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Morpho-metabolites based delineation of root induction protocol for olive cultivars by using darkness and auxins

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Abstract

In vitro rhizogenesis is the last step determining the success of micropropagation of woody plants like olive. Current study has delineated a successful protocol of root induction in proliferated microshoots of four promising olive cultivars by using different levels of auxins (IBA and NAA) and dark period. The effects of these treatments were screened at morphological (rooting percentage, root length, number of roots per shoot and days of root initiation) and biochemical (primary and secondary metabolites) levels by making the detailed evaluations of root parameters and metabolites. Moreover, SEM micrographs of developing roots were generated to get the better understanding about the role of hormones in root architect regulation. Significant impact of increasing levels of hormones was detected in improving the root parameters as well as in triggering the metabolic activities. Our evaluations on morphological and biochemical basis ratified the higher rooting effectiveness of olive media with 2.5 mg L^{-1}supplementation of IBA and NAA; however comparative study marked IBA more promising as compared to NAA. In addition all cultivars showed noteworthy performance on both morphological and biochemical front at 2.5 mg L^{-1} concentration of hormones; while the performance of Arbosana and Arbequina was outstanding. The dark treatment showed only exceptional performance in increasing the rooting percentage, while no promising impact was noticed for other morphological and metabolic parameters. However, comprehensive assessment revealed promising performance of IBA in mediating growth and metabolic activities as compared to NAA.

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Introduction

Successful induction of adventitious roots in microshoots within in vitro culture system is the last step determining the success of micropropagation. Numerous studies explicated the potential impacts of NAA (Naphthalene acetic acid) and IBA (Indolebutyric acid) on the root induction of olive plants (Zuccherelli and Zuccherelli, 2002;Ali *et al.*, 2009;Fabbri *et al.*, 2009;Chaari-Rkhis *et al.*, 2011). Substantial advancements have been made in root induction techniques of olive microshoots over the last two decades, hence even the varieties recalcitrant to cutting and grafting propagation can now be successfully rooted within micropropagation systems (Chaari-Rkhis *et al.*, 2011). The foremost factors attracting the attention of researchers are medium formulations and auxins treatments whose activity varies from cultivars to cultivars. Moreover, auxins are extensively applied in vegetative propagation of many woody plants (Yan *et al.*, 2014) in varying doses according to their effectiveness. Besides, exogenously applied NAA and IBA are marked as the most effective stimulators of root primordial development as well as root morphogenesis (Dubrowsky *et al.*, 2008). Commonly NAA is speculated as stronger stimulant as compared to IBA as it can trigger significant rooting activity even in low concentration. However as compared to NAA, IBA induces early rhizogenesis with more number of root hairs.In woody plants IBA has been widely used for root genesis as compared to other auxins like IAA and NAA (Briccoli Bati *et al.*, 1999; Pop *et al.*, 2011) due to its tendency to induce high number of roots within short time.During the evaluation of comparative roles of different auxins for root induction, Dash *et al.* (2011) noticed more positive results for IBA as compared to NAA and IAA. Auxins synthesized in leaves are actively translocated to other tissues where they play vital part in the development of main root and initiation of adventitious roots (Pop *et al.*, 2011). Exogenous supplementation of auxins in medium increases their concentration in root primordial region where they trigger rhizogenic activity. Moreover, the action of auxins to some extent is light dependent, as initial irradiance causes degradation therefore an initial dark period is recommended to get better root induction (Haslam and Yeung 2011; Manzur *et al.*, 2014). Zacchini and De Agazio (2004) noticed better rooting percentage in olive microshoots subjected to an initial dark treatment of five days. On the other hand the analysis of primary and secondary metabolites during in vitro condition facilitates to evaluate the active role of hormones in regulating the growth and developmental processes as well as to validate the potential role of growth regulator as a supplement of media (Ng *et al.*, 2016). Phytometabolites are categorized as primary which includes carbohydrate, proteins and amino acids and secondary comprising alkaloids, flavonoids, tannins and phenols (Gururani *et al.*, 2015). To date no conclusive evidence is available to demonstrate how metabolic and developmental activities determine the effectiveness of growth regulators during in vitro propagation of plants. The objective of current study is to optimize the root induction protocol of olive cultivars by comprehensively evaluating the effectiveness of IBA and NAA at morphological and biochemical level.

Materials and methods

The microshoots of four olive cultivars Arbosana, Arbequina, Koroneiki and Sorany were evaluated for the optimization of their root induction protocol over olive medium (Rugini, 1984). After culturing the microshoots underwent dark treatment (0 and 5 days). Afterward the plantlets were cultured on OM with varying levels (0, 0.5, 1.5 and 2.5 mgL^{-1}) of auxins IBA and NAA for root induction. The experiment was conducted two times in tri-replicate arrangement using three factorial design, with dark period as factor A, varieties as factor B and auxins as factor C.

Estimation of root morphological parameters

The proliferated microshoots sterilized and used for *in vitro* culture on olive media according to methods described by Zacchini and De Agazio (2004), Ali *et al.* (2007), and Chaari-Rkhis *et al.* (2011). The response of 40 days old olive cultivars was evaluated on the basis of % induced roots, length of primary roots (cm), number of roots per shoot and days of root initiation.

Estimation of root primary metabolites

The amount of Carbohydrates in roots was estimated according to the same method used by Boussadia *et al.*(2010) for leaves. The extraction of these contents was carried out in 80% ethanol at 45°C. The whole system underwent 10 mins centrifugation at 5000 x g and sucrose, glucose, fructose and mannitol were determined by high pH anion-exchange chromatography with pulsed amperometric detection. However, in the leftover starch content was estimated using acid hydrolysis method where the pellet after washing with 80 % ethanol was subjected to 2 hour treatment with 1M HCl at 95°C. At the end starch quantity was calculated with UV-VIS at 340 nm with the help of enzymatic reduction of $NADP^+$. Besides proline content were estimated in 100 mg of fresh root samples of 40 days rooted seedling by following the method described by Bates *et al.* (1973).

Estimation of root secondary metabolites

Total phenolic and tannins contents in roots were determined in the ethanolic extract using the procedure described by Bray and Thorpe (1954). Flavonoids in aqueous extract of olive roots were calculated using the method of Jia *et al.* (1999). Alkaloid contents were estimated using the protocol of Harborne (1973).

Statistical analysis

The data obtained were analyzed using SAS software and the means were compared using LSD at $P \leq 0.05$ (Steel *et al.,* 1997).

Scanning electron microscopy

SEM micrographs of roots of best performing selective plantlets were generated to get the understanding about root architectural development under auxins treatments.

Results

Root growth parameters

Length of Primary root: It was noticed that varieties and hormones significantly ($P \leq 0.01$) increased the length of primary root; however no significant ($P \leq 0.05$) affect was noticed for the duration of dark period (Table 1).

Table 1. Effects of dark period treatments, varieties and hormones levels on various root growth parameters of olive explants.

Treatments	Length of Primary root (cm)	Percentage of induced roots	Number of roots shoot^{-1}	Days of Root initiation
Dark Treatments (D)				
D_0 (0 day)	ns	63.4 ± 5.50^b	ns	ns
D_1 (5 days)		70.1 ±5.91^a		
LSD		3.57		
Variety (V)				
V_1 (Sorany)	2.19±0.256^b	64.9±7.99^c	1.84±0.19^b	ns
V_2 (Koroneiki)	2.32±0.27^b	65.9±8.12^{bc}	2.16±0.18^a	
V_3 (Arbequina)	2.68±0.28^a	67.6±8.37^{ab}	2.20±0.18^a	
V_4 (Arbosana)	2.78±0.32^a	68.6±8.47^a	2.33±0.19^a	
LSD	0.171	1.80	0.175	
Hormones (H)				
IBA (H_I)(mgL^{-1})				
H_0 (0 mgL^{-1})	0.85±0.048^d	12.1±0.45^c	1.03±0.047^d	22.6±0.43^a
H_1 (0.5 mgL^{-1})	2.42±0.087^c	82.4±1.21^b	2.24±0.107^c	14.8±0.29^b
H_2 (1.5 mgL^{-1})	3.05±0.13^b	84.6±1.45^b	2.53±0.077^b	14.1±0.30^b
H_3 (2.5 mgL^{-1})	3.65±0.13^a	87.9±1.51^a	2.73±0.070^a	12.7±0.25^c
LSD	0.198	2.23	0.122	1.0611
NAA (H_N)(mgL^{-1})				
H_0 (0 mgL^{-1})	0.85±0.048^d	12.1±0.45^d	1.03±0.047^d	22.6±0.43^a
H_1 (0.5 mgL^{-1})	2.37±0.086^c	80.0±1.11^c	2.10±0.11^c	15.6±0.29^b
H_2 (1.5 mgL^{-1})	3.01±0.13^b	82.7±1.21^b	2.40±0.085^b	14.6±0.26^{bc}
H_3 (2.5 mgL^{-1})	3.59±0.13^a	85.8±1.47^a	2.65±0.083^a	13.5±0.33^c
LSD	0.2123	2.1222	0.1167	1.1121
Significance				
D	ns	**	ns	ns

V	**	**	**	ns
H	**	**	**	**
D x V	ns	ns	ns	ns
D x H	ns	**	**	ns
V x H	ns	ns	ns	ns
D x V x H	ns	ns	ns	ns

Means followed by the same letter (s) in each column and treatment depicted no significant difference

*, ** indicate significant differences at 0.05 and 0.01 probability levels respectively while 'ns' indicate non-significant difference.

Among cultivars Arbosana and Arbequina depicted significantly high means 2.78 cm and 2.68 cm respectively, as compared to other cultivars. A dramatic increase for both IBA and NAA was noticed at 2.5 mgL^{-1}as compared to other concentrations; however IBA illustrated slightly higher mean value (3.65cm) as compared to NAA (3.59 cm). Besides individual treatments, no significant effect of interactions between dark period, varieties and hormones was noticed on length of primary root.

Table 2. Effect of dark period, varieties and hormonal levels on various Primary metabolites of olive roots during *in vitro* growth.

Treatments	Mannitol mg g^{-1} DW	Starch mg g^{-1} DW	Glucose mg g^{-1} DW	Fructose mg g^{-1} DW	Sucrose mg g^{-1} DW	Proline µg g^{-1} FW
Dark Treatments (D)						
D_0 (0 day)	ns	ns	ns	0.033 ± 0.0028^{a}	0.42 ± 0.039^{a}	39.9 ± 1.78^{b}
D_1 (5 days)				0.022 ± 0.0034^{b}	0.30 ± 0.035^{a}	40.7 ± 1.83^{a}
LSD				0.00178	0.1529	0.3971
Variety (V)						
V_1 (Sorany)	0.94 ± 0.13^{b}	0.36 ± 0.07^{b}	1.01 ± 0.14^{c}	ns	0.30 ± 0.04^{c}	41.4 ± 2.74^{a}
V_2 (Koroneiki)	1.01 ± 0.13^{b}	0.37 ± 0.07^{b}	1.06 ± 0.14^{bc}		0.34 ± 0.05^{b}	40.9 ± 2.61^{a}
V_3(Arbequina)	1.02 ± 0.12^{b}	0.38 ± 0.074^{b}	1.13 ± 0.14^{b}		0.37 ± 0.05^{b}	39.6 ± 2.48^{b}
V_4 (Arbosana)	1.23 ± 0.15^{a}	0.66 ± 0.1^{a}	1.30 ± 0.16^{a}		0.42 ± 0.06^{a}	39.2 ± 2.49^{b}
LSD	0.1665	0.1572	0.0927		0.0339	0.7773
Hormones (H)						
IBA (H_I) (mgL^{-1})						
H_o (0 mgL^{-1})	0.24 ± 0.014^{d}	0.08 ± 0.0096^{d}	0.20 ± 0.011^{d}	0.007 ± 0.0023^{d}	0.05 ± 0.0061^{d}	48.7 ± 0.52^{a}
H_1 (0.5 mgL^{-1})	1.14 ± 0.071^{c}	0.41 ± 0.057^{c}	1.27 ± 0.055^{c}	0.021 ± 0.010^{c}	0.37 ± 0.024^{c}	33.9 ± 0.34^{b}
H_2 (1.5 mgL^{-1})	1.30 ± 0.056^{b}	0.53 ± 0.066^{b}	1.42 ± 0.056^{b}	0.036 ± 0.014^{b}	0.46 ± 0.024^{b}	30.8 ± 0.22^{c}
H_3 (2.5 mgL^{-1})	1.52 ± 0.042^{a}	0.75 ± 0.076^{a}	1.60 ± 0.051^{a}	0.047 ± 0.013^{a}	0.55 ± 0.028^{a}	20.3 ± 0.26^{d}
LSD	0.0745		0.0646	0.00421	0.0277	0.6886
NAA (H_N)(mgL^{-1})						
H_o (0 mgL^{-1})	0.24 ± 0.015^{d}	0.08 ± 0.0096^{d}	0.20 ± 0.011^{d}	0.007 ± 0.0023^{d}	0.05 ± 0.0061^{d}	48.7 ± 0.52^{a}
H_1 (0.5 mgL^{-1})	0.42 ± 0.024^{c}	0.18 ± 0.017^{c}	0.52 ± 0.013^{c}	0.017 ± 0.0017^{c}	0.17 ± 0.016^{c}	46.0 ± 0.66^{b}
H_2 (1.5 mgL^{-1})	0.67 ± 0.025^{b}	0.25 ± 0.017^{b}	0.64 ± 0.014^{b}	0.028 ± 0.0025^{b}	0.25 ± 0.009^{b}	43.1 ± 0.63^{c}
H_3 (2.5 mgL^{-1})	0.88 ± 0.020^{a}	0.29 ± 0.015^{a}	0.81 ± 0.016^{a}	$0.036\pm0..0026^{a}$	0.29 ± 0.01^{a}	23.4 ± 0.25^{d}
LSD	0.0368	0.0237	0.0214	0.00262	0.00815	0.6119
Significance						
D	ns	ns	ns	**	*	*
V	*	**	**	ns	**	**
H	**	**	**	**	**	**
D x V	ns	ns	ns	ns	ns	ns
D x H	ns	*	ns	**	**	*
V x H	*	ns	*	*	*	*
D x V x H	ns	ns	ns	ns	ns	ns

Means followed by the same letter (s) in each column and treatment depicted no significant difference

*, ** indicate significant differences at 0.05 and 0.01 probability levels respectively while 'ns' indicate non-significant difference.

Percentage root induction

All treatments significantly (P≤ 0.01) improved the root induction percentage (Table 1). Comparison of means dictated that increment in percentage made by D_2 (70.1) was statistically significant as compared to D_0 (63.4). Among all cultivars Arbosana showed the highest induction percentage (68.6) as compared to other cultivars. A noteworthy improvement for both IBA and NAA was recorded at 2.5 mgL^{-1}as compared to other levels; however IBA revealed comparatively higher mean percentage (87.9) as compared to NAA (85.8).

Besides, no significant (P≤ 0.01) effect of interaction between darkness and varieties (D × V), and varieties and hormones was noticed on rooting percentage.

Table 3. Effect of dark period, varieties and hormonal levels on various secondary metabolites in roots of olive during *in vitro* growth.

Treatments	Alkaloids mg g^{-1} DW	Flavanoids mg g^{-1} DW	Tannins mg g^{-1} DW	Phenols mg g^{-1} DW
Dark Treatment (D)				
D_0 (0)	1.47±0.056[a]	ns	ns	1.13±0.034[a]
D_1 (5)	1.28±0.051[a]			0.92±0.035[b]
LSD	0.2303			0.1787
Variety (V)				
V_1 (Sorany)	1.20±0.067[d]	0.98±0.062[d]	0.80±0.050[b]	0.93±0.039[c]
V_2 (Koroneiki)	1.32±0.063[c]	1.13±0.054[c]	0.87±0.055[ab]	0.98±0.044[bc]
V_3 (Arbequina)	1.45±0.079[b]	1.23±0.077[b]	0.89±0.054[a]	1.06±0.047[ab]
V_4 (Arbosana)	1.54±0.084[a]	1.35±0.080[a]	0.91±0.054[a]	1.12±0.043[a]
LSD	0.0383	0.0506	0.0743	0.0853
Hormones (H)				
IBA (H_I)(mgL^{-1})				
H_0 (0 mgL^{-1})	0.98±0.039[d]	0.80±0.034[d]	0.68±0.034[d]	0.71±0.015[d]
H_1 (0.5 mgL^{-1})	1.67±0.029[c]	1.39±0.017[c]	0.79±0.041[c]	1.03±0.016[c]
H_2 (1.5 mgL^{-1})	1.87±0.050[b]	1.58±0.036[b]	0.91±0.047[b]	1.11±0.040[b]
H_3 (2.5 mgL^{-1})	2.06±0.048[a]	1.84±0.057[a]	1.03±0.040[a]	1.25±0.036[a]
LSD	0.0476	0.0907	0.0728	0.0675
NAA (H_N)(mgL^{-1})				
H_0 (0 mgL^{-1})	0.98±0.039[d]	0.80±0.044[d]	0.68±0.022[d]	0.71±0.032[d]
H_1 (0.5 mgL^{-1})	1.34±0.045[c]	1.08±0.046[c]	0.82±0.019[c]	0.97±0.039[c]
H_2 (1.5 mgL^{-1})	1.51±0.044[b]	1.27±0.054[b]	0.93±0.034[b]	1.11±0.042[b]
H_3 (2.5 mgL^{-1})	1.68±0.051[a]	1.46±0.066[a]	1.05±0.029[a]	1.22±0.039[a]
LSD	0.0245	0.0461	0.0441	0.0402
Significance				
D	*	ns	ns	*
V	**	**	**	**
H	**	**	**	**
D x V	ns	ns	ns	ns
D x H	*	ns	ns	ns
V x H	**	**	ns	ns
D x V x H	ns	ns	ns	ns

Means followed by the same letter (s) in each column and treatment depicted no significant difference

*, ** indicate significant differences at 0.05 and 0.01 probability levels respectively while 'ns' indicate non-significant difference.

Conversely the significant improvement in rooting percentage was noticed owe to interaction between dark period and hormones (D × H). For interactions (D × H) the highest values of root induction in case of dark treatment were reported at 2.5mgL^{-1} hormonal concentration (Table 4). However, the maximum value (92.1) of IBA and Dark (D × H_I) interaction was greater than the maximum value (88.6) of NAA and Dark (D × H_N) interaction which proved the strong interacting ability of IBA with dark period as compared to NAA. Moreover, no significant effect of three way interaction was reported.

Number of roots per plant

Both cultivars and hormones significantly (P≤ 0.01) enhanced the number of roots in each plant, while no significant(P≤ 0.05)improvement was noticed for dark treatment (Table 1). The comparison of means for the cultivars Arbosana, Arbequina and Koroneiki revealed statistically insignificant difference in their tendency of root induction. On the other hand, all concentrations of hormones illustrated significant improvement in root number, with concentration 2.5 mgL^{-1}being the most effective one. However IBA showed slightly higher mean value (2.73) of root induction as compared to NAA (2.65).Apart from individual treatment, significant effect of interaction between dark period and hormones (D × H) was reported on number of roots. The mean values of D × H interaction indicated that the highest value of root number for dark untreated plant was at 2.5mgL^{-1} hormonal concentration (Table 4).

Table 4. Effect of interaction between Dark treatments and hormones (D x H) on levels of primary and secondary metabolites in the roots of olive.

Dark Treatment (D)	IBA (H_I) (mgL^{-1})	Starch mg g^{-1} DW	Fructose mg g^{-1} DW	Sucrose mg g^{-1} DW	Proline µg g^{-1} FW	Alkaloids mg g^{-1} DW	Percentage of induced roots	Number of roots $shoot^{-1}$
5	0	0.06±0.01	0.006±0.0008	0.03±0.002	49.6±0.80	0.86±0.039	13.5±0.36	1.00±0.09
	0.5	0.24±0.06	0.017±0.0029	0.31±0.022	35.6±0.42	1.59±0.041	86.1±0.67	2.04±0.16
	1.5	0.46±0.09	0.028±0.0050	0.39±0.022	32.4±0.37	1.74±0.058	88.6±1.19	2.36±0.10
	2.5	0.60±0.09	0.039±0.0048	0.46±0.015	22.0±0.32	1.91±0.042	92.1±1.92	2.52±0.07
0	0	0.11±0.01	0.009±0.0005	0.06±0.009	47.3±0.53	1.09±0.036	10.8±0.42	1.05±0.03
	0.5	0.58±0.04	0.026±0.0037	0.44±0.026	32.3±0.53	1.75±0.012	78.8±0.41	2.44±0.10
	1.5	0.59±0.09	0.043±0.0031	0.54±0.022	29.1±0.26	1.99±0.051	80.5±1.68	2.70±0.08
	2.5	0.90±0.10	0.055±0.0023	0.63±0.035	21.0±0.40	2.21±0.048	83.8±1.02	2.92±0.06
	LSD	0.1579	0.00605	0.0398	0.9910	0.0686	3.208	0.1755
	NAA (H_N) (mgL^{-1})							
5	0	0.05±0.01	0.006±0.0008	0.03±0.002	49.6±0.80	0.86±0.039	13.5±0.36	1.00±0.09
	0.5	0.14±0.02	0.013±0.0020	0.11±0.008	46.4±0.73	1.23±0.053	82.9±1.01	1.92±0.17
	1.5	0.21±0.03	0.021±0.0030	0.22±0.004	43.3±0.50	1.43±0.059	85.5±1.31	2.30±0.10
	2.5	0.26±0.02	0.029±0.0028	0.26±0.005	23.6±0.32	1.59±0.071	88.6±2.09	2.43±0.09
0	0	0.11±0.01	0.009±0.0005	0.06±0.009	47.8±0.53	1.09±0.036	10.8±0.42	1.05±0.03
	0.5	0.23±0.02	0.021±0.0020	0.22±0.012	45.6±0.75	1.45±0.049	77.2±1.42	2.39±0.10
	1.5	0.28±0.02	0.035±0.0020	0.27±0.011	43.0±0.85	1.59±0.055	80.0±1.57	2.58±1.12
	2.5	0.32±0.01	0.044±0.0022	0.32±0.011	23.1±0.40	1.78±0.061	83.0±1.65	2.88±0.08
	LSD	0.0341	0.00377	0.0117	0.8805	0.0353	3.0535	0.1678

Means having difference greater than LSD are significant at P ≤ 0.05.

Furthermore, the maximum value (2.92) of IBA and dark interaction (D × H_I) was slightly equal to the maximum value (2.88) of NAA and dark interaction (D × H_N). The analysis of Table 4, revealed slightly higher mean values of interaction D x HI as compared to interaction D × H_N which ratified better interacting tendency of IBA with dark period compared to NAA. Besides, no significant improvement was recorded for the interactions D × V, V × H and D × V × H.

Days of root initiation

It was observed that both darkness interval and varieties showed no significant (P≤ 0.05) decline in duration of root emergence (Table 1). Conversely significant (P≤ 0.01) decline was reported for hormones. Comparisons of means revealed statistically significant decrease in root initiation period for both IBA and NAA at concentration 2.5 mgL^{-1}, however IBA showed less mean value (12.7) as compared to NAA (13.5).

Moreover, no two and three way effect of interactions between dark interval, varieties and hormones was observed.

Root primary metabolites

Mannitol

Both hormones and varieties significantly (P≤ 0.01 and P≤ 0.05 respectively) increased the synthesis of mannitol in root cells, while no significant (P≤ 0.05) alteration was noticed for dark period (Table 2). Among cultivars, the maximum mannitol content (1.23 mg g^{-1} DW)was extracted from the roots of Arbosana. In addition, a remarkable increase in mannitol was reported for both IBA and NAA at 2.5 mgL^{-1} as compared to other concentrations; however IBA showed relatively high mean value (1.52 mg g^{-1} DW) as compared to NAA (0.88 mg g^{-1} DW). Besides individual treatments, significant (P≤ 0.05) effect of interaction between varieties and hormones (V × H) was noticed on root mannitol content. For interaction V × H, the highest mannitol content for all cultivars were reported at 2.5 mg L^{-1} hormonal concentration as shown in Table 5. Furthermore, the maximum value (1.10mg g^{-1} DW) of interaction V × H_I is higher than the maximum value (0.35 mg g^{-1} DW) of interaction V × H_N, which explicates better interacting ability of IBA with cultivars as compared to NAA. On the other hand no significant (P≤ 0.05) impact of interactions D × H, D × V and D × V × H was noticed on root mannitol content.

Table 5. Effect of interactions between varieties and hormones (V x H) on metabolites contents of olive roots.

Varieties (V)	IBA (H_I) (mgL^{-1})	Glucose mg g^{-1} DW	Fructose mg g^{-1} DW	Sucrose mg g^{-1} DW	Proline µg g^{-1} FW	Mannitol mg g^{-1} DW	Alkaloids mg g^{-1} DW	Flavonoids mg g^{-1} DW
Sorany	0	0.18±0.03	0.007±0.0013	0.03±0.006	50.0±0.91	0.19±0.03	0.86±0.080	0.72±0.048
	0.5	1.12±0.11	0.016±0.0047	0.32±0.036	33.8±0.48	0.93±0.17	1.60±0.073	1.41±0.015
	1.5	1.27±0.13	0.031±0.0082	0.40±0.028	30.0±0.41	1.15±0.13	1.74±0.066	1.48±0.085
	2.5	1.48±0.09	0.039±0.0087	0.47±0.039	21.0±0.58	1.50±0.07	1.92±0.092	1.68±0.043
Koroneiki	0	0.19±0.02	0.007±0.0011	0.05±0.009	50.5±0.87	0.24±0.03	0.98±0.088	0.84±0.059
	0.5	1.20±0.12	0.016±0.0038	0.35±0.045	34.3±0.25	1.10±0.15	1.61±0.066	1.35±0.020
	1.5	1.35±0.06	0.031±0.0052	0.42±0.06	31.2±0.29	1.25±0.10	1.74±0.107	1.54±0.066
	2.5	1.50±0.07	0.046±0.0055	0.53±0.043	22.0±0.29	1.45±0.09	2.00±0.089	1.78±0.083
Arbequina	0	0.21±0.02	0.008±0.0017	0.05±0.015	37.3±0.96	0.27±0.02	0.98±0.025	0.73±0.048
	0.5	1.30±0.07	0.021±0.0042	0.36±0.043	34.3±0.29	1.12±0.08	1.74±0.014	1.44±0.013
	1.5	1.40±0.07	0.031±0.0075	0.50±0.042	31.0±0.29	1.28±0.05	1.99±0.094	1.61±0.038
	2.5	1.60±0.06	0.044±0.0061	0.57±0.054	21.0±0.48	1.42±0.05	2.16±0.12	1.89±0.124
Arbosana	0	0.24±0.01	0.008±0.0009	0.06±0.016	47.0±0.41	0.28±0.02	1.09±0.083	0.93±0.078
	0.5	1.45±0.09	0.031±0.0052	0.46±0.051	33.5±0.65	1.40±0.07	1.71±0.043	1.35±0.054
	1.5	1.68±0.06	0.049±0.0031	0.53±0.039	30.8±0.29	1.53±0.08	2.00±0.058	1.69±0.062
	2.5	1.83±0.09	0.059±0.0031	0.62±0.076	22.0±0.25	1.70±0.07	2.15±0.065	2.01±0.141
	LSD	0.1313	0.0086	0.0564	1.4014	0.1514	0.0968	0.18472
	NAA (H_N) (mgL^{-1})							
Sorany	0	0.18±0.03	0.007±0.0013	0.03±0.006	50.0±0.91	0.19±0.03	0.86±0.080	0.72±0.048
	0.5	0.50±0.03	0.015±0.0037	0.14±0.025	46.8±0.49	0.35±0.06	1.14±0.080	0.91±0.099
	1.5	0.62±0.03	0.025±0.0061	0.22±0.009	44.0±0.68	0.60±0.05	1.32±0.060	1.08±0.097
	2.5	0.78±0.03	0.031±0.0072	0.27±0.012	23.0±0.58	0.82±0.06	1.46±0.082	1.22±0.092
Koroneiki	0	0.19±0.02	0.007±0.0011	0.05±0.009	50.5±0.87	0.24±0.03	0.98±0.086	0.84±0.078
	0.5	0.52±0.03	0.012±0.0025	0.17±0.035	47.2±0.75	0.42±0.06	1.30±0.046	1.06±0.085
	1.5	0.63±0.03	0.025±0.0035	0.25±0.019	43.5±0.75	0.68±0.05	1.40±0.046	1.19±0.083
	2.5	0.80±0.04	0.032±0.0048	0.28±0.020	24.5±0.29	0.89±0.04	1.59±0.038	1.34±0.080
Arbequina	0	0.21±0.02	0.008±0.0017	0.05±0.015	47.3±0.96	0.27±0.02	0.98±0.025	0.73±0.105
	0.5	0.53±0.02	0.019±0.0046	0.17±0.037	45.5±1.11	0.44±0.03	1.44±0.070	1.11±0.080
	1.5	0.64±0.03	0.028±0.0066	0.25±0.023	42.5±1.22	0.70±0.05	1.61±0.038	1.36±0.069
	2.5	0.81±0.04	0.038±0.0048	0.29±0.023	23.3±0.48	0.89±0.03	1.76±0.052	1.55±0.091
Arbosana	0	0.24±0.01	0.008±0.0009	0.06±0.016	47.0±1.08	0.28±0.02	1.09±0.083	0.93±0.083
	0.5	0.55±0.02	0.021±0.0024	0.19±0.033	44.5±0.87	0.48±0.02	1.46±0.069	1.22±0.048
	1.5	0.67±0.03	0.035±0.0020	0.27±0.018	42.5±1.25	0.71±0.04	1.69±0.052	1.46±0.080
	2.5	0.82±0.03	0.044±0.0024	0.31±0.023	22.8±0.25	0.92±0.02	1.91±0.052	1.71±0.126
	LSD	0.0433	0.00534	0.01657	1.2452	0.0748	0.0498	0.0938

Mean values having difference greater than LSD are significantly different at $P \leq 0.05$.

Starch

All the treatments with the exception of dark period significantly (P≤ 0.01) increased root starch synthesis during *in vitro* growth on olive media (Table 2). Comparisons of means revealed that among cultivars only Arbosana depicted statistically high starch content (0.66 mg g^{-1} DW) as compared to other cultivars. An abrupt increase in starch synthesis was noticed at 2.5 mg L^{-1} for both IBA and NAA; however the IBA depicted comparatively high mean content (0.75 mg g^{-1} DW) as compared to NAA (0.29 mg g^{-1} DW). Among interactions between dark period, varieties and hormones only the interaction between dark period and hormones (D x H) significantly increased the starch synthesis in roots. For interaction D × H, maximum starch content was recorded for both dark and control at 2.5 mg L^{-1} hormonal level (Table 4). Moreover, the maximum mean values of starch for both D × H_I and D × H_N interactions were noticed in the roots of plants that followed no dark treatment. Correspondingly, the maximum value (0.90mg g^{-1} DW) of D x H_I interaction was higher than the maximum value (0.32mg g^{-1} DW) of D x H_N interaction. Overall analysis of Table 4 revealed relatively higher values of D x H_I as compared to D x H_N which dictate better interaction ability of IBA with dark treatment as compared to NAA.

Glucose

Significant (P≤ 0.01) increment in glucose content was noticed for cultivars and hormones; however no significant (P≤ 0.05) increment was recorded for dark treatment (Table 2). Among cultivars, Arbosana revealed statistically significant level (1.30 mg g^{-1} DW) of metabolite as compared to other cultivars. A considerable rise in glucose synthesis was detected for both IBA and NAA at concentration 2.5 mg L^{-1}; however this rise was more dynamic for IBA (1.60 mg g^{-1} DW) as compared to NAA (0.81 mg g^{-1} DW). Besides among all interactions between dark, varieties and hormones only the interaction between varieties and hormones (V × H) significantly (P≤ 0.01) increased the glucose synthesis. For interaction V x H, all cultivars depicted maximum level of glucose at hormonal concentration 2.5 mg L^{-1} as indicated in Table 5.

Likewise, the maximum value (1.83 mg g^{-1} DW) of V × H_I interaction was higher than the maximum value (0.82 mg g^{-1} DW) of V × H_N interaction. As a whole comparative evaluation of Table 5 revealed slightly higher values of V x H_I as compared to V x H_N, which dictate relatively higher affinity of IBA with cultivars as compared to NAA.

Fructose

Fructose content was significantly (P≤ 0.01) regulated in root cells by dark treatment and hormonal levels while no significant (P≤ 0.05) regulation was monitored for varieties (Table 2). Comparison of means revealed that increase in fructose content made by D_o (0.033 mg g^{-1} DW) was statistically significant as compared to D1 (0.022 mg g^{-1} DW). A dramatic increase in fructose level was noticed for hormones at concentration 2.5 mg L^{-1}; while this increase was more dynamic for IBA (0.047 mg g^{-1} DW) as compared to NAA (0.036 mg g^{-1} DW). On the other hand among all possible interactions between dark, varieties and hormones only the interactions darkness x hormones (D × H) and variety x hormones (V × H) significantly affected fructose content. For interaction D × H, maximum fructose content for dark period was reported at hormonal concentration 2.5 mg L^{-1} as illustrated in Table 4. However, the highest mean value (0.055 mg g^{-1} DW) of D × H_I interaction was slightly greater than the highest mean value (0.044 mg g^{-1} DW) of D × H_N interaction. Overall analysis of Table 4 revealed relatively higher values of D x HI as compared to D x H_N, which revealed high interacting tendency of IBA with dark period as compared to NAA in stimulating fructose metabolism. Correspondingly, for interaction V x H all cultivars showed maximum fructose level at concentration 2.5 mgL^{-1}as described in Table 5. In addition for both V × H_I and V × H_N, Arbosana depicted the highest fructose content, however the maximum value (0.059 mg g^{-1} DW) of V x H_I interaction was higher than the maximum value (0.044 mg g^{-1} DW) of V × H_N interaction.IBA has better interaction tendency with cultivars compared to NAA as revealed by comparatively higher values of V × H_I interaction relative to V × H_N.

Sucrose

All treatments dark period, varieties and hormones significantly (P≤ 0.01) improved sucrose content in the root of olive (Table 2). Comparison of means revealed that improvement in sucrose content made by Do (0.42 mg g^{-1} DW) was statistically higher than D1 (0.30 mg g^{-1} DW). Correspondingly cultivar Arbosana depicted the highest sucrose level (0.42 mg g^{-1} DW) as compared to other cultivars. A remarkable increase sucrose synthesis was reported for both hormones at concentration 2.5 mg L^{-1}, however IBA showed more promising increment (0.55 mg g^{-1} DW) as compared to NAA (0.29 mg g^{-1} DW). Besides individual treatments, among all possible interactions of treatments only D × H and V × H significantly increased the quantity of sucrose. Similarly, for interaction D x H the highest value of sucrose was recorded for dark period treatment at concentration 2.5 mg L^{-1} (Table 4). Correspondingly the maximum value of D × H_I (0.63mg g^{-1} DW) interaction was higher than the maximum value of D × H_N (0.32mg g^{-1} DW) interaction. Moreover, comparative evaluation of Table 4 revealed the higher values of interaction D × H_I as compared to interaction D × H_N, which authenticated the strong interacting tendency of IBA with dark period as compared to NAA. In addition for interaction V × H, all cultivars depicted maximum sucrose contents at hormonal concentration of 2.5 mgL^{-1}. Likewise maximum sucrose content (0.62mg g^{-1} DW) noticed for V × H_I interaction was higher than the maximum sucrose content (0.31mg g^{-1} DW) noticed for V × H_N interaction as shown in Table 5. The highest sucrose content was noticed in Arbosana while the least content was noticed in Sorany. The overall analysis of Table 5 dictated comparatively higher mean value of V × H_I interaction as compared to V × H_N interaction that confirmed the strong interacting tendency of IBA with cultivars as compared to NAA.

Proline

It was noticed that all treatments significantly (P≤ 0.01) altered proline level in the roots of olive plantlets (Table 2). Statistically significant increase in proline content was made by D1 (40.7 µg g^{-1} FW) as compared to Do (39.9 40.7 µg g^{-1} FW).

Among cultivars, the highest level of proline was detected in Sorany (41.4 µg g^{-1} FW). A considerable decrease in proline content was noticed for hormones at 2.5 mg L^{-1} concentration; however this decrease was more prominent for IBA (20.3 µg g^{-1} FW) as compared to NAA (23.4 µg g^{-1} FW). In addition significant (P≤ 0.05) variation in proline level was noticed owe to interaction effect of D × H and V × H. Conversely no significan t (P≤ 0.05) effect was detected on proline amount due to two way interaction D x V and three way interaction D × V × H. For interaction D × H the dark treatment revealed maximum proline content (49.6 µg g^{-1} FW) for control concentration (0 mg $L^{-1}$of hormones) as indicated in TABLE 4. On the other hand the minimum content (21.0 µg g^{-1} FW) detected at 2.5 mg L^{-1} for D × H_I was lower than the minimum content (23.1 µg g^{-1} FW) detected for D × H_N. Comparative overview of Table 4 illustrated comparatively less values of proline for D × H_I interaction as compared to D × H_N interaction that validated strong interacting tendency of IBA in lowering proline amount. In case of interaction V × H all cultivar revealed maximum proline level for control treatment, while least content revealed for 2.5 mgL^{-1} hormonal treatment (Table 5). For interaction V × H_I minimum proline content (21.0 µg g^{-1} FW) observed in Sorany were less than the minimum proline content (22.8 µg g^{-1} FW) observed for interaction V × H_N in Arbosana. Overall analysis of Table 5 showed relatively less values of V × H_I interaction as compared to V x H_N interaction which ratified better interacting tendency of IBA with cultivars in lowering proline level.

Root secondary metabolites

Alkaloids

Varieties and hormones significantly (P≤ 0.01) increased while the dark period made significantly (P≤ 0.05) decreased the level of alkaloids in olive roots within micropropagation system (Table 3). Comparison of means revealed no significant difference in the impact of dark period as compared to control. Among cultivars, the highest alkaloids content (1.54mg g^{-1} DW) was reported in Arbosana. A remarkable increase in alkaloids was traced at 2.5 mg L^{-1} concentration of hormones; however the maximum mean value of alkaloids for IBA (2.06mg g^{-1} DW) was higher than NAA (1.68mg g^{-1} DW).

Moreover, among all possible interactions of treatments only D × H and V × H significantly increased the level of alkaloids. In case of interaction D x H, the dark intervals depicted maximum alkaloids at 2.5 mg L^{-1} hormonal concentration, however maximum alkaloids content was reported in the plants that do not followed dark treatment (Table 4).

In addition the maximum value (2.21mg g^{-1} DW) of alkaloids for D × H_I interaction was higher than the maximum value (1.78mg g^{-1} DW) of D × H_N interaction. Correspondingly, the comparative overview of Table 4 dictates the relative high values of D × H_I interaction as compared to D x H_N interaction that reflects better interacting tendency IBA with dark treatments as compared to NAA. Likewise all cultivars showed the maximum alkaloids content at hormonal concentration of 2.5 mg L^{-1} as illustrated in Table 5. However Arbosana depicted the noteworthy performance for this interaction. Likewise the highest value (2.15mg g^{-1} DW) of V × H_I interaction was greater than the highest value (1.91mg g^{-1} DW) of V × H_N interaction. In Table 5, comparative overview revealed relatively higher values of V × H_I interaction as compared to V × H_N interaction that validated strong interacting affinity of IBA with cultivars as compared to NAA.

Flavonoids

Both varieties and hormones significantly (P≤ 0.01) changed the level of flavonoids within the root system; however no significant (P≤ 0.05) change was reported for dark treatment (Table 3).Statistically significant flavonoids content (1.84mg g^{-1} DW) was isolated from Arbosana as compared to other cultivars. A dynamic increase in flavonoids was observed at 2.5 mg L^{-1} hormonal concentrations; correspondingly this increase was higher for IBA (1.84mg g^{-1} DW) as compared to NAA (1.46mg g^{-1} DW).Moreover among all possible interactions of treatments significant (P≤ 0.01) alteration in flavonoids was only observed for V × H. in case of interaction V × H all cultivars depicted maximum level of metabolite at 2.5mg L^{-1} concentration. Correspondingly the performance of Arbosana cultivar was exceptionally better.

In addition the maximum value (2.01mg g^{-1} DW) of V x H_I interaction was higher than the maximum value (1.71mg g^{-1} DW) of V × H_N interaction. Overall comparative overview of Table 5 revealed relatively higher mean values of V × H_I interaction as compared to V × H_N interaction that ratified better interacting ability of IBA with cultivars as compared to NAA.

Tannins: Varieties and hormones significantly (P≤ 0.01) improved the tannins content of roots; while no significant (P≤ 0.05) improvement was noticed for dark treatment (Table 3). Among cultivars statistically significant contents were reported in cultivars Arbosana (0.91mg g^{-1} DW) and Arbequina (0.89mg g^{-1} DW).In addition a dramatic increase in flavonoids was reported at 2.5 mg L^{-1} hormonal concentration; however contrary to above findings no noteworthy difference was found in the means of IBA (1.03mg g^{-1} DW) and NAA (1.05mg g^{-1} DW).No effect of interactions among treatments was observed for this parameter.

Phenols

All treatments significantly affected the level of phenols in olive roots (Table 3). Comparison of means revealed statistically significant increase in phenols made by Do (1.13mg g^{-1} DW) as compared to D1 (0.92mg g^{-1} DW).Among cultivars maximum phenolic compounds were extracted from Arbosana (1.12mg g^{-1} DW) and Arbequina (1.06mg g^{-1} DW). Moreover, a noteworthy rise in phenols content was observed at 2.5 mg L^{-1} hormonal concentration; while this increment was approximately equally dynamic for both IBA (1.25mg g^{-1} DW) and NAA (1.22mg g^{-1} DW). Besides, no effect of interactions among dark period, varieties and hormones was noticed on phenolic content.

SEM

SEM micrographs were generated to evaluate the root architecture build up in two ways. Firstly, the root micrographs of best performing Arbosana cultivars were compared at 2.5 mgL^{-1} concentration both for IBA and NAA. The comparisons of transversal and cross sections as well as root surface texture revealed at two resolutions 750x and 1500x dictated equally good architectural morphogenesis and build of roots (Fig. 1).

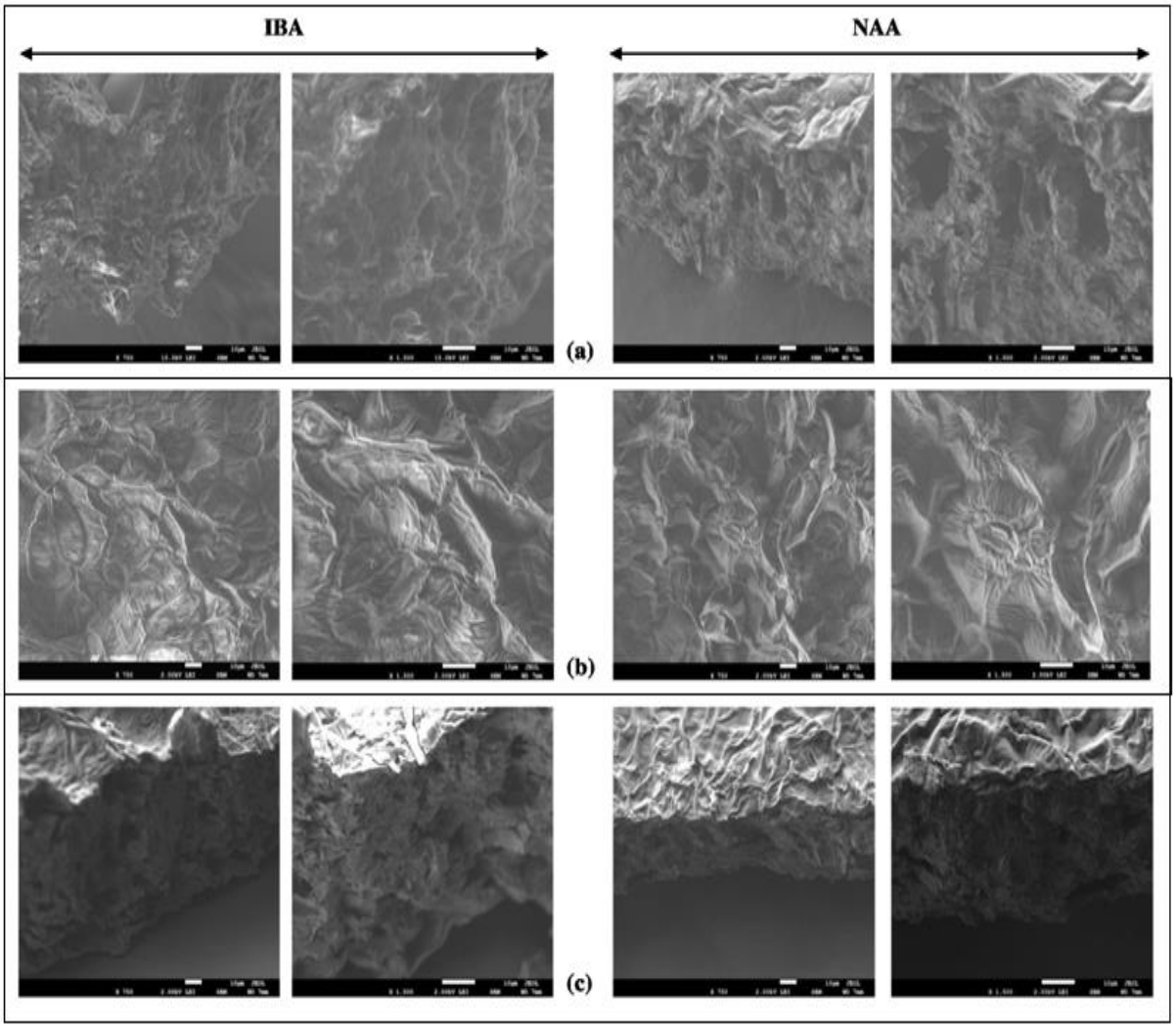

Fig. 1. SEM micrographs illustrating comparative changes in root architecture during *in vitro* root morphogenesis in the presence of growth regulators IBA and NAA.
(a) Growth architect of root transverse section. (b) Growth architect of root Surface. (c) Growth architect of root cross section.

Besides SEM micrographs of Root hair surface texture showed the dense aggregation of fibers at high concentration (2.5 mgL^{-1}) of IBA and NAA as compared to control as illustrated in Fig. 2.

Discussion

The results of above study ratified that the supplementation of growth regulators IBA and NAA in olive medium is a potential necessity for root induction as well as initiation of metabolic activities. It has been noticed that for the induction of *in vitro* rooting at least supplementation of 0.5 mg $L^{-1}$of growth regulator is necessary. Therefore, for the induction of rooting in proliferated seedlings the significance of IBA and NAA as important supplements of olive media cannot be neglected (Chaari-Rkhis *et al.*, 2011). Besides it has been observed that growth regulators and darkness interval synergistically improved the rooting percentage in plants (Zacchini and De Agazio 2004, Manzur *et al.*, 2014). Our findings further authenticated these observations as the highest percentage of rooting was observed in the cultivars exposed to preliminary dark treatments. Formation of adventitious rooting system is highly energy demanding process that needs presence of excess of reserve food for root induction. In fact, this system includes cell division in which definite cells follow morphogenetic pathways to form root primordial development (Manzur *et al.*, 2014).

Therefore, reserve nutrient material and growth regulators such as IBA and NAA exhibit a vital role in determining root growth and morphogenesis. Undoubtedly, endogenous auxin content has dynamic impact on root induction, yet exogenous application whether in lower or higher concentration significantly modulate root induction.

The exogenously supplemented auxins augment the endogenous level at the basal region of cuttings where they initiate rooting signals by optimizing metabolic events (Husen, 2008; Ng *et al.*, 2016).Besides, Yan *et al.* (2014) recorded that lower level of NAA and IBA dramatically enhanced the adventitious root development in *Hemarthria compressa*. However, contrary to their findings we observed increase in root number and length at higher concentration of both auxins (IBA and NAA) in olive.This was in accordance to the theory of Hentig and Gruber (1987) who dictated that increasing hormonal dosages could bring best rooting until the point just below the toxic level. Moreover, in several studies higher concentrations of hormones found to be more effective in inducing rooting in olive microshoots (Zuccherelli and Zuccherelli, 2002;Zacchini and De-Agazio, 2004;Fabbri *et al.*, 2009, Chaari-Rkhis *et al.*, 2011).In fact hormones accelerate the process of cell division and cell elongations thus boost root length that regulates the overall process of growth in plants. Ali *et al.* (2009) reported the highest rooting parameters such as percentage induction, length and number for 1.5 and 1 mgL^{-1} concentrations of IBA and NAA respectively for olive cultivar "Moraiolo". However, outcomes of present study showed contradiction as for both hormones the highest values of root parameters were recorded at 2.5 mg L^{-1}.Correspondingly, higher concentrations of auxins found to be more effectual in inducing root in the micro-shoots of *A. paniculata* (Purkayastha *et al.*, 2008; Dandin and Murthy, 2012). In addition varying degree of responsiveness was observed for all cultivars even at same concentrations of hormones with Arbosana the highest one and Sorany as the lowest one. On the other hand IBA depicts better tendency in mediating various root events within in vitro propagation system as compared to NAA (Ali *et al.*, 2009; Chaari-Rkhis *et al.*, 2011; Yan *et al.*, 2014, Hossein and Urbi, 2016). Complementary results were reported in current study as shown in Table 1.

Zacchini and De-Agazio (2004) reported a remarkable increase in the rooting tendency of olive cultivars after the dark exposure of five days. The action of auxins such as IBA and NAA is associated with light that reveal considerable degradation on exposure to light (Neumann *et al.*,2009).In fact, this fact explicates the higher sensitivity of plantlets to dark period, media components and growth regulators apart from their degradation (Manzur *et al.*, 2014). Correspondingly, in current study both IBA and NAA significantly improved the root induction percentage when plantlets were exposed to a dark period of 5 days (Table 1 and Table 4).

Probably exposure to darkness enhances the activity of oxidases and peroxidases that triggers the process of root initiation. However no promising effect of darkness was noticed for other parameters of roots. To date several rooting protocols have been optimized for the woody plant species by evaluating various culture media, growth regulator combinations and environmental conditions (Dubrowsky *et al.*, 2008;Naija *et al.*, 2008;Jose *et al.*, 2012). However, mostly these studies did not include histological evidences to get the better perceptive of rhizogenesis in microshoots. In present study SEM micrographs were generated for best performing cultivar Arbosana at 2.5 mg L^{-1} hormonal (IBA and NAA) concentration to get the understanding of root architectural changes during the process of rhizogenesis (Fig. 1 and 2).We noticed denser cellular clump like growth pattern along transverse and cross sections of root(Fig. 1) which was an indicator of fast cellular growth and differentiation according to Metivier *et al.* (2007).Moreover, dense aggregation of fibers was noticed along the surface architect of root hairs at higher concentration (2.5 mg L^{-1}) as compared to control (Fig. 2).

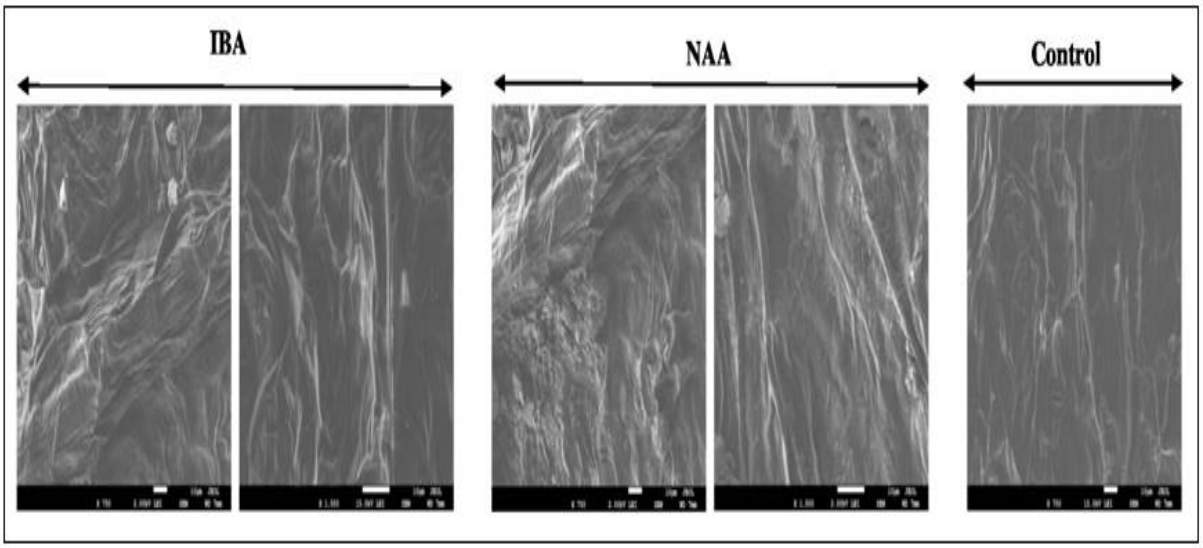

Fig. 2. SEM micrographs of Root hair texture show the dense aggregation of fibers at high concentration (2.5 mgL^{-1}) of IBA and NAA as compare to control.

In current study the activity of hormones was evaluated on the basis of their tendency to regulate primary and secondary metabolism. Variation in the levels of both primary (mannitol, starch, glucose, fructose, sucrose and proline) and secondary (phenols, tannins, alkaloids and flavonoids) metabolites was observed under varying treatments of hormones. In fact the increment in the level of primary metabolites such as carbohydrates probably increases the substrates of specific pathways that are actively involved in the synthesis of secondary metabolites (Ghasemzadeh *et al.,* 2010; Guo *et al.*, 2011). A side by side increase in both primary and secondary metabolic contents was reported under high hormonal doses (Table 2 and Table 3). However, this increase was more dynamic for IBA as compared to NAA. It is common perception that primary metabolism is the front line choice of the plants, however during excessive growth and physiological activities secondary metabolism becomes an optional tool (Xu *et al.*, 2011). Therefore, in current study under IBA application we have observed high level of secondary metabolites, as plants used secondary metabolism as an optional tool to fulfill the cellular deficit created as a result of excessive growth and physiological activities. Astonishingly, proline content showed reciprocated trend as compared to all other metabolites. A subsequent decrease in proline was noticed with increased supplementation of both auxins; however this decrease was more remarkable for IBA relative to NAA. In fact proline guards cellular and sub-cellular machinery and enzymes and regulate the proper assimilation of nutrient ingredients (Ejaz *et al.*, 2012). Therefore this decline can be attributed as stress free root growth of olive plantlets under high concentrations of auxins as stated by Nair *et al.* (2002). According to Aslam *et al.* (2016) an increase in the levels of primary metabolites and secondary metabolites (such as phenols, flavonoids and alkaloids) is to some extent is associated with catabolism of proline. Current diagnostics further authenticated their findings. Outcomes of current study depicted a strong association between the levels of hormones and the synthesis of primary and secondary metabolites. Conclusively, our evaluations on morphological and biochemical basis ratified the higher rooting effectiveness of olive media with 2.5 mg L^{-1}supplementation of IBA and NAA; however comparative study marked IBA more promising as compared to NAA. In addition all cultivars showed noteworthy performance on both morphological and biochemical front at 2.5 mg L^{-1} concentration of hormones; while the performance of Arbosana and Arbequina was outstanding (Table 5). The dark treatment showed only exceptional performance in increasing the rooting percentage, while no promising impact was noticed for other morphological and metabolic parameters. Overall, the present research

has optimized a discernible root induction protocol for olive cultivars by conducting comprehensive screening on both morphological and biochemical fronts. Conclusively, this study will not only provide a novel benefit to olive production industry but also provide a new dimension to researchers for discerning the potential impacts of growth regulators during any kind of micropropagation optimization study.

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