Sensitivity of ZIM-probes and fruit gauges for the determination of plant water status in two olive genotypes

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Figure 1. ZIM-probe clamped to a leaf.

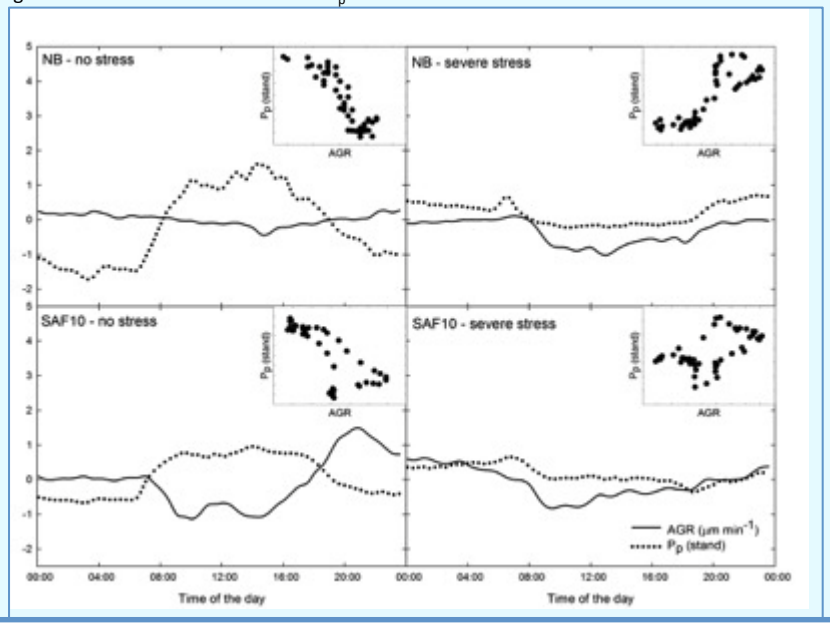
Figure 2. Fruit gauge attached to a fruit.

Two continuous monitoring systems:
- ZIM-probes (Zimmermann et al 2008) on leaves (Fig. 1) for determination of leaf patch pressure (P_p, the inverse of leaf turgor pressure)
- Fruit gauges (Morandi et al. 2007) on fruits (Fig. 2) for the assessment of fruit absolute growth rate (AGR)

Reference: Scholander pressure chamber (1964) for the determination of stem water potential (Ψ_{stem}).

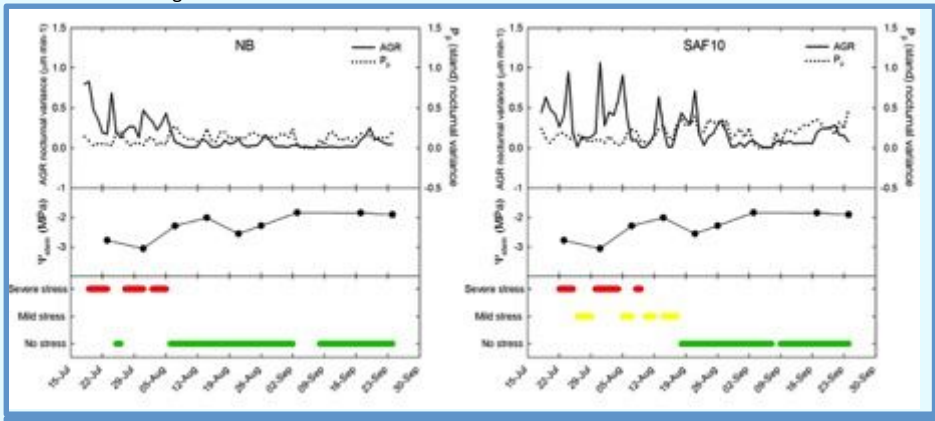
Olive genotypes: **'Nocellara del Belice' (NB)** and **SAF10**

Figure 3. Diel fluctuations of AGR and P_p in NB and SAF10 under no water stress and severe stress.



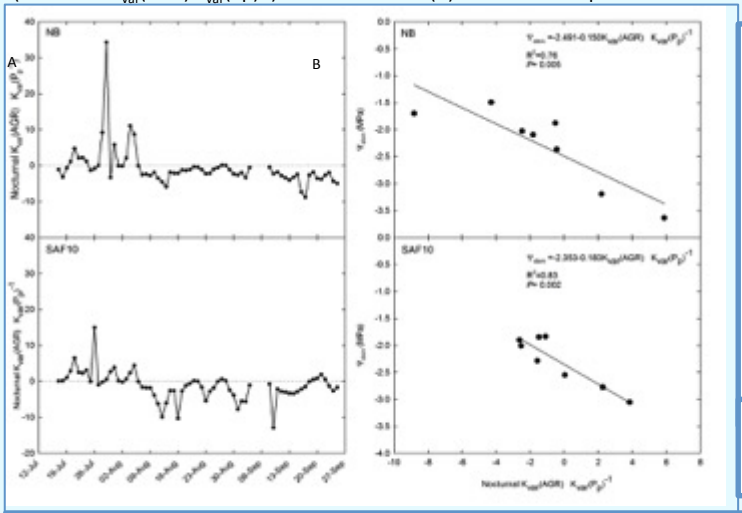
The relationship between AGR and P_p is inverted in conditions of severe water stress.

Figure 4. Nocturnal variance of AGR and P_p in NB and SAF10 genotypes in several conditions of plant water status during summer 2015.



AGR and Pp nocturnal variance change according to tree water stress and appear to be related.

Figure 5. Seasonal fluctuation of the ratio between the nocturnal AGR and P_p coefficients of variation (Nocturnal $K_{var}(AGR)$ $K_{var}(Pp)^{-1}$) in NB and SAF10 (A) and relationship between the ratio and Ψ_{stem}.



The lack of difference between the slopes of the relationships between the two genotypes suggests that the ratio may be a sensitive tool for the determination of plant water status, and for the assessment of

Plant water deficit at: ratio > 0

In conclusion, both ZIM-probes and fruit gauges may be considered promising tools to detect water stress in olive in real-time.

Morandi B., Manfrini L. et al., 2007. A low-cost device for accurate and continuous measurements of fruit diameter. HortScience, 42(6), 1380-1382.
Scholander P.F., Hammel H.T. et al., 1964. Hydrostatic pressure and osmotic potential in leaves of mangroves and some other plants. Proc. of the Nat. Acad. of Sci., 52(1), 119-125.
Zimmermann D., Reuss R et al., 2008. A novel, non-invasive, online-monitoring, versatile and easy plant-based probe for measuring leaf water status. J. of Exp. Bot., *59*(11), 3157-3167.

Stevia rebaudiana: a study of volatiles profile from plants grown in the field, greenhouse and micropropagation *in vitro*

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INTRODUCTION

Stevia rebaudiana Bertoni leaves are natural sources of steviol glycosides, which are used commercially for sweetening and flavouring foods and beverages. Steviol glycosides are natural sweeteners constituting an alternative to synthetic compounds like cyclamates or aspartame. *S. rebaudiana* has been produced mainly for its stevioside compounds but it contains other metabolites with potential therapeutic benefits such as alkaloids, hydroxycinnamic acids, oligosaccharides or essential oils. The chemical profile of samples developed by micropropagation, greenhouse or field conditions, should be characterized to ensure the quality of the samples supplied by *Stevia* producers.

METHODOLOGY

Multiplication rate and fresh weight were determined for plants micropropagated in two different culture media: i) medium A - Murashige and Skoog (MS) without hormones and sucrose, and ii) medium B - MS with 0.5 mg.L^{-1} of kinetin and 20 g.L^{-1}of sucrose. Apart from spontaneous rooting rate determination, induction of plant rooting by auxin shock, using indole-3-butyric acid (IBA) (2 mg.mL^{-1}), was also evaluated.

Acclimatization in greenhouse was performed with hydro atomization nozzles working every 10 minutes. Plants on the field were fertilized with a nutrient solution constituted by N, P_2O_5, K_2O and B.

The essential oil yield was determined for plants in all conditions (*in vitro*, greenhouse, field) using a Clevenger-type apparatus. Volatiles were isolated using a Likens-Nickerson apparatus and analyzed by GC-MS.

Figure:

A - Micropropagated in two different culture media (medium A - Murashige and Skoog (MS) without hormones and sucrose), (medium B - MS with 0.5 mg.L^{-1} of kinetin and 20 g.L^{-1}of sucrose).

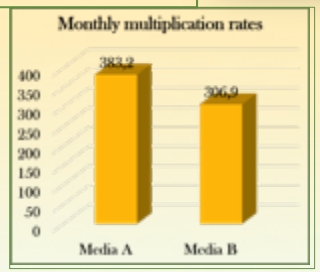
B - Stevia aclimatization in greenhouse.

C - Stevia cultivated in Bragança, Trás-os-Montes, North-eastern Portugal, with defined culture conditions.

D -Extration of essential oil using a Clevenger-type apparatus.

RESULTS AND CONCLUSION

The *in vitro* multiplication rate was 300% per month and the fresh weight after a 4 week subculture was 0.9 g. Spontaneous rooting rate was less than 4% after 4 months but induced rooting achieved 30% of plants with developed root system after 1 week and 70% after 2 weeks. Acclimatization rate was 100% after 2 weeks. The essential oil yield was <0.06% for all samples. Volatiles identification revealed identical composition in all samples, with α-pinene (11-31%), bicyclogermacrene (5–19%), *trans*-β-farnesene (7-15%), β-elemene (6-10%) and β-caryophyllene (3-10%) as major compounds. Quantitative differences were noteworthy.



Graphic 1: The monthly multiplication rates in culture media.

Compounds		IR	Stevia Cultivated	Stevia Greenhouse	Stevia *In Vitro* Medium B	Stevia *In Vitro* Medium A
α-Pinene	MH	930	1,2	1,3	2,5	1,10
Sabinene	MH	958	0,2	0,4	0,3	0,30
1-Octen-3-ol	Oth	961	2,6	4,1	2,7	0,30
β-Pinene	MH	963	11,2	18,6	30,5	14,40
1,8-Cineole	MH	1005	0,7	0,2	0,5	0,00
Limonene	MH	1009	2,0	0,4	0,7	0,50
trans-β-Ocimene	MH	1027	0,5	0,5	0,0	0
Linalool	MO	1074	2,1	1,4	1,3	0,6
Camphora	MO	1102	0,0	0,0	0,6	0
Menthone	MO	1120	0,5	0,0	0,0	0
Menthol	MO	1148	0,2	0,0	0,0	0
α-Terpineol	MO	1159	0,3	0,5	0,5	2
Neryl acetate	MO	1353	0,0	0,0	0,5	0,3
β-Elemene	SH	1388	6,6	9,9	5,7	5,7
trans-β-Caryophylleno	SH	1414	10,4	4,4	3,0	3,50
trans-α-Bergamotene	SH	1434	0,5	0,7	0,6	0,50
α-Humulene	SH	1447	4,2	2,9	4,2	5,70
trans-β-Farnesene	SH	1455	6,6	7,1	14,7	20,30
γ-Muurolene	SH	1469	6,2	4,1	3,3	3,70
Bicyclogermacrene	SH	1487	19,0	13,0	4,8	5,70
δ-Cadiene	SH	1505	1,0	0,4	0,0	0,30
trans-Nerolidol	SO	1549	3,4	4,0	3,1	2,30
Spathulenol	SO	1551	1,3	1,0	0,0	0,30
τ-Cadinol	SO	1616	1,1	0,0	0,0	0,00
α-Cadinol	SO	1626	1,2	0,6	0,4	0,40
% Identification			82,9	75,7	79,8	67,90
Monoterpene hydrocarbons			15,7	21,5	34,4	16,30
Oxygen-containing Monoterpenes			3,2	1,9	2,9	2,90
Sesquiterpene hydrocarbons			54,4	42,5	36,3	45,40
Oxygen-containing Sesquiterpenes			7,1	5,6	3,5	3,00
Others			2,6	4,1	2,7	0,30
TOTAL			82,9	75,7	79,8	67,90

Acknowledgement: The authors are grateful to strategic project PRODER nº 46025 - Gestão Sustentável da Produção de Plantas Aromáticas e Medicinais, and PRODER nº 46207 - Adaptação cultural de hortelã-vulgar e Stévia.

Effect of Fermentation on the Antioxidant Activity of Kalecik Karası (*Vitis vinifera* L.) Winery Pomace

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ABSTRACT

Besides the effects on the quality of wine, phenolic compounds have an important role in viticulture and oenology with their antioxidative effects. Phenolics compounds in winery pomace change with vinification. The studies confirmed that phenolic compounds in grapes and wines have antioxidant, anti inflammatory and anti carcinogenic effects. In this research Kalecik Karası (*Vitis vinifera* L.) grape variety harvested on technological maturity and processed into the wine. Samples was taken in two different times; at harvest day and after pressing. The purpose of the study is to identify the differences antioxidant activity in grape pomace between before and after fermantation. Antioxidant capacity of the pomace was measured spectrophotometrically with TEAC and DPPH methods. As a result of the study the highest antioxidant activity of Kalecik Karası grape pomace was measured in the after fermantation samples.

Key words: Kalecik Karası, wine grape, TEAC, DPPH, winery, pomace.

MATERIAL

Kalecik Karası (*Vitis vinifera* L.) grape variety

Sample 1

Harvest day (Before processing to wine)
Lyophilised grape

Extraction (Waterhouse 2005)
Determine the antioxidant capacity

Sample 2

Pressing day (After fermentation)
Lyophilised grape pomace

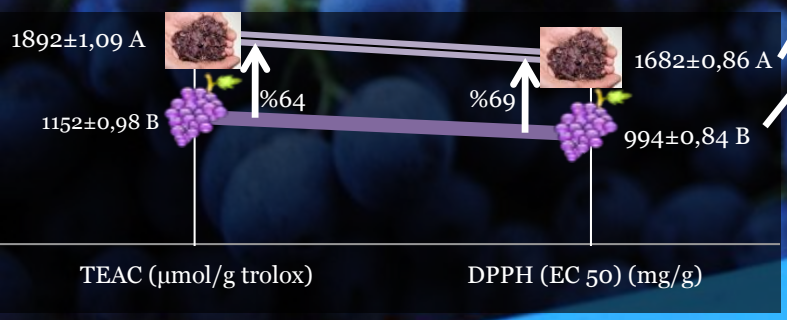
METHOD

TEAC (Re *et al.* 1999)

DPPH (Sanchez Moreno *et al.* 1998)

RESULT

Effect of fermentation on antioxidant activity



Highest antoxidant capacity in “after fermentation” samples

Lower antoxidant capacity in “harvest day ” samples

Smilar to previous research (Girard *et al.* 2001, Sacchi *et al.* 2005, Fanzone *et al.* 2011)

Our study showed that fermentation is effective on increase in atioxidant capacity.

References

•Fanzone, M., Zamora, F., Jofré, V., Assof, M., Gómez-Cardovés, C. and Peña-Neira, A. 2011. Phenolic characterisation of red wines from different grape varieties cultivated in Mendoza province (Argentina). Journal of Food and Agriculture, 92: 704-718.

•Girard, B., Yuksel, D., Cliff, M.A., Delaquis, P. and Reynolds, A.G. 2001. Vinification effects on the sensory, colour, and GC profiles of Pinot noir wines from British Colombia. Food Research International, 34: 483-499.

•Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Biology and Medicine, 26: 1231–1237.

•Sacchi, K.L., Bisson, L. F. and Adams, D. O. 2005. A review of the effect of winemaking techniques on phenolic extraction in red wines. American Journal of Enology and Viticulture, 56: 3.

•Sanchez-Moreno, C. Review: Methods used to evaluate the free radical scavenging activity in foods and biological systems. Food Sci. Technol. Int. 2002 , 8, 121–137.

•Waterhouse, A.L. 2005. Determination of total fenolics, in Handbook of Food Analytical Chemistry, ed. by Wrolstad, R.E., Acree, T.E., Decker, E.A., Penner, M.H., Reid, D.S., Schwartz, S.J., Shoemaker, C.F., Smith, D.M., Sporns, P. John Wiley & Sons, 463–470, New Jersey.

Micropropagation of *Eryngium viviparum* J. Gay: a new way to recover this endangered plant with potential in pharmacology.

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Introduction

s well-known worldwide due to two main reasons: a) this genus are very rich in several organic compounds with great potential in pharmacology and b) because some species are endangered. In this study, we focus on *Eryngium viviparum* (Apiaceae), a small biennial plant that grows in areas subject to seasonal flooding in the NW of Spain and France. It´s classified as endangered and their extinction would mean the loss of potential drugs for human disease. In this work, a micropropagation protocol has been designed by testing the effect of two plant hormones on shoot proliferation.

Materials & Methods

nd culture conditions: *E. viviparum* seeds were collected from its natural habitat in Spain (A Lagoa de Cospeito, Lugo, Galicia). Seedling from germination *in vitro* were cultured in MS medium (Murashige & Skoog, 1962) supplemented with 1 mg L^{-1} 6 - benzyladenine (BAP) and 0.1 mg L^{-1} indole-3- butyric acid (IBA) for 3 subcultures (4 weeks each subculture). Shoots (10) from the above cultures were placed in MS supplemented with two cytokinins (BAP and Kinetin (KIN)) at various concentrations (Table 1) and 0.1 mg L^{-1} IBA.

Explant proliferation rate, number of new shoot proliferated and length parameters were recorded after each subculture period (3). The culture were maintained under cool white light (40 µmol m^{-2} s^{-1}) 16:8 h (light:dark) photoperiod and at 24 ± 2 ºC.

Table 1.-Combination of phytohormones tested.

Treatment	Cytokinins (mg L^{-1})		Auxin (mg L^{-1})
	BAP	KIN	IBA
BAP 1	1,0	0,0	0,1
BAP 1,5	1,5	0,0	
BAP 2	2,0	0,0	
KIN 1	0,0	1,0	
KIN 1,5	0,0	1,5	
KIN 2	0,0	2,0	
BAP 1 + KIN 1	1,0	1,0	

Results & Discussion

ion was observed at all cytokinins concentrations (Fig. 1), proportional to cytokinin concentration. This results disagree with those obtained in other Apiaceae species, describing a significant shoot proliferation decrease at concentrations up to 1 mg L^{-1} BAP or when an auxin no was present in the medium (Thiem *et al*., 2013). Here, *E. viviparum* showed a significant higher proliferation rate at concentrations of 2 mg L^{-1} of cytokinins (as BAP alone or in combination with KIN at 1 mg L^{-1} each), but all in combination with auxin. Also, the highest number of new shoots were obtained at higher concentration of BAP along or in the combination with KIN (Fig. 2). No significant differences were recorded for shoots length (data not shown).

In conclusion, this preliminary results revealed that the designed protocol appears to be successful for *E. viviparum in vitro* proliferation although further research will be need to improve shoot proliferation and to elucidate the role of BAP and IBA.

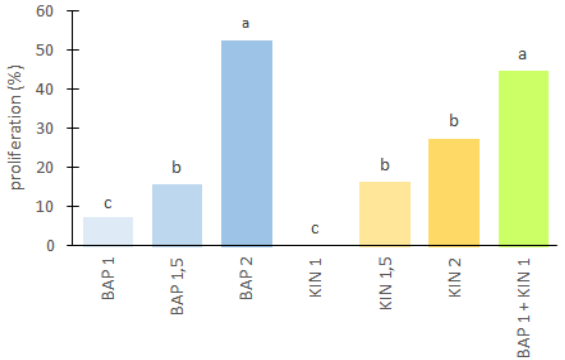

Fig. 1.-Effect of cytokinins at different concentrations on explants proliferation. Treatments denoted by the same letter were not significantly different (p<0.05; Wald test)

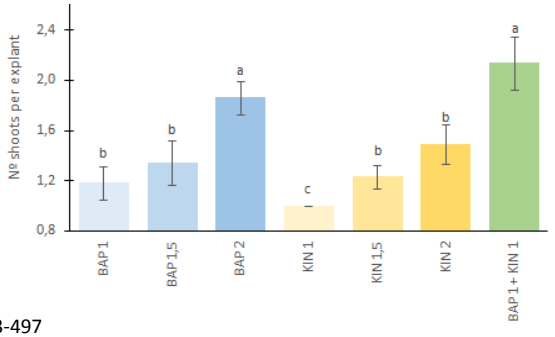

Fig. 2.- Effect of KIN and BAP on number of shoots per explant. Different letters indicate significant differences between concentrations of phytohormone (p<0.05; Wald test)

References

Murashige, T., Skoog., F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue culture. Physiol. Plant. 15:473-497

Thiem, B., Kikowska, M., Krawczyk, A., Wieckowska, B., Sliwinska, E. 2013. Phenolic acid and DNA contents of micropropagated *Eryngium planum* L. Plant Cell Tiss. Organ. Cult. 114:197-206

Molecular evidence of the presence of quarantine Citrus pathogens in the main Algerian citrus growing areas predicts a risk on the citrus patrimony

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Introduction

Citrus represent one of the most important fruit crop in Algeria, covering a total of ca. 70.000 ha and a production of ca. 900.000 tons of fruits. Algeria has a citrus germplasm collection of 256 varieties/clones, which represents a reservoir of genetic resources of inestimable value. This collection is located in the Mitidja area that represents one of the main citrus growing regions in the northern part of the country.

Figure 1: Location of the Mitidja area

Material & methods

Monitoring of the main quarantine pathogens, such as Citrus tristeza virus, and *Spiroplasma citri*, the causal agents of citrus tristeza and stubborn disease was conducted during the last years.

The survey has been conducted using molecular assays (PCR) and serological technique (DTBIA) (1) for the detection of both pathogens. In this context, around 3000 citrus trees were inspected for the presence and distribution of these pathogens in the region.

Molecular assays were performed using the primer pairs T36CP targeting the coat protein gene for the detection of CTV (2).

Whereas, for the *S.citri* detection, the primer pairs SC1 targeting the Spiralin gene were used (3).

In order to perform the molecular characterization of the detected positive samples, cloning was carried out followed by sequence analysis using Mega 6.06 software (4).

Figure 2: Overview of some DTBIA steps

Results

Interestingly, among the sampled trees, the overall infection rate DTBIA assay reached in some areas an infection rate of the 25 % for CTV; whereas, it reach only 2% infection rate for *S. citri*.

Most of the infected CTV trees showed clear cut symptoms in the field including quick decline, however the *S. citri* infected trees evidenced stunting of the tree and leaves.

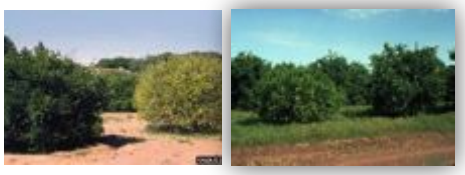

Figure 3: Quick decline of trees and stunting

Among the positive trees, based on the symptoms, expression, age and origin of the trees, some infected plants were selected for further investigations. Molecular assays performed on these selected trees evidenced bands from expected size 672 bp using the primer pairs T36CP targeting the coat protein gene. Whereas the *S.citri* infected trees evidenced bands from 336 bp size using the primer pairs SC1 targeting the Spiralin gene.

Interestingly, for both pathogens the serological trials confirmed the results obtained by PCR assays.

The phylogenic analysis of the obtained nucleotide sequences of the analysed CTV local strains shared a high nucleotide identity with the Spanish CTV mild isolate T385, whereas the detected *S. citri* revealed high nucleotide identity with the Iranian Fasal strain(5) and the Moroccan strain (GII3), both of them were responsible of severe epidemics in some Mediterranean countries.

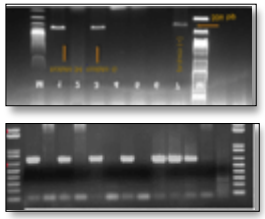

Figure 4: Electrophoretic profile of PCR products using SC1 primers pairs 336bp and T36CP 672bp

(M) DNA ladder 1-5 Algerian samples, lane 6 water control, lane 7 positive control

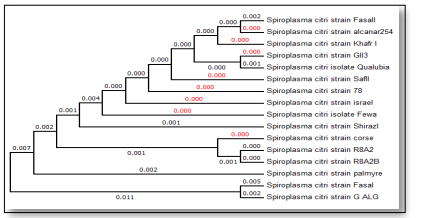

Figure 6 : Phylogenetic tree constructed with the partial spiralin sequences of different isolates from Mediterranean countries

Conclusion

These surveys evidenced a high incidence of CTV infection in some areas (25%), whereas lower was the incidence of the *S. citri* infected trees (2%) in this area.. The presence of isolates from these pathogens that caused outbreaks in some countries of the mediterranean area represents a threat for the Algerian citrus industry. In order to avoid the dispersal of these diseases and preserve the citrus patrimony in the country, several preventive measures such as the use of healthy propagating material, sanitation procedures, vectors and disease monitoring have to be taken by the governmental and scientific institutions.

References

(1) Djelouah K., Frasheri D., Valentini F., D'onghia A. M. and Digiaro M (2014). Direct tissue blot immunoassay for detection of Xylella fastidiosa in olive trees. *Phytopathologia Mediterranea*, 53(3): 559-564.

(2) Larbi D. Belkahla H., Djelouah K. and D'onghia A.M. (2013). Monitoring Citrus Tristeza Virus (CTV) in Algeria and Characterization of Local Isolates DOI: 10.17660/ActaHortic.2015.1065.96

(3) Foissac X., Saillard C., Gandar J., Zreik L. and Bove J. M. (1996). Spiralin polymorphism in strains of Spiroplasma citri is not due to differences in posttranslational palmitoylation. *Journal of bacteriology*, 178(10): 2934-2940

(4) Tamura Koichiro, Stecher Glen, Peterson Daniel, Filipski Alan and Kumar Sudhir (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular biology and evolution*, 30(12): 2725-2729.

(5) Khanchezar A, Izadpanah K, Salehi M and Taghavi Sm. (2010). Novel isolate of Spiroplasma citri from leafhopper Circulifer haematoceps from Fars province. *Iranian Journal of Plant Pathology*, 46(3).

Ozone application to control black Aspergilli contamination and ochratoxin A of Turkish sultana seedless.

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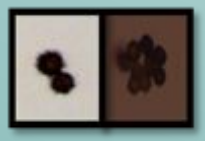
Introduction

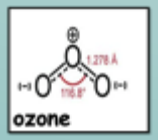
The production of raisins has a great significance in Turkey, where 250,000-300,000 tonnes are produced and 75% of the production is exported to the international market (Çolak, 2012). However, this crop is subject to fungal contamination caused mainly by black Aspergilli,

Among the black Aspergilli group several species can produce Ochratoxin A (OTA), a mycotoxin hazardous to human health (Abarca *et al*, 2003),

To date, there is no effective means of controlling these contaminations. Recently ozone has gained attention as an antimicrobial agent for maintaining food quality during storage (Gabler *et al,* 2010).

The aim of this study was to investigate the effect of ozone application on the black Aspergilli and OTA contamination of Turkish sultana seedless.





Materials and Methods

Sampling

Fungal isolation

Incubation

CFU and OTA analysis were repeated after the treatment.

Ozone (O_3) treatment

- 50 ppm
- 100 ppm
- 150 ppm

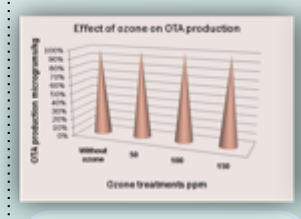
2 hours

CFU and OTA analyses

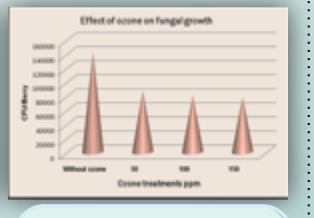
Quality assessment of some horticultural parameters

- Titrable Acidity (TA) was measured by NaOH 0.1N
- Dry matter was measured by weighting the samples (25g) after 2 weeks heating at 65°C.

Result and discussion



Ozone treatment at 50, 100 and 150 ppm reduced OTA production by 66,3%, 67,6% and 69,4% respectively



Ozone treatment at 50, 100 and 150 ppm reduced fungal growth by 28,6%, 34,4% and 36,2% respectively

Quality parameters

O_3 treatment (ppm)	TA %	pH	Dry matter (%)
Without O_3	2,1	4,27	92,551
50	2,62	4,21	92,426
100	2,37	4,24	92,237
150	2,23	4,22	92,105

No significant difference was observed between treated and non treated samples. Also the color of raisins was not affected by O_3 treatment.

Conclusion

- A significant effect of ozone treatment was observed both on Ochratoxin A production and on fungal growth;
- No significant difference was observed among the different concentrations of ozone treatment
- The convenient ozone concentration was 50 ppm which was sufficient to reduce OTA up to 70% and CFU up to 28%.
- Ozone treatment had no influence on some horticultural quality parameters such as, titrable acidity, pH, dry matter and on the color of raisins.

References

66: 504-506
- Gabler F.M., Smilanick J.L., Mansour M.F., Karaca H. 2010. Influence of fumigation with high concentrations of ozone gas on postharvest gray mold and fungicide residues on table grapes. Postharvest Biol. and Technol. 55, 85-90.
- Çolak M. 2012. Heavy metal concentrations in sultana-cultivation soils and sultana raisins from Manisa (Turkey). *Environmental Earth Sciences*, 67(3): 695-712.

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Antennal olfactory responses in *Trissolcus basalis* females using Single Sensillum Recording

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Trissolcus basalis (Wollaston)

Fagopyrum esculentum

Introduction

The sense of smell is essential for many phytophagous insects in locating food, mates, hosts, and oviposition sites. The released blends of volatile compounds by plants help and provide insects with crucial information about their nutritional resources finding and recognition. For *Trissolcus basalis* (Hymenoptera : Platygastridae) which is an egg parasitoid of *Nezara viridula* (Heteroptera : Pentatomidae), a highly polyphagous pest attacking a wide variety of crop plants, previous study has revealed that the wasp females presented behavioural and electrophysiological responses to headspace volatile extracts and a synthetic blend of buckwheat (*Fagopyrum esculentum)* plant volatiles. In this study, we assessed the responses of *T. basalis* females to individual compounds of buckwheat volatiles using Single Sensillum Recording technique (SSR) with the aim to identify the key active buckwheat volatile compounds.

Materials and Methods

SSR bioassay was conducted by mounting *T. basalis* female on a Plasticine block with U-shaped thin copper wire restrains. The insect preparation was positioned in the middle of a charcoal-filtered and humidified main air stream. The reference electrode was a micro-glass electrode inserted into the abdomen of the insect. The recording electrode, an electrochemically sharpened tungsten electrode, was brought in contact with a sensillum in the antenna. The antenna was stimulated with 0.1 s pulses of air containing various test stimuli using Pasteur pipette stimulus cartridges. First the sensillum was stimulated by three mixtures of test compounds (Mixture A, Mixture B and mixture C) which are described in the table 1. If any mixture presents an electrophysiological response after the stimulation, the compounds of this eliciting response mixture were tested individually and randomly.
Data were processed using software (Autospike 32, Syntech, Hilversum, The Netherlands) and the responsiveness of olfactory receptor neurons ORNs was analyzed by comparing the number of action potential before and after 1 second of odor stimulation.

Table 1
Test compounds for the SSR study of *Trissolcus basalis*.

Mixture groups	Compound	Chemical purity (%)
	3-Methyl butanoic acid	97
Mixture A	2-Methyl butanoic acid	98
Buckwheat plant volatiles	Hexanoic acid	98
	α-farnasene	99
	P-benzoquinone	98
	(Z)-3 –Hexen-1-yl acetate	98
	Butanoic acid	98
	1-Nonanol	98
	Geraniol	98
Mixture B	Linalool	97
Common plant volatiles	2-Phenylethanol	99
	Benzldehyde	99.5
	Citral (geranial + neral)	96
	(E)-B-Caryophyllene	98.5
Mixture C	Germacrene-D	40
Common plant volatiles	Geranyl acetate	98
	(Z)-3-hexen-1-ol	98

Results

Among 67 sensilla exhibiting spontaneous firing of action potentials in *T. basalis* females antennae examined, 56 sensilla were found to contain ORNs responsive to plant volatile compounds. The other 11 showed no responses to any of the mixtures tested. Among the three mixtures tested (Table 1), mixture A, containing buckwheat plant volatiles, elicited significant and consistent responses and 44 sensilla showed exclusive responses to this mixture. The responses of these ORNs to the two other mixtures were not significant. Based on their responsiveness to the mixtures, 7 sensillum classes were identified in the tested females (Table 2).

Table 2
Sensillum classes identified in *Trissolcus basalis* females according to their responsiveness to three mixtures of plant volatile compounds: A, B and C. *Hexane was used as control solvent.

sensillum class / Mixtures	NR	A	B	C	AB	AC	ABC
Hexane*							
Mixture A		X			X	X	X
Mixture B			X		X		X
Mixture C				X		X	X
Observed number	11	44	0	1	4	3	4

Among the seven tested buckwheat volatiles, the two major compounds eliciting consistent and significant responses from the largest proportion of the responsive olfactory receptor neurons (ORNs) were 3-methylbutanoic acid (Fig1.) and *p*-benzoquinone (Fig.2)..

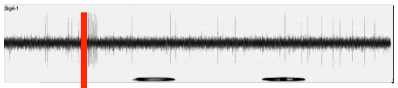

Fig.1

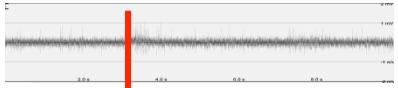

Fig.2

Discussion

SSR shows that the parasitoid *Trissolcus basalis* presents different types of sensilla that seem have a kind of specialized responses for buckwheat volatiles and that 3-methylbutanoic acid and *p*ara-benzoquinone, displayed the highest olfactory activities on the ORNs examined. These olfactory cues might be used by the organism to identify a potential food source.

Cytogenetic and molecular characterization of an interspecific hybrid *Asparagus officinalis* x *A. amarus*

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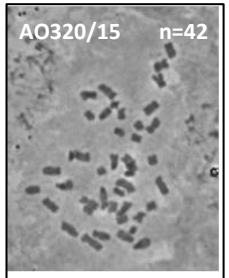
Bundesforschungsinstitut für Kulturpflanzen
Federal Research Centre for Cultivated Plants

Asparagus Virus 1 (AV-1)

- spread worldwide
- transmitted non-persitant manner by aphids
- no symptoms
- detrimental effects on vigor, yield and quality

Introgression-backcrossing to transmit the AV-1 resistance in the *A.officinalis* background

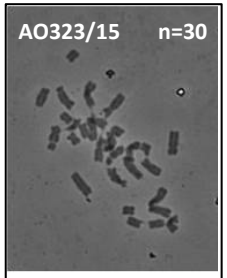


AV-1 resistant

P *A.amarus* (60 chromosomes) x *A.officinalis* (40 chromosomes)

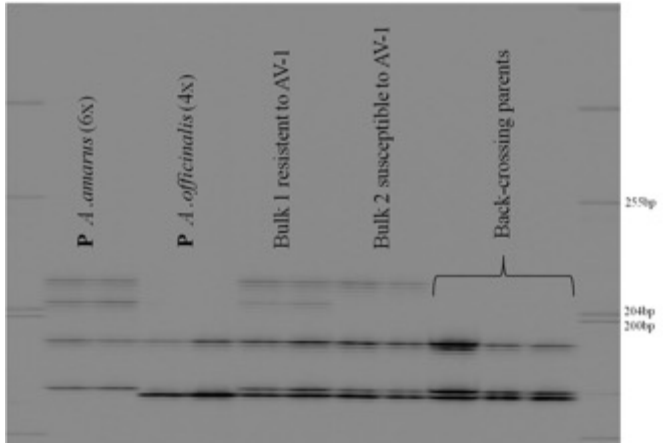
F_1 *AO208/12-S* (50 chromosomes) x *A.officinalis* (40 chromosomes)

BC_1 *AO320/15* *AO323/15*



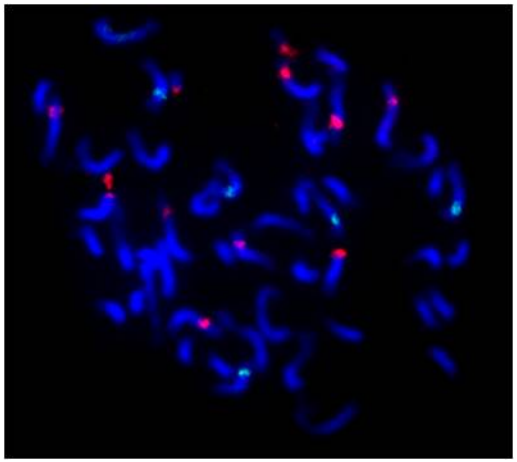
AV-1 susceptible

Bulked Segregant Analysis to identify molecular markers linked to AV-1 resistance



SSR Marker *asp_c4789*

Fluorescence *in-situ* Hybridization (FISH)



F_1 plant AO 208/12:
stained with DAPI (marked all 50 chromosomes blue),
5s rDNA probes (2 green signals) and
18/25s rDNA probes (10 red signals)

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The Effect of Postharvest 1-Methylcyclopropane Treatments on Sugar Content of 'Gloster' and 'Cooper 900' Apples During Cold Storage

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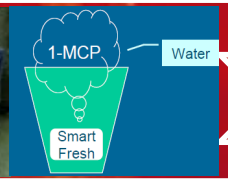
Abstract

Sugars are one of the most important components of apple quality and taste. It has been proved that postharvest 1-MCP technology is very effective for keeping quality for long term storage in many apple cultivars. There is no available data on how this technology effects sugar content of fruit in 'Cooper 900' and 'Gloster' apple cultivar. For this reason fruit harvested at the commercial harvest time and treated with 1-MCP at two different concentrations (625 and 1250 ppb) at room temperature (20±1°C) for 24 hours and then stored at 0±1°C and 85-90% relative humidity conditions for 5 months. Controls were stored without any treatment. As a result, in both cultivars sucrose, glucose and fructose were the dominant sugars. 1-MCP treatments had significant effect on sucrose content but not on other carbonhydrates such as fructose and glucose. However significant differences were observed between the cultivars. It seems that sucrose may be a ripenig related carbonhydrate in these cultivars.

Materials and Methods



Sugar Extraction Method

10gr apple + 20ml pure water → 1min Homogenise → 45min Centrifuge → Supernatant filtration → HPLC

Results

Sucrose Content (mg/kg)

Storage Period (Month)	Cooper 900			Gloster			Storage Period Averages
	Control	625 ppb 1-MCP	1250 ppb 1-MCP	Control	625 ppb 1-MCP	1250 ppb 1-MCP	
0	14761±1262	12303±883	12741±1378	17771±3248	19435±5234	18706±2323	15952 a[2]
1	15565±693	10952±495	10251±3592	10461±1285	16342±1217	18160±4241	15288 a
2	9948±1108	8224±634	10216±1544	15614±635	10514±599	14456±2358	11495 b
3	8158±470	5140±*	6508±705	12903±2927	5533±1921	9284±531	7921 c
4	9193±1108	6265±458	5439±223	8862±1705	7275±152	10181±2201	7869 c
5	9163±307	8320±690	8418±1382	12281±1839	8313±373	12404±2134	9816 bc
1-MCPApplication Avegares				10890 b[2]	9884 b	11397 a	
Variety Averages			9531 b[3]			13249 a	

[1] p≤ 0,05 differences between storage periods, [2] p≤ 0,05 differences between 1-MCP applications, [3] p≤ 0,05 differences between varieties

Glucose Content (mg/kg)

Storage Period (Month)	Cooper 900			Gloster			Storage Period Averages
	Control	625 ppb 1-MCP	1250 ppb 1-MCP	Control	625 ppb 1-MCP	1250 ppb 1-MCP	
0	14386±1109	15170±574	15996±1398	11812±4213	14450±2789	15377±1352	14531 c[1]
1	16715±1673	13305±2441	11509±4039	20008±1238	11893±2777	16698±2320	15020 c
2	21253±699	18450±7970	19897±3521	22346±249	23382±2384	21766±1061	21182 ab
3	20288±1408	18598±*	21647±2278	24392±2911	11032±5627	22121±1672	19679 bc
4	20128±4363	15934±5167	16997±7044	21555±3827	15975±40	22775±3211	18894 bc
5	26324±982	22489±2316	24755±4389	24526±417	28374±2268	22934±868	24900 a

[1] p≤ 0,05 differences between storage periods

Fructose Content (mg/kg)

Storage Period (Month)	Cooper 900			Gloster			Storage Period Averages
	Control	625 ppb 1-MCP	1250 ppb 1-MCP	Control	625 ppb 1-MCP	1250 ppb 1-MCP	
0	40684±1720	35229±2592	39566±2092	35124±871	33579±1833	33314±486	36249 cd[1]
1	42156±1161	40954±1423	31142±12047	32999±4956	27600±4382	33636±2147	34747 d
2	51323±1107	53478±1944	52748±4405	42101±686	43073±3935	41012±3499	47289 ab
3	39578±6657	45847±*	55358±4868	43657±8534	32770±17064	39567±3794	42796 bc
4	50802±4933	51690±3421	39921±12017	38028±4481	38334±4774	41358±6194	43355 abc
5	58144±4323	50523±2324	56143±3049	44401±2550	48172±491	45386±1459	50461 a
Variety Averages			46404 a[2]			38561 b	

[1] p≤ 0,05 differences between storage periods, [2] p≤ 0,05 differences between varieties

Acknowledgement
• 1-MCP used this research was obtained by AgroFresh.

CONTROLLED RELEASE SYSTEM USING LAYER-BY-LAYER ASSEMBLY FOR FRESH-CUT FRUIT APPLICATION

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INTRODUCTION

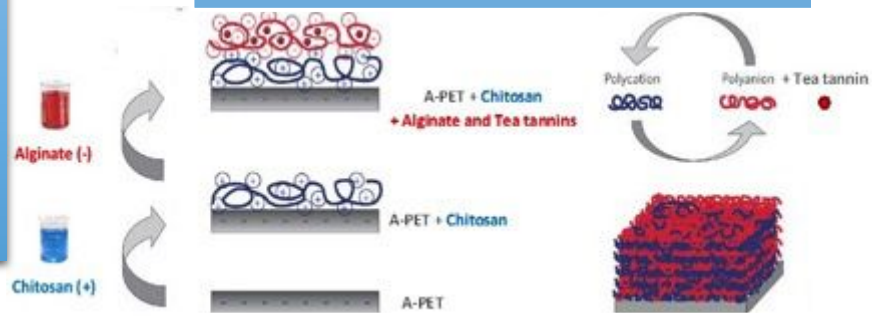
Layer-by-layer (LbL) assembly is a basic technique for the fabrication of multicomponent films on solid supports[1]. Polyelectrolyte multi-nanolayer films can be fabricated through repeat deposition, mainly due to the electrostatic attraction between oppositely charged polyelectrolytes[2]. Chitosan (as antifungal) [3] and alginate including catechin (as antioxidant)[4], were selected as useful polyelectrolytes.

OBJECTIVE

Development and characterization of a controlled release system (CRS) based on Chitosan-Alginate, including catechin, for future application on fresh-cut fruit.

EXPERIMENTAL PROCEDURE

Deposition of 20 bilayers Chitosan (0,2%) - Alginate (0,2% + 0,35% catechin)



MATERIALS and METHODS

In vitro analysis

CRS has been extracted by food simulant (Citric acid solution, at 3,8 pH) for 6, 12, 48 h under stirring.

- Antioxidant capacity (by DPPH);
- Microbiological test on: *Aspergillus niger* (data not shown) and *Penicillium chrysogenum;*
- UV-Visible spectrophotometry.

RESULTS

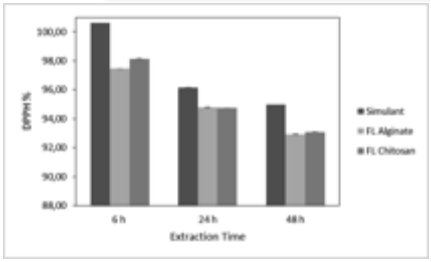


Figure 1. Antioxidant assay of LbL extract expressed as percentage of DPPH decay during the three extraction times (6, 24, 48 h), for different final layer (FL), chitosan or alginate.

Figure 2. Culture of *P. chrysogenum,* front (left) and back (right) of Petri-dish, after 6 days of incubation with different LbL extracts.

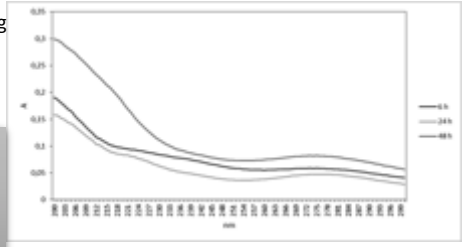


Figure 3. UV-spectra of different LbL extracts at three different time.

- Catechin release was effectively modulated on time;
- Antifungal activity increase after 48 h of extraction by simulant.

Antioxidant and antifungal properties

CONCLUSION

The novel CRS developed showed the migration of chitosan and catechin modulated by time. Chitosan was effect against *P. chrysogenum*, inhibiting the fungal growth. Tea catechin, immobilized into the alginate layers, carried out its antioxidant capacities, that increase according to the extraction time. These characteristics permit the application of CRS on fresh-cut fruit, in order to obtain shelf-life extension.

[1]Decher, G., 1997. Fuzzy nanoassemblies: Toward layered polymeric multicomposites Sci. 277, 1232–1237.

[2]Donath, E., Sukhorukov, G.B., Caruso, F., Davis, S.A., Möhwald, H., 1998. Novel hollow polymer shells by colloid-templated assembly of polyelectrolytes. Angew. Chem., Int. Ed. Engl. 37, 2202–2205.

[3]Alvarez, M.V., Ponce, A.G., Moreira, M.D.R., 2013. Antimicrobial efficiency of chitosan coating enriched with bioactive compounds to improve the safety of fresh cut broccoli. LWT-Food Sci. Technol. 50, 78-87.

[4]Sutherland, B.A., Rahaman, R.M.A., Appleton, I., 2006. Mechanism of action of green tea catechins. With a focus on ischemia-induced. J. Nutr. Biochem. 17, 291-306.

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