

Synthetic Seeds Production by Encapsulated Somatic Embryo of White Olive Cultivar

Mohamed Helmy Abd El-Zaher¹, Sahar Mohamed Abd El-Wahab¹, Shreif Said Saleh² and Nuria Nuri Mustafa Al-Maghrabi^{3*}

¹Horticulture Pomology Department, Faculty of Agriculture, Cairo University, Egypt; ²Medicinal and Aromatic Plants Research Department, Horticulture Research Institute, Agriculture Research Centre, Egypt; ³Post graduate doctor, Tripoli university, Libya

*Corresponding author's e-mail: researchint@yahoo.com

In vitro protocol for the micropropagation has been developed and standardized in vitro propagation methods by culture cotyledons of seeds and embryonic callus induction, encapsulation somatic embryos of white olive cultivar was evaluated. Explants of (cotyledons) of white olive cultivar were subjected to disinfection treatments and used to produce somatic embryo and encapsulated somatic embryo to produce synthetic seeds. Stages of production synthetic seeds are: sterilization stage, callus induction, somatic embryos induction with Rugini media (RM) supplemented with different concentration of kinetin or glutamine or/1 2ip (Poly Vinyl Pyrrolidone) or GA3 or zeatin and encapsulation somatic embryos with Na-alginate with different concentration and time. The results showed that cotyledons cultured on RM were the best part *in vitro* cultured for 6 weeks. The best Sterilization treatments of cotyledons were Clorox 15% for 10 min; HgCl₂ at 0.2 and 0.3 % for 10 min, the highest embryonic calli percentage formed on Rugini Media (RM) with 30 gm sugar and 6mg/L 2,4-D or 15 g Sugar. in conclusion, The highest somatic Embryo formation with Rugini Media (RM) 4(+1mg/L 2iP). The suitable encapsulated somatic embryos with Na –Alginate 4% and solidified by 8% CaNO₃ for 45 minutes with high transparency and solidity%. Synthetic seeds obtained from encapsulated somatic embryogenesis is a suitable method for micro propagation at any time and has the potential for use in the commercial propagation and useful in exchange of sterile material between laboratories, germplasm conservation of white olive cultivar.

Keywords: Encapsulation, olive, somatic embryo, synthetic seed, tissue culture.

INTRODUCTION

Micro-propagation is most significant application of agribiotechnology in the field of horticulture (Suman, 2017). The technique is practical for proliferation of olive (*Olea europaea* L.) tree which is common technique in globally cultivated for decades and its value-added products are consumed (Lambardi and Rugini, 2003; Haq *et al.*, 2021). the protocol has to be found to standardize for mass-multiplication of olive in in-vitro condition by subsequently preparation of synthetic seeds from somatic embryo of grown plantlets in vitro, for increasing demand for promising global olive genotypes and production of a large number of healthy plantlets within a short-period (Micheli *et al.*, 2019; Mazri *et al.*, 2020). The olive tree (*Olea europaea* L.) has several cultivars selected and multiplied initially by farmers mainly

for the size of their fruits and the oil content (Besnard *et al.*, 2018). Currently, 10 million hectares is under olive-farming worldwide, and in European Union as a largest producer of olives products having a share of 75% of global olive production (FAO, 2020). Conventional methods of breeding and multiplication represents a practical and important solution to the crop demand with attention to economic importance, especially the increasing demand for plants needed for new orchards (Ceccarelli, 2015; Lambardi *et al.*, 2023). In vitro propagation techniques are considered as a commercial reality for the multiplication of several olive cultivars (Rugini *et al.*, 2020; Montilon *et al.*, 2021) due to the sanitary quality and the confirmed genetic stability of the regenerated plants (Lopes *et al.*, 2009) despite numerous phenotypic and somaclonal variations observed after several subcultures (Bradai *et al.*, 2019).

Mohamed Helmy Abd El-Zaher, S. M. Abd El-Wahab, S. S. Saleh and N. N. M. Al-Maghrabi. 2024. Synthetic Seeds Production by Encapsulated Somatic Embryo of White Olive Cultivar. Journal of Global Innovations in Agricultural Sciences 12:1109-1114.

[Received 10 Oct 2024; Accepted 10 Nov 2024; Published 17 Nov 2024]



Somatic embryogenesis: Most of the published research reports and established works on somatic embryogenesis in olive considered three main steps, starting with the establishment of embryogenic cultures combining the induction and proliferation of calli, followed by phases of expression and development of structured-embryos ready to be converted into whole plants (Bidabadi and Mohan Jain, 2020; Titouh *et al.*, 2022). However, few works have been done about the germination conditions of olive somatic embryos (Mazri *et al.*, 2020) although regeneration of plantlets has frequently been achieved by introducing the embryos under photoperiod on a standard culture medium based on the special chemical compositions (Rugini, 1984). In addition, the low conversion rates remain the great obstacle of the process in many species, including in olive tree, are caused mainly by deficiencies in the development and maturation of the used embryos (Sánchez Romero, 2019). The encapsulation is a biotechnique which can be considered as a promising the exchange of plant materials between public and private plant tissue culture laboratories (Tripathi *et al.*, 2021) and also to achieve germplasm conservation and the propagules that are derived from in vitro or by micropropagation applied directly in nurseries or in a field (Standardi and Micheli, 2013). The use of sodium alginate as a packaging agent is due to its moderate viscosity, low circulating ability of the solution, low toxicity, fast gelation, low cost and biocompatibility properties (Rai *et al.*, 2009). In overall, the most recent concept involves every meristematic tissue (in vitro or in vivo derived), as long as able to convert in a whole plantlet after encapsulation and possible storage (Standardi and Micheli, 2013).

The synthetic seeds which artificially encapsulated of somatic embryos used for sowing Artificial seeds are advantages hence, better and clonal plants could be propagated similar to seeds (Abbas *et al.*, 2022); preservation of rare plant species for extending biodiversity, more consistent and synchronized harvesting of important agricultural crops, easy of handling, potential long-term storage and low cost of production (Bewket, 2021). In present research the production of synthetic seeds by develop of somatic embryogenesis from cotyledons, encapsulation somatic embryos of white olive cultivar were conducted and evaluated.

MATERIALS AND METHODS

Plant materials: Plant materials of the used the seeds of White olive brought from Libya (Tarhona region belonged Tripoli city). Seeds collected was healthy and true to type of the cultivar. The Parts of mature sprouted (decorated) seed included cotyledon's parts, explants of white olive were subjected to disinfection treatments and used to produce somatic embryos.

Sterilization stage: Seeds removed shill, break woody parts and isolate cotyledons containing sexual embryos. The sexual

embryos were surface sterilized in 5, 10 and 20% by volume commercial sodium hypochlorite solution (NaCl 0.06%) or 0.1, 0.2 and 0.3 % HgCl₂ for 10 min with 2 drops of Tween 80 as a surfactant and wetting agent, followed by three rinses with sterile distilled water and then 70 % ethanol for 1 min followed by three rinses with sterilized distilled water and then germinated on (Rugini media, 1984) (basal salt medium 4.4 g L⁻¹, 30 g L⁻¹ sucrose, 7 g L⁻¹ technical agar).

- Clorox 5 for 10 min
- Clorox 10 for 10 min
- Clorox 15 for 10 min
- HgCl₂ 0.1 for 10 min
- HgCl₂ 0.2 for 10 min
- HgCl₂ 0.3 for 10 min

The Cotyledon's discs were cultured-on Rugini Media (RM), incubated the light/dark (day/night) (16/8 hours/day), and temperature of 27°C for 4 weeks 25-30 days, then taken to the decontamination, Browning and survival percentages % were calculated for 'White olive'.

Callus Induction: After 30 – 35 days cotyledons parts formed were removed and cut into small pieces (10 mm²) and excised using fine sterile forceps and a scalpel and used as explants and placed on callus induction medium (CIM) which consisted of RM (Rugini Media, 1984) of white olive medium supplemented with 2.0, 4.0 and 6.0 mg L⁻¹ 2, 4-dichlorophenoxy acetic acid (2,4-D) with 30g or 15 g sugar with 7g of technical agar as well as a control (without hormones), and incubated at darkness and temperature of 16°C for producing callus after about 6-7 months (repeat this step at 2 weeks as a subculture) and repeat the culture, and repeat the culture for obtaining more callus. Data took as survival explants and callus formation %.

Somatic Embryos Induction: The primary callus derived from cotyledon of (White olive) cut into a small piece (approximately 1 cm³) and Cultured on the following media:

- RM + 1 mg L⁻¹ Kinetin
- RM+2 mgL⁻¹ Kinetin
- RM+3 mgL⁻¹ Kinetin
- RM+ 1mg/l Glutamine
- RM+1 mg/l 2ip (Poly Vinyl Pyrrolidone)
- RM+1 mg/l phenylalanine
- RM+1 mg/l GA3
- RM + 1 mg/l zeatin

Which is RM4 [Rugini media + 15 g sucrose + 3 mg/l 2,4-D + 0.5 mg/l Biotin and incubated at 12/12, day/night illumination at 18 °C for 15-21 days].

Encapsulation Somatic Embryos: For Capsule production, Na Alginate at different concentrations were used at hot water by adding slowly with stirring for 30 min till complete dissolved with two gelling agents added CaCl₂ solutions (Variance Concentrations) for 30 min, CaNo₃ solutions (variance Concentrations) for two times. Also, Transmittance



was determined by spectrophotometer at (450 nm) to determine translucent capsule (Standardi and Michelin, 2013) and solidity was determined by pressure tester. The explants were immersed in Na-alginate (2, 2.5, 3% w/v) and then dropped into calcium chloride solution.

Data and parameters tested

- Decontamination ratio = [No. of no contaminated gar/total gar were cultured] × 100
- Browning= [No. of no browning gar/total gar were cultured] × 100
- Survival percentage = [No. of survived explants / total number of cultured explants] × 100
- Callus % = [Number of explants formed callus/ total number of cultured explants] × 100

Statistical Analysis: Data obtained were designed by Complete Randomized Design (CRD) and using LSD at 5 % to compare the means of treatments and standard division (SD). The data analyzed by Co-State software Version-4 (Statistical Graphics Corporation, 1999).

RESULTS AND DISCUSSION

Steps of production synthetic seeds of White Olive Cultivar

Sterilization treatments of cotyledons

a-Decontamination percentage: Table (1) and Fig. (1) showed that Clorox 15% for 10 min; HgCl₂ at 0.2 and 0.3 % for 10 min gave the highest significant values (100 % for each) but decrease the survival that explain the negative effect of HgCl₂, while the 15 % Clorox gave significant values (100 %) were reflected by Clorox concentration and time of exposure. It seems to be that Clorox affected positively by the moderate concentration that considers suitable or preferable conditions for disinfectant process.

b-The survival percentage: The cotyledons without embryos were culture on Rugini Media (RM) medium free. The results of Table 1 revealed that Clorox 15% for 10 min gave the highest significant survival % (75) but the others disinfectants gave the same lowest significant values ranged between (0 – 30 %), which reflected that the Clorox 15 % seems to be

affected significantly by lower contamination, positively. In observation, increasing browning percentage 66.66%.

Table 1. Effect of concentrations and time of exposure to disinfectant on decontamination; browning and survival percentage of cotyledons of white olive.

Disinfecting treatments	Decontami- nation %	Survival %	Browning %
Clorox 5 for 10 min	0.00	0.00	00.00
Clorox 10 for 10 min	25.00	6.66	10.00
Clorox 15 for 10 min	100.00	75.00	15.00
HgCl ₂ 0.1 for 10 min	90.00	30.00	50.00
HgCl ₂ 0.2 for 10 min	100.00	15.00	65.00
HgCl ₂ 0.3 for 10 min	100.00	15.00	75.00
LSD 5 %	10.01	9.07	8.27



Figure 1. Sterilization treatments of white olive.

c-browning percentage: Concerning the effect of disinfectant solutions, data in Table 1 showed that the lowest with significant effect on browning percentage (15 %) was recorded by the method of disinfecting by 15 % Clorox followed the same material at 10 % as recorded (10 %). The results showed the low level of browning gave highest level of decontamination and low level of survival.

Callus Induction

a-Survival percentage: Data of Table 2 showed that the effect of Rugini Media (RM) media with 2,4-D and sugar at 15 and

Table 2. Effect of sugar strength and 2,4-D concentrations on callus formation of white olive.

	2,4-D treatments	Survival %	Callus %	Color	Siz
Sugar	RUGINI MEDIA (RM) full free	24.00	00.00	0	0
	RUGINI MEDIA (RM) full + 2,4-D 2	80.66	50.00	white	+
	RUGINI MEDIA (RM) full + 2,4-D 4	75.00	70.00	Yellow	++
	RUGINI MEDIA (RM) full + 2,4-D 6	75.00	85.00	White	++
1/2 sugar	RUGINI MEDIA (RM) full free	30.00	00.00	0	0
	RUGINI MEDIA (RM) full + 2,4-D 2	80.00	60.00	white	++
	RUGINI MEDIA (RM) full + 2,4-D 4	85.00	75.00	white	++
	RUGINI MEDIA (RM) full + 2,4-D 6	75.00	85.00	White	++
	LSD 5%	0.974	0.654		



30 g, on the survival percentage of 'white olive' explants (cotyledons pieces). The results showed that MS with 2,4-D at 2,4 and 6 mg/L, plus 15 gm sugar and Rugini Media (RM) with 2,4-D at 2mg/L plus 30gm sugar gave the highest significant survival (30, 80, 85 and 75%, respectively) where reflected that sugar is a determined factor in respect to the effect of 2,4-D on the survival percentage of 'white olive' shoot let's explants, The high concentration of sugar seems to have a negative effect on the survival percentage It may be the explant's at this stage need a low concentration on sugar, in found of 2,4-D, to still life (survival explant).

b-Callus percentage: The callus formation percentage: Data of table (2) and figures (2) illustrated that Rugini Media (RM) media with 6 mg/L, 2,4-D and 30 gm sugar produced the highest significant callus percentage (85 %) From 'white olive' cotyledons pieces explants; also, 85 % callus formed on Rugini Media (RM) with 30 gm sugar and 6mg/L 2,4-D and Rugini Media (RM) with 15 g Sugar. The other treatments failed (zero). It could be concluded that sugar at full concentration (normal) at 30 gm / L. We prefer and suite for forming the callus of 'white olive', as sugar consider an important factor in the plant growth, so it seems to be a stimulant in the callus formation process in white olive cultivar with 2,4 - D.



Figure 2. callus induction cotyledons of white olive.

Somatic embryos induction: The results of Table (3) showed that the effect of Rugini Media (RM)4) +15 gm sugar + 3mg/L,2,4-D + 0.5mg/L Biotin), Kinetin, GA3, 2iP, glutamine, Zeatin and phenylalanine on the embryonic Calli percentage of white olive callus pieces explants.

Data in Table 3 revealed that media of Rugini Media (RM) 4 +1mg/L 2ip (Poly Vinyl Pyrrolidone) and Rugini Media (RM) 4 +1mg/L Kin resulted in the highest significant somatic embryo %(70, 45 and 40 %, respectively), while the other studied media failed (zero%), these results indicated that glutamine, 2ip and zeatin were only positive effected factors on the embryonic calli formation in 'white olive' Somatic

embryogenesis and it may be the 'white olive' explants were more response to these compounds.

Table 3. Effect of various amino acids and plant regulators on somatic embryos formation of white olive cotyledons callus.

Treatments	Average of Callus %	Average of Somatic embryo %
1.Rugini Media (RM) 4 + 1 mgL ⁻¹ Kinetin	75.00	40.0
2.Rugini Media (RM) 4 + 2 mgL ⁻¹ Kinetin	76.00	35.0
3.Rugini Media (RM) 4 + 3 mgL ⁻¹ Kinetin	82.00	33.0
4.Rugini Media (RM) 4 + 1mg/l Glutamine	60.00	70.0
5.Rugini Media (RM) 4 + 1 mg/l 2ip	25.00	45.0
6.Rugini Media (RM) 4 + 1 mg/l phenylalanine	20.00	7.5
7.Rugini Media (RM) 4 + 1 mg/l GA3	6.66	6.5
8.Rugini Media (RM) 4 + 1 mg/l zeatin	6.66	3.0
LSD 5 %	1.082	0.543

The encapsulation stage

a-Synthetic seeds formation

The solidity of the capsules of the synthetic seeds: The results of table (4) and fig. (4) mentioned that the solidity of capsules were determined, when the time increased the solidity increased, the solidity of capsules scored 88.88 % for Alginate 4%+CaNO₃ 8% 45 min& Alginat 4%+CaNO₃ 16% 30 min followed by 77.77 % for Alginate 2%+CaNO₃ 16% 45 min this solidity percentage was more solid and broken after culture on media immediately. So, Alginate 2%+ calcium chloride at 20 % for 30 min was suitable for capsule formation although it was low transference but it was homogenous forms. . These results reflected that the important roles for the concentrations of alginate, CaNO₃ and exposure time, in making the solidity of white olive synthetic seeds.

The transparency of the capsules of the synthetic seeds: Data of table (4) and fig (4) showed that the data the effect of alginate concentrations and source of Ca ion and their concentrations. Using calcium nitrate is the best source of calcium ion because it gave high transparence than calcium chloride Treatments of 4 % alginate +CaNO₃ at 8% for 45 min & 4 % alginate +CaNO₃ at 16 % for 30 gave the best results of transmittance which scored (1.54 and 1.42).



Table 4. Effect of alginate concentrations source of calcium on gelling process.

Encapsulation treatments	Solidity	Transparency
Alginate 2%+CaNO ₃ 8% 30 min	66.66	0.43
Alginate 2%+CaNO ₃ 8% 45 min	0.00	0.00
Alginate 2%+CaNO ₃ 16% 30 min	0.00	0.00
Alginate t 2%+CaNO ₃ 16% 45 min	77.77	0.67
Alginate t 4%+CaNO ₃ 8% 30 min	0.00	0.00
Alginate t 4%+CaNO ₃ 8% 45 min	88.88	1.54
Alginate 4%+CaNO ₃ 16% 30 min	88.88	1.42
Alginate 4%+CaNO ₃ 16% 45 min	0.00	0.00
Alginate 2%+CaCl ₂ 20% 30 min	33.33	0.23
LSD 5 %	9.012	0.282

**Figure 4. encapsulation somatic embryos of white olive.**

Conclusion: A simple protocol of inducing somatic embryos derived from cotyledons of white olive has been developed as Somatic embryo development is associated with a series of molecular events. To produce synthetic seeds, the best part in vitro culture was cotyledons on Rugini Media (RM) for six weeks the best Sterilization treatments of cotyledons were Clorox 15% for 10 min; HgCl₂ at 0.2 and 0.3 % for 10 min with the best survival percentage and the least browning percentage. The highest embryonic calli percentage formed with Rugini Media (RM) with 2,4-D at 2,4 and 6 mg/L, plus 15 gm sugar or Rugini Media (RM) with 2,4-D at 2mg/L plus 30gm sugar. The suitable somatic embryo% with Rugini Media (RM) 4 +1mg/L Zip (Poly Vinyl Pyrrolidone) or Rugini Media (RM) 4 +1mg/L Kin. The suitable encapsulated somatic embryos with Na –Alginate 4% and solidified by 8% CaNO₃ for 45 minutes with high transparency and solidity%. This protocol is used to propagate and preserve white olive cultivar for synthetic seeds production from encapsulated somatic embryogenesis. This type of capsules could be useful

in exchange of sterile material between laboratories, germplasm conservation and direct plant propagation

Authors' Contribution: Mohamed Helmy Abd El- Zaher, Sahar Mohamed. Abd EL-Wahab, Shreif Said. Saleh and Nuria Nuri Mustafa Al -Maghrabi authors are participated in conduction and drafting the research. Also, they had review and approve the final manuscript.

Conflict of interests: Authors declared no conflict of interest.

Acknowledgements: None.

Funding: none.

Ethical statement: ethical rules are arranged based on laboratory care for plant biotechnological studies.

Availability of data and material: all data are available, when journal requests.

Code Availability: none.

Consent for publication: none

SDG's addressed: Zero Hunger and Good Health and Well-being.

REFERENCE

- Abbas, M.K., H.E. Mahood and A.S. Alhasan. 2022. Production of synthetic seeds in vegetable crops: A review. In IOP Conference Series: Earth and Environmental Science 1060:012099.
- Besnard G., J.F. Terral and A. Cornille. 2018. On the origins and domestication of the olive: a review and perspectives. *Annals of Botany* 121:385403.
- Bewket, G.B. 2021. Review on Production and Application of Synthetic Seeds. *Gaolable scientific Journals* 9:189-211.
- Bidabadi, S. and S. Mohan Jain. 2020. Cellular, molecular, and physiological aspects of in vitro plant regeneration. *Plants* 9:702.
- Bradaï F., C. Sánchezromero and C. Martín. 2019 Somaclonal variation in olive (*Olea europaea* L.) plants regenerated via somatic embryogenesis: Influence of genotype and culture age on genetic stability. *Science Horticulture* 251:260-266.
- Ceccarelli, S., 2015. Efficiency of plant breeding. *Crop science* 55:87-97.
- FAO, Food and Agriculture Organization of the United Nations, 2020.
- Haq, I.U., H. Umar, N. Akhtar, M.A. Iqbal and M. Ijaz. 2021. Techniques for Micropropagation of Olive (*Olea europaea* L.): A Systematic Review. *Pakistan Journal of Agricultural Research* 34:188.



- Lambardi, M. and E. Rugini. 2003. Micropropagation of olive (*Olea europaea* L.). In *Micropropagation of woody trees and fruits*. Dordrecht: Springer-Netherlands pp. 621-646.
- Lambardi, M., A. Fabbri, M. Micheli and A. Vitale. 2023. Olive Propagation and Nursery. In *The Olive: Botany and Production*. GB: CABI pp. 228-256.
- Lopes T., A. Capelo, G. Brito, J. Loureiro and C. Santos. 2009. Genetic variability analyses of the somatic embryogenesis induction process in *Olea* spp. using nuclear microsatellites. *Trees* 23: 2936.
- Mazri M.A., R. Naciri and I. Belkoura. 2020. Maturation and conversion of somatic embryos derived from seeds of olive (*Olea europaea* L.) cv. Dahbia: Occurrence of secondary embryogenesis and adventitious bud formation. *Plants* 9:14.
- Micheli, M., A. Standardi and D. Fernandes da Silva. 2019. Encapsulation and Synthetic Seeds of Olive (*Olea europaea* L.): Experiences and Overview. In: Faisal, M., Alatar, A. (eds) *Synthetic Seeds*. Springer, Cham. https://doi.org/10.1007/978-3-030-24631-0_16
- Montilon, V., L. Susca, O. Potere, V. Roseti, A. Campanale, A. Saponari, C. Montemurro, V. Fanelli, P. Venerito and G. Bottalico. 2021. Embryo Culture, in vitro Propagation, and Molecular Identification for Advanced Olive Breeding Programs. *Horticulturae* 8:36.
- Rai, M.K., P. Asthana, S.K. Singh, V. Jaiswal and U. Jaiswal. 2009. The encapsulation technology in fruit plants: a review. *Biotechnology Advances* 27:671-679.
- Rugini E., 1984 In vitro propagation of some olive cultivars with different root-ability, and medium development using analytical data from developing shoots and embryos. *Science of Horticulture* 24:123134.
- Rugini E., L. Baldoni C. Silvestri R. Mariotti, I. Narvaez, N. Cultrera, V. Cristofori, M.A. Bashir, S. Mousavi, E. Palomarios, J.A. Mercado and F. Pliegoalfaro. 2020 *Olea Europaea Olive*, Pp. 343376. In: Litz R.E., F. Pliegoalfaro, And J.I. Hormaza (Eds.) *Biotechnology of fruit and nut crops*. Second edition. CABI International pp. 687.
- Standardi, A. and M. Micheli. 2013. Encapsulation of in vitro-derived explants: An innovative tool for nurseries. *Methods in Molecular Biology* 11013:397-418.
- Statistical Graphics Corporation, COSTAT ,1999. STATGRAPHICS statistical graphics system, STSC., Inc., Englewood Cliffs, NJ.
- Suman, S., 2017. Plant tissue culture: A promising tool of quality material production with special reference to micropropagation of banana. *Biochemical & Cellular Archives* 17:1-26.
- Tripathi, M.K., S. Tiwari, N. Tripathi, G. Tiwari, D. Bhatt, M. Vibhute, N. Gupta, N. Mishra, P. Parihar, P. Singh and A. Sharma. 2021. Plant tissue culture techniques for conservation of biodiversity of some plants appropriate propagation in degraded and temperate areas. *Current Topics in Agricultural Sciences*; BP International Publisher: Bhanjipur, India.
- Titouh, K., K.H. Moussa, N. Boufis and L. Khelifi. 2022. Impact of cultural conditions on germination of olive (*Olea europaea* L.) somatic embryos and plantlets development from the Algerian cultivar Chemlal. *Advances in Horticultural Science* 36:185-191.

