ISSN on-line 1592-1573
ISSN print 0394-6169

AHS

Advances in Horticultural Science

Vol. 30 - n. 2, 2016

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: **Francesco Ferrini**, 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:

ACNP Catalogo Italiano dei Periodici - AGRICOLA - AGRICULTURE Journals - AGRIS -APE Journal - BASE Bielefeld Academic Search Engine - BIOBASE - Biological Abstracts - Biological Science Journals PQ - BIOSIS Preview THOMSON REUTERS - CAB Abstracts - EBSCO - EZB Elektronische Zeitschriften Bibliothek - Universitäts bibliothek Regensburg - Google Scholar - HORTICULTURAL Abstracts -Journal Seek. A Searchable Database of Online Scholarly Journals - JURN - Natural Science Journals PQ - NewJour. Electronic Journals & Newsletters, University of Georgetown - OAISTER oclc - Ornamental Horticulture CABI - Plant Breeding Abstract CABI - Proquest - Scirus ELSEVIER - SciTech Journals - SciVerse SCOPUS ELSEVIER - Searchteam - Ulrich's Periodicals Directory - WoS Web of Science THOMSON REUTERS - WorldCat

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 +39-055-4574910, E-mail: advances@dispaa.unifi.it

CONTENTS

Adv. Hort. Sci., 2016 30(2): 59-67 DOI: 10.13128/ahs-19130

Optical evaluation of the shading properties of climbing fern *Lygodium japonicum* used as a thermal buffering green wall plant

A. Hara [1], H. Takaichi [1], Y. Murata [1], R. Sakata [1], Y. Hara [1], J. Iwase [2, 3], D. Comparini [1, 2, 4], T. Suzuki [1, 4], T. Kawano [1, 2, 4] (*)

[1] *Faculty and Graduate School of Environmental Engineering, The University of Kitakyushu, Kitakyushu, Japan.*
[2] *LINV Kitakyushu Research Center, Kitakyushu, Japan.*
[3] *Kyushu Institute of Technology Collaboration Center, Kitakyushu, Japan.*
[4] *International Photosynthesis Industrialization Research Center, The University of Kitakyushu, Kitakyushu, Japan.*

Key words: building thermal control, environmental measurements, green roof, green wall, rooftop garden, urban agriculture.

Abstract: Recently, thermal properties of the landscaped rooftops and walls have attracted the interest of researchers because of the potential to minimize energy consumption in urban areas and to aid summer-time thermal control. For this reason the creation of a plant-based shade for walls or above buildings is highly important. In this paper we evaluate using *Lygodium japonicum,* one of the many ferns and fern allies traditionally used in Japanese gardening, as a component of thermal-buffering green walls. *Lygodium japonicum*, the only climbing fern species in Japan, is fast-growing, adheres easily to walls and has a climbing nature. A simple thermal analysis of the sun-shading effect of *Lygodium* canopy suggested that local surface temperature above the ceramic tiles placed on the rooftop of a building can be buffered (lowered in daytime and maintained relatively warm at night) by the presence of leafy climbing ferns covering the tiles, possibly due to the reflection and absorbance of solar radiation. Furthermore, the presence of the plants may also slow the night-time release of heat from the building surface. Because plants installed on tall walls or on the tops of buildings are not easily accessed for manual care, we performed a real-time routine monitoring and control of plant growth status using various optical sensors that could be automated and monitored remotely for large-scale applications. For this purpose, the optical properties of a *L. japonicum* canopy under solar incident light have been determined. In order to evaluate the natural shading and growing properties of a green canopy, the incident solar radiation spectrum (*J*), leaf canopy-filtered light spectrum (transmittance, *T*) and leaf-reflectivity spectrum (*R*) were measured. By reading the reflectivity spectrum, concomitant chlorophyll fluorescence signals (*F*) from *Lygodium* leaves were also detected at 760 nm, which corresponds to the O_2-A Fraunhofer line. Our data suggests that the daily change in photosynthetic status (*P*) can be traced by monitoring the change in relative *F* in relation to the estimated heat loss (*H*) and measured *J*, *R*, and *T* using a series of practical equations designed to roughly estimate the gross photosynthetic response within the plant canopy. Using our equations, the photosynthetic capacity in the plant canopy structure could be simply simulated and predictable by optical sensors.

1. Introduction

To aid summer-time thermal control in urban areas, the creation of shade above buildings and/or streets is highly important for a number of reasons. First, shade can provide protection for residents and passersby by buffering the changes in housing thermal conditions inside the building and/or local climate on the streets during heat wave attacks, a serious concern as discovered in the case of the historic, heat-related disaster in Paris in 2003 (Keller, 2013). Second, minimizing energy consumption within buildings has become an important goal of architecture and urban planning in recent years. As a result, several guidelines have been developed depending on the climatic zones, aimed at increasing solar exposure for buildings in cold climates and reducing solar exposure for buildings in warm climates (Okeil, 2010).

(*) Corresponding author: kawanotom@kitakyu-u.ac.jp
Received for publication 23 December 2015
Accepted for publication 19 February 2016

The thermal properties of greened rooftops and walls have recently been extensively documented. A study was performed to compare greened and non-greened walls with and without additional covering shade cloths using model buildings with thermal thin walls (Yamasaki *et al*., 2009). By quantitatively evaluating the cooling load reduction effect under/inside the greened roof/walls, the room temperature of the green-covered building was shown to be significantly lower due to the sun-shielding effect of plants covering the building, and electrical power consumption for air conditioning was also lower in the green building. According to this model, around 40 to 45% of energy could be saved in the greened building compared to the non-greened building. It is natural to conclude that lowering total temperature inside the model buildings is the consequence of the local temperature controls on the surface of the walls or roofs.

As a lack of or excess water could drastically alter the growth status of the plants installed, water consumption by the plants needs to be understood. In addition, the rate of water consumption and air-cooling properties of plants have a close relationship since transpiration by plant leaves plays a key role in local heat removal. Therefore, quantitative evaluation of water consumption by a model wall-greening system is of great importance. Toward this purpose, simple models with net-supported vine plants (such as ivy and morning glory) have reportedly been conducted (Takayama *et al*., 2014).

Like other forms of green infrastructure, green façades in which climbing plants are grown either directly against, or on support structures affixed to external building walls, have recently been gaining the attention of architects as a design feature aimed at reducing internal building temperatures, reducing building energy consumption, and facilitating urban adaptation to a warming climate (Hunter *et al*., 2014). Today, such vertical greenery systems (VGS) are viewed as passive tools for energy savings in buildings (Pérez *et al*., 2014). Accordingly, not only the lowering of building temperature, but also many economic, environmental and social benefits are associated with the use of VGS (Safikhani *et al*., 2014). Furthermore, various new green wall construction methods have been developed to date, although many of these technologies have yet to be evaluated and even to be amended, mainly due to the difficulty of maintaining the active growth and development of plants under stressful conditions such as forced adhesion by artificial supports, exposure to thermal stresses, lack of or excess irrigation and/or fertilization on site and on time (Tachibana *et al*., 2011).

Reports on approaches for the greening of buildings using self-growing plants are increasing day-by-day. For an instance, a Canadian team has designed a prefabricated piece to be used on building envelopes, interior partitions, façades or landscape enclosures, in which the vegetation is integrated within the wall construction instead of being adhered to it (Ardila *et al*., 2009). Accordingly, the designed system by an incorporated conduit system included the self-supporting, self-irrigating and self-fertilizing performance for the growing plants. An urban geographer, Gandy (2010), has explored the work of French botanist Patrick Blanc, who applies his knowledge of botany and related sciences to urban wall design with inspiration from the *mur végetal* (green wall) first made in 1988. Blanc intended to transform the urban sceneries into ravines or rainforests by covering the streets and buildings with ferns and mosses.

It is well known in Japan that, second to mosses, many members of Pteridophytes (encompassing ferns and fern allies) have been traditionally used in Japanese garden design (Kawano, 2015). As encouraged by a French botanist, one of the authors (TK) recently propounded that the use of Japanese fern species on green walls and/or roofs is worth pursuing (Kawano, 2015). For this reason, we would like to discuss the criteria for the plant components of heat buffering green walls which should also be applied to the fern species.

The first criterion is the plants' adhesion to the surfaces of walls or roofs. Among the common garden ferns found in Japan, members of Polypodiaceae such as *Lemmaphyllum microphyllum* Presl (Japanese name: *Mamezuta*), *Lepisorus thunbergianus* (Kaulf.) Ching (Japanese name: *Nokishinobu*), and *Pyrrosia lingua* (Thunb.) Farw (Japanese name: *Hitotsuba*) are epiphytic species often attached to trees and rocks. Therefore, these plants can be effectively used to cover walls. However, *Lemmaphyllum microphyllum* is often exposed to competition on the surface of rocks and walls with neighboring epiphytic higher plants such as *Ficus pumila* L. (Moraceae; Japanese name: *Ōitabi*). Therefore, we would like to emphasize that the second criterion for plant components of thermal buffering green walls is that they should be fast growing. The third criterion for green wall ferns must be an ability to not only adhere, but also climb up the poles and nets, and cover irregular walls of the greening structures. The last criterion is the heat-buffering property of the greenery components.

However, to date, studies on the influence of climbing plant characteristics are still very limited, and even fewer works have investigated the impact of green façade design components on thermal performance (Hunter *et al*., 2014).

As a candidate fern species to be listed as a green wall component, *Lygodium japonicum* (Thunb.) Sw. (Lygodiaceae; Japanese name: *Kanikusa*), the only climbing fern species in Japan, is of great interest since this plant species has a fast-growing, wall adhesive, and climbing nature. The sun-tracking and rotating movements associated with the climbing growth in two *Lygodium* members (*L. articulatum* and *L. scandens*) were briefly described by Charles Darwin (1875). In his book, he concluded that "*As ferns differ so much in structure from phanerogamic plants, it may be worthwhile here to show that twining ferns do not differ in their habits from other twining plants*".

Finally, the heat buffering property under solar radiation must be determined with *L. japonicum.* Through the minimal model tests described here, we attempt to demonstrate that local surface temperature above ceramic roof tiles of a building can be effectively buffered, thus lowering daytime temperature and maintaining relative warmth during the night, by the presence over the tiles of leafy climbing ferns, possibly due to reflection and absorbance of solar rays and prevention of the release of heat from the building surface. However, once plants are installed on tall walls or the top of buildings which are far from accessible for manual daily care, real-time routine monitoring and controls of plant growth status should be automated using various optical sensors. For this purpose, background data for optical properties of a *Lygodium* canopy under solar incident light, reflecting the thermal and growing status, have been investigated.

2. Materials and Methods

Plant materials and experimental set up

Lygodium japonicum is commonly known as "Japanese climbing fern". This native fern grows very rapidly and thus often covers neighboring living trees, rocks and walls in gardens (Fig. 1). For ornamental purposes, *L. japonicum* has been exported out of the country. For instance, this plant was introduced in 1932 in Florida, USA (Gordon and Thomas, 1997). In the Hibikino campus of the University of Kitakyushu (Wakamatsu-ku, Kitakyushu, Japan; 33˚ 53'24'' North latitude, 130˚ 42' 49'' East longitude), semi-wildly propagating *L. japonicum* plants (Fig. 1A-E) directly exposed to sunlight were sampled, replanted in pots, and kept in the greenhouse for three days under fluorescent light to recover prior to experiments.

Model set-up on building roof to measure daytime roof tile temperature with and without Lygodium leaf canopy

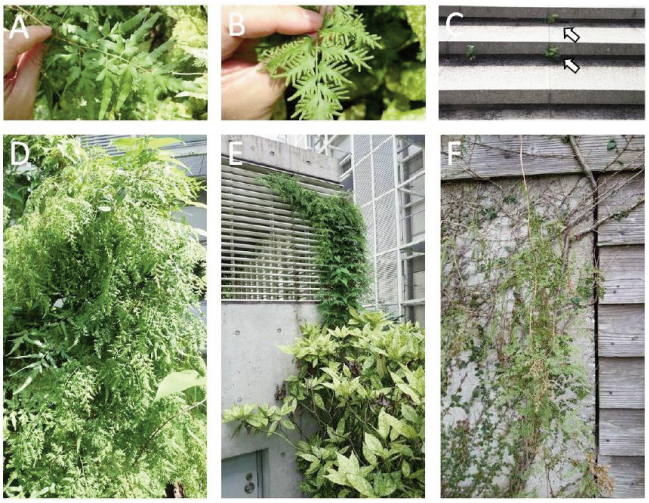

Fig. 1 - The semi-wildly propagating Japanese climbing fern, *Lygodium japonicum,* used in this study. (A) Vegetative leaflet. (B) Reproductive leaflet. (C) Semi-wild plants surviving in the gaps between stones (as indicated by arrows). (D) Aggressive growth of *L. japonicum* winning the competition with other standing plants. (E) Climbing growth of *L. japonicum* on the concrete and aluminum walls, thus naturally greening the building. (F) Even though it came after, *L. japonicum* plants are growing on the concrete wall by rapidly covering over the pre-existing vines of *Ficus pumila* L. Plants were found on Hibikino campus of the University of Kitakyushu, Wakamatsu-ward, Kitakyushu, Japan (A-E), and a private garden in Miyazaki Prefecture, Japan (F).

The experimental was set up on the rooftop of a building to assess the shading properties of the *Lygodium* canopy (Fig. 2A). *Lygodium* plants were potted and placed on the building roof-top, with bunches of leaflets covering ceramic tiles on which pairs of fine thermocouples (thermal sensors) were set.

We previously reported the real-time measurement of rapid and accurate temperature changes in the micro-environments within a plant cell culturing system (Lin *et al*., 2006). The real-time thermo-sensing units employed here have similar set-ups. The units consist of fine thermocouples (KFT-25-200-100, ANBE SMT Co., Japan), an AD/DA 8 channel converter (MR-500, Keyence, Japan), and a PC with a display (Fig. 2 B-D). Each sensory unit, calibrated immediate-

ly before the experiment, possesses small heat capacity, thus enabling immediate and accurate measurements.

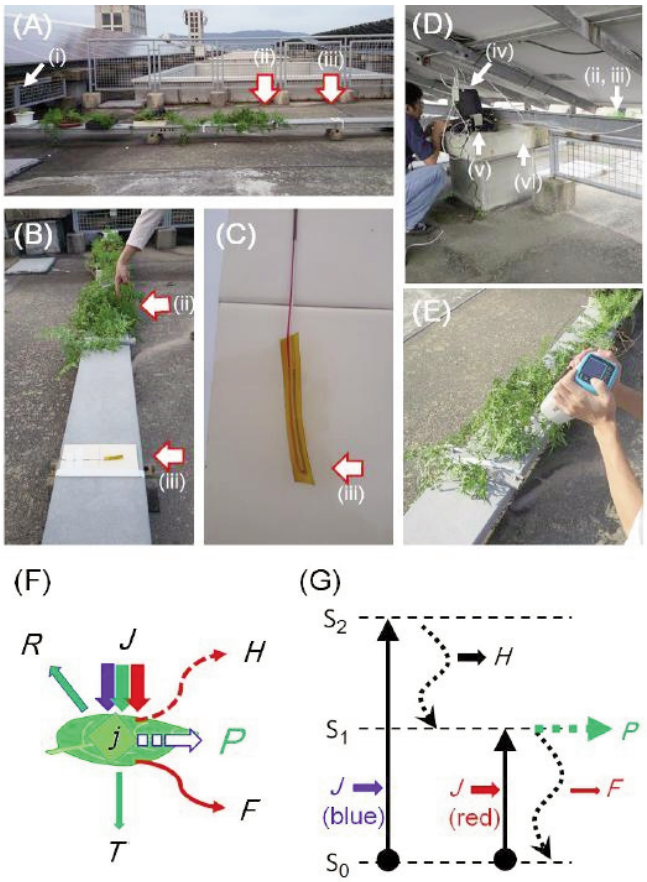

Fig. 2 - Experimental set up to assess the shading properties of the *Lygodium japonicum* canopy on the roof of a building. (A, B) *L. japonicum* plants set up on the roof. (C) One of the fine thermocouples placed on the ceramic tiles. (D) Composition of the monitoring units. (E) Measurement of reflection spectra on the surface of plant leaves using a portable NIR field spectro-radiometer. (i) PC control booth beneath the solar panels. Arrows (ii) and (iii) indicate the positions of the thermocouples. (iv) A note PC, (v) an AD/DA 8-channel converter, (vi) connecting cables. (F) Fate of incident solar light illuminating plant leaves. *J*, incident light; *R*, reflection; *T*, transmittance; *j*, captured light energy; *H*, local heat loss; *F*, fluorescence; and *P*, photosynthesis. Optically, *J*, *R*, *T*, *j* and *F* can be determined. (G) Energy transfer by short wavelength (blue) light and long wavelength (red) light after illumination of chlorophylls.

Solar spectra, canopy-filtered light spectra and leaf-reflectivity spectra

To measure solar and leaf canopy light spectra, a CL-500A Illuminance Spectrophotometer (Konika Minolta, Tokyo, Japan), which covers the range between 360 nm and 780 nm, was used. Spectroscopic analyses of reflectivity on the surface of leaves were carried out using a portable near-infrared (NIR) field spectro-radiometer, FieldSpec HandHeld 2 (ASD Inc., Atlanta, GA, USA), designed for spectral measurements (ranging from 325 nm to 1075 nm) on site (Fig. 2E). In figure 2F and G, the fate of incident solar light illuminating plant leaves and generalized modes of energy transfer by short wavelength (blue) light and long wavelength (red) light after illumination of chlorophylls are illustrated. Based on the experimental design described here, spectroscopic data on incident light (*J*), reflection by leaves (*R*), transmittance through leaves (*T*), light energy captured by leaves (*j*) and chlorophyll fluorescence (*F*) can be non-invasively and remotely monitored.

3. Results and Discussion

Effects of Lygodium japonicum *canopy on ceramic roof tile surface temperature*

The heat-blocking or buffering action by the leafy canopy of *L. japonicum* was assessed by monitoring the changes in local temperature on the surface of model ceramic tiles with and without *L. japonicum* coverage (Fig. 3). Comparisons were made on a cloudy day (2 October 2014) (Fig. 3, top) and a sunny day (3 October 2014) (Fig. 3, bottom). In both cases, daytime temperature was higher on the control tiles without plant canopy. Data clearly suggest that the fluctuation of temperature due to direct exposure to naturally changing solar light intensity could be buffered.

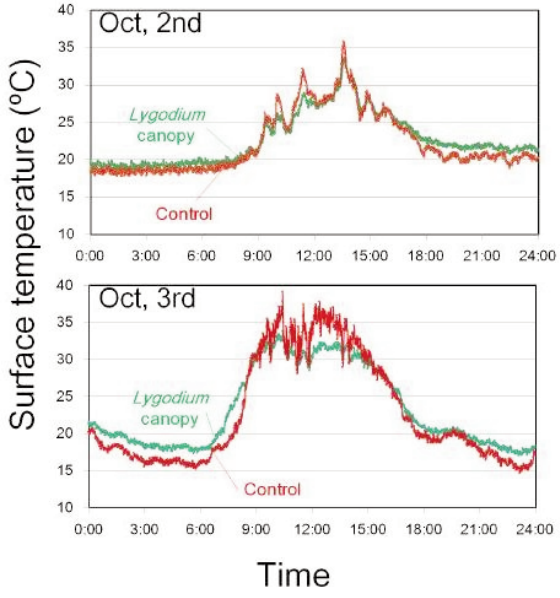

Fig. 3 - Assessment of the sun-shading effect of *L. japonicum* canopy by monitoring of the surface temperature. Typical data, recorded on 2 and 3 October 2014, are shown.

In addition to the action of the plant canopy in the daytime, the nighttime changes in local temperature were also buffered by the presence of the *L. japonicum* leaves, possibly by minimizing the bulk flow of heat-removing air reaching the tile surface, and also by blocking the heat transfer out of ceramic tiles through the highly reflective nature of the leaves in the NIR region. These hypotheses will be the subject of critical experimental examination in future studies.

Filtering of solar rays through Lygodium japonicum *leafy canopy*

Solar radiation above and under the *L. japonicum* leafy canopy were monitored with a hand-held Spectrophotometer (Fig. 4). By subtracting the level of radiation under the canopy from that recorded above the plants, total light filtering performance by *L. japonicum* canopy could be calculated (Fig. 4, bottom). Data from two nearby experimental sites, each with its independent plant, indicated that a majority of solar radiation was filtered by the leaf canopy, possibly through absorption and reflection of light.

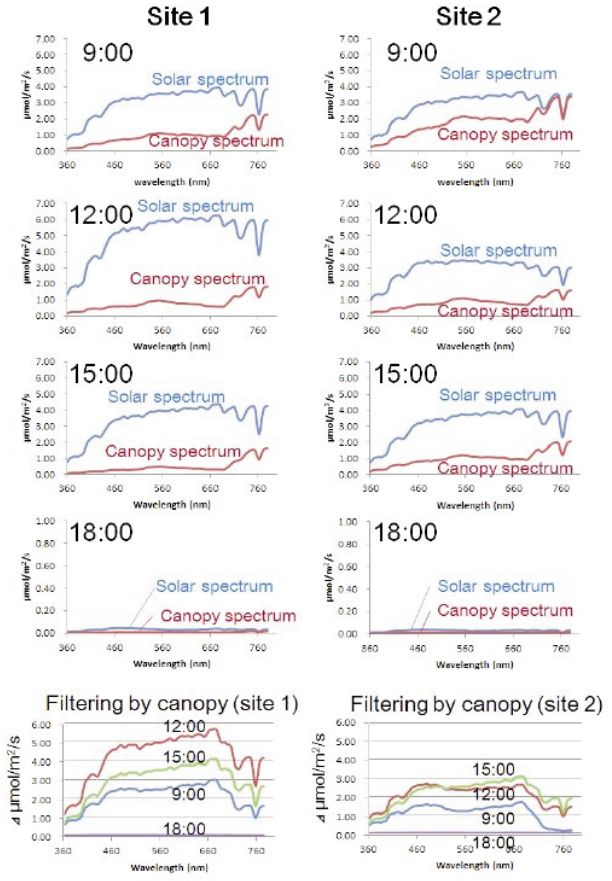

Fig. 4 - Spectrometric analysis of solar radiation and its filtering by *L. japonicum* canopy. Typical data, recorded on 3 October 2014, are shown.

Reflection of solar rays by Lygodium japonicum *leaves*

Reflectivity of *L. japonicum* leaflet surface was measured under sunlight (Fig. 5) (3 October 2014). Due to the presence of chlorophylls, there was always a pair of valleys of reflectivity in the blue and red regions (in the range below 450 nm and around 660 nm), which correspond to the absorbance by chlorophyll *a* and its related metabolites (Kawano *et al*., 1999). In the range of visible light, green-colored light (peaking at around 550 nm) was most highly reflected as expected by the presence of chlorophylls in the leaves. Since green light was also the major light component of the under-canopy radiation (Fig. 4), absorption of green light by plant pigments was shown to be negligible.

In the NIR region, a high rate of solar radiation was reflected upwards (Fig. 5), suggesting that the decrease in NIR radiation below the plant canopy is largely due to reflection. A steep increase in reflectivity spanning from the red region to NIR region represents the so-called “red edge” of reflection. This is a phenomenon commonly observed in various green

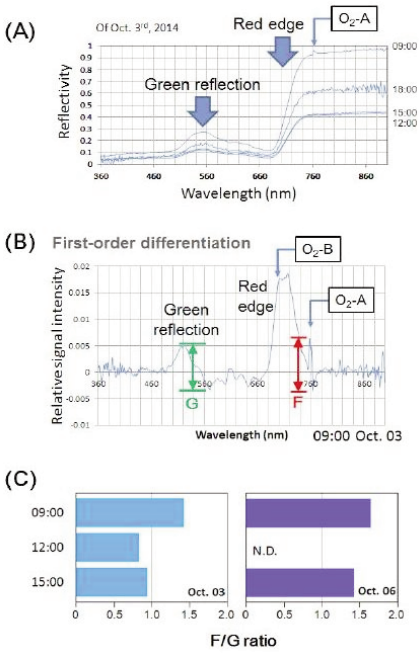

Fig. 5 - Analysis of leaf reflectivity using the leaves of *L. japonicum* plants covering ceramic roof tiles. (A) Typical reflectance spectra from *L. japonicum* leaves recorded at 9:00, 12:00, 15:00 and 18:00 on 3 October 2014 are shown. (B) An example of processed reflectance spectrum. Relative signal intensity was determined after the first order differentiation of the spectral data. (C) Changes in the normalized chlorophyll fluorescence signal. Signals corresponding to reflectance in the green and red-to-NIR regions are labeled as “Green reflection” and “Red edge”, respectively. Chlorophyll-dependent fluorescence signals detected at 760 nm and 679 nm, corresponding to O_2-A and O_2-B Fraunhofer lines, respectively, are labeled.

leafy plants, suggesting that most green plant canopies are capable of creating shade, minimizing the NIR radiation allowed to reach ground level, thus blocking the heating radiation to reach beneath the leaves. In this context, a *L. japonicum* canopy fulfills one of the key criteria as a thermal buffering plant canopy.

Detection of chlorophyll fluorescence signals in the reflectivity spectra

At 760 nm, the wavelength corresponding to the O_2-A solar Fraunhofer line (telluric absorption band) near 760 nm, a spike of signal corresponding to chlorophyll fluorescence was observed especially under morning solar radiation (Fig. 5A) (labelled as O_2-A). However, chlorophyll signal at O_2-B near 687 nm solar Fraunhofer line could hardly be detected since 687 nm coincides with the steep increase in "red edge" reflectance by leaves (Fig. 5A). After first-order differentiation of the reflectance spectra, the red edge reflectance signal no longer interferes with the reading of chlorophyll fluorescence at 687 nm (Fig. 5B) (labelled as O_2-B). In this way, quantification of chlorophylls and estimation of the spread and density of vegetative plant tissue can be non-invasively and even remotely monitored as plant vegetation performance mapped by remote-sensing satellites (Meroni *et al*., 2009; Guanter *et al*., 2010; Mazzoni *et al*., 2012). However, we have to be cautious about the handling of fluorescence data to assess the area of leaf coverage since fluorescence signals can potentially report the status of gross photosynthesis (without considering the rate of respiration) and therefore, it may be altered over the course of the day. The fate of light energy reaching the plant leaf surface can be expressed as follows:

$$J= R+T+H+F+P \quad [1]$$

$$j= J-R-T \quad [2]$$

where *J*, *R*, *T*, *H*, *F*, *P*, and *j* stand for incident light, reflection, transmittance, heat loss, fluorescence, photosynthesis, and captured light energy, respectively. Then, the fate of *j* can be traced as follows:

$$j= H+F+P \quad [3]$$

$$P= j-H-F \quad [4]$$

In this study, we directly and fully monitored *J* (Fig. 4) (solar spectrum), *R* (Fig. 5A), and *T* (Fig. 4) (canopy spectrum), and partial *F,* the intensity of which is proportional to the total *F* (Fig. 5A, B). By definition, *j* can be readily estimated from recorded *J*, *R* and *T*. Therefore, the rate of gross photosynthesis (*P*) under constant or known *j* should be negatively proportional to the rate of heat loss (*H*) + fluorescence (*F*). Assuming that *H* is constant (actual changes in local heat loss in the leaves should be determined in future experiments), changes in *F* indicate the photosynthetic status of the plants. In fact, quantification of fluorescence signal and monitoring of its temporal changes can be readily performed after normalization with green reflection (Fig. 5B, C). Taken together, the data in figure 5C suggest that *L. japonicum* plants are fully ready for photosynthesis only after midday.

Need for the evaluation of photosynthesis

We have recently proposed a series of practical equations designed to describe the collective gross photosynthetic response within the plant canopy (Okamoto *et al*., 2016). Using our equations, the photosynthetic capacity in the plant canopy structure can be simply simulated based on minimal sampling of a single top-positioned leaf through measurement of (i) PI-curve in a horizontally placed single leaf, (ii) state of dark respiration in a single leaf, and (iii) transmittance through a single leaf.

As pointed out by Monsi and Saeki (2005), *T* through layer of leaves can be expressed according to the definition by Beer-Lambert law as follows:

$$T=e^{-ax} \quad [5]$$

where *a* and *x* are absorption coefficient and length of the path within the leaf layer, respectively. For simplification of the model, we assume that the canopy structure consists of uniform leaves. By experimentally determining the value for *T* in a single leaf, we can approximate the total light used for photosynthesis within the canopy structure as follows:

$$\sum_{i=0}^{\infty} JT^i = \lim_{k\to\infty} \sum_{i=0}^{k} JT^i = \frac{J}{1-T} \quad [6]$$

where *i* is the number of leaves (*i*=0 is initial light intensity above the leaves). Today, Michaelis-Menten-type photosynthetic equation proposed by Platt and Jasby (1976) is widely accepted by the plant research community to describe the nature of gross photosynthesis as below:

$$P = \frac{P_{max} J}{K_j + J} \quad [7]$$

Recently, we proposed that photosynthetic light response curves can be generated based on a limited number of experimental data points through application of Platt-Jasby equation by determining P_{max} values and K_j values from least-sized experiments (Nagasawa *et al*., 2015).

By substituting *J* in equation [7] with the total

light used for photosynthesis within the canopy as shown in equation [6], we can obtain the following equation:

$$\sum_{n=1}^{k} P_n = \frac{P_{max}\frac{J}{1-T}}{K_j+\frac{J}{1-T}} \quad [8]$$

where P_n stands for P in the n^{th} leaf in the canopy. Since the collective light yield rapidly converges, k can be replaced with ∞ in a practical sense. This equation can be rewritten to modify the apparent Michaelis constant as follows:

$$\sum_{n=1}^{k} P_n = \frac{P_{max}J}{K_j(1-T)+J} \quad [9]$$

By accurately determining P or total P in the canopy through a model experiment, we can more accurately estimate the local heat loss (H) on the leaf as:

$$H = j-F-P \quad [10]$$

Climbing plants

It is well known that climbing plants, as represented by the tendril-bearing plant families, chiefly belong to higher flowering plant families such as Vitaceae, Bignoniaceae, Passifloraceae and Cucurbitaceae (Fabre, 1855; Darwin, 1875; Gerrath *et al*., 2008), many of which are agriculturally and economically important (Kawano *et al*., 2012). Interestingly, only a few climbing species can be found among the seedless vascular plant lineages, including ferns (Darwin, 1875). Many more climbing fern species may have been lost in the course of evolution, since it is believed that there was a dramatic drop in the diversity and abundance of most fern species, inversely-proportional to the burst of diversification in angiosperms during the Cretaceous period (Schneider *et al*., 2004).

Timing of model experiments

We planned to examine the slowing effects of *L. japonicum* canopy on both local heating during daytime and local cooling during nighttime; for this purpose, early October (2014) was chosen as the timing for model experiments. Although the attempt presented here provides preliminary data in support of the thermal buffering capacity of *L. japonicum* canopy, further model experiments taking place under two extreme conditions, namely in mid-summer and mid-winter, are required in order to fully assess the thermal buffering capacity of this species. Finally, there is great interest in assessing the quantitative heat balance and the radiation balance on the surface of walls or roofs based on the larger scale experiment with special reference to the thermal buffering effect of *L. japonicum* in all seasons in Japan.

Future environmental studies

Green components covering buildings and walls in urban areas are exposed not only to natural environmental stresses but also to artificial stressful conditions, chiefly exposure to polluted air containing ozone (Kadono *et al*., 2006; Tran *et al*., 2013) and related oxidants (Yukihiro *et al*., 2012). Most plants exposed to such oxidative stress readily develop visible symptoms on the leaves reflecting the onset of programmed cell death (Kadono *et al*., 2010). The sensitivity and/or tolerance of *L. japonicum* to such stressful conditions must be studied prior to its wider application in urban greening projects.

4. Conclusions

The minimal thermal analysis of the sun-shading effect of *L. japonicum* canopy was performed by monitoring changes in tile surface temperature. In order to optically monitor the natural shading and growth properties of a green canopy consisting of the leaves of a climbing fern, the following optical approaches have been performed for the first time: here, optical properties of *L. japonicum* under solar incident light, namely, the natural shading and growing properties of green canopy were studied. The incident solar radiation spectrum (J), leaf transmittance (T) spectrum, and leaf-reflectivity spectrum (R) were measured. In the reflectivity spectrum, concomitant chlorophyll fluorescence signal (F) was detected at 760 nm, corresponding to the O_2-A Fraunhofer line. Data suggests that the daily change in photosynthetic status (P) can potentially be traced by monitoring the change in relative F in relation to the estimated heat loss (H) and measured J, R, and T.

Acknowledgements

This work was supported by a grant from the Regional Innovation Strategy Support Program implemented by Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

References

ARDILA A.V.H., RESTREPO M.C.G., MONTOYA L.F.E., SEPULVEDA O.E.C., ACOSTA A.C.R., 2009 - *Green walls: An environmental alternative for the city*. - Proc. 26th Int. Conf. Passive Low Energy Architect., 6.

DARWIN C., 1875 - *The movements and habitats of climbing plants*. 2nd Edition. John Murray, London. UK. French translation by R. GORDON: *Les Mouvements et les habitudes des plantes glimpantes*. Publisher, C. Reinwald. Paris. 1890.

FABRE J.H., 1855 - *De la nature des vrilles des Cucurbitacée*. - Bull. Soc. Bot. France, 2: 512-518.

GANDY M., 2010 - *The ecological facades of Patrick Blanc*. - Architect. Design, 80: 28-33.

GERRATH J.M., GUTHRIE T.B., ZITNAK T.A., POSLUSZNY U., 2008 - *Development of the axillary bud complex in* Echinocystis lobata *(Cucurbitaceae): Interpreting the cucurbitaceous tendril*. - Am. J. Bot., 95: 773-781.

GORDON D.R., THOMAS K.P., 1997 - *Strangers in paradise. Impact and management of nonindigenous species in Florida. Chapter 2. Florida's invasion by nonindigenous plants: History, screening, and regulation*. - Island Press, Washington DC, USA, pp. 21-37.

GUANTER L., ALONSO L., GÓMEZ-CHOVA L., MERONI M., PREUSKER R., FISCHER J., MORENO J., 2010 - *Developments for vegetation fluorescence retrieval from spaceborne high-resolution spectrometry in the O_2-A and O_2-B absorption bands*. - J. Geophys. Res., 115: D19303. doi:10.1029/2009JD013716.

HUNTER A.M., WILLIAMS N.S.G., RAYNER J.P., AYE L., HES D., LIVESLEY S.J., 2014 - *Quantifying the thermal performance of green façades: A critical review*. - Ecol. Engin., 63: 102-113.

KADONO T., TRAN D., ERRAKHI R., HIRAMATSU T., MEIMOUN P., BRIAND J., IWAYA-INOUE M., KAWANO T., BOUTEAU F., 2010 - *Increased anion channel activity is an unavoidable event in ozone-induced programmed cell death*. - PLoS ONE 5: e13373.

KADONO T., YAMAGUCHI Y., FURUICHI T., HIRONO M., GARREC J.-P., KAWANO T., 2006 - *Ozone-induced cell death mediated with oxidative and calcium signaling pathways in tobacco Bel-W3 and Bel-B cell suspension cultures*. - Plant Signal. Behav., 1: 312-322.

KAWANO T., 2015 - *Pteridophyta as active components in the local ecosystems in the gardening, agricultural and horticultural sceneries in Japan*. - Adv. Hort. Sci., 29: 41-47

KAWANO T., ADACHI M., KURATA H., AZUMA R., SHIMOKAWA K., 1999 - *Calcium-dependent catabolism of phaeophorbide a in tomato fruit*. - J. Japan. Soc. Hort. Sci., 68: 810-816.

KAWANO T., KAWANO A., KAWANO M., 2012 - *Simplified exercise for mechanical repositioning of growing runners of forcing-cultured cucumber plants against the supporting tendril tensile strength*. - Environ. Control Biol., 50: 415-423.

KELLER R.C., 2013 - *Place matters: Mortality, space, and urban form in the 2003 Paris heat wave disaster*. - French Historical Studies, 36: 299-330.

LIN C., KADONO T., SUZUKI T., YOSHIZUKA K., FURUICHI T., YOKAWA K., KAWANO T., 2006 - *Mechanism for temperature-shift-responsive acute Ca^{2+} uptake in suspension-cultured tobacco and rice cells*. - Cryobio. Cryotechnol., 52: 83-89.

MAZZONI M., MERONI M., FORTUNATO C., COLOMBO R., VERHOEF W., 2012 - *Retrieval of maize canopy fluorescence and reflectance by spectral fitting in the O_2-A absorption band*. - Remote Sens. Environ., 124: 72-82.

MERONI M., ROSSINI M., GUANTER L., ALONSO L., RASCHER U., COLOMBO R., MORENO J., 2009 - *Remote sensing of solar-induced chlorophyll fluorescence: Review of methods and applications*. - Remote Sens. Environ., 113: 2037-2051.

MONSI M., SAEKI T., 2005 - *On the factor light in plant communities and its importance for matter production*. - Ann. Bot., 95: 549-567.

NAGASAWA K., IWASE J., COMPARINI D., KAWANO T., 2015 - *Empirical and simulative evaluations of white fluorescence-type light emitting diodes as algal growing light sources based on the photosynthetic oxygen evolution by* Synechocystis *spp. PCC6803*. - Environ. Cont. Biol., 53: 169-173.

OKAMOTO Y., HARAGUCHI A., SUZUKI T., KAWANO T., 2016 - *New discussion on Boysen-Jensen's photosynthetic response curves under plant canopy and proposal of practical equations for monitoring and management of canopy photosynthesis*. - Environ. Control Biol., 4: 7-16.

OKEIL A., 2010 - *A holistic approach to energy efficient building forms*. - Energy Build., 42: 1437-1444.

PÉREZ G., COMA J., MARTORELL I., CABEZA L.F., 2014 - *Vertical greenery systems (VGS) for energy saving in buildings: a review*. - Renew. Sustain. Energy Rev., 39: 139-165.

PLATT T., JASBY A.D., 1976 - *The relationship between photosynthesis and light for natural assemblages of coastal marine phytoplankton*. - J. Phycol., 12: 421-430.

SAFIKHANI T., ABDULLAH A.M., OSSEN D.R., BAHARVAND M., 2014 - *A review of energy characteristic of vertical greenery systems*. - Renew. Sustain. Energy Rev., 40: 450-462.

SCHNEIDER H., SCHUETTPELZ E., PRYER K.M., CRANFILL R., MAGALLÓN S., LUPIA R., 2004 - *Ferns diversified in the shadow of angiosperms*. - Nature, 428: 553-557.

TACHIBANA D., NAOKI S., MAKI T., SATO Y., KIKUCHI S., IMAI K., 2011 - *Important issues in green wall planning necessary to create the fine greening of urban areas*. - AIJ J. Technol. Design, 17: 699-702.

TAKAYAMA N., KAWAMURA K., YAMAMOTO H., NOBORI S., TOMINAGA Y., 2014 - *Quantitative assessment of plant water consumption in the summer after creating a green curtain by using ivy morning glory on a south-*

facing wall. - J. Agric. Meteorol., 70: 55-67.

TRAN D., KADONO T., MOLAS M.L., ERRAKHI R., BRIAND J., BILIGUI B., KAWANO T., BOUTEAU F., 2013 - *A role for oxalic acid generation in ozone-induced signalization in* Arabidopsi*s cells*. - Plant Cell Environ., 36: 569-578.

YAMASAKI M., MIZUTANI A., OHSAWA T., 2009 - *Cooling load reduction effect of green roof and green wall in the case of building with thermal thin wall*. - AIJ J. Technol. Design, 15: 155-158.

YUKIHIRO M., HIRAMATSU T., BOUTEAU F., KADONO T., KAWANO T., 2012 - *Peroxyacetyl nitrate-induced oxidative and calcium signaling events leading to cell death*

Adv. Hort. Sci., 2016 30(2): 69-74 DOI: 10.13128/ahs-19131

Some characteristics of tuberose as affected by pre-harvest application of calcium chloride and gibberellic acid

S.N. Mortazavi (*), **F. Bagheri, M. Bahadoran**
Collage of Agriculture, Department of Horticulture, University of Zanjan, Zanjan, Iran.

Key words: bulbous plant, cut flower, ethylene, longevity, postharvest.

Abstract: In the present study the effect of gibberellic acid (GA_3) and calcium chloride ($CaCl_2$) sprays (0, 150, 300 and 450 ml L^{-1}), applied 25 and 15 days before harvesting, on physiological and morphological characteristics of tuberose 'Pearl Double' was studied. Cut flowers were harvested and transported to the laboratory where they were placed in distilled water. The experiment considered some parameters for evaluation, such as relative water content of leaves and petals, water intake, percentage of open florets, electrolyte leakage, ethylene production, chlorophyll and carotenoid content. Results indicate that the best treatment was the combination of 150 ml L^{-1} $CaCl_2$ and 450 ml L^{-1} GA_3 for most of parameters.

1. Introduction

Tuberose (*Polianthes tuberose* L.) is one of the most important cut flowers in tropical and subtropical areas and as cut flowers they are among the most important for flower bouquets, baskets and wreaths (Kendirli and Cakmak, 2007). The florets have a very sweet fragrance and are widely cultivated in India and France as a source of essential oils for the perfume industry. *Polianthes* is also a common garden plant in the spring and it flowers during the summer and early autumn (De Hertogh and Le Nard, 1993). Two major cultivars, white-colored 'Single' and 'Double', are for commercial production (Shen *et al*., 2003). In tuberose, fewer than 50% of the buds normally open after harvest and florets and buds usually drop off after a few days in vase. Postharvest performance is worse in tuberose which has been shipped to distant markets (Waithaka *et al*., 2001). Keeping quality of spikes is only three days for florets and vase life of flowers is only a few days. Since it has delicate flowers and sellers and customers are keen to extend its vase life, it is necessary to improve its postharvest life (Anjum *et al*., 2001). Senescence in cut flowers is affected by three main parameters: the water balance, the supply of carbohydrates, and susceptibility to ethylene (Cortes *et al*., 2011). Treatment with gibberellic acid has also been shown to enhance postharvest life and quality of gerbera cut flowers. Using GA_3 at different concentrations improved membrane stability index, leading to better flower vase life of gerbera cut flowers (Emongor, 2004). Similar effects on membrane stability index have been reported in gladiolus with BA and GA_3 (Singh *et al*., 2008). GA_3 treatment of *sandersonia* flowers delays the senescence-associated increase in protease activity, which by implication delays the breakdown of senescence-associated proteins (Eason, 2002). Calcium (Ca) is an important element which is found in 3% of the earth's crust. It is essential to living organisms and to plant growth and development. Some of these benefits include stronger cell walls, increased postharvest life of flowering plants, and increased disease resistance (Robichaux, 2005). Calcium spray increased the life of rose petals by increasing the relative water content (RWC), maintaining turgidity of leaf cells, avoiding cell wall deformation, and decreasing electrolyte leakage from cells of cut flowers by increasing cell

(*) Corresponding author: mortazavi46@gmail.com
Received for publication 20 January 2016
Accepted for publication 1 April 2016

wall integrity and stability (Mortazavi *et al*., 2007). Floret abscission and short vase life of tuberose are common problems, thus the aim of this study was to determine the effect of foliar application of gibberellic acid and $CaCl_2$ treatments on some morpho-physical parameters that affect the vase life of cut tuberose.

2. Materials and Methods

This experiment was conducted in summer 2012 at the commercial tuberose (double cultivar) farm located in Tarom county of Zanjan province, Iran; latitude 36° 57′ North and longitude 48° 54′ East. About 500 m^2 of this farm is used for experimental treatments. The plants received as foliar spray $CaCl_2$ (from Merck), with molar mass 147.01 g M^{-1}, and gibberellic acid (from Merck), with molar mass 346.37 g M^{-1}, 15 and 25 days before harvest of flowers. $CaCl_2$ (0, 150, 300 and 450 ml L^{-1}) (0, 1, 2 and 3 mM) and gibberellic acid (0, 150, 300 and 450 ml L^{-1}) (0, 0.4, 0.8 and 1.2 mM) were used. Before application, leaves of plants sprayed with distill water, one hour after distil water spired, $CaCl_2$ treatment (four liters) and two hours after that gibberellic acid treatments (four liters) were done until runoff. When most of the two or three lowest inflorescence florets opened, flowers were harvested and immediately transferred to the postharvest laboratory at the Department of Horticulture, Faculty of Agriculture, Zanjan University. Flowering stems then were cut to 65 cm, weighted (fresh weight) and placed in 400 ml distilled water. All experiments were performed in a postharvest room equipped with a controlled environment maintained at 22±1°C, 45±5% relative humidity and light intensity for 12 h/day by cool-white fluorescent lamps.

The following parameters were measured: fresh weight of flowers and numbers of open florets of inflorescences before being placed in distilled water, rate of opening and abscission of florets every three days, water uptake every four days, relative water content (RWC) of leaves and petals, cell membrane injury (CMI) of petals, chlorophyll and carotenoid content of plant tissue, and ethylene production of flowers. Vase life longevity was recorded when at least four open florets on the inflorescence were present.

To measure the RWC, 1 g of petal tissue (fresh weight, F.W.) was immersed in distilled water for 24 h and weighed again (turgid weight, T.W.), then dried at 80°C inside an oven for 48 h (dry weight, D.W.). RWC was calculated using the following equation (Turner, 1981):

$$RWC= (F.W.-D.W.)/(T.W.-D.W.)\times100$$

Cell membrane injury was measured following the Isacc and Urban (1995) method; after seeing the first flowers of inflorescence, measurements were done. One g of petal tissue of top open bud flowers weighted of each plant and washed with distilled water, then were immerged in glass container containing 10 ml of distilled water and were placed inside a benmary (Gemmy Ind. Corp., Taiwan) at 30°C for 60 min and EC was measured (EC_1), then were placed inside a autoclave at 120°C for 20 min, and EC was measured again (EC_2), and CMI was calculated using the following equation:

$$CMI =1-(EC_1/EC_2) \times100$$

Ethylene production of flowers 3, 6, 12 and 24 h after placement in 500 ml distilled water at 23°C was measured by an Ethylene biosynthesis bioconservation device model ICNA56. Treated flowering inflorescence was in distilled water in the laboratory with the same conditions inside the container and packaging (Volume 2.5 liters) were (Almost all the flowers were the same size). Flowers in the tank without meeting with any special hole for injection or sampling were used and thus ethylene concentrations were prepared by flowers. Chlorophyll and carotenoid content were determined by spectrophotometeric method (Arnon, 1949).

The present study was carried out in a complete randomized design with factorial arrangements including 16 treatments and three replications. Data were analyzed by MSTATC software and means were compared using LSD test at 5% level.

3. Results and Discussion

Effect of $CaCl_2$ and gibberellic acid on relative water content of tuberose leaves (RWCL) and florets (RWCF)

Results indicated that interaction of 450 ml L^{-1} gibberellic acid and 150 ml L^{-1} $CaCl_2$ had a significant effect at 5% level on the relative water content of florets compared with the control (Table 1). Our results agree with those of Cortes *et al*. (2011) on *Rosa x hybrid* cv. Grand Gala, Mortazavi *et al*. (2007) on *Rosa* x *hybrida* cv. Iliona and Abdolmaleki *et al*. (2015) on cut rose cv. Dolce Vita. Gibberellic acid reducing water loss via transpiration, increase water

uptake in plant tissues (Emongor, 2004). Calcium spraying increased the life of petals by increasing the RWC, maintaining leaf cell turgidity and avoiding cell wall deformation. Gibberellic acid application together with $CaCl_2$ might increase the efficiency of Ca use in plants.

Effect of $CaCl_2$ and gibberellic acid on water uptake of tuberose (WU)

Results indicated that 450 ml L^{-1} CaC_2 + 450 ml L^{-1} gibberellic acid gave maximum water uptake (Table 1). These results agree with results of Vijaya *et al*. (1999) on cut tuberose, Cortes and *et al*. (2011) regarding *Rosa hybrid* cv. Grand Gala, Dansheng (2003) on cut rose, and Sosa Nan (2007) on sunflower. Calcium interacts with polygalacturonic acid (PGA) groups, forming a structure known as an "Egg box", which causes the contraction of pectins in the pit borders, increasing the diameter and, consequently, water flow (Cortes *et al*., 2011). The increased reducing sugars in flower heads and stems of gerbera cut flowers may increase the osmotic potential of flower head and stem, thus improving their ability to absorb water and maintain their turgidity (Emongor, 2004). Water uptake improved in tuberose by foliar application of gibberellic acid and $CaCl_2$.

Effect of $CaCl_2$ and Gibberellic acid on opening florets of tuberose (OP)

Interaction of 150 ml L^{-1} $CaCl_2$ and 450 ml L^{-1} gibberellic acid yielded the maximum amount of opening florets (Table 1). This result concurred with Halevy *et al*. (2001) who reported that $CaCl_2$ treatment promoted bud opening and delayed senescence in rose cut flowers. The treated flowers stayed turgid and continued their initial postharvest growth for longer periods. Treatment with GA_3 is useful for improving the vase life of cut *N. tazetta* var. *chinensis* flowers (Ichimura and Goto, 2000). Spraying with 200 ml L^{-1} GA_3 increased plant height, early flowering, spike length and number, rachis length, flower weight and length, and total flower yield in a study carried out by Bharathi and Kumar (2009), which was the same as our results. Gibberellins increase hydrolysis of starch, fructans and sucrose into glucose and fructose which are utilized by the flowers for disc floret opening (Emongor, 2004).

Effect of $CaCl_2$ and Gibberellic acid on floret abscission of tuberose (AF)

The lowest percentage of floret abscission (9.83%) was found at 300 ml L^{-1} gibberellic acid while the highest (23.45%) was found at 150 ml L^{-1} $CaCl_2$ (Table 1). Our results were similar to those of Uthairatanakij (2005) who reported $CaCl_2$ significantly reduced the postharvest dropping of orchid buds flower compared to control. Calcium treatment probably increases the strength of cell walls. The abscission of leaves, flowers, and fruits is presumed to be brought about through the weakening of the cell walls in the abscission zone. This weakening may have two components: a solubilizing of the cell wall cementing sub-

Table 1 - Means of interaction of CaC_2 and gibberellic acid on relative water content (RWC) of leaves and florets (%), water uptake (mL), opening and abscission of florets, number of florets, fresh weight of flower (g), vase life (day), chlorophyll content a, b and total chlorophyll content (mg), and carotenoid content (µg) of cut tuberose (*Polianthes tuberosa* L.) after first flower opening of inflorescence

$CaCl_2$ (ml L^{-1})	Gibberellic acid (ml L^{-1})	RWC of least (%)	RWC of floret (%)	Water uptake (ml)	Opening florets (%)	Abscission of florets (%)	Number of florets	Fresh weight of flower (g)	Vase life longevity (day)	Chlorophyll content b (mg)	Total chlorophyll content (mg)	Carotenoid content (µg)
0	0	82.48 g*	76.12 de	111.7 bc	20.77 g	12.24 fgh	35.33 ab	78.46 ef	8.50 h	0.04600 bcdef	0.03267 efg	0.01300 g
	150	92.67 b	70.54 fg	105.6 bcdef	29.43 def	11.14 fgh	32.67 bcd	78.28 ef	9.30 g	0.03767 cdef	0.04867 cde	0.02333 cd
	300	87.51 def	76.66 de	98.89 f	30.15 def	09.83 h	36.33 a	88.27 ab	9.77 def	0.05400 bcdef	0.03567 defg	0.01300 g
	450	94.91 a	91.58 b	112.2 b	37.66 ab	10.70 gh	32.67 bcd	82.46 cde	11.37 b	0.07333 bc	0.01037 a	0.01900 de
150	0	79.39 h	85.82 c	110.6 bcd	31.02 def	23.45 a	33.00 bcd	78.64 def	10.80 c	0.03133 def	0.01733 g	0.01000 h
	150	96.66 a	70.73 fg	103.9 cdef	36.27 abc	18.75 bc	34.00 abc	79.74 def	9.43 fg	0.02100 f	0.02367 fg	0.01267 g
	300	82.27 g	73.75 ef	110.6 bcd	34.50 bcd	16.70 cde	32.00 cd	88.75 a	9.57 efg	0.04600 bcdef	0.03500 defg	0.01833 e
	450	73.99 i	96.10 a	113.3 b	40.74 a	13.72 efg	35.33 ab	82.01 cde	11.83 a	0.16260 a	0.05933 c	0.02233 ab
300	0	86.55 ef	79.45 d	103.9 cdef	30.74 def	20.92 ab	35.00 ab	80.92 def	10.13 d	0.06767 bcd	0.03333 efg	0.01067 h
	150	85.58 f	77.06 de	100.0 ef	27.94 ef	20.22 b	34.67 ab	83.57 bcd	9.90 de	0.03300 def	0.04700 cdef	0.01967 de
	300	89.02 cd	73.29 ef	110.0 bcd	29.82 def	14.23 def	36.00 a	77.50 ef	9.53 efg	0.04233 bcdef	0.05700 cd	0.02133 bc
	450	90.27 c	86.30 c	113.3 b	32.40 cde	13.75 efg	31.00 d	77.77 ef	11.00 bc	0.08000 b	0.08100 b	0.02367 a
450	0	86.48 ef	74.44 ef	103.3 def	29.83 def	17.16 cd	31.00 d	79.24 def	9.47 fg	0.06700 bcde	0.01700 g	0.01333 g
	150	88.33 cde	68.17 g	106.1 bcdef	26.56 f	16.61 cde	35.00 ab	75.88 f	8.70 h	0.02933 ef	0.02533 efg	0.01300 g
	300	87.68 def	71.28 fg	107.8 bcde	30.32 def	12.33 fgh	36.33 a	86.15 abc	9.30 g	0.04333 bcdef	0.03167 efg	0.01667 f
	450	89.49 cd	90.72 b	123.3 a	40.70 a	11.54 fgh	33.67 abc	86.35 abc	11.30 b	0.04833 bcdef	0.03433 defg	0.01633 f

Means in the same column followed by the same letter are not significantly different using LSD test level 5%.

stances, and a hydrolysis of the structural components of the wall. A major part of the cementing properties of walls is presumed to be through the binding of pectic substances by double salt formation with Ca ions (Poovaiah and Leopold, 1973). Gibberellic acid delays flower abscission by decreasing the amount of dry matter (Khan and Chaudhry, 2006).

Effect of $CaCl_2$ and gibberellic acid on number of tuberose florets (NF)

Our results indicate that the interaction of 300 ml L^{-1} gibberellic acid and 450 ml L^{-1} $CaCl_2$ yielded the maximum number of florets, while the interaction of 300 ml L^{-1} $CaCl_2$ and 450 ml L^{-1} gibberllic acid gave the fewest (Table 1). Our results were similar to those found by Parmar *et al*. (2009) on spider lily, Mukhopadhyay and Bankar (1983) on tuberose, and Singh *et al.* (1991) on African marigold (*Tagetes erecta* L.), who reported an increase in number of florets because of role of gibberellic acid on cell elongation and division.

Effect of $CaCl_2$ and gibberellic acid on fresh weight of tuberose florets (FW)

Our results show that the interaction of 150 ml L^{-1} $CaCl_2$ and 300 ml L^{-1} gibberellic acid led to the maximum fresh weight of florets (Table 1), findings which are in agreement with those of Cortes *et al*. (2011) and Dansheng (2003) regarding rose, Sosa Nan (2007) working on sunflower, and Vijaya *et al*. (1999) tuberose. The effect of gibberellic acid on the fresh weight of florets may be a result of its role on increasing cell division (Arun *et al*., 2000).

Effect of $CaCl_2$ and gibberellic acid on vase life of tuberose (VL)

Application of 150 ml L^{-1} $CaCl_2$ and 450 ml L^{-1} gibberellic acid had significant effect on vase life parameter of tuberose (Table 1). Cortes *et al*. (2011) found that using $CaCl_2$ in the vase water of rose cv. Grand Gala gave maximum fresh weight. Loss of cell membrane integrity is characteristic of senescence in plants. Calcium protects the membranes from lipid degradation probably through several mechanisms. Calcium can stabilize the plasma lemma by binding to the negatively charged head groups of PL, which become less prone to degradation by lipolytic enzymes (Cheour *et al*., 1992). Analogous results were found by Uthairatanakij *et al*. (2005) regarding spraying $CaCl_2$ on Dendrobium orchid, and by Robichaux (2005) regarding the effect of calcium chloride, sulfate or nitrate spray on the vase life of rose and poinsettia. Gibberellic acid increases water absorption and relative water content, resulting in vase life longevity. Our results also agree with the findings of Su *et al*. (2001) on tuberose and Emongor (2004) on gerbera flower.

Effect of $CaCl_2$ and gibberellic acid on cell membrane injury of tuberose (CMI)

Results reveal that the minimum cell membrane injury was at 300 and 450 ml L^{-1} gibberellic acid and with interaction of 450 ml L^{-1} $CaCl_2$ and 450 ml L^{-1} gibberellic acid (Fig. 1). The enhancing effect of the application of Ca can be explained on the basis of its role in cell membrane structure. It may be noted that Ca stabilizes cell membranes by connecting various proteins and lipids at membrane surfaces, influences the pH of cells and prevents solute leakage from cytoplasm and increase shoot elongation (Al-Whaibi *et al*., 2010). If low Ca makes the membrane more permeable, it should follow that elevated concentrations make the membrane less permeable (Hepler, 2005).

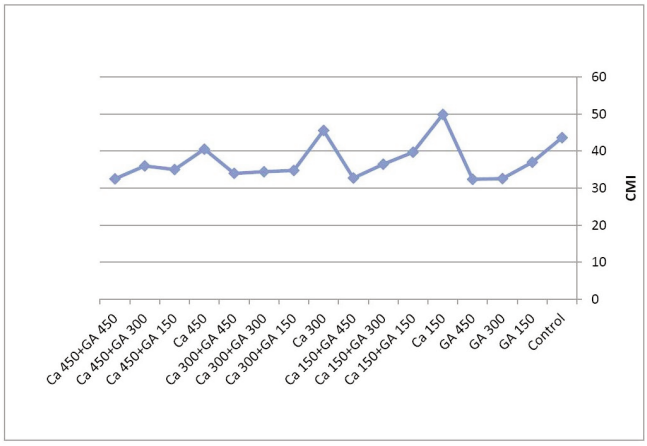

Fig. 1 - Means of interaction of $CaCl_2$ and gibberellic acid on cell membrane injury (CMI) of leaves of cut tuberose.

Effect of $CaCl_2$ and gibberellic acid on ethylene production of tuberose

The lowest values of ethylene production after 3 h were found in 450 ml L^{-1} gibberellic acid and in of 300 ml L^{-1} $CaCl_2$ + 450 ml L^{-1} gibberellic acid; these values were significant compared to control and most of the other treatments. Ethylene production after 6 h of treatment with 300 ml L^{-1} gibberellic acid and after 12 h with 300 ml L^{-1} $CaCl_2$ combined with 150, 300 and 450 ml L^{-1} gibberellic acid, respecitvely, showed the lowest values. After 24 h of treatment, the lowest levels were found with 450 ml L^{-1} $CaCl_2$ + 150, 300

and 450 ml L^{-1} gibberellic acid. Highest ethylene production was found at 3 and 12 hours with treatments of 150 ml L^{-1} $CaCl_2$ + 150 ml L^{-1} gibberellic acid, at 6 hours with treatment 150 ml L^{-1} $CaCl_2$, and 150 ml L^{-1} $CaCl_2$ + 150 ml L^{-1} gibberellic acid at 24 hours (Fig. 2). Pre-harvest treatment of $CaCl_2$ decreased ethylene production, which agrees with the results of Uthairatanakij *et al*. (2005) on orchid and Cortes *et al*. (2011) on rose. Calcium decreased activity and effect of ethylene on cell walls and affected senescence with inhibition of cell membrane injury. The application of calcium spraying gave the result to improve the strength of plant cell wall and delayed the senescence processes by inhibition of ethylene synthesis. In addition, calcium ions also seem to affect ethylene action on cell membranes by inhibiting ion leakage and reducing the effect of ethylene on senescence (Asfanani *et al*., 2008). Gibberellic acid treatments decreased ethylene production compared with the control, findings that agree with the results of Ichimura and Goto (2000) on *Narcissus* and Lers *et al.* (1998) on parsley. Inhibition of ethylene production by gibberellic acid is related to the ethylene production enzyme. Gibberellic acid inhibited ACC enzyme activity and resulted in inhibition of ethylene production (Ben-Arie and Ferguson, 1991).

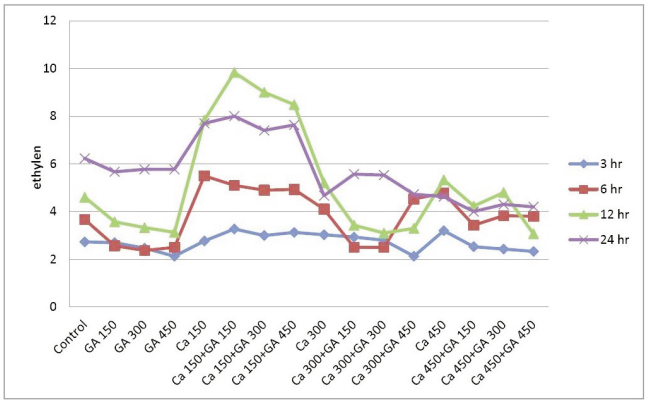

Fig. 2 - Means of interaction of $CaCl_2$ and gibberellic acid on ethylene production after 1, 2, 3 and 4 hours (nL g^{-1} h^{-1}) of cut tuberose.

Effect of $CaCl_2$ and gibberellic acid on chlorophyll and carotenoid of tuberose

Results of this study indicate that the interaction effect of 300 ml L^{-1} $CaCl_2$ and 450 ml L^{-1} gibberellic acid on chlorophyll a content was significant. Also 150 ml L^{-1} $CaCl_2$ and 450 ml L^{-1} gibberellic acid had a significant effect on chlorophyll b content. The lowest chlorophyll b content was found in treatment with 150 ml L^{-1} of gibberellic acid and $CaCl_2$. Total chlorophyll content was highest with 450 ml L^{-1} gibberellic acid. The maximum carotenoid content was recorded at 300 ml L^{-1} $CaCl_2$ and 450 ml L^{-1} gibberellic acid, with the lowest level was found at 150 and 300 ml L^{-1} $CaCl_2$ (Table 1). Calcium treatment caused the leaves to grow greener in color and the stems to grow more (Asfanani *et al.,* 2008). According to the results of Aharoni (1989), Lers *et al*. (1998), Ichimura and Goto (2000), Ferrante *et al*. (2002), and Khan and Chaudhry (2006), yellowing of leaves destruction and decrease of chlorophyll can be delayed by gibberellic acid treatments. Our results reveal that using $CaCl_2$ and gibberellic acid together was better at increasing chlorophyll a, b, and total content than using each one alone. The same results were observed for the content of chlorophyll a, b, and total of faba bean (*Vicia faba* L.) cv. Taraby (Al-Whaibi *et al.,* 2010).

4. Conclusions

Pre-harvest treatments with $CaCl_2$ and gibberellic acid improved some morphological and physiological parameters as well as vase life of cut tuberose. Combining $CaCl_2$ and gibberellic acid had significant effects on some parameters. Floret abscission and low vase life of tuberose are common problems that can be improved by using $CaCl_2$ and gibberellic acid before harvest.

Acknowledgements

The authors of this research with to thank the Department of Horticulture of Zanjan University for its support of this experiment.

References

ABDOLMALEKI M., KHOSH-KHUI M., ESHGHI S., RAMEZANIAN A., 2015 - *Improvement in vase life of cut rose cv. "Dolce Vita" by preharvest foliar application of calcium chloride and salicylic acid*. - Int. J. Hort. Sci. Techn., 2: 55-66.

AHARONI N., 1989 - *Interrelationship between ethylene and plant growth regulator in the senescence of lettuce leaf discs*. - Plant Growth Regulation, 8: 307-317.

AL-WHAIBI M.H., SIDDIQUI M.H., AL-AMRI A., BASALAH M.O., 2010 - *Performance of faba bean under calcium and gibberellic acid application*. - Int. J. P. Dev. Bio., 4: 60-63.

ANJUM M.A., NAVEED F., SHAKEEL F., AMIN S., 2001 - *Effect of some chemicals on keeping quality and vaselife of tuberose (*Polianthes tuberosa *L.) cut flow-*

ers. - J. of Res. (Sci.), 12: 01-07.

ARNON D.I., 1949 - *Copper enzymes in isolated chloroplasts. Polyphenoloxidase in* Beta vulgaris. - Plant Physiol., 24(1): 1-15.

ARUN D.S., ASHOK A.D., RENGASWAMY P., 2000 - *Effect of some growth regulating chemicals on growth and flowering of rose cv. First red under greenhouse conditions*. - Ornamental Horticulture, 3: 51-53.

ASFANANI M., DAVARYNEJAD G.H., TEHRANIFAR A., 2008 - *Effects of pre-harvest calcium fertilization on vase life of rose cut flowers cv. Alexander*. - Acta Horticulturae, 804: 217-221.

BEN-ARIE R., FERGUSON I.B., 1991 - *Ethylene production by growing and senescencing pear fruit cell suspension in response to gibberellins*. - Plant Physiology, 95: 943-947.

BHARATHI T.U., KUMAR S., 2009 - *Effect of growth regulators on growth and flowering parameters of tuberose cv. Suvasini*. - Adv. in P. Sci., 22: 127-128.

CHEOUR F., ARUL J., MAKHLOUF J., WILLEMOT C., 1992 - *Delay of membrane lipid degradation by calcium treatment during cabbage leaf senescence.* - Plant Physiology, 100: 1656-1660.

CORTES M.H., FRIAS A.A., MORENO S.G., PINA M.M., DE LA CRUZ GUZMAN G.H., SANDOVAL S.G., 2011 - *The effects of calcium on postharvest water status and vase life of rosa hybrida cv. Grand Gala.* - Int. J. Ag. & Biol., 13: 233-238.

DANSHENG C., 2003 - *Extension of Ca(2+) on vaselife of cut rosa*. - Guangxi Academy of Sciences, 3: 122-124.

DE HERTOGH A., LE NARD M., 1993 - *The physiology of flower bulbs*. - Elsevier Science Publ., pp. 811.

EASON J.R., 2002 - Sandersonia aurantiaca: *an evaluation of postharvest pulsing solutions to maximise cut flower quality.* - N.Z. J. Crop Hort. Sci., 30: 273-279.

EMONGOR V.E., 2004 - *Effects of gibberellic acid on postharvest quality and vase life of gerbera cut flowers (*Gerbera jamesonii*)*. - J. Acta Agrobotanica, 3: 191-195.

FERRANTE A., HUNTER D.A., HACKETT W.P., REID M.S., 2002 - *Thidiazuron, a potent inhibitor of leaf senescence in* Alstroemeria. - Post. Bio. & Tec., 25: 333-338.

HALEVY A.H., TORRE S., BOROCHOV A., PORAT R., PHILOSOPH-HADAS S., MEIR S., FRIEDMAN H., 2001 - *Calcium in regulation of postharvest life of flowers*. - Acta Horticulturae, 543: 345-351.

HEPLER P.K., 2005 - *Calcium: A central regulator of plant growth and developent*. - Plant Cell, 17(8): 2142-2155.

ICHIMURA K., GOTO R., 2000 - *Effect of gibberellin A_3 on leaf yellowing and vase life of cut* Narcissus tazetta *var.* chinensis *flowers*. - J. Jap. Soc. Hort. Sci., 69: 423-427.

ISACC S., URBAN L., 1995 - *Effect of electrical conductivity and supply rate of the nutrient on stomatal conductance of rose plant leaves.* - Acta Horticulturae, 424: 131-134.

KENDIRLI B., CAKMAK B., 2007 - *Economics of cut flower in greenhouse: case study from Turkey.* - Agri. J., 2: 499-502.

KHAN A.S., CHAUDHRY N.Y., 2006 - *GA_3 improves flower yield in some cucurbits treated with lead and mercury.* - Afr. J. Bio., 5: 149-153.

LERS A., JIANG W., LOMANIEC E., AHARONI N., 1998 - *Gibberellic acid and CO_2 additive effect in retarding postharvest senescence of parsley*. - J. F. Sci., 63: 66-68.

MORTAZAVI N., NADERI R., KHLIGHI A., BABALAR M., ALLIZADEH H., 2007 - *The effect of cytokin and calcium on cut flowers quality in rose (*Rosa hybrida *L.) cv. Iliona.* - J. F. Agr. and Environ., 5: 311-313.

MUKHOPADHYAY A., BANKAR G.J., 1983 - *Regulation of growth and flowering in* Polianthes tuberosa *L. with gibberellic acid and Ethrel spray*. - Sci. Hort., 19: 149-152.

PARMAR A.B., PATEL H.C., CHAVDA J.C., PARMAR J.R., 2009 - *Effect of plant growth regulators on growth and flowering of spider lily (*Hymenocallis speciosa *L.).* - A. J. Hort., 4: 170-172.

POOVAIAH B.W., LEOPOLD A.C., 1973 - *Inhibition of abscission by calcium*. - Plant. Physiol., 51: 848-851.

ROBICHAUX M.B., 2005 - *The effect of calcium or silicon on potted miniature roses or poinsettias.* - M. Sc. Thesis. B.S., University of Louisiana at Lafayette, USA.

SHEN T.M., HUANG K.L., SHEN R.S., DU B.S., 2003 - *Breeding of dwarf tuberose (*Polianthes tuberosa *L.).* - Acta Horticulturae, 624: 73-76.

SINGH A., KUMAR J., KUMAR P., 2008 - *Effect of plant growth regulators and sucrose on post harvest physiology, membrane stability and vase life of cut spikes of Gladiolus*. - J. Plant Growth Regul., 55: 221-229.

SINGH M.P., SINGH R.P., SINGH G.N., 1991 - *Effect of GA_3 and ethrel on growth and flowering of African marigold (*Tagetes erecta *L.)*. - Progr. Hort., 24: 92-95.

SOSA NAN S.J., 2007 - *Effects of pre- and postharvest calcium supplementation on longevity of sunflower (*Helianthus annuus *cv. Superior Sunset)*. - M.Sc. thesis, Lousiana State University, USA.

SU W.R., HUANG K.L., CHANG P.S., CHEN W.S., 2001 - *Improvement of postharvest vase life and flower bud opening in* Polianthes tuberose *using gibberellic acid and sucrose.* - A. J. Exp. Agr., 41: 1227-1230.

TURNER N.C., 1981 - *Techniques and experiment approaches for the measurement of plant water status.* - Plant and Soil, 58: 339-366.

UTHAIRATANAKIJ A., JANSRI S., JITAREERAT P., KANLAYANARAT S., 2005 - *Effect of preharvest calciums spraying on gamma irradiate inflorescences of 'Walter Oumae 4N' Dendrobium.* - International Symposium "New Frontier of Irradiated food and Non-Food Products" 22-23 September, KMUTT, Bangkok, Thailand.

VIJAYA B., VENKATA V., RAO P., REDDY Y.N., 1999 - *Effect of minerals on post-harvest vase-life of cut tuberose (*Polianthes tuberosa *L.) cv. Double.* - Indian Journal of Horticulture, 56(4): 257-261.

WAITHAKA K., REID M.S., DODGE L.L., 2001 - *Cold storage and flower keeping quality of cut tuberose (*Polianthes tuberosa *L.).* - J. Hort. Sci. Biot., 76: 271-275.

Adv. Hort. Sci., 2016 30(2): 75-80 DOI: 10.13128/ahs-19132

Photo-oxidation of ethylene over mesoporous TiO_2/SiO_2 catalysts

A. Licciulli [1](*), R. Nisi [1], S. Pal [1], A. M. Laera [1], P. Creti [2], A. Chiechi [3]

[1] *Dipartimento di Ingegneria dell'Innovazione, Università di Salento, Via Arnesano, 73100 Lecce, Italy.*

[2] *CNR Istituto Microelettronica e Microsistemi, Sezione di Lecce, 73100 Lecce, Italy.*

[3] *Salente SRL, Via Monteroni 147, 73100 Lecce, Italy.*

Key words: gaseous phase photocatalysis, liquid-crystal template, modified storage environment, mixed oxides.

Abstract: Mesoporous TiO_2/SiO_2 catalysts were prepared in order to increase the post-harvest life of climacteric fruits and vegetables reducing ethylene concentration by photo-oxidation. TiO_2/SiO_2 powders were synthesized by sol-gel method using titanium isopropoxide $Ti(O^iPr)_4$ and tetraethoxysilane $Si(OC_2H_5)_4$ as source of metal oxides. Mesoporous SiO_2 framework was used as catalyst support of nanostructured TiO_2 to enhance the photocatalytic efficiency. Different TiO_2/SiO_2 molar ratios were prepared through sol-gel process. A liquid-crystal template route allowed to obtain the mesoporous silica structure, and contemporary TiO_2 insertion in the silica framework. The X-ray diffraction (XRD) analysis demonstrated that silica insertion in TiO_2 framework inhibits the anatase to rutile phase transformation at higher sintering temperature. The photocatalytic efficiency of the catalysts was measured by the photo-oxidation of ethylene gas under UV light irradiation. Complete photo-oxidation of ethylene was observed after 24 h of reaction time. Results show that the silica framework increases the surface area of the composites and make crystalline anatase phase more stable at higher temperature.

1. Introduction

Ethylene gas (C_2H_4) is the ripening hormone of several fruits and vegetables (Hussain *et al*., 2011; Keller *et al.*, 2013). Ethylene is used to promote the uniform ripening of immature fruits such as bananas, but in most cases it doesn't work as beneficial element both in food and horticultural industries. Few ppm of ethylene in the storage atmosphere induce very fast ripening in postharvest which causes undesirable product losses (Keller *et al*., 2013). Thus it is desired to remove or degrade ethylene from the storage environment in order to preserve the postharvest products and keep them fresh for a longer time period. The important role of ethylene as growth regulator of climacteric products was intensively investigated in the last 50 years and now the ripening process associated to high ethylene production is well known. Various methods to remove ethylene have been developed: ventilation, or controlled atmosphere, ethylene oxidation using potassium permanganate. The photocatalytic ethylene oxidation thus represents an innovative way to extend postharvest life of the climacteric fruits and vegetables (Park *et al*., 2009; Keller *et al*., 2013; Ye *et al*., 2013). Among the catalysts able to induce ethylene photo-oxidation, TiO_2 is promising and attractive due to low cost, non-toxicity and high efficiency. The photocatalytic properties of nanostructured TiO_2 have already been highlighted in several applications, such as photocatalytic degradation of both air and water organic pollutants, and self-cleaning surface protection (Pal *et al.*, 2014).

Anchoring TiO_2 nanocrystals on suitable mesoporous substrate brings relevant practical advantages. The immobilization on support materials slows or totally inhibits nanoparticles coarsening, preserving their higher surface area. Secondly, the substrate greatly improves handling, processing and recover of TiO_2 nanopowder. Mesoporous silica, as a catalyst support over nano-sized TiO_2, represent a good choice due to high specific surface area and improved thermal stability of the TiO_2 anatase crystalline phase. Some recent works also demonstrated higher photocatalytic activity of TiO_2/SiO_2 mixed oxide composites (Fu *et al*., 1996; Zhan *et al*., 2014).

(*) Corresponding author: antonio.licciulli@unisalento.it
Received for publication 16 February 2016
Accepted for publication 5 April 2016

The mesoporous SiO_2 is able to reduce the recombination of photogenerated electron-hole pair, while higher surface area increases the efficiency in the heterogeneous photocatalysis.

Over the past thirty years a successful synthetic strategy, named liquid-crystal template, leading to mesoporous materials has been developed allowing to finely tune the porosity (Corma *et al.*, 2006; Xiao *et al.*, 2008). This methodology involves supramolecular aggregates of ionic surfactants that act as templating agents, which are able to direct and control the crystallization of the inorganic nanoparticles (Beck *et al*., 1992). After the surfactant removal by thermal annealing, mesoporous solids with high surface area can be obtained. The morphological and structural properties of the products can be finely tuned by varying process parameters such as pH, temperature, reagent concentrations, surfactants and silica sources.

In this work, TiO_2/SiO_2 binary composites obtained through a liquid-crystal template methodology have been tested as catalyst in UV light assisted ethylene oxidation at room temperature for the application in modified atmosphere packaging of climacteric fruits and vegetables. A titania precursor was added in the reaction medium containing the surfactant and the silica source allowing the one-pot formation of TiO_2 and SiO_2. Structure and morphology of the obtained TiO_2/SiO_2 binary composites with several weight ratios were investigated by using Brunauer-Emmett-Teller (BET) surface area, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and Field emission scanning electron microscopy (FESEM) measurements.

2. Materials and Methods

Synthesis of TiO_2/SiO_2 binary oxides

All the reagents involved in the catalyst synthesis were purchased from Sigma Aldrich and were used without any further modification. The starting colloidal solution was obtained by dissolving 28 g of Pluronic F127 (EO_{106} PO_{70} EO_{106}) in 150 g of distilled water through vigorous stirring. The complete polymer dissolution was reached after addition of a solution of 2M HCl (600 g). Then the required amount of tetraethoxysilane [$Si(OC_2H_5)_4$, TEOS] was added drop wise and continued to stirring for an hour. At this stage, titanium tetraisopropoxide [$Ti(O^iPr)_4$, TTIP] was slowly added and stirred for overnight. The obtained reaction mixture was heated at 100°C for 24 h and after that, a xerogel was obtained by centrifuging. The xerogel was then dried and calcined at 550°C for 6 h with the heating and cooling rate of 1°C/min to remove the organic content. Following this procedure, binary oxide with the TiO_2/SiO_2 weight ratio of 9/1, 8/2, and 7/3 were prepared. For comparison, a sample of pure TiO_2 was also prepared with the same experimental condition. Throughout the text, these samples are represented as TSBA-10, TSBA-91, TSBA-82, and TSBA-73, respectively.

Characterization

Crystalline phases of the TiO_2/SiO_2 powder samples were characterized by X-ray diffraction (XRD) performed on a Rigaku Ultima X-ray diffratometer using Cu*K*α radiation (λ=1.5406 Å) operating at 40 kV/30 mA with the step size of 0.02°. FTIR spectra of the obtained composites were carried out with a JASCO FTIR-6300 over the range 4000-400 cm^{-1} with a resolution of 4 cm^{-1} and accumulating 256 scans for each measurement adopting KBr disc method. Specific surface area of the samples was measured by BET method using a Quantachrome NOVA 2200e surface analyzer. FESEM measurements were performed with a Zeiss scanning electron microscope.

Photocatalytic experimental set up

Photo-oxidation of ethylene gas under UV irradiation in the presence of TiO_2/SiO_2 composite powder samples was performed inside a photocatalytic reactor consisting of a quartz tube of 3L irradiated by 4 UV light sources (Osram Puritec HNS15WG13, 15W, emitting at 254 nm). The pictures of the catalytic reactor have been reported in figure 1. The experimental set up was equipped with flowmeters, vacuum pump, diaphragm pump for gas recirculation, and thermocouples for the temperature control inside the reactor. The TiO_2/SiO_2 powder samples (3 g) were placed in the quartz tube on a rectangular quartz vessel. Before filling the reactor with ethylene gas/air atmosphere, it was kept in vacuum to ensure the purity inside the chamber. Then a gas mixture of air and ethylene (1 equivalent %) was introduced in the reactor with a controlled flow system. All the experiments were performed at atmospheric pressure and the purged gas was continuously circulated in a closed circuit while maintaining room temperature inside the reactor. Ethylene photo-oxidation was monitored at regular intervals by using an Agilent Gas Chromatograph 7820 A equipped with a capillary column (HP-PLOT/Q).

Fig. 1 - Pictures of the experimental set-up reactor for evaluating photocatalysis efficiency.

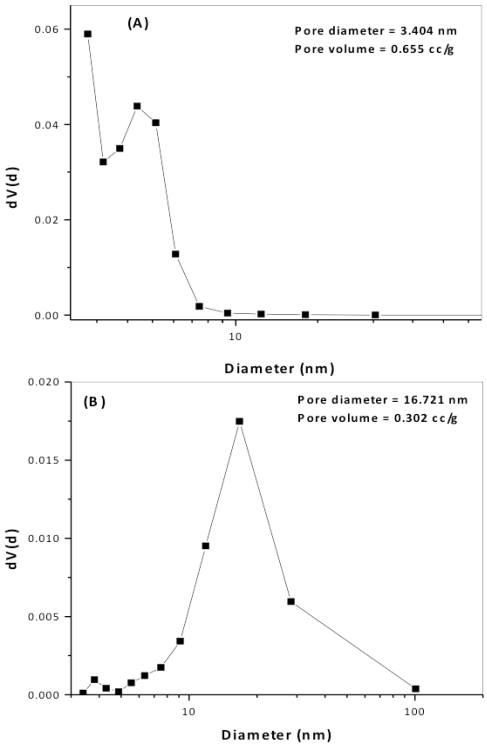

Fig. 2 - Pore size distribution curve of (A) the mesoporous silica (SBA) and (B) TiO_2 (TSBA-10).

3. Results and Discussion

To investigate the effect of silica framework and mesoporosity in the catalyst nanostructure, BET specific surface area measurements were performed on the powder samples. BET measurements showed an increase in specific surface area with the increase in silica content. The sample SBA of pure silica represents pores with an average diameters of 3.4 nm and a specific surface area of ~553 m^2/g, while the pure TiO_2 (TSBA-10) has pores with average diameter of 16.7 nm and a specific surface area of ~59 m^2/g. A great ratio of surface/volume is a crucial parameter to optimize photocatalytic efficiency of nanostructered semiconductors. Intermediate values of specific surface area and average diameters between those recorded for pure silica and TiO_2, have been obtained on the binary TiO_2/SiO_2 oxides (Fig. 2).

The phase transformation of the TiO_2/SiO_2 catalysts was investigated by XRD and the spectra of each sample are presented in figure 3. The average crystalline size was estimated according to the Scherrer's equation, accounting the most intense diffraction peaks of the corresponding anatase and rutile phases.

$$D = k\lambda/\beta\cos\theta \qquad (1)$$

where D is the average crystallite size, k is the shape factor (0.9), λ is the wavelength of X-ray radiation, β is the full line width at half-maxima (FWHM) of the main diffraction peak and θ is the Bragg angle of the corresponding diffraction peak (Pal *et al.*, 2014).

The weight fraction of anatase and rutile contents in the samples were calculated according to the following equations:

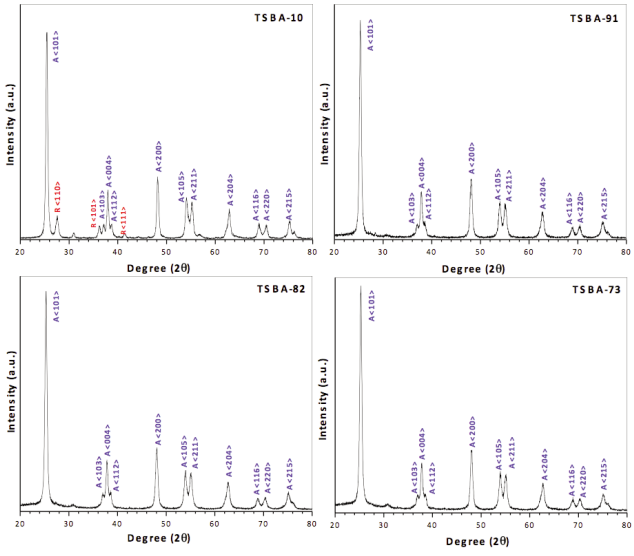

Fig. 3 - XRD spectra of the TiO_2/SiO_2 samples with different TiO_2/SiO_2 weight ratio.

$$W_A = 1/[1+1.26(I_{R110}/I_{A101})] \quad (2)$$
$$W_R = 1/[1+0.8(I_{A101}/I_{R110})] \quad (3)$$

where W_A and W_R are the weighted fraction of anatase and rutile in the mixed phase, and I_{A101} and I_{R110} are the integrated intensity of corresponding anatase (101) and rutile (110) diffraction peaks, respectively (Pal *et al.*, 2014). Phase composition and average crystallite size of the corresponding crystals are summarized in Table 1, where it is observed that TSBA-73, TSB-82 and TSBA-91 samples consist of pure anatase phase whereas in case of TSBA-10, there is a small amount of rutile phase formation (~11 wt%). These data clearly indicate that the mesoporous silica framework helps to stabilize the anatase crystalline phase and prevent the thermodynamically favoured phase transformation in to the rutile phase, as reported by Fu *et al*. (1996).

Table 1 - Anatase (W_A), and rutile (W_R) phase content in different TiO_2/SiO_2 samples and their corresponding crystalline sizes calculated from XRD data

Sample	Phase content		Crystalline size (nm)	
	W_A	W_R	$<D_A>$	$<D_R>$
TSBA-73	1	0	15.749	-
TSBA-82	1	0	15.998	-
TSBA-91	1	0	17.663	-
TSBA-10	0.887	0.111	17.553	13.866

FTIR measurements were carried out to investigate the bonding of SiO_2 and TiO_2 network and they are showed in figure 4. As a reference of pure silica, an FTIR spectrum of SBA is also presented. All the spectra, except for the sample TSBA-10 (100 wt% TiO_2), show intense and distinct vibrational bands at 1092, 805 and 472 cm^{-1} which are assigned to the asymmetric stretching of Si-O-Si (1092 cm^{-1}), Si-O-Si symmetric stretching (805 cm^{-1}), Si-O-Si bending vibration mode (474 cm^{-1}). The broad band around 3300-3600 cm^{-1} and the band at 976 cm^{-1} are attributed to the stretching vibration of H-bonded silanols (Si-OH) with hydroxyl groups of the adsorbed water molecules. Another peak of the adsorbed water is located at the wavenumber of 1634 cm^{-1} due to bending vibration of O-H groups. Furthermore, from the spectra it can be observed that the band at 665 cm^{-1} is absent in SBA sample, whereas it is present in all samples containing TiO_2 and it is therefore attributed to the Ti-O bond. The appearance of broad band at 450-800 cm^{-1} range is also attributed to the presence of TiO_2 It is also interesting to see that the vibrational band at 1092 cm^{-1}, due to asymmetric stretching of Si-O-Si, is gradually decreasing with increasing TiO_2 content in the mixed composite that confirms the variation of TiO_2/SiO_2 ratio.

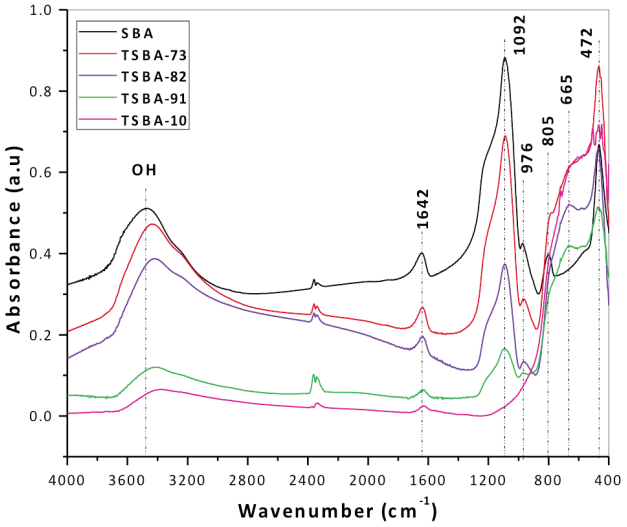

Fig. 4 - FTIR spectra of pure mesoporous silica and of the TiO_2/SiO_2 binary systems.

FESEM measurements on two different samples, one without silica support (TSBA-10) and the other with silica support (TSBA-82) were carried out to investigate the effect of silica insertion in the composite catalyst samples. FESEM images of these two samples are showed in figure 5. The lower magnification images (Fig. 5 a, c) of TSBA-10 and TSBA-82 samples represent the overall structure of these powder samples. Whereas from the images in figure 5 (b, d), it is clearly visible the mesoporous structures. The crystallite size as estimated from the XRD spectra (Table 1) of the corresponding samples matches well with the size as observed in the FESEM images (Fig. 5 b, d).

The photocatalytic efficiency of the powder samples was evaluated by the photo-oxidation of ethylene under UV light illumination. The percentage of ethylene photo-oxidation with reaction time under UV light corresponding to four catalysts is summarized in Table 2. For a better comparison, data reported in Table 2 have been presented as histogram in figure 6. From Table 2 and figure 6, it is clear that the overall photo-oxidation efficiency of TSBA-82 sample is higher respect to the others. It also shows a superior catalytic activity in early reaction times. The higher catalytic activity of TSBA-82 sample can be explained owing to stable anatase crystalline phase, higher specific surface area and having optimum amount of mesoporous silica support.

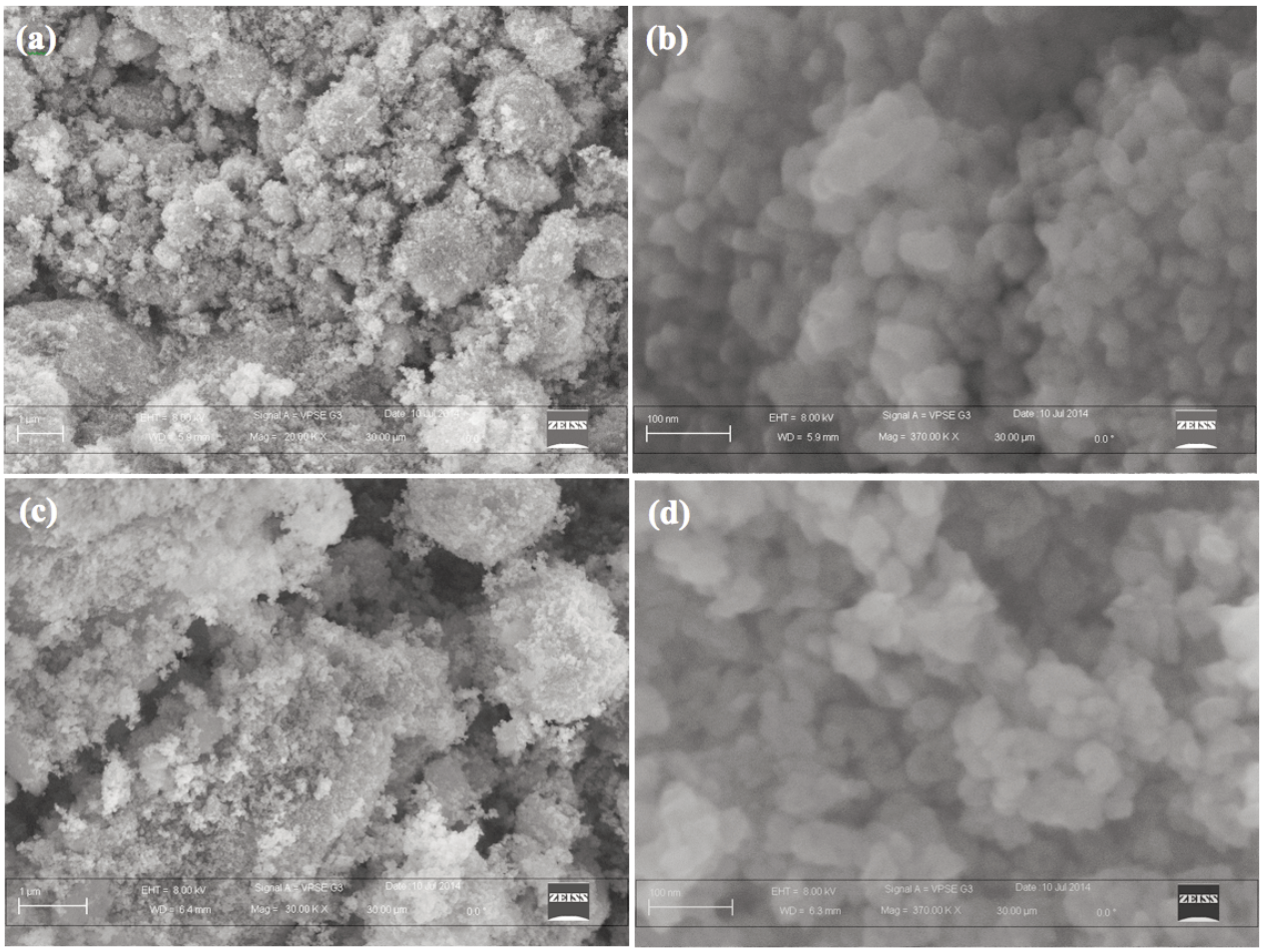

Fig. 5 - FESEM images of (a, b) TSBA-10 and (c, d) TSBA-82 samples.

Table 2 - Percentage of oxidized ethylene for the catalysts TSBA-10, TSBA-91, TSBA-82, and TSBA-73 as a function of the UV light irradiation

Time (h)	Oxidized ethylene (%)			
	TSBA-10	TSBA-91	TSBA-82	TSBA-73
0	0	0%	0%	0%
1	2%	0%	6%	8%
2	8%	5%	15%	6%
3	16%	15%	20%	8%
4	22%	22%	25%	11%
5	26%	31%	30%	13%
24	98%	97%	99%	89%

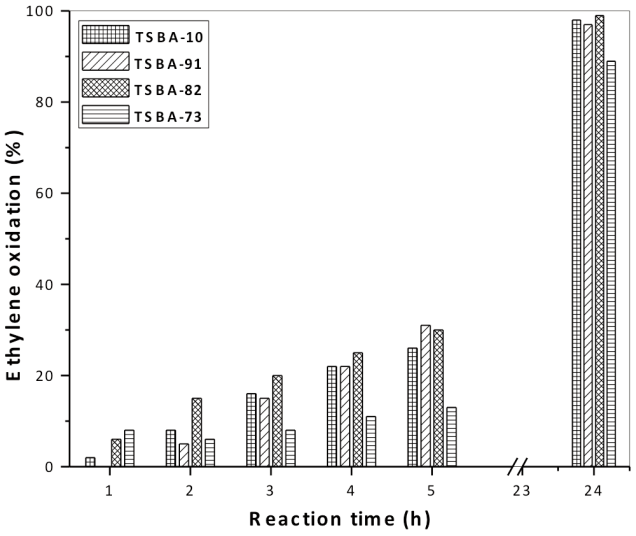

Fig. 6 - Histogram showing the ethylene oxidation efficiency of four catalyst samples with the reaction time under UV light exposure.

4. Conclusions

TiO_2/SiO_2 catalysts, with different TiO_2/SiO_2 weight ratios, were synthesized by liquid crystal template technique, being SiO_2 the mesoporous support

of TiO_2 catalyst. XRD results prove that a more stable anatase phase with silica insertion even at higher calcination temperature is obtained. Increasing the silica content also leads to a higher specific surface area. FESEM images showed the well-defined structure of the catalyst samples. The photocatalysis experiments revealed that, after 24 hours of reaction under UV light, total oxidation of ethylene was achieved in case of TSBA-82 catalyst. The other catalysts, such as, TSBA-0 and TSBA-91 also showed interesting results but the highest activity was observed with the TSBA-82 sample. This higher efficiency was explained with surface area, phase stability and optimum mesoporous support. Overall it can be stated that the TiO_2/SiO_2, rather than pure TiO_2 is a promising candidate in photocatalytic degradation of ethylene. It is therefore expected that TiO_2/SiO_2 can be fruitfully used in several applications requiring the control of ethylene to prevent ripening of fruits and vegetables.

Acknowledgements

Support by MIUR in the framework "Programma Operativo Nazionale R&C 2007-2013" project PON01_01435", "*PRODOTTI ORTOFRUTTICOLI AD ALTO CONTENUTO IN SERVIZIO: TECNOLOGIE PER LA QUALITÀ E NUOVI PRODOTTI"* is gratefully acknowledged by the authors.

References

BECK J.S., VARTULLI J.C., ROTH W.J., LEONOWICZ M.E., KRESGE C.T., SCHMITT K.D., CHU C.T.W., OLSON D.H., SHEPPARD E.W., MCCULLEN S.B., HIGGINS J.B., SCHLENKER J.L., 1992 - *A new family of mesoporous molecular sieve prepared with liquid crystal templates*. - J. Am. Chem. Soc., 114: 10834-10843.

CORMA A., DIAZ-CABANES M.J., MOLINER M., RODRIGUEZ G., 2006 - *Synthesis of micro- and mesoporous molecular sieves at room temperature and neutral pH catalysed by functional analogues of silicatein*. - Chem. Comm., 29: 3127-3139.

FU X., CLARK L.A., YANG Q., ANDERSON A., 1996 - *Enhanced photocatalytic performance of titania-based binary metal oxide: TiO_2/SiO_2 and $TiO_2/ZriO_2$*. - Environ. Sci. Technol., 30: 647-653.

HUSSAIN M., BENSAID S., GEOBALDO F., SARACCO G., RUSSO N., 2011 - *Photocatalytic degradation of ethylene emitted by fruits with TiO_2 nanoparticles*. - Ind. Eng. Chem. Res., 50: 2536-2543.

KELLER N., DUCAMP M.N., ROBERT D., KELLER V., 2013 - *Ethylene removal and fresh product storage: A challenge at the frontiers of chemistry. Toward an approach by photocatalytic oxidation*. - Chem. Rev., 113: 5029-5070.

PAL S., LAERA A.M., LICCIULLI A., CATALANO M., TAURINO A., 2014 - *Biphase TiO_2 microspheres with enhanced photocatalytic activity*. - Ind. Eng. Chem. Res., 53: 7931-7938.

PARK D.R., ZHANG J., IKEUE K., YAMASHITA H., ANPO M., 2009 - *Photocatalytic oxidation of ethylene to CO_2 and H_2O on ultrafine powdered TiO_2 photocatalysts in the presence of O_2 and H_2O*. - J. Catal., 185: 114-119.

XIAO Q., WANG J., ZHOU H., SUN P., YUAN Z., LI B., DING D., CHEN T., 2008 - *Low temperature effect on the morphological control of* mesoporous silican *nanofibers at near-neutral pH: a biomimetic strategy.* - Micropor. Mesopor. Mat., 109: 233-238.

YE S.Y., FANG Y.C., SONG X.L., LUO S.C., YE L.M., 2013 - *Decomposition of ethylene in cold storage by plasma-assisted photocatalyst process with TiO_2/ACF-based photocatalyst prepared by gamma irradiation*. - Chem. Eng. J., 225: 499-508.

ZHAN C., CHEN F., YANG J., DAI D., CAO X., ZHONG M., 2014 - *Visible light responsive sulphated rare earth doped TiO_2@fumed SiO_2 composites with mesoporosity: enhanced photocatalytic activity for methyl orange degradation.* - J. Hazard. Mater., 267: 88-97.

Adv. Hort. Sci., 2016 30(2): 81-85 DOI: 10.13128/ahs-19133

Influence of organics, inorganic and biofertilizers on growth, fruit quality, and soil characters of Himsagar mango grown in new alluvial zone of West Bengal, India

P. Dutta (*), K. Das, A. Patel
Department of Fruits and Orchard Management, Faculty of Horticulture, Bidhan Chandra Krishi Viswavidyalaya, P.O. Mohanpur, 741252 Dt. Nadia, West Bengal, India.

Key words: growth characters, *Mangifera indica*, quality, shelf life, soil microbial population.

Abstract: A field study with organic manures, inorganic manures and biofertilizers alone and in combination was carried out on 11-year-old mango (*Mangifera indica* L.) cv. Himsagar spaced 10 m apart in a randomized block design at the University Research Station, Gayeshpur, BCKV, West Bengal, India. The objective was to determine their effect on growth, soil characters, and fruit quality of mango grown in a new alluvial zone of West Bengal. Respiration, physiological loss in weight (PLW) and shelf life at ambient room temperatures, and soil characters were also assessed. Results revealed that among the eight treatments, biofertiliser (*Azotobacter* + PSM) along with 50% inorganic fertilizer significantly increased the growth characters of mango trees. This treatment also increased the physico-chemical character of fruit while biofertilizers alone improved the fruit quality, viz. total soluble solids, total sugar and β-carotene. Fruits treated with biofertilizers also had increased shelf life with lower PLW and respiration rate. Soil microbial population and other soil characters were improved by application of biofertilizer as well. It is concluded that biofertilizer application in mango gives better growth, fruit quality, and soil health.

1. Introduction

Mango (*Mangifera indica* L.) is the most important tropical fruit and considered king of all the fruits in India. The fruits are a rich source of iron and various anti-oxidants. In addition, mango is a rich source of vitamin A, E and selenium, which help to protect against heart disease and other such related aliments. Nowadays, continuous use of chemical fertilizer without organic manure causes problems of soil health and fruit quality. Fruit qualities are being deteriorated through the use of chemical fertilizers (Huyskens-Keil and Schreiner, 2003). Organic farming is currently gaining gradual momentum worldwide with growing awareness of health and environmental issues in agriculture and consumers demanding the production of organic fruit, thus offering an attractive source of rural income. Organic farming in India has attracted many farmers throughout the country and different fruit crops like banana, papaya, pineapple and sapota have been successfully tested. According to Neuhoff *et al*. (2011) working with oranges and Ilic *et al*. (2013) tomato, organically grown fruits are rich in various minerals such as P, K, Ca and Mg. Soil microbes were found to increase in organic systems compared to the conventional system of planting (Dutta and Kundu, 2011). However, the influence of organic, inorganic, and biofertilizer on growth characters and fruit quality of mango and soil properties are not well documented. Hence, a study was initiated to evaluate their effect on fruit quality and soil properties.

2. Materials and Methods

The study was conducted at the University Research station, Gayeshpur, Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India on 11 years old tree of mango cv. Himsagar with 10×10 m spacing. The following eight treatments were imposed: Vermicompost at 5 kg/plant/year, FYM at 10 kg/plant/year, inorganic fertilizer (NPK at 1000:500:1000 g/plant/year), 50% Vermicompost +

(*) Corresponding author: pallab_bckv@rediffmail.com
Received for publication 9 August 2015
Accepted for publication 11 April 2016

50% inorganic fertilizer, 50% FYM+50% inorganic fertilizer, Biofertilizer (Azotobacter at 150 g/plant) + PSM at 100 g/plant), Biofertilizer + 50% inorganic fertilizer, and control (no treatment). Treatments were applied separately in split doses, once at fruit set and another at harvest The experiment was laid out in a randomized block design with three replications. Mature fruits were harvested and brought to the laboratory for physico-chemical analyses following all standard procedures as described by Ranganna (2000).

Total soluble solids were determined using a hand refractometer (0-32°brix). Total soluble sugar content was analysed using Fehlings' A and B solution, according to the method described by Ranganna (2000) and expressed as percentage. In this method, for inversion of room temperature an aliquot of clarified and diluted solution was transferred for a flask. 10 ml of HCl (1:1) was added and allowed to stand at room temperature for 24 hrs. The solution was then neutralized with concentrated NaOH solution and made to volume. An aliquot was taken and the total soluble sugars were determined as invert sugars using Fehling's A and B solution. Titratable acidity (% malic acid) was estimated by titrating fruit juice (5 ml) to pH 8 against 0.1 M NaoH using phenolphthalene as indicator. Total carotenoids were estimated by the method described by Ranganna (2000). Five grams of fresh sample were taken, a few crystals of anhydrous sodium sulphate were added, and then crushed in 10 ml acetone with the help of a mortar and pestle. The supernatant was decanted into a beaker. The process was repeated twice or thrice and the combined supernatant was transferred to a separating funnel out on standing. Petroleum ether (10 to 15 ml) was added in the separating funnel and rinsed, the pigment was then transferred to the petroleum ether phase by diluting the acetone with water or water containing 5% sodium sulphate. The extraction of the acetone phase with a small volume of petroleum ether was repeated, if necessary, until no more colour was extracted. The lower layer was discarded and the upper layer was collected in a 100 ml volumetric flask. The petroleum ether extract was filtered through anhydrous Na_2SO_4 and the volume was made up to 100 ml with petroleum ether. The optical density was recorded at 452 nm using petroleum ether as blank containing 3 ml acetone per 100 ml and expressed as µg 100 g^{-1} pulp. As carotenoids are light sensitive, all steps were performed under subdued light. Shelf life was determined at ambient room temperature (34±1°C). The CO_2 evolution of fruits was determined by titration of residual $Ba(OH)_2$ in the solution with standardized N/10 HCl as described by Mitra *et al*. (1971). Growth characters such as plant height, plant spread, and trunk girth were measured after fruit harvest. Soil properties were recorded as per the standard procedure given by Black (1965). Soil microbial population was counted using the method described by Collin and Lyne (1985). Statistical analysis was carried out according to the standard procedures.

3. Results and Discussion

Growth parameters

Table 1 reveals that different nutrient treatments significantly increased plant height, canopy, spread, and trunk girth. Biofertilizer + half inorganic fertilizer gave maximum (6.72 m) plant height, canopy spread (6.37×6.92 m), and trunk girth (79.32 cm), followed by Vermicompost (2.5 kg/plant/year) + half chemical fertilizer (RDF-NPK at 1000:500:1000 g/plant/year);

Table 1 - Growth characters of mango cv. Himsagar as influenced by inorganic and organic manures

Treatments	Plant height (m)	Canopy spread (m)		Trunk girth (cm)
		E-W	N-S	
1. Vermicompost (5 kg/plant)	5.99	5.49	5.12	69.72
2. FYM (10 kg/plant)	6.10	5.97	6.14	72.11
3. Inorganic fertilizer (NPK- 1000:500:1000 g/plant/yr.)	6.00	5.97	6.11	73.72
4. 50% Vermicompost + 50% Inorganic fertilizer	6.24	6.12	6.31	70.47
5. 50% FYM + 50% Inorganic fertilizer	6.11	6.00	6.09	73.15
6. Biofertilizer (*Azotobacter* @ 150g/plant + PSM @ 100 g/plant)	5.99	5.84	5.91	70.37
7. Biofertilizer + 50% Inorganic fertilizer	6.72	6.37	6.92	79.32
8. Control	5.97	5.82	5.90	71.41
SEM±	1.01	0.72	1.11	2.40
CD (P=0.05)	3.11	1.92	3.31	3.14

the lowest measurements for these three parameters were found in untreated control plants. Korwar *et al*. (2006) in Aonla and Shukla *et al*. (2009) in guava obtained similar results. These findings may be due to a better nutritional environment: application of organic matter improves soil health by improving the physico-chemical and biological activities (Schnitizer, 1991) and biofertilizer was found to enhance the rate of mineralization and availability of the nutrients, further enhancing plant growth (Sahoo and Singh, 2005).

Physico-chemical composition of fruit

Different nutrient treatments significantly improved the physico-chemical composition of fruits (Table 2). Biofertilizer + half of inorganic fertilizer produced maximum fruit weight (285.15 g), yield (57.20 kg/plant), fruit length/breath (9.14/8.19 cm), followed by biofertlizer alone. Also with regard to fruits, control trees gave minimum results. Unlike the physical characters, the bio-chemical composition of fruits was more effected by biofertilizer alone.

Biofertilizer (*Azotobacter* @ 150 g/plant + PSM @ 100 g/plant/year) gave maximum total soluble solids (19.80°brix, total sugar (16.00%) and β-carotene (6123 µg/100 g) with minimum (0.16%) acidity. Fruits treated with inorganic fertilizers showed minimum total solids (18.20°brix) and β-carotene (4792 µg/100 g) with maximum (0.31%) acidity. Results revealed that fruits grown under organic manure/biofertilizer had better fruit quality. The increase in physico-chemical parameters in fruits due to bio-fertilizer might be because of their role in nitrogen fixation, production of phytohormone-like substances and increased uptake of nitrogen as reported by Dutta and Kundu (2012). Furthermore, micro-organisms are an important component of soil environment (Arshad and Frankemberger, 1992). Thus, utilization of biofertilizer could be a better preposition for improving biological attributes of soil, which in turn may increase quality and productivity potential of various crops as reported by Allen *et al*. (2002).

Soil nutrient status and soil bacterial population

Different nutrient treatments significantly increased the soil pH and soil organic carbon (Table 3). Biofertilizer alone gave maximum (6.70) soil pH while inorganic fertilizer-treated soil the minimum

Table 2 - Fruit quality of Himsagar mango as influenced by inorganic and organic manures

Treatments	Fruit weight (g)	Yield (kg/plant)	Fruit length/breadth (cm)	TSS (°Brix)	Total Sugar (%)	Acidity (%)	β Carotene (µg/100g)
1. Vermicompost (5 kg/plant)	250.00	50.95	8.40/7.29	18.70	16.12	0.14	5720
2. FYM (10 kg/plant)	151.42	50.35	8.31/7.31	18.90	15.91	0.17	5824
3. Inorganic fertilizer (NPK- 1000:500:1000 g/plant/yr)	248.43	51.75	8.00/7.44	18.20	15.33	0.31	4792
4. 50% Vermicompost + 50% Inorganic fertilizer	265.14	53.92	9.11/8.12	19.00	15.11	0.25	5012
5. 50% FYM + 50% Inorganic fertilizer	270.00	54.98	9.00/8.15	19.10	15.23	0.26	5170
6. Biofertilizer (*Azotobacter* @ 150 g/plant + PSM @ 100 g/plant)	270.40	54.12	8.99/85.16	19.80	16.00	0.16	6123
7. Biofertilizer + 50% Inorganic fertilizer	285.15	57.20	9.14/8.19	19.60	15.92	0.18	5814
8. Control	240.40	48.00	8.10/7.60	18.00	14.94	0.19	4914
SEM±	1.15	0.70	1.01/0.72	0.51	0.07	0.01	4.31
CD (P=0.05)	3.72	2.10	3.11/1.97	1.37	0.21	0.03	12.39

Table 3 - Soil characters as influenced by inorganic and organic manures

Treatments	pH	EC (1:2.5) (dSm-1)	Bulk density (g/cc)	OC (%)	Soil microbial population (Bacteria) (cfug-1 soil)
1. Vermicompost (5 kg/plant)	6.57	0.263	1.21	0.90	1.6×10^6
2. FYM (10 kg/plant)	6.59	0.137	1.29	0.84	5.9×10^6
3. Inorganic fertilizer (NPK- 1000:500:1000 g/plant/yr.)	6.00	0.171	1.49	0.61	4.3×10^5
4. 50% Vermicompost + 50% Inorganic fertilizer	6.45	0.214	1.12	0.72	5.1×10^6
5. 50% FYM + 50% Inorganic fertilizer	6.42	0.219	1.41	0.74	5.4×10^6
6. Biofertilizer (Azotobacter @ 50 g/plant + PSM @ 100 g/plant)	6.70	0.198	1.31	0.78	8.3×10^6
7. Biofertilizer + 50% Inorganic fertilizer	6.42	0.197	1.27	0.74	6.0×10^6
8. Control	6.10	0.111	1.69	0.60	6.9×10^5
SEM±	0.13	0.70	0.03	0.02	4.72
CD (P=0.05)	0.39	2.10	0.09	0.07	14.43

(6.00). Organic carbon content of soil also varied significantly. Vermicompost or FYM-treated soil gave maximum (0.90/0.84%) content of organic carbon, followed by biofertilizer-treated soil (0.78%). Soil organic carbon was at the lowest level in untreated control. The increase in organic carbon of soil may be due to the addition of organic matter through organic manure or microbes and recycling of organic materials in the form of crop residue, which brings the soil pH nearer to neutral and increases the nutrient availability. Our results are in close conformity with earlier findings (Dutta and Kundu, 2012). The effective conductivity (EC) of soil solution depends on the presence of soluble salts. The concentrations of these salts affect the growth and absorption of water. Different treatments significantly influenced the EC of soil. Application of Vermicompost led to the highest EC (0.263 dSm^{-1}) in this study, whereas the control plot gave the lowest. This could be due to the fact that Vermicompost contains salts, mostly Cl and SO_4, but not at toxic levels (Masciandaro *et al*., 1998). Bulk density varied due to different treatments, with the untreated control giving maximum bulk density (1.69 g/cc) of soil. The soil microbial population varied with the different treatments: the maximum ($8.3x10^6$ $cfug^{-1}$ soil) was found with Biofertilizer (*Azotobacter* at 50 g/plant + PSM at 100 g/plant). Soil applied with biofertilizer showed more soil bacteria, whereas least was formed by Vermicompost (Table 3). Similar results were obtained by Dutta *et al*. (2010) in litchi.

PLW, shelf life and respiration of fruit

Physiological loss in weight (PLW) of fruit varied significantly among the different treatments (Table 4). Untreated fruit had the highest (18.95%) PLW while the lowest was found with fruit grown in biofertilizer (11.31%). Fruits grown in biofertilizer showed maximum (10 days) shelf life with minimum respiration rate (109.72 mg/hr/kg fruit) during storage. Untreated control fruit recorded maximum respiration and PLW with minimum shelf life (5 days). Improvement of shelf life due to application of biofertilizer was previously reported in mango (Dutta and Kundu, 2012).

4. Conclusions

The present study reveals that application of organic and biofertilizer are more beneficial for quality mango production and increase soil health. Therefore, this approach can be spread among growers to improve the quality in mango orchards.

References

ALLEN M.F., JASPER D.A., ZAK J.C., 2002 - *Microorganism*, pp. 257-278. - In: PERROW M.R., and A.J. DAVY (eds.) *Handbook of ecological restoration. Volune I. Principles of Restoration. Part 4. Manupulation of the biota*. Cambridge University Press, Cambridge, UK, pp. 445.

ARSHAD M., FRANKEMBERGER JR. W.T., 1992 - *Microbial production of plant growth regulators*, pp. 307-348. - In: MEETING F.B. Jr. (ed.) *Soil microbial ecology. Application in agricultural and environmental management.* Marcel Dekker, Inc. New York, USA.

BLACK C.A., 1965 - *Method of soil analysis.* - Amer. Soc. of Agron. Inc. Publisher, Madison, Wisconsin, USA, Vol. 9, Parts 1 & 2, pp. 1572.

COLLIN C.H., LYNE P.M., 1985 - *Microbiological methods*. - Butterworth Co., London, UK, pp. 450.

DUTTA P., KUNDU S., 2011 - *Effect of organic manure and biofertilizers on organic production of mango*. -

Table 4 - PLW, shelf life and respiration of fruit at the end of storage life as affected by inorganic and organic manures

Treatment	PLW (%)	Shelf life (days)	Respiration CO_2 evolution (mg/hr/kg fruit)
1. Vermicompost (5 kg/plant)	12.62	8	120.41
2. FYM (10 kg/plant)	12.44	9	118.32
3. Inorganic fertilizer (NPK- 1000:500:1000 g/plant/yr.)	17.92	6	170.41
4. 50% Vermicompost + 50 % Inorganic fertilizer	14.31	7	150.37
5. 50 % FYM + 50 % Inorganic fertilizer	14.45	2	159.37
6. Biofertilizer (Azotobacter @ 150 g/plant + PSM @ 100 g/plant)	11.31	10	109.72
7. Biofertilizer + 50% Inorganic fertilizer	13.00	8	122.14
8. Control	18.95	5	163.14
SEM±	1.10	0.61	4.11
CD (P=0.05)	3.42	1.82	12.12

Proceedings of the third scientific conference of ISOFAR, 1: 475-478.

DUTTA P., KUNDU S., 2012 - *Effect of biofertilizers on nutrient status and fruit quality of Himsagar mango grown in new alluvial zones of West Bengal*. - J. Crop Weed Sci., 8(1): 72-74.

DUTTA P., KUNDU S., BISWAS S., 2010 - *Integrated nutrient management in litchi cv. Bombai in new alluvgial zone of West Bengal*. - Indian J. Hort., 67: 181-184.

HUYSKENS-KEIL S., SCHREINER M., 2003 - *Quality of fruit and vegetable*. - J. Appl. Bot., 77: 147-151.

ILIC Z.S., KAPOULAS N., MILENKOVIC L., 2013 - *Micronutrient composition and quality characteristics of tomato (*Lycopersicon esculentum*) from conventional and organic production*. - Indian J. Agric. Sci., 83: 651-655.

KORWAR G.R., PRATIBHA G., RAVI V., PALANIKUMAR D., 2006 - *Influence of organics and inorganics on growth, yield of aonla (*Emblica officinalis*) and soil quality in semi-arid tropics*. - Indian J. Agric. Sci., 76: 457-461.

MASCIANDARO G., CECCANTI B., GARCIA C., 1998 - *Changes in soil biochemical and cracting properties induced by living much system*. - Canadian J. Soil Sci., 77: 579-589.

MITRA D., GUHA J., CHAUDHURI S.K., 1971 - *Studies in Botany*. - Vol, II, Moulik library, Kolkata, India.

NEUHOFF D., VLATSCHKOV V., RAIGON D., 2011 - *Comparison of the quality of conventionally and organically grown oranges in Spain*. - Proceedings of the third scientific conference of ISOFAR, pp. 487-490.

RANGANNA S., 2000 - *Manual of analysis of fruits and vegetables Products*. - Tata McGrow Hill Publishing Co. Ltd. 3rd Ed. New Delhi, India.

SAHOO S.K., SINGH D.B., 2005 - *Effect of different level of biofertilizers on growth, yield and quality of strawberry (*Fragania ananassa *Duch) cv. Sweet Charley*. - The Orissa J. Hort., 33: 82-85.

SCHNITIZER M., 1991 - *Soil organic matter in the next 75 years*. - Soil Sci., 151: 41-59.

SHUKLA A.K., SAROLIA D.K., BHAVANA K., KAUSHIK R.A., MAHAWER L.N., BAIRWA H.L., 2009 - *Evaluation of Substrate dynamics for integrated nutrient management under high density planting of guava cv. Sardar*. - Indian J. Hort., 66: 461-464.

Adv. Hort. Sci., 2016 30(2): 87-94 DOI: 10.13128/ahs-19134

Kentucky bluegrass (*Poa pratensis* L.) silicon-treated turfgrass tolerance to short- and long-term salinity condition

S. Esmaeili, H. Salehi (*)
Department of Horticultural Science, College of Agriculture, Shiraz University, Shiraz, Iran.

Key words: chlorophyll content, proline content, salt stress, Si, turfgrass.

Abstract: The effects of short- and long-term salinity condition were investigated on silicon-treated and control plants of Kentucky bluegrass (KBG) (*Poa pratensis* L.) in a greenhouse study. Salt stress solely affected visual quality at ≥15 dS m^{-1} concentrations while Si application increased salt tolerance of KBG after 45 days. In long-term salinity stress, Si had no effect on salt tolerance of KBG at ≥15 dS m^{-1} concentration. Si increased morphological parameters including height and number of shoots, and physiological parameters including relative water content (RWC) and chlorophyll content of leaves. In addition, fresh and dry weights of roots and shoots in response to high salt concentrations declined, but showed an increase with Si treatment. Proline content and electrolyte leakage (EL) increased under high salinity levels. In response to the Si treatment, Na concentration in the shoots significantly decreased at the 5 dS m^{-1} salinity level. With increasing salinity levels, the concentration of K in roots and shoots decreased while the amount of K in both Si-treated roots and shoots reduced. Overall, Si alleviative effects were more pronounced in 45 days after turfgrasses being salinity treated.

1. Introduction

Kentucky bluegrass (*Poa pratensis* L.), a native to Europe, is the most commonly used cool-season turfgrass in the temperate and subarctic regions of North America, and it is also recognized for its ability to create a high-quality turf (Fry and Huang, 2004). Saline soils reduce growth due to osmotic and ion stresses (Marschner and Part, 1995; Munns, 2002). Salinity causes stress in plants in two ways, and plants respond in two distinct phases through time: a rapid response to the increase in external osmotic pressure, and a slower response due to the accumulation of Na^+ in leaves (Munns and Tester, 2008).

It is generally accepted that silicon can positively affect growth and health status of plants under biotic (Adatia and Besford, 1986; Ma, 2004) and abiotic (Barceló *et al.*, 1993; Ranganathan *et al.*, 2006) stresses. Acceptable results of silicon application against NaCl stress have been shown in rice (Matoh and Kairusmee, 1986; Yeo *et al.*, 1999), wheat (Ahmad *et al.*, 1992; Tuna *et al.*, 2008; Tahir *et al.*, 2010; Chen *et al.*, 2014), and barley (Liang *et al.*, 1996; Liang, 1999). Possible mechanisms for salt tolerance with the utilization of silicon have been proposed. These include accumulation of silicon in leaves resulting in reduced transpiration (Matoh and Kairusmee, 1986), turgor enhancement (Romero-Aranda*et al.*, 2006), formation of Na complexes in roots (Ahmad *et al.*, 1992), increased photosynthetic activity and protection of plasmatic membranes and chloroplast ultrastructures (Liang *et al.*, 1996; Liang, 1998; Shu and Liu, 2001), protection of plant tissues from free radicals through increasing the activity of antioxidative enzymes (Liang, 1999; Liang *et al.*, 2003; Zhu *et al.*, 2004), and alleviation of specific ionic effects (Rafiq, 1990) by reducing Na uptake (Liang, 1999; Epstein, 2001; Gong *et al.*, 2003).

Gong *et al.* (2003) also observed improved water economy and dry matter yield of plants with Si application. Silicon application is reported to enhance leaf water potential in wheat under drought stress (Liang, 1999). The authors suggested that a double layer, comprised of silica and cuticle on leaf epidermal tissue, is responsible for this higher water potential. Silicon application enhances water use efficiency, heat/salt tolerance, and resistance to pathogens and

(*) Corresponding author: hsalehi@shirazu.ac.ir
Received for publication 23 March 2015
Accepted for publication 24 April 2016

heavy metals (Yeo *et al.*, 1999; Zwieniecki *et al.*, 2001; Gao *et al.*, 2004; Liang *et al.*, 2005 a, b; Wang *et al.*, 2005; Guo *et al.*, 2007; Liang *et al.*, 2007). It has been highly recommended to use Si in turfgrass management (Datnoff and Rutherford, 2003; Datnoff and Rutherfood, 2004). In another study on *Poa pratensis* L. 'Baron', Chai *et al.* (2010) reported that Si application under salinity condition raised the transfer of K^+ from roots to shoots, but inhibited the absorption and transfer of Na^+, which may contribute to better turf quality and growth with Si treatment under saline conditions.

Chen *et al.* (2014) also found that Si can enhance plant salt tolerance by alleviating the salt-induced osmotic stress. Mateos-Naranjo *et al.* (2013) showed that in wheat, the alleviative effects were more pronounced in the osmotic stress phase than the ion toxicity phase. These results clearly showed that Si can enhance plant salt tolerance by alleviating the salt-induced osmotic stress.

The purpose of the present study was to evaluate the short- and long-term effects of salinity on growth and physiological parameters of Kentucky bluegrass with and without silicon treatment.

2. Materials and Methods

Plant materials and growth conditions

The experiment was conducted for a period of 90 days in the greenhouse of the Department of Horticultural Science, College of Agriculture, Shiraz University, Shiraz, Iran. Plastic pots (20 cm in diameter and 10 cm in depth) were filled with 1.3 kg of a mixture of 1:1 sand and perlite, then seeds were cultured. The field capacity and permanent wilting point for the potting mixture were 10% and 4%, respectively. During the germination stage, turfgrasses were irrigated daily with 150 ml deionized water until plants were adequately established. Then, irrigation was carried out every two days with 200 ml deionized water. Before treatments began, the turfgrass was clipped to 5-7 cm every two weeks. The nutrient solution of a commercial whole fertilizer (Cristalone) was used weekly with a concentration of 0.1%. The EC of the nutrient solution was 1 dS m^{-1}. Mean relative humidity, daily temperature and light condition of the greenhouse were categorized as 45±5%, 24±4°C and 29 Wm^{-2} (16/8 h day/night), respectively.

Treatments

Silicon was applied in the form of potassium silicate (K_2SiO_3) at a concentration of 1 mM as foliar application weekly from 1 September to 1 December 2009. Salinity treatment began two weeks after the silicate treatment. Different salt concentrations were prepared by adding 1 NaCl: 1 $CaCl_2$ (w/w) to deionized water to obtain desired EC values; saline water of 5, 10, 15 and 20 dS m^{-1} along with deionized water as the control was applied (200 ml per pot) every two days. To prevent salinity shock, salinity levels were increased stepwise by 5 dS m^{-1}.

Measurements

Physiological parameters were measured twice: at 45 and 90 days following commencement of salinity treatments. Chlorophyll content, Relative water content (RWC), electrolyte leakage and proline content were measured.

Chlorophyll content was measured according to the method of Saini *et al.* (2001). Half a gram of fresh leaf material, taken from the youngest fully expanded leaf, was extracted with 80% acetone and read using a spectrophotometer at 645 and 663 nm wavelengths. Chlorophyll content was calculated using the following formula:

$$mgChl/g\ f.w. = [(20.2(OD\ 645\ nm) + (8.02(OD663nm)]*V/\ (f.w.*1000)$$

where V is the final solution volume in ml and f.w. is tissue fresh weight in mg.

Relative water content was measured using the methods of Nepomuceno *et al.* (1998) and Sairam *et al.* (2002). The value of RWC was determined by the following equation:

$$RWC\ (\%) = [(f.w.-d.w.)/(t.w.-d.w.)]\times 100$$

where f.w. is the fresh weight, d.w. is the dry weight and t.w. is the turgid weight.

Electrolyte leakage was determined according to the methods described by Saadalla *et al.* (1990).

Samples of 0.1 g of fresh leaves were weighed and washed three to four times with deionized water and immersed in a test tube containing 15 ml deionized water, then maintained at 25°C for 24 h. The tubes were then shaken for 15 min and the conductivity of the solution was measured (EC1) using an electrical conductivity meter (Metrohm 644, Swiss). The test tubes were then placed in an autoclave at 0.1 MPa for 10 min to kill the plant tissue and release all of the electrolytes. The tubes were cooled to 25°C, shaken, and their solution conductance measured again (EC2). The electrolyte leakage was calculated as EC1/EC2 and expressed as percent.

Proline content was calculated according to the

Bates *et al.* (1973) method. A half gram of fresh leaves was homogenized with 10 ml of 3% aqueous sulfosalicylic acid and filtered through Whatmans no. 2 filter paper. Two ml of filtrate was mixed with 2 ml of acid-ninhydrin and 2 ml of glacial acetic acid in a test tube. The mixture was placed in a water bath at 100°C for 1 h. The reaction mixture was extracted with 4 ml toluene, and the absorbance was measured at 520 nm with a spectrometer (UV-120-20, Japan). Standard curves of proline were used for the calculation of proline amount in the samples.

For fresh and dry weight determination of shoot and root systems and further chemical analysis, leaves and roots were washed three to four rinses in distilled water and then dried at 70°C for 48 h. The dried leaves and roots were ground to powder using an electric mill (AR 10, Molinex, China) and subsequently stored in polyethylene bottles at room temperature. One gram of leaf sample was ashed in a furnace at 550°C for 5 h. The ash was then dissolved in 10 ml 2N HCl and diluted to the volume of 100 ml with distilled water. Potassium and sodium contents were determined using a flame photometer (PFP7, Jenway, England) (Champan and Pratt, 1982).

Statistical analysis

The experiment was conducted in a complete randomized design with three replications. Data were analyzed using MSTAT-C software. Means were compared using the least significant difference (LSD) test at (P<0.05) level.

3. Results

The turfs treated with silicon, even at high concentration of salinity, maintained their visual quality and turf performance while those without silicon at 20 dSm^{-1} concentration died after 45 days. During long-term salinity exposure, either with or without Si, Kentucky bluegrass lost turf performance at 15 and 20 dS m^{-1} concentrations (data not shown). Salt stress reduced chlorophyll content during short-term salinity. Silicon treatment increased chlorophyll content extensively both under non saline irrigation and various concentrations of salty solutions (Fig. 1).

Under different salinity exposures, RWC increased through Si treatment. Results were significantly different in the presence and absence of silicon under non saline conditions. Significant differences were observed between salinity control groups, treated with Si of otherwise (Fig. 2).

As shown in figure 3, increased salinity resulted in remarkably enhanced electrolyte leakage. Silicon reduced electrolyte leakage after 45 days of salinity. In lower concentrations of salinity, silicon reduced

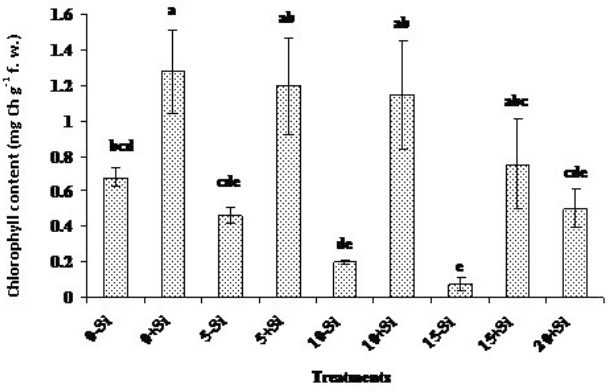

Fig. 1 - Effects of different salinity and Si levels on chlorophyll content of *Poa pratensis* after 45 days. 0: control, 5, 10, 15 and 20 dS m^{-1} as salinity levels; Si: K_2SiO_3 1 mM. Data are mean ± SE at P<0.05 using LSD test.

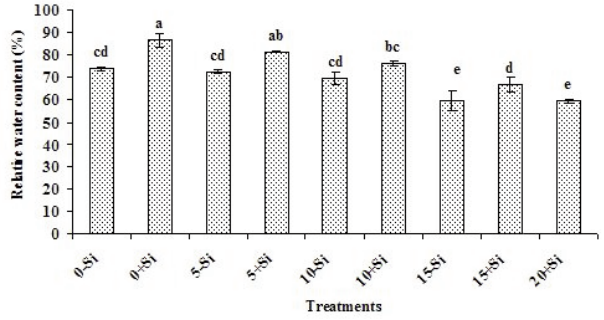

Fig. 2 - Effects of different salinity and Si levels on relative water content in of *Poa pratensis* after 45 days. 0: control, 5, 10, 15 and 20 dS m^{-1} as salinity levels; Si: K_2SiO_3 1mM. Data are mean ± SE. at P<0.05 using LSD test.

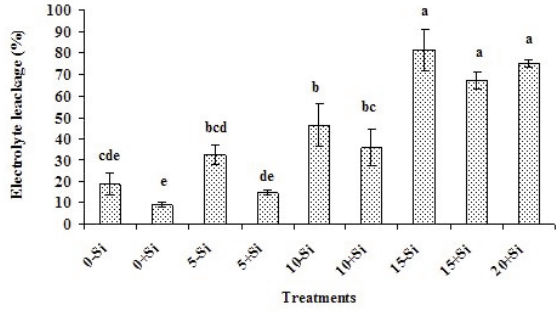

Fig. 3 - Effects of different salinity and Si levels on electrolyte leakage of *Poa pratensis* after 45 days. 0: control, 5, 10, 15 and 20 dS m^{-1} as salinity levels; Si: K_2SiO_3 1 mM. Data are mean ± SE. at P<0.05 using LSD test.

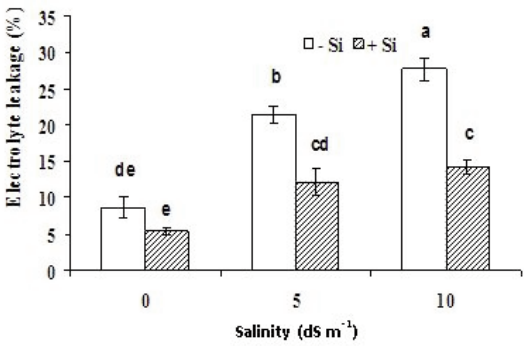

Fig. 4 - Interaction effects of different salinity and Si levels on electrolyte leakage of *P. pratensis* after 90 days. Data are mean ± SE at P ≤0.05 using LSD test.

electrolyte leakage even after 90 days (Fig. 4).

Proline concentration increased dramatically after 45 and 90 days of salt stress, although plants with Si application had less proline content (Figs. 5 and 6).

Higher concentrations of saline irrigation showed more reduction in shoot number of *P. pratensis*.

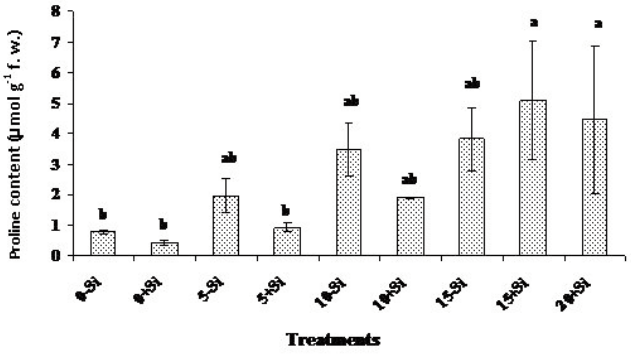

Fig. 5 - Effects of different salinity and Si levels on proline content of *Poa pratensis* after 45 days. 0: control, 5, 10, 15 and 20 dS m^{-1} as salinity levels; Si: K_2SiO_3 1 mM. Data are mean ± SE. at P<0.05 using LSD test.

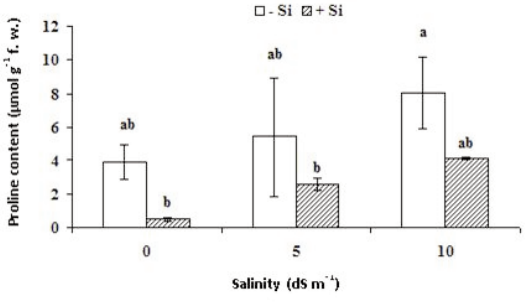

Fig. 6 - Interaction effects of different salinity and Si levels on proline content of *P. pratensis* after 90 days. Data are mean ± SE at P ≤0.05 using LSD test.

Silicon markedly increased shoot numbers at lower concentrations (Table 1). A dramatic reduction in visual quality based on shoot density and percentage of green leaf canopy area (GLCA) was observed after 45 days when *P. pratensis* was cultured at different

Table 1 - Interaction effects of different salinity and Si levels on growth parameters, shoot and root Na and K concentrations of *Poa pratensis*

		Treatments		
		Salinity (dS m^{-1})		
Indicator		0	5	10
Shoot length (cm)	-Si	24.57 ab	20.93 bc	17.37 c
	+Si	29.27 a	20.53 bc	16.4 c
Shoot number in pot	-Si	337 ab	121.3 c	62.67 c
	+Si	378.7 a	340.7 ab	258.7 b
Shoot fresh weight (g)	-Si	22.57 ab	10.28 cd	3.413 d
	+Si	29.43 a	16.90 bc	8.313 cd
Root fresh weight (g)	-Si	46.67 ab	23.57 cd	20.61 d
	+Si	50.20 a	34.96 bc	18.58 d
Shoot dry weight (g)	-Si	6.116 ab	3.506 bc	0.947 c
	+Si	9.791 a	6.925 ab	2.033 c
Root dry weight (g)	-Si	14.28 ab	6.372 c	6.109 c
	+Si	21.28 a	12.45 bc	5.744 c
Shoot Na concentration (g)	-Si	360.6 c	948.0 a	826.2 ab
	+Si	234.3 c	562.1 bc	554.6 bc
Root Na concentration (g)	-Si	40.22 cd	102 ab	118.3 a
	+Si	29.06 d	71.48 bc	81.15 ab
Shoot K concentration (g)	-Si	542.2 ab	347.2 bc	175.6 c
	+Si	861.4 a	374.3 bc	216.3 c
Root K concentration (g)	-Si	12.16 c	3.966 d	2.242 d
	+Si	26.15 a	18.37 b	13.41 c

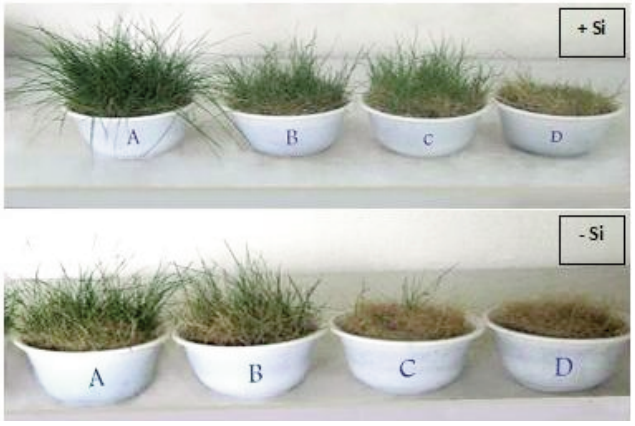

Fig. 7 - Comparison of different concentrations of salts (A: 5 dS m^{-1}, B: 10 dS m-1, C: 15 and D: 20 dS m^{-1} with Si (+Si) and without Si (-Si) in *P. pratensis* 45 days after beginning the treatments.

levels of salinity (Fig. 7).

Shoot fresh and dry weight of *P. pratensis* showed the most prominent decrease at 5 dS m^{-1} salt concentration (Table 1). Also, a significant decline in root fresh and dry weight was observed at 5 dS m^{-1} (Table 1). Si application partially enhanced shoot fresh and dry weight. In addition, Si had a greater impact on root fresh weight with non-saline irrigation (Table 1). In the leaves and roots, Na^+ content significantly increased at low concentrations of salty irrigations after 90 days. In contrast, shoot K^+ content was significantly less when salinity level reached 10 dS m^{-1}. Si treatment increased shoot K^+ content in comparison to saline irrigations without Si application. Root K^+ content reduced markedly at 5 dS m^{-1}, compared to the control. Turfs treated with Si had a higher concentration of K^+ in the roots (Table 1).

4. Discussion and Conclusions

Kentucky bluegrass (KBG) is generally considered to be a salt sensitive turf. In our research, KBG had no tolerance at periodically extended concentrations higher than 15 dS m^{-1} salinity, regardless of silicon application; the salinity tolerance threshold of KBG resulted to be 10dS m^{-1}. Silicon could increase salinity tolerance at higher concentrations.

The silicon remedy was more pronounced during short-term saline conditions, findings that are consistent with previous reports. Silicon could increase the amount of chlorophyll and photosynthesis and consequently, growth. Si protects plasmatic membranes and chloroplast ultrastructures (Liang *et al.*, 1996; Liang, 1998; Shu and Liu, 2001), stimulates H^+-ATPase activity, and increases K^+ in shoots (Liang *et al.*, 1996; Liang *et al.*, 2003; Liang *et al.*, 2005 a). Furthermore, it improves the activity of antioxidant enzymes, reducing the damage of reactive oxygen species (ROS) (Liang, 1999; Liang *et al.*, 2003; Zhu *et al.*, 2004) and reduces Na^+ root uptake, alleviating specific ion effects (Epstein, 2001; Gong *et al.*, 2003; Liang *et al.*, 2003).

It has been reported that the accumulation of Si in plants enhances the strength and rigidity of the tissues (Ma and Yamaji, 2006; Neethirajan *et al.*, 2009). An increased Si supply improves the structural integrity of crops and may also improve plant tolerance to disease, drought, and metal toxicities (Yeo *et al.*, 1999; Richmond and Sussman, 2003; Ma *et al.*, 2004). These findings are in agreement with our study. Some researchers hypothesized that Si deposition in the cell wall of root endoderm may contribute to the maintenance of the apoplastic barrier and thereby improve plant tolerance to disease and drought stress (Lux *et al.*, 2002; Lux *et al.*, 2003; Hattori *et al.*, 2005).

Electrolyte leakage was influenced more in the short-term salinity stress than long-term salinity stress. It appears that plant adaptability to lower salinity levels in the short term increases resistance to salt. As shown in figures 5 and 6, a significant increase in proline content was observed at 15 dS m^{-1}. However, silicon only slightly affected proline levels compared to the turfgrasses treated with saline and non-saline waters. Proline often accumulated in grasses under salinity stress, however this amount of content was insufficient for osmotic adjustment in grasses (Marcum, 2002).

Foliar application of Si increased the unsaturated fatty acid ratios [(18:2+18:3)/18:1] in glycolipids and phospholipids and also proliferated the amount of membrane lipids in strawberries (Wang and Galletta, 1998). Agarie *et al.* (1998) noted that Si increased membrane stability of rice under drought and heat stresses, which prevented the structural and functional deterioration of cell membranes. In concordance, it appears that Si plays an important role in maintaining the integrity, stability and function of cell membranes in Kentucky bluegrass under salt stress.

Ashraf and Foolad (2007) reported an increase in the amount of proline primarily in cytosols under salinity stress. They found that plant tolerance to salinity stress and proline accumulation are positively related. However, the relationship is not universal and might be cultivar dependent.

Moreover, Bartels and Sunkar (2005) and Ashraf and Foolad (2007) reported other possible roles attributable to proline besides osmotic adjustment in stressed plants, such as acting as a hydroxyl scavenger, the stabilization of membrane and protein structure, serving as a sink for carbon and nitrogen during stress recovery, and the buffering of cellular redox potential under stressful conditions.

As shown in figure 2 RWC reduced significantly under higher salinity levels in short-term salt stress. A probable explanation for an increase in RWC under Si application may be the prevention of transpiration.

Marcum and Murdoch (1990) reported that shoot water content of *Zoysia matrella* (L.) Merrill, *Z. japonica* L., *Paspalum vaginatum* Swarts and *C. dactylon* decreased during a one month salinity stress in solution culture, and suggested that osmotic adjustment was not achieved exclusively by solute accumulation.

Si is known to decrease Na uptake (2004). The present results clearly show that Na uptake could be reduced through Si treatment. Similar results have been achieved in investigations by Epstein (2001), Liang *et al.* (2003), Gong *et al.* (2003, Chai *et al.* (2010), and Bae *et al*. (2012).

Shoot growth and leaf firing decreased as salinity levels increased (Fig. 7 and Table 1). Horst and Taylor (1983) stated that growth declines to 50% when the concentration of salt reaches 11 dS m^{-1} in 44 cultivars of Kentucky bluegrass, which was approximately similar to the value obtained in this study. An adverse result was reported by Alshammary *et al.* (2004) who stated that shoot growth decreased by 50% at 5.5 dS m^{-1} concentration of salinity (a mixture of NaCl and $CaCl_2$) in Kentucky bluegrass.

Under high salinity levels, cell expansion could be reduced by accumulation of salts in cell walls which would effectively reduce cell turgor and consequently retard growth (Oertli, 1968; Flower and Yeo, 1986). A decrease in growth at higher sodium concentrations due to a decrease in the uptake of K^+ and Ca^{2+} has also been reported (Sairam and Tyagi, 2004).

Si increased chlorophyll content, RWC, and visual quality under non saline water and low concentrations of salinity in both short- and long-term exposures. Proline content and electrolyte leakage increased in response to increasing salinity levels. K^+ concentration in shoots and roots increased as a result of Si application. Shoot number and shoot length dramatically decreased under higher salinity levels while Si application increased shoot number and shoot length under low concentration of salt stress. In general, silicate fertilizer is recommended for turfgrass management against environmental stresses such as salinity, high or low temperatures, drought and heavy metals.

References

ADATIA M.H., BESFORD R.T., 1986 - *The effects of silicon on cucumber plants grown in recirculating nutrient solution*. - Ann. Bot., 58: 343-351.

AGARIE S., HANAOKA N., UENO O., MIYAZAKI A., KUBOTA F., AGATA W., KAUFMAN P.B., 1998 - *Effects of silicon on tolerance to water deficit and heat stress in rice plants* (Oryza sativa *L.) monitored by electrolyte leakage*. - Plant Prod. Sci., 1: 96-103.

AHMAD R., ZAHEER S.H., ISMAIL S., 1992 - *Role of silicon in salt tolerance of wheat* (Triticum aestivum L.). - Plant Sci., 85: 43-50.

ALSHAMMARY S.F., QIAN Y.L., WALLNER S.J., 2004 - *Growth response of four turf grass species to salinity*. - Agric. Water Manag., 66: 97-111.

ASHRAF M., FOOLAD M.R., 2007 - *Roles of glycine betaine and proline in improving plant abiotic stress resistance*. - Environ. Exp. Bot., 59: 207-216.

BAE E.J., LEE K.S., HUH M.R., LIM C.S., 2012 - *Silicon significantly alleviates the growth inhibitory effects of NaCl in salt-sensitive 'Perfection' and 'Midnight' Kentucky bluegrass (*Poa pratensis L.*).* - Hort. Environ. Biotechnol., 53: 477-483.

BARCELÓ J., GUEVARA P., POSCHENRIEDR C., 1993 - *Silicon amelioration of aluminium toxicity in teosinte (*Zea mays L. *ssp. Mexicana).* - Plant Soil, 154: 249-255.

BARTLES D., SUNKAR R., 2005 - *Drought and salt tolerance in plants*. - Crit. Rev. Plant Sci., 24: 23-58.

BATES L.S., 1973 - *Rapid determination of free proline for water-stress studies*. - Plant Soil, 39: 205-207.

BRADBURY M., AHMAD R., 1990 - *The effect of silicon on the growth of* Prosopis juliflora *growing in saline soil*. - Plant Soil, 125: 71-74.

CHAI Q., SHAO X., ZHANG J., 2010 - *Silicon effects on* Poa pratensis *responses to salinity*. - Hort. Science, 45: 1876-1881.

CHAMPAN H.D., PRATT P.F., 1982 - *Methods of plant analysis,* pp. 60-193. In: CHAMPAN H.D., and P.F. PRATT D.F. (eds.) *Methods of analysis for soils, plants and water*. Chapman Publishers, Riverside, CA, USA.

CHEN D., YIN L., DENG X., WANG S., 2014 - *Silicon increases salt tolerance by influencing the two-phase growth response to salinity in wheat (*Triticum aestivum L.*)*. - Acta Physiol. Plant., 36: 2531-2535.

DATNOFF L.E., RUTHERFORD B.A., 2003 - *Accumulation of silicon by bermudagrass to enhance disease suppression of leaf spot and melting out*. - Environ. Exp. Bot., 53: 29-37.

DATNOFF L.E., RUTHERFORD B.A., 2004 - *Effects of silicon on leaf spot and melting out in bermudagrass*. - Golf Course Manage., 5: 89-92.

DATNOFF L.E., SEEBOLD K.W., CORREA VICTORIA F.J., 2001 - *The use of silicon for integrated disease management: Reducing fungicide applications and enhancing host plant resistance*, pp. 171-184. - In: DATNOFF L.E., G.H. SNYDER, and G.H. KORNDORFER (eds.) *Silicon in agriculture*. Elsevier Science, Amsterdam, The Netherlands, pp. 424.

EPSTEIN E., 1999 - *Silicon*. - Annu. Rev. Plant Physiol., 50: 641-664.

EPSTEIN E., 2001 - *Silicon in plants: Facts vs. concepts*, pp. 1-15. - In: DATNOFF L.E., G.H. SNYDER, and G.H. KORNDORFER (eds.) *Silicon in agriculture*. Elsevier Science, Amsterdam, The Netherlands, pp. 424.

FLOWERE T.J, YEO A.R., 1986 - *Ion relations of plants under drought and salinity*. - Aust. J. Plant Physiol., 13: 75-91.

FRY J.D., HUANG B., 2004 - *Applied turfgrass science and physiology*. - John Wiley and Sons, Hoboken, NJ, USA, pp. 320.

GAO X., ZOU C., WANG L., ZHANG F., 2004 - *Silicon improves water use efficiency in maize plants.* - J. Plant

Nutr., 27: 1457-1470.

GONG H.J., CHEN K.M., CHEN G.C., WANG S.M., ZHANG C.L., 2003 - *Effect of silicon on growth of wheat and its antioxidative enzymatic system*. - Chin. J. Soil Sci., 34: 55-57.

GUO W., ZHU Y.G., LIU W.J., LIANG Y.C., GENG C.N., WANG S.G., 2007 - *Is the effect of silicon on rice uptake of arsenate related to internal silicon concentrations, iron plaque and phosphate nutrition*? - Environ. Pollut., 148: 251-257.

HATTORI T., INANGA S., TANIMOTO E., LUX A., LUXOVA M., LUX A., 2005 - *Application of silicon-induced changes in viscoelastic properties of sorghum root cell walls*. - Plant Cell Physiol., 44: 743-749.

HORST G.L., TAYLOR R.M., 1983 - *Germination and initial growth of Kentucky bluegrass in soluble salts*. - Agron. J., 75: 679-681.

IWASAKI K., MAIER P., FECHT M., HORST W.J., 2002 - *Leaf apoplastic silicon enhances manganese tolerance of cowpea* (Vigna unguiculata). - J. Plant Physiol., 159: 167-173.

LIANG Y.C., 1998 - *Effects of Si on leaf ultrastructure, chlorophyll content and photosynthetic activity in barley under salt stress*. - Pedosphere, 8: 289-296.

LIANG Y.C., 1999 - *Effects of silicon on enzyme activity and sodium, potassium and calcium concentration in barley under salt stress*. - Plant Soil, 209: 217-224.

LIANG Y.C, CHEN Q., LIU Q., ZHANG W.H., DING R.X., 2003 - *Exogenous silicon (Si) increases antioxidant enzyme activities and reduces lipid peroxidation in roots of salt-stressed barley* (Hordeum vulgare *L.).* - J. Plant Physiol., 160: 1157-1164.

LIANG Y.C, DING R.X., 2002 - *Influence of silicon on micro distribution of mineral ions in roots of salt-stressed barely as associated with salt tolerance in plants*. - Sci. China (Series C), 45: 298-308.

LIANG Y.C., SHEN Q.R., SHEN Z.G., MA T.S., 1996 - *Effects of silicon on salinity tolerance of two barley genotypes*. - J. Plant Nutr., 19: 173-183.

LIANG Y.C., SUN W.C., ROMHELD V., 2005 a - *Effects of foliar- and root-applied silicon on the enhancement of induced resistance to powdery mildew in* Cucumis sativus. - Plant Pathol., 54: 678-685.

LIANG Y.C., SUN W.C., ZHU Y.G., CHRISTIE P., 2007 - *Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants, a review*. - Environ. Pollut., 147: 422-428.

LIANG Y.C., ZHANG W.H., CHEN Q., DING R.X., 2005 b - *Effects of silicon on H^+-ATPase and H^+- PPase activities, fatty acid composition and fluidity of tonoplast vesicles from roots of salt-stressed barley* (Hordeum vulgare L.). - Environ. Exp. Bot., 53: 29-37.

LUX A., LUXOVA M., ABE J., TANIMOTO E., HATTORI T., INANAGA S., 2003 - *The dynamics of silicon deposition in the sorghum root endodermis*. - New Phytol., 158: 437-441.

LUX A., LUXOVA M., HATTORI T., INANAGA S., SUGIMOTO Y., 2002 - *Silicification in sorghum (*Sorghum bicolor*) cultivars with different drought tolerance*. - Physiol. Plant., 115: 87-92.

MA C.C., LI Q.F., GAO Y.B., XIN T.R., 2004 - *Effects of silicon application on drought resistance of cucumber plants*. - Soil Sci. Plant Nutr., 50: 623-632.

MA J.F., 2004 - *Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses*. - Soil Sci. Plant Nutr., 50: 11-18.

MA J.F., YAMAJI N., 2006 - *Silicon uptake and accumulation in higher plants*. - Trends Plant Sci., 11: 392-397.

MARCUM K.B., 2002 - *Growth and physiological adaptations of grasses to salinity stress*. - Handbook of Plant and Crop Physiology, pp. 623-636.

MARCUM K.B., MURDOCH C.L., 1990 - *Growth responses, ion relations, and osmotic adaptations of eleven C_4 turfgrasses to salinity*. - Agron. J., 82: 892-896.

MARSCHNER H., PART I., 1995 - *Nutritional physiology,* pp. 18-30. - In: MARSCHNER H. (ed.) *Mineral nutrition of higher plants*, 2nd ed., Academic Press Limited, London, UK, pp. 890.

MATEOS-NARANJO E., ANDRADES-MORENO L., DAVY A.J., 2013 - *Silicon alleviates deleterious effects of high salinity on the halophytic grass* Spartina densiflora. - Plant Physiol. Biochem., 63; 115-121.

MATOH T., KAIRUSMEE E., 1986 - *Salt-induced damage to rice plants and alleviation effect of silicate*. - Soil Sci. Plant Nutr., 32: 295-304.

MUNNS R., 2002 - *Comparative physiology of salt and water stress*. - Plant Cell Environ., 25: 239-250.

MUNNS R., TESTER M., 2008 - *Mechanisms of salinity tolerance*. - Annu. Rev. Plant Biol., 59: 651-681.

NEETHIRAJAN S., GORDON R., WANG L., 2009 - *Potential of silica bodies (Phytoliths) for nanotechnology*. - Trends Biotech., 27:461-467.

NEPOMUCENO A.L., OOSTERHUIS D.M., STEWART J.M., 1998 - *Physiological responses of cotton leaves and roots to water deficit induced by polyethylene glycol*. - Environ. Exp. Bot., 40: 29-41.

OERTLI J.J., 1968 - *Extracellular salt accumulation, a possible mechanism of salt injury in plant.* - Agrochemica, 12: 461-465.

RAFIQ M., 1990 - *Soil resources and soil related problems in Pakistan. Soil physics: application under stress environments*. - Proceedings of the International Symposium on Applied Soil Physics in Stress Environments, Islamabad, Pakistan, 22-26 January 1989. Pakistan Agricultural Research Council (PARC), pp. 16-23.

RANGANATHAN S., SUVARCHALA V., RAJESH Y., SRINIVASA PRASAD M., PADMAKUMARI A., VOLETI S., 2006 - *Effects of silicon sources on its deposition, chlorophyll content, and disease and pest resistance in rice*. - Biol. Plant., 50: 713-716.

RICHMOND K.E., SUSSMAN M., 2003 - *Got silicon? The non-essential beneficial plant nutrient*. - Curr. Opin. Plant Biol., 6: 268-272.

ROMERO-ARANDA M.R., JOURADO O., CUARTERO J., 2006 - *Silicon alleviates the deleterious salt effects on tomato plant growth by improving plant water status*. - J. Plant. Physiol., 163: 847-855.

SAADALLA M.M., SHANAHAN J.F., QUICK J.S., 1990 - *Heat tolerance in winter wheat: I. Hardening and genetic effects on membrane thermostability*. - Crop Sci., 30: 1243-1247.

SAINI R.S., SHARM K.D., DHANKHAR O.P., KAUSHIK R.A., 2001 - *Laboratory manual of analytical techniques in horticulture*. - Published by Agrobios, India, pp. 49-50.

SAIRAM R.K., RAO K.V., SRIVASTAVA G.C., 2002 - *Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration*. - Plant Sci., 163: 1037-1046.

SAIRAM R.K., TYAGI A., 2004 - *Physiology and molecular biology of salinity stress tolerance in* plants. - Curr. Sci. (Bangalore), 86: 407-421.

SHU L.Z., LIU Y.H., 2001 - *Effects of silicon on growth of maize seedlings under salt stress*. - Agro-Environ. Prot., 20: 8-40.

TAHIR M.A., RAMATULLAH A., AZIZ T., ASHRAF M., 2010 - *Wheat genotypes differed significantly in their response to Si nutrition under salinity stress.* - J. Plant Nutr., 33: 1658-1671.

TUNA A.L. KAYA C., HIGGS C.D., MURILLO-AMADOR B., AYDEMIR S., GIRGIN A.R., 2008 - *Silicon improves salinity tolerance in wheat plants*. - Environ. Exp. Bot., 62: 10-16.

WANG L., QING N., LI M., ZHANG F., ZHUANG J., YANG W., LI T., WANG Y., 2005 - *Biosilicified structures for cooling plant leaves: a mechanism of highly efficient mid infrared thermal emission*. - Appl. Phys. Lett., 87: 194105.

WANG S.Y., GALLETTA G.J., 1998 - *Foliar application of potassium silicate induces metabolic changes in strawberry plants*. - J. Plant Nutr., 21: 157-167.

YEO A.R, FLOWERS S.A., RAO G., WELFARE K., 1999 - *Silicon reduces sodium uptake in rice* (Oryza sativa L.) *in saline condition and this is accounted for by a reduction in the transpirational by pass flow*. - Plant Cell Environ, 22: 559-565.

ZHU Z., WEI G., LI J., QIANG Q., YU J., 2004 - *Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt-stressed cucumber (*Cucumis sativus L.*)*. - Plant Sci., 167: 527-533.

ZWIENIECKI M.A., MELCHER P.J., HOLBROOK N.M., 2001 - *Hydrogel control of xylem hydraulic resistance in plants*. - Science, 291: 1059-1062.

Adv. Hort. Sci., 2016 30(2): 95-102 DOI: 10.13128/ahs-19135

Ultrastructural changes in potato (*Solanum tuberosum*) under NaCl mediated salinity stress *in vitro*

B. Al-Safadi (*), M. Nakar
Department of Molecular Biology and Biotechnology, Atomic Energy Commission of Syria, PO Box 6091, Damascus, Syria.

Key words: flavanone glycoside, NaCl, *Solanum tuberosum*, trichomes, ultrastructural changes.

Abstract: Histological analysis was employed to investigate the way potato plants (*Solanum tuberosum* cv. Draga and Spunta) face salinity stress. Different concentrations of NaCl (50, 100, 150, 200 and 250 mM) were used on potato plantlets growing *in vitro* to simulate salinity stress condition. Potato plants treated with 50 and 100 mM concentrations of NaCl went into the osmotic stage, and responded with changes: the flavone naringin was created and accumulated in the cells of the aerial parts, and a different type of trichome was observed, in addition to the original types, in potato plants treated with concentration 100 mM. This new type of trichome appeared similar to type B trichomes therefore they were called "type B-like trichomes". While no substance was exudated from these trichomes in cv. Draga, the trichomes, in cv. Spunta, green droplets were noted on the glandular vesicle. Furthermore, the non glandular trichomes had some swollen stem cells, and branched ones were also observed. Thanks to these new trichomes, the plants had increased leaf pubescence.

1. Introduction

Plants frequently face adverse environmental conditions, such as drought, salinity, chilling, freezing, and high temperatures which may delay growth and development, reduce productivity and, in severe instances, cause plant death (Krasensky and Jonak, 2012). Salt shock is an extreme form of salt stress resulting from the sudden exposure of plants to high levels of salinity (Shavrukov, 2013). Cellular responses to stress include adjustments in the membrane system, modifications in the cell wall architecture, and changes in cell cycle and cell division. These responsive mechanisms help plants cope with their surrounding environment and tolerate these stresses (Sairam and Tyagi, 2004; De Oliveira *et al*., 2013).

Plant defense mechanisms against biotic and abiotic stresses can be either constitutive (continuous) or inducible under stress conditions (Freeman and Beattie, 2008). In the case of low level salinity stress, for example, the cells undergo osmotic phase due to the none lethal salinity. As a result, they accumulate organic non-toxic solutes such as sugars, proline, mannitol, sorbitol, and amino acids in the vacuoles of the cytoplasm, even if cells do not produce such compounds in normal conditions (Läuchli and Grattan, 2007; De Oliveira *et al*., 2013). These solutes are called osmolytes, and the reason cells amass them is to achieve osmotic balance and to protect enzyme activity. Furthermore, they can play a role as scavenger of oxygen-free radicals produced by salinity (Shannon, 1997; Sairam and Tyagi, 2004; Zhu, 2007; Etehadnia, 2009; De Oliveira *et al*., 2013).

Other compounds can also play a role in plant tolerance to stresses. The flavonoid compounds, for example, perform as free radical scavengers and antioxidants against oxidative damage during exposure to various biotic and abiotic stresses such as heavy metals, drought, salinity, excess solar radiation etc. (Tattini *et al*., 2000; Ali and Abbas, 2003; Brown, 2005; Zhu, 2007; Chutipaijit *et al*., 2009; Samantal *et al*., 2011).

Trichomes comprise another type of plant defense response and they play mechanical and chemical roles in controlling water loss by transpiration, increasing tolerance to extreme temperatures, protecting plants against attacks by herbivores, and

(*) Corresponding author: ascientific@aec.org.sy
Received for publication 10 September 2015
Accepted for publication 13 May 2016

reducing excessive radiation (Gonzales *et al*., 2008; Kang *et al*., 2010; Adebooye *et al*., 2012). *Solanum* species are well-known for employing their trichomes (especially the glandular types) against insects, for example, the resistance of wild potato cultivars against Colorado potato beetle, potato tuber moths, the parasitoid *Copidosoma koehleri* (Blanchard) (Pelletier *et al*., 2013), green peach aphid (Myzus persicae Sulzer) (Vallejo *et al*., 1994), and potato leafhopper (Medeiros and Tingey, 2006). The role of potato trichomes in abiotic stress tolerance has not been reported. However, Tattini *et al*. (2002) found an integrated role of glandular trichomes and flavonoid glycosides in the mechanisms of acclimation of *Phillyrea latifolia* to excess solar radiation.

Tuber-bearing *Solanum* species have two major types of trichomes: glandular and non glandular. Glandular trichomes are recognized as type A or type B and have been extensively studied (Pelletier *et al*., 2013). Type A has a short stalk and a four-lobed glandular head which contains phenolic compounds, while type B is characterized by its long stalk and small glandular vesicle which continuously exudate acyl sugar secretions. Wild potato *Solanum berthaultii* bears all types of trichomes, some, or none (Pelletier *et al*., 2013), but some commercial *Solanum tuberosum* cultivars, for example Elba and Allegeny, have non-glandular and type A glandular trichomes only (Medeiros and Tingey, 2006).

The aim of the present study was to use histological analysis to investigate how potato (*Solanum tuberosum*) responds to salinity shock.

2. Materials and Methods

Plant material

Tubers from the potato cultivars Draga and Spunta were obtained from General Organization for Seed Multiplication (Aleppo, Syria). Draga variety, a cross SVP 50-2017 x MPI 19268 (HZPC, Netherlands) is an early variety, consistent performer, with medium yield, medium to large uniform tubers, round shape, cream-coloured skinned with creamy-white flesh and medium depth eyes (EUROGROW, 2011).

Spunta variety, a cross Béa x USDA 96-56 (Higgins Agriculture Ltd) is a medium-early ripening variety, with very high yield, long, slightly kidney-shaped, bulky tubers and shallow eyes (Ahdb, 2011).

Explants (nodal segments containing one or two buds) were prepared as previously described (Al-Safadi and Arabi, 2003, 2007).

Salinity stress

In order to simulate salinity stress *in vitro*, the explants were grown in tubes containing MS medium (Murashige and Skoog, 1962) with different concentrations of NaCl (50, 100, 150, 200, 250 mM) and grown as described by Al-Safadi and Arabi (2007).

Histo-anatomical study

Six plants from each salinity treatment were examined. The whole potato plant (shoot, mature leaves, root) and peels of orange fruit were prepared as follows: The samples were fixed in Carnoy's solution (3 Ethyl alcohol: 1 acetic acid) for 2 h, then transferred to 70% alcohol and stored at 4°C until analyses. Cross-sections were prepared manually by using a blade razor. Some sections were stained with Safranin O for 5 min (Al-khatib *et al*., 1995; Tiță *et al*., 2010); for sample examination, a bright field microscope (Nikon Eclipse 80i) was used and photos were captured by a digital camera (Nikon DS-Ri1).

Identification of the crystals

Solubility. Five different plants from each salinity treatment were used. Two leaves per plant were taken and immersed in hot water (90°C) for 10 min, and grapefruit juice was diluted with water (50%) and heated to (90°C) for 10 min. The crystals were then studied as cross-sections under Nikon Eclipse 80i microscope to observe whether any dissolution occurred.

Reacting to ferric chloride. Since physiological parameters showed no significant differences (data not shown) between plants treated with 50 mM and those treated with 100 mM NaCl, and for simplicity purposes, only leaves and cross-sections of plants treated with 100 mM of NaCl, plus the control, were soaked in a solution of 1% Ferric Chloride and heated at 90°C for 3-4 min, then left at room temperature for 10 min, to observe the change in colors.

Crystallization. To obtain and compare the sugar crystals under a Nikon Eclipse 80i microscope, Carnoy's solution was added for 2 h to the samples for comparison: potato leaves and cross-sections of control and those treated with 100 mM NaCl, the pericarp of orange fruit and grapefruit juice.

3. Results

Our first observation when we studied the tissues of plants treated with 150, 200 and 250 mM of NaCl

was that most of the cells in studied plant parts (roots, shoots, leaves) suffered plasmolysis. This means that the cells were already dysfunctional and the plants were no longer in the tolerance stage but had shifted to the toxic stage, when ions accumulate up to fatal levels in the cytoplasm and cells cannot overcome the damage from accumulating solutes (Lauchli and Grattan, 2007; De Oliveira *et al*., 2013). On the contrary, at concentrations of 50 and 100 mM, plasmolysis was limited, therefore we focused on these two treatments to detect changes in the cytoplasm. Consequently, when referring to treated plants we intend those treated with concentrations of 50 and 100 mM only.

Studying cross-sections of the stems and leaves of the treated plants from both cultivars (Draga and Spunta) revealed some interesting and curious structures which did not exist in the tissues of control plants. Scattered cells, with rosette-shaped crystals inside, were observed in the cortex layer of the stems and in the spongy mesophyll layer of the leaves (Fig. 1 a, b).

The crystals resembled the shape of flavanone glycosides found in citrus (hesperidin and naringin) when crystallized. Hesperidin is a sweet tasting glycoside usually found in the mesocarp layer (the white pith, Albedo) of the pericarp (the peel) of orange fruit *Citrus auratium* (Hendrickson and Kesterson, 1956; Al-khatib *et al.*, 1995), while naringin is a principal flavonoid in grapefruit and gives it its bitter taste and can be found in the juice (Kesterson and Hendrickson, 1953). Both glucosides are very similar in appearance to crystals under the microscope, as the crystal needles agglomerate in a rosette pattern and have very close chemical structures (Kesterson and Hendrickson, 1953; Hendrickson and Kesterson, 1956).

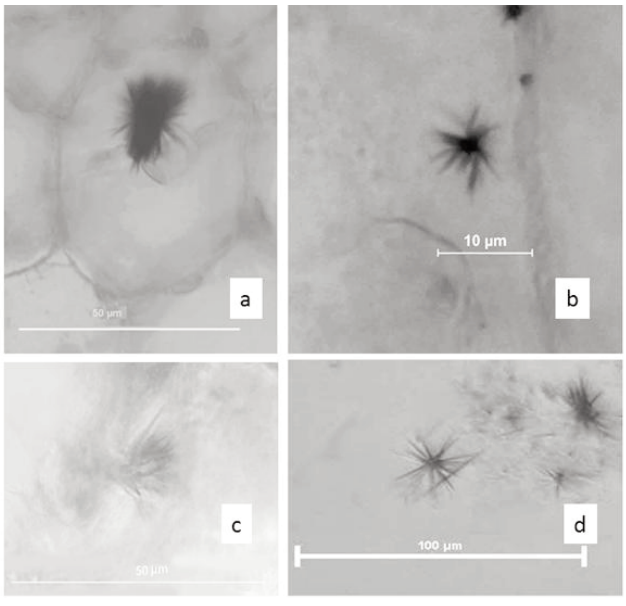

Fig. 1 - Rosette-shapedcrystals with caramel color inside cells of the stem's cortex of treated plants with salt concentration 100 mM. A) in Draga B) in Spunta C) Pale Hesperidin crystal in the mesocarp layer of the peel of orange fruit, D) Tan naringin crystals in the juice of grapefruit; the resemblance between the two structures is obvious.

To confirm our analysis, we took cross-sections of the pericarp of orange fruit and grapefruit juice treated with Carnoy's solution. Comparing the results to those in the treated potato, we found the two shapes to be similar (Fig. 1 c, d). These crystals were not seen in tissues not fixed with Carnoy's solution. This is considered to be further evidence that these were crystals of a flavanone glycoside dissolved in the cytoplasm and only could be seen under microscope when crystallized by adding alcohol, acetic acid or cooling (Kesterson and Hendrickson, 1953; Hendrickson and Kesterson, 1956; Al-khatib *et al*., 1995).

Moreover, in the control plants, these crystals were not found in the tissues before or after treating with Carnoy's solution or cooling.

The crystals, in addition to their rosette shape, had specific Naringin's crystal features: i) brownish tan color while Hesperidin crystals are colorless or pale yellow as seen in the mesocarp layer of the peel of orange fruit (Fig. 1 a-d) (Kesterson and Hendrickson, 1953; Hendrickson and Kesterson, 1956); ii) when interacted with ferric chloride, the color of the leaves of treated plants changed from pale grey to light caramel. On the contrary, when the leaves of the control plants were immersed in ferric acid no difference in color could be seen (Fig. 2 a, b). The color of the crystals in the cross sections of the leaves changed from brownish caramel to a very dark red wine color (Fig. 3 a, b). The interaction of ferric chloride with Naringin, especially at a sufficient level of concentration, caused the change in color which gets darker with the increase in concentration, becoming black at very high concentrations (Kesterson and Hendrickson, 1953; Sinclair, 1972; Radhakrishnan *et al.*, 2013); iii) when the leaves were immersed in hot water for 10 min, the crystals in the cross sections and grapefruit juice started diffusing (Fig. 3 c-e); it is known that Naringin's crystals are much more soluble in hot than cold water, unlike Hesperidin which does not dissolve in hot water (Kesterson and Hendrickson, 1953; Hendrickson *et al*., 1954; Hendrickson and Kesterson, 1956). Therefore, we assume that the crystals were

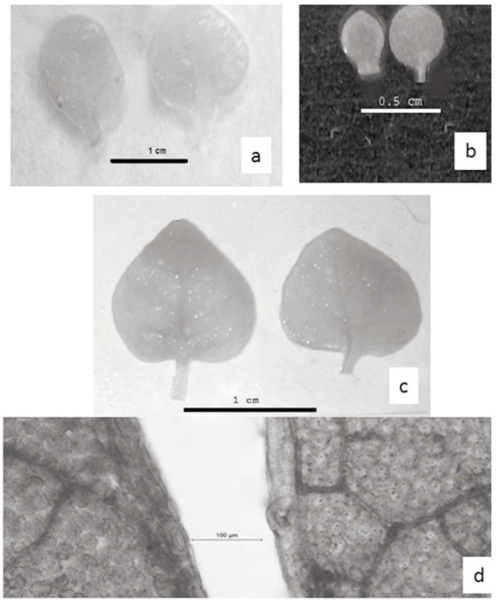

Fig. 2 - Leaves of treated plants, the leaf immersed in ferric acid (left) and not immersed (right) (differences in color are noticeable): A) Draga, B) Spunta C) Draga control plant leaf: immersed in ferric acid (left) and not immersed (right) (no changes in color could be seen). D) microscope photo showing parts of 'Spunta' control plant leaf (left) and treated plant leaf (right) , both were immersed in ferric acid and dark red dots (naringin crystals) could be seen only in the cells of treated plant leaves.

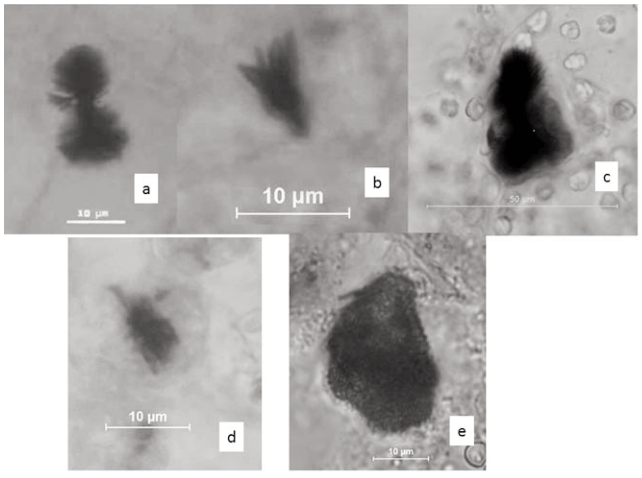

Fig. 3 - Dark red crystals from the leaves of treated plants after ferric acid reaction: A) from cultivar Draga; B) from cultivar Spunta; C-D) Naringin crystals with melted edges after boiling in water: C) from cultivar Draga; D) from cultivar Spunta, E) from grapefruit juice.

Naringin.

The control plants and those treated with the 50 mM concentration had only straight non-glandular and Type A trichomes on the aerial parts. However, surprisingly, type B-like trichomes were observed in addition to the other type of trichomes in plants treated with the 100 mM concentration. While they had type B trichomes, there was no evidence that they secreted any substance in 'Draga' plants,

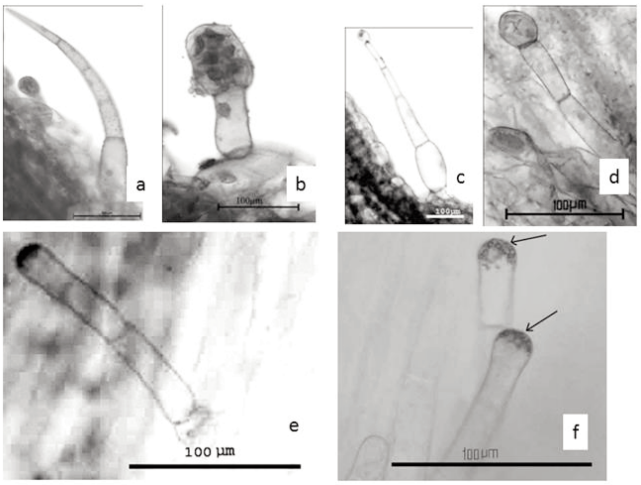

Fig. 4 - Photos of trichomes colored with safranin O leaves of: A-B) Control plants; A. conical straight non-glandular, B) type A. C-D) 'Draga' plants treated with 100 mM salt concentration; C) a photo of whole type B-like trichomes, D) empty glandular vesicle of the type B-like trichomes without any secrets . E-F) 'Spunta' plants treated with 100 mM salt secretions; E) a photo of whole type B-like trichomes; F) the glandular vesicle of type B-like trichomes secreting green droplets (arrows). Bars=100 µm.

although green droplets were noticeable on glandular vesicles in 'Spunta' plants (Fig. 4).

In control plants and those treated with 50 mM NaCl, non-glandular trichomes were straight, linear with conical shape, and the size of the stem cells were gradually reduced from the base cell to the top. However, in the 100 mM NaCl treatment, the trichomes were very long and branched with swollen stem cells on very large base cells (Fig. 5).

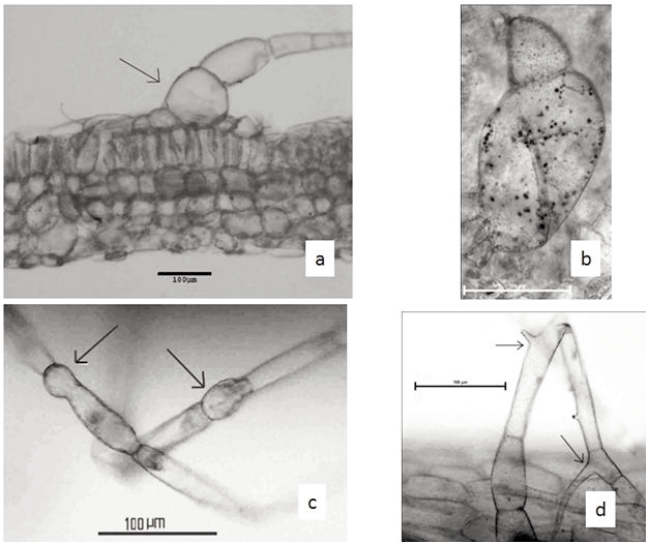

Fig. 5 - Photos of non-glandular trichomes on the adaxil surface of safranin O.colored leaves of plants treated with 100 mM salt concentration; A) branched trichomes (Draga) (arrows), B) two non-glandular trichomes with swollen stem cells (Draga) (arrows), C-D) a non- glandular trichome with very large base cell (arrow) compared to the adjacent cells: C) from Draga, D) from Spunta. (arrows). Bars=100 µm.

4. Discussion and Conclusions

In this study we found that potato plants treated with low concentrations of salinity (50 and 100 mM) went into the osmotic stage. Consequently, cells in the aerial parts created a new substance, which was identified as 'Naringin' (the rosette-shaped crystals we found in the cortex) (Fig. 2 a, b). Naringin is a flavanone-7-*O*-glycoside between the flavanone naringenin and the disaccharide neohesperidose. It is mainly found in grapefruit to which it gives its typical bitter flavor (Belajova and Suhaj, 2004).

Flavonoids, such as catechin, epi-catechin, erodictyol, kaempeferol, and naringenin in different amounts, are very common in the cultivated potato *Solanum tuberosum* (Brown, 2005). It has been reported for rice (Chutipaijit *et al*., 2009), barley (Ali and Abbas, 2003) and many other plants (Samantal *et al*., 2011) that flavonoids accumulate in the tissues of stressed-plants. Furthermore, Gupta and Huang (2014) reported that anthocyanin (also a flavonoid) is accumulated in plants exposed to salt stress. Flavonoids become more soluble in the form of flavonoid glycoside and thus more effective (Chutipaijit *et al*., 2009; Samantal *et al*., 2011).

Therefore, we assume that the flavonone naringenin in treated potato became available in larger quantity than normal, as a result of the salinity stress. In potato, naringenin is a byproduct of the pathway of anthocyanin and flavonoles biosynthesis (Gramene, 2016). It is unclear, from our work, how naringin was synthesized and accumulated in the tissues of potato plants treated with NaCl. However, it is likely that under stress conditions, potato plants produced naringin through the naringenin glycoside biosynthesis pathway (Caspi *et al*., 2016), thus making it possible to visualize naringin under the microscope as crystals.

Our study also revealed that potato plants treated with 100 mM concentration resulted in an additional type of trichomes, to the original types (non-glandular and type A) (Fig. 4 c-f). These new trichomes were similar in shape (long stalk with glandular vesicle) to those in the wild type potato *Solanume berthaultii* (Vallejo *et al*., 1994), hence, we called them "type B-like trichomes". Here, we suggest several hypotheses to explain why potato plants formed these trichomes.

With regard to the genetics of type B trichome-density inheritance, we found that most of the studies in the literature were carried out on diploid potatoes. However, some researchers (Gibson, 1979; Mehlenbacher *et al*., 1983; Mehlenbacher *et al*., 1984; Vallejo *et al*., 1994; Jansky *et al*., 1999) have convergent conclusions, for example: a) the absence of type B trichomes is controlled by a few genes, at least one of them being recessive due to structural genomic differentiation; b) most of the variation among individuals of offspring of back-crosses studied were not due to heritable genetic differences; c) the inheritance of type B trichome density could be influenced by non nuclear genetic factors such as maternal cytoplasmic DNA, as suggested by Vallejo *et al*. (1994), and Jansky *et al*. (1999) who presumed that the cytoplasm in the somatic tetraploid hybrids they studied could repress the gene expression; d) in contrast, the droplet size of type B trichomes was highly heritable.

Therefore, we wonder if the genes responsible for the presence of type B trichomes were silenced in one way or another in cultivated potatoes, due to structural genomic differentiation during the hybridization, segregation and recombination processes. Especially in our case, 'Draga' and 'Spunta' are tetraploids with 2n = 4x = 48 chromosomes (Caprutoi *et al*., 2000, 2003). Additionally, the expression of these genes could be affected by changes in the cytoplasm (Vallejo *et al*., 1994; Jansky *et al*. 1999; Hanson and Bentolila, 2004). Therefore, we hypothesize (although more genetic studies are called for on this subject) that at least one of the genes that control the presence of type B trichomes was silenced or changed epigenetically, which affected its expression and, subsequently, the other genes could not be expressed during the evolution of the potato cultivars.

In the current study we did not investigate the genes involved in formation or activation of trichomes under salinity stress. However, other studies have looked into the *WRKY* transcription factors (TFs) family in plants which comprises numerous members that regulate genes involved in seed germination, seed dormancy, trichomes development, lignin biosynthesis, and both biotic and abiotic stress responses (Pnueli *et al*., 2002; Guillaumie *et al*., 2010; Wang *et al*., 2010). Some of the *WRKY* genes have been reported to be activated in response to various abiotic stresses including high salinity (Jianchao *et al*., 2015).

Furthermore, it has been documented that salinity could induce modifications in the genome, like other abiotic stresses, by making changes in the histone modification pattern, thus activating some genes and/or silencing others (Kapazoglou and

Tsaftarism, 2011; Kim *et al*., 2012; Pecinka and Scheid, 2012). It could also induce irregularities in the mitotic division and aberrations in the mitotic chromosomes (Barakat, 2003). Salinity may thereby change the genome epigenetically (changes in the chromatin) and genetically (changes in DNA). It is also possible that salinity affected the resultant proteins of those genes; it has been reported that salinity can make changes in the protein patterns (Barakat, 2003; Kim *et al*., 2012).

On the other hand, without regard to genetic reasons, Gonzales *et al*. (2008) referred to some reports which presumed that plants under salt stress could use the glandular trichomes as facultative salt glands and to eliminate excessive salt (Gonzales *et al*., 2008). In our case, it is possible that a conversion from non-glandular to glandular trichomes under salt stress occurred. However, as we indicated in the results section of this work, we did not observe any substance exudates from the type B-like trichomes in 'Draga', while in 'Spunta', these trichomes exuded some kind of green droplets. If the droplets had the same chemical makeup as the exudate from the glandular vesicle of type B trichomes (sucrose esters viscous droplets) (Pelletier *et al*., 2013), it would mean that they may form an irregular local layer on the surface of the leaves; it is known that these trichomes secrete droplets continuously (Pelletier *et al*., 2013; Wollenweber *et al*., 2005). This layer would play a role of additional covering to coat and reduce the absorbance of radiation and water loss.

Furthermore, we detected some non glandular trichomes with swollen stem cells (Fig. 5 a, b, c). The same results were reported by Kang *et al*. (2010) when they studied trichome distortion caused by hairless mutation of tomato (*Solanum lycopersicum*). They found that type I trichomes (equivalent to type B trichomes in potato) on the *hl* mutant were crooked and had highly swollen stem cells (Kang *et al*., 2012). In contrast, Gomes *et al*. (2011), after treating *Salvinia auriculata* Aubl. with different doses of NaCl salt, found that the trichomes became more slender as the dose was increased.

We also noticed branched trichomes (Fig. 5 d) which are probably caused by the extensions in the swell of stem cells, giving the appearance of branching. Kang *et al.* (2010) reported the same observation in tomato affected by hairless mutation (Kang *et al*., 2012). Also, irregularity in trichome shape was reported in some plants which were exposed to salt stress (Adebooye *et al*., 2012). Nevertheless, the branched trichomes are more effective in protecting the plant from losing additional amounts of water by forming a shield against sunlight, thus maintaining a good quantity of water to adjust the turgor pressure in cells. Somehow, the branched trichomes and type B-like trichomes contributed to increasing leaf pubescence, which is a familiar phenomenon among some plants subjected to drought or salinity stress (Gonzales *et al*., 2008; Makbul *et al*., 2011; Adebooye *et al*., 2012).

In our study, potato gained new traits: naringin (the flavanone glycoside), branched non-glandular trichomes and type B-like trichomes. The new trichomes allow these plants to have increased leaf pubescence and perhaps different chemicals on the leaf surface, making them more resistant to insects.

We believe more research must be carried out on how salinity and drought could be exploited to produce more resistant plants. After all, as some research has pointed out, salinity does not always cause negative effects in plants (Shannon and Grieve, 1999).

In conclusion, potato plants (*Solanum tuberosum* L.) have mechanisms to help them tolerate salinity stress at the cellular, biochemical, and physiological levels. In the present study, some changes were observed in potato plants growing *in vitro*, including the creation of the "flavanone glycoside" naringin in the cells of the aerial parts (playing the role of antioxidant) and the formation of a new type of trichome. Further research is needed to study the role of these cellular changes in potato plants reacting to salinity stress.

Acknowledgements

The authors would like to thank the Director General of AECS and the Head of Molecular Biology and Biotechnology Department for their support. The assistance of N. Tafesh and I. Karajoli in conducting the experiments is much appreciated.

References

ADEBOOYE O.C., HUNSCHE M., NOGA G., LANKES C., 2012 - *Morphology and density of trichomes and stomata of* Trichosanthes cucumerina *(Cucurbitaceae) as affected by leaf age and salinity*. - Turk J. Bot., 36: 328-335.

AHDB, 2011 - *Spunta potato variety*. - http://varieties.ahdb.org.uk/varieties/view/SPUNTA.

ALI R.M., ABBAS H.M., 2003 - *Response of salt stressed*

barley seedlings to phenylurea. - Plant Soil Environ., 49: 158-162.

AL-KHATIB A., BAGDADI W., HAMAD I., SOLIMAN M., 1995 - *Botany for freshmen*. - University of Damascus' press, Damascus, Syria.

AL-SAFADI B., ARABI M.I.E., 2003 - In vitro *induction, isolation and selection of potato mutants resistant to late blight.* - J. Genet. Breed., 57: 359-364.

AL-SAFADI B., ARABI M.I.E., 2007 - In vitro *induction, isolation, and selection of potato mutants tolerant to salinity.* - Adv. Hort. Sci., 21(3): 127-132.

BARAKAT H., 2003 - *Interactive effects of salinity and certain vitamins on gene, expression and cell division*. - Int. J. Agri. Biol., 5: 219-225.

BELAJOVA E., SUHAJ M., 2004 - *Determination of phenolic constituents in citrus juices: method of high performance liquid chromatography*. - Food Chemistry, 86: 339-343.

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

CARPUTO D., BASILE B., CARDI T., FRUCIANTE L., 2000 - *Erwinia resistance in backcross progenies of* Solanum tuberosum *x* S. tarijense *and* S. tuberosum *(+)* S. commersonii *hybrids*. - Potato Res., 43: 135-142.

CARPUTO D., PARISI M., CONSIGLIO F., IOVENE M., CARUSO G., MONTI L., FRUSCIANTE L., 2003 - Aneuploid hybrids *from 5X-4X crosses in potato: chromosome number, fertility, morphology and yield*. - Amer . J. of Potato Res., 80(2): 93-101.

CASPI R., BILLINGTON R., FERRER L., FOERSTER H., FULCHER CA., KESELER I.M., KOTHARI A., KRUMMENACKER M., LATENDRESSE M., MUELLER L.A., ONG Q., PALEY S., SUBHRAVETI P., WEAVER D.S., KARP PD., 2016 - *The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of Pathway/Genome Databases*. - Nucleic Acids Res., 42: D471-480.

CHUTIPAIJIT S., CHA-UM S., SOMPORNPAILIN K., 2009 - *Differential accumulations of proline and flavonoids in indica rice varieties against salinity*. - Pak. J. Bot., 41: 2497-2506.

DE OLIVEIRA A.B., ALENCAR N.L.M., GOMES-FILHO E., 2013 - *Comparison between the water and salt stress effects on plant growth and development,* pp. 67-94. - In: AKINCI Ș. (ed.) *Responses of organisms to water stress.* InTech, Open Access Publisher, pp. 180.

ETEHADNIA M., 2009 - *Salt stress tolerance in potato genotypes*. - PhD Thesis, University of Saskatchewan Saskatoon.

EUROGROW, 2011 - *Draga potato variety*. - http://www.eurogrow.co.nz/varieties/draga.

FREEMAN B.C., BEATTIE G.A., 2008 - *An overview of plant defenses against pathogens and herbivores*. - The Plant Health Instructor, APS.

GIBSON R.W., 1979 - *The geographical distribution, inheritance, and pest-resisting properties of sticky-tipped foliar hairs on potato species*. - Potato Res., 22: 223-236.

GOMES M.A., SUZUKI M.S., DA CUNHA M., TULLII C.F., 2011 - *Effect of salt stress on nutrient concentration, photosynthetic pigments, proline and foliar morphology of* Salvinia auriculata *Aubl*. - Acta Limnol. Bras., 23: 164-176.

GONZALES W.L., NEGRITTO M.A., SUAREZ L.H., GIANOLI E., 2008 - *Induction of glandular and non-glandular trichomes by damage in leaves of* Madia sativa *under contrasting water regimes*. - Acta Oecologica, 33: 128-132.

GRAMENE, 2016 - Solanum tuberosum *Pathways: flavonoid biosynthesis*. - http://pathway.gramene.org.

GUILLAUMIE S., MZID R., MÉCHIN V., LÉON C., HICHRI I., DESTRAC-IRVINE A., TROSSAT-MAGNIN C., DELROT S., LAUVERGEAT V., 2010 - *The grapevine transcription factor WRKY2 influences the lignin pathway and xylem development in tobacco*. - Plant Mol Biol., 72(1-2): 215-234.

GUPTA B., HUANG B., 2014 - *Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization*. - Int. J. Genom., pp. 18.

HANSON R.M., BENTOLILA S., 2004 - *Interactions of mitochondrial and nuclear genes that affect male gametophyte development*. - The Plant Cell, 16: 154-169.

HENDRICKSON H., KESTERSON J.W., 1954 - *Hesperidin, the principal glycoside of oranges*. - Fla. Agr. Exp. Sta. Tech. Bul., 545: 1-43.

HENDRICKSON H., KESTERSON J.W., 1956 - *Purification of Naringin*. - Fla Agr Exp Sta J., 524: 149-152.

JANSKY S., AUSTIN-PHILLIPS S., McCARTHY C., 1999 - *Colorado potato beetle resistance in somatic hybrids of diploid interspecific solanum clones*. - HortScience, 34: 922-927.

JIANCHAO M., JING L., JIANMEI X., BINGBING D., XIAODONG H., JIANQUAN L., 2015 - *Genome-wide Identification of WRKY genes in the desert poplar* Populus euphratica *and adaptive evolution of the genes in response to salt stress.* - Evol. BioInform., 11(S1): 47-55.

KANG J.H., SHI F., JONES A.D., MARKS M.D., HOWE G.A., 2010 - *Distortion of trichome morphology by the hairless mutation of tomato affects leaf surface chemistry*. - J. Exp. Bot., 61: 1053-1064.

KAPAZOGLOU A., TSAFTARISM A., 2011 - *Epigenetic chromatin regulators as mediators of abiotic stress responses in cereals,* pp. 395-414. - In: SHANKER A., and B. VENKATESWARLU (eds.) *Abiotic stress in plants. Mechanisms and adaptations*. InTech Europe, Rijeka, Croatia, pp. 420.

KESTERSON J.W., HENDRICKSON H., 1953 - *Naringin, a bitter principal of grapefruit*. - Fla. Agr. Exp. Sta. Tech. Bul., 511: 1-35.

KIM J., TO T.K., ISHIDA J., MATSUI A., KIMURA H., SEKI M., 2012 - *Transition of chromatin status during the process of recovery from drought stress in* Arabidopsis thaliana. - Plant Cell Physiol., 53: 847-856.

KRASENSKY J., JONAK C., 2012 - *Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks.* - J. Exp. Bot.63(4): 1593-1608.

LÄUCHLI A., GRATTAN S.R., 2007 - *Plant growth and development under salinity stress*, pp. 1-32. - In: JENKS M.A., P.M. HASEGAWA, and JAIN S.M. (eds.) *Advances in molecular breeding toward drought and salt tolerant crops*. Springer, Dordrecht, The Netherlands, pp. 817.

MAKBUL S., GULER S.N., DURMUSN., GUVEN S., 2011 - *Changes in anatomical and physiological parameters of soybean under drought stress*. - Turk. J. Bot., 35: 369-377.

MEDEIROS A.H., TINGEY W.M., 2006 - Glandular trichomes *of* Sulanum berthaltii *and its hybrids with* Solanum tuberosum *affect nymphal emergence, development, and survival of* Empoasca faba (*Homoptera: Cicadellidae*). - J. Econ. Entomol., 994:1483-1489.

MEHLENBACHER S.A., PLAISTED R.L., TINGEY W.M., 1983 - *Inheritance of* glandular trichomes *in crosses with* Solanum berthaultii . - American Potato Journal, 60: 699-708.

MEHLENBACHER S.A., PLAISTED R.L., TINGEY W.M., 1984 - *Heritability of trichome density and droplet size in interspecific potato hybrids and relationship to aphid resistance*. - Crop Sci., 24: 320-322.

MURASHIGE T., SKOOG F., 1962 - *A revised medium for rapid growth and bioassays with tobacco tissue cultures*. - Physiol. Plant, 15: 473-497.

PECINKA A., SCHEID O.M., 2012 - *Stress-induced chromatin changes: A critical view on their heritability*. - Plant Cell Physiol., 53: 801-808.

PELLETIER Y., HORGAN F.G., POMPON J., 2013 - *Potato resistance against insect herbivores: Resources and opportunities*, pp. 439-462. - In: ALYOKHIN A., C. VINCENT, and P. GIORDANENGO (eds.) *Insect pests of potato*. Global perspectives on biology and management. Academic Press, Elsevier B.V., Oxford, UK, pp. 616.

PNUELI L., HALLAK-HERR E., ROZENBERG M., COHEN M., GOLOUBINOFF P., KAPLAN A., MITTLER R., 2002 - *Molecular and biochemical mechanisms associated with dormancy and drought tolerance in the desert legume Retama raetam*. - Plant J., 31: 319-330.

RADHAKRISHNAN I., SAMPATH S., KUMAR T. S., 2013 - *Isolation and characterization of enzyme Naringinase from* Aspergillus flavus. - Intl. J. of Adv. Biotec. and Res., 4: 208-212.

SAIRAM R.K., TYAGI A., 2004 - *Physiology and molecular biology of salinity stress tolerance in plants*. - Current Science, 86: 407-421.

SAMANTAL A., DAS G., DAS S.K., 2011 - *Roles of flavonoids in plants*. - Int. J. Pharm. Sci. Tech., 6: 12-35.

SHANNON M.C., 1997 - *Adaptation of plants to salinity*. - Adv. Agron., 60: 75-120.

SHANNON M.C., GRIEVE C.M., 1999 - *Tolerance of vegetable crops to salinity*. - Sci. Hortic., 78: 5-38.

SHAVRUKOV Y., 2013 - *Salt stress or salt shock: which genes are we studying?* - J. Exp. Bot., 64: 119-127.

SINCLAIR W.B., 1972 - *Composition of peel, Rag, and seeds*, pp. 200-280. - In: SINCLAIR W.B. (ed.) *The grapefruit: its composition, physiology, and product*. University of California Press, USA, pp. 660.

TATTINI M., GRAVANO E., PINELLI P., MULINACCI N., ROMANI, A., 2000 - *Flavonoids accumulate in leaves and glandular trichomes of* Phillyrea latifolia *exposed to excess solar radiation*. - New Phytologist, 148: 69-77.

TIȚĂ M.G., LUPULEASA D., MOGOŞANU G.D., 2010 - *Histo-anatomical researches on the leaf of* Fraxinus excelsior *L. species*. - Curr. Health Sci. J., 36: 232-234.

VALLEJO R.L., COLLINS W.W., MOLL R.H., 1994 - *Inheritance of A and B glandular trichome density and polyphenol oxidase activity in diploid potatoes*. - J. Amer. Soc. Hort. Sci., 119: 829-832.

WANG H., AVCI U., NAKASHIMA J., HAHN M.G., CHEN F., DIXON R.A., 2010 - *Mutation of WRKY transcription factors initiates pith secondary wall formation and increases stem biomass in dicotyledonous plants*. - Proc. Natl. Acad. Sci. USA, 107: 22338-22343.

WOLLENWEBER E., DORSAM M., DORR M., ROITMAN J.N., VALANT-VETSCHERA K.M., 2005 - *Chemodiversity of surface flavonoids in Solanaceae*. - Z Naturforsch C., 60: 661-670.

ZHU J.K., 2007 - *Plant salt stress*. - Encyclopedia of life sciences, John Wiley & Sons, Ltd, UK.

Adv. Hort. Sci., 2016 30(2): 103-109 DOI: 10.13128/ahs-19136

Effect of H_2O_2 pretreatment on the response of two seashore paspalum (*Paspalum vaginatum* Sw.) cultivars (Salam and Seaspray) to cold stress

S. Soufi [1 (*)], K. Ben Hamed [2], M. Arbaoui [1], N. Elbey [1], S. Rezgui [1], C. Abdely [2], T. Bettaeib [1]

[1] *Laboratoire des Sciences Horticoles, Département de Production Végétale, Institut National Agronomique de Tunisie, 43, Avenue Charles Nicolle, 1082 Tunis, Mahrajène, Tunisie.*

[2] *Laboratoire des Plantes Extremophiles, Centre de Biotechnologie de Borj Cedria, BP 901, 2050 Hammam Lif, Tunisia.*

Key words: cold stress, hydrogene peroxide, *Paspalum vaginatum*.

Abstract: Seashore paspalum is a warm season grass that requires few maintenance inputs. Expanded use of seashore paspalum could play a key role in making recreational sites more sustainable and environmentally. However, one key barrier to widespread Seashore paspalum use is a relative lack of winter hardiness. Under severe stress conditions, the antioxidant capacity may not be sufficient to minimize the harmful effect of oxidative injury. The search for signal molecules that mediate the stress tolerance is an important step in better understanding how plants acclimate to the adverse environment. This study aims to screen the responses of two *Paspalum vaginatum* cultivars (Salam and Seaspray) to local weather conditions and to study how to enhance its cold tolerance by a foliar pretreatment by hydrogen peroxide at low concentrations of 10 mM under controlled conditions. The current study provides evidence that exogenous H_2O_2 decreases the endogenous content of H_2O_2 and malondialdehyde in the first three days of exposure to cold stress in pretreated 'Seaspray' plants. in comparison to their control and pretreated 'Salam'. These results indicate that pretreatment with 10 mM H_2O_2 could improve the tolerance of seashore paspalum to cold stress, especially Seaspray cultivar which showed better response to cold stress compared to 'Salam'. Exogenous H_2O_2 could constitute a signaling molecule that significantly increases peroxidase relative density, and decreases MDA and H_2O_2 content.

1. Introduction

Seashore paspalum is among compatible warm-season turfgrasses used for recreational sites, such as golf courses, which requires low insecticide and fertilizer applications and is tolerant to salt (Duncan, 1997). Seashore paspalum is found between 35° N-S latitudes in the Americas and expands to several islands of the Caribbean-Atlantic-Pacific rim and the Mediterranean-African coastal areas. This species lacks winter hardiness (Duncan, 1997); it is sensitive to freezing or chilling due to its tropical and subtropical origins (Allen and Ort, 2001). A remarkable physiological distruption known as chilling injury is exihibited when plants of this species are exposed to temperatures below about 10 to 12°C (Lyons, 1973).

Low temperature may disrupt major components of photosynthesis including thylakoid electron transport, the carbon reduction cycle and controls stomatal conductance (Allen and Ort, 2001). It constitutes one main issue for the management of warm-season turfgrasses in tropical areas, usually causing yellowing and withering during the winter season (Xia *et al*., 2000). Exposure of plants to unfavorable growing conditions such as high temperature, heavy metals, drought, water availability, air pollutants, nutrient deficiency, or salt stress increases the production of reactive oxygen species (ROS) such as superoxide (O2.-), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH) and singlet oxygen (1O2). Usually ROS production can be coupled with development of oxidative injury and disruption of metabolic functions in plants (Mittler, 2002). The major generation site of reactive oxygen species (ROS) are reaction centers of PSI and PSII in chloroplast thylakoids (Asada *et al*., 1999). ROS may affect several cellular functions by damaging nucleic acids, oxidizing proteins, and causing lipid

(*) Corresponding author: sihemsoufi@yahoo.fr
Received for publication 22 December 2015
Accepted for publication 18 May 2016

peroxidation (LPO) (Foyer and Noctor, 2005). Depending on the delicate equilibrium between ROS production and scavenging at the proper site and time, ROS will act as damaging, or as signaling molecules (Gratão *et al*., 2005). Oxidative damage occurs when production of ROS exceeds the capacity of these scavenging systems. Components of the antioxidant defense system can be divided into enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GD), monodehydroascobate reductase (MDHAR), glutathione reductase (GR), and dehydroascorbate reductase (DHAR). Non-enzymatic antioxidants are ascorbate (AsA), glutathione (GSH) (both water soluble), carotenoids and tocopherols (lipid soluble) (Munné-Bosch and Falk, 2004). Among ROS, H_2O_2 is considered to be the best suited signaling molecule due to its stability and longer half-life. It is able to cross biological membranes and diffuses from cell to cell or can be transported long distances from its sites of origin in plants depending on the availability of environmental stimuli. Prasad *et al*. (1994) and Wahid *et al*. (2007) found that pre-treated H_2O_2 at the appropriate concentrations improved salt-tolerance and chilling-tolerance in wheat and maize seedlings respectively (Neto *et al*., 2005). These authors suggest that H_2O_2 signals the activation of antioxidants in seed. Hydrogen peroxide is thus hypothesized to be implicated in enhancing the chilling resistance of plants associated with the function of ROS scavenging systems.

The aim of the present study is to investigate whether H_2O_2 supply enhances low temperature resistance of seashore paspalum turfgrass. For this purpose, we used two cultivars of *Paspalum vaginatum*, Salam and Seaspray, the latter having better response to cold stress than the former (Arbaoui *et al*., 2010).

2. Materials and Methods

Field study

The study was carried out in Tunisia at INAT Tunisia (National Agronomy Institute) (36°82' N; 10°17' E; 13 m) in 2011-2012 on two mature turfs of seashore paspalum (*Paspalum vaginatum* Swartz cv. Salam and Seaspray). The swards were established in a semiarid area on slit-loam soil (40% clay, 30% silt and 25% sand) with 40 g of P_2O_5 45%, 40 g of K_2SO_4 as basic mineral fertilizer and pH 7.9. During the trial period, regular mowing and irrigation were applied to maintain a healthy turf. The experimental design was randomized block for each species with four replicates each of 1 m^2 area (Fig. 1). The conditions of relative humidity, total rainfall, and air temperature are reported in Table 1 from the beginning of the vegetative period to the end of the trial.

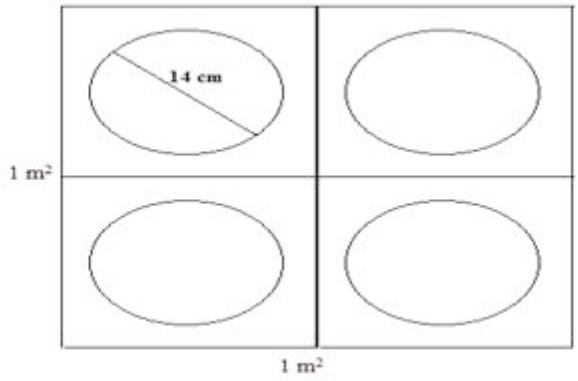

Fig. 1 - Schematic representation of one of the three experimental units with four replicates per species.

Table 1 - Total rainfall, relative humidity, and air temperature from the beginning of the vegetative period to the end of the trial (Centre for Water Resources Management, INAT Tunisia)

Period	Total rain fall (mm)	Average of RH (%)	Temperature Max (°C)	Temperature Min. (°C)	Average of air temperature (°C)
Septembre	11.5	64.2	26.0	16.6	20.6
Octobre	3.80	62.8	21.5	12.1	16.8
Novembre	6.50	67.4	16.7	9.8	12.3
Decembre	3.80	54.5	13.3	6.3	10.1
Janury	9.20	58.7	10.9	3.8	6.3
February	8.40	68.7	10.7	3.8	7.3
March	3.20	65.6	13.4	4.6	9.0
April	11.2	64.8	17.4	8.7	13.0

Each plot was photographed in winter on minimum green coverage and after winter on spring green up with a digital camera; the images were taken at a height of 1.20 m with an angle of 90°. Spring green up was the date when new stolon growth started to occur (recovery) in ecotypes. Paspalum coverage was determined using Environment for Visualizing Images (ENVI version 4.7, ITT Corporation, NY) (Fig. 2). Photochemical efficiency (Fv/Fm) was evaluated during winter, subsequent low temperature, and recovery. Leaf water content (LWC) and Dry weight (DW) were also measured at the end of the trial.

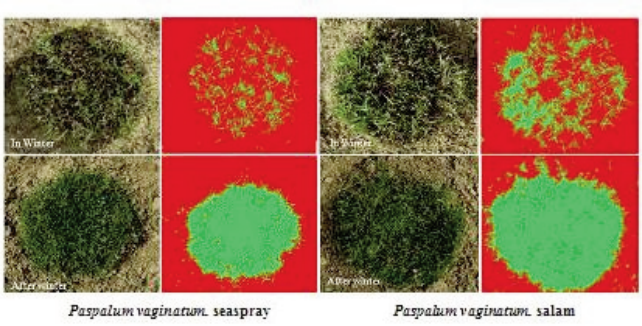

Fig. 2 - Paspalum grass coverage during and after winter. Data on minimum green coverage were collected in winter in December and spring green up data were collected in March.

Laboratory study

Plant materials and growth conditions. Seashore paspalum (*Paspalum vaginatum* cv. *S*alam and Seaspray) were collected from a two-year field plot at INAT, Tunisia. Plants were transplanted into plastic pots (0.5 L volume) filled with peat and positioned to grow for one and a half months in a greenhouse at 32°C/26°C (day/night). Before the beginning of the experiment, *Paspalum vaginatum* salam and seaspray were sprayed with 30 ml of 0, 10 mM H_2O_2 three times daily for two days and left in a normal chamber for 12 h for full absorption of the H_2O_2 solution (Fig. 3). These plants were then transferred to a growth chamber with temperature 18°C/8°C (day/night), irradiance of 2000 lux, 11 h photoperiod, and relative humidity 70%. Chlorophyll fluorescence was measured before exposure of plants to low temperature. Leaves were sampled before initiation (0 days) and after 3 and 6 days of exposure to low temperature stress. Samples from each treatment were immediately frozen in liquid nitrogen and stored at -80°C until biochemical analysis. The final harvest occurred after four months of exposure to low temperature to determine dry weight (DW), fresh weight (FW), and water content (WC).

Growth and water status in seashore Paspalum

Leaves of seashore paspalum samples were excised after four months of chilling treatment and used for fresh weight (FW) and dry weight (DW) determinations. Dry weight was obtained after drying leaves in an oven at 75 C until constant weight.

These data were used to calculate water content (WC) as follows:

$$WC= (FW-DW) \times 100 \times DW^{-1}.$$

Chlorophyll fluorescence

Chlorophyll fluorescence was measured on fully expanded young leaves by using an FIM 1500 fluorescence induction monitor (Analytical Development, Hoddesdon, England). Each leaf was excised, immediately clipped into a leaf clip (32 mm wide × 80 mm long), and the shutter plate closed to induce dark-adaptation for 30 min at room temperature. An array of six high-intensity light emitting diodes (LEDs) provided red light with a peak wavelength of 650 nm to

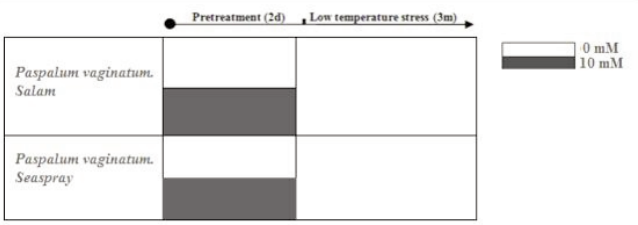

Fig. 3 - Schematic representation of the experimental design procedure. Paspalum cultivars were first sprayed then left 48 h for full absorption of different concentrations of H_2O_2 (0 mM (distilled water) and 10 mM H_2O_2). Plants appertaining to each treatment were subsequently exposed to cold stress.

illuminate the exposed leaf surface (4 mm diameter). Maximum intensity of the illuminating light was approximately 3000 µmol m^{-2} s^{-1} at leaf surface. Measured fluorescence parameters included *F*0 (initial fluorescence measured at the onset of illumination), *F*m (maximum fluorescence), *F*v (variable fluorescence = *F*m−*F*0), and *F*v/*F*m (ratio of variable to maximum fluorescence indicating the quantum yield). Measurements of three leaves from the same pot were averaged to obtain a mean.

Physiological assays to determine oxidative damage

H_2O_2 determination. The concentrations of H_2O_2 were estimated following the method of Ferguson (Ferguson *et al*., 1983): 100 mg fresh leaf was ground with 3 ml acetone for 30 min at 4°C, and the sample was then filtered through eight layers of gauze cloth. After the addition of 0.15 g active carbon, the sample was centrifuged twice at 3000 *g* for 20 min at 4°C. Next, 0.2 ml 20% $TiCl_4$ in HCl and 0.2 ml ammonia were added to 1 ml of the supernatant. The post-reacted compound was centrifuged at 3000 *g* for 10 min; the supernatant was discarded and the pellet was dissolved in 3 ml 1 mol L^{-1} H_2SO_4 and the absorbance value was determined at 410 nm. The standard curve was made using H_2O_2, and the H_2O_2 content in leaf was calculated from the absorbance at 410 nm compared with the standard curve.

Lipid peroxidation. Membrane lipid peroxidation was assessed by measuring the content of malonyldialdehyde in tissue. Fresh leaf samples were homogenized in 0.1% (w/v) TCA (Trichloroacetic acid) solution. The homogenate was centrifuged at 15000 *g* for 10 min. An aliquot of the supernatant was added to

0.5% TBA (Thiobarbituric acid) in 20% TCA (Trichloroacetic acid). The mixture was heated at 90°C for 30 min in a shaking water bath, and then cooled in an ice bath. The samples were centrifuged at 10 000 g for 5 min, and the absorbance of the supernatant was read at 532 and 600 nm (Hernandez *et al*., 2000). The MDA concentration was calculated as the difference of absorbance at 600 nm and 532 nm.

Protein extraction. Aliquots of frozen fresh leaves were ground to a fine powder with liquid nitrogen and extracted (100 mg FW) at 4°C in 100 mM Tris-HCl buffer (pH 8.0) containing 10 mM EDTA, 50 mM KCl, 20 mM $MgCl_2$, 0.5 mM PMSF, 1 mM DTT, 0.1% (v/v) Triton X-100, and 10% (w/w) PVP. The homogenate was centrifuged at 14000 *g* for 30 min at 4°C, and the supernatant was utilized for protein content and the determination of antioxidative enzyme activities. Three replicates per treatment were used. Protein concentration was determined according to Bradford (1976), using bovine serum albumin as standard.

Native gel electrophoresis and enzyme activity staining. Samples of crude *Paspalum vagintum* leaf extracts were separated by gel electrophoresis in 10% (POD) polyacrylamide slab gel, at pH 8.9 under native conditions according to Davis (1964). POD isoforms were visualized on gels according to Vallejos (1983).

The gel was first incubated in a 0.1 M sodium acetate buffer (pH 4.0) containing 1% (v/v) guaiacol for 30 min. Then, in a solution containing a final concentration of 4.7 mM 3-amino-9-ethylcarbazole, 38 mM N,N-dimethyl formamide, 0.1 M sodium acetate buffer (pH 5.0), 0.1 M $CaCl_2$, 3 mM and H_2O_2 the revelation was achieved.

Statistical analysis

Statistical differences were assessed using ANOVA of SAS (9.0) by t-test. The experimental design was a randomized complete block design with three replications. All data were statistically analyzed using least significant difference (LSD) to separate entry means. When the interaction was significant, a subroutine (PDMix800, SAS) was used to compare means at (p<0.05%).

3. Results

Local weather conditions in field induced changes in turfgrass coverage, in photochemical efficiency of PSII, and growth parameters

Data on minimum green coverage collected in winter (December) indicated that turfgrass coverage was significantly (p<0.05) altered by local weather conditions (Fig. 4, Table 1). With spring green up (March) turfgrass coverage of *Paspalum vaginatum* 'Seaspray' and 'Salam' exhibited an increase of 44.12% and 75%, respectively (Fig. 2, 4). Photochemical efficiency of phtosystem II (*Fv/Fm)* followed a significant (p<0.05) similar pattern of decrease then increase through the winter and spring green up. It decreased significantly (p<0.05) and was most pronounced in the second measurement time (Table 2). The percentages of decrease were, respectively, 15 and 6.5%. By the end of the trial, DW and WC were slightly different in both Paspalum cultivars and were not significantly affected (Table 2).

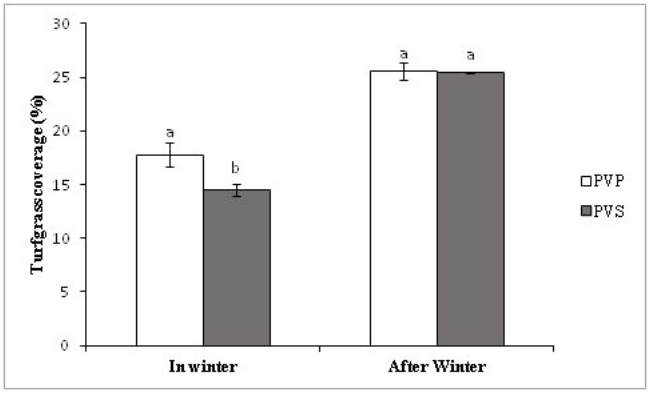

Fig. 4 - Turfgrass coverage on minimum green coverage (in winter) and on maximum green coverage (spring green up) in Paspalum vaginatum Salam (PVS) and Seaspray (PVP). Data are the mean ± SE of three plants.

Table 2 - Changes in photochemical efficiency of PSII (Fv/Fm ratio), dry weight (DW), and water content (WC) in *Paspalum vaginatum* 'Salam' and *Paspalum vaginatum* 'Seaspray' under local weather conditions

Date	'Salam'	'Seaspray'
	Fv/Fm	
D1	0.71 b	0.72 b
D2	0.70 b	0.71 b
D3	0.79 a	0.80 a
	DW (g)	
	21.30 a	20.28 b
	WC (%)	
	247.73 a	238.61 b

Data are the mean ± SE of twelve replications (n=12). Different letters indicate significant differences at a p<0.05 probability level. Measurements were taken three times during the trial D1= December; D2= January; D3= March).

Effect of pretreatment with H_2O_2 and long-term cold stress on growth and photosytem II efficiency of Paspalum vaginatum *'Salam' and 'Seaspray'*

The effect of foliar pretrement using low concentrations of H_2O_2 on biomass production (DW) and water content (WC) of both seashore cultivars is

shown in figure 5. Cold stress exposure reduced the DW of pretreated cultivar Salam compared to the control with no significant difference observed between pretreated and untreated 'Seaspray' ($p>0.05$). Biomass production (DW) in pretreated cultivars and their controls were above 300% and leaves did not wilt during the cold stress process however not significant levels were noted ($p>0.05$). These results seem to be independent of stress exposure and chemical pretreatment of these cultivars. The photochemical efficiency of PSII *(F*v/*F*m) was monitored three times throughout the cold exposure (Table 3) and the *Fv/Fm* ratio remained above 0.80 in both control and pretreated seashore cultivars at 10 mM ($p<0.05$).

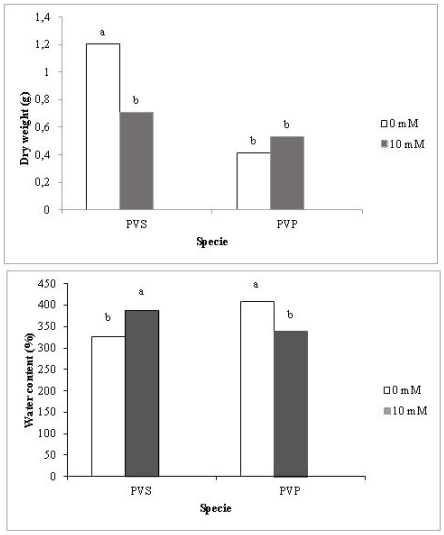

Fig. 5 - Dry weight and water content of *Paspalum vaginatum* 'Salam' (PVS) and *Paspalum vaginatum* 'Seaspray' (PVP). Values expressed as mean ± SE (n=3). Different letters indicate significant differences at probability level $p<0.05$.

Table 3 - Changes in Fv/Fm ratio in pretreated plants with 10 mM H_2O_2 and in control leaves of *Paspalum vaginatum* 'Salam' and *Paspalum vaginatum* 'Seaspray' during cold stress exposure

Genotype	Date	0 mM	10 mM
Paspalum vaginatum 'Salam'	D1	0.78 b	0.80 ab
D2	D2	0.81 a	0.80 a
D3	D3	0.81 a	0.80 ab
Paspalum vaginatum 'Seaspray	D1	0.80 ab	0.80 ab
	D2	0.81 a	0.80 a

Data are the mean ± SE of six replications (n=6). Different letters indicate significant differences at a probability level $p<0.05$. Measurements were taken three times during stress time (Dates 1, 2, and 3) under controlled conditions (See Materials and Methods).

Changes in H_2O_2, MDA and protein content

H_2O_2 pretreatment, exposure duration to cold stress, and the types of paspalum cultivars significantly affected the production of H_2O_2 and MDA content ($p<0.05$). In cultivar Salam, H_2O_2 content in control and pretreated plants did not differ significantly during cold stress, whereas MDA content decreased by 54% in pretreated plants when compared to control ones. In 'Seaspray' prior to cold stress (0 day), H_2O_2 content was higher in pretreated plants than in controls (Fig. 6). During the third day of cold stress, H_2O_2 content decreased by 83.77% compared to their controls, before it increased again after 6 days of cold stress. H_2O_2 pretreated plants lowered (77%) significantly ($p<0.05$) the MDA content in 'Seaspray', reaching the minimum levels during the third day of cold exposure as compared to the controls (Fig. 6).

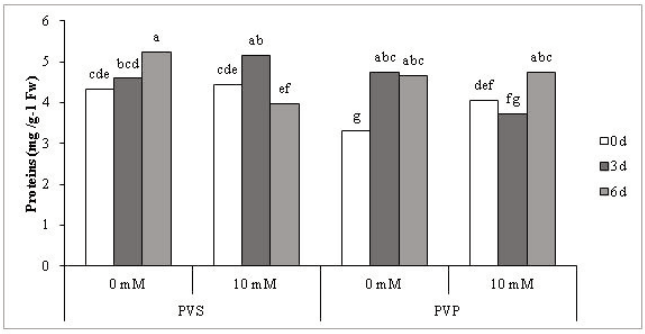

Fig. 6 - Protein content of *Paspalum vaginatum* 'Salam' and *Paspalum vaginatum* 'Seaspray' leaves in control plants (0 mM) and plants pretreated with 10 mM H_2O_2 after 0, 3, and 6 days of cold stress exposure. Values expressed as mean ± SE (n=3). Treatment ($p<0.001$), Species ($p<0.001$), Time ($p<0.001$) and interaction ($p<0.001$). Different letters indicate significant differences at probability level $p<0.05$.

After six days of stress exposure, MDA content increased slightly but was lower than MDA content recorded on the first day (0 d). Protein content in the leaves of 'Salam' changed slightly and remained at constant concentrations during the stress period. On the other hand, protein content in 'Seaspray' pretreated with 10 mM H_2O_2 varied significantly ($p<0.05$) compared to their controls (Fig. 7).

Chemical pretreatment and cold stress exposure altered relative density of Peroxydase isoforms

Peroxidase activity was assessed on polyacrylamide gel to distinguish the different POD isoforms, and to quantify their activities using image j software. Paspalum cultivars show different POD activity reactions under cold stress conditions. POD relative density in all pretreated *Paspalum vaginatum* 'Salam' (Fig. 8) plants increased with increased levels of cold exposure, compared to controls. However, POD relative density in pretreated *Paspalum vaginatum* sea-

spray was much higher compared to control plants and then decreased during the third day of stress (Fig. 8).

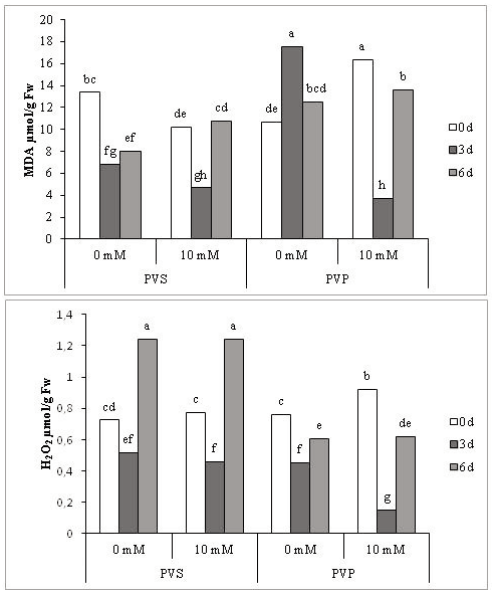

Fig. 7 - H_2O_2 and MDA contents of *Paspalum vaginatum* 'Salam' (a) and *Paspalum vaginatum* 'Seaspray' (b) leaves in control plants (0 mM) and plants pretreated with 10 mM H_2O_2 after 0, 3, and 6 days of cold stress exposure. Values expressed as mean ± SE (n=3). Treatment ($p<0.001$), Species ($p<0.001$), Time ($p<0.001$) and interaction ($p<0.001$). Different letters indicate significant differences at probability level $p<0.05$.

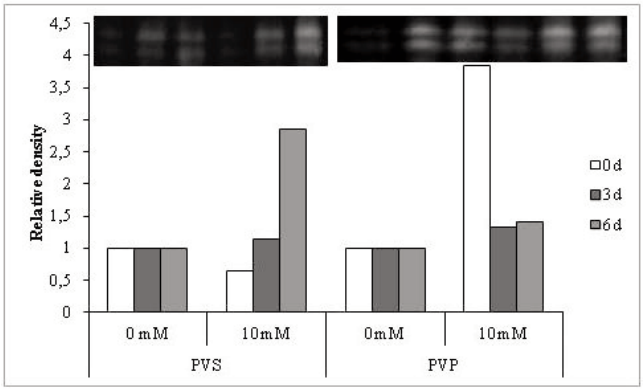

Fig. 8 - POD isoforms in gel, relative density in control plants (0 mM) and plants pretreated with 10 mM H_2O_2 under cold stress condition.

4. Discussion and Conclusions

Results showed that both cultivars were affected by environmental conditions and could have an impact on turfgrass coverage and on *Fv/Fm*. Lower *Fv/Fm* ratio values were close to 0.7 (date 1), suggesting an altered performance of PSII. Remarkably, both paspalum cultivars required longer to recover, reaching 0.8 (*Fv/Fm)* (date 3). A close relationship has been reported between low temperatures and photosynthetic rates. The recovery of photosynthetic activity under cold stress was faster in other species such as sorghum, maize and pennisetum, while it was slightly slower in soybean, and ryegrass (Taylor and Rowley, 1971).

Some of the excessive energy is quenched into chlorophyll fluorescence to minimize damage to photosynthetic systems, particularly in photosystem II (PSII) and subsequent electron carriers (Krause and Weis, 1991; Araboui *et al*., 2010). Changes in membrane structure were considered as the primary lesion of stress injury and may lead to a loss of membrane permeability and metabolic disfunction (Lyons, 1973; Montillet *et al*., 2005). The peroxidation of lipids is also a damaging process that can be taken as a single parameter to determine the level of lipid destruction under various stresses. During lipid peroxidation, products are formed from polyunsaturated precursors that include hydrocarbon fragments; such as ketones, MDA, and compounds related to them (Grag and Machanda, 2009). The increase of MDA indicated the deterioration of peroxidation and membrane injury induced by ROS under cold stress. Our findings suggest that within three days of cold stress exposure, 10 mM of H_2O_2 remarkably decreased MDA in cultivar Seaspray. He *et al*. (2009) shown that H_2O_2 pretreatment enhanced the membrane stability of wheat seedlings, as revealed by a greatly reduced membrane damage rate (MDA) and malondialdehyde (MDA) content. This was associated with a slight increase in protein content. Improving tolerance of both Paspalum cultivars to chilling stress also leads to the over production of ROS such as O_2.- and H_2O_2 in plant tissues (Desikan *et al*., 2003). ROS are extremely active molecules that may damage membranes and other cellular components to avoid cold stress or other stress-induced injuries. However, ROS could be considered as a signal for molecules that mediate responses to various stimuli. Compared to the other ROS, H_2O_2 can be the most suited to act as a signaling molecule due to its higher stability and longer half-life. The fluctuation of H_2O_2 level in plants should spatially and temporally reflect changes in the environment (Desikan *et al*., 2003, 2004).

Our study provides evidence that exogenous H_2O_2 resulted in a significant decrease ($p<0.05$) of the endogenous content of H_2O_2 and MDA in the first three days of exposure to cold stress in pretreated 'Seaspray' plants. The higher H_2O_2 content (0 d) in pretreated 'Seaspray' was followed by a systematic

high relative density of POD isoforms. With further cold stress, relative densities of POD decreased but remained higher than control plants. This type of response would permit pretreated Seaspary Cv. plants to still upright to overcome the wilting and mechanical weakness imparted by cold stress.

In conclusion, our results have demonstrated that pretreatment with 10 Mm of H_2O_2 could improve the tolerance of seashore paspalum to cold stress, especially cultivar Seaspray which showed a better response to cold stress than 'Salam'. Exogenous H_2O_2 could constitute a signaling molecule that significantly increases H_2O_2 detoxifying and POD activity, and decreases MDA and H_2O_2 content.

The meaning of presented results can be important for fundamental and practical sciences. These results would help to understand what are plant reactions to cold stress. I will be also possible to apply low concentrations of hydrogen peroxide in golf course and sport fields, making these fields more viable and sustainable.

References

ALLEN D.J., ORT D.R., 2001 - *Impacts of chilling temperatures on photosynthesis in warm-climate plants*. - Trend. Plant Science, 6: 36-42.

ARBAOUI S., ARBAOUI M., TISSAOUI T., HARABAOUI Y., BETTAIEB T., 2010 - *Evaluation par imagerie numérique de la couverture végétale et du comportement végétatif et physiologique en conditions hivernales, de quelques graminées à gazon des régions chaudes*. - Revue INAT, 25,

ASADA K., 1999 - *The water-water cycle in chloroplasts: scavenging of active oxygen's and dissipation of excess photons.* - Annu. Rev. Plant Physiol. Plant. Mol. Biol., 50: 601-639.

BRADFORD M.M., 1976 - *A rapid and sensitive method for the quantification of microgram quantities of protein utilising the principal of protein-dye binding*. - Anal. Biochem., 72: 248-254.

DESIKAN R., CHEUNG M.K., CLARKE A., GOLDING S., SAGI M., FLUHR R., ROCK C., HANCOCK J., NEILL S., 2004 - *Hydrogen peroxide is a common signal for darkness- and ABA-induced stomatal closure in* Pisum sativum*.* - Funct. Plant. Biol., 30: 913-920

DESIKAN R., HANCOK J.T., NEILL S.J., 2003 - *Oxidative stress signaling,* pp. 121-148. - In: HIRT H., and K. SHINOZAKI *(*eds*.). Plant responses to abiotic stress: topic in current genetics*. Springer-Verlag, Berlin, Heidelberg, New York, pp.

DUNCAN R.R., 1997 - *Environmentally compatibility of seashore paspalum (saltwater couch) for golf courses and other recreational uses. II. Management protocols.* - Int. Turfgrass. Soc. Res. J., 8: 1230-1239.

FERGUSON I.B., WATKINS C.B., HARMAN J.E., 1983 - *Inhibition by calcium of senescence of detached cucumber cotyledons*. - Plant. Physiol., 71: 182-186.

FOYER C.H., NOCTOR G., 2005 - *Oxidant and antioxidant signaling in plants: a re-evaluation of the concept of oxidative stress in a physiological context*. - Plant, Cell and Environment, 28: 1056-1071.

GARG N., MANCHANDA G., 2009 - *ROS generation in plants: boon or bane?* - Plant. Biosys., 143: 8-96.

GRATAO P.L., POLLE A., LEA P.J., AZEVEDO R.A., 2005 - *Making the life of heavy metal stressed plants a little easier*. - Funct. Plant. Biol., 32: 481-494.

HE L., GAO Z., LI, 2009 - *Pretreatment of seed with H2O2 enhances drought tolerance of wheat (*Triticum aestivum *L.) seedlings*. - Afr. J. Biotech., 8(22): 6151-6157.

HERNANDEZ J.A., JIMENEZ A., MULLINEAUX P., SEVILLA F., 2000 - *Tolerance of pea (*Pisum sativum *L.) to long-term salt stress is associated with induction of antioxidant defences*. - Plant. Cell. Environ., 23: 853-862.

KRAUSE G.H., WEIS E., 1991 - *Chlorophyll Fluorescence and Photosynthesis: The Basics.* - An. Rev. Plant. Physiol. and Plant Mol. Biol., 42: 313-349.

LYONS J.M., 1973 - *Chilling injury in plants*. - Ann. Rev. Plant. Physiol., 24: 445-466.

MITTLER R., 2002 - *Oxidative stress, antioxidants and stress tolerance*. - Trends. Plant. Sci., 7: 405-410.

MUNNE-BOSCH J., FALK J., 2004 - *New insights into the function of tocopherols in plants*. - Planta*,* 218: 323-326.

NETO A.D.A, PRÌSCO J.T., ENEAS-FILHO J., JAND-VENES R., GOMES-FILHO E., 2005 - *Hydrogen peroxide pre-treatment induces salt stress acclimation in maize plants*. - J. Plant Physiol., 162: 1114-1122.

PRASAD T.K., ANDERSON M.D., MARTIN B.A., STEWART C.R., 1994 - *Evidence for chilling-induced oxidative stress in maize seedlings and a regulatory role for hydrogen peroxide*. - Plant. Cell., 6: 65-74.

TAYLOR A., ROWELEY J.A., 1971 - *Plants under climatic stress: low temperature, high light effects on photosynthesis*. - Plant. Physiol., 47: 713-718.

VALLEJOS C.E., 1983 - *Enzyme activity staining*, pp. 469-516. - In: TANKSLEY S.D., and T.J. ORTON (eds.) *Isozymes in plant genetics and breeding. Part. A.* Elsevier Science Publishers, Amsterdam, The Netherlands.

WAHID A., PREVEEN M., GELANI S., BARSA S.M.A., 2007 - *Pretreatment of seed with H_2O_2 improves salt tolerance of wheat seedlings by alleviation of oxidative damage and expression of stress proteins*. - J. Plant. Physiol., 164(3): 283-294.

XIA H.P., AO H.X., LIU S.Z., 2000 - *Reasons why turfgrass becomes yellow and wither in winter of south China and some preventive strategies-taking turf in Guangzhou as an example*. - Grassl. China, 5: 64–67 (Abstract in English).

Adv. Hort. Sci., 2016 30(2): 111-118 DOI: 10.13128/ahs-19137

The usefulness of apricot gum as an organic additive in grapevine tissue culture media

S. Khorsha, M. Alizadeh (*), K. Mashayekhi
Horticulture Department, Faculty of Plant Production, Gorgan University of Agricultural Sciences and Natural Resources, Golestan, P.O. Box 386, Gorgan, Iran.

Key words: apricot gum, callus culture, carrot, grapevine, rooting, stevia, tissue culture.

Abstract: The growth and morphogenesis of cultured plant tissues can be improved by small amounts of some organic elements. In addition to being a natural source of carbon, organic additives may contain natural vitamins, phenols, fiber, hormones and also proteins. Hence, the physiological effects of apricot gum on the regeneration capacity and growth rate of three different plant species i.e. carrot (as a model plant), stevia (as an herbaceous plant), and grapevine (as a woody plant) were examined. The proliferated callus cultures of carrot and *in vitro*-derived microcuttings of stevia and grapevine were inoculated on their respective standardized proliferation media supplemented with 2.0-6.0 g/l apricot gum. The growth parameters of treated samples were measured and compared to gum-free medium. Earlier callus initiations with greater fresh weight, volume, as well as improved pigmentation were recorded in media fortified with apricot gum. The usefulness of gum application was also obvious in both stevia and grapevine with respect to better shoot multiplication and rooting parameters. Due to positive effects of apricot gum, longer vines with a higher number of lateral shoots, internodes and leaf area were achieved. Overall, the gum at the rate of 4.0 g/L was found to be a logical concentration with respect to encouraging response in all three species. Owing to promising results evolved in the present research, the application of gum in commercial tissue culture protocols is highly recommended. However, further studies are needed to exploit plant derived gums as an alternative carbon source in plant tissue culture media.

1. Introduction

The degree of success in any technology employing plant cell, tissue or organ culture is related to quite a few major factors. A significant factor is the choice of nutritional components and growth regulators (Gamborg, 1991). A plant tissue culture medium is composed of necessary and optional components required for plant growth, which vary according to the plant species, cultivar, or explant type that is used and must be experimentally defined for each particular case. Moreover, all the nutrients in a medium should be present in optimum concentrations to ensure the best possible growth of explants (George, 1993). Under *in vitro* conditions, an intact plant requires macronutrients, micronutrients, plant growth regulators, vitamins, amino acids and other nitrogen supplements and sugars (Gamborg, 1991). Another important component in plant tissue culture media is the carbon source because it supplies energy to the plants, especially when they are not ready to photosynthesize their own food during the early stage of tissue culture (Al-Khateeb, 2008 b). Carbon source can be in the form of simple or complex sugars (Akter *et al.,* 2007). Normally, sucrose is used as the carbon source in plant tissue culture. A range of other organic additives have been used in plant tissue culture to promote the growth of the plants, including coconut milk, banana pulp, potato homogenate and juice, honey, date palm syrup, corn extract, papaya extract, guar gum, and isubgol (Islam *et al.*, 2003; Jain and Babbar, 2005; George *et al.*, 2008; Murdad *et al.,* 2010; Nambiar *et al.,* 2012). Such additives are commonly known as organics with undefined compositions (Torres, 1989). The advantages of adding such organic materials to medium have already been reported by some researchers, for example, the organic additives help to produce more PLBs, shoots and leaves in orchid (Akter *et al.,* 2007), increase the size of date palm somatic embryos (Al-Khateeb, 2008 a), and also promote growth and development of asymbiotic seeds and regeneration

(*) Corresponding author: mahdializadeh@gau.ac.ir
Received for publication 9 May 2016
Accepted for publication 25 May 2016

of *Cymbidium* plantlets (Tawaro *et al.,* 2008). The reasons for application of organic additives into culture medium, in addition to being a natural source of carbon, are because they contain natural vitamins, phenols, fiber, hormones, and also proteins (Gnasekaran *et al.,* 2010). It was mentioned in a report by Al-Khateeb (2008 a) that organic additives contained not only sugar but also other nutrients such as proteins, lipids and minerals.

In recent years, plant-derived polymers have evoked remarkable attention in various industries due to their diverse applications as food emulsifiers, stabilizers and thickeners, pharmaceuticals, cosmetics, textiles, and in art. They are also used as gelling agents in gels and bases in suppository (Nussinovitch, 1996). These polymers are biocompatible, biodegradable and are preferred to semi synthetic and synthetic excipients because of their lack of toxicity, low cost, soothing action, and non irritant nature (Deogade *et al.*, 2012). Environmental-friendly processing and local availability, especially in developing countries, are considered additional advantages for application of these natural products (Jain and Babbar, 2005). The Rosaceae family, *Prunus* genus, consists of peach, plum, apricot, cherry, and almond trees, all of which can produce exudate gums. Herbal exudate gums normally secrete from bark, branch and fruit of trees due to their protection impact against mechanical damage or microbial attacks. Gum secretion may also occur due to adaptation to climate of some trees, called physiological gummosis (Simas *et al.*, 2008; Simas-Tosin *et al.*, 2009). A large number of complex natural additives can be very effective in providing an undefined mixture of organic nutrients and growth factors. In the context of carbohydrates, gums are usually considered to be non-starch, water-soluble polysaccharides with commercial importance. Gums are typically more or less sticky in nature and are translucent and amorphous substances which are degradation products of the cell wall of woody species which exude from trees. Natural gums (gums obtained from plants) are hydrophilic carbohydrate polymers having high molecular weights, generally composed of monosaccharide units joined by glucosidic bonds (Khorsha, 2014). While thorough reports on gum structure (Saniewski *et al.*, 2001; Lluveras-Tenorio *et al.*, 2012), its rheological properties (Wang *et al.*, 2008), and its applications in different industries (Verbeken *et al.*, 2003) were previously reported, the influence of such natural products as organic addenda in plant tissue culture media has yet to be studied. Therefore, the present investigation was conducted to evaluate the effectiveness of apricot gum as an organic additive on growth and *in vitro* regeneration of three different plant species: carrot (as a model plant), stevia (as herbaceous species) and grapevine (as woody plant). The results of the current study should be applicable to other plant species following minor modifications.

2. Materials and Methods

The present research work was conducted in the plant tissue culture laboratory of the Horticulture department, Faculty of Plant Production, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran. The physiological effects of apricot gum on regeneration capacity and growth rate of three different plant species i.e. carrot (as model plant), stevia (as herbaceous plant) and grapevine (as woody plant) were examined. The apricot gum was obtained dried on bark and shoots of apricot trees immediately after secretion, collected from a commercial apricot orchard (Gonabad, Khorasan, Eastern Iran) through scraping it from bark surfaces. The bark residues were removed from the collected mass and the clean, pure gum was powdered prior to addition to the culture medium.

Plant materials and in vitro *culture establishement*

Carrot. Healthy, undamaged roots of carrot (*Daucus carrota* cv. Nantes), 3-4 cm diameters long, were selected and washed thoroughly with normal tap water (30 min) and surface sterilized with ethanol (70% v/v for 40 s) followed by sodium hypochlorite solution (35% v/v plus two drops of tween-20 for 15 min). The clean, sterilized roots were cut transversely, employing a scalpel, to prepare root explants as slices (10×10 mm and 1mm thickness). Each explant was prepared in such a way to consist of xylem, secondary phloem and a small part of cambium tissues (Hall, 1991).

Stevia. Stevia (*Stevia rebaudiana*) mother plants were procured from Golsaran-e-Shomal Corporation, as tissue cultured pot plants and were transferred to our laboratory. Single node explants were disinfected with $HgCl_2$ (0.1% for 6 min) and washed three times with sterilized distilled water inside a laminar hood cabinet. The single node explants were cultured and *in vitro* culture was established following an already standardized protocol (Taherian, 2012).

Grapevine. Single node explants of grapevine (*Vitis vinifera* cv. Laal) were utilized following the pre-

viously standardized micropropagation protocol developed by Alizadeh *et al.* (2010). Laal is an Iranian, commercially grown variety of grapevine. Single node explants (2-4 cm length) were pre-washed in 0.1-0.2% commercial detergent (JAM dish-washing liquid, Iran) followed by stirring in Mancozeb (2 g/L) solution for 45 min. The explants were surface disinfected using 60% (v/v) NaOC1 solution (5% available chlorine) for 30 min. After four to five rinses in sterile distilled water, single node cuttings (2-4 cm) were inoculated for *in vitro* culture establishement.

Culture media and incubation conditions

Basal MS (Murashige and Skoog, 1962) medium was used during the whole experiment for all three plant species. The various media along with their growth regulators for each plant species are reported in Table 1. The pH was adjusted to 5.8 prior to the addition of 0.8% agar, and the media were autoclaved at 121°C and 15 PSI for 15 min. Carrot root cultures were kept in growth chamber in darkness and low light intensity for 7-14 days and the explants were investigated for callus formation. The callus mass was sub-cultured on fresh medium supplemented with apricot gum. The stevia and grapevine single node cultures were incubated at 25±2°C under continuous light (50 µmol m^{-2} s^{-1}). The established and sprouted cultures were sub-cultured as double-node explants on proliferation media (Table 1) supplemented with different concentrations of apricot gum.

Table 1 - Different culture media and growth regulators for each plant species

Plant species	*In vitro* establishment	Shoot proliferation	Rooting
Carrot	2,4-D (1.0 mg/l)	-	-
Stevia	Hormone-free medium	IBA (2.0 mg/l)	IBA (2.0 mg/l)
Grapevine	BA (2.0 mg/l) + NAA (0.2 mg/l)	IBA (2.0 mg/l)	IBA (2.0 mg/l)

Measured parameters

Due to the utilization of explants with different origins, dissimilar parameters were recorded in each species. In the case of carrot callus cultures, days to callus initiation, callus color, firmness, volume and fresh/dry weight were recorded 30 days after caulogenesis. In the case of stevia and grapevine, initially the *in vitro* cultures were established as explained in above. Then, the double-node explants procured from *in vitro* proliferated cultures were inoculated on their respective proliferation media supplemented with different concentrations of apricot gum. The parameters such as days taken to bud sprouting, number of leaves, leaf area, total chlorophylls and cartenoides, intermodal length, shoot length, days to root initiation, number of roots, root length, rooting percentage and root/shoot fresh and dry weights were measured in both stevia and grapevine regenerated cultures.

Experimental design and data analysis

The present experiment was conducted as a complete randomized design with four replications. The percentage data were transformed using root square method (√ % + 0.5) prior to analysis. The results were analyzed using SAS software (SAS Institute Inc., 2003) and the mean values were compared by least significant difference (LSD) test in $p<0.01$ probability.

3. Results

The present research was undertaken to ascertain the likely positive effects of apricot gum as an organic additive to plant tissue culture media. The gum was primarily supplemented to carrot callus cultures and, owing to its positive response, was tested in two other plant species, stevia and grapevine, for which *in vitro* culture propagation protocols were already standardized in our laboratory (Alizadeh *et al.*, 2010; Taherian, 2012).

Iran is the world's second producer of apricot (FAOSTAT, 2012) and apricot gum is readily available throughout the country at a very low price. In order to optimize the concentrations of the apricot gum, some preliminary tests were performed and the rate of 2.0-6.0 g/L was found to be a logical range because the higher concentration formed a dense solution that was difficult to dispense in culture vessels. Furthermore, in high concentration, it negatively interacted with agar solidification (Khorsha, 2014).

Carrot callus growth

The application of gum surprisingly enhanced the performance of *in vitro* callus initiation and further growth of carrot explants. Furthermore, it significantly reduced the time taken to callus initiation and among the treatments caulogenesis occurred even less than two weeks following inoculation of the explants on medium supplemented with 6.0 g/L apricot gum. The callus initiation in control explants was found to start normally after 5 weeks, therefore, precocious callus formation (nearly 20 days earlier than control) is considered a promising result for *in vitro* application of apricot gum. In addition, callus morphological traits such as callus volume, color, and tissue firmness were also affected by gum treatments

(Table 2). The callus mass produced on control medium (30 days after inoculation) were light brown in color and had intermediate firmness. However, with gum application the color was greenish and the tissue mass volume was increased corresponding to gum concentration (Fig. 1). The callus fresh weight was also improved in all the treatments, however the dry weight was the same and was determined to be lower than control media (Table 3). It is worth noting that the treatment with 6.0 g/L apricot gum may be toxic for carrot callus; however callus fresh weight was not statistically significant compared to 2 g/L level (Table 3).

Due to the positive and promising results with carrot callus cultures, the effect of apricot gum was evaluated on *in vitro* proliferation of an herbaceous medicinal plant, stevia, for which its proliferated cultures were already established in our laboratory.

Table 2 - The qualitative traits of carrot callus as affected by gum application 30 days after inoculation

Qualitative traits	Apricot gum concentration (g/l)			
	Control	2.00	4.00	6.00
Callus volume	<30%	30-50%	50-100%	≥100%
Callus color	Light brown	Beige to light green	Beige to greenish	Beige to pistachio green
Tissue firmness	+++	+++	++++	++++

+++ indicates medium hadness, ++++ indicates medium hardness and fragile callus tissue.

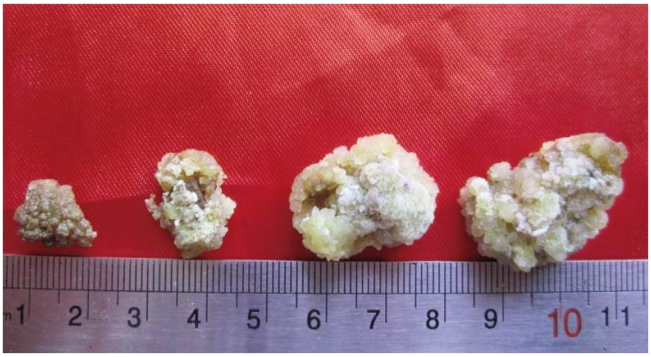

Fig. 1 - The effect of apricot gum on *in vitro* growth and proliferation of carrot callus cultures 30 days after inoculation (left to right: Control, 2.0, 4.0, 6.0 g/l apricot gum).

Table 3 - The growth parameters of carrot callus as affected by gum application 30 days after inoculation

Qualitative traits	Apricot gum concentration (g/l)			
	Control	2.00	4.00	6.00
Days to callus initiation	35.75 a	29.80 b	19.60 c	13.70 d
Callus fresh weight (g)	0.121 c	0.269 cb	1.01 a	0.364 b
Callus dry weight (g)	21.54 a	10.08 b	11.32 b	13.66 b

Means in the same row followed by different letters are significantly different at $P<0.01$ using LSD-Test.

Stevia shoot multiplication and rooting

Stevia is an herbaceous plant with rapid *in vitro* proliferation. In our laboratory, stevia cultures were normally sub-cultured at three- to four-week intervals. The growth parameters of stevia shoot proliferation and rooting four weeks after inoculation are shown in Table 4. It is clear that apricot gum added to stevia medium was particularly beneficial. The gum significantly reduced the time taken to root initiation and the micro-cuttings inoculated on medium containing gum (6.0 g/L) achieved root initiation in less than a week (6.5 days only), while the control plantlets came into rooting after at least 10 days. Root length also increased in the presence of gum, however, the number of roots were not considerably different among treatments. *In vitro* plantlet length and leaves were reduced on gum-supplemented media, but instead the leaf area and lateral shoots were enhanced (Table 4). It seems the lower concentrations of apricot gum (2.0 and 4.0 g/L) led to better vegetative response in stevia cultures. A similar trend was also recorded with respect to stevia leaf pigments. Thus, at the rate of 2.0 g/L, gum was more effective in pigmentation and production of dark green leaves. Overall, it may be stated that apricot gum had a positive influence on most of the *in vitro* traits measured in stevia tissue-cultured plants.

Table 4 - The growth parameters of stevia shoot proliferation and rooting 28 days after inoculation

Growth trait	Apricot gum concentration (g/l)			
	Control	2.00	4.00	6.00
Plantlet length (cm)	20.00 ab	10.95 bc	22.72 a	8.60 c
Number of shoots	2.25 b	3.75 ba	3.25 ba	5.25 a
Number of leaves	68.25 a	27.00 b	36.75 b	47.5 ab
Average leaf area (cm^3)	0.06 c	1.75 a	1.16 ab	0.68 bc
Days to root initiation	10.75 a	10.25 a	7.25 a	6.50 a
Number of roots	35.00 a	29.50 a	35.00 a	17.50 a
Root length (cm)	0.56 b	2.21 a	1.86 a	0.85 b
Chlorophyll a (mg.g F.W.)	7.50 c	14.45 a	10.96 b	10.68 b
Chlorophyll b (mg.g F.W.)	4.33 ab	5.43 a	2.77 b	3.87 ba
Total chlorophyll (mg.g F.W.)	11.91 b	19.97 a	13.76 b	14.72 b
Carotenoid (mg.g F.W.)	3.15 b	5.95 a	4.25 b	4.53 ba

Means in the same row followed by different letters are significantly different at $P<0.01$ using LSD-Test.

Grapevines shoot multiplication and rooting

It has previously been reported that shoot proliferation and rooting occur simultaneously in grapevine (Alizadeh *et al.*, 2010), hence, in the present experiment, it was possible to measure related

parameters collectively in a single medium (shoot proliferation *cum* rooting medium supplemented with apricot gum). Furthermore, the results of our grapevine experiment were more obvious and noticeable than for the other species. These distinct effects on grapevine shoot proliferation and rooting are reported in figure 2 and Table 5. In the case of rooting parameters, the gum considerably reduced the time taken to root initiation (6 days against 14 days in control explants) and the rooting percentage was also improved, reaching nearly 100 % in the case of 6.0 g/L gum-supplemented media. Root length also increased: the greatest length was found in medium containing 4.0 g/L gum. However, all gum treatments showed longer roots compared to control explants. A similar trend was also observed in stevia samples (Table 4). Shoot proliferation was vigorously enhanced following application of gum to the media (Fig. 2 and 3). Thanks to the positive effects of apricot gum, longer vines with more lateral shoots, internodes and greater leaf area were achieved (Fig. 3). Although all three concentrations of gum were found to be effective with regard to the aforementioned vegetative traits as compared to gum-free medium, the efficiency of 4.0 g/L was evident.

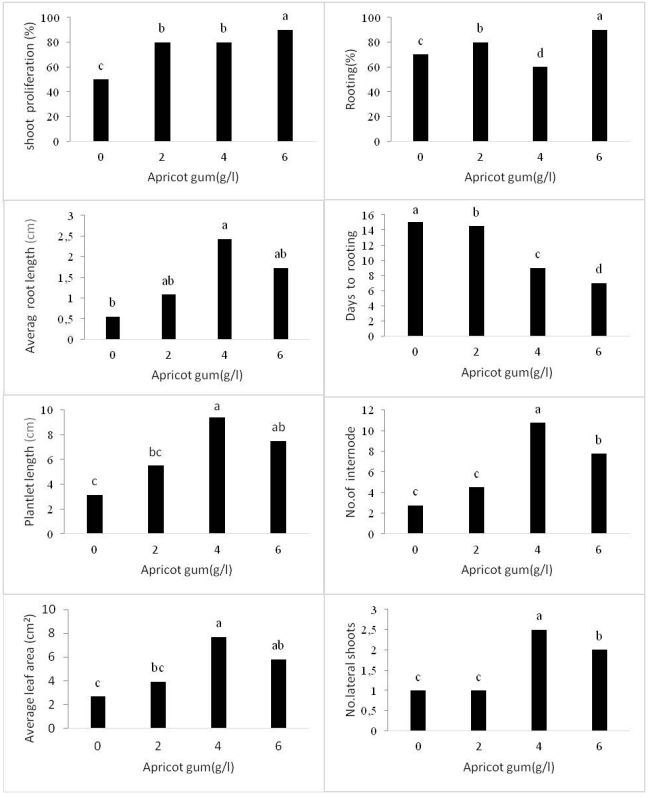

Fig. 2 - The effect of apricot gum on *in vitro* growth performance of grapevine explants.

Table 5 - The effect of apricot gum on certain growth parameters of grapevine 30 days after inoculation

Growth trait	Apricot gum concentration (g/l)			
	Control	2.00	4.00	6.00
Appearance of the first leaf	17.25 a	7.00 b	5.00 c	4.75 c
Number of shoots	1.00 c	1.00 c	2.5 a	2.00 b
Number of leaves	3.00 b	4.75 b	9.5 a	9.50 a
Number of roots	20.25 b	32.5 b	77.25 a	65.75 a
Internode length (cm)	0.94 c	1.02 bc	1.41 a	1.29 ab

Means in the same row followed by different letters are significantly different at $P<0.01$ using LSD-Test.

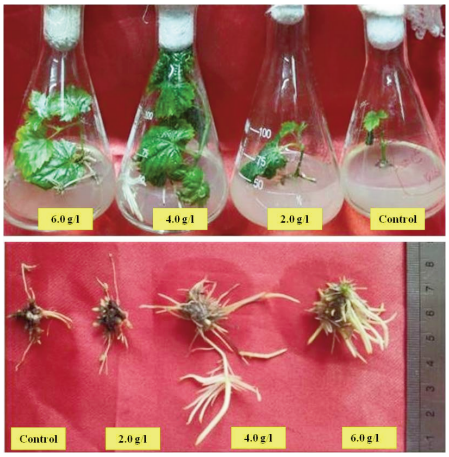

Fig. 3 - The effect of apricot gum on *in vitro* shoot proliferation (above) and rooting (below) of grapevine.

4. Discussion and Conclusions

Cultured plant tissues need a continuous supply of carbohydrates from the medium to encourage growth and survival *in vitro* (Kozai, 1991). Therefore, sugars such as sucrose, glucose, and sorbitol are generally added as a carbon source (Kadota and Niimi, 2004). Furthermore, reliable callus proliferation and subsequent plant regeneration are important for efficient micropropagation and genetic manipulation of plant tissues (Khorsha, 2014). Carbohydrates play an important role in *in vitro* cultures as an energy and carbon source, as well as an osmotic agent (George *et al.*, 2008). There are numerous reports on the effects of various carbon sources on *in vitro* callus growth and regeneration. For example, Huang and Huang (1999) demonstrated that sorbitol acts as a carbon source, providing energy for callus growth and plantlet regeneration. Other studies suggest that it acts only as an osmotic regulator to adjust osmotic pressure in calli (Al-Khayri and Al-Bahrany, 2002; George *et al*., 2008). The capacity of various carbohy-

drates to support growth of Japanese morning glory callus was examined and it was found that sucrose was the most effective compound but glucose, fructose, trehalose, maltose, cellobiose, raffinose and soluble starch were also found to be significant (Hisajima and Thorpe, 1985).

Apricot gum and callus growth and proliferation

Gums are typically more or less sticky in nature and translucent and amorphous. Chemical analysis of apricot gum has already been performed by Lluveras-Tenorio *et al.* (2012). They found total sugars (60%), galactose (43%), mannose (4%), arabinose (44%), xylose (7%) and ramnose (1%) in apricot gum. It is clear that such composition may be different in each lot, from one tree to another, and the results may not be reproducible in different tissue culture laboratories. However, the present study was performed in five replications and positive responses were found with carrot callus growth. The callus volume produced on media fortified with 4.0-6.0 g/L apricot gum was at least three to four times that of gum-free medium (Fig. 1). The enhanced callus growth induced by apricot gum may be considered a significant logical reason for application of gum in plant tissue culture media, especially in species for which indirect regeneration (plant regeneration from callus) is to follow.

Apricot gum and shoot/root proliferation

Different types of basal media used in plant tissue culture vary with regard to the concentration of macro and microelements, greatly affecting the *in vitro* growth and multiplication of shoots. Apart from this, type and concentration of carbon sources in the medium affect the physiology and differentiation of tissues (Lipavska and Konradova, 2004) while serving as osmotic and energy source (George *et al.*, 2008). In our experiment, the utilization of apricot gum caused reasonable shoot proliferation both in stevia and grapevine (Table 4, Fig. 3), not to mention its very low cost and economic advantages, making it useful in commercial tissue culture laboratories seeking economical and feasible protocols for *in vitro* propagation of horticultural crops. There are some reports on the utilization of such low-priced and organic materials as a potential plant source with a high amount of sucrose and other sugars which could possibly be used as an alternative carbon source, for example molasses (Dhamankar, 1992), sugar cane juice (Buah *et al*., 2011), and date palm syrup (Al-khateeb, 2008 a). Furthermore, in a review presented by Yaseen *et al.* (2012), factors determining the efficacy of a carbon source including its type, concentration, and their mutual interaction are reported. In our study, apricot gum was exploited, although the application of almond gum or sweet cherry gum would also be possible, since these types of gum are also readily available throughout the country at similar price. However, there may be some differences in *in vitro* responses of gums from different origins. With regard to concentration, for tissue culture media, 4.0-6.0 g/L may be considered as a feasible range, because beyond this dose the solubility of gum would be difficult even with heat treatments. Moreover, in high concentrations the culture media cannot be solidified properly (Khorsha, 2014). The mutual interaction between gum and explants is also an important matter. Although, in the present experiment all three plant species showed positive responses for most of the measured parameters, however grapevine *in vitro* shoot proliferation was greatly enhanced in gum-supplemented media (Fig. 3).

Rooting is considered a difficult step in micropropagation of many woody plants (George, 1993) and it is regulated by a number of physiological, biochemical, and genetic factors (Pawlicki and Welander (1995). Generally, rooting occurs in an auxin-enriched medium. On the other hand, Hassan *et al.* (2009) mentioned that sucrose at the highest level considerably increased rooting of palm tissue cultures. The results of the present research on rooting parameters of stevia and grapevine correspond with those of Al-Khateeb (2008 b) on date palm. He observed an enhanced root formation as the sugar concentration increased (60 g/L and above).

In conclusion, the present study considered addition of apricot gum as a complex organic addendum in tissue culture of carrot callus, stevia and grapevine. It was found that addition of gum not only was without negative effects, but its usefulness was definitely confirmed. Apricot gum significantly increased *in vitro* growth and proliferation of carrot callus tissues. Furthermore, shoot/root vegetative parameters of gum-fortified media were actually promising in stevia and grapevine. Overall, gum at the rate of 4.0 g/L was found to be a logical concentration with respect to the positive response in all three species. Owing to the promising results found with the present research, the application of gum in commercial tissue culture protocols is highly recommended. However, further studies would be required to fully exploit plant-derived gums as an alternative carbon source.

Acknowledgements

The authors are grateful to Golsaran-e-Shomal tissue culture Company for providing stevia mother plants. The help and cooperation of Mr. F. Salahi for his assistance in grapevine sample collection is fully acknowledged.

References

AKTER S., NASIRUDDIN K.M., KHALDUN A.B.M., 2007 - *Organogenesis of Dendrobium orchid using traditional media and organic extracts*. - J. Agric. Rural Dev., 5(1&2): 30-35.

ALIZADEH M., SINGH S.K., PATEL V.B., 2010 - *Comparative performance of* in vitro *multiplication in four grape (*Vitis *spp.) rootstock genotypes*. - Int. J. Plant Production, 4: 41-50.

AL-KHATEEB A.A., 2008 a - *Comparison effects of sucrose and date palm syrup on somatic embryogenesis of date palm* (Phoenix dactylifera L.). - Am. J. Biochem. and Biotech., 4(1): 19-23.

AL-KHATEEB A.A., 2008 b - *Regulation of* in vitro *bud formation of date palm (Phoenix dactylifera L.) cv. Khanezi by different carbon sources*. - Bioresource Technology, 99 (14): 6550-6555.

AL-KHAYRI J.M., AL-BAHRANY A.M., 2002 - *Callus growth and proline accumulation in response to sorbitol and sucrose-induced osmotic stress in rice*. - Biologia Plantarum, 45: 609-611.

BUAH J.N., TACHIE-MENSON J.W., ADDAE G., ASARE P., 2011 - *Sugarcane Juice as an Alternative Carbon Source for in vitro Culture of Plantains and Bananas*. - Am. J. Food Techn., 6: 685-694.

DEOGADE U.M., DESHMUK V.N., SAKARKAR D.M., 2012 - *Natural gums and Mucilage's in NDDS: Applications and recent approaches*. - Intern. J. Pharmaceutical Techn. Res., 4(2): 799-814.

DHAMANKAR V.S., 1992 - *Molasses, a source of nutrients for* in vitro *sugar cane culture*. - Sugar Cane, 4: 14-15.

FAOSTAT, 2012 - *http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor*.

GAMBORG O.L., 1991 - *Media preparation*, pp. 1-24. - LINDSEY K. (ed.) *Plant tissue culture manual.* - Kluwer Academic Publishers, Dordrecht, The Netherlands.

GEORGE E.F., 1993 - *Plant propagation by tissue culture*. - Exegetics Ltd., Edington, England, pp. 574.

GEORGE E.F., HALL M.A., KLERK G.D., 2008 - *The components of plant tissue culture media II: organic additions, osmotic and pH effects and support systems*. - Plant Propagation by Tissue Culture, pp. 115-173.

GNASEKARAN P., XAVIER R., UMA R.S., SREERAMANAN S., 2010 - *A study on the use of organic additives on the proto-corm like bodies (PLBs) growth of* Phalaenopsis violacea *orchid*. - J. Phytology, 2(1): 29-33.

HALL R.D., 1991 - *The initiation and maintenance of callus cultures of carrot and tobacco*, pp. 1-24. - In: LINDSEY K. (ed.) *IPlant tissue culture manual*. Kluwer Academic Publishers, Dordrecht, The Netherlands.

HASSAN M., AHMED Z., MUNIR M., MALIK S.I., SHAHZAD K., 2009 - *Effect of sorbitol in callus induction and plant regeneration in wheat*. - African J. Biotech., 8(23): 6529-6535.

HISAJIMA S., THORPE T., 1985 - *Carbohydrate utilization and activities of various glycosidases in cultured Japanese morning glory callus*. - Plant Tissue Culture Letters, 2(1): 14-21.

HUANG H.J., HUANG D.Q., 1999 - *Special functions of sorbitol on rice somatic cell culture*. - Chinese J. Rice Sci., 13: 248-250.

ISLAM M.O., RAHMAN M., MATSUI S., PRODHAN A., 2003 - *Effects of complex organic extracts on callus growth and PLB regeneration through embryogenesis in the Doritaenopsis orchid*. - JARQ, 37(4): 229-235.

JAIN R., BABBAR Sh.B., 2005 - *Guar gum and isubgol as cost-effective alternative gelling agents for in vitro multiplication of an orchid,* Dendrobium chrysotoxum*.* - Current Sci., 88(2): 292-295.

KADOTA M., NIMII Y., 2004 - *Influences of carbon sources and their concentrations on shoot proliferation and rooting of* Hosui japanease *pear*. - HortScience, 39(7): 1681-1683.

KHORSHA S., 2014 - *Feasibility of application of apricot gum in grapevine tissue culture and micropropagation media*. - MSc thesis, College of Plant Production, Gorgan University of Agricultural Sciences and Natural resources, Gorgan, Iran.

KOZAI T., 1991 - *Micropropagation under photoautotrophic conditions*, pp. 447-469. - In: DEBERGH P.C., and R.H. ZIMMERMAN (eds.) *Micropropagation: technology and applications*. Kluwer Academic Publ., Dordrecht, The Netherland.

LIPAVSKA H., KONRADOVA H., 2004 - *Somatic embryogenesis in conifers: The role of carbohydrate metabolism*. - In Vitro Cell Developmental Biology-Plant, 40: 23-30.

LLUVERAS-TENORIO A., MAZUREK J., RESTIVO A., COLOMBINI M.P., BONADUCE I., 2012 - *Analysis of plant gums and saccharide materials in paint samples: comparison of GC-MS analytical procedures and databases*. - Chemistry Central Journal, 6: 115-125.

MURASHIGE T., SKOOG F., 1962 - *A revised medium for rapid growth and bio-assays with tobacco tissue cultures*. - Physiologia Plantarum, 15: 473-497.

MURDAD R., LATIP M.A., AZIZ Z.A., RIPIN R., 2010 - *Effects of carbon source and potato homogenate on* in vitro *growth and development of Sabah's endangered orchid:* Phalaenopsis gigantea. - Asia-Pacific J. Mol. Biol. Biotech., 18(1): 199-202.

NAMBIAR N., TEE C.S., MAZIAH M., 2012 - *Effects of organic additives and different carbohydrate sources on proliferation of proto-corm like bodies in*

Dendrobium alya *Pink*. - Plant Omics, 5(1): 10-16.

NUSSINOVITCH A., 1997 - *Hydroclloid applications. Gum technology in the food and other industries*. - Springer Science, pp. 361.

PAWLICKI N., WELANDER M., 1995 - *Influence of carbohydrate source, auxin concentration and time of exposure on adventitious rooting of the apple rootstock Jork 9*. - Plant Sci., 106(2): 167-176.

SANIEWSKI M., JUNICHI U., MARCIN H., KENSUKE M., JERZY P., 2001 - *Gum in apricot (*Prunus armeniaca *L.) shoots induced by methyl jasmonated*. - Acta Agrobotanica, 2: 27-34.

SAS INSTITUTE INC., 2003 - *The SAS System for Windows,* Release 8.2. SAS Institute Inc., Cary, NC, USA.

SIMAS F.F., GORIN P.A.J., WAGNER R., SASSAKI G.L., BONKERNER A., IACOMINI M., 2008 - *Comparison of structure of gum exudate polysaccharides from the trunk and fruit of the peach tree (*Prunus persica L.*)*. - Carbohydrate Polymers, 71: 218-228.

SIMAS-TOSIN F.F., WAGNER R., SANTOS E.M.R., SASSAKI G.L., GORIN P.A.J., IACOMINI M., 2009 - *Polysaccharide of nectarine gum exudate: Comparison with that of peach gum.* - Carbohydrate Polymers, 76: 485-487.

TAHERIAN Z., 2012 - *Study of the role of culture medium on callus regeneration and secondary metabolites of* Stevia rebaudisana. - MSc thesis, College of Plant Production, Gorgan University of Agricultural Sciences and Natural resources, Gorgan, Iran.

TAWARO S., SURANINPONG P., CHANPRAME S., 2008 - *Germination and regeneration of* Cymbidium findlaysonianum *Lindl. on a medium supplemented with some organic sources*. - Walailak J. Sci. Techn., 5(2): 125- 135.

TORRES K.C., 1989 - *Tissue culture techniques for horticultural crops*. - Springer US, pp. 224.

VERBEKEN D., DIERCKX S., DEWETTINCK K., 2003 - *Exudate gums: occurrence, production, and applications*. - Appl. Microb. Biotech., 63: 10-21.

WANG C.Y., CHIOU C.Y., WANG H.L., KRISHNAMURTHY R., VENKATAGIRI S., TAN J., YEH K.W., 2008 - *Carbohydrate mobilization and gene regulatory profile in the pseudobulb of Oncidium orchid during the flowering process*. - Planta, 227(5): 1063-1077.

YASEEN M., AHMAD T., SABLOK G., STANDARDI A., HAFIZ I.A., 2012 - *Review: role of carbon sources for in vitro plant growth and development*. - Molecular Biology Report, 40(4):2837-2849.