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RESEARCH ARTICLE

The Role of Rooting Cofactors in Improvement of Rooting Response in Difficult-to-Root Cuttings of Nerium Oleander Var. White (Vs .Pink var.)

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Abstract

In the current study, it has been identified that white oleander is impossible or difficult-to-root (0.00%) while, pink oleander is slightly difficult -to-root cuttings (6.67 %) in absence of supplied auxin (treated with distilled water). However, to improve the capability of rooting response of white oleander cuttings (difficult-to-root variety), six treatments have been employed including identified (e.g. *o*-coumaric acid) and un-identified co-factors (e.g. whitetope seeds extract), individually and in combination with IBA and the results revealed the followings:-

- Some of these treatments were at high level of significance ($P_{0.05}$), verifying an original successful and economical acceptance by vegetative propagation of difficult-to-root cuttings (white oleander) that caused (induced) to root and increased vegetative traits, when supplied individually or in combination with IBA, the highest values are represented in the following treatments:-
- *O*-Coumaric acid in combination with IBA was induced rooting percentage (33.33%), average roots no.(23.67), chlorophyll content (28.03 SPAD unit) and length of branches (15.00 cm)
- Whitetope seeds extract in combination with IBA, induced rooting response (20%), roots no. (28.60)/cutting, leaves no. (39.30) and leaf surface area (15.80) cm^2
- IBA, Whitetope seeds extract, were induced rooting percentage (6.67%).
- As a comparison with pink oleander, all treatments that have been tested were stimulated rooting when supplied in combination with IBA , at a rate higher than white oleander except d/H_2O, *o*-Coumaric acid and whitetope extract treatments, where the response is zero. However, the maximum response in rooting % was found with combination between IBA and *o*- Coumaric acid followed by IBA+whitetope extract (33.33%). However, the discussion was focused on a)phenolic compounds as auxin protectors from destruction by IAA-O enzyme b) *o*-coumaric acid as anti-oxidant by activation of electronic conjugation c) plant extracts containing substances considered as precursor for its conversion into non enzymetic anti-oxidant such as GSH, proline. etc and its role in AsA-GSH cycle, or substances that enhances CAT, SOD enzymes that activates the anti-oxidant defense mechanisms and consequently enhances rooting response.

Keywords:- *Anti-oxidants, Co-factor, Difficult-to-root cuttings, IAA-O, IBA, Improvement of rooting response, Inhibitors, Nerium Oleandr, o-Coumaric acid and whitetope seeds extract.*

Introduction

Hardwood tree species, such as pecan (*Carya spp.)*, Oak (*Quercus spp.*) and black willow (*Salix nigra*), are important resources for the forest products industry worldwide and to the international trade of lumber and logs [1].The latter, described the fundamental mechanisms that triggers or regulates the initiation and development of ARF on stem cuttings from woody species is a complex physiological, genetical and environmental process and is still largely unknown. Generally, difficult-to-root cuttings of woody plants (e.g. White oleander) may be attributed to many reasons such as:-

First: Presence of inhibitors as it was the case in Pecan cuttings [2] and white oleander [3].

Second: Presence of anatomical barriers such as sclerenchyma circle in stem cortex

,resin ducts, secretory ducts or aggregation of vascular tissues [2,4] that retared root penetration toward epidermis during development phase.

Third: Increasing of IAA-oxidase activity [5] during root initiation phase prevents the accumulation of IAA.

Fourth: Decline of IAA content [6].

Fifth: Oxidative stress (new stress hormone) [7], supplying of cuttings by auxin enhance the enzymatic and non- enzymatic anti-oxidant mechanisms.

Sixth: Decline of rooting co-factors [8]. There is a wide spectrum of chemical substances both naturally and synthetic enhances rooting response induced- by auxin .Some terms such as, "auxin-synergists" and rooting co-factors has been employed to described these compounds .The later deals with the naturally occurring phenolic compounds [9]. It is noteworthy, not all co-factors, at least those present in non-woody cuttings are of phenolic nature [10]. Co-factors are formed in leaves [11] buds [12] or cotyledons [13], translocated and accumulated at cuttings bases then interact with auxin [14].

Some of these co factors are identified such as phenolic compounds (e.g. coumaric acid) [10], amino acids (e.g. Tryptophan [9], polyamines [15], Calcium [6], or ABA as presumed by [16]. In addition, to other UN-identified co- factors (e.g. Agueous or alcoholic etc. Plant extracts of easy-to-root plants). The aim of this study is to improve the ability of rooting response of difficult-to-root cuttings of white oleander by supplying *o*-Coumaric acid in addition to plant extract such as whitetope seeds extract as source of un- identified rooting co- factors, individually and in combination with IBA.

Materials and Methods

Stem cuttings *of Nerium oleander* Var. white and pink (15 cuttings/ treatment) were taken at 17/2/2014 from stock plants of (13-year old) free of diseases and insects, grown in Al- Tohmazia nursery / Babylon municipal. However, the length of the cuttings are (12-15) cm and diameters (0.5-1) cm, consist of pair of leaves (after removal of the free half of each leaf), two nodes with small bud at each node [17].Cuttings were treated by dipping the bases 3 cm in tested solutions for 24h in controlled cabinet with standard conditions [18] continuous light, at irradiance of 1500-1800 lux, temp. 25$\pm$ 1C ^{0}, and relative humidity 60-70 %).However after treatment, cuttings were cultivated in perforated black plastic boxes (25 × 17 × 8 cm) filed with peat moss + river loam soil (1:1) (v:v) that already treated with 5% Robin fungicide at average of (25-50) m^2 to prevents cuttings bases from fungal infection. Plastic boxes were kept in wooden lath house / college of science /Univ. Of Babylon after full irrigation .The later repeated once every 3 days until the end of experiment at 17/5/2014 [19].

Moreover, solutions were prepared at the following concentrations:-

IBA, 5× 10^{-4} M, *o*-Coumaric acid, 10^{-3} M and Whitetope seeds extract , 0.02% In addition, to combinations between IBA with the above substances, as well as distilled water as control treatments. Finally, completely randomized design (CRD) was employed and L.S.D was depended by using Genstat program for comparison between treatments mean for 3 replicates (15 cuttings/ treatments) on $P_{0.05}$ level [20].

Results

Table (1) shows the influence of different chemical substances and plant extracts on rooting response in terms of percentage of rooted cuttings of two varieties of *Nerium oleander* L. (white and pink). Obviously, cuttings of white var. is impossible to induce adventitious roots (0.00%) in absence of exogenous auxin (control treatment =d/H_2O) whereas, for pink var. is (6.70 %).However, the above result denoted white var. is absolutely difficult whereas pink var. is slightly difficult to root cuttings. Moreover application of auxin (IBA) caused (established) rooting response (6.70%) in white var. And promotes (33.33%) in pink Var. The result that deals with difficult to root cuttings (white var.) revealed the followings:-

- Treatment that absolutely not induce rooting response is d/H_2O and *o*-Coumaric acid (0.00%, dead cuttings).
- Treatment that induced the lower limit of response (6.67%) included: - IBA and whitetope seeds extract. The above percentage (6.67%) is represents one cutting out of 15 cuttings/ treatment. So it is statistically negligible for all triats.

- Combination between IBA +whitetope seeds extract induced 20% of rooting response.
- Treatment were caused the higher induction of rooting response (33.33%) for white oleander (difficult- to root cuttings) represented by combination between IBA+ O-Coumaric acid.

On the other hand, results that deals with pink var. revealed that all treatments have promontory effect except o-Coumaric acid when supplied individually, have response equal to 0.00%. However, the promotion having different levels, some at 6.67% of rooting response such as, d/H_2O and whitetope seeds extract (negligible), some at 33.33% represent for IBA and the combination (IBA + whitetope seeds extract). In addition, the highest degree of induction 60.00% represent by combination between IBA + *o*-Coumaric acid. On the other hand, the best rooting co-factors that supplied for pink var. is represented by combination between IBA with *o*-Coumaric acid, that approaches its maximal level (60.00%) and to lower level (33.33%) with whitetope seeds extract.

Table 1: Effect of chemical treatments on rooting response of rooted cuttings and roots number, length and branches number and length of *Nerium oleande*r var. white and pink

Treatments	Rooting (%)		Roots no. /cuttings		Roots length /cutting		Branches No. /cutting		Branches length/cutting	
	White	Pink	White	Pink	White	Pink	White	Pink	White	Pink
Distilled water	0.00	6.67	__	__	__	__	__	__	__	__
IBA	6.67	33.33	__	13.00	__	9.00	__	2.33	__	8.67
o-Coumaric acid	0.00	0.00	—	—	—	—	—	—	—	—
Whitetope Seeds extract	6.67	6.67	—	—	—	—	—	—	—	—
IBA+o-Coumaric Acid	33.33	60.00	23.67	26.00	9.00	12.33	2.67	3.00	15.00	16.00
IBA+Whitetope Seeds extract	20.00	33.33	28.76	10.33	9.00	9.17	2.67	3.00	14.67	10.67
$LSD_{0.05}$	1.84	4.42	1.26	3.78	0.49	1.47	0.18	0.54	0.69	2.06

Discussion

In this study, white oleander has been identified as absolutely difficult- to-root cuttings whereas, pink oleander as (slightly hard)-to-root cuttings (slightly hard) .However, rooting response in terms of percentage of rooted cuttings of white var. is (0.00%) while, for pink var. Is (6.67%) in absence of auxin is (with d/H_2O) Table (1). Physiologically, it is well known that auxin has the priority in their promotion of adventitious root formation (ARF) in cuttings. Table (1) revealed that application of IBA for *Nerium oleander* cuttings can't caused (established) rooting response (6.67 % = 0.0 ignored statistically) in white var. (difficult-to-root) and promotes (33.33 %) in pink var.

This may be attributed to the presence of inhibitors as it was the case in pecan [2] and in white oleander [3]. The results of the current study confirmed the decline of free IAA level as well as bound-IAA in leaves and stem of stock plant of white oleander compared to pink oleander (Appendix-1).However, such decline was coincided with increasing IAA-oxidase activity in leaves and stem of stok plant (white Var.) compared to its decline in (pink Var,) (Appendix-1) .Obviously, supplying of auxin exogenously can easily solve such problem , but some plant species are impossible to induce ARF even when supplied with inductive auxin treatment (IAT). These findings were confirmed with different spp. such as *Citrus sinensis* L. [21] and *Quercus rubra* [1]. In this case auxin acts as new stress hormone [7].Such findings may be attributed to the absence of non- auxin substances such as, rooting co-factors [22]. For improvement the ability of rooting response of difficult-to-root variety (white oleander) cuttings, six treatments has been employed, which are represents the best positive results were obtained from our previous studies that deals with rooting response on mung bean fresh and aged cuttings [23].

However, understanding ageing concept (e.g. decline or lossing the response of plant tissues to auxin) and its control by physic-chemical means considered as the key to discover the reasons of difficulty in rooting particularly in woody cuttings [24]. It is noteworthy, those two out of six treatments that involved in current study has been caused the higher induction of rooting response in white oleander (Hard-to- root cuttings Table (1). These are:-

- *O-Coumaric* acid in combination with IBA induced rooting percentage (33.33%), roots number cutting^{-1} (23.67), root length (9.00 cm) and branches number cutting^{-1} (2.67).
- Whitetope seeds extract in combination with IBA induced rooting percentage (20%), roots number cutting $^{-1}$ (28.60), root length (9.00 cm), and branches number cutting $^{-1}$ (2.67).

Surprisingly, the above rooting percentage in treatment (a) and (b) are caused an increase in rooting percent of 379% and 199.8% respectively over the control treatment (IBA alone =6.67%).Such treatments at high level of significance ($P_{0.05}$) that verifying an original successful and economical acceptance for vegetative propagation of white oleander as example of difficult-to- root cuttings.

On the other hand, *o*-Coumaric acid as identified Co-factor had no effect in ARF when supplied individually for both varieties (white and pink oleander (rooting response = 0.00%).On the same direction, [25] confirmed the same findings , that o-coumaric acid had no effect on processes that occurred during ageing of mung bean cuttings that leds to diminish rooting response.

Whereas *o*-Coumaric acid in combination with IBA induced rooting percentage (33.33%) in white oleander with an increase in most traits specially chlorophyll content (28.03)SPAD (appendix 2)and branches length (15 cm) (Table-1).Such increase was coincided with increase of (IAA-Oxidase / IAA)ratio (1.71 / 1.46) in leaves and decline of (IAA/ Cytok) ratio(1.46/ 13.67) in leaves (appendix-3) as well as increase of (C/N)ratio (6.34/ 2.88) (Table 2).

Although, the above ratios were differs in some treatments and between oleander varieties, but collectively direct the metabolism for increasing the cytokinin level that enhances shoot development. Consequently, [26] was pointed out the necessity of structural formula of phenols in their capability to induce ARF. However, o-Coumaric acid is characterized by high acidity compared to cinnamic acid for example, because of presence of OH-group at *ortho-position*. The function of this position is to push electrons and hence, leads to increase the active electronic conjugations that induce the area of OH-group, ring, and side group in the direction of (COOH) group [27]. The above explanation gave strong character for o-Coumaric acid as anti-oxidant.

It is, because of having a high scavenging character of oxidizing electron by persisting it longer inside the ring. This was rised through ARF in terms of hight rooting percentage (33.33%) in white oleander (Hard-to- root) [28].Was reported that phenolic compounds act as inhibiters for oxidative system of IAA via trapping process for free radicals that formed on positive in dole -3-acetic acid (Indoly cation radical =IAA^{+}). Thereafter prevent its continuous oxidation .Generally, it is well known, that phenolic compounds acts as protectors from destruction by IAA-O. Consequently, the accumulation of total phenols increase into 45% during the 1st 24 h of rooting and continue to day -4 in mung bean till the formation of root primordia [29].

Such increase was coincided with increasing IAA level and declining of IAA-O during initiation phase [30]. Whereas, increasing IAA-O activity that caused declining of IAA level considered necessary during the growth of development phase involves conversion of root primordia into visible roots [31]. Although, phenolic compounds considered as rooting co-factors [10], but it can't acts alon more than control (d/H_2O) unless supplied in combination with auxin (IBA + O-Coumaric acid).

However, o-Coumaric acid inhibits the other traits under the current study in both varieties when supplied alone (rooting % =zero). While, in combination with IBA caused the highest rooting percentage in white oleander (33.33%) of in pink oleander (60%) .On the other hand, whitetope seeds extract in combination with IBA was caused induction of rooting percentage (20%), in white oleader, which represent 199.8% over control.

In addition to increase roots number (28.67 root/cutting), leaves number (39.33 leaf /cutting), leaf area (15.80 cm^2), SOD, CAT, As A and proline (Data not presented), compared to combination between (IBA + o-Coumari acid).

However, whitetope seeds extract have no effect when supplied individually, and the rooting % is very low (6.67%) for both varieties. Whereas, when supplied in combination with IBA induce 20% and 33.33% in white and pink varieties respectively .This case suggest the presence of un-identified co-factors in whitetope seeds extract, but it acts when auxin supplied, according to the concept of Rhizocaline complex theory [14]. The above proposal has been agreed with [32] using combination between (IBA + Anise (*Pimpinella anisum*) or whitetope (*Cardaria draba*) extract to improve rooting response of slightly hard –to-root cuttings of *Citrus aurantium* represented by 200% over control treatment (IBA).

Additional confirmation in the same trend has been obtained by [33] using IBA with extract of ginger as well as [34] using IAA + salicylic acid, both with mung bean cuttings. Finally all combination were employed in the current study has been promoted significantly an increase in rooting response compared to IBA when supplied individually. This confirming that, although IBA has the priority in ARF but it doesn't acts alone in absence of endogenous rooting co-factors in difficult-to-root cutting. However, the significant increase that obtained by supplying *O*-Coumaricacid or Whitetope seeds extract with IBA simultaneously represent the role of un-identified co- factors (e.g. whitetope seeds extract or other extracts that acts as anti-oxidant (e. g. *o*-Coumaric acid).

The same as with supplied cysteine during its conversion into glutathione (GSH) and its role as non-enzymatic anti-oxidant in defense mechanisms such as GSH- AsA cycle [35, 36].Clearly. The current study revealed decline in As A level as well as proline in leaves and stems of stock plants (white oleander) compared to (pink oleander), represented as non-enzymatic antioxidant (Table 1).

Table 1: Free and bound IAA concentration, IAA-Oxidase activit, Ascorbic acid and proline content in stem and leaves of Nerium oleander (white and pink varieties) in stock plants (initial amounts)

Varieties	Free IAA (Mm)		Bound IAA (Mm)		IAA-oxidzse (mg unoxidized IAA/h/g f.wt		Ascorbate (mg/g f.wt.)		Proline content (mg/g f.wt.)	
	Leaves	stem	leaves	stem	leaves	Stem	Leaves	Stem	Leaves	Stem
Nerium oleander Var.white	0.23	0.27	0.48	0.11	3.57	2.31	2.43	1.85	2.75	0.52
Nerium oleander Var.pink	1.19	1.33	0.64	0.14	1.99	1.23	0.96	2.78	2.69	1.05
$LSD_{0.05}$	0.02	0.02	0.03	0.02	1.10	1.23	0.16	0..22	0..22	0.02

Table 2: Effect of chemical treatments on protein content carbohydrate content in leaves and cuttings bases and chlorophyll content (SPAD unit) of *Nerium oleander* var. white and pink

Treatments	Protein content (mg/g F.wt.) in				Carbohydrate content (Mg/g F.wt.) in				Chlorophyll content (SPAD) in leaves	
	Leaves		cuttings base		leaves		cuttings base			
	withe ص	Pink	With	pink	in withe ص	Pink	withe	Pink	ص withe	Pink
Distilled water	—	—	—	—	—	—	—	—	—	17.83
IBA	__	4.74	__	0.58	__	9.99	__	10.19	15.10	35.50
o-Coumaric acid	—	—	—	—	—	—	—	—	—	—
Whitetope Seeds extract	—	—	—	—	—	—	—	—	—	—
IBA +o-Coumaric Acid	2.88	3.46	2.22	1.41	6.34	8.71	6.89	19.19	28.03	36.43

IBA+Whitetope Seeds extract	2.33	1.42	1.36	2.02	10.95	10.20	7.17	4.75	23.70	29.33
$LSD_{0.05}$	0.26	0.77	0.16	0.48	0.22	0.22	0.18	0.54	2.80	8.39

Table 3: Effect of chemical treatments on free IAA, free cytokine in) in leaves and cuttings bases and IAA-Oxidase activity of *Nerium oleander* var. white and pink

Treatments	Free IAA (Mm) Leaves		Free IAA (Mm) in cuttings base		Free zeatin (Mm) Leaves		IAA-oxidase Activity (mg unoxidized IAA/ h/g f.wt. in			
							leaves		Cutting base	
	withe	Pink	With	pink	in with	pink	withe	Pink	withe	Pink
Distilled water	__	__	__	__	__	__	__	__	__	__
IBA	2.74	5.21	2.79	3.71	9.96	13.71	__	0.97	__	1.47
o-Coumaric acid	—	—	—	—	—	—	—	—	—	—
Whitetope Seeds extract	—	—	—	—	—	—	—	—	—	—
IBA+o-Coumaric Acid	1.46	7.63	2.34	9.73	13.67	6.20	1.71	0.66	2.55	0.59
IBA + Ascorbic acid	1.10	10.68	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
IBA+Whitetope Seeds extract	6.25	9.57	3.40	6.18	3.89	13.45	1.18	3.33	3.73	3.33
$LSD_{0.05}$	0.44	1.31	0.58	1.73	0.98	2.95	0.08	0.23	0.13	0.38

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