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Mother plant luminescence and zeatin concentration in the *in vitro* establishment of an olive plant

Roseane Maidana Moreira[1,*], Márcia Wulff Schuch[1], Zeni Fonseca Pinto Tomaz[1], Carlos Gustavo Raasch[1] and Josiane Vergara Casarin[1]

[1]Universidade Federal de Pelotas, Programa de Pós Graduação em Agronomia, Fruticultura de Clima Temperado, Departamento de Fitotecnia, Caixa Postal 354, CEP 96010

ABSTRACT

This study aimed to evaluate the effect of the presence and absence of light provided to the mother plant and zeatin concentrations *in vitro* establishment, to reduce oxidation and promote the establishment of olive explants. The trials were carried out at the Fruit Tree Propagation Lab, Federal University of Pelotas, Brazil. In the first experiment, plants of olive 'Arbequina' were subjected to the absence and presence of light and established on WPM with 0 and 2mg.L^{-1} zeatin. In the second experiment, plants of olive 'Leccino', 'Arbequina' and 'Frantoio' 'were submitted to the absence of light, and established *in vitro* on WPM with 0, 4 and 8 mg.L^{-1} Zeatin. Mother plants of olive tree 'Arbequina' in conditions of absence of light are the most suitable *in vitro* establishment. The concentration of 2 mg.L^{-1} of zeatin promotes survival of explants of olive 'Arbequina'. The concentrations 0, 4 and 8 mg.L^{-1} of zeatin) results no difference between Leccino and Arbequina cultivars for the variable fungal contamination.

Key words: *Olea europaea* (L), micropropagation, oxidation, cytokinin.

INTRODUCTION

Olive growing has been the basis of the Mediterranean region economy, and the world's largest commercial plantations are located in the European Community countries. Spain, for instance, is the main olive growing country, with production of 8.014.000 tons in 2010, in 2.092.800 hectares; followed by Italy, Greece among others (FAO 2011).

According to the International Olive Council (2017) olive oil and table olive imports grew by 38% and 26% respectively, from October/2016 and January/ 2017, in Brazil. This increase in olive oil and table olives consumption is probably due to the benefits it brings to health and to its more accessible prices to consumers.

According to Coutinho et al. (2015), olive trees culture expansion in the country is growing, mainly in the states of Rio Grande do Sul, Minas Gerais, São Paulo and Santa Catarina, where there are crops under production, olives processing and olive oil packing. The cultivation of this fruit tree represents a reduction in expenses, since the country is fully dependent on table olives and olive oils imports. However, Vieira Neto et al. (2010) report that Brazil lacks technology applied to olive tree cultivation, and needs more studies on their culture characteristics.

Commercially, olive trees are grown vegetatively through cuttings and enxertia; however, as the demand for seedlings is increasing, micropropagation can provide an adequate supply of plants. According to Rugini et al. (2001), this technique makes the reproduction of genetically homogeneous plants possible, and that most olive trees cultivars have brought promising results.

The objective of adding vegetal regulators to the culture medium is to make up for the endogenous content deficiencies in the explants (Donini et al., 2008). The most used cytokinin for the proliferation of shoots in olive trees is the zeatin use in high concentrations (Grigoriadou et al., 2002).

According to Andrade et al. (2000), the accumulation of polyphenols and oxidation products around the excised surface, modifies the cultivation medium composition, making metabolites absorption difficult. Several antioxidants such as the ascorbic acid, citric acid and activated charcoal are used in the culture medium as an alternative to control native and woody species oxidation whose tissues are rich in these compounds. Some authors suggest pre-treatments with the vegetal material donor plant to prevent or minimize oxidation. According to Grattapaglia and Machado (1998), plants under light produce more phenols.

Being phenolic oxidation one of the problems faced by woody species, making *in vitro* establishment difficult and, consequently, preventing advances in the micropropagation area, the objective of tis work was to evaluate the effect of the presence and absence of light on the mother plant. It also evaluated the zeatin concentrations in *in vitro* establishment, to develop a method capable of reducing oxidation and promoting the establishment of olive tree explants.

MATERIALS AND METHODS

The two experiments were conducted from June to September, 2013, at the Fruit Plants Propagation Laboratory from Faculdade de Agronomia Eliseu Maciel Phytotechnology Department, Pelotas Federal University, RS.

For the *in vitro* establishment of the olive tree, four-year-old mother plants from the UFPel Germoplasm Active Bank were kept in 90-liter vases, under a semi-hydroponic system, irrigated with nutritive solution, formulated by

Schuch and Peil (2012), according to the culture needs. To reduce *in vitro* contamination, mother plants were pulverized every two days, for the minimum of three applications, with Kasumin® (bactericidal) and Cercobin® (fungicide), in the dosages of 3 ml.L^{-1} and 0.7 g.L^{-1}, respectively.

Olive trees herbaceous shoots were collected, with two buds, which had their leaves removed at collection. First, explants were disinfected, using alcohol at 70%, under agitation, for 1 minute, and then immersed in sodium hypochlorite at the concentration of 2.5% of active chlorine, by adding two drops of Tween 20 for 15 minutes in contact with the explants, under agitation. Next, the disinfected material was washed three times with autoclaved, sterilized, distilled water, in a laminar flow chamber, for later explants isolation.

In the first experiment, the study evaluated the *in vitro* establishment of explants from an 'Arbequina' olive tree, under different mother plant's maintenance conditions.

The experiment a 2x2 (luminescence x zeatin) factorial design. Treatment variation sources were the following luminescence conditions: absence of light and presence of light and zeatin concentrations (0 e 2 mg.L^{-1}).

The experimental design was entirely randomized with 4 treatments and 4 replications each, including 12 tubes with one explant.

Mother plants from the 'Arbequina' olive tree were maintained in a greenhouse. Out of these plants, five were kept under a black plastic canvas (absence of light) and fiver to the presence of light (natural) for a period of 25 days, for later *in vitro* establishment.

The culture medium used for the in vitro nodal segments establishment included salts and WPM vitamins (Lloyd and Mccown 1980) with 2 mg.L^{-1} of zeatin and WPM without the addition of zeatin, plus 100 mg.L^{-1} of mio-inositol, 30 g.L^{-1} of saccharose. The pH was adjusted to 6.0 prior to the inclusion of agar in the concentration of 6 g.L^{-1} and, then, autoclaved at 121oC and 1.5 atm for 20 minutes in test tubes (150 x 20 mm) with 10 ml of culture medium.

Explants were kept in the dark for a week to reduce phenolic oxidation. Later, they were transferred to a growth room with photoperiod of 16 hours of light and 8 hours of dark, with radiation of 27µmol.$m^{-2}s^{-1}$ and temperature of 25±2ºC.

In the second experiment, zeatin concentrations were assessed during olive tree cultivars *in vitro* establishment.

The experiment adopted a 3x3 (cultivars x zeatin) factorial design. Treatment variation sources included the Leccino, Arbequina and Frantoio cultivars and zeatin concentrations (0. 4 and 8 mg.L^{-1}). The experiment design was entirely randomized, with nine treatments and four replications each, with 12 test tubes with one explant.

Mother plants from the olive trees 'Leccino', 'Arbequina' and 'Frantoio' were submitted to absence of light for 25 days for posterior *in vitro* establishment in culture medium with from different concentrations of zeatin.

The culture medium used for the *in vitro* nodal segments establishment included salts and WPM vitamins (Lloyd and Mccown 1980) with different concentrations of zeatin (0. 4 and 8 mg.L^{-1}), according to the respective treatments.

Assessments in both experiments were carried out at days 7, 14, 21 and 28 of cultivation in regards to bacterial contamination percentage, indicated by the presence of visible bacteria in the culture medium, percentage of fungal contamination indicated by the presence of visible fungi, present in the culture medium and percentage of oxidized explants.

At 45 days of cultivation, the material was assessed regarding its survival indicated by the green coloration of the nodal segment and to the establishment percentage, determined by the development of foliar primordia and the presence of shoots.

Data were submitted to an analysis of variance and treatment means were compared statistically by the Tukey test ($p < 0.05$), through the statistical program WinStat (Machado and Conceição 2010). Data in percentages were transformed in arcsine of where x is the percentage obtained.

RESULTS AND DISCUSSION

Results showed that there was no interaction between mother plant luminescence and zeatin concentrations for oxidation percentage in the first experiment.

Lower oxidation percentage (1.04%) was verified in olive tree explants whose mother plants were submitted to a period of 25 days without light (Figure 1) and established in culture medium with 2 mg.L^{-1} de zeatin (Table 1). According to Grattapaglia and Machado (1998), the maintenance of branches in the dark stimulates etiolation, allowing the collection of adequate explants.

There was no significant difference for the bacterial contamination variable. As for the fungal contamination percentage, there was a significant difference only for days, with the lowest contamination percentage at 7 and 14 days of cultivation, and the highest percentage (9.89%) at 28 days (Table 2). These data are considered low in relation to those found by Dias et al. (2013) in a study carried out with activated charcoal and etiolation in the in vitro establishment of pomegranate trees (*Punica granatum* L.). These authors observed 51.56% of fungal contamination. 1t 10 days of in vitro cultivation. In an experiment with blueberry trees (Vacciniun spp.), Rosa et al. (2009) found no relation in regards to fungal and bacterial contaminations with the period in which the explants remained under the dark, with no influence on the total result of the contamination.

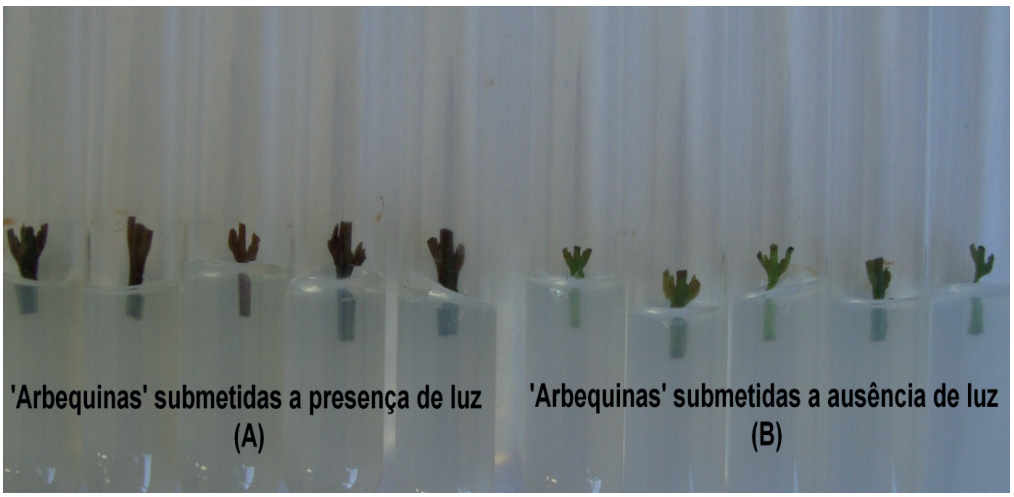

Figure 1. 'Arbequina' olive tree explants originated from mother plants submitted to the presence of light showed oxidation symptoms at 14 days of *in vitro* cultivation (A) and explants originated from mother plants submitted to absence of light remained green (B). FAEM/UFPel, Pelotas, RS, Brazil. 2014.

Table 1. Percentage of olive tree explants oxidized and established *in vitro*, after different conditions of mother plant luminescence, 2013. Pelotas, RS, Brazil. 2014.

Zeatin concentrations ($mg.L^{-1}$)	Oxidation (%)	
	Mother plant luminescence conditions	
	Presence of light	Absence of light
0	65.62 aB[1]	13.54 bA
2	72.39 aA	1.04 bB

[1]Uppercase letters compare columns and lowercase lines by the Tukey test at 5% of probability.

Table 2. Percentage of olive tree explants contaminated by fungi, established *in vitro* after different conditions of mother plant luminescence, 2013. Pelotas, RS, Brazil. 2014.

Days	Fungal contamination (%)
7	1.04 b[1]
14	1.56 b
21	7.29 a
28	9.89 a

[1]Means followed by distinct letters differ from each other by the Tukey test at 5% of probability.

At 45 days of cultivation, it became evident that mother plants from the 'Arbequina' olive tree, submitted to the presence of light showed higher percentage of oxidized explants for both tested zeatin concentrations (Table 3).

Table 3. Percentage of olive tree explants oxidized at 45 days of *in vitro* cultivation, after different conditions of mother plant luminescence, 2013. Pelotas, RS, Brazil. 2014.

Zeatin concentrations (mg.L-1)	Oxidation (%)	
	Mother plant luminescence conditions	
	Presence of light	Absence of light
0	81.24 aB[1]	14.58 bA
2	91.67 aA	2.08 bB

[1]Uppercase letters compare columns and lower case compare lines by the Tukey test at 5% of probability.

According to Gratapaglia and Machado (1998), plants submitted to light, especially woody plants, synthesize greater amount of phenols that intoxicate the explants, when oxidized *in vitro*.

In regards to the survival variable, assessed at 45 days of cultivation, the highest percentage of surviving explants observed was in the culture medium with 2 $mg.L^{-1}$ pf zeatin obtained from mother plants submitted to absence of light (Table 4).

Table 4. Percentage of explants from surviving olive trees at 45 days of *in vitro* cultivation after different conditions of mother plants luminescence, 2013. Pelotas, RS, Brazil. 2014.

Zeatin Concentrations ($mg.L^{-1}$)	Survival (%)	
	Mother plant luminescence conditions	
	Presence of light	Absence of light
0	4.16 aA[1]	0.00 aB
2	0.00 bA	18.75 aA

[1]Uppercase letters compare columns and lowercase compare lines.

Roussos and Pontikis (2002), working with olive tree explants, observed that the addition of 1-2 $mg.L^{-1}$ of zeatin or 0.1-0.2 $mg.L^{-1}$ of TDZ (thidiazuron) showed greater number of shoots.

For the olive tree explants establishment variable, there was significant difference only for mother plant submission conditions, in which the highest percentages of established explants (Figure 2) came from plants submitted to absence of light for 25 days (Table 5).

Table 5. Percentage of olive tree explants established at 45 days of *in vitro* cultivation, submitted to different conditions of mother plant luminescence, 2013. Pelotas, RS, Brazil. 2014.

Mother plant luminescence conditions	Establishment (%)
Absence of light	69.79 a[1]
Presence of light	3.12 b

[1]Means followed by distinct letters differ from each other by the Tukey test at 5% of probability.

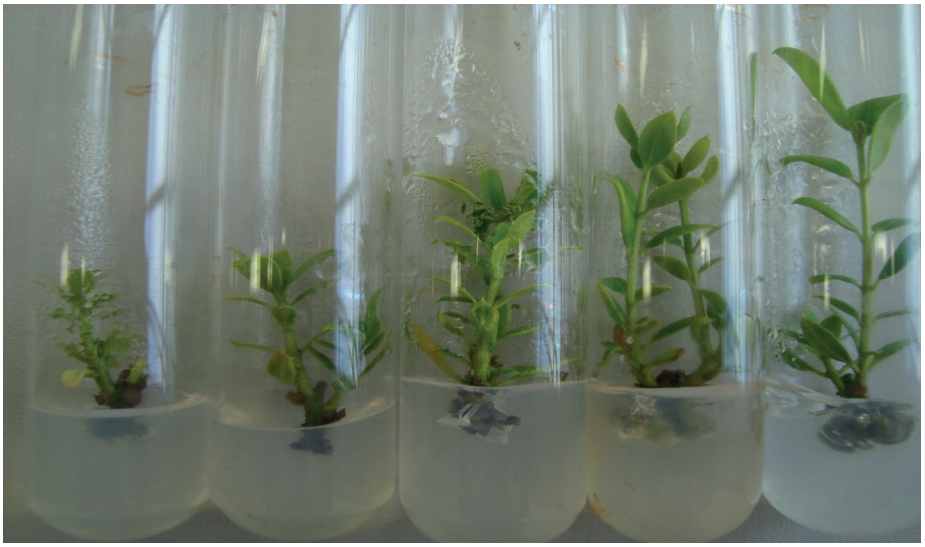

Figure 2. 'Arbequina' olive tree explants established at 45 days of *in vitro* cultivation. FAEM/UFPel, Pelotas, RS, Brazil. 2014.

In an *in vitro* establishment work with the blueberry cultivar 'Florida', Silva et al. (2007) also verified high percentages of explants establishment removed from plants that remained for 15 days in the dark.

In regards to the second experiment, there was an interaction among cultivars and zeatin concentrations. However, there was no significant difference for the cultivation days' factor.

Table 6 shows the low oxidation percentage of explants for Leccino, Arbequina and Frantoio cultivars for both zeatin concentrations. This is probably due to the pre-treatment given to the explants donating mother plants, 25 days under the absence of light (Figure 3).

Pelizza et al. (2013), during the *in vitro* establishment of blue berry cultivars Bluecrop, Duke and Misty also verified a low percentage of oxidized explants and different behaviors among them.

As for the bacterial contamination variables, the Leccino, Arbequina and Frantoio cultivars showed no significant difference with the tested zeatin concentrations (Table 7). Cultivar Leccino showed the lowest percentage of bacterial contamination compared to the others (8.33%), in the absence of zeatin.

In regards to fungal contamination, there was no significant difference among the zeatin concentrations for the Leccino and Arbequina cultivars. Among the tested cultivars, cultivar Frantoio showed the lowest percentage of fungal contamination in a culture medium with 8 $mg.L^{-1}$ of zeatin (Table 8). Silva et al. (2007) attribute these fungal

Table 6. Percentage of 'Leccino', 'Arbequina' and 'Frantoio' olive trees explants, oxidized and established *in vitro* in different Zeatin concentrations, 2013. Pelotas, RS, Brazil. 2014.

Cultivars	Oxidation (%)		
	Zeatin concentrations (mg.L^{-1})		
	0	4	8
Leccino	1.04 aB[1]	1.04 aA	0.00 bB
Arbequina	2.08 aA	0.00 aA	3.12 aA
Frantoio	0.00 bB	1.04 aA	0.00 bB

[1]Uppercase letters compare columns and lowercase compare lines.

Figure 3. Mother plants from the 'Leccino', 'Arbequina' and 'Frantoio' olive trees kept under a plastic black canvas (A) in a semi- hydroponic (B) to prevent the passage of light for 25 days. FAEM/UFPel, Pelotas, RS, Brazil. 2014.

Table 7. Percentage of the 'Leccino', 'Arbequina' and 'Frantoio' olive trees explants contaminated by bacteria and established *in vitro* in different concentrations of Zeatin 2013. Pelotas, RS, Brazil. 2014.

Cultivars	Bacterial Contamination (%)		
	Zeatin concentrations (mg.L^{-1})		
	0	4	8
Leccino	8.33 aB[1]	15.62 aB	16.14 aA
Arbequina	23.43 aA	25.52 aA	18.22 aA
Frantoio	18.74 aA	20.31 aAB	22.39 aA

[1]Uppercase letters compare columns and lowercase compare lines.

Table 8. Percentage of the 'Leccino', 'Arbequina' and 'Frantoio' olive trees explants contaminated by fungi and established *in vitro* in different concentrations of Zeatin, 2013. Pelotas, RS, Brazil. 2014.

Cultivars	Fungal Contamination (%)		
	Zeatin concentrations (mg.L^{-1})		
	0	4	8
Leccino	24.99 aA[1]	25.00 aA	31.24 aA
Arbequina	22.91 aB	26.04 aA	29.16 aA
Frantoio	31.25 aA	20.83 aA	18.23 bB

[1]Uppercase letters compare columns and lowercase compare lines.

contamination results to the environment condition of the mother plants. The closed and dark environment could probably have induced an increase in the air relative humidity, which favored the development of fungi.

At 45 days of *in vitro* cultivation, explants oxidation, survival and establishment for the factors studied showed no significant effect according to the analysis of variance, with an average of 3.93% of oxidation, 8.10% of survival and 40.74% of establishment (Figure 4).

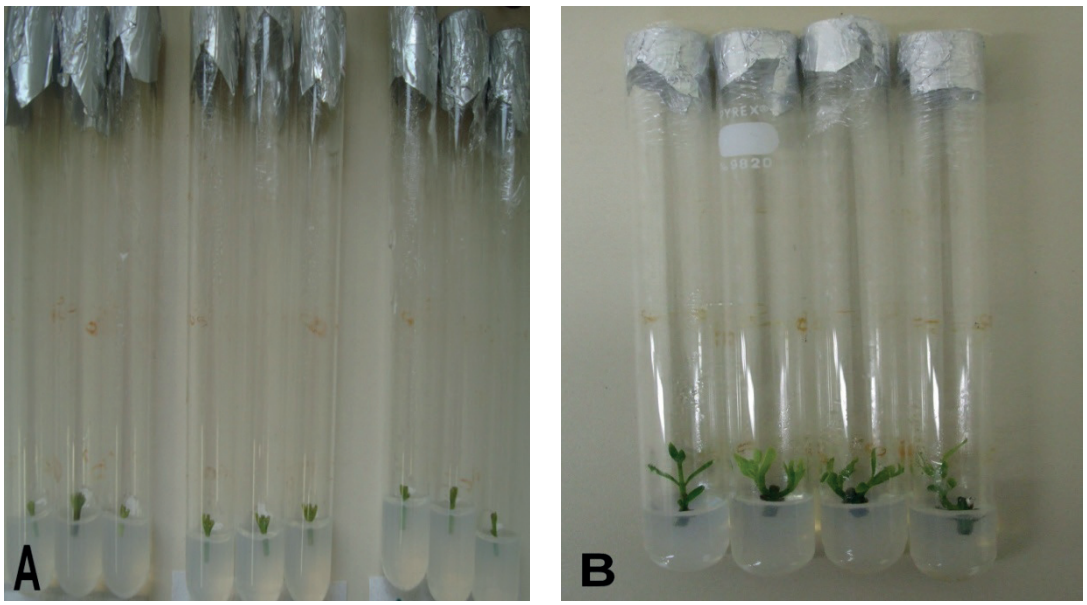

Figure 4. Explants from the 'Leccino', 'Arbequina' and 'Frantoio' olive trees inoculated *in vitro*, in different concentrations of Zeatin (0.4 and 8 mg.L^{-1}) (A) and established (B). FAEM/UFPel, Pelotas, RS, Brazil. 2014.

However, as a woody plant, the percentages of established explants (40,74%) may be considered a satisfactory result, considering the oxidation problem presented by the other species and the difficulty in establishing the material *in vitro*, which is an essential factor for the next step, the multiplication phase.

CONCLUSIONS

Mother pants from the 'Arbequina' olive tree, under the absence of light conditions, are the most indicated for *in vitro* establishment.

Zeatin concentration of 2 mg.L^{-1} favors the survival of the 'Arbequina' olive tree explants.

Zeatin concentrations (0, 4 and 8 mg.L^{-1}) showed no differences in regards to fungal contamination between the Leccino and Arbequina cultivars.

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REFERENCES

Andrade MW, Luz JMQ, Lacerda AS and Melo PRA (2000) Micropropagação da aroeira (*Myracrodruon urundeuva* Fr. All). Ciência e Agrotecnologia 24: 174-180.

Coutinho EF, Jorge RO, Haerter JA and Costa VB (2015) Oliveira: Aspectos técnicos e cultivo no Sul do Brasil. Embrapa, Brasília, 181p.

Dias MM, Nietsche S and Pereira MCT (2013) Carvão ativado e estiolamento no estabelecimento *in vitro* de romãzeira. Tecnologia & Ciência Agropecuária 7: 1-5.

Donini LP, Schuch MW, Ribeiro MF, Souza JA and Soares GC (2008) Estabelecimento *in vitro* de oliveira cv. 'Arbequina' para início da micropropagação. Ciência Rural 38 (6): 1769-1772.

Food and agriculture organization of the united national (2011) FAO. Faostat - Estatistical database. http://faostat.fao.org/site/339/default.aspx. Acessado em 13 dez. 2013.

Grattapaglia D and Machado MA (1998) Micropropagação. Torres AC, Caldas LS and Buso JA (eds.) Cultura de Tecidos e Transformação Genética de Plantas. Serviço de Produção de Informação, Brasília, pp.183-260.

Grigoriadou K, Vasilakakis M and Eleftherios EP (2002) *In vitro* propagation of the Greek olive cultivar Chondrolia Chalkidikis. Plant Cell, Tissue and Organ Culture 71: 47-54.

International Olive Council (2017) Publications. Market Newsletter 114, March. http://www.internationaloliveoil.org/. Acessed 20 Abr. 2017.

Lloyd G and Mccown B (1980) Commercially-feasible micropropagation of mountain laurel, kalmia latifolia, by use os shoot-tip culture. Combined Proceedings International Plant Propagators Society 30: 421-427.

Machado AA and Conceição AR (2010) Sistema de análise estatística para Windows. WinStat. Versão 1.0. UFPel.
Pelizza TR, Silveira FN, Muniz J, Grimaldi F, Rufato L and Kretzschmar AA (2013) Estabelecimento in vitro de mirtileiro: cultivares Bluecrop, Duke e Misty. Plant Cell Micropropagation 9(1-2): 24-29.

Rosa LPP, Etcheverria C, Dávila ES and Martins CR (2009) Efeito de antibiótico e do período de escuro no estabelecimento *in vitro* de mirtilo *Vacciniun* spp. Revista da Faculdade de Zootecnia, Veterinária e Agronomia 16(2): 265-277.
Roussos PA and Pontikis CA (2002) *In vitro* propagation of olive (*Olea europaea* L.) cv. Koroneiki. Plant Growth Regulation 37: 295-304.

Rugini E, Muganu M, Biasi R, Aversa G, Pannelli G, Maggini F, Bignami C, Barba M, Romagnoli, B, Martelli GP, Venzi L and Zamboni EL (2001)'organizzazione di un moderno vivaismo olivicolo alla base della produzione di piante certificate. Frutticoltura 5: 11-24.

Schuch MW and Peil RMN (2012) Soilless cultivation systems: A new approach in fruit plants propagation in southern Brazil. Acta Horticulturae 952: 877-883.

Silva LCD, Schuch MW, Souza JAD, Erig AC and Antunes LEC (2007) Efeito da iluminação e pré-lavagem das brotações de mirtilo cv. Florida no estabelecimento *in vitro*. Revista Brasileira de Agrociência.13 (1): 127-129.

Vieira Neto JV, Cançado GMA, Oliveira AF, Mesquita HA, Lúcio AD and Silva LFO (2010) Fertilizantes na produção de mudas de oliveira 'Arbequina' . Scientia Agraria 11(1): 49-55.

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