

## EFFECT OF DIFFERENT CONCENTRATIONS OF AUXINS ON IN VITRO ROOTING OF OLIVE CULTIVAR 'MORAILO'

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### Abstract

The inability to induce adventitious roots is often a limiting factor in conventional cuttings and tissue culture. In this study, several criteria were taken into consideration in determining the best rooting treatment in olive cultivar Moraiolo. Among the indolebutyric acid (IBA) and Naphthaleneacetic acid (NAA) hormones, tested for maximum percentage of rooted shoots, root number, root length and quality of roots, IBA at 1.5 mg l<sup>-1</sup> concentration proved to be the best one for rooting of Moraiolo cultivar of olive producing maximum root initiation in 86.67% shoots, 5.03 number of roots per rooted explant and 4.95 cm root length. The roots produced on IBA were longer with better quality shoots whereas NAA produced poor response with necrotic leaves and leaf abscission.

### Introduction

The application of tissue culture on mass clonal propagation of plants is the widest utilization of this technology (Thorpe, 2006). Micropropagation of plants has largely been through proliferation of axillary shoots derived from shoot or meristem tips followed by rooting of elongated shoots generally on a low salt medium with auxin, although rooting can often occur on hormone free or charcoal containing medium. Increase in volume associated with callus and adventitious root growth must be the result of either cell division or cell enlargement, or both. Auxin, known to be involved in cell enlargement, was long thought to be the controlling factor in the rooting process. Two types of evidence support this reasoning: (a) the increased content of endogenous auxin in the base of cutting during rooting induction (Blachova, 1969) and (b) the rooting response of many plants to exogenous auxin. Success in micropropagation is thus mostly dependant on the production of good quality adventitious roots, whose formation has three distinct phases viz., induction, initiation and expression (Kevers *et al.*, 1997; De Klerk *et al.*, 1999). Generally, micropropagation of woody plants has lagged parallel application to herbaceous species, because of more recalcitrant problems in culture (McCown, 2000). Nevertheless, microculture has been successfully applied to a wide range of woody plants. For rooting of olive, various auxins have been used, in particular indolebutyric acid, Naphthaleneacetic acid and indoleacetic acid (Cozza *et al.*, 1997; Tanimoto, 2005; Jimenez, 2005), though the type and concentration requirement of each of these varies with different varieties (Soumendra *et al.*, 2000; Grigoriadou *et al.*, 2002; Saida *et al.*, 2005). The present study was carried out to find the best concentration and type of auxin for rooting of Moraiolo cultivar of olive in order to establish the best protocol for rooting of this cultivar.

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## Materials and Methods

Microcuttings of Olive (*Olea europaea* L.) cv. 'Moraiolo' from stock cultures of Plant Tissue Culture Lab of PMAS Arid Agriculture University, Rawalpindi maintained on olive medium (OM; Micheli *et al.*, 1998) supplemented with zeatin 4 mg l<sup>-1</sup>, sucrose 30 g l<sup>-1</sup>, agar 6.5 g l<sup>-1</sup> and subcultured every 40 days were used as experimental material (Table 1). For this experiment, proliferated uniform shoots (more than 1.5 cm) from sub-apical part up to 4<sup>th</sup> node bearing two leaves at the top portion were excised and transferred individually to each culture jar containing rooting medium consisting of OM medium (OM half macro & micro elements) supplemented with sucrose 15 g l<sup>-1</sup> and agar 6.5 g l<sup>-1</sup>. Varying levels of IBA and NAA compared were (a) No auxin (control), (b) IBA (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg l<sup>-1</sup>) and (c) NAA (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg l<sup>-1</sup>). The pH of rooting medium was adjusted to 5.8 before autoclaving. Plants were incubated at 25 ± 1°C under 16 h light (2,000 lux) with white fluorescent tubes (Philips TL 40W/54). The experiment was CRD (Completely Randomized Design) consisting of three replications per treatment and five shoots per replication. Data was recorded on the number of rooted shoots, the number of roots per shoot and root length after four weeks. Statistical analysis of the data was carried out by using analysis of variance (ANOVA) and differences among treatment means were compared by using Least Significance Difference (LSD) Test at 5% probability level.

## Results and Discussion

**Rooting % age:** Both the concentration and type of auxins used markedly influenced percentage of root formation (number of rooted shoots). IBA produced rooting in maximum 86.67% shoots at T<sub>4</sub> (1.5 mg l<sup>-1</sup>) whereas NAA produced rooting in only 40 % shoots (T<sub>5</sub>) when used at concentration of 1.0 mg l<sup>-1</sup> (Table 2). The results showed that auxin is essential to induce rooting in the olive microcuttings as no rooting was observed in the absence of auxin. Bellamine *et al.* (1998) reported that auxin exerts primary role in root formation by its involvement in successive and interdependent phases. Application of IBA at 1.5 mg l<sup>-1</sup> may have triggered the early anticlinal cell division and root primordia formation than NAA. Sabatini *et al.*, (1999) reported that differentiation of phloem ray parenchyma cells into root primordia depends upon the type and concentration of auxin. Further, it has been reported by Blakesley & Chaldecott (1997) that differentiating cells require the most appropriate auxin to become competent to respond to the organogenic signal. Barcelo *et al.*, (1999) and Wynne & McDonald (2002) reported that IBA enhanced the cell division of first root initials in Avocado. It is also clear from the results that rooting percentage showed the tendency to increase with increasing concentration of IBA, however, the higher concentration (>1.5 mg l<sup>-1</sup>) than the optimal exerted its inhibitory effect on the number of rooted shoots. The results also indicate that with NAA the adverse effects occur at relatively low concentration as compared to IBA. Table 2 reveals that if the concentration of NAA was raised from 0.5 mg l<sup>-1</sup> to 1.0 mg l<sup>-1</sup>, there was only slight increase in the number of rooted shoots, whereas the concentration higher than 1.0 mg l<sup>-1</sup> resulted in decrease in rooting percentage. Sugiyama (1999) reported that the effect of an auxin on rooting is promotory at low concentrations and inhibitory at supra-optimal concentrations.

**Table 1. Composition of olive medium (OM).**

Ingredient	Olive medium (OM) mg l <sup>-1</sup>
KNO <sub>3</sub>	1772.00
NH <sub>4</sub> NO <sub>3</sub>	412.50
Ca(NO <sub>3</sub> ) <sub>2</sub> . 4H <sub>2</sub> O	1300.00
MgSO <sub>4</sub> . 7H <sub>2</sub> O	731.00
KH <sub>2</sub> PO <sub>4</sub>	340.00
FeSO <sub>4</sub> . 7H <sub>2</sub> O	27.80
Na <sub>2</sub> EDTA	37.50
MnSO <sub>4</sub> . 4H <sub>2</sub> O	16.90
H <sub>3</sub> BO <sub>3</sub>	12.40
ZnSO <sub>4</sub> . 7H <sub>2</sub> O	14.30
Na <sub>2</sub> MoO <sub>4</sub> . 2H <sub>2</sub> O	0.25
CuSO <sub>4</sub> . 5H <sub>2</sub> O	0.25
CoCl <sub>2</sub> . 6H <sub>2</sub> O	0.02
KI	0.83
Myo-inositol	100.00
Glycine	2.00
Thiamine. HCl	0.50
Pyridoxin. HCl	0.50
Nicotinic acid	5.00
Biotin	0.05
Folic acid	0.50
Glutamine	1178.00

**Table 2. Effect of growth regulators (IBA & NAA) on *In vitro* rooting of olive cv. 'Moraiolo'.**

Treatments	Rooting % age	No. of roots per shoot	Root length (cm)
<b>IBA (mg l<sup>-1</sup>)</b>			
T <sub>1</sub> 0.0	0.00 g	0.00 k	0.00 l
T <sub>2</sub> 0.5	26.67 def	1.00 h	1.95 f
T <sub>3</sub> 1.0	46.67 bc	3.20 b	3.50 b
T <sub>4</sub> 1.5	86.67 a	5.03 a	4.95 a
T <sub>5</sub> 2.0	53.33 b	3.13 b	3.30 d
T <sub>6</sub> 2.5	40.00 bcd	2.07 d	1.86 g
T <sub>7</sub> 3.0	20.00 ef	1.15 g	0.85 i
<b>NAA (mg l<sup>-1</sup>)</b>			
T <sub>8</sub> 0.5	20.00 ef	2.30 c	2.35 e
T <sub>9</sub> 1.0	40.00 bcd	3.18 b	3.35 c
T <sub>10</sub> 1.5	33.33 cde	1.90 e	1.95 f
T <sub>11</sub> 2.0	20.00 ef	1.34 f	1.26 h
T <sub>12</sub> 2.5	13.33 fg	0.66 i	0.47 j
T <sub>13</sub> 3.0	13.33 fg	0.35 j	0.20 k
LSD <sub>5%</sub>	14.22	0.075	0.017

Means followed by the same letter are not significantly different at *p*<0.05.

**Number of roots per rooted explant:** A synergism was found between the rooting percentage and number of roots per rooted explant (Table 2). The results show that IBA produced maximum number of roots (5.03) per rooted explant (Fig. 1) at T<sub>4</sub> (1.5 mg l<sup>-1</sup>) while the maximum number of roots per explant produced by NAA was only 3.18 at 1.0 mg l<sup>-1</sup> concentration (Fig. 2). Benelli *et al.*, (2001) and Tanimoto (2005) have proved that IBA is the most effective auxin in olive rhizogenesis as compared to NAA. The reason for these differences in root inducing ability may be the slow and continuous release of IAA from IBA (Krieken *et al.*, 1993; Liu *et al.*, 1998) and release of IBA through hydrolysis of conjugates (Epstein & Muller, 1993). These IBA conjugates were reported to be superior to free IBA in serving as an auxin source during later stages of rooting (Staswick *et al.*, 2005). Muller (2000) found the IAA levels 20-30 times higher in IBA treated explants than in explants cultured without IBA. Garcia *et al.* (1994) also reported that in avocado microcuttings, IBA increased not only the endogenous IAA but also indole-3-acetyl-aspartic acid (IAAsp) that are required for normal growth of root meristemoids as the root formation proceeded. The inferior effect of NAA on the root number may be due to the reason that NAA is more persistent than IBA, remains present in the tissue and may block further development of root meristemoids (De Klerk *et al.*, 1997; Nanda *et al.*, 2004). Hausman (2003) has shown that in tissue culture media, IAA is photo-oxidized rapidly (50 % in 24 h) while IBA oxidized slowly (10 %) and NAA is very stable. Higher than optimal concentration of both the auxins led to a decrease in the number of roots per rooted explant. Baker & Wetzstein (2004) have reported that higher concentration of auxin induces the higher level of degradative metabolites in tissues thus blocking the regeneration process.

**Root length (cm):** The results showed that the trend set forth by the previous two characters has been maintained here (Table 2). IBA produced the longest roots (4.95 cm) at T<sub>4</sub> (1.5 mg l<sup>-1</sup> IBA). The maximum root length produced by NAA was 3.35 at T<sub>9</sub> (1.0 mg l<sup>-1</sup> NAA). These results indicate a positive correlation between the rooting percentage, number of roots per rooted explant and root length. Like other developmental processes, cell elongation involves sequential changes in levels and/or activity of enzymes (Cosgrove, 1999). The enzymes involved in cell enlargement processes are triggered by the auxin. Wada *et al.*, (1998) reported that IBA promotes root length by influencing the synthesis of enzymes concerned in cell enlargement. Muller (2000) reported that the superior effects of IBA on root elongation as compared to NAA might be due to the several factors, such as its preferential uptake, transport, metabolization and subsequent gene activation. It was observed that type and concentration of auxin strongly influenced the quality of the shoot and the root system at the end of the rooting period. Roots developed with NAA were shorter in length. Moreover, necrotic leaves and leaf abscission were noticed on NAA (Fig. 3). The intensity of these side effects was proportional to the NAA concentration whereas IBA produced longer roots with better quality shoots (Fig. 4). The results also revealed that root length tend to reduce with higher than optimum concentration of IBA and NAA. Kollmeier *et al.*, (2000) reported that root elongation phase is very sensitive to auxin concentration and it is inhibited by high concentration of auxin in the rooting medium. It is possible that supra-optimal concentration of auxins inhibit root elongation through enhancement of ethylene biosynthesis. Taiz & Zeiger (2002) reported that roots may require a less concentration of auxin to grow, but root growth is strongly inhibited by its higher level because at this level, auxin induces the production of ethylene, a root growth inhibitor.



Fig. 1. Profused rooting with good quality shoots produced by IBA.



Fig. 2. Fewer number of roots with low quality shoots produced by NAA.



Fig. 3. Rooted shoots bearing necrotic leaves at the end of rooting period at NAA.



Fig. 4. Better quality roots with healthy shoots on IBA.



Fig. 5. Successful acclimatization of rooted shoots.

**Acclimatization:** The *In vitro* rooted plants with good quality shoot were successfully acclimatized (Fig. 5). Acclimatization was achieved by direct transfer of plants to pots containing mixture of soil, sand (1:1) and placed in glasshouse having light intensity of 4000-10000 lux, 90-95% humidity with temperature ranging from 26-28°C, a simple procedure already proposed by Roussos & Pontikis (2002) that highly simplified the acclimatization process.

### Conclusion

IBA proved to be better rooting hormone for Moraiolo cultivar of olive in terms of rooting percentage, number of roots per rooted explant and root length as compared to NAA. Naphthaleneacetic acid proves to be less effective for rooting of this cultivar of olive due to being more stable in nature as compared to Indolebutyric acid.

## References

Blachova, M. 1969. Changes in the level of endogenous gibberellins and auxin proceeding the formation of adventitious roots of isolated epicotils of pea plant. *Flora*, 160: 493-499.

Barcelo, A.M., C.L. Encina and E.S. Perez. 1999. Micropropagation of adult olive. *Pl. Cell Tissue Organ Cult.*, 36(3): 321-326.

Baker, C.M. and H.Y. Wetzstein. 2004. Influence of auxin type and concentration on peanut somatic embryogenesis. *Pl. Cell Tissue Organ Cult.*, 36(3): 361-368.

Bellamine, J., C. Penel, H. Greppin and T. Gaspar. 1998. Confirmation of the role of auxin and calcium in the late phases of adventitious root formation. *Pl. Growth Reg.*, 26(3): 191-194.

Benelli, C., A. Fabbri, S. Grassi, M. Lambardi and E. Rugini. 2001. Histology of somatic embryogenesis in mature tissues of olive (*Olea europaea* L.). *J. Pl. Biotech.*, 76(1): 112-119.

Blakesley, D. and M.A. Chaldecott. 1997. The role of endogenous auxin in root initiation. *Pl. Growth Reg.*, 13(1): 77-84.

Cosgrove, D.J. 1999. Enzymes and other agents that enhance cell wall extensibility. *Crit. Rev. Pl. Physiol. Pl. Mol. Biol.*, 50: 391-417.

Cozza, R., D. Turco, C. Briccoli-Bati and B. Bitonti. 1997. Influence of growth medium on mineral composition and leaf histology in micropropagated plantlets of *Olea europaea*. *Pl. Cell Tissue Organ Cult.*, 51: 215-223.

De Klerk, G.J., J.T. Brugge and S. Marinova. 1997. Effectiveness of indoleacetic acid, indolebutyric acid and naphthaleneacetic acid during adventitious root formation *In vitro* in Malus 'Jork 9'. *Scient. Hort.*, 31: 115-119.

De Klerk, G.J., W.M. Van Der Krieken and J.C. de Jong. 1999. The formation of adventitious roots: new concepts, new possibilities. *In vitro Cell. Dev. Biol. Plant*, 35: 189-199.

Epstein, E. and J.L. Muller. 1993. Indole-3-butyric acid in plants: Occurrence, synthesis, metabolism and transport. *Physiologia Plantarum*, 88(2): 382-389.

Garcia, M.L.G., C.S. Romero, A.B. Munoz, A. Heredia and F.P. Alfaro. 1994. Levels of endogenous indole-3-acetic acid and indole-3-butyric acid during adventitious rooting in Avocado microcuttings. *J. Exp. Bot.*, 45(6): 865-870.

Grigoriadou, K., M. Vasilakakis and E.P. Eleftheriou. 2002. *In vitro* propagation of the Greek olive cultivar Chondrolia Chalkidikis. *Pl. Cell Tissue Organ Cult.*, 71(1): 47-54.

Hausman, J.F. 2003. Changes in peroxidase activity, auxin level and ethylene production during root formation by poplar shoots raised *In vitro*. *Pl. Growth Reg.*, 13(3): 263-268.

Jimenez, V.M. 2005. Involvement of plant hormones and plant growth regulators on *In vitro* somatic embryogenesis. *Pl. Growth Reg.*, 47(2-3): 91-110.

Kevers, C., J.F. Hausman, O. Faivre-Rampant, D. Evers and T. Gaspar. 1997. Harmonal control of adventitious rooting: progress and questions. *Angew. Bot.*, 71: 71-79.

Kollmeier, M., H.H. Felle and W.J. Horst. 2000. Is basipetal auxin flow involved in inhibition of root elongation. *Pl. Physiol.*, 122: 945-956.

Krieken, W.M., H. Breteler, M.H. Visser and D. Mavridou. 1993. The role of the conversion of IBA into IAA on root regeneration in apple: Introduction of a test system. *Pl. Cell Rep.*, 12(4): 203-206.

Liu, Z.H., W.C. Wang and Y.S. Yen. 1998. Root formation and indole-3-acetic acid. *Bot. Bull. Acad. Sin.*, 39: 113-118.

McCown, B.H. 2000. Woody shrubs and trees. In: *Plant Tissue Culture: Techniques and Experiments*. (Ed.): R.H. Smith, 2<sup>nd</sup> ed. Academic Press, New York. p. 123-134.

Micheli, M., M. Mencuccui and A. Standardi. 1998. Encapsulation of *in vitro* proliferated buds of olive. *Adv. Hort. Sci.*, 12: 163-168.

Muller, J.L. 2000. Indole-3-butyric acid in plant growth and development. *Pl. Growth Reg.*, 32(2-3): 219-230.

Nanda, R.M., P. Das and G.R. Rout. 2004. *In vitro* clonal propagation of *Acacia mangium* and its evaluation of genetic stability through RAPD marker. *Ann. For. Sci.*, 61: 381-386.

Roussos, P.A. and C.A. Pontikis. 2002. *In vitro* propagation of olive (*Olea Europaea* L.) cv. Koroneiki. *Pl. Growth Reg.*, 37: 295-304.

Sabatini, S., D. Beis and H. Wolkenfelt. 1999. An auxin dependent distal organizer of pattern and polarity in the *Arabidopsis* root. *Scient. Hort.*, 99: 463-472.

Saida, S., P. Chatelot, O. Noureddine, F. Dosba and B. Ilham. 2005. Micropropagation of eight Moroccan and French olive cultivars. *J. Hort. Sci.*, 40(1): 193-196.

Soumendra, K.N., S. Pattnaik and P.K. Chand. 2000. High frequency axillary shoot proliferation and plant regeneration from cotyledonary nodes of pomegranate (*Punica granatum* L.). *Scient. Hort.*, 85: 261-270.

Staswick, P.E., B. Serban and M. Rowe. 2005. Characterization and rooting ability of indole-3-acetic acid conjugates formed during rooting of mung bean cuttings. *Pl. Physiol.*, 91: 1080-1084.

Sugiyama, M. 1999. Organogenesis *in vitro*. *Scient. Hort.*, 116(2): 61-64.

Taiz, L. and E. Zeiger. 2002. Mineral Nutrition: *Plant Physiology*. 2<sup>nd</sup> ed. Sinaver Associates Inc. Pub. p. 67-86.

Tanimoto, E. 2005. Regulation of root growth by plant hormones: Roles for auxin and Gibberellin. *Crit. Rev. Pl. Sci.*, 24(4): 249-265.

Thorpe, T.A. 2006. History of plant tissue culture. In: *Plant Cell Culture Protocols*. (Eds.): B.M. Loyola-Vargas, F. Vazquez-Flota. 2<sup>nd</sup> edn. Totowa, NJ.: Humana Press Inc. 9: 32.

Wada, S., E. Tanimoto and Y. Masuda. 1998. Cell elongation and metabolic turnover of the cell wall as affected by auxin and cell wall degrading enzymes. *Pl. Cell Physiol.*, 9(2): 369-376.

Wynne, J. and M.S. McDonald. 2002. Adventitious root formation in woody plant tissue: The influence of light and indole-3-butyric acid. *In vitro Cell Dev. Biol.*, 38(2): 210-212.

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