RESEARCH ARTICLE

PLANT GROWTH PROMOTING RHIZOBACTERIA IMPROVES GROWTH IN *ALOE VERA*

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Received-08.08.2017, Revised-22.08.2017

Abstract*:* Sustainableagriculture involves the use of biofertilizers and biopesticides to reduce the application of chemical fertilizers. Microbial consortium-based sustainable and economicbionutrient package for *Aloe vera* has been developed to reduce reliance on chemical fertilizers. Consortium includes *Acinetobacter radioresistens* SMA4, *Bacillusthuringiensis* SMA5, *Brevibacteriumfrigoritolerans* SMA23 and *Pseudomonas fulva* SMA24. In the earlier studies all these four bacterial strains have been found to possess multiple plant growth promoting attributes. Consortium used in this study increased all biometric parameters in *Aloe vera*such as plant biomass, root weight, shoot weight and gel content. Increase in aloinA content was also observed in this study in plants treated with PGPR.

Keywords: *Aloe vera*, Consortium, PGPR, Aloin, Biofertilizer

INTRODUCTION

Aloe belongs to family*Liliaceae*, is well known for its marvelous medicinal properties. *Aloe vera*is a unique plant which is a rich source of many chemical compounds and plays an important role in the international market. *Aloe vera*plant is traded in the medicinal drug market for flavoring liquid formulations (Rajendran*et al*., 2007). Chemistry of this plant revealed the presence of more than 200 different biologically active substances including vitamins, minerals, enzymes, sugars, anthraquinones or phenolic compounds, lignin, saponins, sterols, amino acids and salicylic acid (Vogler and Ernst, 1999; Durej*aet al.*, 2005; Park and Jo, 2006; Chauhan *et al*., 2007).The gel present inside the leaves of *Aloe* plant contains phenolic compounds like aloin-A (barbaloin), aloesin, isoaloeresin D and aloeresin E used in the treatment of tumors, diabetes, ulcers and cancer (Ishii *et al*., 1990; Okamura *et al*., 1996; Park *et al*., 1998). Aloin-A (barbaloin, $C_{21}H_{22}O_9$) is the major phenolic compound reported from the plant (Groom and Reynolds, 1987). Demand for this medicinal plant is increasing worldwide. So, there is need to find associated microbes which can promote plant growth without having any negative impact on soil and environment.
Earlier reports have shown the effect of arbuscular mycorrizal fungi and *Azotobacter* on the growth and barbaloin content ofthe plant (Tawaraya*et al.*, 2007; Pandey and Banik, 2009). In the present study, the effect of PGPR is reported for plant growth parameters and the aloin-content in *A. barbadensis.*
The use of beneficial soil plant growth promoting rhizobacteria (PGPR) for improving crop production requires the selection of rhizosphere-competent bacterial strains with multiple plant growth promoting attributes (Nautiyal*et al.*, 2008; Hynes *et al*., 2008).PGPR promote plant growth directly by either facilitating resource acquisition (nitrogen, phosphorus and essential minerals) or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development inthe forms of biocontrol agents (Glick, 2012).

MATERIAL AND METHOD

Plant growth-promoting rhizobacteria used in this study has been isolated from *Aloe vera* rhizosphere. The four PGPR strains, *Acinetobacter radioresistens* SMA4, *Bacillusthuringiensis* SMA5, *Brevibacteriumfrigoritolerans* SMA23 and *Pseudomonas fulva* SMA24 were found to possess various plant growth promoting attributes such as phosphate solubilisation, IAA production, Siderophore production and Antifungal assay. The identification of the isolates was done on the basis of morphological, Biochemical and 16S rRNA studies (Meena*et al*., 2014; Meena*et al*., 2017). The 16S rDNA sequences of the isolates were deposited in NCBI GenBank under the accession numbersJQ618289, KC663437, KC986860 and JQ618289 respectively. The cultures were maintained on nutrient agar slants at 6 °C in a refrigerator with regular subculturing. For inocula preparation, the cultures were grown separately in nutrient broth at 28 ± 2 °C. To obtain bacterial cultures in mid log phase, flasks were incubated for 24h up to a cell density of 8×10^9CFU ml^{-1}on a rotary shaker at 30 °C. Bacterial cells were harvested by centrifugation (7000 rpm for 20 min). After removal of the culture medium, the bacterial pellet was washed in sterile water and centrifuged again (7000

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rpm for 20 min). Bacterial cells were then resuspended in sterile saline solution and cell density was adjusted to get approximately8 $\times 10^9$ CFU ml^{-1}(Van *et al*., 2000).

Experimental design and green house treatments

The two efficient plant growth promoting bacteria from the in vitro experiments were analysed for studying the efficacy on plant growth promotion in vivo under greenhouse condition using pot culture experiments. The experiment was arranged in a complete randomized design (CRD) with three replications per treatment.

Pot preparation

All experiments reported in this paper were conducted in the greenhouse at the Department of botany, Kurukshetra University Kurukshetra. Each PGPR isolate was grown in nutrient broth at 30 °C in an orbital shaker (150 rev min^{-1}) for 24 h. Cultures were centrifuged in 50 ml sterile plastic tubes at 6000 × g for 15 min. The pellets were re-suspended in nutrient broth to obtain a final concentration of 10^8 ml^{-1}colony forming units (CFU). The liquid cultures of each isolate were used for individual inoculations. About 10 treatments were designed for experiment which includes individual inoculant and in combination. Consortium was prepared by mixing all individual cultures of isolates of equal cell density 10^8 CFU ml^{-1} into 250 ml sterilized flask and was used as a mixed inoculum (Mamta*et al*., 2011). Experiments were conducted in the greenhouse (uncontrolled conditions) during March-August, 2014. Unsterile loamy soil (pH, 7.6; total organic C, 0.12%; available N, 42.8 mg kg^{-1}; available P, 6 mg kg^{-1}; available K, 15.8 mg kg^{-1}; total Ca, 0.6 m Eq 100 g^{-1}; total Mg, 0.10 m Eq 100 g^{-1}) was thoroughly mixed, passed through a 2 mm sieve and dried at sunlight for 7 days. Ethanol disinfected earthen pots (20 cm diameter × 25 cm height) were filled with 5.0 kg of soil. Roots of tissue cultured plantlets were sterilized by dipping in 2% NaOCl solution for 10 min and then washed three times with sterile distilled water. Roots were dipped into the selected bacterial suspension (10^8 CFU ml^{-1}). Experiments were performed in a completely randomized block design. Plantlets were planted under two different soil conditions and ten different treatments; (i) *Acinetobacter radioresistens*SMA4(ii) *Bacillus thuringiensis.* SMA5 (iii) *Brevibacteriumfrigoritolerans SMA23* (iv) *Pseudomonas fulva* SMA24 (v) SMA4+SMA5 (vi) SMA4+SMA23 (vii) SMA5+SMA24 (viii) SMA5+SMA23 (ix) SMA4 + SMA5 + SMA23 (x) SMA5 + SMA23 + SMA24 (xi) SMA4 + SMA5 + SMA24 (xii) Mixture of all PSB.

Quantitative high-performance liquid chromatography (HPLC) analysis of aloin-A

Leaves of harvested *A. barbadensis* were washed with running tap water and then twice with distilled water. The inner gel was mechanically separated from the outer cortex of the leaf with the help of a knife. Total gel was extracted from all the leaves of a plant, then freeze dried (Heto dry winner, Model DW 1-0-110, Allerd, Denmark). The 0.5 g of each freeze-dried gel sample was extracted with 10.0 ml methanol for 24 h at 4 °C (Okamura *et al*.,1996). The suspension was centrifuged at 4000 × g for 10 min at 4 °C. The supernatant was subjected to HPLC (WATER Corporation, USA, Lichrocart® C- 18 coloumn, flow rate of 0.8 ml min^{-1}, 800 psi, run time 35.0 min). The mobile phase was 0.1% acetic acid/acetonitrile (60:40). Analytes were detected at wavelength 290 nm with a photodiode array detector and identified by their retention time and by spiking the sample with standard aloin-A (Sigma-Chemical, USA). Quantification was done by reference to the peak area percentage obtained for the standard compound.

RESULT AND DISCUSSION

Plant Growth Promoting Rhizobacteria (PGPRs) are able to exert a beneficial effect upon plant growth. In rhizosphere, beneficial interactions between plant and microbes are main determinant for plant growth and development (Jeffries *et al.*, 2003).

Effect of PGPR on plant growth

The PGPR treatments (applied individually or as a consortium) increased all parameters of *A. barbadensis*in un-amended soil with the consortium treatment yielding better results than each individual treatment. In plants treated with single PGPR strains, maximum stimulatory effects on various biometric parameters were obtained by *Pseudomonas fulva* SMA24 followed by*Bacillus thuringiensis* SMA5, *Brevibacteriumfrigoritolerans* SMA23 and *Acinetobacter radioresistens* SMA4. SMA5significantly ($P \leq 0.05$) increased Plant biomassby 105.72%, root weight by 111.67%, shoot weight by 137.23%, total number of leaves weight by 63.23%, total gel volume by 193% as compared to the control plants (Table 1).The individual PGPR treatment also showed significant stimulatory effects on plant growth. The application of PGPR as a consortium increased all biometric parameters of *A. barbadensis*plants grown in pots more than individual plant growth promoting rhizospherictreatments (Table 1). Compared to control plants, an increase of over 213% in total gel volume was observed in plants treated with the consortium. In individual treatments, the degree of stimulation varied with respect to the type of growth parameter. Maximum increase in plant biomass (130.03%) and root weight (111.49%) was shown by T_9treated plants after consortium(Table 1). The plants treated with SMA4 and SMA23 showed smaller increases in growth parameters than SMA24 and SMA5 treated plants. The increase in growth parameter may be due to increase in availability of nutrients such as nitrogen and phosphorus, and increase in Indole acetic acid production by PGPR.

Similarly, promotion in plant height, number of tillers, plant dry weight and grain yields of various crop plants in response to inoculation with PGPR were reported by other workers (Chen *et al*., 2008; Khalid *et al*., 2004; Biswas *et al*., 2000; Hilali*et al*., 2000).

Table 1. Growth Enhancement in *Aloe vera* plant due to PGPR inoculation

	Treatments	**Plant Biomass (g pot^{-1})**	**Root wt. (g pot^{-1})**	**Shoot wt. (g pot^{-1})**	**Leave wt. (g pot^{-1})**	**Gel wt. (g pot^{-1})**
	Control	180.40	40.24	120.78	26.60	8.52
T1	**SMA4***	270.99	60.55	210.06	46.37	19.11 19,11
T2	**SMA5***	352.00	55.83	300.57	41.61	24.95
T3	**SMA23***	301.87	62.78	239.43	39.65	23.06
T4	**SMA24***	371.13	85.18	286.53	43.42	25.02
T5	**SMA4+SMA5**	372.00	65.52	305.04	74.44	22.63
T6	**SMA4+SMA23**	350.64	64.75	286.59	52.30	23.94
T7	**SMA5+SMA24**	402.26	86.49	310.68	54.09	24.45
T8	**SMA5+SMA23**	310.81	60.15	250.82	41.84	13.89
T9	**SMA4+SMA5+SMA 23**	415.60	72.20	343.98	74.42	18.96
T10	**SMA5+SMA23+SM A24**	330.18	51.38	279.51	52.28	25.42
T11	**SMA4+SMA5+SMA 24**	382.24	60.56	240.48	56.29	16.61
T12	**SMA4+SMA5+SMA 23+SMA24**	**435.54**	**90.39**	**345.55**	**74.59**	**26.71**
SEm		1.11	0.28	0.53	1.00	0.90
CD		3.08	0.78	1.47	2.76	2.50

Effect of PSB on aloin-A content

*Aloe*plants grown in soil treated with PGPR strains showed higher aloin-A content than plants grown in soil treated with control (Fig. 1). The increase in the aloin-A content was due to both the increase in biomass of *Aloe*plants and the increased biosynthesis of aloin-A as compared to control plants. The PGPR consortium treated plants grown in pots showed maximum yield of aloin-A and also the highest increase in thealoin-A content per plant. Amongst individual PGPR treatments, a maximum increase 184% was observed with *P. fulva*treated plants followed by *Bacillusthuringiensis* SMA5 (152.6%)(Fig. 1). Lower values were obtained with plants treated with SMA4 and SMA23. A significant ($P \leq 0.01$) positive correlation was found between PGPR attributes and aloin-A biosynthesis (mg g−1) in all plants grown in pots.Similar reports are observed by Gupta *et al*. (2014) that use of PGPRconsortium in *Aloe vera* greatly influences the aloin-A production due to higher plant biomass.

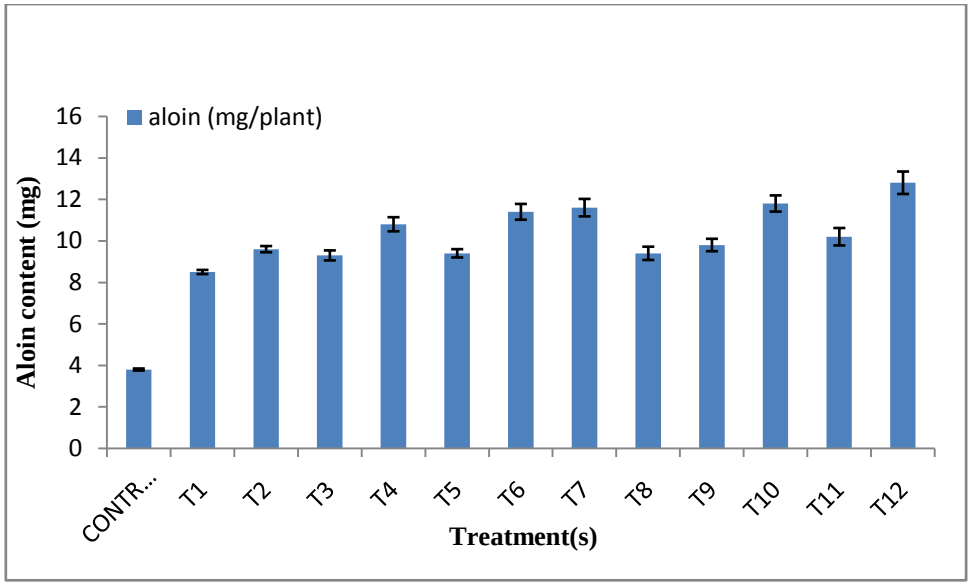

Fig. 1. Influence of PGPR treatment on the aloin-A content. Error bars represent standard deviation. Bars with same letter are not significantly different according to LSD at P ≤0.05.

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