



EFFECT OF CYTOKININS ON *IN VITRO* SHOOT PROLIFERATION OF OLIVE CULTIVARS "EARLIK" AND "BARI ZAITOON-2"

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ABSTRACT

In olive, micro-propagation is an effective technique for mass multiplication of disease free, true to type plants, but shoot proliferation in mature tissues of olive is a major difficulty encountered during culture establishment. Thus an experiment was designed with an objective to study the effect of different cytokinins (BAP and Zeatin) on shoot proliferation of two olive cultivars (Earlik and BARI Zaitoon-2). In olive Rugini medium, BAP and Zeatin were added alone and in combination at a concentration of 1 mg L⁻¹ and 2 mg L⁻¹. Experiments were arranged according to completely randomized design (CRD) with three replications per treatment. Results showed that both the olive cultivars performed well when BAP + Zeatin were supplemented to the medium at 2+2 mg L⁻¹ concentration. However, the cultivar "BARI Zaitoon-2" had the highest shooting percentage (90.67 %) and number of nodes per shoot (8.33) in treatment containing BAP (2 mg L⁻¹) + Zeatin (2 mg L⁻¹), whereas, "Earlik" at same concentration had more shoot length (9.10 cm) and number of shoots per explant (3.67). Thus, it was concluded that the findings of these results will help in the future for developing shoot proliferation protocols for other olive cultivars. Moreover, these protocols can also be further be used in other woody plants micro-propagation.

Key word: BAP, cytokinin, micro-propagation, olive, shoot proliferation and Zeatin.

INTRODUCTION

Olive (*Olea europaea* L.) one of the important fruit crop that originated in the Mediterranean region and consumed by people all over the world. It is considered as the oldest fruit tree of mankind. Cultivation of olive trees has expanded from the Mediterranean basin to various regions of North and, South and Central Asia, South America and Australia. It is a highly nutritious crop so consumption of olive oil and fruit is increasing day by day (Afridi *et al.*, 2015). Propagation of olive is commonly done through both sexually by seeds and asexually by cutting, layering, budding and grafting (Rostami and Shahsava, 2012). As it is a highly important crop throughout the world, thus there is an utmost demand of increasing olive tree production. However, this demand cannot be fulfilled through conventional asexual propagation methods like grafting and cutting (Hamooh and Shah, 2017). With an aim to develop other alternative propagation methods which can overcome or limit the problems related to conventional methods, several studies has been conducted on micro-propagation of olive. The purpose of doing micro-propagation in olive is to produce a large number of plants in a shorter period. Furthermore, plants produced under *in vitro* sterile conditions are free from any insect pest and disease (Afridi *et al.*, 2015). Micro-propagation technique in olive is successful in producing plants of valuable and high quality genotypes. For olive micro-propagation, olive medium proposed by Rugini (1984) has been examined to be effective for a wide range of olive cultivars (Rkhis *et al.*, 2011). But still, establishment of contaminant free axenic culture and shoot proliferation in mature tissues is the major hurdle that encounter during olive micro-propagation (Mangal *et al.*, 2014). Hence, to improve initial shoot growth, various types

of cytokinins such as TDZ, BAP, metatopolin and Zeatin alone or in combination with other type are added to Rugini olive medium (Kole, 2011). However among all these types of cytokinins, Zeatin at rate 4.56 to 45.62 mM is accepted as the only hormone (cytokinins) that is able to induce shoot proliferation in olive explants (Peixe *et al.*, 2007).

OBJECTIVES

This present research has been conducted with an aim to evaluate best concentration of cytokinins for shoot proliferation with an objective to find out best performing olive cultivar.

MATERIAL AND METHODS

For *in vitro* shoot proliferation, 2-3 cm binodial shoot tips were excised from 10 mature, 30 years old healthy trees of "Earlik" and "BARI Zaitoon-2" cultivated in an orchard of the Barani Agricultural Research Institute, Chakwal. These excised explants were cultured in Rugini Olive Medium (ROM) (Table 1) supplemented with cytokinins (Benzylaminopurine and Zeatin) alone or in combination of concentration of 1 mg L⁻¹ and 2 mg L⁻¹. Rugini olive medium (ROM) with different concentration of BAP and Zeatin was previously used in different olive cultivars by Hamooh and Shah (2017). Media pH was maintained until 5.8 before autoclaving. Explants were cultured in a conical flask containing 20 mL media. Each flask contained three explants. After culturing, explants were incubated in a growth chamber at 23±2°C and 1200-1500 flux light respectively. After four weeks, data regarding shooting percentage shoot length (cm), number of shoots per explant and number of nodes per shoots was recorded for both olive cultivars.

Effect of different cytokinins on shoot proliferation of olive cultivars were analyzed by analysis of variance (ANOVA)

using “Statistix 8.1” software. Difference among means was compared through LSD (least significant difference) at the 5% probability level.

Table 1: Composition of Rugini Olive Medium (ROM)

Stocks	Components	Rugini media Conc. (1000 mL)
A	Ca(NO ₃) ₂ .H ₂ O	13 g
B	NH ₄ NO ₃ and KNO ₃	17.72 g
C	KI	166 mg
	COCL ₃ .6H ₂ O	4 mg
D	KH ₂ PO ₄ ,	34 g
	H ₃ BO ₄ ,	1.24 g
	NaMoO ₄ .2H ₂ O	25 mg
E	MgSO ₄ .7H ₂ O	73.1 g
	MnSO ₄ .5H ₂ O	1.69 g
	ZnSO ₄ .7H ₂ O	1.43 g
	CuSO ₄ .5H ₂ O	25 mg
F	FeSO ₄ .7H ₂ O	5.76 g
	Na.EDTA	7.45 g
Vitamins		
1	Mio inositol	2 g/100 mL
2	Nicotinic acid	500 mg/100 mL
3	Pyridoxin	50 mg/100 mL
4	Thiamine	50 mg/100 mL
5	Biotin	5 mg/100 mL
6	Folic acid	50 mg/100 mL
7	Glycine	2 mg/L
8	Glutamine	1.178g/100 mL

RESULTS

Results related to the effect of different cytokinins on shoot proliferation showed that in both olive cultivars “BARI Zaitoon-2” and “Earlik”, maximum shoot length was obtained when 2 mg L⁻¹ BAP was used in combination with 2 mg L⁻¹ Zeatin (Table 2). However, along with cytokinins concentration, genetic variability also affects the shoot length. As cultivar “Earlik” produced longer shoots (9.1 cm) as compared to “BARI Zaitoon-2” where shoots reached up to only 6.5 cm long. It was observed that in cultivar “BARI Zaitoon-2” number of shoots per explant was significantly similar in treatments where Zeatin was used alone or in combination with BAP (Table 2) whereas in cultivar “Earlik” combination of BAP + Zeatin (2 mg L⁻¹) produced the maximum number of shoots per explant (Table 2). However, in both cultivar, significantly equal number of shoots per explant (3.67 and 3.33 were observed at BAP + Zeatin (2 mg L⁻¹) concentration respectively. Statistical analysis revealed that in cultivar “BARI Zaitoon-2” Zeatin alone and in combination with BAP was effective in producing the maximum number of nodes per shoot and greater number of nodes (8.33) were achieved in BAP + Zeatin (2 mg L⁻¹) (Table 2). Likewise the cultivar “Earlik” also showed the same response and had 6.66 nodes per shoot at BAP + Zeatin (2 mg L⁻¹) treatment (Table 2). Shooting percentage like other shoot proliferation parameters was examined to be highest in BAP + Zeatin (2 mg L⁻¹) in both cultivar “BARI Zaitoon-2” (90.67 %) and “Earlik”

(83.67 %). However cultivar “BARI Zaitoon-2” was genetically superior by having more shooting percentage as compared to cultivar “Earlik”.

DISCUSSION

Olive is on the oldest, long lived, evergreen fruit tree of the world (Lambardi *et al.*, 2012). Its product, olive fruit and olive oil is main constituent of Mediterranean people’s diet and also consumed in a large quantity in all over the world (Preedy and Watson, 2010). Olive tree can be used for fuel production, cosmetic industry, medicine, soap production and wool treatment. Oil extracted from its fruit are commonly used for cooking, salad purpose and preserving of other fruits (Lambardi *et al.*, 2012). Furthermore oil has important significant in religion, therapy, beauty and decoration (Radha and Mathew, 2007). Olive propagation through tissue culture is an effective technique for mass production but it is not much successful in it due to difficulties in disinfection of explants, explant oxidation and labor intensive *in vitro* shoot establishment process (Lambardi *et al.*, 2012). Thus to overcome the difficulty in shoot proliferation, presently different types of cytokinins had used in Rugini olive medium (ROM).

Cytokinins are a plant growth regulator that encourages shoot proliferation, improves growth and development and controls the enzymatic activity in order to activate transcription process. Up till now, more than 200 types of both natural and synthetic cytokinins have been discovered (Niaz *et al.*, 2014). Optimum concentration of cytokinins in growing media under *in vitro* conditions improves growth by increasing bud formation/shoot initiation (morphogenesis). Endogenous cytokinins concentration varies in different plant parts like its maximum concentration is present in meristematic tissues and actively growing areas such as roots, young leaves, growing fruits and seeds. Different types of cytokinins have different response on plants in inducing shoots (Niaz *et al.*, 2014). Difference in activity of various types of cytokinins could be due to their different rate of uptake from nutrient medium, variation in rate of translocation towards meristematic tissues or it may depend upon its stability as in various metabolic process cytokinins degrade or it combines with amino acids or sugar to produce inert (biologically inactive) compounds. Moreover, it was noticed that effective working of cytokinins was affected by cytokinins receptors affinity related to shoot induction (Verma *et al.*, 2011). In the present study, BAP was effective alone or in combination with Zeatin in both olive cultivars which attributed due to its stability as it doesn’t easily degraded or breakdown thus it remain in a nutrient media. Still, it also conjugated with other materials but its amount is relatively smaller as compared to other plant growth regulators (Buah *et al.*, 2010). Although BAP was considered best in inducing shoot as it increased shoot proliferation in various crops like a peach (Arab *et al.*, 2014), kiwi fruit (Akbaş *et al.*, 2007), hazelnut (Thomson and Deering, 2011) and persimmon (Kochanová *et al.*, 2011). But in olive cultivars, Zeatin improves shoot induction in both

Table 2: Effect of different concentration of cytokinins on shoot proliferation of different olive cultivars

Cytokinins (mg L ⁻¹)	"BARI Zaitoon-2"			"Earlik"				
	Shoot length (cm)	Number of shoots per Explant	Number of nodes per shoot	Shooting (%)	Shoot length (cm)	Number of shoots per Explant	Number of nodes per shoot	Shooting (%)
Control	0.00 e	0.00 c	0.00 e	0.00 d	0.00 f	0.00 e	0.00 d	0.00 d
BAP (1 mg L ⁻¹)	2.83 d	1.00 bc	2.00 d	48.67 c	3.16 e	1.00 d	1.33 cd	41.66 c
BAP (2 mg L ⁻¹)	3.16 d	2.33 ab	2.33 d	56.66 c	3.86 de	2.33 c	2.33 c	52.00 bc
Zeatin (1 mg L ⁻¹)	4.20 c	2.66 a	4.33 c	57.00 c	4.76 d	2.66 bc	4.00 b	46.33 bc
Zeatin (2 mg L ⁻¹)	5.23 b	3.33 a	5.00 bc	70.67 b	5.83 c	2.33 c	4.33 b	55.66 b
BAP (1 mg L ⁻¹) + Zeatin (1 mg L ⁻¹)	5.50 b	3.00 a	6.00 b	82.00 a	7.06 b	3.33 ab	5.33 ab	76.33 a
BAP (2 mg L ⁻¹) + Zeatin (2 mg L ⁻¹)	6.53 a	3.33 a	8.33 a	90.67 a	9.10 a	3.67 a	6.66 a	83.66 a

"Earlik" and "BARI Zaitoon-2" cultivars respectively. In olive best performance of Zeatin as compared to other cytokinins was due to its predominance in mature tissues. Zeatin is a naturally occurring cytokinins that present in two types of isoforms, cis and trans Zeatin. It is the one of the major translocatable form of cytokinins that present in cytokinins. Thus, it rapidly and effectively transposes material to meristematic tissues (Ling *et al.*, 2013). Furthermore, it encourages cell division, shoots formation at axillary buds and promotes root formation. It also reduces shoot and leaf senescence (Haddadi *et al.*, 2013). Combination BAP (2 mg L⁻¹) + Zeatin (2 mg L⁻¹) were effective in increasing shoot multiplication rate in both "BARI Zaitoon-2" and "Earlik". The reason behind this is that the composition of nutrient media is in agreement with the plant growth requirements. As the shoot multiplication under in vitro condition is largely depends on plant genotype and cytokinins concentration (Radmann *et al.*, 2011) so cytokinins concentration in medium must be optimized in order to get effective shoot proliferation (Khan *et al.*, 2010). According to the results, cultivar "BARI Zaitoon-2" performed better under different cytokinins concentrations by having more shooting percentage (%) and number of shoots per explant. The different varietal response to different cytokinins was due to genetic factors. It may also be related to cellular sensitivity or receptor affinity to different plant growth regulators. Moreover, it also depends on plant metabolism, rate of hormone uptake and its transportation towards different growing point of explants (Ling *et al.*, 2013).

CONCLUSION

In the present research, BAP and Zeatin in combination at concentration of 2 mg/L was effective in shoot proliferation of both olive cultivars "Earlik" and "BARI Zaitoon-2". Furthermore, in future this combination will be used in other olive cultivars in order to improve their shoot proliferation rate.

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