Adv. Hort. Sci., 2022 36(3): 185-191 DOI: 10.36253/ahsc-12488

AHS
Advances in Horticultural Science

Impact of cultural conditions on germination of olive (*Olea europaea* L.) somatic embryos and plantlets development from the Algerian cultivar Chemlal

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OPEN ACCESS

Key words: Germination, micropropagation, olive, plant regeneration, somatic embryo.

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Citation:
TITOUH K.,HADJ MOUSSA K., BOUFIS N., KHELIFI L., 2022 - *Impact of cultural conditions on germination of olive (Olea europaea L.) somatic embryos and plantlets development from the Algerian cultivar Chemlal.* - Adv. Hort. Sci., 36(3): 185-191.

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 28 December 2021
Accepted for publication 13 June 2022

Abstract: The *in vitro* propagation techniques are currently a commercial alternative for the production of plants with good quality in several plant species, including the olive tree (*Olea europaea* L.). Somatic embryogenesis is the process practically used for the application of several biotechnological tools of improvement and *in vitro* plant regeneration via the germination of somatic embryos. Our work aims to evaluate the effect of the chemical and hormonal composition of the culture medium on the germination of olive somatic embryos (cv. Chemlal) as well as the micropropagation of the obtained plantlets before their acclimatization to natural conditions. The results indicated that the production of olive plants by somatic embryogenesis depends strongly on the genotype of the somatic embryos (cell line) and more on the culture conditions, particularly the presence of growth regulators. Indeed, a solid OM medium supplemented with hormones (BA and IBA) permitted an advanced root emergence and germination allowing the production of well-developed plants with several leaves. In addition, an OM medium supplemented with Zeatin and IBA allowed better reactivity of micro-cuttings producing well-developed shoots with several emitted roots which facilitates their further acclimatization to natural conditions.

1. Introduction

The olive tree (*Olea europaea* L.) a diploid dicotyledonous species of the *Oleaceae* family, includes several cultivars selected and multiplied initially by farmers mainly for the size of their fruits and the oil content (Besnard *et al.,* 2018). Conventional methods of breeding and multiplication represent an important solution to the crop problems, especially the increasing demand for plants needed for new orchards. However, these

techniques have become unable to achieve significant results because of the long juvenile period of the species (Rugini *et al.,* 2020) as well as the recalcitrance of certain main cultivars, such as 'Chemlal' in Algeria, to semi-hardwood cuttings (Fabbri *et al.,* 2004). In this context, *in vitro* propagation techniques are currently a commercial reality for the multiplication of several olive cultivars (Rugini *et al.,* 2020) due to the sanitary quality and the confirmed genetic stability of the regenerated plants (Lopes *et al.,* 2009) despite some phenotypic changes and somaclonal variations observed after several subcultures (Leva, 2009; Bradaï *et al.,* 2016, 2019).

Somatic embryogenesis is a morphogenetic process through which somatic cells produce a bipolar structure morphologically similar to the zygotic embryo called a 'somatic embryo' able to develop into a whole plant (Neumann *et al.,* 2009). Actually, this process has become a common technique for *in vitro* regeneration allowing the application of several biotechnological tools of improvement and conservation such as genetic transformation, *in vitro* selection and cryo-preservation of various species and interesting genotypes (Sánchez-Romero, 2021). Most of the established works on somatic embryogenesis in olive consider three main steps starting with the establishment of embryogenic cultures combining the induction and proliferation of calli, followed by a phase of expression and development of structured embryos ready to be converted into whole plants. 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 chemical compositions 'MS' (Murashige and Skoog, 1962) or 'OM' (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 (Merkle *et al.,* 1995; Sánchez-Romero, 2019) but also by unfavorable conditions to their germination (Bradaï *et al.,* 2016).

The objective of our study is to evaluate the effect of the chemical and hormonal composition of the culture medium on the germination of somatic embryos of the main Algerian olive cultivar 'Chemlal'. In addition, the micropropagation of the obtained plantlets as well as their acclimatization to natural conditions were tested.

2. Materials and Methods

Establishment of the embryogenic cultures

The embryogenic cultures used were induced from radicles of zygotic embryos of the cultivar 'Chemlal' according to a modified method of Cerezo *et al.* (2011). Radicles isolated after disinfection of the seeds extracted from the stones of mature olives were cultured on solid OMc medium (Cañas and Benbadis, 1988) supplemented with 0.5 mg l^{-1} of Zeatin and 5 mg l^{-1} of indole-3-butyric acid (IBA) for three weeks. Subsequently, the explants were transferred to the same OMc medium without Zeatin and containing 0.5 mg l^{-1} of IBA for four weeks. Finally, the obtained calli were maintained by monthly subcultures on a solid ECO basal medium (Cerezo *et al.,* 2011) supplemented with 0.1 mg l^{-1} of Zeatin, 0.1 mg l^{-1} of Benzylaminopurine (BA) and 0.05 mg l^{-1} IBA in addition to 0.55 g l^{-1} of glutamine and 1 g l^{-1} of casein hydrolyzate. All the media were supplemented with 20 g l^{-1} of sucrose, 50 mg l^{-1} of Myo-Inositol and solidified with 6 g l^{-1} of agar after adjusting the pH to 5.74 with NaOH or HCl (1 N). The cultures were incubated in total darkness at a temperature of 25±2°C.

Pro-embryos exceeding 2 mm in size were isolated from calli of two embryogenic lines (C2 and C3) after one month of culture in suspension on a liquid ECO medium supplemented with hormones in darkness with stirring at 100 rpm. These immature embryos were transferred for maturation on solid ECO free-hormones medium and supplemented with 1 g l^{-1} of activated charcoal for two months in total darkness at 25±2°C.

Germination of somatic embryos

Mature embryos with a perfectly bipolar or cotyledonary structure, were germinated individually in test tubes over two different media: OM (Rugini, 1984) and MS (Murashige and Skoog, 1962) solidified with 6 g l^{-1} of agar. In addition, the effect of a hormonal combination consisting of 1 mg l^{-1} BA and 0.1 mg l^{-1} IBA was tested. The two media were supplemented with 20 g l^{-1} of sucrose, 100 mg l^{-1} of Myo-Inositol. At least 16 mature somatic embryos were incubated for more than eight weeks under a 16 h light photoperiod (35 µmol m^{-2} s^{-1}) at a temperature of 25±2°C. Germination, root emergence, shoot

length as well as the number of leaves per obtained plantlet after eight weeks of culture were observed.

Micropropagation of shoots and acclimatization of regenerated plantlets

In order to maintain the maximum number of plants in culture; micropropagation of the shoots resulting from the germination of somatic embryos was applied. Therefore, the shoots were divided into uni-nodal micro-cuttings and cultured on two different culture media: OM and DKW (Driver and Kuniyuki, 1984) as modified by Revilla *et al.* (1996). The two culture media were supplemented with 20 g l^{-1} of sucrose, 100 mg l^{-1} of Myo-Inositol in addition to hormonal balances composed of 2 mg l^{-1} of BA or Zeatin combined with 0.1 mg l^{-1} of IBA. The different media were solidified with 6 g l^{-1} of agar. At least 16 micro-cuttings having two leaves were cultured individually in test tubes containing 15 ml of the micropropagation medium for eight weeks under a 16 h light photoperiod at a temperature of 25±2°C. The reactivity of the micro-cuttings and the number of reactive buds, shoot length, number of leaves as well as the number of roots emitted were observed.

The obtained plants showing an acceptable length with several leaves and well-developed roots were acclimatized in the laboratory under a photoperiod for about two months on a humidified mixture of sand/potting soil/perlite at a rate of 2/2/1 (v/v/v). Subsequently, the reactive plantlets were transferred to natural conditions under greenhouse on a substrate rich in organic matter and frequently irrigated before being permanently planted in the field.

Data analysis

Statistical analyses of the data (Analysis of variance and tests) were carried out using the "XLSTAT" program version 2016.02.27444. In case of a significant difference, the separation of means was performed by Fisher's LSD (Least Significant Difference) test. The percentages were analyzed by the chi-square test. The results were presented as a mean ± standard deviation or as a percentage relative to the total of introduced explants. A significance level of 5% was considered in all analyses. The letters in the tables indicate homogeneous groups.

3. Results

Germination of somatic embryos

From the first days under photoperiod, the white-opaque somatic embryos of olive showed greening of their stem part and yellowing of the root part (Fig. 1 A) followed by its elongation preceding an increase in size of the two cotyledonary leaves and their separation each one from the other (Fig. 1 B) before the emergence of a small shoot (Fig. 1 C). Indeed, the germination capacity of embryos and the plants development varied from one cell line to another and were significantly influenced by the culture conditions, particularly the presence of growth regulators (Table 1). Thus, more embryos of the C2 line germinated on OM medium while the germination of the two lines was similar on the MS medium. However, the presence of hormones allowed a significant improvement in the embryos germination of both lines. In fact, the best germination rates were obtained on OM medium supplemented with BA and IBA (OM_1) with 56.3 and 37.5% respectively for C2 and C3 while the low germination rate of 25% was recorded on MS without hormones (MS_0) (Table 1).

In addition, embryos of C2 germinated with root emergence from the first week of culture (Fig. 1 A) while no reactivity was observed before two weeks for C3 embryos. Likewise, the germination and the root emergence were faster on the OM medium than

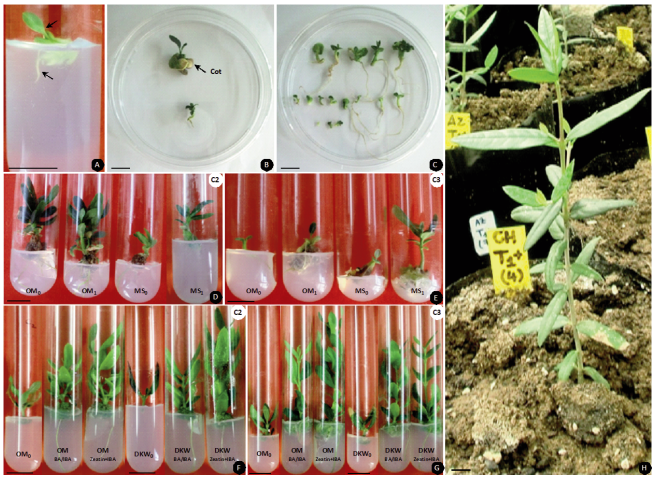

Fig. 1 - Germination of mature somatic embryos from two lines of embryogenic olive callus, cv. Chemlal, and micropropagation of the obtained shoots after 8 weeks of culture on different culture media. (A) Somatic embryo germinated on solid medium. (B) Swelling of cotyledonary leaves of embryos. (C) Different stages of embryos germination. (D and E) Plantlets obtained after germination. (F and G) Plantlets obtained after the micropropagation of shoots obtained from germination of somatic embryos. (H) Acclimated plantlets. (→: the arrows indicate the emergence of the root, shoot and the two cotyledonary leaves. Bar corresponds to 1 cm. Cot: Cotyledons).

Table 1 - Effect of the chemical composition (OM and MS) of the culture medium and the presence of hormones (without hormones '0' or with hormones '1') on germination and root emergence of somatic embryos of two lines of embryogenic olive calli, cv. Chemlal, after eight weeks of culture

Germination medium	Germination rate (%)		Average time of germination (Days)		Average time of root emergence (Days)		Average length of plantlet (cm)		Average number of leaves/plantlet	
	C2	C3	C2	C3	C2	C3	C2	C3	C2	C3
OM_0	50.0 b	31.3 d	16.8±3.8 b'	21.0±0.0 de'	12.3±3.5 a''	17.5±4.9 cd''	1.6±0.1 **b**	0.6±0.1 **d**	6.0±1.6 **a'**	2.0±0.0 **c'**
OM_1	56.3 a	37.5 c	14.0±4.4 a'	18.7±4.0 bc'	10.5±4.0 a''	15.4±3.1 b''	2.3±0.8 **a**	0.9±0.2 **c**	6.3±1.6 **a'**	2.3±0.6 **c'**
MS_0	25.0 e	25.0 e	24.5±4.9 f'	21.7±1.2 e'	21.0±0.0 e''	18.7±4.0 d''	0.8±0.1 **cd**	0.8±0.1 **cd**	2.5±0.7 **c'**	2.0±0.0 **c'**
MS_1	31.3 d	31.3 d	21.0±0.0 de'	19.3±4.7 cd'	17.5±0.7 cd''	16.3±4.0 bc''	0.9±0.1 **c**	1.9±0.1 **b**	2.5±0.7 **c'**	4.5±0.7 **b'**

The different small letters of the same format in columns indicate the homogeneous groups of a significant difference at level of 5%.

on MS, especially in the presence of growth regulators which accelerated significantly germination of embryos. Therefore, the embryos emitted their roots after 10.5 and 15.4 days and germinated after 14 and 18.7 days on the OM_1 medium respectively for C2 and C3 (Table 1). Consequently, early germination and rooting of C2 embryos resulted in well-developed plants with an average length of 1.6 and 2.3 cm with 6 and 6.3 leaves per plantlet respectively on OM_0 and OM_1 (Table 1, Fig. 1 D) while the MS_1 medium was more beneficial for the C3 plants reaching 1.9 cm in length with 4.5 leaves (Table 1, Fig. 1 E).

Micropropagation of shoots and acclimatization of regenerated plantlets

Reactivity of micro-cuttings and shoot development. The micropropagation of shoots resulting from the germination of somatic embryos was significantly influenced by the callus line as well as the chemical and hormonal composition of the culture medium (Table 2). In fact, micro-cuttings of the C2 line were more reactive than those of C3 regardless of the culture conditions, although the presence of hormones was essential for the development of the shoots given the low reactivity recorded on the control media. In addition, the best result of reactivity (100%) of the explants of both lines was obtained with the balance Zeatin/IBA and also the C2 cuttings on the combination BA/IBA in presence of which only 62.5 and 87.5% of the C3 cuttings reacted respectively on OM and DKW (Table 2). Elsewhere, the DKW medium in particular supplemented with hormones (Zeatin/IBA) accelerated the bud reaction and improved the number of reactive buds per explant while the cuttings introduced particularly on the free-hormones OM medium reacted late with less sprouted buds (Data not shown).

The development of the obtained shoots depended directly on the culture conditions and was significantly influenced by the reactivity degree of the cut-

Table 2 - Effect of the micropropagation medium (OM and DKW) and the presence of hormones (BA/IBA or Zeatin/IBA) on the reactivity, development and rooting of shoots from uni-nodal micro-cuttings obtained after germination of somatic embryos of two lines of embryogenic olive calli, cv. Chemlal, after eight weeks of culture

Composition of the micro-propagation medium	Hormonal combinations	Reactivity and shoot development						Rooting			
		Reactivity rate (%)		Average length of shoot (cm)		Average number of leaves/shoot		Rooting rate (%)		Average number of roots	
		C2	C3	C2	C3	C2	C3	C2	C3	C2	C3
OM	Control	25.0 d	12.5 d	0.7±0.3 g	0.9±0.0 fg	1.5±0.7 d'	2.0±0.0 cd'	0.0 e'	0.0 e'	0.0±0.0 f''	0.0±0.0 f''
	BA/IBA	100.0 a	62.5 c	2.0±0.7 de	1.9±0.2 de	2.9±1.0 cd'	3.1±0.9 cd'	31.3 d'	31.3 d'	1.0±0.0 e''	1.5±0.7 bc''
	Zeatin/IBA	100.0 a	100.0 a	3.7±1.0 b	5.2±2.1 a	6.9±3.2 b'	9.9±3.1 a'	62.5 a'	43.8 c'	1.5±0.0 bc''	1.7±0.6 b''
DKW	Control	18.8 d	12.5 d	0.5±0.0 g	0.7±0.0 g	2.0±0.0 cd'	2.0±0.0 cd'	0.0 e'	0.0 e'	0.0±0.0 f''	0.0±0.0 f''
	BA/IBA	100.0 a	87.5 b	1.7±0.3 ef	3.1±1.0 bc	3.4±1.1 c'	2.7±0.4 cd'	43.8 c'	31.3 d'	1.3±0.6 cd''	1.5±0.7 bc''
	Zeatin/IBA	100.0 a	100.0 a	2.7±0.2 cd	3.3±1.4 bc	6.6±1.1 b'	6.8±2.4 b'	56.3 a'	50.0 b'	1.3±0.5 d''	2.0±0.8 a''

The different small letters of the same format in columns indicate the homogeneous groups of a significant difference at level of 5%.

ting particularly the number of reactive buds. In cuttings with two active buds; one shoot regularly grown more than the other (Fig. 1 F and G). Moreover, the cuttings cultured on OM medium supplemented with hormones especially Zeatin/IBA and which sprouted early, produced well-developed shoots of 3.7 and 5.2 cm in length with 6.9 and 9.9 leaves respectively for C2 and C3 while the shoots obtained on the control media were the least developed, with less than 1 cm of length and a maximum of 2 leaves per plantlet (Table 2, Fig. 1 F and G).

Rooting of developed shoots. The presence of hormones in the culture medium was essential for the formation of a basal callus on the micro-cuttings before the emission of roots, while the chemical composition contributed more in the development of the induced calli due that the majority of calli generated on OM were generally larger compared to those obtained on DKW (Data not shown, Fig. 1 F and G).

Root emission was significantly influenced by the genotype (cell line) as well as chemical and hormonal composition of the propagation medium (Table 2). Indeed, more rooting was observed with micro-cuttings of the line C2 particularly on the OM medium supplemented with Zeatin/IBA allowing 62.5 and 43.8% rooting with 1.5 and 1.7 roots emitted by cutting respectively for the two lines while the chemical composition of DKW was more beneficial to C3 explants with 50% of rooting and an average of 2 roots per plantlet (Table 2). However, the appearance of roots occurring from the 3rd to the 7th week of culture was often faster on DKW compared to OM medium especially in the presence of BA with IBA. Furthermore, the substitution of BA by Zeatin in the added hormonal combination reduced the rooting time by more than a week in the cuttings of both lines, which improved the length of the emitted roots (Data not shown). Therefore, the plantlets showing well-developed shoots and roots were easily acclimatized in the laboratory and exhibit normal growth and phenotype even after transfer to natural field conditions (Fig. 1 H).

4. Discussion and Conclusions

Germination of somatic embryos

Germination of olive somatic embryos was frequently achieved on media based on the chemical formulations OM and MS with a reduced concentration of mineral salts, similar to those used for the culture of zygotic embryos (Sánchez-Romero, 2019). Rugini (1988) indicated that germination and development of olive plantlets from somatic embryos is faster on OM medium than on MS. Indeed, Rugini and Caricato (1995) observed the germination of embryos of cultivars 'Canino' and 'Moraiolo' after 1 to 2 weeks on OM free-hormones medium, whereas Shibli *et al.* (2001) didn't note reactivity in embryos of the cultivar 'Nabali' before two weeks of incubation on MS medium, which agree with our results indicating a faster germination on OM medium compared to MS one.

Several studies reported low rates of embryo germination varying with genotype but depending more on the quality of the used embryos. Therefore, Jafarzadeh-Bajestani *et al.* (2011) obtained less than 6% of germination on MS medium with embryos of the cultivar 'Zard' while a desiccation step for three days improved conversion to 50% and formation of rooted plantlets with 6 to 8 leaves. Furthermore, culturing embryos of the cultivar 'Picual' on a cellulose acetate semi-permeable membrane during the first month of maturation (Cerezo *et al.,* 2011) allowed an adequate dehydration, good structuring of the embryos and a significant improvement in germination and quality of the obtained plantlets. Therefore, the low germination capacity observed in this study was probably due to the use of embryos directly after their maturation without passing a desiccation phase allowing the synchronization of their germination (Merkle *et al.,* 1995).

Germination of olive somatic embryos was often achieved in the absence of hormones, although Rugini (1995) recommended the addition of Zeatin to solid MS medium to boost emergence. Thus, embryos of the cultivars 'Chetoui', 'Chemlali' and 'Arbequina' (Trabelsi *et al.,* 2003) and 'Picholine Marocaine' (Brhadda *et al.,* 2008) germinated easily on OM and MS media supplemented with 0.5 mg l^{-1} of Zeatin and resulted to well-developed plantlets, while a free-hormones medium rich in sucrose induced cell proliferation of the embryos. Conversely, Toufik *et al.* (2017) observed that the presence of growth regulators, in particular Zeatin alone in the OM-based medium, was not essential for the germination of embryos of the cultivar 'Dahbia' and can even inhibit the root emergence and cause a strong explant necrosis although the addition of NAA with GA3 allowed up to 45% conversion from mature embryos according to Mazri *et al.* (2020). Therefore, the inclusion of growth regulators especially cytokinins to the germination

medium is directly determined by the genotype and degree of maturity of the embryos (Merkle *et al.,* 1995). In this sense, Bradaï *et al.* (2016) indicated that well-matured and structured or cotyledonary embryos of 'Picual' germinated easily in the absence of hormones, unlike globular embryos whose germination varies between 30 and 70%.

The regenerated plants in this study showed a normal phenotype (phyllotaxis, leaf shape, etc.) during their *in vitro* maintenance as well as an easy acclimatization to natural conditions. Leva (2009) indicated that plantlets regenerated by somatic embryogenesis show a stable phenotype similar to that of the mother plants. Moreover, despite their fragility, these plants acclimatize easily to natural conditions and show normal growth under greenhouse and good development after transfer to the field.

Micropropagation of shoots and acclimatization of regenerated plantlets

The *in vitro* multiplication of plants regenerated by somatic embryogenesis was rarely practiced because their acclimatization was usually carried out directly after germination (Bradaï *et al.,* 2016). In fact, the DKW medium (Driver and Kuniyuki, 1984) as modified by Revilla *et al.* (1996) and supplemented with a hormonal balance rich in cytokinins was often used although the chemical formulation of OM medium containing Zeatin was commonly recommended for the rapid stimulation of axillary buds of several olive cultivars (Lambardi *et al.,* 2013) due to its nutritional content, particularly in microelements (Rugini *et al.,* 2020). Cerezo *et al.* (2011) indicated that the micro-cuttings taken after germination of embryos of the cultivar 'Picual' respond easily on DKW medium supplemented with BA and IBA by developing shoots of about 1.4 cm in height with formation of a basal callus often accompanied by the emission of one or more roots. Nevertheless, Bradaï *et al.* (2016) observed that shoots multiplication from somatic embryos was influenced more by the age of the cell culture than by its genotype (callus line). These authors obtained more developed rooted shoots from young cultures while less sprouted buds with older lines giving small shoots often without roots. According to Bhojwani and Razdan (1996) *in vitro* shoots need to reach a minimal size to root easily as small plants may not survive during the acclimatization period. Therefore, our results confirm the importance of the genotype, the significant effect of the DKW chemical formulation and the presence of Zeatin in addition to an auxin for the multiplication and rooting of shoots resulting from the germination of somatic embryos.

In conclusion, our study is a contribution to the optimization of the *in vitro* regeneration of olive tree by somatic embryogenesis and describes for the first time an efficient regeneration of whole plants without morphological abnormalities in the main olive cultivar in Algeria 'Chemlal' via embryogenic cultures induced from juvenile material, radicles of zygotic embryos. The obtained results show that the development of plantlets by germination of somatic embryos depends strongly on the genotype and the chemical and hormonal composition of the used culture medium. A solid OM medium supplemented with hormones allowed a faster germination resulting in well developed shoots. Subsequently, multiplication of the shoots on OM or DKW media containing Zeatin with IBA generated whole and rooted plantlets easily acclimatized to natural conditions.

Acknowledgements

The authors gratefully acknowledge funding support from the National Higher School of Agronomy (ENSA) and the Algerian Ministry of Higher Education and Scientific Research (MESRS).

References

BESNARD G., TERRAL J.F., CORNILLE A., 2018 - *On the origins and domestication of the olive: a review and perspectives*. - Ann. Bot., 121: 385-403.

BHOJWANI S.S., RAZDAN M.K., 1996 - *Plant tissue culture: theory and practice, a revised edition*. - Elsevier, The Netherlands, pp. 767.

BRADAÏ F., PLIEGO-ALFARO F., SÁNCHEZ-ROMERO C., 2016 - *Long-term somatic embryogenesis in olive (*Olea europaea *L.): Influence on regeneration capability and quality of regenerated plants*. - Sci. Hortic., 199: 23-31.

BRADAÏ F., SÁNCHEZ-ROMERO C., MARTÍN C., 2019 - *Somaclonal variation in olive (*Olea europaea *L.) plants regenerated* via *somatic embryogenesis: Influence of genotype and culture age on genetic stability*. - Sci. Hortic., 251: 260-266.

BRHADDA N., WALALI L.D.M., ABOUSALIM A., 2008 - *Effet du sucre sur l'embryogenèse somatique de l'olivier (*Olea europaea *L.) cv. 'Picholine Marocaine'*. - Biotechnol. Agron. Soc. Environ., 12(3): 245-250.

CAÑAS L.A., BENBADIS A., 1988 - In vitro *regeneration from cotyledon fragments of the olive tree (*Olea

europaea *L.)*. - Plant Sci., 54: 65-74.

CEREZO S., MERCADO J.A., PLIEGO-ALFARO F., 2011 - *An efficient regeneration system via somatic embryogenesis in olive*. - Plant Cell Tiss. Organ Cult., 106: 337-344.

DRIVER J.A., KUNIYUKI A.H., 1984 - In vitro *propagation of paradox walnut rootstock*. - HortScience, 19: 507-509.

FABBRI A., BARTOLINI G., LAMBARDI M., KAILIS S., 2004 - *Olive propagation manual*. - Landlinks Press, Collingwood, VIC, Australia, pp. 141.

JAFARZADEH-BAJESTANI M., KHODAI-KALAKI M., MOTAMED N., NOORAYIN O., 2011 - *Genetic transformation of olive somatic embryos through* Agrobacterium tumefaciens *and regeneration of transgenic plants*. - Afr. J. Biotechnol., 10(28): 5468-5475.

LAMBARDI M., OZUDOGRU E.A., RONCASAGLIA R., 2013 - In vitro *propagation of olive (*Olea europaea *L.) by nodal segmentation of elongated shoots,* pp. 33-44. - In: LAMBARDI M., E.A. OZUDOGRU, and S.M. JAIN (eds.) *Protocols for micropropagation of selected economically-important horticultural plants, Methods in molecular biology*. Humana Press, Totowa, NJ, USA, pp. 994.

LEVA A.R., 2009 - *Morphological evaluation of olive plants propagated* in vitro *culture through axillary buds and somatic embryogenesis methods*. - Afr. J. Plant Sci., 3(3): 37-43.

LOPES T., CAPELO A., BRITO G., LOUREIRO J., SANTOS C., 2009 - *Genetic variability analyses of the somatic embryogenesis induction process in* Olea *spp. using nuclear microsatellites*. - Trees, 23(1): 29-36.

MAZRI M.A., NACIRI R., BELKOURA I., 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(1489): 14.

MERKLE S.A., PARROT W.A., FLINN B.S., 1995 - *Morphogenic aspects of somatic embryogenesis*, pp. 155-204. - In: THORPE T.A. (eds.) In vitro *embryogenesis in plants. Current plant science and biotechnology in agriculture*, Vol. 20. Springer, Dordrecht, Germany, pp. 560.

MURASHIGE T., SKOOG F., 1962 - *A revised medium for rapid growth and bio assays with tobacco tissue cultures*. - Physiol. Plantarum., 15: 473-497.

NEUMANN K.H., KUMAR A., IMANI J., 2009 - *Plant cell and tissue culture - a tool in biotechnology basics and application*. - Springer, Berlin, Germany, pp. 333.

REVILLA M.A., PACHECO J., CASARES A., RODRIGUEZ R., 1996 - In vitro *reinvigoration of mature olive trees (*Olea europaea *L.) through micrografting*. - In Vitro Cell Dev.Biol. - Plant, 32: 257-261.

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*. - Sci. Hortic., 24: 123-134.

RUGINI E., 1988 - *Somatic embryogenesis and plant regeneration in olive (*Olea europaea *L.)*. - Plant Cell Tiss. Organ Cult., 14: 207-214.

RUGINI E., 1995 - *Somatic embryogenesis in olive (*Olea europaea *L.)*, pp. 171-189. - In: JAIN S.M., P.K. GUPTA, and R.J. NEWTON (eds.) *Somatic embryogenesis in woody plants-Angiosperms*. Vol. 2. Springer, Dordrecht, Germany, pp. 513.

RUGINI E., BALDONI L., SILVESTRI C., MARIOTTI R., NARVAEZ I., CULTRERA N., CRISTOFORI V., BASHIR M.A., MOUSAVI S., PALOMO-RIOS E., MERCADO J.A., PLIEGO-ALFARO F., 2020 - Olea europaea *Olive*, pp. 343-376. - In: LITZ R.E., F. PLIEGO-ALFARO, and J.I. HORMAZA (eds.) *Biotechnology of fruit and nut crops*. Second edition. CABI International, pp. 687.

RUGINI E., CARICATO G., 1995 - *Somatic embryogenesis and plant recovery from mature tissues of olive cultivars (*Olea europaea *L.) "Canino" and "Moraiolo"*. - Plant Cell Rep., 14: 257-260.

SÁNCHEZ-ROMERO C., 2019 - *Somatic embryogenesis in* Olea *spp*. - Plant Cell Tiss. Organ Cult., 138: 403-426.

SÁNCHEZ-ROMERO C., 2021 - *Somatic embryogenesis in olive*. - Plants., 10(433): 6.

SHIBLI R.A., SHATNAWI M., ABU-EIN, Al-JUBOORY K.H., 2001 - *Somatic embryogenesis and plant recovery from callus of 'Nabali' Olive (*Olea europaea *L.)*. - Sci. Hort., 88: 243-256.

TOUFIK I., BELKOURA I., GUENOUN F., 2017 - *Improving germination conditions of somatic embryos derived from radicle explants of Moroccan olive cultivar Dahbia*. - Eur. J. Biotech. Biosci., 5(3): 51-56.

TRABELSI E.B., BOUZID S., BOUZID M., ELLOUMI N., BELFELEH Z., BENABDALLAH A., GHEZEL R., 2003 - In vitro *regeneration of olive tree by somatic embryogenesis.* - J. Plant Biol., 46(3): 173-180.