cellar management and bottling. Electronic labeling of wine for high value products using RFID systems and for the fight against forgery are principal areas for application, but do not involve cross reference to information about plant health or identity or the previously mentioned databases.

In conclusion, even if IT solutions can support management procedures with regard to the spread of pathogens in plant material and fight forgery, much still has to be done in order to create a virtual environment for grape and wine production, changing this fragmented agricultural "internet of things" into a coherent "internet of trees", in which regulations and best practices may converge in harmonized electronic labeling and databases, without losing the link between plants and food. Indeed, the relationship between plants and food is not just a simple question of input/output, but rather a complex system in which plant pathogens and their control play an important role. This link is promoted by the European Food Safety Authority (EFSA), the agency that provides scientific advice and communication on existing and emerging risks associated with the food chain and the Authority's work covers all matters with a direct or indirect impact on food safety, including plant protection and plant health as included in the general objective and mission of the EFSA.

References

BANDINELLI R., TRIOLO E., LUVISI A., PAGANO M., GINI B., RINALDELLI E., 2009 - *Employment of radiofrequency technology (RFId) in grapevine nursery traceability*. - Adv. Hort. Sci., 23(2): 75-80.

CUNHA C.R., PERES E., MORAIS R., OLIVEIRA A.A., MATOS S.G., FERNANDES M.A., FERREIRA P.J.S.G., REIS M.J.C.S., 2010 - *The use of mobile devices with multi-tag technologies for an overall contextualized vineyard management*. - Computers and Electronics in Agriculture, 73: 154-164.

LUVISI A., PAGANO M., BANDINELLI R., RINALDELLI E., GINI B., SCARTÒN M., MANZONI G., TRIOLO E., 2011 - *Virtual vineyard for grapevine management purposes: a RFID/GPS application*. - Computers and Electronics in Agriculture, 75: 368-371.

LUVISI A., PANATTONI A., TRIOLO E., 2012 - *Electronic identification-based Web 2.0 application for plant pathology purposes*. - Computers and Electronics in Agriculture, 84: 7-15.

PEETS S., GASPARIN C.P, BLACKBURN D.W.K., GODWIN R.J., 2009 - RFID tags for identifying and verifying agrochemicals in food traceability systems. - Precision Agriculture, 10: 382-394.

Adv. Hort. Sci., 2013 27(3): 112

RFID microchips as a tool for traceability in grapevine nurseries: pre- and post-grafting implants

F. Mannini, I. Gribaudo
Plant Virology Institute CNR, Grugliasco Unit, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy, i.gribaudo@ivv.cnr.it

Radio Frequency Identification (RFID) technology has been used for some years for the identification and traceability of various products. This approach has been successfully adopted also in the agrifood sector to guarantee a reliable traceability system. In grapevine nurseries, the storage of data in a field log is an essential step in the plant production process. Specific regulations require the presence of colored labels indicating the type, origin and other features of the material. This system is inexpensive and simple, but has several drawbacks, thus the application of RFID technology in grapevine nurseries has been studied. The insertion of RFID tags (microchip) in grafted and ungrafted cuttings would allow to store and retrieve a large quantity of data concerning the single plant, including the application of hot water treatment against phytoplasma diseases.

In March 2011, microchips (0.21 x 1.20 cm) were inserted in the pith of two types of grapevine (*Vitis* spp.) propagation materials: grafted cuttings (a) and grafted rootlings (b), subjected or not to hot water treatment (50 °C for 45 min). In (a) the microchips were inserted in the rootstock cuttings (Kober 5BB and S.O.4) after direct drilling of pith, before grafting the cuttings with scions of 'Barbera' and 'White Muscat'. In (b) the microchips were inserted in grafted rootlings of three 'Nebbiolo' clones, a few cm below the grafting point, through a "U" cut performed laterally by a specially designed machine. Tags were read through a palmtop computer and an appropriate software program allowed management of the stored data.

After the first year of cultivation in the nursery, the grafted cuttings (a) were planted in a vineyard in spring 2012. There was some variability in the nursery take among treatments also related to the quality of the plant materials, whereas tagging accuracy (readable microchips) in September 2012 was very good (about 90% successful read rate). The grafted rootlings (b) were planted in a vineyard in spring 2011. In the following summer more than 90% of microchips were readable, whereas this percentage decreased to 50% in summer 2012 under more humid conditions, indicating that microchip insertion in grafted rootlings can encounter some problems.

In conclusion, electronic tagging of grapevine propagation materials proved technically feasible, although the cost of microchips (still too high compared to the average price of grapevine rootlings) continues to hinder their routine adoption in nurseries. Nevertheless, electronic tagging could be advisable for "basic" material (that used by nurseries to establish their mother-vine vineyards) as it has greater commercial value.

Adv. Hort. Sci., 2013 27(3): 113-114

Competitiveness of the wine sector: considerations on future scenarios

G. Scalabrelli, A. Toffanin
Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy, giancarlo.scalabrelli@unipi.it

For a critical assessment of the issues relating to the future viticulture nursery system in order to identify the strengths and weaknesses is essential to examine the relationship between factors of the supply chain and the extrinsic factors able of modulating the interactions between the whole wine system. The uncertainty of the evolution over time of the forecasts suggests examining these aspects for hypothetical scenarios which involves the following interfaces: Consumer (market)/ Producer, Grower/Nursery, Research/Nursery/Grower/Consumer. Other aspects might modulate the above relations: social ethics, energy cost, globalization, economic situation, climate change, Pathogens emergencies.

Consumer (market)/producer. What is certain is that enlargement of the European Community to the East and the lack of restrictions on the spread of viticulture in new emerging countries (Latin America, South Africa and Oceania), will pose competition problems on traditionally wine countries such as Italy and France which will fight fiercely to defend the current position revenue linked to the prestige of wine-growing areas. There is no doubt that the new emerging countries, at least for several years, will base their market strategy on the best ratio between cost and price, with condition that it will be possible to maintain rapid and streamlined trades between countries, leaving the market share of high-quality wines. The dominant effects of the recession could lead to the reduction in the purchasing power of the population and thus to decrease the consumption of luxury goods, within the framework of the poorer population groups. The decrease in consumption will vary depending on the role attributed to the wine (food or hedonistic) and the ability to reduce other purchases luxury or essential goods, although the pleasure being part of main needs (but individual) will favors the consumption of high quality wine. New dietary needs could emerge, to supplement the daily diet, and especially locally or from neighboring basins (localism, joint buying groups). Environmental sensitivity could increase the need for produce in an environmentally-friendly way (Scalabrelli, 2010) and with less wasted energy and CO_2 production due to long-range transport. There is, however, to take into account many motivational aspects regarding the future evolution of wine consumption. We already feel the first symptoms of new trends that tend to favor wines with special features of healthiness, peculiarity and uniqueness. In fact, a part of the population does not seek more homogeneous characteristics and wines already granted who find themselves generally in varietal wines produced in a specified area or a specific brand. The uncertainty on these issues lies in sizing the scope of this question.

Grower/Nursery. The expected decline in demand should not have immediate effects on the structural consistency of the companies, although it might affect the renewal of new vineyards and delay the activation of new business ventures. Consequently, with the exception of the physiological abandonment of vineyard areas, it could be envisaged a slight decline in demand for new plantations with possible surplus of material produced. In a first stage the survival of nursery companies will not be at risk, but those more efficient and better organized will have more chances of remain on the market. In a longer period, the continuing demand for wine (or diversification), might request to redirect the offer to new sales channels (or production), although it is hardly predictable as the globalized market might move and what changes might be required in order to produce and sell. Companies will have to carefully follow the market and consumer demands, develop new solutions to meet the new population requirements. For example, produce in an environmentally-friendly, organic, biodynamic will be no longer just slogans, if the research will have clarified and deepened aspects and come up with appropriate techniques, but the needs imposed by the consumer. For productive diversification, there is currently a clear differentiation between traditional varieties and the advent of new varieties obtained by genetic improvements. The tendency to preserve the local varieties and to use the traditional ones appears a goal practiced by small farm meanwhile the possible use of new varieties obtained by genetic improvement will be probably a strategy of the globalized system. Of course biodiversity conservation would not be exhaustive unless their characteristics and performance will be known in view to utilise this source for possible direct cultivation or for genetic improvement. In this contest the nurseries could have new opportunities either on the production of autochthon varieties or propagating new material obtained by genetic improvement.

Research/Nursery/Grower/Consumer. The critical economic situation it is not expected to influence promptly the work in progress for the clone selection, previously started, altogether new projects initiatives could be restricted unless, a reversal trend towards investment of funds for research will take place. The local germplasm collection and its use are

prospected although this aspect is not universally shared, as worldwide is focusing attention on few international grapevine varieties. In any case the biodiversity of grapevine germplasm is considered a resource (Scalabrelli, 2007; D'Onofrio and Scalabrelli, 2010). Another area of great scientific interest is the study of varietal behavior, in view of climate change and, above all, thanks to the progress made with the grape genome sequencing (Jaillon *et al*., 2007) genetic improvement to obtain varieties more resistant to biotic and abiotic stress, can take advantages of the assisted breeding techniques. We could have, therefore, on world market engineered plants produced exclusively from several countries or companies that can find spread depending on the acceptance or rejection by the consumers. It is clear that a fundamental part of research will be carried out to clarify whether GMOs can pose risks to health or the environment. On this basis a possible clarification it might also change the current hostile attitude of some countries or part of the population. The strategy choice to cultivate or not genetically modified vines could also result in disruption in the spread of viticulture in different countries, but this could not occur before ten years. In our opinion, it should be advisable the introduction of specific genes within the genus *Euvitis*. Moreover another field of research is the intensification of the studies to understand the mechanisms that regulate the plants functioning and the role of symbiotic microorganisms which could facilitate the cultivation and the development of environmentally friendly techniques (biodynamic method?). Beneficial microorganisms, genetically modified or not, will be useful for finding biotechnological solutions, especially to control biotic and abiotic stress and for a biofertilization in balance with the aimed productions. In presence of a high critical situation of funding we need to wisely manage the ethical aspects of research and ensure adequate and rational fund investment. It would be desirable for local projects carry out a "Shared Research", involving the various components of the "Wine chain", a new need this to satisfy, especially if the State and the Regions will not be able to devote resources to this sector, as committed to supporting consumption and to invest on social safety nets

References

JAILLON O., AURY J.-M., NOEL B., POLICRITI A., CLEPET C., CASAGRANDE A., CHOISNE N., AUBOURG S., VITULO N., JUBIN C., VEZZI A., LEGEAI F., HUGUENEY P., DASILVA C., HORNER D., MICA E., JUBLOT D., POULAIN J., BRUYÈRE C., BILLAULT A., SEGURENS B., GOUYVENOUX M., UGARTE E., CATTONARO F., ANTHOUARD V., VICO V., DEL FABBRO C., ALAUX M., DI GASPERO G., DUMAS V., FELICE N., PAILLARD S., JUMAN I., MOROLDO M., SCALABRIN S., CANAGUIER A., LE CLAINCHE I., MALACRIDA G., DURAND E., PESOLE G., LAUCOU V., CHATELET P., MERDINOGLU D., DELLEDONNE M,, PEZZOTTI M., LECHARNY A., SCARPELLI C., ARTIGUENAVE F., PÈ M.E., VALLE G., MORGANTE M., CABOCHE M., ADAM-BLONDON A.-F., WEISSENBACH J., QUÉTIER F., WINCKER P., - *The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla*. - Nature, 449: 463-467.

D'ONOFRIO C., SCALABRELLI G., 2010 - *Un database viticolo universale*. - Italus Hortus, 17(3): 328-333.

RUSSO A., CARROZZA G.P., VETTORI L., FELICI C., CINELLI F., TOFFANIN A., 2012 - *Plant Beneficial microbes and their application in plant biotechnology,* pp. 57-72. - In: AGBO E.C. (ed.). *Innovations in biotechnology*. InTech Europe, Rijeka, Croatia, pp. 474.

SCALABRELLI G., 2007 - *I vitigni autoctoni*. - Locus. Felici Editore, Pisa, 7: 45-52.

SCALABRELLI G., 2010 - *Quale viticoltura per il futuro*? - Italus Hortus, 17(3): 680-686.

Adv. Hort. Sci., 2013 27(3): 115-121

Effect of mulching and plant density on out-of-season organic potato growth, yield and quality

G. Caruso[1], D. Carputo, S. Conti, C. Borrelli, P. Maddaluno, L. Frusciante
Dipartimento di Agraria, Università degli Studi di Napoli Federico II, Via Università, 100, 80055 Portici (NA), Italy.

Key words: biodegradable mulch, plant leaf area and dry matter, production, *Solanum tuberosum* L., tuber vitamin C.

Abstract: Research was carried out on potato (*Solanum tuberosum* L., cv. Spunta) growing in the field in the Campania region (southern Italy) in 2007 and 2008, adopting organic farming practices, in order to evaluate the effects of two mulching treatments (black biodegradable film and bare soil) and six plant densities (12.5, 10.0, 8.3, 7.1, 6.2 and, as a control, 5.3 plants per m^2) on growth, yield and quality of "new potato" winter-spring and summer-autumn crops. Only in the case of the summer-autumn crop cycle, mulching resulted in a higher yield, plant dry matter and leaf area compared with the bare soil control, while in both crop cycles this latter treatment induced a delay in harvest. The winter-spring cycle gave a higher production of 40-70 mm tubers, while the summer-autumn cycle resulted in a higher vitamin C content. For the winter-spring crop cycle, the plant density of 8.3 plants·m^{-2} resulted in the highest yield for food-use tubers, whereas the highest production of seed tubers was obtained with a density of 12.5 plants·m^{-2}. The plant density of 8.3 plants·m^{-2} also resulted in the highest plant dry matter and leaf area. For the summer-autumn crop cycle, the 10 plants·m^{-2} density gave the highest production of 40-70 mm calibre tubers, as well as the highest plant dry matter and leaf area. In this cycle, the 6.3 plants·m^{-2} density resulted in the highest production of 70-80 mm calibre tubers. In terms of cost effectiveness, the choice of biodegradable mulching could save the expense of manual weed control and, in the case of the summer-autumn crop cycle, it is also associated with a higher yield. Overall, tuber yield increased with plant density but the final production was also affected by the crop cycle. This may depend on the different environmental conditions and duration which characterized each cultural cycle and, therefore, affected the vegetative development of organic new potatoes.

1. Introduction

Potato (*Solanum tuberosum* L.) is widely cultivated in Italy, with a total field area of 58,398 ha devoted to this crop in 2012, mainly in Sicily (11,236 ha), Campania (9,467 ha) and Calabria (6,115 ha) (ISTAT, 2012). In the southern Italian regions of Campania, Sicily and Apulia out-of-season crop production is traditionally practised and the early potato crop covers a total of 14,011 ha (ISTAT, 2012), that is 93.1% of the total acreage under this crop (15.051 ha) in Italy. In addition, nearly all of the Italian potato production is obtained from conventional farming practice, although in the last nine years there has been a small increase in the area devoted to organic potato production. This latter accounted for about 730 ha in 2003 and 981 ha in 2011 (1.18 and 1.58% of the total area, respectively) (CCPB, 2012).

In the Mediterranean area, out-of-season potato yield obtained from winter-spring or summer-autumn crop cycles is mainly channelled into the highly profitable export market. However, for both conventional and organic crops the availability of certified seed tubers is a limiting factor. Indeed, this crop frequently gives a modest yield because small and/or immature tubers are used (Delaplace *et al*., 2008) which are still conditioned by apical dominance owing to the brief interval between seed tuber harvest and their use for planting. In the case of the summer-autumn potato crop, a possible method to improve yield is to obtain seed tubers from an early crop (winter-spring cycle) in order to harvest the product before aphid proliferation (Monti and Struik, 1999) and to limit pathogen infections (Hospers-Brands *et al*., 2008). Under organic farming practice, Saucke and Döring (2004) found that, in addition to effective weed control, the use of biodegradable mulch films helps reduce aphid infestation on leaves and potato virus Y incidence in tubers. Moreover, within each microenvironment it is important to choose the planting time which best suits the requirements of the specific cultivar (Frusciante *et al*., 1999; Caruso *et al*., 2010) in order to obtain good results.

As a general precaution, the choice of seed tubers of the correct size should be preferred. In fact, seed potatoes should be neither too big, requiring to be cut in order to limit the possible diffusion of infections (Franc and Banttari, 1984), nor undersized to avoid slow initial growth and tuberization (Mustonen, 2004). Indeed, shoot vigour

[1] Corresponding author: gcaruso@unina.it
Received for publication 22 March 2013
Accepted for publication 2 July 2013

and development rate are inversely correlated to seed tuber size, which provides meristematic resources (MacKerron *et al*., 1988): when the latter are limited, scarce shoot emission and hence smaller within-plant resource competition occurs. This leads to the formation of fewer large tubers (Hide *et al*., 1997), which is not desirable for the production of either food-use or seed tubers.

Crop mechanization (Green, 1962), aimed at reducing production costs, has led to an enlargement of between-row and in-row plant spacings. Hence, the current trend is to use spacings of 70-80 cm between the rows and 20-30 cm along the rows for conventional potato crops grown from ripe tubers in February-March. In this regard, contrasting results have been published: in some areas both lower and higher in-row spacings resulted in yield reduction (Masarirambi *et al*., 2012) while in other environments a higher plant density resulted in a higher production but reduced tuber calibre (Turajizadeh *et al*., 2011; Fontes *et al*., 2012), the latter effect being desirable for "seed" production. Moreover, increasing in-row spacing leads to greater tuber size (Tarkalson *et al*., 2011). In mild climates, winter-spring and summer-autumn crops are respectively grown from commercial "seed" potatoes or from locally (re)produced tubers. At the time of planting for out-of-season potato production, the seed tubers from these two crop cycles are still not completely mature and they are conditioned by apical dominance. In these cases, it may be interesting to increase plant density to balance the scarce shooting capacity of physiologically immature propagation material.

To our knowledge, no conclusive research has yet been published regarding the use of mulching and its interaction with plant density in organic farming practice in southern Italy. Thus, we planned our field trials with the aim of testing the effect of biodegradable mulching and of plant density on the production of "seed" and food potatoes from winter-spring crops and on the production of food potatoes only from summer-autumn crops. Trials were carried out in the Campania region (southern Italy) on organic potato crops, cv. Spunta.

2. Materials and Methods

Research was carried out in San Gennaro Vesuviano (Naples) in 2007 and 2008 on potato cv. Spunta, grown in the field under organic farming practices on a sandy-loam soil (Table 1); temperature and rainfall values are reported in Table 2 as means of the data from the first and second experimental years.

Twelve experimental treatments were compared. These were obtained from the factorial combination of two mulching treatments (black biodegradable film and bare soil) and six plant densities (12.5, 10.0, 8.3, 7.1, 6.2 and, as a control, 5.3 plants per m^2). The experimental treatments were randomized in a split-plot design with three replicates, assigning mulching treatments to the main plots and plant densities to the elementary plots; the latter had a 18.00 m^2 (6.00 x 3.00 m) surface area.

The soil was prepared by forming raised beds which were mulched with a 15-µm thick biodegradable black film made from corn starch. Potatoes were planted in double rows for all treatments except for the control crop which was grown in single rows. The six different plant densities were obtained using the following spacings: 0.40 m between the rows and 0.20, 0.25, 0.30, 0.35 or 0.40 m along the rows for 12.5, 10.0, 8.3, 7.1, 6.2 plants·m^{-2} treatments; 0.75 m between the rows and 0.25 m along the rows for the 5.3 plants·m^{-2} treatment (control).

Table 1 - Soil characteristics

On 100 g of air-dried and 2 mm sieved soil		
Coarse sand	g	35.3
Fine sand	g	45.5
Silt	g	7.8
Clay	g	11.4
Organic matter (Walkley-Black method)	g	1.18
Total nitrogen (N) - Kjeldhal method	g	0.1
Available phosphate (P_2O_5) - Olsen method	mg	1.7
Available potassium (K_2O) - ammonium acetate method	mg	54.0
Total lime (Dietrich-Früling)	g	traces
pH		7.4

Table 2 - Temperature and rainfall values (means of 2007 and 2008) during winter-spring and summer-autumn crop cycles in San Gennaro Vesuviano (Naples)

Month	Dates intervals	Temperature (°C)		Rainfall (mm)
		Minimum	Maximum	
January	21-31	9.3	14.9	13.4
February	1-10	9.5	15.2	42.2
	11-20	8.1	14.3	37.1
	21-28	10.9	17.3	17.5
March	1-10	11.3	16.0	68.1
	11-20	11.3	17.2	33.3
	21-31	8.9	16.6	65.2
April	1-10	11.8	19.8	25.7
	11-20	13.3	21.3	27.4
	21-30	13.7	22.4	21.0
May	1-10	15.2	24.1	10.6
	11-20	15.8	24.4	29.4
August	21-31	23.8	33.1	0.0
September	1-10	20.8	30.0	15.7
	11-20	18.8	27.6	35.4
	21-30	16.2	24.5	23.7
October	1-10	17.6	24.6	28.0
	11-20	16.4	24.0	6.0
	21-31	15.5	21.6	14.8
November	1-10	14.5	20.4	9.0
	11-20	10.6	15.4	24.3
	21-30	12.2	16.6	76.4
December	1-10	10.9	15.1	63.0

Plantings took place on the following dates: 24 January for the winter-spring crop using certified "seed" tubers (50±2 g) and 20 August for the summer-autumn crop using "seed" tubers obtained from the previous crop cycle (30-40 mm). Tubers were kept at 7°C until use and showed no pre-sprouting at planting time. Potato crops were preceded by common bean and fava bean on the same plots.

The organic farming system was managed in compliance with EC Regulation 834/2007 and the farming practices were: fertilization before planting with 100 kg·ha^{-1} of N, 85 of P_2O_5 and 210 of K_2O with Biolsa 6-5-13; drip fertigation with 50 kg·ha^{-1} of nitrogen as 8.5 N hydrolyzed animal epithelium; weed control by hand; plant protection treatments with copper, azadirachtin and rotenone.

New potato tubers were harvested when the aerial part of the plant showed the initial symptoms of senescence (leaf yellowing) and wilting.

Tubers from the winter-spring crop were harvested on 18 May 2007 and 21 May 2008. The summer-autumn crops were harvested on 4 December 2007 and 2 December 2008. Undamaged tubers of regular shape were classified as "marketable" and graded as follows: the 30-40 mm and 40-70 mm grades (which are usually addressed to the large scale retail channel and packaged in boxes or bags, respectively) and the 70-80 mm grade (which is usually channelled to the local market).

At the time of harvest, the following determinations were made in each plot: number of failures; number of shoots per plant; number and weight of tubers; tuber mean weight on a 50-unit sample; tuber grading and classification into the three calibre classes. Tubers falling into the 30-40 mm class were classified as "seed" tubers in the case of the winter-spring crop or as food tubers in the case of the summer-autumn crop.

The weight of tubers unsuitable for the market was also recorded in order to monitor total biomass production for each treatment. Plant biomass was calculated as the sum of the aboveground plant biomass at the end of the experiment plus the total tuber production. Dry residue was assessed after dehydration of the fresh samples in an oven at 70°C under a vacuum until they reached constant weight. Leaf area was measured at the end of the cycle, using a bench top LI-COR leaf area meter.

In order to evaluate the quality of tubers produced both from the winter-spring and from the summer-autumn crop, samples of 20 tubers per plot were randomly collected at harvest time and transferred to the laboratory, where the following determinations were made:

- dry residue, in an oven at 70°C under vacuum until steady weight;
- vitamin C content, using a Waters 600E HPLC system equipped with a Waters 486 UV detector set to 410 nm λ and a Biorad column mod. HPX87H at 35°C.

Data were processed by analysis of variance and mean separations were performed through the Duncan multiple range test, with reference to 0.05 and 0.01 probability levels, using SPSS software version 15.

3. Results and Discussion

From statistical processing of the data, no differences were detected between 2007 and 2008, therefore only the mean values of the two research years are shown.

In the case of winter-spring crops, mulching did not significantly affect yield but the crop cycle of the mulched crops was four days and a half shorter compared with the bare soil treatments (Table 3). Similar results were published by Boyd *et al*. (2001) and Döring *et al*. (2005) who reported that mulching did not produce any increase in yield and tuber size but it only caused an increase in soil temperature (Xing *et al*., 2012). Conversely, Maletta *et al*. (2006) reported that mulching resulted in increased yield and tuber size in organic potato.

Plant density significantly affected yield (Table 3): maximum production was obtained with a plant density

Table 3 - Yield results and growth indices of winter-spring potato crop

Treatment	Failures %	Actual density no.·m^{-2}	Shoots no.·pt^{-1}	Tubers no.·m^{-2}	Tuber mean weight g	Yield t·ha^{-1}	Crop duration days	Plant growth indices (maximum values)	
								Dry matter g·m^{-2}	LAI m^2·m^{-2}
Mulching									
Biodegradable film	4.7	7.9	1.08	25.1	110.6	26.8	114.5	696.3	2.1
Bare soil	4.6	7.9	1.09	25.1	107.0	26.0	119.0	663.9	2.0
	NS		NS	NS	NS	NS	*	NS	NS
Plant density									
5.3 pt·m-2	4.0	5.1	1.10	16.9 f	123.3 a	20.8 d	117.2	546.4 d	1.6 d
6.3	5.3	5.9	1.15	19.8 e	121.5 ab	24.1 c	117.0	622.3 c	1.9 c
7.1	4.6	6.8	1.15	22.5 d	117.5 bc	26.4 b	116.8	681.0 b	2.1 b
8.3	3.7	8.0	1.12	26.1 c	112.0 c	29.2 a	116.7	748.5 a	2.3 a
10.0	5.0	9.5	1.03	29.9 b	97.4 d	29.1 a	116.5	744.0 a	2.3 a
12.5	5.4	11.8	1.00	35.5 a	81.3 e	28.8 a	116.3	738.3 a	2.2 ab
	NS		NS				NS		

* = significant at p≤0.05; NS = not significant; whitin each column, means followed by different letters are significantly different according Duncan test at p≤0.05.

of 8.3 plants·m^{-2}, though it was not significantly different from 12.5 and 10.0 plants·m^{-2}. The control plant density of 5.3 plants·m^{-2} gave the lowest yield. Similar results were reported by Fontes and coworkers (2012), who found that reducing the in-row spacing from 50 to 29 cm led to a higher tuber number and greater yield. However, Masarirambi *et al*. (2012) reported a yield decrease when plant density increased from 3.7 to 7.4 plants·m^{-2}. Due to the early plantings, the "seed" tubers used to start the winter-spring crops had still not reached their full physiological maturity. Consequently, they produced plants with only one shoot and a small number of tubers which did not vary between the treatments. Therefore, the productive results were affected both by the number of tubers per square meter and by the tuber mean weight: the highest number of tubers was recorded at the highest plant density while the highest tuber mean weight was obtained with 5.3 plants·m^{-2}. No difference in precocity among the treatments was recorded. Total yield increased with increasing plant density up to 8.3 plants·m^{-2}, because the apical dominance effect induced tubers to produce only one shoot, thereby reducing dramatically the competition between plants and within each plant.

Growth parameters varied similarly to yield in response to mulching and plant density (Table 3). Dry matter and leaf area were not significantly affected by mulching, while plant density increases up to 8.3 plants·m^{-2} resulted in the highest values of both dry matter and leaf area, reflecting the mulching effect on total yield.

The relative proportion of tubers classified into the different calibre classes (Table 4) was not significantly different between the mulched or bare soil treatments, but significant effects were recorded in response to the different plant densities. The highest densities of 12.5 and 10.0 plants·m^{-2} resulted in the highest incidence of 30-40 mm grade tubers, which are suitable for uncut "seed" use and may therefore be considered the best choice as seed potatoes for the following summer-autumn crop. The plant densities of 6.3 and 7.1 plants·m^{-2} produced the highest proportion of 40-70 mm grade tubers, which are suitable for the food market, whereas plant density did not significantly affect the incidence of 70-80 mm calibre tubers. These results are in accordance with previously published studies reporting an increase in the small- and medium-tuber size class when the in-row plant spacing was reduced from 50 to 29 cm (Fontes *et al*., 2012) and plant density from 8.0 to 5.3 plants·m^{-2} (Turajizadeh *et al*., 2011).

As a quality indicator of potato tubers, the dry residue was not significantly affected by mulching or by plant density (Table 4). Vitamin C content was higher in tubers obtained from mulched plots, whereas it did not vary in response to the different plant densities. Similar results were published by Gram (1951) who found that tubers from straw-mulched crops had significantly higher ascorbic acid content. Moreover, in more recent years Dvorak *et al*. (2012) reported no significant effects of mulching on potato tuber ascorbic acid content under different soil or climatic conditions. These contrasting results suggest that the actual ascorbate content of potato tubers may result from an interaction of multiple factors. However, the tuber vitamin C values detected in our research fall in the range reported by Brown (2005).

After discarding about 10% of virus-infected tubers, the 30-40 mm tubers produced by the winter-spring crops were used as seed potatoes for the following summer-autumn crops. These seed tubers were to be planted about three months after harvest, before they could reach their full physiological maturity, similarly to the commercially available seed potatoes used for the winter-spring crops. As discussed

Table 4 - Grading and quality indicators of "seed" and food-use potato tubers produced from winter-spring crop

Treatment	Seed-use grade				Food-use grades		Tuber quality indicators	
	30-40 mm				40-70 mm	70-80 mm		
	Yield t·ha^{-1}	% of total yield	Tuber mean weight g	Potential Use [z] ha	% of total yield	% of total yield	Dry residue %	Vitamin C mg·100 g^{-1} f.w.
Mulching								
Biodegradable film	8.9	33.2	49.1	3.4	58.7	8.0	18.5	23.0
Bare soil	8.8	34.0	50.6	3.3	58.0	7.9	18.8	22.2
	NS	NS	NS	NS	NS	NS	NS	*
Plant density								
5.3 pt·m-^{2}	6.7 e	32.2 bc	50.4 ab	2.5 e	59.8 ab	8.0	18.5	22.7
6.3	7.6 d	31.4 c	51.8 a	2.7 de	60.5 a	8.1	18.6	22.5
7.1	8.7 c	32.8 b	51.1 ab	3.2 cd	59.1 ab	8.1	18.7	22.9
8.3	9.8 b	33.4 b	50.3 ab	3.6 bc	58.6 b	8.0	18.9	22.3
10.0	10.3 ab	35.5 a	48.9 bc	4.0 ab	56.6 c	7.9	18.6	22.7
12.5	10.5 a	36.4 a	47.0 c	4.2 a	55.7 c	7.9	18.8	22.5
						NS	NS	NS

[z] Using the traditional 75 x 25 cm spacings (5.3 plants·ha^{-1}) in the following summer-autumn crop; * = significant at p≤0.05; NS = not significant; whitin each column, means followed by different letters are significantly different according Duncan test at p≤0.05.

above, their immature state caused each "seed" to originate only one shoot and, consequently, the number of tubers per plant did not differ among the treatments. Therefore, yield in the different plots was only affected by the number of tubers per unit surface area and by the tuber mean weight (Table 5).

In the summer-autumn cycle, mulching increased crop precocity and affected tuber production favourably, as compared with bare soil treatments (Table 5). This result may be explained by the lower failures and higher tuber mean weight recorded in mulched plots, though these variables were not significantly affected by mulching. It was suggested that mulching resulted in higher potato yield due to the reduction of soil nutrient loss caused by runoff (Rees *et al*., 2002) and to the prevention of excessive soil heating (Dvorak *et al*., 2012).

Plant density significantly affected tuber production (Table 5) and the highest yields were recorded with 10.0 and 12.5 plants·m^{-2}, whereas the control plots (5.3 plants·m^{-2}) gave the lowest production. At the same time, tuber mean weight was affected by competition among plants and it increased at lower plant densities. No significant effect of plant density on precocity was recorded. Total production increased with plant density up to 10.0 plants·m^{-2}, i.e. even over the 8.3 density threshold recorded for the winter-spring crop. In fact, the latter showed greater plant expansion (Tables 3 and 5) due to the longer crop cycle, causing presumably greater plant competition for available resources. Moreover, both growth indexes (plant dry matter and leaf area) attained higher values with mulching compared with bare soil, whereas they were not significantly affected by plant density (Table 5).

Tubers harvested in the autumn were graded only with regard for the food market (Table 6). Mulched plots pro-

Table 5 - Yield results and growth indices of summer-autumn potato crop

Treatment	Failures %	Actual density no.·m^{-2}	Shoots no.·pt^{-1}	Tubers no.·m^{-2}	Tuber mean weight g	Yield t·ha^{-1}	Crop duration days	Plant growth indices (maximum values)	
								Dry matter g·m^{-2}	LAI m^2·m^{-2}
Mulching									
Biodegradable film	14.5	6.9	1.0	20.9	114.1	24.7	105.0	582.1	1.8
Bare soil	15.7	7.0	1.0	20.4	112.0	20.7	109.3	496.5	1.5
	NS		NS	NS	NS	*	*	*	*
Plant density									
5.3 pt·m-^{2}	15.1	4.5	1.0	13.6 f	125.7 a	17.1 e	107.7	372.3 d	1.2 d
6.3	14.9	5.4	1.0	16.2 e	124.5 a	20.2 d	108.4	475.6 c	1.4 c
7.1	15.0	6.0	1.0	18.2 d	118.5 b	21.6 d	108.0	510.2 c	1.5 c
8.3	15.2	7.0	1.0	20.9 c	115.5 b	24.1 c	107.1	570.7 b	1.7 b
10.0	15.3	8.5	1.0	24.7 b	110.0 c	27.1 b	106.4	642.9 a	1.9 a
12.5	15.2	10.6	1.0	30.9 a	84.5 d	26.1 a	105.8	663.8 a	2.0 a
	NS		NS				NS		

* = significant at p≤0.05; NS = not significant; whitin each column, means followed by different letters are significantly different according Duncan test at p≤0.05.

Table 6 - Grading and quality indicators of food-use potato tubers produced from summer-autumn crop

Treatment	Food use tuber grades			Tuber quality indicators	
	30-40 mm % of total yield	40-70 mm % of total yield	70-80 mm % of total yield	Dry residue %	Vitamin C mg·100 g^{-1} f.w.
Mulching					
Biodegradable film	26.4	62.4	11.2	17.8	21.7
Bare soil	28.4	59.2	12.4	18.0	21.4
	*	*	*	NS	NS
Plant density					
5.3 pt·m-^{2}	26.2 d	60.8 bc	13.0 d	17.9	21.5
6.3	26.0 d	58.5 d	15.5 a	18.0	21.6
7.1	27.0 c	59.9 c	13.1 b	17.7	21.4
8.3	27.9 b	61.0 b	11.1 c	17.8	21.7
10.0	28.6 a	62.1 a	9.3 d	18.0	21.5
12.5	28.9 a	62.5 a	8.6 e	17.8	21.6
				NS	NS

* = significant at p≤0.05; NS = not significant; whitin each column, means followed by different letters are significantly different according Duncan test at p≤0.05.

duced a higher percentage of medium-sized tubers (40-70 mm) whereas the crops grown on bare soil produced a higher percentage of small and large tubers (30-40 mm and 70-80 mm, respectively).

The highest plant densities of 12.5 and 10 plants·m^{-2} led to the highest proportion of 30-40 mm calibre tubers, as well as of the 40-70 mm calibre. Moreover, the percentage of the largest tubers increased at lower plant densities, whereas this parameter was not significantly affected in the case of the winter-spring crop cycle.

The quality indicators of potato tubers (dry residue and vitamin C) were not significantly affected by the experimental factors during the summer-spring crop cycle (Table 6).

Tuber dry residue and vitamin C contents of tubers harvested in the autumn were as much as 4 and 5% lower compared with tubers harvested in the spring. These results were in accordance with previous research (Ierna, 2010) reporting higher values of yield and tuber dry residue, but lower tuber mean weight in spring tubers compared to autumn ones.

Finally, the winter-spring crop cycle resulted in lower tuber mean weight and higher yield, compared with the summer-autumn crop cycle.

4. Conclusions

In the case of the winter-spring cycles, the use of biodegradable mulching induced an anticipation of the harvest but it was not found to have significant effects on tuber production.

The use of biodegradable mulching for the summer-autumn crop cycle gave better production results and an anticipation of the harvest; moreover, it also had positive effects on the prevention of water logging caused by abundant rainfall in November.

Since chemical weed control is not permitted in organic farming, in both crop cycles mulching allowed easier and cheaper weed control than that carried out by hand for bare soil cultivation.

Unlike conventional "mature potato" crops which are usually grown with a plant density of 5-6 seed tubers per m^2, in the case of "new potato" crops a higher yield was obtained by increasing plant density both in the winter-spring and summer-autumn cycles. In this respect, the summer-autumn cycle allowed the highest plant density (up to 10.0 plants·m^{-2}) compared with the winter-spring cycle (up to 8.3 plants·m^{-2}) because of the lower plant vegetative growth. In the case of the winter-spring cycle, a plant density of 12.5 plants·m^{-2} density produced the highest amount of "seed" potatoes (30-40 mm) to be used for the next summer-autumn crop cycle.

Acknowledgements

The authors wish to thank Dr. Mark Walters for editing the manuscript and Mr Roberto Maiello for his assistance with laboratory analyses. This research was funded by the Campania Regional Authority.

References

BOYD N.S., GORDON R., ASIEDU S.K., MARTIN R.C., 2001 - *The effects of living mulches on tuber yield of potato (*Solanum tuberosum *L.)*. - Biol. Agric. Hortic., 18(3): 203-220.

BROWN C.R., 2005 - *Antioxidants in potato*. - Amer. J. Potato Res., 82(2): 163-172.

CARUSO G., CARPUTO D., FRUSCIANTE L., 2010 - *Research on potato seed-use practices: planting times and seed tuber weight in relation to cultivar*. - Adv. Hort. Sci., 24(2): 149-153.

CCPB, 2012 - *Statistical data 2012*. - Consorzio per il controllo dei prodotti biologici, Bologna, www.ccpb.it.

DELAPLACE P., BROSTAUX Y., FAUCONNIER M.L., DU JARDIN P., 2008 - *Potato (*Solanum tuberosum *L.) tuber physiological age index is a valid reference frame in post-harvest ageing studies*. - Postharvest Biol. and Technol., 50(1): 103-106.

DÖRING T.F., BRANDT M., HESS J., FINCKH M.R., SAUCKE H., 2005 - *Effects of straw mulch on soil nitrate dynamics, weeds, yield and soil erosion in organically grown potatoes*. - Field Crop Res., 94(2-3): 238-249.

DVORAK P., TOMASEK J., KUCHTOVA P., HAMOUZ K., HAJSLOVA J., SCHULZOVA V., 2012 - *Effect of mulching material on potato production in different soil-climatic conditions*. - Romanian Agric. Res., 29: 201-209.

FONTES P.C.R., NUNES J.C.S., MOREIRA M.A., 2012 - *Graded potato yield in response to interplant spacing and fertilizer recommendation criteria*. - Biosci. J., 28(3): 404-412.

FRANC G.D., BANTTARI E.E., 1984 - *The transmission of potato virus S by the cutting knife and retention time of infectious PVS on common surfaces*. - Amer. Potato J., 61: 253-260.

FRUSCIANTE L., BARONE A., CARPUTO D., RANALLI P., 1999 - *Breeding and physiological aspects of potato cultivation in the Mediterranean region*. - Potato Res., 42: 265-277.

GRAM M.R., 1951 - *Reduced ascorbic acid content of potatoes grown with and without straw mulching and irrigation in eastern Nebraska.* - Nebraska Agric. Expt. Sta. Res. Bull., 40.

GREEN H.C., 1962 - *Row widths and inter-row cultivation of potatoes*. - Eur. Potato J., 5: 57-67.

HIDE G.A., WELHAM S.J., READ P.J., AINSLEY A.E., 1997 - *Effects of planting mixtures of different sizes of potato seed tubers on the yield and size of tubers*. - J. Agric. Sci., 128: 173-180.

HOSPERS-BRANDS A.J.T.M., GHORBANI R., BREMER E., BAIN R., LITTERICK A., HALDER F., LEIFERT C., WILCOCKSON S.J., 2008 - *Effects of presprouting, planting date, plant population and configuration on late blight and yield of organic potato crops grown with different cultivars*. - Potato Res., 51(2): 131-150.

IERNA A., 2010 - *Tuber yield and quality characteristics of potatoes for off-season crops in a Mediterranean environment*. - J. Sci. Food Agric., 90(1): 85-90.

ISTAT, 2012 - *Annual crop data 2012*. - Istituto Nazionale di Statistica, Rome, www.istat.it.

MACKERRON D.K.L., MARSHALL B., JEFFERIES R.A., 1988 - *The distribution of tuber sizes in droughted and irri-*

gated crops of potato. II. Relation between size and weight of tubers and the variability of tuber-size distributions. - Potato Res., 31(2): 279-288.

MALETTA M., HENNINGER M., HOLMSTROM K., 2006 - *Potato leaf hopper control and plastic mulch culture in organic potato production*. - HortTechnology, 16(2): 199-204.

MASARIRAMBI M.T., MANDISODZA F.C., MASHINGAIDZE A.B., BHEBHE E., 2012 - *Influence of plant population and seed tuber size on growth and yield components of potato (*Solanum tuberosum*)*. - Intern. J. Agric. Biol., 14(4): 545-551.

MONTI L., STRUIK P.C., 1999 - *Recent developments in potato research: scientific highlights of the EAPR conference in Sorrento, Italy, May 1999*. - Potato Res., 42: 381-395.

MUSTONEN L., 2004 - *Yield formation and quality characteristics of early potatoes during a short growing period*. - Agric. and Food Sci., 13(4): 390-398.

REES H.W., CHOW T.L., LORO R.J., LAVOIE J., MONTEITH J.O., BLAAUW A., 2002 - *Hay mulching to reduce runoff and soil loss under intensive potato production in northwestern New Brunswick, Canada*. - Canadian J. Soil Sci., 82(2): 249-258.

SAUCKE H., DÖRING T.F., 2004 - *Potato virus Y reduction by straw mulch in organic potatoes*. - Annals Appl. Biol., 144(3): 347-355.

TARKALSON D.D., KING B.A., BJORNEBERG D.L., TABERNA J.P., 2011 - *Evaluation of in-row plant spacing and planting configuration for three irrigated potato cultivars*. - Amer. J. Potato Res., 88(3): 207-217.

TURAJIZADEH H., NADERI M.R., GOLPARVAR A.R., SOLEIMANI A., 2011 - *Effect of planting pattern and phosphorus on growth and yield of potato grown in Freidan region (Esfahan) of Iran*. - Res. Crops, 12(2): 532-538.

XING Z.S., TONER P., CHOW L., REES H.W., LI S., MENG F.R., 2012 - *Effects of hay mulch on soil properties and potato tuber yield under irrigation and non irrigation in New Brunswick, Canada*. - J. Irrig. Drain. Engin.-Asce, 138(8): 703-714.

Adv. Hort. Sci., 2013 27(3): 122-126

Application of sucrose on tomato seedlings improves transplant quality, crop establishment, cold and dark hardiness

Jamal Javanmardi [(1)], **Shandiz Emami**
Department of Horticultural Sciences, Faculty of Agriculture, Shiraz University, Shiraz, Iran.

Key words: chilling tolerance, survival in dark, transplant establishment, transplant hardening, transplanting.

Abstract: Transplant production is an important part of the vegetable production process. Therefore, improving transplant quality and resistance to adverse environmental conditions is important. Effects of sucrose solutions (0, 5, 10, 15, 20 and 25%), applied to the foliage of tomato (*Solanum lycopersicum* L.) cultivars Calj-N3 and Rio-Grande were studied. Treatments were applied to developing seedlings at every other irrigation for six weeks. Application of 25% sucrose increased fresh and dry weights of shoots, fresh weight of roots, and shoot and root dry weight percentages. Application of 25% sucrose led to a 13 and 18% higher survival to chilling temperature in 'Calj-N3' and 'Rio-Grande' tomato seedlings, respectively. The highest transplant survival percentages in darkness were found at 10% sucrose and higher. Seedlings sprayed with 15% sucrose solution had the highest transplant establishment in the field and flowering was approximately four to five days earlier. A 15% sucrose treatment is thus preferred.

1. Introduction

Transplanting vegetables has benefits over direct seeding. Transplanting increases crop season length, reduces expenses of vegetable production, and decreases the risk of chilling and other potential risks (Schrader, 2000).

Tomato (*Solanum lycopersicum* L.) is used for the fresh and processing market. In most open field tomato production areas use of transplants is beneficial because of a short growing season. Cool temperatures are an early season problem in some areas and they result in delays in production and economic loss (Shukry and El-Otaby, 2011). Strategies to increase tomato chilling resistance are necessary to produce quality transplants and increase stress resistance by appropriate treatments.

It was found that tomato seedlings are able to take up sugar solution applied to foliage; although up-take depends on environmental conditions (Berrie, 1960). Multiple applications of sugars to foliage on tomato in a dark room increases growth rate and especially when application is at fairly high temperatures (Went and Carter, 1948). Tomato plants treated with a 10% aqueous sucrose solution daily up to three days before transplanting, produced more adventitious roots (Smith and Zink, 1951). Application of 70 $g \cdot l^{-1}$ sucrose resulted in improved establishment in the field and shoot growth as a result of producing more adventitious roots (Percival and Fraser, 2005). In other investigations on non-vegetable plants such as young trees of silver birch, cherry, and red oak, applications of sugar ≤ 50 $g \cdot l^{-1}$ in water as a root drench significantly enhanced root vigor by week 12 (Percival, 2004). Sucrose injection to soybeans increased leaf area and pod numbers but suppressed photosynthesis (Abdin *et al.*, 1998).

There is very little information in the literature on the application of sucrose on transplants to improve chilling resistance and other transplant quality characteristics. The present study was undertaken to investigate effects of sucrose application on tomato transplants, as a commercially important vegetable established from transplants, and evaluate effects on increasing chilling resistance and other traits under field conditions. Foliar application was chosen because roots take up very little sugar, stems take it up mainly through wounds, but intact leaves absorb it readily; apparently through the whole surface (Went and Carter, 1948).

2. Materials and Methods

The study was carried out in a 200 m^2 area, at an altitude of 1810 m above sea level. The regional mean relative humidity was 46% and the mean monthly minimum and maximum temperatures during the growing season were 15.6 and 30.7 °C, respectively. Soil samples were analyzed prior to the start of the experiment. The loamy soil had an EC of 2.41 $mmhos \cdot m^{-1}$ and a pH of 7.6 (soil extract 1:2). The soil was plowed and disked to turn under existing plant

[1] Corresponding author: javanm@shirazu.ac.ir
Received for publication 11 March 2013
Accepted for publication 8 July 2013

material. Fertilization was according to soil test result and crop need to supply nutrient requirements for tomato production (Jones, 2007).

The tomato cultivars Calj-N3 and Rio-Grande were used. Seeds were sown in 300 ml plastic pots filled with peat and perlite (1:1 v:v) and irrigated every other day. Plantlets emerged after one week. Pots were irrigated with water for the first two weeks after sowing. Afterwards, they were fertigated with a complete NPK fertilizer as recommended by Papadopoulos (1991) for tomato transplant production. Greenhouse conditions for transplant production were 24±4°C and 45±5% relative humidity.

Seedling foliage was treated with six sucrose solutions (0, 5, 10, 15, 20 and 25% concentrations) starting two weeks after emergence. One drop of tween 20 was added to each solution to increase leaf surface absorption. Application covered all leaf surfaces at every other irrigation interval until seedlings were moved to the field at the sixth week after seed sowing. Leaves were washed using a mist system between each sucrose application.

For the field experiment, six-week-old seedlings were transplanted into the field at a 40×60 cm spacing in rows covered with black polyethylene mulch. Plots were fertigated through tape irrigation with starter fertilizer 10-10-10 NPK for the first week after transplanting to help stand establishment according to Masson *et al.* (1991). Cultural practices until the end of the experiment were carried out as described by Papadopoulos (1991). The field experiment was repeated in two subsequent years.

Seedlings at the end of the sixth week from seed sowing were divided into four groups. Group A was used to determine vegetative characteristics including leaf chlorophyll content (Saini *et al.*, 2001), transplant shoot and root fresh and dry weights, and shoot and root dry matter percentage. Group B was stored in darkness at 20°C for 96 h to determine effects of treatment on darkness survival. Group C was placed in an incubator at 0°C for 96 h to determine effects of sucrose on resistance to chilling. Group D was transplanted into the field to determine degree of field establishment, leaf number preceding first flower and the days from transplanting to first flower formation.

Group A, B and C experiments were arranged in a completely randomized design; group D (factors after transplanting into field) was arranged in a completely randomized block design over two years. All treatments consisted of six replicates of 10 plants. Data analysis was performed using the SPSS12 (SPSS Inc., Chicago, IL) computer software for Windows. If differences were significant, means were separated with least significant difference (LSD) test.

3. Results and Discussion

The effects of treatments on all measured traits were always significant except for leaf number, leaf chlorophyll content, fruit set percent and yield; for group D the effects of year and its interaction with treatments for all measured traits were not significant (data not shown).

Fresh and dry weights of shoots and roots, shoot and root dry matter percentage increased with increasing sucrose concentration in both cultivars (Table 1). The highest values were for the 25% sucrose concentration. Similar results in terms of increased dry matter of shoots and roots were found in seedlings of a non-vegetable plant, birch (*Betula pendula* Roth.), after a root drench application of sucrose and fructose (Percival and Fraser, 2005). Accordingly, it seems that sucrose could be absorbed by aerial and underground plant organs and used in metabolism for growth and development. Ritchie (1987) reported that root growth potential is not a physiological process. However, it integrates many important physiological processes in seedlings and has become a popular and useful indicator of seedling vigor.

Table 1 - Vegetative characteristics of two tomato cultivars, sprayed with aqueous sucrose solution for a 6-week transplant raising period

Tomato cultivar	Sucrose concentration (%)	Shoot fresh weight (g)	Shoot dry weight (g)	Shoot dry weight percentage	Root fresh weight (g)	Root dry weight (g)	Root dry weight percentage	Leaf number preceding the first flower	Days from transplanting to first flower
Calj-N3	0	4.25±0.20 e	0.33± 0.01 e	7.83±0.19 f	0.21±0.01 g	0.018±0.048 a	8.55±0.05 ef	9.67±0.33 a	27.33±0.88 a
	5	4.57±0.23 de	0.35±0.03 e	7.55±0.29 fg	0.24±0.02 ef	0.021±0.002 a	8.79±0.12 de	9.33±0.33 ab	26.33±0.33 b
	10	4.74±0.20 cd	0.52±0.02 c	10.89±0.15 cd	0.42±0.03 cd	0.037±0.002 a	8.86±0.12 cd	8.67±0.33 c	24.67±0.33 d
	15	4.72±0.24 cd	0.52±0.03 c	10.94±0.23 bcd	0.50±0.05 bc	0.046±0.005 a	9.27±0.11 ab	7.33±0.33 ef	23.33±0.33 ef
	20	5.02±0.04 c	0.59±0.01 b	11.73±0.31 ab	0.61±0.04 a	0.057±0.005 a	9.40±0.15 a	7.00±0.00 f	23.00±0.00 ef
	25	6.22± 0.15 a	0.74±0.02 a	11.97±0.18 a	0.64±0.02 a	0.060±0.003 a	9.36±0.07 ab	7.33±0.33 ef	22.67±0.33 f
Rio-Grande	0	3.24±0.19 g	0.22±0.02 f	6.70±0.26 h	0.11±0.01 h	0.008±0.001 b	7.52±0.19 h	9.67±0.33 a	27.33±0.33 a
	5	3.55±0.16 fg	0.24±0.03 f	6.84±0.49 gh	0.15±0.02 gh	0.012±0.002 ab	7.96±0.30 g	9.33±0.33 ab	25.67±0.33 bc
	10	3.84±0.16 f	0.38±0.02 de	10.00±0.26 e	0.31±0.03 e	0.026±0.002 a	8.26±0.09 f	9.00±0.00 bc	25.00±0.57 cd
	15	4.26±0.20 e	0.42±0.02 d	9.78±0.74 e	0.40±0.05 d	0.036±0.005 a	8.95±0.04 cd	8.00±0.00 d	23.67±0.33 e
	20	5.02±0.09 c	0.51±0.03 c	10.19±0.50 de	0.51±0.03 b	0.046±0.004 a	9.10±0.12 bc	7.67±0.33 de	23.33±0.33 ef
	25	5.54±0.19 b	0.62±0.02 b	11.15±0.58 bc	0.53±0.01 b	0.048±0.001 a	9.12±0.05 abc	7.33±0.33 ef	23.33±0.33 ef
LSD value		0.37	0.053	0.81	0.075	0.042	0.28	0.59	0.86

Data are mean ± S.E. of six replicates. Different letters indicate significant differences for each column at P≤ 0.05 by LSD test.

Any physiological problem affecting seedlings should show up as a decrease in the seedling's ability to produce roots. Thus, sucrose spray is an applicable method to increase root fresh weight and thereby increase transplant vigor.

Survival in dark

Tomato growers often purchase seedlings from distant transplant producers, risking deterioration of transplants during transportation. Transportation conditions, status of flower development at time of transport, planting conditions after transport, and unfavorable combinations of these conditions often result in flower abortion and delayed fruit development of the first truss (Kubota *et al.*, 2004). Low-temperature storage in darkness has been reported as a way to preserve seedling quality (Leskovar and Cantliffe, 1991; Kaczperski and Armitage, 1992; Kaczperski *et al.*, 1996). Nearly all plant food reserves are stored as starch or sugars. These are produced by photosynthesis and consumed by respiration to sustain plant growth and metabolism. Cold storage affects photosynthesis and respiration in two ways. First, absence of light interrupts photosynthesis, and second, low temperature decreases respiration rate. The net effect is that seedlings very slowly burn up their supply of reserve carbohydrates in storage (Ritchie, 1987). Therefore, survival in darkness and seedling quality are important for transplant transportation. Low temperature and dim light during transportation maintain transplant quality at an acceptable level (Kubota and Kroggel, 2006). Our results showed that by increasing sucrose concentration, survival of transplants in darkness was increased. The highest transplant survival percentage in darkness was found with 10% concentration of sucrose and was not cultivar dependent (Fig. 1). Kubota *et al*. (1997) reported reductions in levels of soluble sugars and starch in dark-stored broccoli (*Brassica oleracea* L. Botrytis 'Group Green Duke') plantlets. In spite of interruption of the supply of photosynthates in darkness, amounts of soluble sugars increase through degradation of starch in shoots (Sato *et al.*, 2004). Accordingly, it is concluded that foliar application of sugars can be used as an energy source for respiration in darkness with low, or no, decrease in transplant quality.

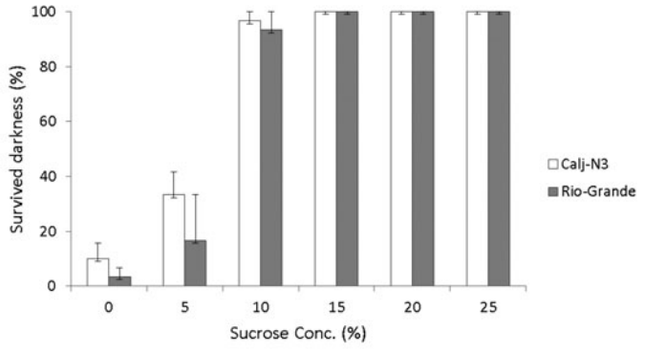


Fig. 1 - Effect of sucrose foliar spray on the percent of survived 'Calj-N3' and 'Rio-Grande' tomato transplants in darkness.

Chilling temperature survival

Seedling survival percentage increased as sucrose concentration increased in both cultivars (Fig. 2). Application of 25% sucrose solution produced the best survival in response to chilling. Compared to controls there was 13 and 18 times greater chilling resistance in 'Calj-N3' and 'Rio-Grande' seedlings, respectively. Since carbohydrate reserves undergo a net loss during low temperature storage and hardiness development requires an expenditure of metabolic energy (Ritchie, 1987), providing sucrose as an available source of energy through foliar application can help transplants survive low temperatures. Sucrose has been reported as a metabolite for stabilizing membranes in plant tissues during chilling (King *et al.*, 1988). Sugar is able to maintain dry mass of plantlets under a wider range of environmental conditions during low temperature storage (Kubota, 2005).

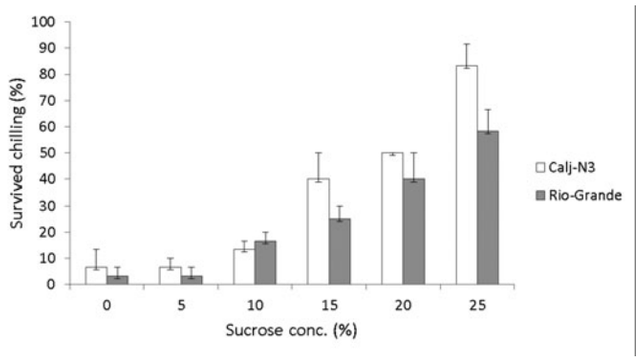


Fig. 2 - Effect of sucrose foliar spray on the percent of 'Calj-N3' and 'Rio-Grande' tomato cultivar transplants that survived chilling temperatures.

Leaf chlorophyll content

Leaf chlorophyll content was not different in cultivars due to treatment (data not shown). The same result was observed in birch (Percival and Fraser, 2005) where sugar feeding at 25 $g \cdot l^{-1}$ level had no significant effect on carotenoid and chlorophyll concentrations.

Field establishment efficacy

High transplant establishment percentage in the field results from quality transplants and successful transplanting. Tomato seedlings treated with 15 or 25% sucrose had the highest and similar transplant establishment rates (Fig. 3). This could be related to the greater root systems produced by those plants. Root growth is considered a useful indicator of seedling vigor (Ritchie, 1987).

Number of leaves and days to first flower

A standard indicator for flowering time is the number of leaves produced on the primary shoot before first flowers are initiated (Koornneef *et al.*, 1991). In tomato, the number of leaves produced before flowers is genetically controlled, but mediated by environmental conditions. Usually only from six to 11 leaves are required below the first inflorescence in tomato (Kinet and Peet, 1997), with

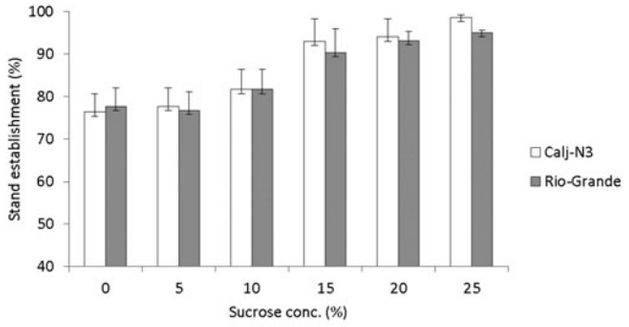


Fig. 3 - Effect of sucrose foliar spray on the percent of successful stand establishment of 'Calj-N3' and 'Rio-Grande' tomato cultivar transplants.

the lower number being found in an early crop. The 'Calj-N3' and 'Rio-Grande' seedlings treated with 15 and 20% sucrose solution, respectively, produced flowers sooner than other treatments (Table 1). In those concentrations there were fewer leaves than in the controls. It may be that sucrose application provides additional carbohydrates required for flower initiation. Flowering was observed to occur approximately four to five days earlier in cultivars treated with ≥15% sucrose (Table 1).

Fruit set percent and yield

Analysis of variance for both cultivars over two years did not show differences for fruit set percent and fruit yield (data not shown). It seems the effect of sucrose application on seedlings might not extend beyond flowering. Fruit set (and eventually yield) is affected by environmental factors and plant growth regulators during the fruit set period (Kinet and Peet, 1997).

4. Conclusions

Overall, transplant quality of horticultural crops is generally defined by physiological potentials (growth and developmental characteristics and photosynthetic ability), visual quality (color and morphology), genetic uniformity, and pathogen status (Kubota *et al.*, 2002). In the present study, in most cases the 15-25% sucrose treatment gave the best results. The main detracting factor for the 25% sucrose concentration was sticky leaf surfaces after application, which may attract insects. Washing leaves to remove stickiness and applying this amount of sugar is probably not economical. Therefore, the 15% level is recommended, which can be applied through overhead mist or irrigation system in commercial transplant production operations. Overhead irrigation systems wash excesses sucrose off leaves, reducing problems. The results from this investigation can be useful for transplant production companies and growers. Adverse environmental conditions do not always allow immediate transplanting into the field and sucrose-treated seedlings can be held for a few days, thus allowing flexibility in crop scheduling and labor management.

References

ABDIN A.O., ZHOU X., COULMAN B.E., CLAUTIER D., FARIS M.A., DONALD L., 1998 - *Effect of sucrose supplementation by stem injection on the development of soybean plants*. - J. Exp. Bot., 49(329): 2013-2018.

BERRIE A.M.M., 1960 - *The effect of sucrose sprays on the growth of tomato*. - Physiologia Plantarum, 13(1): 9-19.

JONES J.B., 2007 - *Tomato plant culture: in the field, greenhouse, and home garden*. - CRC Press, Taylor and Francis Group, Boca Raton, FL, USA, pp. 400.

KACZPERSKI M.P., ARMITAGE A.M., 1992 - *Short-term storage of plug-grown bedding plant seedlings*. - HortScience, 27(7): 798-800.

KACZPERSKI M.P., ARMITAGE A.M., LEWIS P.M., 1996 - *Performance of plug-grown geranium seedlings preconditioned with nitrogen fertilizer or low-temperature storage*. - HortScience, 31(3): 361-363.

KINET J.M., PEET M.M., 1997 - Tomato, pp. 207-258. - In: WIEN H.C. (ed.) *The physiology of vegetable crops.* CAB International, Wallingford, UK, pp. 663.

KING A.I., JOYCE D.C., REID M.S., 1988 - *Role of carbohydrates in diurnal chilling sensitivity of tomato seedlings*. - Plant Physiol., 86(3): 764-768.

KOORNNEEF M., HANHART C.J., VEEN J.H., 1991 - *A genetic and physiological analysis of late flowering mutants in* Arabidopsis thaliana. - Mol. Gen. Genet., 229(1): 57-66.

KUBOTA C., 2005 - *Low temperature storage of plants under dim light*, pp. 205-212. - In: KOZAI T., F. AFREEN, and S.M.A. ZOBAYED (eds.) *Photoautotrophic (sugar-free medium) micropropagation as a new micropropagation and transplant production system.* Springer Verlag, The Netherlands, pp. 316.

KUBOTA C., KROGGEL M., 2006 - *Air temperature and illumination during transportation affect quality of mature tomato seedlings*. - HortScience, 41(7): 1640-1644.

KUBOTA C., KROGGEL M., SOLOMON D., BENNE L., 2004 - *Analyses and optimization of long distance transportation conditions for high quality tomato seedlings*. - Acta Horticulturae, 659: 227-234.

KUBOTA C., RAJAPAKSE N.C., YOUNG R.E., 1997 - *Carbohydrate status and transplant quality of micropropagated broccoli plantlets stored under different light environments*. - Postharvest Biology and Technology, 12(2): 165-173.

KUBOTA C., SEIYAMA S., KOZAI T., 2002 - *Manipulation of photoperiod and light intensity in low temperature storage of eggplant plug seedlings*. - Scientia Horticulturae, 94(1-2): 13-20.

LESKOVAR D.I., CANTLIFFE D.J., 1991 - *Tomato transplant morphology affected by handling and storage.* - HortScience, 26(11): 1377-1379.

MASSON J., TREMBLAY N., GOSSELIN A., 1991 - *Effects of nitrogen fertilization and HPS supplementary lighting on vegetable transplant production. II. Yield*. - J. Amer. Soc. Hort. Sci., 116(4): 599-602.

PAPADOPOULOS A.P., 1991 - *Growing greenhouse tomatoes in soil and in soilless media*. - Agriculture Canada Publication, Ottawa, Canada.

PERCIVAL G.C., 2004 - *Sugar feeding enhances root vigor of young trees following containerization*. - J. of Arboric., 30(6): 357-364.

PERCIVAL G.C., FRASER G.A., 2005 - *Use of sugars to improve root growth and increase transplant success of birch (*Betula pendula *Roth.)*. - J. of Arboric., 31(2): 66-77.

RITCHIE G.A., 1987 - *Some effects of cold storage on seedling physiology*. - Tree Planters' Notes, 38: 11-15.

SAINI R.S., SHARME K.D., DHANKHAR O.P., KAUSHIK R.A., 2001 - *Laboratory manual of analytical techniques in horticulture*. - Agrobios, India.

SATO F., YOSHIOKA H., FUJIWARA T., HIGASHIO H., URAGAMI A., TOKUDA S., 2004 - *Physiological responses of cabbage plug seedlings to water stress during low-temperature storage in darkness.* - Scientia Horticulturae, 101(4): 349-357.

SCHRADER W.L., 2000 - *Using transplants in vegetable production*. - The Regents of the University of California, ANR Publication, 8013: 5.

SHUKRY W.M., EL-OTABY L.M., 2011 - *Increased chilling tolerance and altered protein patterns in tomato seedlings following hardening of seeds or system in application.* - Acta Botanica Hungarica, 53(3-4): 389-408.

SMITH P.G., ZINK F.W., 1951 - *Effect of sucrose foliage sprays on tomato transplants*. - Proceedings Amer. Soc. Hort. Sci., 58: 168-178.

WENT F., CARTER M., 1948 - *Growth response of tomato plants to applied sucrose*. - American Journal of Botany, 35(2): 95-106.

Adv. Hort. Sci., 2013 27(3): 127-132

Physiological responses of c_4 grasses to prolonged heat stress

A. Pompeiano[(1)]*, M. Volterrani, and L. Guglielminetti****

* *Laboratory of Plant Physiology, Center of Agricultural Sciences, Federal University of Alagoas, Maceio, BR 104, Km 85, s/n, Rio Largo, AL 57100-000, Brazil.*

** *Department of Agriculture, Food and Environment, University of Pisa, Italy.*

Key words: *Cynodon*, heat shock proteins, total soluble sugars, *Zoysia.*

Abstract: C_4 grasses are best adapted to the transition, warm-arid, and warm-humid climatic zones and have the ability to acquire thermotolerance by exposure to acute heat stress. Exposure to sub-lethal temperatures results in changes in physiological, biochemical, metabolic, and molecular processes. The response of two warm-season grasses to prolonged heat stress was investigated. Plants of hybrid bermudagrass (*Cynodon dactylon* × *C. transvaalensis* 'Tifway') and Japanese lawn grass (*Zoysia japonica* Steud. 'Meyer') were exposed for 168 h to supraoptimal temperature conditions (47°C) in controlled-environment chamber. Compared with zoysiagrass, bermudagrass showed greater damage. Metabolite profiles were affected by prolonged heat exposure, with significant differences between these species. Consistent differences were found in total soluble sugars accumulation over the study period and severity of plant organ senescence. Bermudagrass roots were more affected, as compared to leaves. Leaf proteins expression determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis showed an early degradation in zoysiagrass, as thermal exposure proceeded. A significant net decline in protein content was observed after 48 h of exposure, while in bermudagrass an analogous decline was not detected until 96 h of treatment. Although heat stress is not considered a detrimental factor to C_4 grass species, the two species showed significant differences in their physiological response to continuous high temperatures.

1. Introduction

Higher plants exhibit a photosynthetic limitation as a function of temperature. Warm-season grasses, characterized by the Hatch-Slack pathway (C_4), evolved a RuBisCO (Ribulose-1,5-bisphosphate carboxylase/oxygenase) type that more efficiently utilizes high CO_2 at high temperature, and have an optimum growth temperature range of 27 to 35°C, which is approximately 10°C higher than in C_3 plants (Leegood, 1993). C_4 grasses are best adapted to the transition, warm-arid, and warm-humid climatic zones and usually have the ability to acquire thermotolerance by exposure to acute heat stresses (DiPaola and Beard, 1992).

Plants compensate for their sessile nature by developing specific responses to abiotic conditions. Exposure to sub-lethal temperatures results in changes in physiological, biochemical, metabolic, and molecular processes, developing an active regulatory mechanism for maintaining cellular homeostasis, as well as enhanced tolerance (Kislyuk *et al.*, 2004, 2007; Guy *et al.*, 2008; Kumar *et al.*, 2013). The severity of damage to cellular and subcellular structures mainly depends on the intensity, duration, and rate of temperature increase. Changes in protein metabolism have been correlated with the thermotolerance mechanism. In particular, transcription and translation of specific heat shock proteins (HSPs) are induced or enhanced when plants are exposed to supraoptimal temperature, playing a crucial role in adaptive mechanism. Most of the HSPs are molecular chaperones, functioning by binding and stabilizing proteins at intermediate stages of folding, assembly, degradation, and translocation across membranes (Xu *et al.*, 2011).

Furthermore, carbohydrate metabolism was found to be affected by heat shock on *Arabidopsis*, with an accumulation of maltose, sucrose, galactinol, myo-inositol, raffinose and monosaccharide cell-wall precursors (Rizhsky *et al.*, 2004). Induction of the triose phosphate and starch hydrolytic pathways of carbohydrate metabolism provides precursors, leading to raffinose biosynthesis and accumulation of galactinol and raffinose. Soluble sugars are known osmolytes that are beneficial during heat stress conditions, providing protection of cell membranes during stress exposure (Diamant *et al.*, 2001).

Bermudagrass (*Cynodon* spp. Rich.) and zoysiagrass (*Zoysia* spp. Willd.) provide excellent surfaces for functional, recreational, and ornamental areas, including golf course fairways, ornamental lawns and parks. The ability of these perennial, warm-season grasses to tolerate heat

[1] Corresponding author: onaiepmop@gmail.com

Received for publication 2 May 2013
Accepted for publication 17 September 2013

stress is reported to be excellent compared to cool-season grasses (Beard, 1973). In an investigation, conducted to determine the relative effects of drought and heat on cool-season tall fescue (*Festuca arundinacea* Schreb.) and Manilagrass [*Zoysia matrella* (L.) Merr.], the superior heat and drought tolerance recorded for zoysiagrass has been associated with its ability to maintain photochemical activity and cellular membrane stability (Du *et al.*, 2008). This characteristic was also associated with maintenance of more active antioxidant enzymes and lower membrane lipid peroxidation (Du *et al.*, 2009). Additionally, comparing the ability of three warm-season turfgrass species to mitigate heat accumulation during a prolonged 60-day summer drought, zoysiagrass increased rates of leaf damage and maintained significantly higher canopy temperatures during drought conditions, as compared to bermudagrass and St. Augustinegrass (*Stenotophrum secundatum* (Walt.) Kuntze), suggesting these latter species have an enhanced heat dissipation through greater evapotranspiration (Steinke *et al.*, 2009). Amongst the species, few significant differences were observed during cultivar comparisons, although Manilagrass tended to accumulate and retain heat more quickly than cultivars of Japanese lawn grass (*Zoysia japonica* Steud.). The differential heat tolerance after prolonged stress, exhibited by bermudagrass as compared to *Poa pratensis* (L.), was attributed to a higher accumulation of organic acids, amino acids, soluble sugars, and inositol (Du *et al.*, 2011).

Differences in photosynthetic response in C_4 plants do exist. RuBisCO in *Cynodon dactylon* constantly exhibited a higher catalytic turnover rate at 16, 28, and 40°C than in *Zoysia japonica*, with analogous activation energy of 50.1-51.2 kJ mol^{-1} (Sage, 2002). However, it exceeded the photosynthetic capacity above 25°C, and was not a limiting factor at warm temperature.

Limited work has been done to examine and compare physiological responses in protein induction and degradation, as well as carbohydrate metabolism under heat stress in C_4 grasses. The objectives of the present research were (i) to evaluate the heat tolerance of bermudagrass and zoysiagrass standard cultivars using controlled environment and heating procedures, and (ii) to determine differences in stress responsive metabolite accumulation in C_4 standard cultivars to short- and long-term heat stress.

2. Materials and Methods

Experimental conditions

The research was carried out at the department of Agriculture, Food and Environment, University of Pisa, Italy. On 22 February 2011, plants of *Cynodon dactylon* × *C. transvaalensis* Burtt. Davy cv. 'Tifway' and *Zoysia japonica* Steud. cv. 'Meyer', selected as standard cultivars, were collected and clonally propagated as phytomers (1- to 2-cm segments of stolon obtained from mature plants, containing root tissue, crown, and shoot material) into a sphagnum moss peat-based growing medium, mixed with volcanic sand, into 160-hole seed trays, with a cell volume of 5 cm^3. Plants were established at 23°C (±4°C) day/night temperatures for 32 weeks in a greenhouse. During the active growing season a mineral solution (8N-7P-19.9 K + 4 Ca^{-2} Mg) at 1.3 g l^{-1} was supplied three times per week. The fertilization program was periodically adjusted according to the physiological age and state of the grass. Irrigation was applied as needed to prevent wilting and plant material was maintained at a cutting height of 2.5 cm throughout the entire pre-treatment phase. Mature plants were then acclimated in a growth chamber for four weeks before treatments were applied, and maintained at 22±1°C with a 12-h photoperiod and a light intensity of 90 µmol $m^{-2}s^{-1}$.

All treatments were performed in parallel. The control was maintained in a growth chamber, and stress was applied by exposing plants to 47±1°C with 45% relative humidity and 12-h photoperiod of 90 µmol $m^{-2}s^{-1}$ photosynthetically active radiation. Temperature was monitored with a datalogger (Campbell Scientific, Logan, UT, USA), and plants exposed to heat stress were sub-irrigated daily to avoid drought stress. For each species, five plants were removed from the heat chamber at target times (set at 6 h, 24 h, 48 h, 96 h, and 168 h), and finally transferred again to the growth chamber for assessment of vitality after three weeks. Biometric response to heat stress was evaluated as fresh weight (FW) (expressed in percentage compared to t0= 100%) regrowth in the growth chamber for three weeks.

Analysis of carbohydrates

Samples (0.5 g FW) were ground to a powder and extracted as described by Tobias *et al.* (1992) in order to quantify glucose, fructose, and sucrose (total soluble sugars, total soluble carbohydrates). Samples were assayed by coupled enzymatic assay methods (Guglielminetti *et al.*, 1999) measuring the increase in A_{340}. The accuracy of the method was tested using standards with known amounts of carbohydrates. Incubations of samples and standards were carried out at 37°C for 30 min. The reaction mixtures (1 ml) were as follows. Glucose: 150 mM Tris-HCl, pH 7.6, 3 mM $MgCl_2$, 2 mM ATP, 0.6 mM NADP, 1 unit Glc6P dehydrogenase, 1 unit hexokinase; fructose was assayed as described for glucose plus the addition of 2 units of phosphoglucose isomerase; the increase in A_{340} was recorded. Sucrose was first broken down using 85 units of invertase (in 50 mM Na-acetate, pH 4.6) and the resulting glucose was assayed as described above.

Recovery experiments evaluated losses taking place during the extraction procedures. Two tests were done for each metabolite by adding a known amount of authentic standards to the samples prior to the extraction. The concentration of the added standards were similar to those estimated to be present in the tissues in preliminary experiments. The percentage of recovery ranged between 94 and 107% depending on the sugar. Data were corrected on the basis of the recovery percentages obtained for

each sample, and expressed as µmoles hexoses equivalent g^{-1} FW.

Analysis of pigments

Pigments were extracted by incubating tissues (50-100 mg FW) in 1.5 ml 80% acetone for one week at 4°C in darkness. The absorbance of extracts was measured spectrophotometrically at 470.0, 663.2, and 646.8 nm. These absorbance values were used for calculation of chlorophyll *a*, chlorophyll *b*, and carotenoids contents in accordance with Pompeiano *et al.* (2013).

Protein extraction and separation

Leaves were collected at the experimental target times previously reported and extracted in 50 mM Tris-HCl buffer (pH 7.6 containing 10 mM DTT and 10% glycerol). Protein quantification was performed according to Guglielminetti *et al.* (1997). Equal amounts of protein (2 µg) were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on 12.5% polyacrylamide gels followed by conventional silver nitrate staining.

Statistical analysis

The experiment was replicated for a total of four experimental replications. The statistical analyses of biometric and growth traits were performed using one-way analysis of variance (ANOVA) to determine whether significant differences among cultivars and groups existed. When significant differences were found, the means were compared using the Least Significant Difference (LSD) test. Significant differences for all statistical tests were evaluated at the level of p= 0.05. All computations were performed with R 2.15.0 (R Development Core Team, 2012) and R package *agricolae* (de Mendiburu, 2012). For the photosynthetic pigments and soluble carbohydrates data, the Student-Newman-Keuls (SNK) test was used for *a posteriori* multiple comparison of means.

3. Results

Heat tolerance

Both species exhibited an increasing susceptibility, expressed as percentage of fresh weight canopy regrowth relative to the control, following prolonged exposure to heat stress (Fig. 1). No significant difference between the two species was detected after 6 h of treatment. Bermudagrass showed significant (p< 0.001) higher resistance to heat stress than zoysiagrass at all target times after the first 6 h of imposed stress. Moreover, our data showed that, after 6 and 24 h of exposure at sub-lethal temperature, recovery of 'Tifway' was greater as compared to t0, although these differences were not significantly different *vs.* the control. No significant difference was observed until 96 h of heat stress for this species. A prolonged exposure to sub-lethal temperature revealed a significant decline, although canopy recovery at the last target time, 168 h, still displayed 49.8% of regrowth as compared to t0. In contrast, a rapid, sharp decline was clearly evident in 'Meyer' after 6 h of treatment, with 36.3% of regrowth as canopy recovery. No vitality was detected following 96 h of exposure.

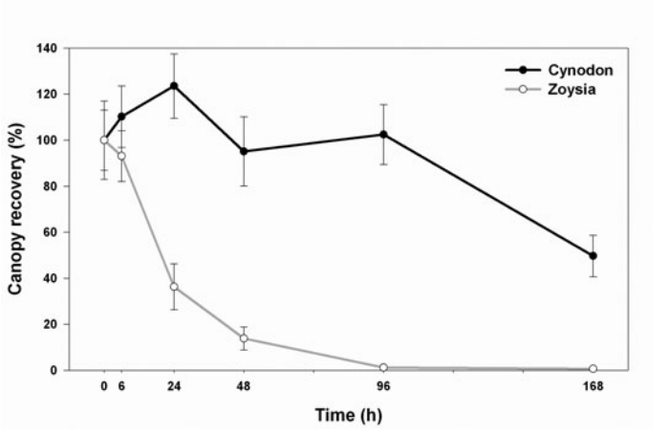


Fig. 1 - Changes in canopy recovery after heat treatments as percentage of living fresh tissues at control time. Error bars represent standard error of the mean (n=4).

Photosynthetic pigments

Clear differences in photosynthetic pigments were noticed between control and heat shock-stressed plants (Fig. 2). Under heat stress conditions, significant (p < 0.05) changes in chlorophyll *a, b* and carotenoid contents were observed, although the species had different behaviors. In bermudagrass, a progressive decline was detected from the initial hours of stress, particularly evident in chlorophyll *b* levels. Chlorophylls were completely degraded after 168 h of exposure, while the degradation of carotenoids was less severe at the end of the treatment (-64.7% compared to the control). In contrast, zoysiagrass pigments showed a sharp increase and attained a peak level at 48 h of heat exposure, resulting in a significant (p< 0.001) difference between treatments. Thereby indicating a significant decline under increasing heat stress as compared with the control. In bermudagrass, changes in photosynthetic pigments did

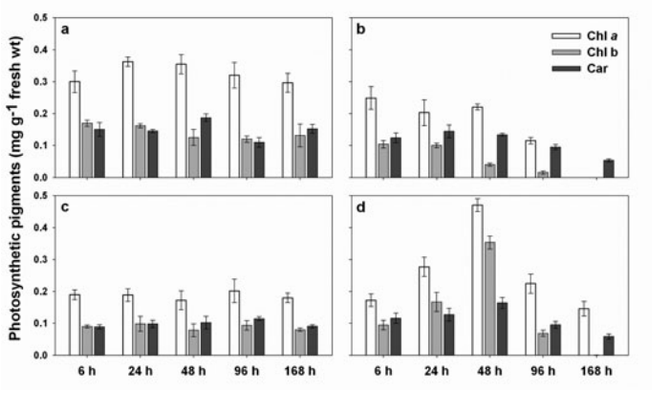


Fig. 2 - Leaf photosynthetic pigments in (a) bermudagrass control, (b) bermudagrass heat shock, (c) zoysiagrass control, and (d) zoysiagrass heat shock observed over time. Error bars represent standard error of the mean (n=4).

not lead to any significant difference in the carotenoids-to-chlorophylls ratio at 48 h exposure, whereas in zoysiagrass the ratio significantly ($p< 0.01$) increased under heat stress.

Soluble carbohydrates

Analysis of total soluble sugars data showed differential responses to heat stress in the two warm season grasses (Figs. 3, 4). With few exceptions, total soluble sugars (TSS) levels in the controls remained constant throughout the experiment. Under control and heat stress conditions, sucrose comprised the majority of the total sugar concentration in all the tissues analyzed.

In bermudagrass leaves, heat stress stimulated a significantly ($p< 0.001$) greater TSS production in the initial 48 h as compared with the control (Fig. 3). Although a pronounced peak after 24 h of stress was observed (with a concentration of 49.8 µmol g^{-1} FW), TSS declined gradually thereafter till 16.0 µmol g^{-1} FW, 42.2% lower than the control (Fig. 3B). Glucose and fructose concentrations generally remained constant throughout the investigation, and no significant differences were detected between the control and treated samples. Zoysiagrass leaves reduced significantly their TSS content soon after the beginning of the heat stress, with a sudden contraction (-52.4%) found at the 6 h sampling. A gradual decrease in TSS was observed when treatment was prolonged; the minimum concentration of 9.8 µmol g^{-1} FW at 96 h (-74.7% compared to the control) was reached, and a plateau level was attained at the last target time.

Bermudagrass roots contained significantly ($p< 0.001$) higher TSS than zoysiagrass under both control and heat stress conditions. Overall, TSS content decreased considerably in plants exposed to heat stress. The averages of all the independent observations were 64.6 and 32.7% lower than those of the control plants in bermudagrass and zoysiagrass, respectively (Fig. 4). Under heat conditions, sucrose levels showed a pronounced decline (-79.0% *vs.* the control) 6 h after the beginning of treatment. This metabolite significantly increased during the first 24 h to 36.8 µmol g^{-1} FW, but later decreased constantly with the treatment (Fig. 4 B). Significant decreases in glucose and fructose contents were recorded in bermudagrass roots exposed to stress. In 'Meyer', TSS content decreased considerably in both treatments compared to bermudagrass. Moreover, levels of TSS showed a sharp decline after 48 h of exposure to heat stress.

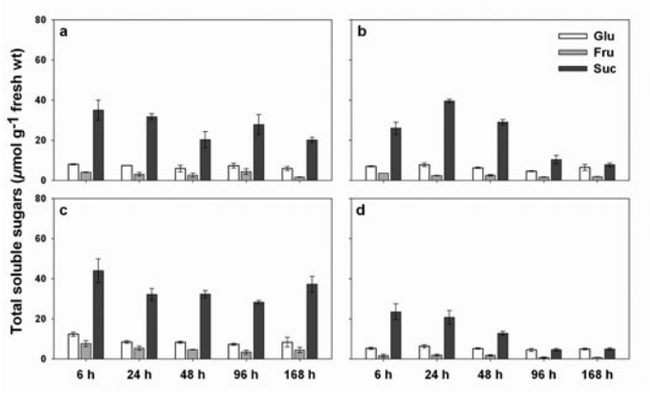


Fig. 3 - Glucose, fructose, and sucrose (as hexose equivalents) leaf contents in (a) bermudagrass control, (b) bermudagrass heat shock, (c) zoysiagrass control, and (d) zoysiagrass heat shock over time. Error bars represent standard error of the mean (n=4).

Protein expression during heat stress

In both C_4 grasses, soluble protein expressions were significantly affected in response to heat stress. In bermudagrass leaves, protein synthesis mostly ceased after 96 h of heat shock exposure, whereas in Japanese lawn grass degradation occurred after 48 h of treatment (Fig. 5). However, a few bands of zoysiagrass leaf protein SDS-PAGE persisted till the end of the treatment period. Degradation of a large band, corresponding to the large subunit of RuBisCO (about 50 kD), occurred after 48 h of heat exposure in zoysiagrass, while in bermudagrass it was no longer detectable at 96 h of heat stress.

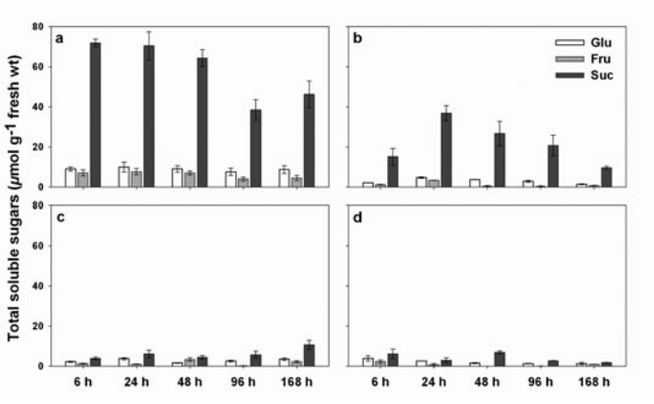


Fig. 4 - Glucose, fructose, and sucrose (as hexose equivalents) root contents in (a) bermudagrass control, (b) bermudagrass heat shock, (c) zoysiagrass control, and (d) zoysiagrass heat shock over time. Error bars represent standard error of the mean (n=4).

4. Discussion and Conclusions

In open fields usually plants are simultaneously exposed to combined drought and heat effects, two interacting abiotic stresses that limit growth and quality. Since initial reports, 'Meyer' has been identified as a heat and drought tolerant species (Forbes and Ferguson, 1947; Dunn, 1989). In the Midwest transition zone, it was observed to lose color in response to extreme midsummer heat, yet still leaving a playable surface (Dunn, 1998). However, a lower dehydration rate and drought resistance were attributed to zoysiagrass when compared to *Cynodon* spp., due to a limited root system, higher ET rate, and slower rate of epicuticular wax production under stress conditions (Beard and Sifers, 1997). The present study indicates that zoysiagrass has a moderate tolerance to prolonged heat stress compared to bermudagrass. After the initial 6 h period of stress, zoysiagrass canopy recovery exhibited a sharp decline, while bermudagrass maintained an unaltered plant regrowth response until 96 h of exposure.

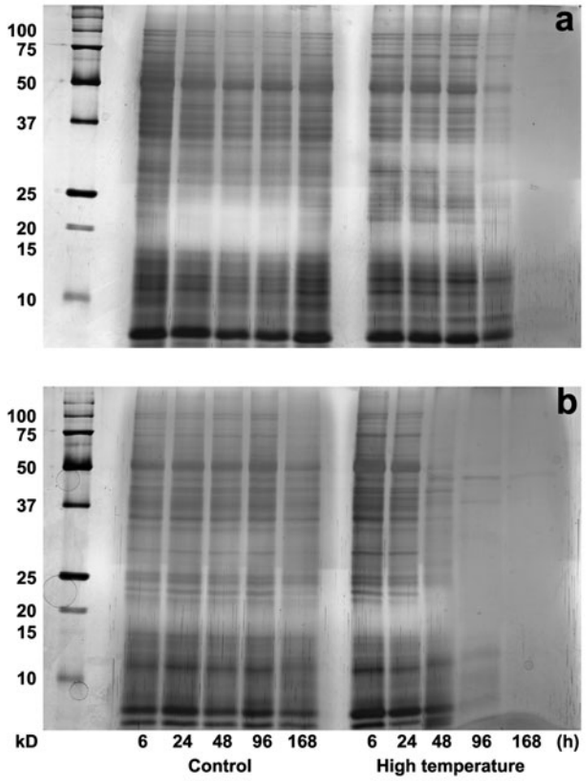


Fig. 5 - Representative SDS-PAGE profiles of leaf soluble proteins for (a) bermudagrass and (b) zoysiagrass, respectively with control (left) and exposure to high temperature (right) for 168 h. Equal amounts of protein were loaded in each lane. Standard molecular weights in kD are reported.

Physiological characterization of C_4 plants subjected to prolonged heat stress revealed significant differences between the species. While in bermudagrass a gradual net decline of photosynthetic pigments was observed throughout the experimental period, zoysiagrass enhanced chlorophyll synthesis during the first 48 h of heat stress, followed by a severe leaf senescence induced by a prolonged exposure. The increasing chlorophylls and carotenoids contents might reflect an adaptive physiologic response to the abiotic stress. It may be associated with a higher photosynthetic performance, as well as with a better dehydration tolerance of 'Meyer' in comparison to 'Tifway' (Kim, 1987). Moreover, under heat stress conditions, carotenoids resulted more stable than chlorophylls in both species, as shown by the chlorophyll *a, b*:carotenoids ratios in the treated plants, in agreement with Wahid (2007) observations.

Changes in TSS content exhibited consistent differences in the timing and severity of plant organ senescence induced by the heat treatment. Compared with bermudagrass, zoysiagrass had greater damage. Moreover, bermudagrass root tissues were more affected than leaves, which had a peak of soluble sugars after 24 h of exposure. Previous studies showed similar results in soybean (Djanaguiraman and Prasad, 2010; Djanaguiraman *et al.*, 2011) and, as observed in our study, this effect occurred despite a concomitant loss of chlorophyll pigments, usually attributed to membrane damage. Causes of this significant accumulation of soluble sugars are unknown, although degradation of starch (Geigenberger *et al.*, 1998) could be involved.

Examination of the pattern of leaf proteins subjected to SDS-PAGE showed an early degradation in zoysiagrass as thermal exposure proceeded. A significant net decline was observed after 48 h of exposure, while in bermudagrass an analogous decline in soluble protein was not detected until 96 h of treatment. Overall, bermudagrass showed a greater ability to cope with high-temperature stress, as indicated by the persistence of the band, corresponding to the large subunit of RuBisCO. In accordance with previous reports (Hashimoto *et al.*, 1989; Veerasamy *et al.*, 2007), our results showed that chlorophyll breakdown was related to protein degradation, as a progressive decline of both occurred under prolonged stress conditions.

In summary, although heat stress is not a detrimental factor for C_4 grass species, which usually respond positively to complex and simultaneous environmental conditions occurring in the field, the two species showed significant differences in their physiological response to continuous exposure to high temperature. Compared to bermudagrass, zoysiagrass showed a greater susceptibility to heat stress: differences in chlorophyll breakdown, TSS content and proteins expression revealed a different ability to species-specifically modulate its response to supraoptimal temperatures. Considering all the observed physiological parameters, bermudagrass provided a less marked response to heat stress, manifesting an enhanced thermotolerance not detected in zoysiagrass. For this species, the time-course experiment of metabolite changes during heat shock showed, in contrast, a sudden response, associated with a significant decline.

References

BEARD J.B., 1973 - *Turfgrass: science and culture.* - Prentice-Hall, Englewood Cliffs, New Jersey, USA, pp. 658.

BEARD J.B., SIFERS S.I., 1997 - *Genetic diversity in dehydration avoidance and drought resistance within the* Cynodon *and* Zoysia *species.* - Int. Turfgrass Soc. Res. J., 8: 603-610.

DE MENDIBURU F., 2012 - Agricolae*: statistical procedures for agricultural research.* - R package version 1.1-4, pp. 60.

DIAMANT S., ELIAHU N., ROSENTHAL D., GOLOUBINOFF P., 2001 - *Chemical chaperones regulate molecular chaperones* in vitro *and in cells under combined salt and heat stresses.* - J. Biol. Chem., 276: 39586-39591.

DIPAOLA J.M., BEARD J.B., 1992 - *Physiological effects of temperature stress,* pp. 231-267. - In: WADDINGTON D.V., R.N. CARROW, and R.C. SHEARMAN (eds.). *Turfgrass. Agronomy monographs, No. 32.* ASA, CSSA, & SSSA, Madison, WI, USA.

DJANAGUIRAMAN M., PRASAD P.V.V., 2010 - *Ethylene production under high temperature stress causes premature leaf senescence in soybean.* - Funct. Plant Biol., 37: 1071-1084.

DJANAGUIRAMAN M., PRASAD P.V.V., BOYLE D.L., SCHAPAUGH W.T., 2011 - *High-temperature stress and soybean leaves: leaf anatomy and photosynthesis.* - Crop Sci., 51: 2125-2131.

DU H., WANG Z., HUANG B., 2008 - *Differential responses of tall fescue and zoysiagrass to heat and drought stress.* - Acta Horticulturae, 783: 207-214.

DU H., WANG Z., HUANG B., 2009 - *Differential responses of warm-season and cool-season turfgrass species to heat stress associated with antioxidant enzyme activity.* - J. Am. Soc. Hortic. Sci., 134: 417-422.

DU H., WANG Z., YU W., LIU Y., HUANG B., 2011 - *Differential metabolic responses of perennial grass* Cynodon transvaalensis × Cynodon dactylon *(C_4) and* Poa Pratensis *(C_3) to heat stress.* - Physiol. Plant., 141: 251-264.

DUNN J.H., 1989 - *Observations on 'Meyer' zoysiagrass after 20 years of research: a look at zoysiagrass's past - and future as a transition area turfgrass.* - Golf Course Management, 57: 64-76.

DUNN J.H., 1998 - *Go easy on nitrogen for zoysiagrass root growth: extra nitrogen fertilizer reduces root growth in Midwest research.* - Golf Course Management, 66: 65-67.

FORBES I., FERGUSON M.H., 1947 - *Observations on the* Zoysia *grasses.* - Greenkeepers Rep., 15: 7-9.

GEIGENBERGER P., GEIGER M., STITT M., 1998 - *High-temperature perturbation of starch synthesis is attributable to inhibition of ADP-glucose pyrophosphorylase by decreased levels of glycerate-3-phosphate in growing potato tubers.* - Plant Physiol., 117: 1307-1316.

GUGLIELMINETTI L., LORETI E., PERATA P., ALPI A., 1999 - *Sucrose synthesis in cereal grains under oxygen deprivation.* - J. Plant Res., 112: 353-359.

GUGLIELMINETTI L., WU Y., BOSCHI E., YAMAGUCHI J., FAVATI A., VERGARA M., PERATA P., ALPI A., 1997 - *Effects of anoxia on sucrose degrading enzymes in cereal seeds.* - J. Plant Physiol., 150: 251-258.

GUY C., KAPLAN F., KOPKA J., SELBIG J., HINCHA D.K., 2008 - *Metabolomics of temperature stress.* - Physiol. Plant., 132: 220-235.

HASHIMOTO H., KURA-HOTTA M., KATOH S., 1989 - *Changes in protein content and in the structure and number of chloroplasts during leaf senescence in rice seedlings.* - Plant Cell Physiol., 30: 707-715.

KIM K.S., 1987 - *Comparative drought resistance mechanisms of eleven major warm-season turfgrasses.* - Texas A&M University, College Station, Texas, pp. 134.

KISLYUK I.M., BUBOLO L.S., KAMENTSEVA I.E., KOTLOVA E.R., SHERSTNEVA O.A., 2007 - *Heat shock increases thermotolerance of photosynthetic electron transport and the content of chloroplast membranes and lipids in wheat leaves.* - Russ. J. Plant Physiol., 54: 456-463.

KISLYUK I.M., BUBOLO L.S., PALEEVA T.V., SHERSTNEVA O.A., 2004 - *Heat-induced increase in the tolerance of the wheat photosynthetic apparatus to combined action of high temperature and visible light: CO_2 fixation, photosynthetic pigments, and chloroplast ultrastructure.* - Russ. J. Plant Physiol., 51: 455-463.

KUMAR R., GOSWAMI S., SHARMA S., SINGH K., GADPAYLE K., SINGH S.D., PATHAK H., RAI R., 2013 - *Differential expression of heat shock protein and alteration in osmolyte accumulation under heat stress in wheat.* - J. Plant Biochem. Biotechnol., 22: 16-26.

LEEGOOD R.C., 1993 - *Carbon dioxide-concentration mechanism*, pp. 47-72. - In: LEA P.J., and R.C. LEEGOOD (eds.). *Plant biochemistry and molecular biology.* John Wiley & Sons, Chichester, UK, pp. 522.

POMPEIANO A., VOLPI I., VOLTERRANI M., GUGLIELMINETTI L., 2013 - *N source affects freeze tolerance in bermudagrass and zoysiagrass.* - Acta Agric. Scand. Sect. B Soil Plant Sci., 63: 341-351.

R DEVELOPMENT CORE TEAM., 2012 - *R: a language and environment for statistical computing.* - R Foundation for Statistical Computing, Vienna, Austria.

RIZHSKY L., LIANG H., SHUMAN J., SHULAEV V., DAVLETOVA S., MITTLER R., 2004 - *When defense pathways collide. The response of* Arabidopsis *to a combination of drought and heat stress.* - Plant Physiol., 134: 1683-1696.

SAGE R.F., 2002 - *Variation in the* k_{cat} *of Rubisco in C_3 and C_4 plants and some implications for photosynthetic performance at high and low temperature.* - J. Exp. Bot., 53: 609-620.

STEINKE K., CHALMERS D.R., THOMAS J.C., WHITE R.H., 2009 - *Summer drought effects on warm-season turfgrass canopy temperatures.* - Appl. Turfgrass Sci., pp. 1-6.

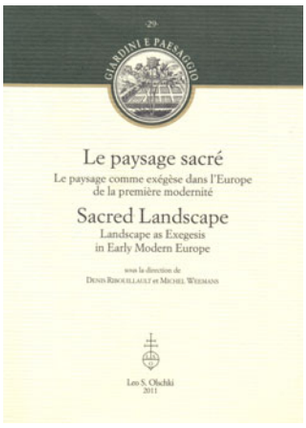
TOBIAS R.B., BOYER C.D., SHANNON J.C., 1992 - *Alterations in carbohydrate intermediates in the endosperm of starch-deficient maize (*Zea mays *L.) genotypes.* - Plant Physiol., 99: 146-152.

VEERASAMY M., HE Y., HUANG B., 2007 - *Leaf senescence and protein metabolism in creeping bentgrass exposed to heat stress and treated with cytokinins.* - J. Am. Soc. Hortic. Sci., 132: 467-472.

WAHID A., 2007 - *Physiological implications of metabolite biosynthesis for net assimilation and heat-stress tolerance of sugarcane (*Saccharum officinarum*) sprouts.* - J. Plant Res., 120: 219-228.

XU Y., ZHAN C., HUANG B., 2011 - *Heat shock proteins in association with heat tolerance in grasses.* - Int. J. Proteomics, 11.

BOOK REVIEWS



LE PAYSAGE SACRÉ - SACRED LANDSCAPE. *Ribouillat D.,* and *M. Weemans*. Giardini e Paesaggio, vol. 29. Leo S. Olschki, Florence (Italy), 2011, pp. XXXII + 368. ISBN 978-88-2-6126-72. € 48.00.

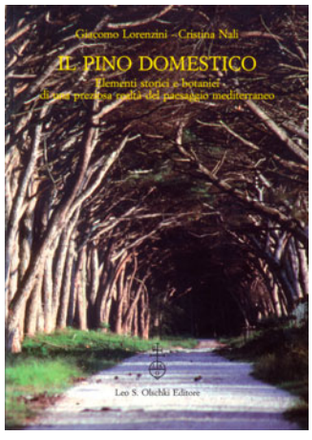
This is the 29th volume in the "Giardini e Paesaggio" series. Unlike other studies that have been influenced by the idea of an "autonomous landscape", this volume underlines the importance of the sacred aspect of nature in the representation and creation of landscapes in the early modern period in Europe. The work, examining areas from literature to gardens and painting to microarchitecture, suggests that landscape in the period under study should be considered as an interpretation of the artists in the "Book of Nature".

The various contributions - in English and in French - that make up the volume are enriched with images from the period that have been carefully assembled by highly regarded international experts in the field of landscape history, stimulating the debate surrounding the concept of scared landscapes. It is necessary to underline the importance of this topic and the efforts undertaken by various scholars to reveal the deep relationship between landscape and sacredness.

This volume is a significant addition to the vast literature on the evolution of landscapes through history, from a scientific and technical, as well as historical and cultural perspective and offers the reader meaning and understanding with regard to the sacred values that make up the environments considered.

Francesco Ferrini

IL PINO DOMESTICO. Elementi storici e botanici di una preziosa realtà del paesaggio mediterraneo. *Lorenzini G.,* and *C. Nali*. Giardini e Paesaggio, vol. 37. Leo S. Olschki Editore, Florence (Italy), 2013, pp. 96. ISBN 978-88-222-6252-3. € 15.00.



This volume, as the authors describe, aims to attract the reader's attention to a "friend" in trouble. The "friend" is the pine tree. It deserves, according to the authors, attention and support as it has always been generous with man, and is symbolic of the Italian landscape.

The book consists of 96 pages, divided into three parts and a premise, and is written not only for scholars in the field but also to those who, to varying degrees, are interested in this species and to the typical landscapes it has created in our country.

The authors are well known experts in the field as, for many years, they have carried out studies and taught many courses on the topic.

The first part of the book is devoted to the natural history of pine and its dissemination, the second to breeding for productive purposes and the third to the place that this species occupies in mythology, history, tradition and art. The appendix is of particular interest as it presents curiosities (and not only) about this species.

The volume includes illustrations and both color and black and white photos, as well as a bibliography to conclude the book. The authors offer through this work a key upgrade on the subject, from a scientific and technical perspective and with regard to our historical and cultural heritage. The book is of great value for all researchers working in the field of arboriculture and for those who have an interest in basic physiology, applied ecology, and biology to preserve this precious and much-loved species.

Francesco Ferrini

MANUALE DI ORTOFRUTTICOLTURA. Innovazioni tecnologiche e prospettive di mercato. *Sansavini S.*, and *P. Ranalli* (eds.). Ministero delle politiche agricole e forestali. Edizioni Agricole de Il Sole 24 Ore, Bologna, 2012. pp. xxiv + 668. ISBN 978-88-506-5360-7. € 50.00.

This volume, carefully edited by Silviero Sansavini and Paolo Ranalli and with contributions by renowned experts in the field, offers considerable breadth.

The text covers a wide range of agricultural products, from fruits to vegetables, with particular emphasis on integrated and organic productions, as well as on the market and its prospects. The up-to-date knowledge regarding the fruit and vegetable sector, and in particular pertaining to genetic improvement and new varieties, make the "Handbook of horticulture " an immediate tool for operators, students and researchers. The presence of numerous mainly color illustrations and the orderly succession of tables render each treated topic easy to consult and encourage further investigation.

The text consists of the following five parts: Part One, "Stato dell'arte dell'Ortofrutticoltura italiana" (Italian horticulture: state of art); Part Two, "Frutticoltura" (Fruitculture); Part Three, "Orticoltura" (Horticulture); Part Four, "Produzione integrata e biologica: caratteristiche, disciplinari, gestione" (Integrated and organic production: characteristics, disciplines and management); Part Five, "Il mercato e le prospettive future" (The market and future perspectives).

Enrico Rinaldelli

PRODUZIONE ED IMPIEGO DELLE PIANTE OFFICINALI. *P. Catizone, L. Barbanti, I. Marotti, and G. Dinelli.* PÀTRON Editore, Bologna, 2013. pp. 348. ISBN 978-88-555-3233-4. € 44.00.

It's not easy to write a book on medicinal plants. As explained on the back cover of the book: "the term medicinal plants defines a large group of plant species that have been used in pharmaceutical laboratories, but in a broader sense also includes plants for aromas, cosmetics, dyes, biocides and other agricultural purposes".

After a period of declined interest, due to the belief that chemistry would rapidly produce active principles via synthetic processes, in recent years there has been swift development in the study of these plants, which has led to substantial progress in knowledge. The acquisition of this not only scientific and technical knowledge prompted the authors to bring together a text that is both useful for experts in the field and for other readers, such as students, who look this difficult subject for the first time. The subject of medicinal plants calls for multidisciplinary knowledge ranging from botany, arboriculture and natural science to chemistry, pharmaceutical sciences and medicine. Indeed, the authors have presented in a concise manner a mass of knowledge and shed innovative light on the subject to eliminate the sense of uncertainty for those who are approaching it for the first time (and not only) and are dealing with the heterogeneous reality that is the world of herbs.

After a general introductory part, the text is divided into seven chapters: the first is dedicated to cultivation, the second examines economic aspects, the third regulatory framework, the fourth focuses on metabolism and active principles, while the fifth to seventh chapters examine in detail cultivation and post-harvest processes and utilization. A special part consists of detailed records of individual species (two to three pages each) accompanied by photos of plants, flowers and seeds.

The 348-page book also includes an appendix with an extensive glossary, many of which are medical terms.

This manual is undoubtedly of great value for students, technicians and all researchers working in the field, but it can be considered interesting for growers of medicinal species at amateur level as well.

Cinzia Silori