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Effect of Various Auxin Treatments (Indole Butyric Acid and Naphthalene Acetic Acid) on Root Initiation of Olive Cultivars, Coratina and Carolea

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The study was conducted to assess appropriate concentration of indole butyric acid (IBA) and naphthalene acetic acid (NAA) for the rooting of olive cultivars Coratina and Carolea. Semi-hard wood cuttings were treated with various concentrations of IBA (0, 1000, 2000, 3000, 4000 ppm) and NAA (0, 500, 1000, 1500, 2000 ppm) and inserted in sand medium under mist conditions. IBA treated cuttings showed better results than NAA regarding all rooting parameters. The results showed that the use of IBA at 3000 and 2000 ppm concentration is the most effective treatment for the rhizogensis and subsequent survival of olive cultivars, Coratina and Carolea, respectively. Highest value of root index was (90.95) obtained at T_3 (3000 ppm IBA) followed by (55.57) at T_4 (4000 ppm IBA). At T_2 (2000 ppm IBA) and T_1 (1000 ppm IBA) the values of root indices were 48.23 and 47.05, which were statistically at par. Direct relationship was found between number of sprouts per cutting and number of roots per cutting, the maximum number of sprouts was found in those treatments which produced maximum roots per cutting.

Key Words: Olive, Indole butyric acid, Naphthalene acetic acid, Root initiation, Hard wood cuttings.

INTRODUCTION

Olive (*Olea europaea* L.) belongs to family Oleaceae. The native home of the olive is considered to be Asia Minor, from where it spread to Europe and North African countries. The world area under this crop is 10 million hectares from which 2.6 million tones oil is produced annually[1]. According to survey, about 98 % of olive trees are grown in the Mediterranean countries, Italy and Spain alone accounting for 385 million trees[2]. Olive fruit comprises of around 20-34 % oil and has very less cholesterol. It contains 80 % unsaturated fatty acid against 20 % saturated ones along with high oleic acid percentage[3]. Propagation of olive through seed is not a desirable method due to segregation and also their seedlings require longer time to come

into bearing[4]. Top working of European olive onto wild olive resulted in very low percentage of graft success and had proven unsuitable for commercial requirements[5]. Clonal propagation of olive through stem cuttings is an inexpensive method which can speed up the olive nursery production cycle and the new plants bear fruit in fewer years[6-8]. Olive cuttings are hard to root but it can be influenced by use of hormones like auxins[4,9,10] which have a wide variety of effects on plant growth and morphogenesis[11]. Moreover, it has been reported that the auxins also enhance the survival percentage of rooted cuttings which otherwise don't survive in green house during the process of hardening[4,7,12,13]. The rooting ability of stem cuttings through auxins differs widely among the olive cultivars[14-17]. Hence, the present study is aimed at assessing the appropriate concentration of indole butyric acid (IBA) and naphthalene acetic acid (NAA) for the rooting of selective olive cultivars Coratina and Carolea.

EXPERIMENTAL

The experiment was carried out at Horticulture Research Farm, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan during the year 2006. Semi-hard wood cuttings were taken from one and a half year old branches of olive trees of same phenotype. The length of each cutting was 10 to 12 cm with 4 to 6 nodes having 3 to 4 leaves at upper end. The basal portion of these cuttings was treated with talc powder blended with different concentrations of IBA (0, 1000, 2000, 3000, 4000 ppm) and NAA (0, 500, 1000, 1500, 2000 ppm) while in control treatment; the basal end was only treated with talc powder. The cuttings were planted in coarse sand in green house under polythene sheet covering with natural day length in the month of February. The intermittent mist was given with hand spray pump thrice a day to maintain relative humidity at 80 to 90 % inside the plastic tunnel. The treatments were arranged according to randomized complete block design (RCBD) with three replications per treatment and fifteen cuttings per replication. After 80 d of plantation, the cuttings were taken out of the sand and data was collected for parameters like rooting percentage of cuttings, number of roots per cuttings, length of roots of each cutting, root index, number of sprouts per cutting and survival percentage of rooted cuttings. The data thus collected was statistically analyzed by using analysis of variance (Anova) technique and differences among treatment means were compared by using least significant difference (LSD) Test at 5 % probability level[18].

RESULTS AND DISCUSSION

Rooting percentage of cuttings: Maximum rooting percentage (80.95 %) was obtained at T_3 (3000 ppm IBA) followed by 79.78 % at T_2 (2000 ppm IBA). Both T_3 and T_2 remained statistically higher than all other treatments

(Table-1). At T_1 (1000 ppm IBA) and T_4 (4000 ppm IBA), the values of rooting percentage were 56.45 and 60.83 %, which showed that the rooting percentage decreases below 2000 ppm and above 3000 ppm of IBA concentration. On the other hand, minimum rooting percentage (32.18 %) was recorded at T_8 (2000 ppm NAA) while at T_0 (control) it was 38.78 %. It was noted that there was non significant effect of NAA on the rooting percentage of both varieties. The highest rooting percentage (45.52 %) with NAA was obtained at T_6 (1000 ppm NAA). Rooting percentage at T_5 (500 ppm NAA) and T_7 (1500 ppm NAA) were 36.45 and 38.85 % which were statistically at par. The results showed that the increase in concentration of NAA from 1000 ppm tends to reduce the rooting percentage in both varieties.

TABLE-1
EFFECT OF DIFFERENT CONCENTRATIONS OF IBA AND NAA ON ROOTING PERCENTAGE OF OLIVE CULTIVARS CORATINA AND CAROLEA

	Rooting percentage		Means
	Coratina	Carolea	
T_0 (control)	42.00 def	35.57 ef	38.78 cd
T_1 (1000 ppm IBA)	53.23 cd	59.67 bc	56.45 b
T_2 (2000 ppm IBA)	73.00 ab	86.57 a	79.78 a
T_3 (3000 ppm IBA)	82.00 a	79.90 a	80.95 a
T_4 (4000 ppm IBA)	59.67 bc	62.00 bc	60.83 b
T_5 (500 ppm NAA)	37.57 ef	35.33 ef	36.45 d
T_6 (1000 ppm NAA)	42.14 def	48.90 cde	45.52 c
T_7 (1500 ppm NAA)	44.47 def	33.23 f	38.85 cd
T_8 (2000 ppm NAA)	33.23 f	31.13 f	32.18 d
Means of Varieties	51.92 a	52.48 a	
	Treatments	Interaction (T × V)	Varieties
$LSD_{5\%}$ (34 d.f)	10.13	14.33	5.85

Means followed by same letter are not significantly different $p < 0.05$.

IBA is more effective than NAA for rhizogensis and root initiation (Table-1) because of its stability, its slow transport from site of application at the base of cuttings and its conversion to IAA in the cuttings[19]. By exogenous application of IBA, the status of indigenous hormone was increased in the cuttings at the point of root initiation resulting in enhanced rooting ability. The cells of pericycle are stimulated to divide by the application of IBA resulting in the formation of root apex and hence induced rooting[11].

Significant interaction was observed among varieties and auxins applied. Maximum rooting (82 %) was obtained in cultivar Coratina at 3000 ppm IBA while in Carolea maximum rooting percentage (86.57 %) was obtained at 2000 ppm. This might have happened due to variation in genetic make up and internal physiology (carbohydrate level, mineral concentration and indigenous hormonal status) of cuttings of different varieties[14,20,21].

Number of roots per cutting: Increase in the concentration of IBA also increased the number of roots per cutting (Table-2). Maximum number of roots per cutting (9.33) were recorded at T_4 (4000 ppm IBA) followed by 8.83 at T_3 (3000 ppm IBA), both these treatments are statistically at par. While at T_2 (2000 ppm IBA) and T_1 (1000 ppm IBA) the root number were 5.83 and 4.17. Minimum number of roots per cutting (3.00) was found at T_0 (control). Maximum number of roots per cutting (4.67) with NAA was observed at T_7 (1500 ppm NAA) followed by 4.33 roots per cutting at T_6 (1000 ppm NAA).

TABLE-2
EFFECT OF DIFFERENT CONCENTRATIONS OF IBA AND NAA ON NUMBER OF ROOTS PER CUTTING OF OLIVE CULTIVARS CORATINA AND CAROLEA

	Number of roots per cutting		Means
	Coratina	Carolea	
T_0 (control)	3.00 e	3.00 e	3.00 d
T_1 (1000 ppm IBA)	4.00 e	4.33 e	4.17 cd
T_2 (2000 ppm IBA)	4.67 e	7.00 cd	5.83 b
T_3 (3000 ppm IBA)	7.67 c	10.00 ab	8.83 a
T_4 (4000 ppm IBA)	10.67 a	8.00 bc	9.33 a
T_5 (500 ppm NAA)	3.67 e	3.00 e	3.33 cd
T_6 (1000 ppm NAA)	3.67 e	5.00 de	4.33 bcd
T_7 (1500 ppm NAA)	4.67 e	4.67 e	4.67 bc
T_8 (2000 ppm NAA)	3.33 e	3.67 e	3.50 cd
Means of Varieties	5.03 a	5.41 a	
	Treatments	Interaction (T × V)	Varieties
$LSD_{5\%}$ (34 d.f)	1.549	2.189	0.894

Means followed by same letter are not significantly different $p < 0.05$.

It was observed that increase in the NAA concentration up to 1500 ppm enhanced rooting but further increase in concentration had negative effect on number of roots per cutting. It was also revealed that the effect of IBA was prominent than NAA on both varieties because NAA is sensitive to auxin degrading enzyme system[19,22]. The increase in number of roots per cutting is due to the lateral root formation, which is highly dependent upon IBA availability[16]. IBA stimulates the individual quiescent cells in pericycle to differentiate and proliferate to form lateral roots primordium. The cells in the lateral root primordia differentiate and elongate causing the lateral roots to emerge through primary root epidermis causing an increase in the number of root.

In Coratina, maximum number of roots per cutting (10.67) was observed at 4000 ppm IBA as compared to 10.00 at 3000 ppm IBA in case of Carolea. Different olive varieties differ in their response to auxin concentrations for rooting[9,15,20].

Length of roots of each cutting: Maximum root length (10.28 cm) was recorded at T_3 (3000 ppm IBA) followed by 8.25 cm at T_2 (2000 ppm IBA). Root lengths at T_4 (4000 ppm IBA) and T_1 (1000 ppm IBA) were 5.95 and 5.65 cm, respectively which indicated that there was a decline in root length when concentration of IBA was increased above 3000 ppm or decreased below 2000 ppm. The cuttings treated with NAA showed maximum root length of 6.30 cm at T_6 (1000 ppm NAA), while at T_5 (500 ppm NAA) and T_7 (1500 ppm) NAA) the root lengths recorded were 5.95 and 5.97 cm that were statistically at par. At T_8 (2000 ppm NAA) it was 5.05 cm, which reveals that NAA effects positively up to concentration of 1000 ppm and above this there are negative effects on root length. Minimum root length (4.95 cm) was observed at T_0 (control).

TABLE-3
EFFECT OF DIFFERENT CONCENTRATIONS OF IBA AND NAA ON AVERAGE LENGTH OF ROOTS OF EACH CUTTING OF OLIVE CULTIVARS CORATINA AND CAROLEA

	Average length of roots of each cutting (cm)		Means
	Coratina	Carolea	
T_0 (control)	5.00 cd	4.90 d	4.95 e
T_1 (1000 ppm IBA)	5.67 cd	5.63 cd	5.65 cde
T_2 (2000 ppm IBA)	8.17 b	8.33 b	8.25 b
T_3 (3000 ppm IBA)	10.20 a	10.37 a	10.28 a
T_4 (4000 ppm IBA)	5.90 cd	6.03 cd	5.97 cd
T_5 (500 ppm NAA)	5.97 cd	5.93 cd	5.95 cd
T_6 (1000 ppm NAA)	6.33 c	6.27 cd	6.30 c
T_7 (1500 ppm NAA)	5.933 cd	6.00 cd	5.97 cd
T_8 (2000 ppm NAA)	5.13 cd	4.97 cd	5.05 de
Means of Varieties	6.48 a	6.49 a	
	Treatments	Interaction (T × V)	Varieties
$LSD_{5\%}$ (34 d.f)	0.984	1.392	0.568

Means followed by same letter are not significantly different $p < 0.05$.

During root elongation and growth stage, IBA is required for cell division and elongation, IBA induces cell enlargement by extruding protons actively into cell wall region and resulting in decrease in pH activating wall loosening enzyme that promotes the breakage of key cell wall bonds and increases cell wall extensibility, hence causing an increase in cell size[11]. Auxin treatment also consequences in the mobilization and redistribution of nitrogen in the stem cuttings during rooting process which lead to enhanced metabolism resulting in increased growth and development of roots[8]. The results regarding average root length showed that the interaction among both varieties in relation with hormones applied, was statistically non significant. This might be due to the almost uniform internal physiology (carbohydrates level,

mineral concentrations and endogenous hormonal status) of cuttings obtained from different sources[23].

Root index: Root index is the product of number of root per cutting and average root length per cutting. Highest value of root index was (90.95) was obtained at T_3 (3000 ppm IBA) followed by (55.57) at T_4 (4000 ppm IBA). At T_2 (2000 ppm IBA) and T_1 (1000 ppm IBA) the results were 48.23 and 47.05 which were statistically at par. Highest root index value (27.29 and 27.86) for NAA treated cuttings were found at T_6 (1000 ppm NAA) and T_7 (1500 ppm NAA) respectively both were statistically placed at same level. Lowest value (14.85) was observed at T_0 (control).

It indicated that 3000 ppm IBA concentration produced strongest root system (Table-4). The increase in IBA concentration upto 3000 ppm has positive effect on root index and further increase in concentration tended to reduce root index value in both varieties. IBA is more effective than NAA regarding root index as IBA treated cutting had more number of roots per cutting and more average lengths than cuttings supplied with NAA.

TABLE-4
EFFECT OF DIFFERENT CONCENTRATIONS OF IBA AND NAA ON ROOT INDEX OF OLIVE CULTIVARS CORATINA AND CAROLEA

	Root index		Means
	Coratina	Carolea	
T_0 (control)	15.0 d	14.7 de	14.85 de
T_1 (1000 ppm IBA)	22.7 cde	24.4 cde	23.55 cde
T_2 (2000 ppm IBA)	38.2 cd	58.3 bc	48.23 c
T_3 (3000 ppm IBA)	78.2 b	103.7 ab	90.95 ab
T_4 (4000 ppm IBA)	62.9 bc	48.2 cd	55.57 b
T_5 (500 ppm NAA)	22.0 cde	17.8 de	19.88 de
T_6 (1000 ppm NAA)	23.2 d	31.4 d	27.29 d
T_7 (1500 ppm NAA)	27.7 de	28.0 de	27.86 de
T_8 (2000 ppm NAA)	17.1 d	18.2 d	17.67 d
Means of Varieties	34.12 a	38.3 a	
	Treatments	Interaction (T × V)	Varieties
LSD $_{5\%}$ (34 d.f)	1.265	1.790	0.731

Means followed by same letter are not significantly different $p < 0.05$.

Number of sprouts per cutting: Table-5 depicted that there was a significant effect of IBA on number of sprouts per cutting. Maximum numbers of sprouts per cutting (0.887) were found at T_3 (3000 ppm IBA) closely followed by 0.874 at T_4 (4000 ppm IBA) and they were statistically placed above all other treatments. At T_1 (1000 ppm IBA) and T_2 (2000 ppm IBA) the number of sprouts were 0.607 and 0.738, respectively. So, the increase in IBA concentration had positive effects on number of sprouts per cuttings up to the level of 3000 ppm. Minimum number of sprouts per cuttings

(0.483) were noted at T_8 (2000 ppm NAA) and 0.490 at T_0 (control) which were statistically at par. Highest number of sprouts (0.605) with NAA was observed at T_5 (500 ppm NAA) followed by 0.538 and 0.533 at T_6 (1000 ppm NAA) and T_7 (1500 ppm NAA), respectively. So it is concluded that there is non-significant effect of various NAA concentrations on number of sprouts per cutting.

TABLE-5
EFFECT OF DIFFERENT CONCENTRATIONS OF IBA AND NAA ON NUMBER OF SPROUTS PER CUTTING OF OLIVE CULTIVARS CORATINA AND CAROLEA

	Number of sprouts per cutting		Means
	Coratina	Carolea	
T_0 (control)	0.530 fg	0.450 g	0.490 d
T_1 (1000 ppm IBA)	0.660 de	0.553 de	0.607 c
T_2 (2000 ppm IBA)	0.790 bc	0.687 cd	0.738 b
T_3 (3000 ppm IBA)	0.970 a	0.803 bc	0.887 a
T_4 (4000 ppm IBA)	0.897 ab	0.850 ab	0.874 a
T_5 (500 ppm NAA)	0.660 de	0.550 efg	0.605 c
T_6 (1000 ppm NAA)	0.533 efg	0.543 efg	0.538 cd
T_7 (1500 ppm NAA)	0.600 def	0.507 fg	0.553 cd
T_8 (2000 ppm NAA)	0.497 fg	0.470 g	0.483 d
Means of Varieties	0.681 a	0.601 b	
	Treatments	Interaction (T × V)	Varieties
$LSD_{5\%}$ (34 d.f)	0.0908	0.1285	0.0524

Means followed by same letter are not significantly different $p < 0.05$.

It was observed that the number of sprouts per cutting is directly related with the number of roots per cutting so the maximum number of sprouts was found in those treatments which produced maximum roots per cutting. The effect of NAA on number of sprouts per cutting is inferior to IBA because application of synthetic NAA to stem cuttings can inhibit bud development, sometimes to the point at which no shoot growth will take place even though root formation has been adequate because NAA is easily translocated from base of stem cuttings to the aerial parts where it may have inhibiting effects[19]. Maximum number of sprouts per cutting (0.970) was observed in Coratina at 3000 ppm IBA in comparison with 0.870 sprouts per cutting in Carolea at 4000 ppm IBA concentration. This might have happened due to different internal physiology of cuttings of both varieties[7,9,16].

Survival percentage of rooted cuttings: The maximum survival percentage of rooted cuttings was found at T_3 (3000 ppm IBA) resulting in 90.68 %, followed by 84.48 % at T_2 (2000 ppm IBA) then 80.75 % at T_4 (4000 ppm IBA) (Table-6). These three treatments remained statistically higher than all other treatments. Minimum survival percentage (36.33 %) was found at

T_0 (control). Maximum survival percentage (53.67 %) with NAA was recorded at T_8 (2000 ppm IBA). Other treatments, T_5 (500 ppm NAA), T_6 (1000 ppm NAA) and T_7 (1500 ppm NAA) resulted in 46.75, 46.67 and 41.50 percent survival which were statistically at par.

TABLE-6
EFFECT OF DIFFERENT CONCENTRATIONS OF IBA AND NAA ON SURVIVAL PERCENTAGE OF OLIVE CULTIVARS CORATINA AND CAROLEA

	Survival percentage		Means
	Coratina	Carolea	
T_0 (control)	31.67 d	41.00 d	36.33 c
T_1 (1000 ppm IBA)	41.17 d	48.67 cd	44.92 bc
T_2 (2000 ppm IBA)	76.00 ab	92.97 a	84.48 a
T_3 (3000 ppm IBA)	89.40 a	91.97 a	90.68 a
T_4 (4000 ppm IBA)	81.50 ab	80.00 ab	80.75 a
T_5 (500 ppm NAA)	49.17 cd	44.33 cd	46.75 bc
T_6 (1000 ppm NAA)	40.00 d	41.33 d	40.67 bc
T_7 (1500 ppm NAA)	39.33 d	43.67 cd	41.50 bc
T_8 (2000 ppm NAA)	43.67 cd	63.67 bc	53.67 b
Means of Varieties	54.66 a	60.84 a	
	Treatments	Interaction (T × V)	Varieties
LSD $_{5\%}$ (34 d.f)	15.12	21.38	8.727

Means followed by same letter are not significantly different $p < 0.05$.

Survival percentage was directly related with root index as T_3 (3000 ppm IBA) had maximum survival percentage of rooted cuttings because they produced stronger root systems (maximum root number and highest average root length) which helped these cuttings to survive the earlier phase. Moreover, roots are the site for the synthesis of gibberrlic acid (GA) which might have boosted the shoot development and ultimately survival percentage. IBA treated cuttings had more survival percentages than NAA treated cuttings. Because of instability of NAA[4] and high incidence of death in cuttings treated with NAA[7]. Maximum survival percentage (89.40 %) was observed in cultivar Coratina at 3000 ppm IBA concentration as compared to 92.67 % survival at 2000 ppm IBA in cultivar Carolea. This variation in varieties in response to different concentrations of IBA regarding survival percentage was in conformity with[4,7,12,13].

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