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EFFECT OF DIFFERENT MEDIA AND GROWTH REGULATORS ON *IN VITRO* SHOOT PROLIFERATION OF OLIVE CULTIVAR 'MORAIOLO'

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Abstract

The effect of Woody Plant Medium (WPM) and Olive Medium (OM) with various concentrations of Zeatin & Benzylaminopurine (BAP) solely or in combinations with each other, was investigated on *In vitro* shoot proliferation of the olive cultivar 'Moraiolo'. Olive medium proved to be the most effective one, resulting in better and morphologically superior microshoots as compared to woody plant medium. Zeatin (3.0 mg l^{-1}) in combination with 0.5 mg l^{-1} BAP resulted in highest number of microshoots per explant (0.84), with 2.25 cm shoot length and 1.88 number of nodes on olive medium as compared to its single use. The best interaction of both media with cytokinins occurred when 3.0 mg l^{-1} Zeatin was used in combination with 0.5 mg l^{-1} BAP.

Introduction

In vitro multiplication efficiency in *Olea europea* species is widely dependent on culture medium (Rugini, 1984; Cozza *et al.*, 1997; Grigoriadou *et al*., 2002; Santos *et al*., 2003), growth regulators (Rugini & Fedeli, 1990; Chaari *et al*., 2003) and genotype (Mencuccini & Rugini, 1994; Soumendra *et al*., 2000; Zuccherelli & Zuccherelli, 2002). In particular a specific medium for recalcitrant olive varieties has been defined by undertaking an analysis of mineral elements in embryos or young shoots (Rugini, 1984). In spite of the extensive studies, the *In vitro* propagation of *Olea europea* species is still limited due to poor growth, slow lateral bud outgrowth and variable rooting ability of the explants (Rugini & Fedeli, 1990; Sarmiento *et al*., 2001; Saida *et al*., 2005). The problem is compounded by intraspecific variation in tissue culture responses between different cultivars. Here the effect of Olive Medium OM (Micheli *et al*., 1998) and Woody Plant Medium WPM (Lloyd & McCown, 1980) and growth regulators (Zeatin & BAP) on the shoot proliferation of 'Moraiolo' cultivar of olive has been studied to find the best one for its micropropagation.

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 OM medium supplemented with zeatin 4 mg l^{-1}, sucrose 30 g l^{-1}, agar 6.5 g l^{-1} and subcultured every 40 days were used as experimental material. For this experiment, single uni nodal segments 10-15 mm in length, with two opposite leaves having an axillary bud, were implanted individually to culture jar containing shoot proliferation media *viz*., OM and WPM. Composition and comparison of both media is given in Table 1 & 2 respectively. Both media were supplemented with sucrose 30 g l^{-1}, agar 6.5 g l^{-1}

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Table 1. Basal nutrient medium composition of olive medium (OM) and woody plant medium (WPM).

Ingredient	Olive medium (OM) mg l^{-1}	Woody plant medium (WPM) mg l^{-1}
KNO_3	1772.00	--
NH_4NO_3	412.50	400.00
$Ca(NO_3)_2$. $4H_2O$	1300.00	556.00
$CaCl_2$. $2H_2O$	--	96.00
$MgSO_4$. $7H_2O$	731.00	370.00
KH_2PO_4	340.00	170.00
K_2SO_4	--	990.00
$FeSO_4$. $7H_2O$	27.80	27.80
Na_2EDTA	37.50	37.30
$MnSO_4$. $4H_2O$	16.90	22.30
H_3BO_3	12.40	6.20
$ZnSO_4$. $7H_2O$	14.30	8.60
Na_2MoO_4. $2H_2O$	0.25	0.25
$CuSO_4$. $5H_2O$	0.25	0.25
$CoCl_2$. $6H_2O$	0.02	--
KI	0.83	--
Myo-inositol	100.00	100.00
Glycine	2.00	2.00
Thiamine. HCl	0.50	1.00
Pyridoxin. HCl	0.50	0.50
Nicotinic acid	5.00	0.50
Biotin	0.05	--
Folic acid	0.50	--
Glutamine	1178.00	--

and varying levels of zeatin and BAP (Table 3). The pH of both the culture media was adjusted to 5.8 before autoclaving. Plants were incubated at 25 ± 1ºC under 16-h light (2,000 lux) with white fluorescent tubes (Philips TL 40W/54). Data were recorded after four weeks on total number of shoots per proliferating explant, shoot length (cm) and number of nodes per shoot as well. The experiment was bifactorial (Media x Growth Regulators) randomized in CRD (Completely Randomized Design) with three replications per treatment and six shoots per replication. 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

Effect of different culture media (OM & WPM) and growth regulators (Zeatin & BAP) on number of shoots per proliferated explant of olive cv. 'Moraiolo': OM medium proved to be superior to WPM medium (Table 4) by producing maximum number of shoots per proliferated explant (0.84), significantly higher than that on WPM medium (0.51). OM medium has higher level of nitrogen, phosphorus and glutamine as compared to WPM medium. Nitrogen may function as a signal molecule of plant growth *via* increased gene expression for enzyme responsible for the uptake and utilization of

Table 2. Comparison of OM & WPM Media for the Mineral Elements.

Mineral elements	OM mg l^{-1}	WPM mg l^{-1}
N	545.12	208.418
P	77.18	38.59
K	781.43	492.14
Ca	219.70	120.172
Mg	70.91	35.00
I	0.635	--
S	102.432	236.802
Cl	0.0059	45.984
Fe	5.89	5.89
Na	4.641	4.635
Mn	4.157	5.485
B	2.195	1.097
Zn	3.246	1.952
Mo	0.099	0.099
Cu	0.0635	0.0635
Co	0.00496	--
Myo	100	100
Glycine	2.00	2.00
Thiamine	0.50	1.00
Pyridoxine	0.50	0.50
Nicotinic acid	5.00	0.50
Biotin	0.05	--
Folic acid	0.50	--
Glutamine	1.178 g	--

Table 3. Different levels of Zeatin and BAP with olive and woody plant medium.

Treatments	OM (OM macro, micro elements) medium		WPM (WPM macro, micro elements) medium	
	Zeatin mg l^{-1}	BAP mg l^{-1}	Zeatin mg l^{-1}	BAP mg l^{-1}
T_1	1.0	0.0	1.0	0.0
T_2	2.0	0.0	2.0	0.0
T_3	3.0	0.0	3.0	0.0
T_4	4.0	0.0	4.0	0.0
T_5	1.0	0.5	1.0	0.5
T_6	2.0	0.5	2.0	0.5
T_7	3.0	0.5	3.0	0.5
T_8	4.0	0.5	4.0	0.5
T_9	1.0	1.0	1.0	1.0
T_{10}	2.0	1.0	2.0	1.0
T_{11}	3.0	1.0	3.0	1.0
T_{12}	4.0	1.0	4.0	1.0
T_{13}	1.0	1.5	1.0	1.5
T_{14}	2.0	1.5	2.0	1.5
T_{15}	3.0	1.5	3.0	1.5
T_{16}	4.0	1.5	4.0	1.5

Table 4. Effect of different culture media (OM & WPM) and growth regulators (Zeatin & BAP) on number of shoots per proliferated explant of olive cv. 'Moraiolo'.

Treatments Zeatin + BAP mg l^{-1}		Mean number of shoots per proliferated explant		Mean
		OM	WPM	
T_1	1.0 + 0.0	0.21 s	0.13 u	0.17 O
T_2	2.0 + 0.0	0.35 p	0.23 r	0.29 M
T_3	3.0 + 0.0	0.69 k	0.51 l	0.60 G
T_4	4.0 + 0.0	1.51 c	0.97 g	1.24 C
T_5	1.0 + 0.5	1.31 e	0.89 i	1.09 E
T_6	2.0 + 0.5	1.61 b	1.03 f	1.31 B
T_7	3.0 + 0.5	2.05 a	1.50 c	1.78 A
T_8	4.0 + 0.5	1.47 d	0.90 i	1.19 D
T_9	1.0 + 1.0	0.45 n	0.29 q	0.37 J
T_{10}	2.0 + 1.0	0.70 k	0.37 o	0.53 I
T_{11}	3.0 + 1.0	0.49 m	0.21 s	0.35 K
T_{12}	4.0 + 1.0	0.35 p	0.10 v	0.22 N
T_{13}	1.0 + 1.5	0.75 j	0.38 o	0.57 H
T_{14}	2.0 + 1.5	0.93 h	0.46 n	0.70 F
T_{15}	3.0 + 1.5	0.45 n	0.17 t	0.31 L
T_{16}	4.0 + 1.5	0.20 s	0.07 w	0.13 P
Mean		**0.84 A**	**0.51 B**	

$LSD_{5\%}$, Medium = 0.004, Interaction (M x T) = 0.016, Treatments = 0.012
Means followed by the same letter are not significantly different $p<0.05$.

nitrate (Mashayekhi, 2000). Growth on a poor nitrogen source is not sufficient to cause the induction of nitrate reductase and nitrite reductase enzymes essentially required for the consumption of nitrate (Avilla *et al.*, 1998). Reduced nitrogen forms especially the glutamine is present in OM medium and is absent in WPM medium. There is growing evidence for the usefulness of these supplemented amino compounds in the culture medium which can enhance cell division, differentiation, growth and development of multiple shoots *In vitro* (Ramage, 1999; Sotiropoulos *et al.*, 2005). It has been reported that the number of shoots produced by explant was correlated with the amount of phosphorus absorbed by explants of many tree species (Sharma & Thorpe, 1999). Phosphate has been known to be rapidly consumed by shoot forming explants corresponding to both the initiation and the growth of shoots but not by non-shoot forming explants (Smith *et al.*, 2000). In tobacco culture, over 50% of the phosphorus pool was consumed by day 20 during shoot initiation, indicating that this process requires high energy inputs (Bar-Yousef *et al.*, 1995). The remaining 50% of the phosphorus pool was consumed over the following 15 d during the growth of leafy shoots.

Statistical analysis showed the significant interaction between the culture media (OM & WPM) and growth regulators (Zeatin & BAP) at $p<0.05$ for the number of shoots per proliferated explant. The results indicate that OM + T_7 (3.0 mg l^{-1} Zeatin and 0.5 mg l^{-1} BAP) produced maximum number of shoots (2.05) per explant whereas WPM medium produced only 1.50 number of shoots per explant at same concentration of cytokinins. These results reveal that the eliciting interaction for the maximum number of shoots per explant occurs between growth regulators and OM medium at T_7 (3.0 mg l^{-1} Zeatin and 0.5 mg l^{-1} BAP). Ahmad *et al.*, (2002) reported that the effect of growth regulators can be strongly modified by the medium on which the cultures are grown. Calcium level is

higher in OM medium as compared to WPM medium and has been suggested to play a major role in various physiological processes like cytokinin signal transduction in Funaria cells (Mencuccini, 2003). Increases in the internal concentration of calcium of cytokinin treated cells led to more cell division. Since these increases were detected in bud forming tissues, so the hormonal stimulation of bud formation must lie further down the signal transduction pathway. Regarding the variation of different combinations of Zeatin and BAP in the culture media (OM & WPM), the number of shoots per explant increased with increasing concentrations up to certain extent and then fell. In both OM and WPM medium, Zeatin showed an increasing trend up to T_4 (4.0 mg l^{-1} Zeatin) when used as single growth hormone. However, the number of shoots per proliferated explant decreased drastically at 4.0 mg l^{-1} Zeatin combined with 0.5 mg l^{-1} BAP (T_8) in both media. The same trend was found in other combinations when BAP concentration was increased by 1.0 mg l^{-1} and 1.5 mg l^{-1} along with different levels of Zeatin in the media. The results indicate that high concentration of growth regulators significantly reduced shoot multiplication in both OM and WPM media.

Selection of concentration and combination of plant growth regulators is critical to shoot regeneration. Combination of Zeatin with BAP at T_7 (3.0 mg l^{-1} Zeatin and 0.5 mg l^{-1} BAP) proved to be more efficacious by raising the number of shoots (1.78) per explant as compared to Zeatin alone or with less or more concentration of BAP. These results are in line with Rugini *et al*., (1999) who also reported that olive microshoot development is promoted by Zeatin and is improved by its combination with BAP. The combined use of Zeatin and BAP has also proved better for shoot multiplication in several other woody species (Grigoriadou *et al.*, 2002; Roussos & Pontikis, 2002; Saida *et al.*, 2005). In present study, BAP at concentration of 0.5 mg l^{-1} has shown the synergistic effect on shoot regeneration with Zeatin when used at 3.0 mg l^{-1} as compared to its other combinations.

Effect of different culture media (OM & WPM) and growth regulators (Zeatin & BAP) on shoot length (cm) of olive cv. 'Moraiolo': Both the culture media (OM & WPM) differed significantly for the shoot length (Table 5). OM medium provided the maximum shoot length 2.25 cm while 1.24 cm long shoots were observed with WPM medium. The shoots developed in both the media were also morphologically different from each other. OM medium produced healthy green and more uniform shoots (Fig. 1), whereas those on WPM medium were small, claviform and anomalous (Fig. 2). The improvement of shoot growth and morphology attained during preceding proliferation stage on OM medium were probably effective in shoot elongation, which nevertheless proved unsatisfactory on WPM medium. OM medium is enriched in potassium as compared to WPM medium which has been reported to influence the flux of other minerals such as nitrogen, phosphorus and carbon, and enhances the translocation of photosynthates which in turn enhance the quality of shoots (Goncalves *et al.*, 2005). WPM medium is higher in chloride level which has been reported to result in growth depression in Kochia plants due to inhibited nutrient uptake, transport and utilization of nutrients (Karimi *et al.*, 2005). Moreover, magnesium deficiency was also observed due to higher concentration of chloride owing to low osmotic potential. WPM medium has also resulted in chlorotic shoots which according to Taiz & Zeiger (2002) are the typical symptoms of calcium deficiency.

Table 5. Effect of different culture media (OM & WPM) and growth regulators (Zeatin & BAP) on shoot length (cm) of olive cv. 'Moraiolo'.

Treatments Zeatin + BAP mg l^{-1}		Mean shoot length (cm)		Mean
		OM	WPM	
T_1	1.0 + 0.0	1.05 s	0.68 y	0.87 N
T_2	2.0 + 0.0	2.01 j	1.13 r	1.57 I
T_3	3.0 + 0.0	2.82 e	1.27 p	2.04 F
T_4	4.0 + 0.0	1.54 m	0.96 u	1.25 K
T_5	1.0 + 0.5	2.70 f	1.14 r	1.92 G
T_6	2.0 + 0.5	3.41 b	1.62 l	2.51 C
T_7	3.0 + 0.5	4.70 a	3.01 c	3.86 A
T_8	4.0 + 0.5	3.01 c	2.17 i	2.59 B
T_9	1.0 + 1.0	1.49 n	0.87 w	1.18 L
T_{10}	2.0 + 1.0	2.61 g	1.55 m	2.08 E
T_{11}	3.0 + 1.0	1.90 k	0.91 v	1.41 J
T_{12}	4.0 + 1.0	1.00 t	0.59 z	0.79 P
T_{13}	1.0 + 1.5	2.29 h	1.18 q	1.73 H
T_{14}	2.0 + 1.5	2.90 d	1.39 o	2.14 D
T_{15}	3.0 + 1.5	1.48 n	0.79 x	1.13 M
T_{16}	4.0 + 1.5	1.01 t	0.63 z	0.82 O
Mean		**2.25 A**	**1.24 B**	

$LSD_{5\%}$, Medium = 0.001, Interaction (M x T) = 0.016, Treatments = 0.004
Means followed by the same letter are not significantly different $p<0.05$.

OM medium is also high in phosphorus level as compared to WPM medium while iodine is only present in OM medium. Phosphorus is easily absorbed by the olive plants and is a possible trailer for the induction of substances such as growth regulators, amino acids and macroelements which in turn exert positive effect on shoot growth (Maalej *et al.*, 2006). Zheng-Hua (2002) reported that the final shoot growth was proportional with the initial phosphate level in the cultures. Lieben & Laszlo (2005) while investigating the effect of various ions in sugar metabolism in tobacco leaf disc found that the iodine ions, in combination with several other ions, significantly increased the utilization of sugar and helped in better shoot growth. Iodine also helps in the breakdown and better utilization of organic acids especially the biotin and folic acids, which are present in OM medium only, by increasing the enzymatic decomposition of these substances in plants during normal growth and differentiation process (Umaly & Poel, 1997).

Same trend was put forth for interaction between the culture media (OM & WPM) and growth regulators (Zeatin & BAP) as seen for preceding character of number of shoots per proliferated explant (Table 3). Better response was observed on OM + T_7 (3.0 mg l^{-1} Zeatin and 0.5 mg l^{-1} BAP) by producing the maximum 4.70 cm long shoots. The maximum shoot length produced by WPM was 3.01 cm at the same treatment i.e., T_7. Several studies have explored the relationship between mineral composition and plant growth regulators in culture media (Preece, 1995; Carl & Richard, 2002; Maalej *et al.*, 2006). Mineral composition may also affect the sensitivity of explants to plant growth regulators (Karimi *et al.*, 2005). A linear relationship was observed between exogeneous BAP supply and the concentration of potassium in tobacco leaf discs (Tomas *et al.*, 2001). In sunflower leaves, cytokinin treatments changed the potassium selectivity of cells (Letham, 1997). The hypothesis that minerals affect cell sensitivity to plant growth regulators is supported by regeneration studies on *Oryza sativa* L. somatic embryogenesis (Toriyama & Hinata, 1995).

Fig. 1. Healthy, green and more uniform shoots on OM medium.

Fig. 2. Small, claviform & anomalous shoot growth on WPM medium.

Concerning the variation with different concentrations of Zeatin and BAP in the OM and WPM media, depression in shoot length was observed with sub-optimal and supra-optimal concentrations. Like the shoot number, an increasing trend was also observed in shoot length up to T_3 (3.0 mg l^{-1} Zeatin) in both the media when Zeatin was used as a single growth hormone. However, an abrupt decrease in shoot elongation was seen at T_8 when 4.0 mg l^{-1} Zeatin was supplemented with 0.5 mg l^{-1} BAP in both the media. It may be inferred from these results that supra-optimal concentration of cytokinins shows the poor interaction with the salts of both the media. This has in turn depressed the shoot length in both the media. These results are in sequence with the preceding parameter of shoot multiplication in which the high level of cytokinins significantly reduced the number of shoots per proliferated explant.

Similar to the preceding parameter, treatments differed significantly as regards their effects on shoot length. Combination of Zeatin and BAP at T_7 (3.0 mg l^{-1} Zeatin and 0.5 mg l^{-1} BAP) produced the maximum shoot length (3.86 cm) followed by T_8 (4.0 mg l^{-1} Zeatin and 0.5 mg l^{-1} BAP) producing 2.59 cm long shoots. Reduced shoot length (0.79 cm) was observed when Zeatin and BAP both were having high concentration in T_{12}. However, an increase in shoot length (2.08 cm) was again observed when the concentration of Zeatin and BAP balance the cytokinins requirement at T_{10} (2.0 mg l^{-1} Zeatin and 1.0 mg l^{-1} BAP). The types of cytokinin and their concentrations significantly influenced the shoot elongation by their effect on cell division and cell expansion. The results show that shoot elongation response to joint use of Zeatin and BAP was more effective than to Zeatin alone. The effectiveness of various combinations of cytokinins on micropropagation of woody plants has been reported by Higuchi *et al*., (2004). However, shoot elongation phase is highly sensitive to higher concentrations of growth regulators (Kadota & Niimi, 2003).

Effect of different culture media (OM & WPM) and growth regulators (Zeatin & BAP) on number of nodes per shoot of olive cv. 'Moraiolo': Number of nodes per microshoot was counted considering that every node could be used as a new explant for further subculture. The same pattern existed for the number of nodes per shoot as for shoot number and shoot length (Table 6). OM medium produced maximum 1.88 nodes per shoot whereas 1.18 nodes per shoot were observed on WPM. Chemical composition of the culture medium has been reported to affect all types of morphogenic responses, including axillary bud proliferation (Soumendra *et al.*, 2000), caulogenesis (Nas & Read, 2006), plant regeneration (Ramsay & Galitz, 2003), and embryogenesis (Maalej *et al.*, 2006). Magnesium, which is comparatively high in OM medium, plays an essential role in cell elongation which in turn increases the number of nodes per shoot by activating several enzymes especially those involved in the transfer of phosphates (Shaul, 2002). These phosphates are involved in the initiation and growth of shoots (Bar-Yousef *et al.*, 1995). WPM medium is comparatively low in zinc level and it has been reported by Taiz & Zeiger (2002) that zinc deficient plants may be characterized by a reduction in internodal growth, as a result plants display a rosette habit of growth containing less number of nodes per shoot. OM medium constitutes the higher level of boron and nicotinic acid. Boron plays role in cell elongation, nucleic acid synthesis, and cell differentiation (Lauchli, 2002). Gupta (1997) reported an interactive effect of boron with other nutrients suggesting that high magnesium may induce a high boron requirement. Boron deficiency affects mitosis and DNA synthesis thus reducing the number of regenerants (Bolanos *et al.*, 2004). In pea meristem, cellular division does not proceed without an external supply of nicotinic acid which indicates that this vitamin is directly involved in cellular division in the meristem (Donald, 1998).

Table 6. Effect of different culture media (OM & WPM) and growth regulators (Zeatin & BAP) on number of nodes per shoot of olive cv. 'Moraiolo'.

Treatments		No. of nodes per shoot		Mean
Zeatin + BAP mg l^{-1}		OM	WPM	
T_1	1.0 + 0.0	1.01 r	0.70 x	0.85 O
T_2	2.0 + 0.0	1.35 m	0.91 s	1.13 J
T_3	3.0 + 0.0	1.89 i	1.05 q	1.47 G
T_4	4.0 + 0.0	2.01 g	1.21 o	1.61 E
T_5	1.0 + 0.5	1.32 n	0.85 t	1.09 K
T_6	2.0 + 0.5	3.01 c	1.90 i	2.46 C
T_7	3.0 + 0.5	4.35 a	2.52 d	3.43 A
T_8	4.0 + 0.5	3.19 b	2.11 f	2.65 B
T_9	1.0 + 1.0	1.19 o	0.78 v	0.99 M
T_{10}	2.0 + 1.0	1.49 l	0.92 s	1.21 I
T_{11}	3.0 + 1.0	1.11 p	0.73 w	0.92 N
T_{12}	4.0 + 1.0	0.99 r	0.62 y	0.81 P
T_{13}	1.0 + 1.5	1.98 h	1.10 p	1.54 F
T_{14}	2.0 + 1.5	2.33 e	1.58 k	1.96 D
T_{15}	3.0 + 1.5	1.69 j	1.05 q	1.37 H
T_{16}	4.0 + 1.5	1.21 o	0.81 u	1.01 L
Mean		**1.88 A**	**1.18 B**	

$LSD_{5\%}$, Medium = 0.0041, Interaction (M x T) = 0.016, Treatments = 0.012
Means followed by the same letter are not significantly different $p<0.05$.

The trend observed in the previous parameters of shoot multiplication and shoot length was maintained here for the interaction between culture media (OM & WPM) and growth regulators (Zeatin & BAP). Better response was established on OM + T_7 (3.0 mg l^{-1} Zeatin + 0.5 mg l^{-1} BAP) by producing the maximum 4.35 number of nodes per shoot (Fig. 3) while 2.52 nodes per shoot were produced as a result of interaction of WPM salts and cytokinins (Fig. 4) on T_7 (3.0 mg l^{-1} Zeatin and 0.5 mg l^{-1} BAP). Various reports indicate that the exogeneous supply of growth regulators may affect the uptake and utilization of mineral nutrients (Preece, 1995; Ibanez *et al.*, 2003; Kadota & Niimi, 2003; Tefera & Wannakrairoj, 2006). Mobilization of nutrients by cytokinins has also been reported by Taiz & Zeiger (2002). They demonstrated that nutrients are preferentially transported to, and accumulated in cytokinin treated tissues. It has been postulated that the plant growth regulators cause nutrient mobilization by creating a new source sink relationship (Endres *et al.*, 2002). Presence of essential organic acids, biotin and folic acid, in OM medium and their absence in WPM medium plays an important role in directing and transporting cytokinins (Alban *et al.*, 2000). The enhanced effect of interaction between the salts of OM medium and growth regulators may be the result of prompt availability of cytokinins. Scott *et al.*, (2000) also reported that folates, a class of pteridine compounds are essential for normal growth and differentiation. They further suggested that folic acid coenzymes are involved in one carbon transfer reactions such as those necessary for the biosynthesis of methionine, serine, deoxythymidylic acid, and purines essential for the cell differentiation.

Fig. 3. Elongated shoots with more number of nodes as a result of interaction of OM medium and growth regulators (Zeatin & BAP).

Fig. 4. Stunted shoot growth with less number of nodes produced as a result of interaction of WPM and growth regulators (Zeatin & BAP).

Significantly higher number of nodes (3.43) was developed by the combined use of Zeatin and BAP at T_7 (3.0 mg l^{-1} Zeatin and 0.5 mg l^{-1} BAP) as compared to the single use of Zeatin (Table 5). It is also evident from the results that sub-optimal level < 3.5 of the combination of Zeatin and BAP could not yield adequate number of nodes per shoot. Similarly, when the medium had a higher than optimal level of both growth regulators (Zeatin & BAP), it has also posed the deleterious effects on the number of nodes per shoot. Zeatin has been widely accepted as the preferred cytokinin capable of inducing satisfactory shoot growth and number of nodes per shoot in olive cultured explants (Peixe *et al*., 2007). However, the combined use of Zeatin and BAP has also been considered to have the synergistic effect on the morphogenesis of olive (Grigoriadou *et al*., 2002). Tomas *et al*., (2001) reported that cytokinins are derived from adenine and accelerate the process of morphogenesis by producing two immediate effects on undifferentiated cells: the stimulation of DNA synthesis and increased cell division. The results also indicate that excessive supply of Zeatin and BAP has concealed the balance of cytokinins and thus inferred the inhibitory effect on the number of nodes per shoot. These results are in line with those of Tomas *et al*., (2001) and Higuchi *et al*., (2004) who reported that the production of ethylene by the excessive cytokinins caused the inhibition of internode elongation and number of regeneration of tobacco discs and suggested this as the example of the interdependence of hormonal regulatory pathways.

Conclusion

Olive medium, a high ionic salt showed better interaction with the zeatin (3.0 mg l^{-1}) and BAP (0.5 mg l^{-1}) combination in comparison to a low ionic salt woody plant medium in terms of total number of shoots per proliferated explant, shoot length (cm) and number of nodes per shoot of olive cultivar Moraiolo. It has been observed that the combined use of zeatin and BAP proved better than the single use of these growth regulators.

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