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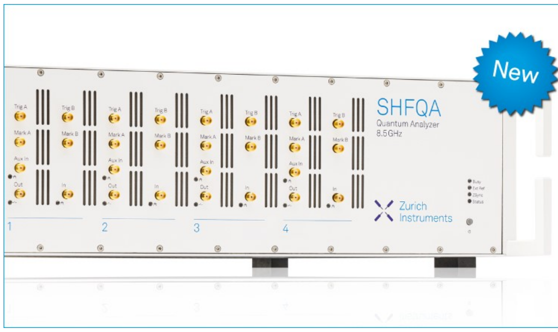

The Role of Ascorbic Acid and Iba in Improvement of Rooting Response of Difficult to Root Cuttings of Acacia Spp

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Abstract. Acacia leprosa is difficult- to- root cuttings, Acacia cyanophylla L. is slightly hard-to-root cuttings in absence of supplied auxin. However, improvement of root ability of Acacia leprosa cuttings involved employment of four treatments (distilled water, IBA , Ascorbic acid (AsA) and (AsA in combination with IBA) and the results revealed the followings AsA alone is the best treatment that caused (induced) rooting and increased vegetative traits when supplied individually rather than in combination whit IBA of difficult-to- root cuttings (Acacia leprosa). AsA when supplied individually (alone) increased to 20.00 % of rooting percentage ,average of roots number/ cutting (21.76) , length (18.80 cm), branches number /cutting(4.93) and length (15.67 cm) in Acacia leprosa, compared to distilled water treatment that developed (0.00 %) for all traits (dead cuttings).In addition , IBA when supplied in combination with AsA ,reduced rooting percentage into 0.00% in Acacia leprosa and 26.67 % in Acacia cyanophylla L. In contrast, with Acacia cyanophylla L, all treatments (except distilled water) that have been tested were stimulated rooting whether individually or in combination with IBA , at rate higher than Acacia leprosa. However, the maximum rooting percentage was found with AsA when supplied individually (66.67%). Furthermore, the discussion was focused on considering AsA as anti-oxidant by activating the electronic conjugation system and its role in AsA –GSH cycle. Collectively, AsA inducing rooting response by increasing IAA level via inhibiting IAA- oxidase or GH3 enzyme activity.

Keywords. IBA, Ascorbic acid , Difficult-to-root-cuttings, Acacia leprosa and rooting response

INTRODUCTION

Tree species with hardwood, such as Pecan (*Carya spp.*), Oak (*Quercus spp.*) are important resources for forest industry worldwide and to the international traded by lumber and logs (Pijut et al., 2011). Generally , the fundamental mechanisms that trigger or regulate the initiation and development of adventitious root formation (ARF) on stem cuttings from woody species is a complex physiological , genetic and environmental process and is still unknown (Druege et al.,2019).

Ascorbic acid (AsA), promotes significantly rooting response in aged mung bean (*Vigna radiate* L.) cuttings ($P_{0.01}$) at high concentrations (200 – 500 $mg.L^{-1}$) more than fresh cuttings . These findings were coincided with increasing (56.80%) IAA content (Shaheed, et al., 2010). In addition, (AL-Ryahi, 2011) obtained the same results by using the same cuttings (Mung bean), involving the role of AsA in detoxification of cadmium as anti- oxidant in

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resisting oxidant stress. It is noteworthy, that (Shaheed et al., 2009) found that the decline in rooting response of aged mung bean cuttings (62.30%) was coincided with declining of total AsA (66.80) . Seemingly, AsA may acts against difficulty –to-root cuttings as it was the case in stressful plants under heavy metals toxicity or ageing phenomenon (e.g. decline or less of plant tissue response to auxin (Shaheed, 2017). The aim of this study was to improve the rooting ability of difficult –to-rootcuttings of *Acacia spp* by supplying AsA individually and in combination with IBA.

MATERIALS AND METHODS

Cuttings of *Acacia cyanophylla* L. and *Acacia leprosa* L. varieties were taken in 10 February 2018 from stock plants (10-year old) which are free from disease and injuries insect , grown in house garden. The length of cuttings are (12-15cm) and diameters was (0.3 -1 cm) with a pair of leaves .Cuttings were treated by dipping the bases (3 cm) in tested solutions for 24 h controlled cabinet with standard conditions (Anyasi ,2011) continuous light , at irradiance of 1500-1800 lux , temperature 25 $-^{+}1$ and relative humidity (60 -70%). Furthermore, Cuttings after treatment were cultivated in perforated black plastic boxes (25 ×17 × 8 cm) filled with (peat moss + river loam soil) volume ratio (1:1) that already treated with 5% Robin fungicide at average of (25-50 m^2) to prevent cuttings bases from fungal infection. Plastic boxes were kept in lath house /AL-Musaib Technical college / AL-Furat Al-Awsat technical University. The later repeated once (irrigation) every 3 days until the end of experiment in 1/ 10/ 2018(Bojja , et al ., 2018). Moreover, solutions were prepared at following concentrations :-

1. Distilled water
2. IBA, 5×10^{-4} M.
3. (Ascorbic acid, 750 mg L^{-1}).
4. (IBA, 5×10^{-4} M + Ascorbic acid ,750 mg L^{-1}) .

Consequently, combination between IBA and AsA was prepared by doubling the concentration and reducing the volume into half (1/2) of required volume of each substance .Finally , complete randomized design (C.R.D) was employed and L. S. D. was depended by using Genstat program for comparison between treatments means for 3 replicates (15 cuttings/ treatment) on the 5% probability level (Glantz , 2005)

RESULTS AND DISCUSSION

Acacia leprosa has been identified as acacia is absolutely difficult-to-root cuttings whereas, Acacia cyanophylla L. is slightly hard- to-root cuttings. Table (1) shows that rooting percentage of Acacia leprosa and Acacia cyanophylla .L. were 0.00% in absence of supplied auxin (distilled water) .

Controlling the root ability by improving the rooting response of difficult-to- root cuttings (Acacia leprosa) involved four treatments (distilled water, indole butyric acid (lBA), ascorbic acid (AsA) and combination between lBA + AsA . Excision of cutting from mother plants greatly modified plant hormone homeostasis in the isolated shoot segment, while signals are reinforced by exogenous auxin (Della Rovere et al., 2013) it is widely accepted that auxin is an effective inducer of ARF (Pacurar et al., 2014). Results of table (1) revealed that supplying of auxin (IBA) for acacia cuttings did not cause (establish) rooting percentage = 0.00% with other vegetative traits because cutting were dead) in Acacia leprosa (difficult- to- root) and promotes (53.33%), in Acacia cyanophylla L(Radhi and Hussein, 2017). This may be attributed to the presence of inhibitor as it was the case in Pecan (wally et al.,1980) and in Acacia leprosa, or decline of endogenous IAA content (Hartmann et al., 1990), that confirmed in white oleander (difficult – to-root) for both free and bound IAA(Shaheed & Radhi, 2019). However, exogenous supply of auxin can easily solve such problem, but some species are impossible to induce ARF even when supplied with inductive auxin treatment (IAA) such as Citrus sinensis L. (Khudari & Thewaini, 1957) and Quercus rubra (Pijut et al., 2011). Auxin acts in this case as new stress hormone (Bielach et al ., 2017). These findings may be attributed to the absence of non- auxin substances called rooting Co-factors (Genve , 1990). Alternatively, it may be related to increasing IAA-oxidase activity (Gemici et al., 2002), during root initiation rather than root growth and development phase (Husen, 2012). The latter case (IAA-O activity) was confirmed in white oleander(difficult–to-root) vs. pink oleander is (slightly hard -to-root cuttings in absence of supplied auxin in leaves (2.03 vs. 0.46) and in cutting base (3.03 vs. 0.67) Mg unoxidized 1AA / h/g F.w.t. (Shaheed & Radhi , 2019).

It is noteworthy, that one out of four treatments that involved in the current study. The higher induction ofrooting response in *Acacia leprosa* , (Hard-to-root) , table (1) is Ascorbic acid when supplied individually(alone) induced rooting percentage (20.00%), average of root number / cutting (21.67) , root length (18.80 cm) , number of branches /cutting (4.93) and branches length/ cutting (15.67 cm). In contrast, IBA when supplied in combination with AsA rooting response was reduced into 0.00% It is noteworthy, that AsA treatment having high AsA supplied individually, is the unique treatment that caused the highest induction of rooting percentage not only in *Acacia leprosa*. (20.00%) but in *Acacia cyanophylla* L. (66.67%) also (Table-1). However, the above responses were declined when IBA was supplied in combination with AsA into (0.00%) in *Acacia leprosa*, and (53.33%) in *Acacia cyanophylla* L. Meanwhile, mean root number and root length per cutting in both *Acacia leprosa* and *Acacia cyanophylla* L. are approx. equal (0.00 and 18.13 roots) and
(0.00 and 14.58 cm) respectively (table 1) . Moreover, AsA caused approx. an equal increase (highest values) of protein content in leaves both varieties (5.04 & 4.86 mg /g f. wt.) respectively. (Data not presented).

Clearly, IBA acts a severe antagonist to AsA when supplied simultaneously in combination. The above results were confirmed by (AL-Ryahi, 2014) she denoted the absence of synergistic action between AsA and IBA in mung bean cuttings. In other words, AsA acts a lone in difficult- to- root cuttings such as *Acacia leprosa* with no need to IBA and caused the highest percentage of rooting response in both varieties. AsA may acts as plant hormone (Forti and Elli, 1995) or as co- enzyme (Davey et al., 2000) or as anti- oxidants (Thomas at al., 1992 ; padh , 1990). The latter case was confirmed by (Shaheed et al,. 2010) about the significant increase in rooting response if fresh and beyond that limit in aged mung been cuttings particularly at high concentrations of AsA 200-500 mg L^{-1} (AsA, employed in the current study at 750 mg L^{-1}). These results were denoted, that ascorbate is capable to stop ageing process completely as it (ageing) was considered as physiological stress (Suhaib et al., 2018). It was attributed to AsA as anti- oxidant having electronic conjugation system between carbon atoms no.(1) and no(3) [3 carbon atoms and 3 groups of Oxygen]. Similarly, cysteine converts into Galutathime (GSH) and its role as non-enzymatic anti-oxidant in defines Mechanism(e.g. GSH –AsA cycle) (Sharma et al., 2012) .

Recently, a strong transcriptional regulation of auxin responsive GRETCHEN $HAGEN_3$ (GH_3) genes was monitored in cuttings base of several plant sp.(review in Druege et al., 2016). However GH_3 genes may encode IAA-amido synthetases, , conjugating IAA to amino acids , but may also have other function in AFR (Gufierrez et al., 2012).

Moreover, recent study of Cano et al., (2018) should that the poor ARF of specific correlated with higher levels of DcGH3-1 transcript and of IAA-ASP at the expense of IAA in the stem base of the cutting during adventitious root induction when compared to a good -rooting cultivar, could be partially recovered by chemical inhibition of GH_3 enzyme activity. Additional results, confirmed a significant decline in AsA as well as proline levels (initial amount in leaves of stock plants (*Acacia leprosa*) compared to (*Acacia cyanophylla* L.), represented as non – enzymatic anti-oxidant s(Appendix 1).

Collectively, application of AsA caused increasing of endogenous anxin (lAA) (Shaheed et al., 2010) Via inhibiting (IAA-oxidase enzyme) (Stange , 1984). Thereafter, accumulation of IAA at the right level, at the right place and at the right time will induces ARF.

TABLE 1. Effect of ascorbic acid and IBA on rooting percentage of rooted cuttings, roots number and length and branches number and length of Acacia leprosa and Acacia cyanophylla L

Treatments	**Rooting (%)**		**Roots no. cutting^{-1}**		**Roots length cutting $^{-1}$**		**Branches no cutting^{-1}**		**Branches length cutting^{-1}**	
Varieties	**Acacia** a	**Acacia** *a*	**Acacia**	**Acacia** *A*	**Acacia** a	**Acacia** *a*	**Acacia** a	**Acacia** *a*	**Acacia** a	**Acacia** *a*
Distilled watter	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
IBA	0.00	53.33	0.00	15.10	0.00	18.10	0.00	2.93	0.00	9.68

Treatments	Rooting (%)		Roots no. cutting^{-1}		Roots length cutting $^{-1}$		Branches no cutting^{-1}		Branches length cutting^{-1}	
Varieties	Acacia	Acacia	Acacia	Acacia	Acacia	Acacia	Acacia	Acacia	Acacia	Acacia
Ascorbicacid (AsA)	20.00	66.67	21.67	19.00	18.80	23.77	4.93	5.61	15.67	19.90
IBA+AsA	0.00	26.67	0.00	18.13	0.00	14.58	0.00	3.80	0.00	16.70
L.S.D.$_{0.05}$	0.47	0.66	0.67	0.95	0.67	0.94	0.62	0.88	0.59	0.83

Appendix 1. Ascorbic acid (mg/g f. wt.)and proline (m/g f. wt.)contents in leaves of Acacia leprosa and Acacia cyanophylla .L in stock plants (initial amounts)

Varieties	AsA	Proline
Acacia leprosa	1.82	0.61
Acacia cyanophylla L.	2.68	1.53
LSD $_{0.05}$	0.21	0.02

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