VOLATILES COMPOUNDS COMPOSITION: STEVIA FIELD PLANTS IN MEDITERRANEAN CONDITIONS, GREENHOUSE PLANTS AND PLANTS *IN VITRO*.

GOES H[2], BARROSO M[1,2], RODRIGUES MA[1,2], ARROBAS M[1,2], PEDRO L[3], SOUSA MJ[1,2]

[1]Mountain Research Centre - Escola Superior Agrária-Instituto Politécnico de Bragança
Campus de Santa Apolónia-Apartado 117, 5301-855 Bragança, Portugal
[2] Polytechnic Institute of Bragança - Instituto Politécnico de Bragança, Campus de Santa Apolónia,5300-253 Bragança, Portugal
[3]Sciences Faculty of Lisbon – Faculdade de Ciências de Lisboa, Campo Grande
1300-234 Lisboa, Portugal
joaos@ipb.pt

INTRODUCTION

A major concern about public health is the control of obesity. The search for new natural sweeteners, as an alternative to synthetic compounds like cyclamates or aspartame, brought *Stevia rebaudiana* (Compositae), a sweet plant native to South America, to the attention of the scientific community. *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 characterization of the chemical profile of micropropagated plants, as well as greenhouse and field grown plants, is important to ensure the quality of the plants to supply to Stevia growers.

MATERIAL AND METHODS

Multiplication rate and fresh weight were determined for plants micropropagated in MS media A with sucrose (20g.L-1) and without growth regulators and MS media B with kinetin (0.5mg.L-1) and sucrose (20g.L-1). Apart from spontaneous rooting rate determination, induction of plant rooting by auxin shock, using IBA (2mg.mL-1), was also evaluated.

RESULTS

Greenhouse Material and methods

Acclimatization in greenhouse was performed with hydro atomization nozzles working every 10 minutes. Plants on the field were fertilized by a nutrient solution with N, P2O5, K2O and B.

MICRO PROPAGATION

In vitro multiplication rate was 300% per month, fresh weigh after a 4 week subculture was 0.9g. 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.

Figure 1: Media A (left) and Media B (right)

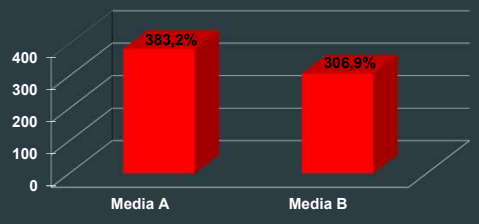

Figure 2: The monthly multiplication rates in culture media.

Figure 3: Percentage at rate of spontaneous rooting, after 4 months in culture

ACCLIMATIZATION

Acclimatization was performed in greenhouse with 360º hidroatomizing spires working each 10 minutes and the acclimation rate in the greenhouse was of 100% for both medium after 2 weeks.

Figure 4: Greenhouse apparatus

Figure 3: Greenhouse Stevia

EXTRACTONS

There were extraction of essential oils with Clevenger apparatus for Yield purposes with average yield values below 0.6 %. In Clevenger, six samples were from Vilariça region and two from micro propagation, one was MS medium without growth regulators and the other was MS medium with 0.5 mg/L kinetin.

In LN the extractions from four different sources, that are represented in figure 6. The essential oil identification was between 75,5 and 83% for the three samples and the results are not very different in the compounds. 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.

Cultivated Stevia shows the higher value in the sequiterpenes hydrocarbons (54,4%), oxygen-contaning monoterpenes (3,2%) and oxygen-contaning sequiterpenes (7,1%). *In vitro* Stevia Media A and B the value are only very different in monoterpene hydrocarbons and other compounds, but the other value are very similar or exactly the same. In general cultivated and greenhouse Stevia have higher values for sequiterpenes and the *in vitro* Stevia have higher values for monoterpenes.

Compounds		Stevia Cultivated	Stevia Greenhouse	Stevia *In Vitro* (Media A)	Stevia *In Vitro* (Media B)
α-Pinene	MH	1.2	1.3	1,1	2.5
Sabinene	MH	0.2	0.4	0.3	0.3
1-Octen-3-ol	Oth	2.6	4.1	0,3	2.7
β-Pinene	MH	11.2	18.6	14.4	30.5
1,8-Cineole	MH	0.7	0.2	0.0	0.5
Limoneno	MH	2.0	0.4	0,5	0.7
trans-β-Ocimene	MH	0.5	0.5	0.0	0.0
Linalool	MO	2.1	1.4	0,6	1.3
Camphor	MO	0.0	0.0	0.0	0.6
Menthone	MO	0.5	0.0	0.0	0.0
Menthol	MO	0.2	0.0	0.0	0.0
α-Terpineol	MO	0.3	0.5	2.0	0.5
Neryl acetate	MO	0.0	0.0	0,3	0.5
β-Elemene	SH	6.6	9.9	5,7	5.7
β-Caryophyllene	SH	10.4	4.4	3.5	3.0
trans-α-Bergamotene	SH	0.5	0.7	0,5	0.6
α-Humulene	SH	4.2	2.9	5,7	4.2
trans-β-Farnesene	SH	6.6	7.1	20,3	14.7
γ-Muurolene	SH	6.2	4.1	3,7	3.3
Bicyclogermacrene	SH	19.0	13.0	5,7	4.8
δ-Cadinene	SH	1.0	0.4	0,3	0
trans-Nerolidol	SO	3.4	4.0	2,3	3.1
Spathulenol	SO	1.3	1.0	0,3	0
T-Cadinol	SO	1.1	0.0	0.0	0
α-Cadinol	SO	1.2	0.6	0.4	0.4
% identification		82.9	75.7	67.,9	79.8
Monoterpene hydrocarbons		15.7	21.5	16,3	34,4
Oxygen-containing Monoterpenes		3.2	1.9	2,9	2,9
Sesquiterpene hydrocarbons		54.4	42.5	45,4	36,3
Oxygen-containing Sesquiterpenes		7.1	5.6	3.0	3,5
Others		2.6	4.1	0,3	2,7

Figure 6: Table of the essential oils in the diferents samples

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