

Pseudomonas fluorescens and its ability to promote root formation of olive microshoots

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Abstract

Root formation and root architecture of olive microshoots, inoculated or not with *Pseudomonas fluorescens* P19 or P21, were evaluated by measuring of length and the numbers of adventitious and lateral roots. Three-four nodal *in vitro* shoots were treated with different population densities (0, 10⁵, 10⁸ CFUml⁻¹) of rhizobacteria. The density of 10⁸ CFUml⁻¹ induced almost two times increase in number and length of roots per explant, in contrast to that achieved by 10⁵ CFUml⁻¹. Both strains depending on the L.Tryptophan concentration of the bacterial media, strongly affected root growth and architecture. By application of L. Tryptophan up to 10mg l⁻¹ in the media, the length and numbers of adventitious and lateral roots were increased. Bacterial treatments were more efficient than IBA. Moreover different inoculation methods revealed that co-inoculation of the soil and microshoots increased the numbers and length of the roots.

Keywords: IAA producing rhizobacteria; Micropropagation; Olive

Introduction

Plant growth promoting rhizobacteria (PGPR) have been studied for the past century to increase plant productivity (Egamberdiyeva, 2007). It has been postulated PGPR producing plant growth regulators play a critical role in plant growth promotion (Ahmad et al., 2005).

Auxin is a central regulator in many processes during plant growth development. Bacterial IAA producers (BIPs) have the potential to interfere with any of these processes by input of IAA into the plant's auxin pool. Many plant growth bacteria, such as *Azotobacter Paspali* (Surette et al., 2003), *Azospirillum brasilense* (Dobbelaere et al., 2001), *Pseudomonas putida* (Leveau and Lindow, 2005) which stimulate the growth of roots can produce at least small amounts of the auxin indole 3-acetic acid (IAA). This is the first report of auxin producing rhizobacteria on rooting of olive microshoots.

Materials and Methods

Plant source and Culture condition

Uni-nodal segments of sterilized shoots of *Olea europea* L. (cv. *Rowghani*) were cultured in DKW (Driver and Kuniyuki, 1984) medium supplemented with 2-isopentenyl adenine (4 mg l⁻¹). Culture were kept at 23±2 °C and 16h photoperiod. After 60 days, sterile raised shoots (3-4 nod) were used for rooting experiences.

Bacterial source

Two IAA producing *Pseudomonas fluorescens* strains including P21 and P19 were received from National Institute for Genetic Engineering and Biotechnology.

Bacteria colonies were grown in King B (King et al., 1954) broth supplemented with different concentration of L. Tryptophan (0, 2, 5, 10 mg l⁻¹) from a filter-sterilized stock solution.

Rooting experiences

Effect of population density of bacteria (Table 1), inoculation methods (Table 2) and Tryptophan concentrations (Table 3) on root formation were examined. In all experiments three-four nodal of *in vitro* shoots were treated by dipping the base of shoots in different solutions of IBA or rhizobacteria for 15 minutes. Treated microshoots were transferred to the jiffy pots and kept in the transparent boxes in 24-25 °C and 16 h/8 h photoperiod. Thirty days after treatments, length and the numbers of roots per explant were measured.

Table 1. Effect of population density of rhizobacteria on root formation. Different letters columns indicate significant differences ($p \leq 0.05$).

Treatment	Length(cm)	Number/explant
Control	0.58 (b)	2.42 (bcd)
Rhizobacteria (CFUml ⁻¹)		
P19 (10 ⁵)	0.34 (b)	2.25 (bcd)
P19 (10 ⁸)	0.91 (ab)	3.14 (abc)
P21 (10 ⁵)	0.38 (b)	2.73 (abc)
P21 (10 ⁸)	1.7 (a)	4.34 (a)

Control: Basal bacterial medium with L. Tryptophan (10mg l⁻¹).

Table 2. Effects of various inoculation methods on length and numbers of adventitious roots. Different letters columns indicate significant differences ($p \leq 0.05$).

Treatment	Length (cm)	Number/explant
Control*	0.72 (c)	0.45 (b)
Method I**	10.79 (bc)	2.33 (ab)
Method II***	20.72 (ab)	3.70 (a)
Method III****	25.90 (a)	4.09 (a)

*Control: basal bacterial medium; **Inoculation of microshoots by P21 just before transferring to the jiffy pots; ***Co-inoculation of the soil and microshoots by P21 just before transferring to the jiffy pots; ****Co-inoculation of the soil and microshoots with a week interval; In all methods bacteria were grown in the presence of L. Tryptophan (10 mg l⁻¹) for 72 h and the bacterial population was 10⁸ CFU ml⁻¹.

Table 3. Effects of IBA and rhizobacteria at different L.Tryptophan concentration on root formation of microshoots. Different letters columns indicate significant differences ($p \leq 0.05$).

Treatment	Try (mgL^{-1})	Length (cm)	Number of A*	Number of L*
Control*	0	1.80 (g)	1.05 (cd)	0.22 (d)
	2	2.659 (efg)	1.40 (bcd)	0.20 (d)
	5	1.48 (fg)	1.36 (bcd)	0.00 (d)
	10	1.11 (fg)	1.20 (cd)	0.11 (d)
Rhizobacteria	P19	0	4.65 (cde)	2.35 (bcd)
		2	7.50 (bc)	2.90 (ab)
		5	7.90 (b)	3.40 (a)
	P21	10	12.84 (a)	3.68 (a)
		0	6.00 (bcd)	2.57 (ab)
		2	7.36 (bc)	2.52 (abc)
	IBA (mgL^{-1})	5	12.55 (a)	3.45 (a)
		10	10.90 (a)	3.04 (ab)
		0	0.32 (g)	0.79 (d)
	100	3.57 (def)	3.10 (ab)	
	300	3.88 (def)	3.44 (a)	

*Control: Basal bacterial médium; A= adventitious roots; L= Lateral roots.

Results and Discussion

Both bacterial strains solution promoted rooting of microshoots in 10^8 CFU mgL^{-1} density. This density induced almost the double number and length of roots per explant, in contrast to that achieved by 10^5 CFU mgL^{-1} density and bacterial basal medium (Table 1). It was shown that the practical use of PGPR as microbial fertilizers, and their efficiency is strongly dose-dependent because they should be survive in microhabitats associated with the root surface, in competition with the other microbiota (Barea et al., 2005).

Different inoculation methods indicated co-inoculation of the soil and microshoots increased the numbers and length of roots (Table 2). Probably in this way root colonization established better. Generally, microorganisms isolated from rhizosphere and rhizoplane of various plants are more active in producing auxins than those from root-free soil (Kampert et al., 1975).

In L. Tryptophan treatments, formation and growth of roots depended on L. Tryptophan concentrations. The concentration of 5 mgL^{-1} of L. Tryptophan in P21 medium and 10 mgL^{-1} in P19 medium induced root formation/development more than the other treatments. Both bacteria improved the roots length significantly compared to IBA (Table 3).

P21 and P19 can produce high level of IAM and IAA which can promote root formation of microshoots. At the same concentration of L. Tryptophan, P21 produce almost double amount of auxin, in contrast to that achieved by P19 (Data not shown), For this reason shoot treated by P21 (at Tryptophan 5 mgL^{-1}) showed root development similar to P19 (at Tryptophan 10 mgL^{-1}).

Root length and lateral root number taken from P21 and P19 treatments showed significant increase compared to IBA, because plants inoculated with bacteria received auxin continuously. Auxin in concentration more than 10^{-8} molar can stimulate the lateral root formation. Lateral branching in root and shoot systems represent a major determinant of plant architecture. Several lines evidence indicate that indole acetic acid is required at

several stages of lateral root development (Casimiro et al., 2001). Therefore, plant morphogenic effects may also be a result of different ratios of plant hormones produced by roots as well as by rhizosphere bacteria.

In general for some olive cultivars, micropropagation have not been very successful, because the formation of adventitious roots in microshoot are difficult. The common rooting method in olive micropropagation is *in vitro* rooting (Lambardi and rugini, 2003; Mendoza de Gyves et al., 2008). It was found that shoot rooting of hard rooted of olive cultivars is possible by using auxin *ex vitro* (Leva et al., 2004). Our results showed with this method, auxin could be substituted by auxin producing rhizobacteria.

References

- Ahmad, F., Ahmad, I., Khan, M.S., 2005. Indole Acetic Acid Production by the Indigenous Isolates of Azotobacter and Fluorescent Pseudomonas in the Presence and Absence of Tryptophan, Turkish Journal of Biology, 29: 29-34.
- Barea, J.M., Jose´ Pozo, M., Rosario Azco´n, Azco´n-Aguilar, C., 2005. Microbial co-operation in the rhizosphere, Journal of Experimental Botany, 56: 417. 1761-1778.
- Casimiro, I., Marchant, A., Bhalerao, R.P., Beeckman, T., Dhooge, S., Swarup, R., Graham, N., Inzé, D., Sandberg, G., Casero, P.J., Bennett, M., 2001. Auxin Transport Promotes Arabidopsis Lateral Root Initiation. The Plant Cell, 13: 843-852.
- Dobbelaere, S., Croonenborghs, A., Thys, A., 2001. Response of agronomically important crops to inoculation with Azospirillum. Australian Journal of Plant Physiology, 28: 1-9.
- Driver, D., Kuniyuki, A., 1984. In vitro propagation of paradox walnut rootsock. Horticultural Science, 19: 507-506.
- Egamberdiyeva, D., 2007. The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils, Agriculture, ecosystems and environment. Applied soil ecology, 36: 184-189.
- Kampert, M., Strzelczyk, E., Pokojnska, A., 1975. Production of auxins by bacteria isolated from pine roots (Pinus sylvestris L.). Acta Microbiologica polonica, 7: 135-143.
- King, E.O, Ward, M.K., Raney, D.E., 1954. Two simple media for the demonstration of pyocyanin and fluorescin. Journal of laboratory and clinical medicine, 44: 301-307.
- Lambardi, M., Rugini, E., 2003. Micropropagation of olive (Olea europaea L.) In Micropropagation of woody Trees and Fruits. Kluwer Ac. Pub.' Netherland, Pp: 621-646.
- Leva, A.R., Petruccielli, R., Polsinelli, L., 2004. In vitro propagation From the laboratory to the production line. Olivae, 101: 18-26.
- Leveau, J.H.J., Lindow, S., 2005. Utilization of the Plant Hormone Indole-3-Acetic Acid for Growth by Pseudomonas putida Strain 1290, Applied and Environmental Microbiology, 71: 5. 2365-2371.
- Mendoza-de Gyves, E., Mira, F.R., Ruiu, Rugini, E., 2008. Stimulation of node and lateral shoot formation in micropropagation of olive (Olea europaea L.) by using dikegulac. Plant cell tissue and organ culture, 92: 233-238.
- Surette, M.A., Sturz, A.V., Lada, R.R., Nowak, J., 2003. Bacterial endophytes in processing carrots (Daucus carota L. var. sativus): their localization, population density, biodiversity and their effects on plant growth. Plant and Soil, 253: 381-390.