International Journal of Basic Medical Sciences and Pharmacy (IJBMSP)
Vol. 6, No. 1, June 2016, ISSN: 2049-4963
© IJBMSP
www.ijbmsp.org

Comparison of Different Parameters for the *In Vitro* Propagation of Various Cultivors of Olive (*Olea Europaea L.*)

Naveed Ahmad[1], Numan Khan[1], Fahad Ali1[1,2]*, Khalil Ur Rahman[1,2], Sulaiman Bahadar[3], Muhammad Ismail Khan[2], Sami Ullah Jan[1], Raheem Bahadar[5], Ziafat Rehman[3], Rabia Zardad[3], Muhammad Hubab[3], Saira Zahoor[4], Azam Hayat[3], Muhammad Anwar[6]

[1]Department of Biotechnology and Genetic Engineering, Kohat University of Science and Technology, Kohat, Pakistan.

[2]Department of Genetics, Hazara University, Mansehra, Pakistan.

[3]Department of Microbiology, Abbottabad University of Science and Technology, Havialian Abbottabad, Pakistan.

[4]Department of Biochemistary, Hazara university mansehra, pakistan.

[5]Department of Pharmacy, COMSATS Institute of information Technology, Abbottabad, Pakistan

[6]Department of Zoology, Kohat University of Science and Technology, Kohat, Pakistan

Email: fahadali.biotechnologiest@gmail.com

Abstract – Olive (*Olea europaea L.)* is most important fruit species and usually propagated by shoot and leaf cutting under the mist. By traditional methods the propagation of some fruit species is difficult to multiply and now make possible by micropropagation, regarding the olive micropropagation. In our study *Uslu, Coratina, Nocellara, Pendolino, Leccino* veriaty of Olive plants were taken sample were collected from its shoot. Then MS Media were used for inoculation along with growth regulators like BAP, NAA, GA3, coconut water. Then treat with Mancozeb + Metalaxyl and chlorox containing NaOCl to control the contamination. Then inoculate and the growth condition were apply. For fungal contamination, among all concentrations treatment with 7.5g/250mL Mancozeb for 1 hour prevented fungal contamination up to 90 %. For chlorox treatment, among all concentrations 20% chlorox treatment for 5 minutes was found to be the most effective to minimize browning and maximize sterilization. The growth regulator BAP, NAA and coconut water not show proliferation. And GA3 regenerate the plant growth. In light the plant produce more shoots than the dark one. Auxiliary buds have high regeneration capacity than the apical buds. Juivinail is batter then old explant in shoot sprouting.

Keywords – *Olive Plant, In vitro, Propagation, Parameters, Cultivators.*

1. Introduction

Olive micropropagation is known to be started in mid 1970s, and much work was done by tissue culturists to optimize the cultural media formulations. They tried to develop a protocol enough for all the cultivars. They modified the MS medium [1]. In Many countries like Italy and Spain olive is very important because it is a source of many economically important products and besides that it has a remarkable history. [2, 3]. In recent times, efforts have been directed at producing hybrid cultivars with qualities such as resistance to disease, quick growth and larger or more consistent crops. Bari Zaitoon 1 and 2 are recently approved cultivars grown in Pakistan. Its cultivation has been extended throughout the world. Currently, the area of olive cultivation is approximately 9.7 million hactres. With the European Union responsible for 75% of olive oil production, and Spain and Italy are the most important producer countries (4). One of the most important cultivars of olive in northern Greece is Chondrolia Chalkidikis. The positive aspects of olive tree are its long life span (hundreds of years), a prolong juvenile period for most cultivars, a wide biodiversity with the consequent variability in the fruit which influences quality aspects of the olive oil, including aroma and taste [5, 6]. Olive has many different good characteristics like tolerating slight unfavorable environmental conditions like high summer temperatures and drought. It can adapt to different soils like clay and sandy ones. It has long life [7].

Micropropagation has several benefits over traditional techniques; micropropagation of Olive is done to get a large number of desired plants in minimum possible time. Moreover, the plants obtained in this way are free of any disease or pest. It doesn't require a vast field but can be done in a small test tube in lab. High quality of olive tree cultivars can be produced through this technique [8, 9, 10, 11]. Olive has been propagated through tissue culture since 1980s [12, 13, 14, 15]. Tissue culture is a common cloning

technique for rapid micro propagation of many fruit trees in present times. Rugini contributed a lot to improve the propagation of some of the olive cultivars but they cannot be applied to all cultivars [16]. Much advancement in the olive micropropagation has been made in the past decade [9, 17, 18; 19]. But these improvements have been in some of the olive cultivars and not in all. So generally, olive micropropagation has not been very successful [9, 20]. But after the development of auxenic culture that encountered in the successful in vitro micro propagation of olive cultivars from their mature tissues [20, 21].

2. Material and Methods

2.1. Plant Material

The *in vitro* micropropagation performed in the current study was carried out on olive plant material taken from mature, field grown; healthy olive trees (*Olea europaea* L.) cultivated in the orchards of the Horticulture Research Institute, at NARC, Islamabad. Following important cultivars were used as source of explants:

S No	Name of Variety	Explant material used	Origin
1	*Uslu*	Suckers, Apical shoots	Turkey
2	*Coratina*	Apical shoots	Italy
3	*Nocellara*	Apical shoots	Italy
4	*Pendolino*	Suckers, Apical shoots	Italy
5	*Leccino*	Suckers, Apical shoots	Italy

2.2. MS Media used

MS media was used for inoculation of explants. Different growth regulators were tried in the MS like BAP, NAA, GA3, coconut water along with plain MS.

2.3. Explant Collection

The explants were prepared from the branches of mature olive trees in which different size (thick and thin), different types from different cultivars, the nodal explant and apical explant from the shoot, juvenile were collected. And sterilized by using different chemicals.

2.4. Mancozeb Treatment

They were washed in detergent for about 5 minutes. They were agitated in a vessel on an electric stirrer with detergent. The foam was removed under tap water. They were then agitated with a fungicide (Mancozeb + Metalaxyl). Different amount in grams of the fungicide was tested for sterilization starting from 5.5 g up and slightly increasing up to 8 g in 250mL of distilled water on an electric stirrer for 1 hour. After treatment with Mancozeb, they were brought to laminar flow hood but the vessel was first sprayed with 100% ethanol.

2.5. Chlorox Treatment

To control bacterial contamination, chlorox containing NaOCl sodium hypochlorite a bleaching agent was applied. A few drops of surfactant tween20 were added to chlorox solution to decrease the surface tension. Different concentrations of chlorox starting from 50% to 0% were tested. The duration of treatment with chlorox was 5 minutes. After treatment with chlorox, they were washed with autoclaved distilled for water 3-4 times. Before cutting the ends that were browned due to chemical treatment, the explants were sprayed with 100% ethanol to ensure sterilization.

2.6. Sterilization of Equipments and Explants inoculation

The most serious problem with micropropagation of plants is microbial contamination mainly fungal or bacterial. All the equipments (forceps, Petri plates etc), the media and the explants should be sterilized. The inoculation was done near the flame to minimize contamination.

2.7. Growth conditions

The cultures were placed in the growth rooms at 16 hours photoperiod, 30 ˚C temperatures, some under cool white fluorescent light and some under dark conditions. They were observed daily, the contaminated cultures were disposed and readings taken in a note book, (the number of contaminated, browned, regenerated, having callus and showing no response).

3. Results and Discussion

3.1. Microbial Contamination

The first problem with cultures was of contamination mainly fungal contamination. Among different concentrations, treatment with 7.5g/250mL Mancozeb for 1 hour prevented fungal contamination up to 90 %. So this concentration was retained in the rest of experiments.

Table 1: Effect of different concentrations of fungicide (Mancozeb) on micro-cuttings
Treatment duration= 1 hour

Test No	Fungicide (g)/250 mL	Plants Material	Contamination Rate (%)	Browning rate (%)	Survival rate (%)
T1	5.5	Apical shoots/ Suckers	70	10	20
T2	6	Apical shoots/ suckers	60	20	20
T3	6.5	Apical shoots/ suckers	50	15	35
T4	7	Apical shoots/ suckers	30	10	60
T5	7.5	Apical shoots/ suckers	10	0	90
T6	8	Apical shoots/ suckers	10	10	80

Table 2: Effect of Different Concentrations of NaOCl
Treatment Duration = 5 min

Test #	NaOCl (%)	Plant Material	Contamination rate (%)	Browning rate (%)	4. Surviva l rate (%)
T1	50%	Apical shoots/ suckers	85.0	13.0	5. 2.0
T2	40%	Apical shoots/ suckers	75.0	13.0	12.0
T3	30%	Apical shoots/ suckers	50.0	20.0	30.0
T4	25%	Apical shoots/ suckers	30.0	10.0	60.0
T5	20%	Apical shoots/ suckers	11.0	7.0	82.0

Table 3: Response of Explants

Basal Media	Growth regulators	Number of explants	Age of explant	Response of explants %age					
				Nodal segments			Shoot tips		
				Leccino	Pendolino	Uslu	Leccino	Pendolino	Uslu
MS	NAA+BAP	100	Juvenile	40	30	45	30	20	35
			Old	20	20	40	10	15	20
MS	GA3	100	Juvenile	60	55	70	40	30	50
			Old	40	30	50	35	20	30
MS	2iP	100	Juvenile	40	30	50	30	25	40
			Old	30	15	35	20	10	25
MS	Coconut water	100	Juvenile	40	40	55	35	30	40
			Old	25	30	40	20	15	30
MS	Plain	100	Juvenile	30	25	40	25	20	30
			Old	15	20	30	15	10	25

Treatment with chlorox checked bacterial contamination. Among all concentrations, 20% chlorox treatment for 5 minutes was found to be the most effective to minimize browning and maximize sterilization.

The temperature 30˚C of the growth room temperature from was optimum as regeneration was observed in some cultures for the first time. So this temperature was retained for further cultures.

3.2. Response of Explants

At first the medium used for initiation of cultures that was supplemented by NAA 3.5mg/L + BAP 0.05mg/L did not show any proliferation. Another medium MS supplemented with coconut water 50ml/L did not show any satisfactory results. The plain MS media gave regeneration in about two weeks. But the rate of regeneration was very much less. Media with GA3 0.5mg/L also gave regeneration of explants.

3.3 Light VS Dark

Shoot sprouting appeared only in the cultures kept in light. Although, calli did appear in the cultures kept in dark but no shoots. It was proved that light promotes more shoots as compare to dark that did not induce any shoot at all.

Figure 1: Cultures kept in dark showed no response

Figure 2: Shoot sprouted in cultures kept in lght

3.4. Apical VS Nodal segments

Shoot sprouting was mostly observed in the auxiliary buds of nodal segments. Apical buds showed less regeneration. This confirmed that the auxiliary buds have good capacity to regenerate in microshoots.

Figure 3: Apical segments with no response

Figure 4: Nodal segments with sprouted microshoots

3.5. Juvenile VS Old explants

In case of these two types, the juvenile explants were better in shoot sprouting. Although regeneration did appear in other explants type but to a very less extent. This showed that juvenile segments had greater capacity of shoot sprouting in vitro.

Figure 1 Shoot Sprouting in juvenile Explant

Figure 2 Shoot Sprouting in old Explant

4. Conclusion

Working for 90 days with the in vitro micropropagation of olive and reading literature about it made a few things clearer. Comparing a few parameters about olive micropropagation like type and age of explants, treatment conditions like light and dark, temperature and sterilization of explants, it became obvious that between nodal and apical explants, the former showed good response in shoot sprouting. And among juvenile and old shoots, juvenile were better. The best results of shoot sprouting obtained at 30˚C temperature. Shoots sprouted in cultures kept in light and did not in dark conditions. 7.5 gm Mancozeb per 250mL water for 1 hour and 20% chlorox for 5 minutes were the best among different treatments for sterilization and survival of explants. And finally MS medium with 0.05mg/L GA3 and plain MS medium were gave better results as compare to MS medium with 1.5mg/L NAA and 3.5mg/L BAP or MS medium + coconut water.

References

[1] T. Murashige and F. Skoog , “A Revised Medium for Rapid Growth and Bioassays with Tobacco Tissue Culture”, Physiol. Plant., 15: 473–497, 1962.

[2] R. Ciferri, “Data and Hypotheses on the Origin and Evolution of the Olive”, Olearia., (3-4): 3-10, 1950.

[3] G. Bartolini and R. Petruccelli. “Classification, Origin, Diffusion and History of the Olive. ed., Rome: FAO, 2002.

[4] FAOSTAT (2009) http://faostat.fao.org/.

[5] G. Roselli, P. Mariotti, and A. Tessa, “Characterization of progeny of olive by chemical analysis and sensory oils”, Proocedings of an National Symposium: Germplasm and typical olive oil held at Perugia , 278-283, 2003.

[6] L. Annarita, “Morphological Evaluation of Olive Plants Propagated In vitro Culture through Axillary buds and Somatic Embryogenesis methods”, Afr J. Plant Sci., 3 , 037–043, 2009.

[7] E. Rugini, R. Biasi, and R. Muleo, “Olive (Olea europea var. sativa) Transformation In: Jain, S.M., Minocha S.C. ed., Molecular Biology of Woody Plants. 2. Dordrecht: Kluwer Academic Publishers, 245-279, 2000.

[8] B.C. Briccoli and N. Lombardo, “Propagation in vitro of cv Nocellara etnea. Proceedings conference. “The olive mediterranean state and prospective of the culture and research”, Italy: Consenza, 249-256, 1995.

[9] J.L. Garcia, J. Troncoso, R. Sarmiento, and A. Troncoso, “Influence of Carbon Source and Concentration on the In vitro Development of Olive”, Plant cell tiss Org.,69 : 95-100, 2002.

[10] G. Zuccherelli, and S. Zuccherelli, “In Vitro Propagation of 50 Olive Cultivars”, Acta Hort., 586: 931–934, 2002.

[11] A. Chaari, M. Maalej, N. Drira, and A. Standardi, “Micropropagation of Olive Tree Oleaeuropaea L. ‘Oueslati’. Turk J Agric For.35: 403-412, 2011.

[12] E. Rugini, “In vitropropagation of some (OleaeuropaeaL.) Cultivars with different Root ability and Medium Development using Analytical data from Developing shoots and Embryos”, Sci Hort., 24: 123-134, 1984.

[13] P. Fiorino, and A.R. Leva, “Investigations on the micropropagation of the olive (Olea europaea L.) and influence of some mineral elements on the proliferation and rooting of explants”, Olea., 17:101-104, 1986.

[14] A.R. Leva, R. Petruccelli, R. Muleo, R. Goretti, and G. Bartolini, “Influence of trophic factors, and regulatory conditions in vitro culture of different olive cultivars. Proceeding of a National Symposium “The Olive Mediterranean: Status and Perspectives of Culture and Research” held at Consenza. 239-248, 1995.

[15] K. Grigoriadou, M. Vasilakakis, and P.E. Eleftheriou, “In Vitro Propagation of the Greek Olive Cultivar ‘Chondrolia Chalkidikis’”, Plant Cell Tiss Org., 71: 47–54, 2002.

[16] E. Rugini, P.P. Gutierrez, and P.L. Sampinato, “New perspective for Biotechnologies in Olive Breeding: Morphogenesis, in vitro selection and gene Transformation”, Acta Hortic., 474: 107–110, 1999.

[17] A.R. Leva, R. Petrucceclli, and L. Polsinelli, “In vitro Propagation from the Laboratory to the Production Line”, Olivae., 101: 18-26, 2004.

[18] A. Zacchini, and M.D. Agazio “Micropropagation of a Local Olive Cultivar for Germplasm Preservation”, Biol Plantarum., 48 (4), 589-592, 2004.

[19] D.G.E. Mendoza, F.R. Mira, Ruiu. E. Rugini, “Stimulation of Node and Lateral Shoot Formation in Micropropagation of Olive (Oleaeuropaea L.) by using dikegulac”, Plant cell tiss Org., 92: 233-238, 2008.

[20] M. Lambardi, and E. Rugini, “Micro Propagation of Olive (Olea europaea L.). In Bienstock D.M, Ishii K., Micropropagation of Woody Trees and Fruits. Netherland: Kluwer Academic Publisher, 621-646, 2003.

[21] M. Peyvandi, F. Farahani, Z. Noormohamadi, O. Banihashemi, M. Hosseini, and S. Ataee, “Mass Production of Oleaeuropeal. (cv. Rowghani) through Micropropagation”, Gen and Applied Plant Physiol.,35 (1–2):35–43, 2009.