OnLine Journal of Biological Sciences 2 (7): 438-440, 2002
ISSN 1608-4127

In vitro Shoot Development from Juvenile Cuttings of Field-Grown Olive (*Olea europaea* L.) cv. Leccino

Muhammad Ramzan Khan, Hamid Rashid and Azra Quraishi
Agricultural Biotechnology Institute, National Agricultural Research Centre, Park Road, Islamabad, Pakistan

Abstract: Effects of different growth regulators on uni-nodal explants of field-grown olive (*Olea europaea* L.) cv. Leccino were investigated. Single-node cuttings were obtained from 8th bud pair. Disinfection protocols were standardized by using 0.1% mercuric chloride for five min followed by 50% Clorox for 15 min. sterilized cuttings were tested on MS medium supplemented with various plant growth regulators viz. gibberellic acid kinetin benzylaminopurine, and isopentenyladenine. These growth regulators were used singly in different combinations ranging from 1.0 to 8.0 μM l^{-1} *In vitro*. GA_3 @ 4 μM l^{-1} was found to be more effective in enhancing shoot elongation. A higher concentration of kinetin, BAP and 2iP induced more callusing and hyperhydrated microshoots. Inclusion of polyvinylpyrrolidone @ 0.2 g/l in media was found to be successful to eliminate phenolic exudates.

Key words: *In vitro*, *Olea europaea*, explant, disinfection, growth regulators

Introduction

Olive is one of the oldest and most important crops found in the Mediterranean area and its cultivation is presently expanding into areas of South America, Australia and South Africa (Rugini and Fedeli, 1990; Rugini and Lavee, 1992).

Thousands of different olive genotypes are currently under cultivation and a high level of morphological variability is present. Nevertheless, to optimize the future sustainability of this crop certain problems, which severely limit the broad application of this crop such as low efficiency production and high cultivation cost must be solved.

Olive breeding through conventional and modern genetic methods has not yet resulted in significant improvement of this crop (Mencuccini and Rugini, 1993; Lavee, 1990; Rugini and Caricato, 1995). Failure to achieve this objective is due to its long juvenility phase (10-15 years), slow developmental stages and self-incompatibility in many olive cultivars (Canas *et al.*, 1987a,b; Canas and Benbadis, 1988; Cozza *et al.*, 1997; Lavee, 1990; Mencuccini *et al.*, 1991; Rugini, 1988). Thus breeding of this species is difficult and time consuming, severly restricting breeders from quickly developing and propagating varieties.

In vitro propagation presents a very important alternative, also because it has higher rates of multiplying clean (pest and disease-free) planting material and the small amount of space required to multiply large number of plants. Micropropagation techniques were developed during the past two decades and are now well established (Batrolini *et al.*, 1990; Cozza *et al.*, 1997; Dimassi-Theriou, 1994; Rama and Pontikis, 1990; Rugini, 1984,1988; Rugini and Fontanazza, 1981; Troncoso *et al.*, 1999). Micropropagation from axillary buds is not a common practice in many olive cultivars (Cozza *et al.*, 1997; Rugini and Caricato, 1995; Rugini and Lavee, 1992). This is because growth is slow, characterized by poor proliferation and delayed and insufficient *In vitro* rooting. Most recently *In vitro* propagation from axillary buds was reported in olive (Otero and Docampo, 1998). Growth regulators and media manipulations are the key factors for *In vitro* proliferation and regeneration in olive (Cozza *et al.*, 1997; Dimassi-Theriou, 1994). The aim of this experiment was to study the effects of different growth regulators on shoot development from juvenile cuttings of field-grown olive (*Olea europaea* L.) cv. Leccino.

Materials and Methods

Research work was conducted at National Agricultural Research Centre, from Sep. 2001 to Jan. 2002. Agricultural Biotechnology Institute, NARC provided the experimental facilities. The explants of olive (*Olea europaea* L.) cv. Leccino were kindly provided by Horticultural Research Institute, NARC, Islamabad.

Explants were obtained from the growing shoot apices of 10-15 years old field-grown Leccino plants. Single-node cuttings were obtained from 8th bud pair. Expanded leaves were removed and 4-5 cm long pieces were washed thoroughly in running tape water for 2-3 h and immersed for 30 min in a solution of ascorbic acid (100 mg l^{-1}) plus citric acid (150 mg l^{-1}). Explants were disinfected by immersion in 0.1% mercuric chloride ($HgCl_2$) for 5 min and rinsed three times with sterile distilled water. For complete sterilization explants were dipped in 50% Clorox (NaOCl) for 15 min and again washed with sterile distilled water, 5 min for each wash. The explants were then placed on MS (Murashige and Skoog, 1962) media supplemented with μM (0, 1, 2, 4 and 8) of GA3, Kn, BAP and 2iP. Medium in all the treatments contained 0.2 g l^{-1} polyvinylpyrrolidone (PVP) to prevent browning by phenolic exudates. The pH of the medium was adjusted to 5.8 prior to autoclaving at 121 °C for 20 min. For solidification gelrite of Sigma Chemical Co @ 2g l^{-1} was used. Culture conditions were 23-25 °C, 16 hr photoperiod at 48 $\mu mols^{-1}m^{-2}$. Visual observations were taken after every week and subculture period was maintained after every 20-25 days. After 3 subcultures in the same media the data were collected for shoot height, extent of callus formation and incidence of hyperhydration.

Results and Discussion

Among different growth regulators tested for shoot growth, GA_3 affected the shoot height greatly (Fig. 1a, b). GA_3 @ 4 μM gave the maximum shoot height (32mm) (Fig. 1c). On the other hand BAP was least effective in enhancing the shoot height. Shoot height was reduced with an increase in the concentration levels of different cytokinins (Kn, BAP and 2iP). Hyperhydration incidence was increased with BAP, with a maximum of 40% at 8 μM. GA_3 and 2iP treated cultures as well as the control did not experience any hyperhydricity symptoms. Increased concentration of BAP also enhanced callusing at the base portion of shoots. Kinetin showed low callusing at higher concentration. No callusing was recorded in the control, 2iP and GA_3 treated cultures throughout the period of study (Table 1). Dismassi-Theriou (1994) reported a shoot height of 10.4 mm in 'Kalamon' olive on woody plant medium (WPM) containing 4.4 μM BA, 4.9 μM IBA (indolebutyric acid) and 0.3 μM GA_3. Zeatin riboside was reported effective in the micropropagation of 'Dolce Agogia' olive and was used @ of 45.6 μM in combination with 0.5 μM IBA and 0.3 μM GA_3 (Rugini and Fontanazza, 1981). In contrast to these results Rugini (1984) reported that zeatin was the most effective cytokinin for shoot proliferation. These findings are in exact corroboration with Shibli *et al.* (2000) who reported a height of 48 mm in 'Nabali' olive on MS medium containing 5 μM GA_3. Shoot proliferation was also reported to be genotype (cultivar) dependent (Rama and Pontikis,

Corresponding Author: Mr. Muhammad Ramzan Khan, Agriculture Biotechnology Institute, National Agriculture Research Centre, Islamabad, Pakistan

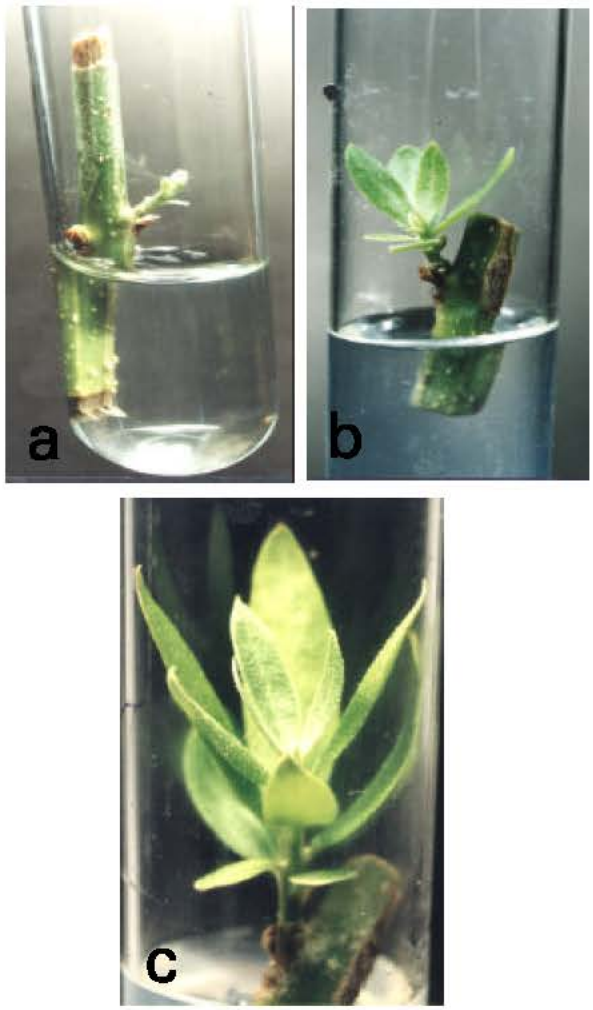

Fig. 1: *In vitro* shoot development of olive cv. Leccino from cuttings obtained from field-grown plants. a) Single-node cuttings from juvenile material cultured on MS + 4 μM l^{-1} GA_3 after 17 days. b) Shoot of olive cv. Leccino developed 40 days after subculture. c) Shoot of olive cv. Leccino 70 days after subculture

1990; Rugini and Fontanazza, 1981). BA at 5.0 to 7.5 μM was reported to give 1.2 to 1.8 shoots per explant, respectively and did not affect shoot height in 'Kalamon' olive (Rama and Pontikis, 1990).

A higher concentration of Kinetin, BAP and 2iP induced more callusing and hyperhydrated microshoots. Hyperhydricity was reported to show high correlation with and increased concentration of cytokinins used in the medium (Shibli *et al.*, 1997). Though cytokinins in high concentration can stimulate shoot development, they can also have negative effects such as toxicity, reduction of leaf size, internode length and occasionally in rooting (Grattapaglia and Machado, 1990). Garcia Berenguer (1991) also observed negative effects of high cytokinin concentration (up to 1.2 mg l^{-1}) in the culture medium of olive cv. Picual. The formation of basal calli is probably due to cytokinin accumulation in shoots.

Culture contamination in the explants was between 50-60% and could not be reduced by the disinfection procedures used. PVP @ 0.2 g l^{-1} was found to be successful to eliminate the phenolic exudates. These findings are in consistent with Otero and

Table 1: Influence of different growth regulators on shoot height, incidence of hyperhydration and callusing in olive (*Olea europaea* L.) cv. Leccino

Cytokinin con (μM)	Shoot height (mm)	Hyperhydration (%)	Callusing
Control	14	0.0	-
GA_3			
1	30	0.0	-
2	32	0.0	-
4	29	0.0	-
8	29	0.0	-
Kinetin			
1	24	3.0	-
2	18	10.0	-
4	15	14.0	+
8	13	14.0	++
BAP			
1	26	0.0	-
2	17	6.0	-
4	14	14.0	+
8	14	20.0	+++
2iP			
1	27	0.0	-
2	28	0.0	-
4	25	0.0	-
8	25	2.0	-

Data represent mean of 10 replicates/treatment in two repeated experiments

Docampo (1998), Rugini (1986) and Canas and Benbadis (1988), where explants from adult cuttings suffered from rapid oxidation, despite preventive treatments. Tannin exudation (which causes blackening, explant necrosis and low incidence of sprouting) was stopped at all stages of micropropagation (Rugini, 1984). The use of ascorbic acid and citric acid at the end of sterilization does not improve the explant survival (Martino *et al.*, 1999; Khan *et al.*, 2002).

Based on these results, it is concluded that the single-node cuttings from the 8th bud pair of shoots were suitable for the *In vitro* culture of olive cv. Leccino. GA_3 affected the shoot height greatly. This cultivar is also suitable for cryopreservation, which makes it possible to conserve this valuable olive genetic resource for future.

Acknowledgments

Pakistan Oilseed Development Board (PODB) is funding this project. Sincere appreciation and gratitude to Dr. Hafeez-ur-Rehman SSO, HRI and M. Riaz SA, Agricultural Biotechnology Institute (ABI) NARC, Islamabad, for valuable comments and suggestions which helped to improve the quality of this work.

References

Batrolini, G., A.R. Leva and A. Benelli, 1990. Advances in *In vitro* cultures of olive: Propagation of cv. Maurino. Acta. Hort., 286: 41-44.

Canas, L.A., L. Carramolino and M. Vicente, 1987a. Vegetative propagation of the olive tree from *In vitro* cultured embryos. Pl. Sci., 50: 85-90.

Canas. L.A., A.M. Wyssman and M.C. Benbadis, 1987b. Isolation, culture and division of olive (*Olea europaea* L.) protoplasts. Pl. Cell Rep., 6: 369-371.

Canas. L.A. and M.C. Benbadis, 1988. *In vitro* regeneration from cotyledon fragments of the olive tree (*Olea europaea* L.) Pl. Sci., 54: 65-74.

Cozza, R., D. Turco, C.B. Bati and M.B. Bitoni, 1997. Influence of growth medium on mineral composition and leaf histology in micropropagated plantlets. Plant Cell, Tiss. Org. Cult., 51: 215-223.

Dimassi-Theriou, K., 1994. *In vitro* propagation of 'Kalamon' olives (*Olea europaea sativus* L.). Adv. Hort. Sci., 8: 185-189.

Garcia Beregure, A., 1991. Tesis Doctoral. Univ. Cordoba, Depto. Agronomia, Espana, pp: 195.

Grattapaglia, D. and M. Machado, 1990. In: Tecnicas e aplicacoes da culture de tecidos de plantas. (eds) Torres, A.Y. Caldas L., pp: 99.

Khan, M.R., H. Rashid and A. Quraishi, 2002. Development of aseptic protocols in field-grown olive (*Olea europaea* L.) cv. Pendollino. Asian J. Pl. Sci., 3: 220-221.

Lavee, S., 1990. Aims, methods and advances in breeding of new olive (*Olea europaea* L.) cultivars. Acta Hort., 286: 23-36.

Martino, L., A. Cuozzo and L. Brunori, 1999. Establishment of meristem tip culture from field-grown olive (*Olea europaea* L.) cv. Moraiolo. Agric. Med., 129: 193-198.

Mencuccini, M., C. Corona and D. Mariotti, 1991. Plant regeneration and first attempt of *In vitro* genetic improvement of olive (cv. Moraiolo). Acta Hort., 300: 261-264.

Mencuccini, M. and E. Rugini, 1993. *In vitro* regeneration from olive cultivar tissues. Plant Cell, Tiss. Org. Cult., 32: 283-288.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol. Pl., 15: 472-493.

Otero, M.L. and D.M. Docampo, 1998. Micropropagation of olive (*Olea europaea* L.) cv. Arbequina from juvinile cuttings. ϕYTON. International J. Exp. Bot., 63: 133-140.

Rama, P. and C.A. Pontikis, 1990. *In vitro* propagation of olive (*Olea europaea sativa* L.) Kalamon. J. Hort. Sci., 65: 347-353.

Rugini, E. and C. Fontanazza, 1981. *In vitro* propagation of 'Dolce agogia' olive. Hort. Sci., 16: 482-493.

Rugini, E., 1984. *In vitro* propagation of some olive (*Olea europaea* L.) cultivars with different root-ability and medium development using analytical data from developing shoots and Embryos. Sci. Hort., 24: 124-134.

Rugini, E., 1986. Olive (*Olea europaea* L.). In: Bajaj, Y.P.S. (eds.), Biotechnology in agriculture and forestry, Trees I. Springer-Verlag, Heidelberg, Berlin, pp: 253-267.

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

Rugini, E. and E. Fedeli, 1990. Olive (*Olea europaea* L). as oilseed crop, pp 593-641. *In*: Bajaj Y.P.S. (ed.) Biotechnology in Agriculture and Forestry. Legumes and oilseed. 10 Crop I. Springer-Verlag, Berlin, Heidelberg, New York.

Rugini, E. and S. Lavee, 1992. Olive, pp. 371-382. *In*: Hammer-Schlag, F.A. and R.E. Litz (eds.). Biotechnology of perennial fruit crops. CAB International, Wallingford, Oxon, UK.

Rugini, E. and G. Caricato, 1995. Somatic embryogenesis and plant recovery from mature tissues of olive cultivars (*Olea europaea* L.) 'Canino' and 'Moraiolo'. Pl. Cell Rep., 14: 257-260.

Shibli, R.A., M.A.L. Smith and R. Nast, 1997. Iron source and cytokinin mitigate the incidence of chlorosis and hyperhydration *In vitro*. J. Pl. Nutr., 20: 773-781.

Shibli, R.A., A.E. Abdullah and H.A.J. Karim, 2000. Influence of different growth regulators on *in vitro* multiplication and rooting of 'Nabali' olive (*Olea europaea* L.). Plant Tissue Cult., 10: 93-102.

Troncoso, A., J.M. Linan, M.M. Cantos Acebedo and H.F. Rapoport, 1999. Feasibility and anatomical development of an *In vitro* olive cleft-graft. J. Hort. Sci. Biotech., 74: 584-587.