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In vitro germination and development of Arbequina and Coratina olive cultivars

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Abstract: *In vitro* culture method was carried out during 2014-2015 seasons at the laboratory of Pomology Department, Faculty of Agriculture Cairo University and Biotechnology Lab., Pomology Dept., National Research Centre, to evaluate the germination percentage and development of embryos isolated from seeds of Arbequina and Coratina olive cultivars. Embryo germination and development were determined by *in vitro* culture using 1/3 strength MS medium. The embryo was cultured on the following media (Agar, Agar+Sucrose, Agar+Sucrose+MS and Agar+Sucrose+ MS+ GA_3). The highest germination percentage was 72.66% in Coratina with Agar. The germination started in short period of time (6 days) with agar media for Coratina cv. The tested cultivars showed high growth performance after germination, Arbequina superiority with Agar +Sucrose +MS in leaf number (5.66). Also, Agar+ Sucrose +MS media gave the highest plant height (3.50 cm) with Coratina cv.
Keywords: Olive, *In vitro*, Embryo culture, Germination percentage.

Introduction

Olive (*Oleaeuropaea* L.)one of the most ancient cultured trees. It was originated in the eastern side of the Mediterranean basin, and spread to other countries. Until recently the genetic improvement of olive tree basedon theselectionofindividuals adapted to local conditions and constrains [1]. To date the used method to improve olive cultivars is by classical crosses in breeding programs between existing cultivars. A few cultivars from these breeding programs have been reported recently[2, 3, 4].

Barranco *et al*[5] stated that the main cause of the few programs to improve cultivars conducted to date is the prolonged duration of juvenile phase in olive[6], and the slow, and low germination in seeds of olive Lagarda *et al*[7].

Due to the heterozygosis of the generated individuals and the added possibility of genetic recombination after controlled cross pollination, seed is a great important for breeding in olive Troncoso*et al* [8].

However, seed germination in olive is very slow, for example seeds of Manzanillo cv. take 3 years for germination of only 17% Acebedo*et al*[9].This could be attributed to the nature of olive seeds which are covered by thick stony endocarp. Also, embryo dormancy has been reported by many authors Lagarda*et al* [10]and Crisosto and Sutter[11].

Numerous treatments have been applied to improve seed germination, including mechanical and chemical scarification [11] and Bandino*et al* [12]and soaking in solutions of phyto-hormones Lagarda and Martin [13].However, low success had been obtained [12].

In vitro embryo culture has been used successfully in many fruit species to overcome such germination problems [14]. Germination of isolated embryo of olive cultivars had higher germination percentage in comparison with stonless seeds Acebedo*et al* [9]; Garcia *et al.*[15] and Maalej*et al*[16]. Also, Acebedo*et al* [9] reported that the isolated olive embryos germinated uniformly within 10-14 days.

Therefore this study aim to evaluate the germination percentage and development *in vitro* of embryos isolated from seeds of Arbequina and Coratina olive cultivars.

Materials and Methods

The present study was carried out during 2014-2015 seasons at the laboratory of Pomology Department, Faculty of Agriculture, Cairo University and Biotechnology Lab., Pomology Dept., National Research Centre.

a. Plant materials

Fruits of olive cultivars (Arbequina and Coratina) were harvested when the color begin to change from yellow green to violet. The fruit pulp was removed and the sclarified endocarps were broken according to Sotomayor-Leon and Caballero [17].

b. Surface sterilization of olive seeds

After elimination of the endocarp, the stoneless seeds were surface sterilized under sterile conditions with 20% commercial sodium hypochlorite solution for 15 min. then the seeds were transferred to 0.1% $HgCl_2$ for 10 mint Maalej*et al* [18] and Kiani*et al* [19].Finally, the seeds were washed three times with sterile water.

c. Embryo isolation

The sterilized seeds were soaked for 48h in sterilized water to promote swelling and faciliting the embryo isolation. Embryo was isolated by cutting off two lateral sections, and freeing the embryo from remaining seed tissue [9].

d. Media composition

The isolated embryos were cultured on one of the following media:

1- Agar.

2- Agar+Sucrose.

3- Agar+Sucrose+MS.

4- Agar+Sucrose+MS+GA_3

MS medium Murashige and Skoog[20] was used at 1/3 strength and the sucrose was added at 30 g per liter. The pH was adjusted at 5.8. The media was solidified by adding 6% agar and autoclaved.

e. Embryo culture

The isolated embryos were placed individually in sterile jars each containing 30 ml of culture media. The jars were placed in growth chamber at 25°C with 16 h photoperiod. 100 jars were used for each cultivar.

The following parameters were recorded.

a. Seed characteristics

Including seed weight (g), stoneless weight (g), and the percentage of empty seeds.

b. Germination percentage

The emergence of roots and opening of cotyledons was the sign of germination, the emerged platelets were counted and germination percentage was calculated.

c. Time for the first emergence

d. Mean germination time: The time in days between the first emergence and complete germination.

e. Seedling growth

Growth measurements were performed for each plant; stem and root length (cm) and plant fresh weight (g) were measured.

Experimental design and data analysis

This study followed the randomized complete design with 5 replicates, 20 jars for each one and data were subjected to analysis of variance (ANOVA) according to Snedecor and Cochran [21] using MSTAT C statistical package Freed *et al*[22] software, and means of the treatments were compared by Least Significant Difference (L.S.D.) according to Duncan [23] at significance level of 0.05.

Results and Discussions

a. Seed characteristics

Data presented in Table (1) showed that Coratina cv. had higher seed weight (0.46 g) compared with Arbequina (0.26 g). Also Coratina had higher stonless seed weight (0.050 g) compared with Arbequina (0.041 g).

The number of the empty seeds also varied among the studied cultivars, Arbequina cv. had low percentage of empty seeds (10.66%).

Table 1.Seed characteristics of Arbequina and Coratina olive cultivars.

Cultivar	Seed weight	Stoneless weight	Empty seed %
Arbequina	0.26	0.041	10.66
Coratina	0.46*	0.050*	16.33*

Means within each column are significant (*) or not significantly (NS) at *P*< 0.05

Acebedo *et al*[9] reported that there is a high variation in seed characteristics *i.e*. seed weight, stoneless seed weight and empty seed percentage. These characteristics explain partially the difference in seed germination capacity between olive cultivars.

b. Effect of different media on germination percentage

As shown in Table (2) Coratina olive cv. recorded high germination percentage (52.58%) compared with Arbequina that showed the lowest germination percentage (49.08%). while the highest germination percentage obtained from Agar + Sucrose + MS media (62.83%) flow it Agar+Sucrose + MS +GA3 media (59.5%) while the lowest germination percentage obtained from Agar +Sucrose media (21.83%) .

Table 2.Effect of different media on Germination %, of Arbequina and Coratina olive cultivars.

Cultivars	Media				Mean
	Agar	Agar+ Sucrose	Agar+ Sucrose+ MS	Agar+Sucrose+MS+GA_3	
Arbequina	45.66	15.66	71.00	64.00	49.08 **B**
Coratina	72.66	28.00	54.66	55.00	52.58 **A**
Mean	59.16 **B**	21.83 **C**	62.83 **A**	59.50 **AB**	

Means within each column with the same letter are not significantly different at *P*< 0.05

c. Effect of different media on first germination time:

Concerning the time required for germination process, data in Table (3) showed that, embryo germination started in short period of time (5days for Coratina with Agar +Sucrose media and 6 days for

Arbequina with Agar media without any addition). However, the highest long period were recorded with Agar +Sucrose +MS+GA3 media 9 days with both Arbequina and Coratina cultivars. Generally Coratina took low period to emergence 7.08 days.

Table 3. Effect of different media on first emergence of germination of Arbequina and Coratina olive cultivars .

Cultivars	Media				Mean
	Agar	Agar+Sucrose	Agar+Sucrose+MS	Agar+Sucrose+MS+GA_3	
Arbequina	6.00	9.00	8.00	9.00	8.00 **A**
Coratina	7.00	5.00	7.00	9.00	7.00 **A**
Mean	6.50 **B**	7.00 **AB**	7.50 **AB**	9.00 **A**	

Means within each column with the same letter are not significantly different at *P*< 0.05

d. Effect of different media on mean germination time

Table (4) showed that, the mean germination time (time for complete germination) were shorter in Coratina cv. (8 day) compared with Arbequina cv. (14.5 day). Whereas, long time in both cultivars Arbequina and Coratina cvs. 16 days with Agar + Sucrose + MS media, while a short time with Agar without any addition7.5 days.

Acebedo *et al*[9] studied the germination of isolated embryo of 10 olive cultivars; isolated embryos of all cultivars had higher germination percentage. Similar results were obtained by Maalej *et al* [16] and Kiani *et al* [19]. However, some studies reported that Olive seed germination may continue erratically for 3 years Sotomayor-Leon and Caballero[17] and Acebedo *et al*[9].Even the increase of germination rate and uniformity were obtained from stonelss seeds after removing the hard endocarp [11]. However germination rate of stonless seeds of many olive cultivars still slow reported [24].Acebedo *et al* [9] reported that the mean germination time was longer in the stoneless seeds (55 to 95 days) while the isolated embryos germinated uniformly within 10-14 days. Zienkiewicz *et al*[25] studied in the complete course of seed germination, they reported that olive cotyledons started to greenish around the 4th day, 26 days later the seedlings were ready for growing in pots.

Table 4.Effect of different media on mean germination time of Arbequina and Coratina olive cultivars.

Cultivar	Media				Mean
	Agar	Agar+ Sucrose	Agar+ Sucrose+ MS	Agar+Sucrose+MS+GA_3	
Arbequina	9.00	15.00	21.00	13.00	14.50 **A**
Coratina	6.00	7.00	11.00	8.00	8.00 **B**
Mean	7.50 **C**	11 **B**	16.00 **A**	10.50 **B**	

Means within each column with the same letter are not significantly different at *P*< 0.05

e. Effect of different media on seedlings growth:

1- Leaves number

Data in Table (5) showed that, the two tested of olive cultivars showed good growth over the period of the experiment. Arbequina cv. had high number of leaves (3.72) compared with Coratina (3.21). The highest leaf number showed with Agar+Sucrose+ MS media followed by Agar+Sucrose+MS+GA_3, while Agar media recorded the lowest value in leaves number.

Table 5. Effect of different media on leaf number of Arbequina and Coratina olive cultivars

Cultivars	Media				Mean
	Agar	Agar+ Sucrose	Agar+ Sucrose+ MS	Agar+Sucrose+MS+GA_3	
Arbequina	2.00	3.00	5.66	4.23	3.72 **A**
Coratina	2.00	2.00	4.66	4.18	3.21 **A**
Mean	2.00 **C**	2.5 **C**	5.16 **A**	4.21 **B**	

Means within each column with the same letter are not significantly different at *P*< 0.05

2- Plant height

As shown in Table (6) Arbequina olive cv. recorded higher plant height (2.30 cm) compared with Coratina (1.83 cm). While the higher plant height was recorded with Agar+Sucrose+MS media and the lowest plant height was recorded with Agar media. Acebedo *et al*[9] reported that difference in shoot growth was recorded among the tested cultivars. *In vitro* cultures help the breeders to overcome the problems associated with seed germination and increase the size of progenies for evaluation state. These results indicted the possibility of obtaining seedling with high survival percentage; Garcia *et al*[15] reported that the survival of olive seedlings after *in vitro* culture reached up to 85%. Kiani *et al*[19] reported that, percentage of seedlings formation of olive cultivars from *in vitro* embryo culture ranged from 70.1% in Zard cv. to 79% in Dezful cv. Similar results were reported by Sahijram *et al* [26] in mango and Jaskani *et al* [27] in citrus.

Table 6. Effect of different media on plant height of Arbequina and Coratina olive cultivars

Cultivars	Media				Mean
	Agar	Agar+Sucrose	Agar+Sucrose+MS	Agar+Sucrose+MS+GA_3	
Arbequina	1.00	2.16	3.03	3.00	2.30 **A**
Coratina	1.00	1.00	3.50	1.84	1.83 **A**
Mean	1.00 **C**	1.58 **BC**	3.27 **A**	2.42 **AB**	

Means within each column with the same letter are not significantly different at *P*< 0.05

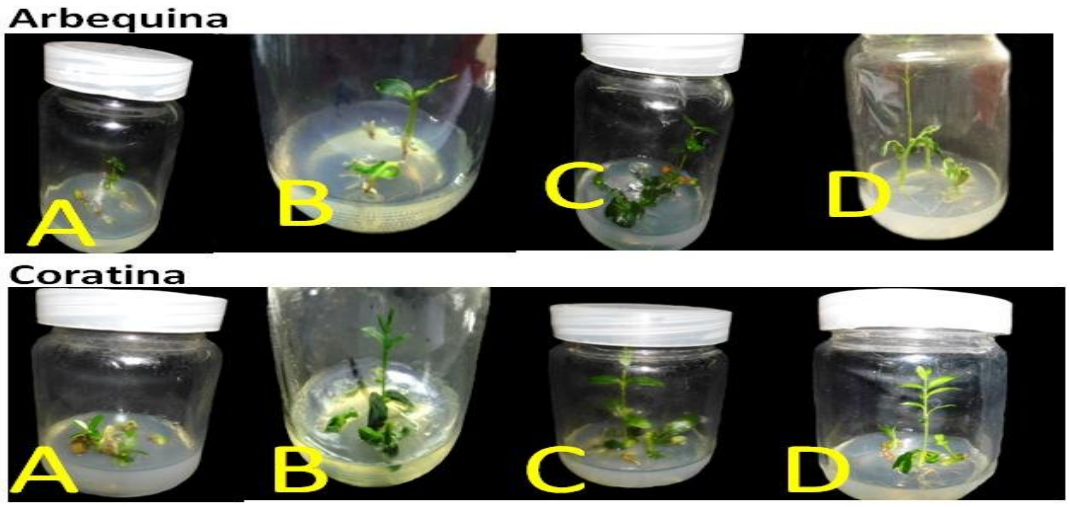

Fig. 1: Effect of different media on development of Arbequina and Coratina olive embryo. A: Agar, B: Agar+ Sucrose, C: Agar+ Sucrose+ MS, D: Agar+Sucrose+MS+GA_3

References

1. Rallo, L. Selección y mejoragenéticadelolivo en España. Olivae, 1995, 59 (12): 46-53.
2. Lavee, S. 'Kadesh' table olive. HortScience, 1978,13:62–63.
3. Lavee, S., Haskal, A., Wodner, M. 'Barnea' a new olive cultivar from first breeding generation. Olea, 1986, 17:95–99.
4. Bellini E.; Parlati M.V. and Giordani E. Three new olive cultivars obtained by cross-breeding. ActaHortic., 2002,586: 221-223.
5. Barranco D , R. Rallo and AI Kiran, , L. León. Seedling vigour as a preselection criterion for short juvenile period in olive breeding. Crop and Pasture Science, 2006,57 (4), 477-481
6. Rugini, E. *In vitro* culture of the olive: An overview of the present scientific status. Acta Hortic.,1990, 286: 93-96.

7. Lagarda A, Martin GC, Kester DE. Influence of Environment, Seed Tissue, and Maturity on 'Manzanillo' Olive Seed Germination. Hortscience,1983a, 18(6):868-869.
8. Troncoso, A.; Cantos, M.; Linan, J.; Troncoso, J. and Rapoport H. F. *In vitro* development and germination of immature olive embryos. J. Hortic. Sci. Biotechn., 2003,78 (5): 728-733
9. Acebedo, M. M.; Laver, S.; Linnan, J. and Troncoso, A., *Invitro* germination of embryos for speeding up seedling development in olive breeding programs. Sci. Hortic.,1997, 69: 207-215.
10. Lagarda A, Martin GC, Polito VS. Anatomical and morphological development of 'manzanillo' olive seed in relation to germination. Am. Soc. Hortic. Sci,1983b,108:868-869.
11. Crisosto, C. and Sutter, E.G. Improving Manzanillo olive seed germination. HortScience,1985, 20: 100-102
12. Bandino, G.; Sedda, P. and Mulas, M. Germination of olive seeds as affected by chemical scarification, hot water dip and Gibberellic acid treatments. Acta Hortic.,1999, 474:35-38.
13. Lagarda A, Martin G.'Manzanillo' Olive seed dormancy as influenced by exogenous hormone application and endogenous adscisic acid concentration. Hortsciences, 1983, 18(6):869-871.
14. Ramming D.W. The use of embryo culture in fruit breeding. Hortscience,1990, 25:393-398.
15. García, J. L.; Troncoso, J.; Sarmiento, R. and Troncoso, A. Influence of carbon source and concentration on the *invitro* development of olive zygotic embryos and explants raised from them. Plant Cell Tiss. Org., 2002, 69 (1) 95-100.
16. Maalej, M.; Drira N.; Chaari-Rkhis A. and Trigui A. *Invitro* germination of seeds of three Tunisian olive varieties. ActaHortic., 2002a ,586:903-906.
17. Sotomayor-Leon, E.M. and. Caballero, J.M. An easy method of breaking olive stone to remove mechanical dormancy ActaHortic., 1990, 286: 113-116.
18. Maalej, M.; Drira, N.; Chaari-Rkhis, A., and Trigui, A. Preliminary results of somatic embryogenesis from young zygotic embryos of olive tree. ActaHortic., 2002b, 586:899-902
19. Kiani, M.; Zamani, Z. and Ebadi, A. *In vitro* germination of three olive cultivars. ActaHortic., 2006,725:333-336.
20. Murashige, T. and Skoog, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant, 1962,15:473-97.
21. Snedecor, G. W. and Chochran, W.G. Statistical Methods, Seventh Edition, Ames: Iowa State University Press, 1980,507 p.
22. Freed, R.; Eisensmith, S.P.; Goetz, S.; Reicosky, D.; Smail, V.M. and Wollberg, P. (1990) MSTAT-C A Microcomputer Program for the Design, Management and Analysis of Agronomic Research Experiments. https://www.msu.edu/~freed/disks.htm
23. Duncan, D. B. "Multiple Range and Multiple F-Tests. Biometrics, 1955, 11: 1-42.
24. Rinaldi, L.M.R.Germination of seeds of olive (*Oleaeuropaea* L.) and ethylene production: Effects of harvesting time and thidiazuron treatment. J. Hort. Sci. Biotech., 2000, 75: 727-732.
25. Zienkiewicz, A.; Jiménez-López, J.C.; Zienkiewicz, K.; de Dios, Alché, J. and Rodríguez-García, M.I. Development of the cotyledon cells during olive (Oleaeuropaea L.) in vitro seed germination and seedling growth. Protoplasma, 2010,248 (1): 1-15.
26. Sahijram, L.; Bollamma, K.T.; Naren, A.; Soneji, J. R.; Dinesh, M.R. and Halesh, G.K. *In vitro* hybrid embryo rescue in mango (*Mangiferaindica* L.) breeding. Indian J. Hortic., 2005,62 (3): 235-237.
27. Jaskani, M. J.; Khanb, I. A. and Khana, M.M. Fruit set, seed development and embryo germination in interploid crosses of citrus Sci. Hortic., 2005,107 (1): 51-57.

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