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Vol. 9(50), pp. 3632-3637, 11 December, 2014
DOI: 10.5897/AJAR2014.9239
Article Number: 456AE6848972
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

A comparative study to improve rooting of English lavender stems cuttings

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Received 13 October, 2014; Accepted 18 November, 2014

An experiment was conducted to compare the ability of two bacteria strains from *Agrobacterium rhizogenes* (DSM30200 and A4), yeast extract (0.5, 1 and 2 g/L) and indole butyric acid (IBA) (50, 100 and 150 mg/L) on rooting of the terminal cuttings of *Lavandula angustifolia* L plant. *A. rhizogenes* strains were more effective than most of the other treatments in increasing rooting percentage, roots number and increase of cuttings length except using IBA at 100 and 150 mg/L, as no significant differences were found between them. In contrast, the controls cuttings and cuttings treated with yeast at higher concentrations produced the lowest rooting percentage. Same trend treatments which showed higher rooting characteristics also recorded higher percentage of total carbohydrates, phenol and N percentage. On commercial scale use of two examined strains of *A. rhizogenes* for rooting of terminal cuttings of *L. angustifolia* can reduce the use of synthetic auxins like IBA.

Key words: *Agrobacterium*, yeast extract, IBA, lavender, stem cutting.

INTRODUCTION

Rooting of stem cutting is an important horticultural tool for the propagation of ornamental, medicinal and aromatic plants. Factors affecting the rooting of cuttings can include application of auxins, seasonal timing at which cuttings are collected, type of cuttings, wounding, physiological and histological factors as well as an imbalance of endogenous phytohormones. Synthetic auxins are known to stimulate adventitious root formation on cuttings, but the world was going to reduce the use of synthetic chemical compounds as a result for its risks to the environment. Indole butyric acid (IBA) is the most widely used among the root promoting auxinic compounds because it is non-toxic and effective over a wide range of species (Couvillon, 1988). *Lavandula angustifolia* L. "Munstead" (English lavender) family Lamiaceae is one of the medicinal and aromatic plants which can commercially cultivate on Egypt especially in the calcareous soils in the north coast of Egypt and other soils. It is a hardy perennial shrub rich in aromatic essential oils and there is an increase in demand for this plant to be used in pharmaceutical preparations, culinary herbs, cosmetics industry, etc.

Also, it is a very beautiful ornamental pot plant and has ability for cut and formation. Lavender plants are commercially propagating by stem cuttings, but have a poor rooting ability (Andrade et al., 1999). The seeds have a poor germination percentage (Takano et al., 1990). As well, *Agrobacterium rhizogenes* is one of the

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plant growths promoting rhizobacteria which have recently been used to stimulate root growth. *A. rhizogenes*, the causative agent of hairy root syndrome is a common soil organism capable of entering a plant through a wound and causing proliferation of root (McAfee et al., 1993). Also, the importance of yeast in some physiological properties may be due to that it contains after the composition of a wide group of amino acids and vitamins. In addition, yeast is a natural source of many growth substances as a protective agent, and most of nutritional elements (Na, Ca, Fe, K, P, S, Mg, Zn and Si) and contained cytokinin as well as some organic compounds (Nagodawithana, 1991). It was also investigated for improving growth and flowering of some crops (Ahmed, 1998; El-Mogy et al., 1998; Fathy et al., 2000).

The aim of this study was to investigate the effect of using two strains from *A. rhizogenes*, yeast extracts and evaluate and compare both of them with different concentrations from IBA to establishment of a commercial propagation protocol for this plant as well as minimizing the use of synthetic auxins.

MATERIALS AND METHODS

Experimental design

Terminal cuttings of *L. angustifolia* L. "Munstead" (English lavender) (15 cm long) were obtained from the Experimental Station Of Ornamental Plants, Faculty of Agriculture, Mansoura University, Egypt in January. Cuttings basal part of 0.5 mm each (fresh wounded) was dipped in 0.5 ml bacterial suspension for 24 h in complete darkness (Damiano et al., 1995) and planted in a plastic black pots 5 cm in diameter at a depth of 3 to 5 cm approximately each contained peat moss:vermiculite 1:1 (v/v). Planted pots were placed on the soil surface in a greenhouse with mist irrigation unit.

Bacterial strains and cultivation

Two strains of virulent *A. rhizogenes* (DSM30200 and A4) were obtained from Cairo Mircen Laboratory, Egypt. The bacterial cells were aerobically cultured on Luria Bertani (LB) medium supplemented with 10.0 g/L tryptone, 5.0 g/L yeast extract, 10.0 g/L NaCl and 15.0 g/L agar at Ph 7 and left for 3 days at 27 ± 1°C. From the previous cultured bacteria transformation was done to inoculate 5 ml of LB liquid medium and incubated overnight at 27 ± 1°C under continuous shaking at 120 rpm. One milliliter (1 ml) of overnight grown *Agrobacterium* culture was used to inoculate 25 ml of LB medium shaken till an optical density reading of 0.6 at 600 nm wave lengths, followed by centrifugation under cooling at 4200 rpm for 20 min (Draper et al., 1988). The supernatant was discarded and *Agrobacterium* cells precipitate pallet was suspended in 25 ml of Murashige and Skoog (1962) (MS) liquid basal medium.

Yeast extract and indole butyric acid (IBA) treatments

Yeast extract was used at concentrations of 0.5, 1 and 2 g/L. Long with IBA at 50, 100 and 150 mg/L plus a control treatment (cuttings dipped on distilled water).

Statistical analysis

Simple experiment in randomize complete block design was used. All experiments were repeated twice and similar trends of data were obtained. The number of terminal cuttings in each of the different treatments was 15. Data of percentages were either converted by arcsin transformation prior to analysis of variance (ANOVA) ($p < 0.05$). Multiple comparison test was performed using Duncan's multiple range test ($a = 0.05$) following ANOVA. All statistical analyses were performed using Costat version 6.303 (copyright 1998 - 2004 CoHort software, Monterey, CA).

RESULTS AND DISCUSSION

Adventitious root formation and rooting parameters

Adventitious roots induced on the treated terminal cuttings were evaluated through the measurements of four physical root parameters including cuttings rooting percentage, average roots number, roots length and increase in cuttings length. It was clear from data in Table 1 and Figure 1 that the highest rooting percentage of 88.89% was obtained when lavender cuttings were treated with the two bacteria strains (DSM30200 and A4). This extensive root formation is caused by *A. rhizogenes* which induces formation of adventitious roots at sites of infection, resulting in a hairy-root phenotype and expression of *A. rhizogenes* genes encoded by transfer-DNA (T-DNA), a fragment of DNA originating from a root-inducing plasmid (Ri) (Moore et al., 1979). Also, this bacterium transfers its T-DNA which is a portion of the large plasmid called the root-inducing plasmid (pRi) to susceptible plant cells where the T-DNA, if integrated into the nuclear genome of the plant cell, will encode genes that direct the synthesis of auxin (indole-3-acetic acid) and/or increase the sensitivity of the transformed plant cells to auxin plus to the endogenous production of auxin and/or an increase in auxin sensitivity can lead to the formation of hairy roots (Hatta et al., 1996). But, it was observed that there were no significant differences between cuttings treated with bacteria strains, yeast extract at 0.5 and IBA at all its concentrations as they ranged from 44.44 to 55.56%.

On the other hand, the lowest rooting percentage of 22.23% was tabulated for cuttings treated with 2 g/L yeast extract. Data in the same Table 2 and Figure 2 showed that the DSM30200 bacteria strain produced the highest roots number of 6.44 roots when compared with most of the other treatments. Followed by 4.33, 4.45 and 4.56 roots when A4 bacteria and IBA at 100 and 150 mg/L were used, respectively, no significant differences were shown between them. Exogenous IBA may induce changes in enzyme activities [peroxidase and indole acetic acid (IAA) oxidase] and in their effectors contents (phenolic) allowing the establishment of the favorable endogenous hormone balance. It is well known that applied auxins induce modifications in their own metabolism, mostly by conjugation, and in other

Table 1. Effect of Agrobacterium strains, yeast extract and indole butyric acid (IBA) concentrations on rooting parameters of English lavender stem cuttings.

Rooting substance		Rooting	Roots	Roots	Increase in cuttings
Treatment	**Concentrations**	**percentage**	**number ± SD**	**length ± SD**	**length (cm) ± SD**
Control	0	33.34 ± 0.00^{b}	0.56 ± 0.88^{bc}	0.72 ± 1.15^{b}	3.17 ± 1.41^{cd}
Bacteria strains	DSM30200	88.89 ± 6.24^{a}	6.44 ± 3.5^{a}	3.73 ± 2.46^{a}	3.94 ± 1.42^{abc}
	A4	88.89 ± 6.22^{a}	4.33 ± 5^{abc}	3.17 ± 3.28^{ab}	4.39 ± 2.62^{abc}
Yeast (g/L)	0.5	44.45 ± 12.91^{ab}	0.33 ± 0.71^{c}	0.67 ± 1.66^{b}	3.5 ± 1^{bcd}
	1	33.33 ± 0.00^{b}	0.89 ± 1.45^{bc}	0.83 ± 1.69^{b}	3.5 ± 1.22^{bcd}
	2	22.23 ±12.83^{b}	1.11 ± 1.70^{bc}	1 ± 1.85^{ab}	2.83 ± 1.66^{cd}
IBA (mg/L)	50	55.55 ± 12.24^{ab}	1 ± 1.32^{bc}	0.87 ± 1.06^{b}	1.94 ± 1.13^{d}
	100	55.56 ± 12.91^{ab}	4.45 ± 5.98^{abc}	2.89 ± 4.34^{ab}	5.61 ± 2.42^{a}
	150	44.44 ± 6.24^{ab}	4.56 ± 7.23^{ab}	2.56 ± 3.91^{ab}	5.39 ± 2.57^{ab}

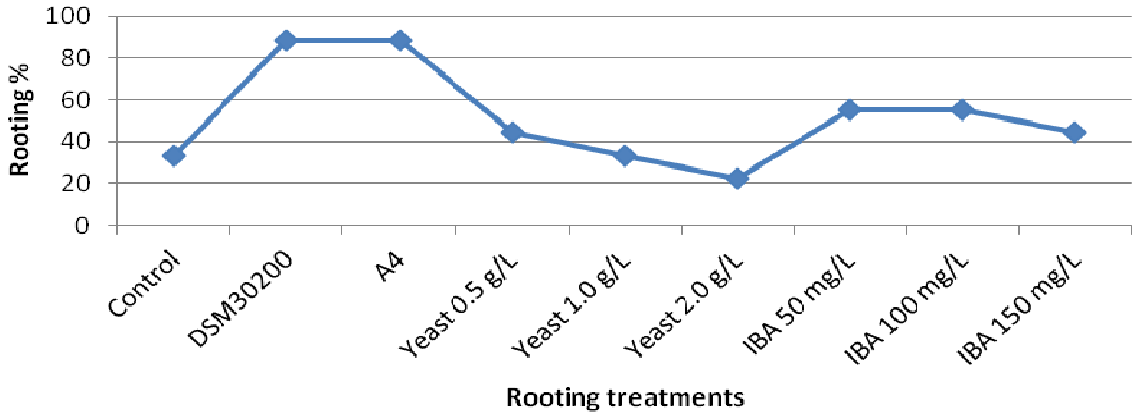

Figure 1. Effects of *Agrobacterium* strains, yeast extract and indole butyric acid (IBA) concentrations on rooting percentage of English lavender stem cuttings.

hormones such as cytokinins (Ribnicky et al., 1996; Gaspar et al., 1997).

It was obvious that cuttings treated with 0.5 g/L yeast gave the lowest roots number and length of 0.33 roots and 0.67 cm. No significant differences were recorded between the previous result and cuttings treated with 2 g/L yeast, 100 and 150 mg/L IBA, as they were 1, 2.89 and 2.56 cm, respectively. Also, the two bacteria strains and IBA at 100 and 150 mg/L, recorded 3.94, 4.39, 5.61 and 5.39 cm increase in cuttings length, respectively and no significant differences were shown between them. The control, yeast at all its concentrations and 50 mg/L IBA gave the lowest increase in cuttings length.

Changes in chemical constituents involved in formed adventitious roots

As shown in Table 2, the adventitious roots formation process and root parameters on cuttings of lavender under study were activated corresponding with the effect of tested treatments. The treatments have the ability to make an increasing effect on nitrogen contents, carbohydrates and phenols succeeded to form adventitious roots of higher qualities. This was in agreement with Samaan et al. (2014). But, it was extremely clear that *A. rhizogenes* strain DSM30200 is significantly still higher than all of the other treatments on these parameters, especially in carbohydrates percentage as shown in Figure 3, followed by using IBA at the higher concentration of 150 mg/L in N percentage, as no significant difference was found between them. The positive relationship between changes in chemical constituents in basal part of the treated cuttings and root parameters using *A. rhizogenes* could be attributed to certain evidences supported by the effective role of this biochemical compound to exert an influence on the rooting of cuttings. Carbohydrates in cutting provide

Table 2. Effect of *Agrobacterium* strains, yeast extract and indole butyric acid (IBA) concentrations on chemical constituents involved in formed adventitious roots of English lavender stem cuttings.

Rooting substance		N % ± SD	Carbohydrates % ± SD	Total phenol % ± SD
Treatment	**Concentrations**			
Control	0	1.91 ± 0.03^{e}	33.23 ± 0.25^{f}	0.25 ± 0.003^{h}
Bacteria strain	DSM30200	2.35 ± 0.05^{a}	36.56 ± 0.42^{a}	0.37 ± 0.01^{a}
	A4	2.13 ± 0.01^{c}	35.53 ± 0.21^{c}	0.34 ± 0.004^{c}
Yeast (g/L)	0.5	1.81 ± 0.04^{f}	31.23 ± 0.16^{i}	0.29 ± 0.002^{f}
	1	1.98 ± 0.03^{d}	31.97 ± 0.15^{h}	0.24 ±0.005^{i}
	2	2.22 ± 0.03^{b}	34.3 ± 0.01^{e}	0.27 ±0.007^{g}
IBA (mg/L)	50	2.03 ± 0.02^{d}	32.7 ± 0.2^{g}	0.31 ± 0.001^{e}
	100	2.09 ± 0.09^{c}	34.90 ± 0.03^{d}	0.33 ± 0.003^{d}
	150	2.31 ± 0.02^{a}	36.04 ± 0.06^{b}	0.36 ± 0.005^{b}

Figure 2. Effect of *Agrobacterium* strains, yeast extract and indole butyric acid (IBA) concentrations on rooting parameters of English lavender stem cuttings.

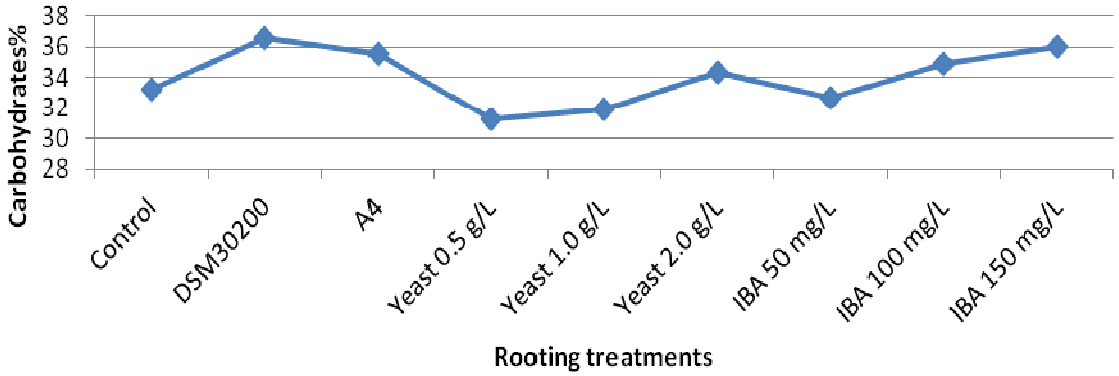

Figure 3. Effects of *Agrobacterium* strains, yeast extract and indole butyric acid (IBA) concentrations on carbohydrates percentage of English lavender stem cuttings.

sufficient amount of food for roots formation (Bleasdale, 1984). In the same point, the former authors reported that the rooting capacity of many cuttings has been correlated with carbohydrates content. Tahir et al. (1998) confirmed the important of carbohydrates accumulation in guava stem cuttings and other hardwood cuttings to produce number of roots and shoots. Sufficient of both carbohydrates and nitrogenous basis works with others in synthesis of the building blocks for nucleic acids (DNA and RNA). These biochemical compounds are the main source in synthesis of proteins as well as carbohydrates and fats metabolism which they all are necessary for normal cell division (Cannon et al., 2002). In that respect, Galle (1965) previously worked on leaf cuttings of cauliflowers (*Brassica oleraca*) and found a marked increase in the RNA content in tissues of cuttings basal part occurred during the period in which root primordia were formed. If the nitrogen level in cuttings decreased below a certain level, root formation was decreased in spite of a high level of carbohydrates. A similar result was cleared in the studies of Hambrick et al. (1985) with *Rosa multiflora* cuttings. He showed also that effect of non-rooting was related to carbohydrates content in cuttings as for C/N ratio, similar relationship of carbohydrates and C/N ratio with root qualities was found. High C/N ratio in hard wood stem cuttings is favorable to good rooting (Mahros, 2000; Hussein, 2008). They added that C/N ratio maybe an important factor influencing the root ability and their levels were positively related to rooting percentage on cuttings under study.

In testing, the biological activity of compounds structurally related to rooting co-factors, since early times Hess (1962) found that the phenol compounds catechol reacts synergistically with IAA in root production in the mung bean bioassay. Bouillenne and Bouillene (1955) suggested that auxidation of an orthodihydroxy phenol is one of the first step leading to root initiation tests using ultraviolet (UV) spectrum analysis and infrared spectroscopy indicated that the rooting factor is a complex structure of high molecular weight (an indole-phenol complex). They reported also that this factor may react at the base of the cuttings with specific enzyme initiating cell division leading to adventitious root formation (Hartmann and Kester, 1968; Fadle and Hartmann, 1967).

Generally, it could be concluded that English lavender terminal stem cuttings infected the basal part with the two strains of *A. rhizogenes* resulting in the best rooting percentage, average roots number and roots length. Moreover, these treatments also increased cuttings length.

Conflict of Interest

The author(s) have not declared any conflict of interest.

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