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SYNERGISTIC EFFECT OF PLANT GROWTH PROMOTING RHIZOBACTERIA AND KINETIN ON MAIZE

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ABSTRACT

Plant growth promoting rhizobacteria (PGPR) colonize the plant roots and promote plant growth and yield by different mechanisms. A study was conducted in a series of jar and pot trials where effect of four different PGPR isolates alone and in combination with kinetin on maize (Z*ea mays* L.) was evaluated under controlled and natural conditions. Results of jar trial indicated that PGPR isolates had positive interaction with the kinetin and significantly increased shoot and root length. However, the synergistic effect of PGPR isolates and kinetin was significantly better. Data of pot trial further confirmed the results of jar trial where combined use of bacterial strains with kinetin increased plant height (53%), shoot fresh weight (48%), root length (50%), shoot dry weight (133%), root fresh weight (37%) and root dry weight (70%) as compared to the sole application of kinetin. While interaction of PGPR with kinetin also improved the nitrogen, phosphorus and potassium contents in plant tissues as compared to control. Study revealed that plant growth promoting capabilities of PGPR could be improved if they are supplemented with exogenous kinetin.

Key words: rhizobacteria, growth regulator, cytokinins.

INTRODUCTION

Compared to bulk soil the rhizosphere is rich in nutrients for microbial growth due to rhizodeposition of root exudates. As a result, the number of soil bacteria around roots of plant is many times greater compared to bulk soil (Morgan *et al.,* 2005). The rhizosphere provides organic substances to a large and diverse community of soil microorganism that survives on root exudates (Nihorimbere *et al.,* 2011). On the basis of their effects on plant these rhizobacteria can be largely divided into beneficial, deleterious, and neutral bacteria (Bais *et al.,* 2006). Rhizobacteria that live in plant rhizosphere and have advantageous effects on growth and development of plant via different direct and/or indirect mechanisms are referred as plant growth promoting rhizobacteria (PGPR) (Nadeem *et al.,* 2010; Laslo *et al.,* 2012). Indirect plant growth promotion occurs by reducing deleterious effects of pathogens (Glick and Bashan, 1997) through production of antibiotic, release of siderophore, synthesis of antifungal metabolities and induced systemic resistance (Recep *et al.,* 2009). In case of direct plant growth promotion, the bacteria assist the host plant by facilitating nutrient uptake or by providing active growth substances/phytohormones (Glick *et al.,* 1995). The direct mechanisms of plant growth promotion include atmospheric nitrogen fixation, phosphorus solubilization, reducing the stress induced ethylene level by enzymatic activity and/or phytohormones production (Zahir *et al.,* 2003; Asghar *et al.,* 2004; Peralta *et al.,* 2013). Phytohormones (plant growth regulators) are low molecular weight biologically active compounds which regulate the normal physiological and developmental processes of plants (Chiwocha *et al.,* 2003). The main groups of phytohormones include auxins, gibberellins, cytokinins, abscisic acid and ethylene (Khalid *et al.,* 2006). Cytokinins are class of physiological important and versatile phytohormones (Mazid *et al.,* 2011) which promote cell division, involved in cell differentiation, effect apical dominance, related to axillary bud growth and leaf senescence, enhance plant defense and also involved in long distant signaling (Giron *et al.,* 2012). Cytokinins are very crucial phytohormone for plant meristemic activity and morphogenesis and their deficiency result in stunted shoots with smaller apical meristems (Werner *et al.,* 2001). Exogenous application of cytokinins improves the plant growth (Zahir *et al.,* 2001) by delaying senescence and by preventing degradation of chlorophyll and photosynthetic proteins (Wingler *et al.,* 1998). Exogenously applied cytokinins also promote flower production and reduce the flower abortion (Nagel *et al.,* 2001). Cytokinins are also produced by plant growth promoting rhizobacteria (Salamone *et al.,* 200) but due to different factors the microbes cannot produce sufficient amount of cytokinins to improve the plant growth significantly. If PGPR are supported with exogenous application of different plant growth regulators, like kinetin, this strategy may results to overcome suboptimum levels of cytokinin in plants. This scenario advocates that combined use of PGPR and exogenously applied cytokinin could further improve the plant growth promoting capability of rhizobacteria. To investigate this, the present study was carried out to

segregate the effect of kinetin and PGPR compared to their combined effect and to screen out the best combination of PGPR with kinetin to improve the growth of maize.

MATERIALS AND METHODS

The present study was planned to evaluate the role of kinetin and PGPR alone as well as in combinations on growth and development of maize. The experiment was conducted under controlled conditions using jars in growth room and repeated under natural conditions using pots in the wire house.

Jar Trial: Pre-isolated strains of PGPR from maize rhizosphere were used for inoculation of maize. Inoculum was prepared by growing the selected isolates in glucose peptone broth media. Flasks containing glucose peptone broth were inoculated with selected isolates and incubated at 28 ± 1°C for 72 hours. Bacterial cells were harvested by centrifugation at 4500 rev min^{-1} for 20 minutes. Then cells were washed and suspended in sterilized phosphate buffer saline (pbs) and uniform cell density (10^7-10^8 CFU mL^{-1}) was achieved by maintaining optical density of (OD=0.45) at 535 nm. The inoculum of each isolate was injected into sterile peat (100 ml kg^{-1}) and was incubated for 24 hrs at 28 ± 1°C before using it for seed coating. For seed inoculation, seed dressing was carried out with inoculated peat mixed with clay and 10% sugar solution. In case of the un-inoculated control, the seeds were coated with the same but autoclaved inoculum suspension. The surface sterilized seeds of maize inoculated with four different isolates were sown in sterilized jars containing 500 g sand. Kinetin (10^{-4} M) was applied at emergence stage of seedlings. Total 10 treatments were applied where four different PGPR isolates were used alone as well as in combination with kinetin, keeping one treatment as sole application of kinetin and control where neither PGPR nor kinetin was applied. Treatments were allocated according to completely randomized design (CRD) with 3 replications. For nutrient, half strength Hoagland's solution was applied. Experiment was carried out under complete control conditions and was harvested after one month and data regarding shoot and root length were recorded.

Pot Trial: To further verify the results of jar trial, a pot trial was carried out in wire house under natural conditions to evaluate the interaction of PGPR and kinetin on maize *(Zea mays* L.*).* Pots were filled with 12 kg soil having organic matter 0.60%, pH 7.7, extractable potassium 110 mg kg^{-1}, available phosphorus 6.20 mg kg^{-1} and total nitrogen 0.06%. Experiment was repeated with same set of treatments and following same statistical design as explained in jar trial. Seeds were inoculated as described for jar trial. Fertilizer NPK @ 175, 160, 100 kg ha^{-1} respectively were applied. Data regarding the different growth parameters were recorded after 80 days. Root and shoot samples were analyzed for NPK content. Data of both jar and pot trials were analyzed by using Statistix-9 computer software (Copyright 2005, Analytical Software, USA). Data were analyzed by following CRD and means were compared by least significant difference (Steel *et al*., 1997)

RESULTS AND DISCUSSION

The study was conducted to evaluate the effect of exogenously applied cytokinin (kinetin) and four different PGPR isolates alone as well as in combination with kinetin on growth and development of maize. Separate use of kinetin improved maize growth but the effect of PGPR isolates was more prominent regarding plant growth promotion. However, when kinetin was applied in combination with these four PGPR isolates, the maize growth was further enhanced. The plant growth promotion effect of all four PGPR isolates was variable either these were applied alone and/or in combination with kinetin. Results of jar experiment shown in the Fig. 1 indicated that the exogenous applied kinetin and four different PGPR isolates significantly ($p<0.05$) improved the shoot and root length of maize as compared to control. Separate application of kinetin improved the shoot and root length upto 16 and 23%, respectively, over control whereas the best PGPR isolate (S1) enhanced the shoot and root length upto 60 and 97%, respectively, over control. While, the synergistic effect of kinetin with PGPR isolate (S1) further increased the shoot and root length upto 98 and 197%, respectively, as compared to control which was upto 70 and 142%, respectively, more as compared to separate use of kinetin. This improvement in shoot and root length by the use kinetin might be attributable to improved meristematic activity by kinetin (Werner *et al.,* 2001). The role PGPR in plant growth promotion is well documented and the increase in shoot and root length under control conditions might be due to different plant growth promoting activities like synthesis of phytohormones (Zahir *et al.,* 2003) and nutrient availability (Peralta *et al.,* 2013). Laslo *et al.* (2012) reported that bacteria isolated from maize rhizosphere had different plant growth promoting and biocontrol activities which support our results that the bacteria used in our study might have one or more plant growth promoting traits which enhanced the shoot and root length under control conditions. Shaharoona *et al*. (2006) also reported similar results that PGPR isolates improved shoot and root length under controlled conditions.

Results of pot experiment presented in table-1 further justified that kinetin and four different PGPR isolates significantly ($p<0.05$) improved the maize growth and development under natural conditions. Data depicted that alone use of kinetin increased the plant height, shoot fresh and dry weight of maize upto 24, 12

and 19%, respectively, as compared to control. From the PGPR isolates, S1 improved the plant growth by increasing plant height, shoot fresh and dry weight of maize upto 60, 17 and 41%, respectively, as compared to control. However, the improvement in plant height, shoot fresh and dry weight of maize by combined use of kinetin and PGPR isolate S1 was upto 90, 66 and 178%, respectively, as compared to control which was upto 53, 48 and 133%, respectively, more as compared to sole application of kinetin. Similarly, combined use of kinetin and PGPR isolate S1 showed promising results by increasing root length, root fresh and dry weight of maize upto 63, 115 and 151%, respectively, as compared to control while this increase in root length, root fresh and dry weight was upto 50, 37 and 70%, respectively, compared to alone use of kinetin.

Current results of our study are in line with the finding of Pan *et al.* (1999), they reported that both PGPR and kinetin had positive effect on corn growth and development but PGPR had more pronounced effect as compared to kinetin. The improvement in maize growth by kinetin might be due to involvement of kinetin in nutrient mobilization (Sakakibara, 2005) and leaf longevity (Kim *et al.,* 2006). The improvement in shoot length and root length of maize might be due to possible physiological role of kinetin in plant morphogenesis (Igari *et al.,* 2008) and might be due to increase in number of actively reproducing plant cells in inflorescence meristems (Leibfried *et al.,* 2005). The plant growth was more pronounced in response to PGPR application which might be due to multiple direct and indirect mechanisms of actions like increasing nutrient availability, synthesis of phytohormones and suppression of harmful microbes in rhizosphere (Saharan and Nehra, 2011). As, Arruda *et al*. (2013) reported that bacteria isolated from maize rhizosphere had ability to produce indole acetic acid (IAA), siderophores and solubilize phosphates. Our results regarding role of PGPR in growth promotion of maize are also in agreement with Nezarat and Gholami (2009), they reported different PGPR strains improved the shoot fresh and dry weight of maize in axenic and field conditions.

Table 1: Synergistic effect of PGPR and kinetin on growth of maize in pot trial

Treatments	Shoot parameters			Root parameters		
	Length (cm)	Fresh wt. (g)	Dry wt.(g)	Length (cm)	Fresh wt. (g)	Dry wt. (g)
Control	14.06 g	30.02 g	7.34 f	40.32 g	22.42 g	3.25 f
Kinetin	17.49 f	33.61 f	8.76 e	43.82 f	35.05 d	4.80 e
S1	22.44 e	35.19 e	10.36 d	46.21 e	31.02 f	6.01b-d
S2	23.04 de	33.12 f	10.64 d	46.79 e	33.86 e	5.69 cd
S3	23.06 c-e	35.03 e	10.29 d	46.99 de	34.96 de	6.48 b
S4	23.64 cd	37.94 d	10.51 d	47.84 d	35.04 d	6.09 b-d
S1+Kinetin	26.71 a	49.71 a	20.39 a	65.70 a	48.15 a	8.17 a
S2+Kinetin	23.24 c-e	45.73 b	16.14 c	61.67 b	44.39 b	6.57 b
S3+Kinetin	24.07 c	45.07 bc	16.80 bc	56.46 c	42.30 c	5.57 d
S4+Kinetin	25.30 b	44.34 c	17.19 b	56.18 c	45.35 b	6.20 bc

Means sharing the same letter (s) are statistically non-significant according to Duncan's multiple range test ($p<0.05$)

Table 2. Synergistic effect of PGPR and kinetin on NPK contents of maize in pot trial

Treatments	Shoot			Root		
	N (%)	P (%)	K (%)	N (%)	P (%)	K (%)
Control	1.47 j	0.18 e	1.24 i	1.46 j	0.12 g	1.20 j
Kinetin	1.52 h	0.20 e	1.30 h	1.72 g	0.14 f	1.28 i
S1	2.10 e	0.24 ad	1.45 e	1.52 i	0.20 cd	1.41 e
S2	1.93 f	0.21 ce	1.43 f	1.73 f	0.17 e	1.36 g
S3	1.51 i	0.22 be	1.42 f	1.79 e	0.16 e	1.39 f
S4	1.79 g	0.23 ae	1.40 g	1.66 h	0.14 f	1.33 h
S1+Kinetin	2.89 a	0.28 a	1.61 a	2.23 a	0.24 a	1.56 a
S2+Kinetin	2.78 b	0.25 ad	1.53 b	2.01 b	0.21 bc	1.43 d
S3+Kinetin	2.58 c	0.26 ac	1.49 c	1.95 d	0.22 b	1.49 b
S4+Kinetin	2.39 d	0.27 ab	1.51 d	1.96 c	0.19 d	1.45 c

Means sharing the same letter (s) are statistically non-significant according to Duncan's multiple range test ($p<0.05$)

Data presented in table-2 showed that the application of kinetin and PGPR isolates not only increased the growth and development of maize but also significantly (*p<0.05*) improved the nutrient use

efficiency of maize as compared to control. Results of chemical analysis clearly depicted that the best combination where kinetin and PGPR isolate S1 were applied increased shoot nitrogen (96%), root nitrogen (53%), shoot phosphorus (56%), root phosphorus (100%), shoot potassium (30%) and root potassium (30%) contents as compared to control.

This improvement in nutrient uptake by maize in response to kinetin might be attributed to transduction of nutritional signals and influence on nutrient status via root-shoot communication (Sakakibara, 2005 & 2006). Arruda *et al*. (2013) also reported that inoculation of maize seeds with PGPR isolates improved the nitrogen, phosphorus and potassium contents in shoot and roots. This improvement in uptake of nitrogen, phosphorus and potassium might be due to more availability of nutrients to plants by phosphorus solubilization (Ranjan *et al.*, 2013), siderophore production (Sayyed *et al.*, 2010) and improved root growth (Karnwal, 2012) which ultimately results in more uptake of nutrients from soil. Moreover, improvement in plant growth and development by combined use of PGPR and kinetin might be due to regulation of phytohormones balance i.e. auxin/ cytokinin ratio in micro-environment of rhizosphere which may directly influenced the PGPR and plant growth.

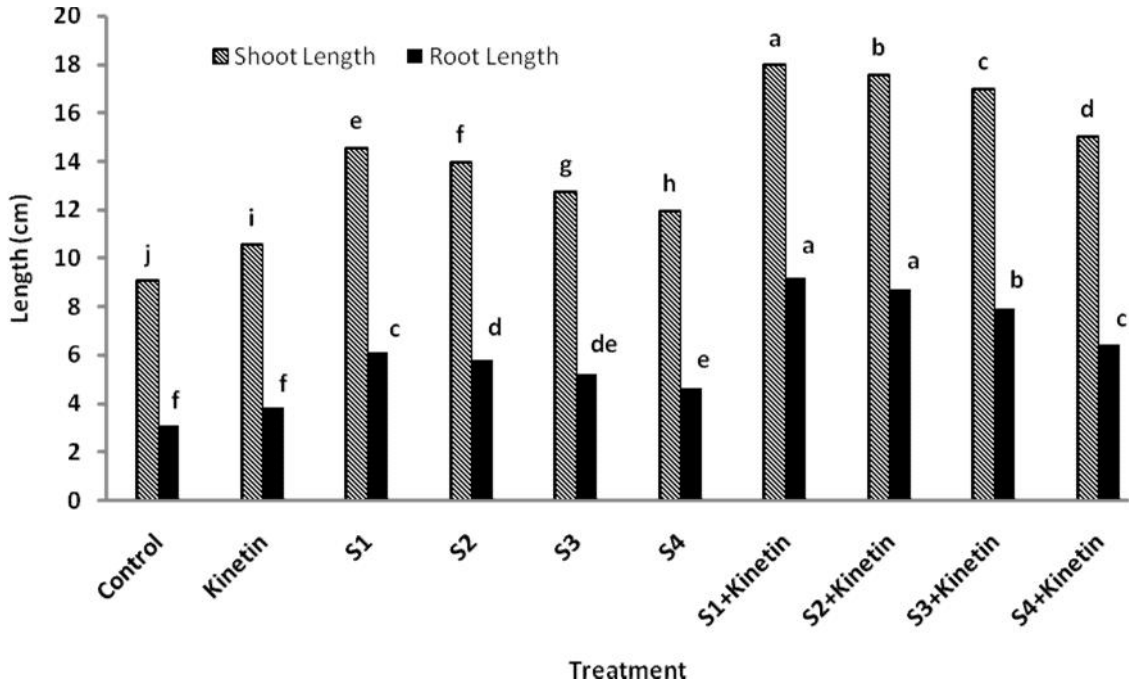

Fig. 1. Effect PGPR and kinetin on shoot and root length of maize under controlled conditions. Bars sharing the same letter (s) are statistically non-significant according to Duncan's multiple range test ($p<0.05$).

Conclusion: Our study revealed that if PGPR are supplemented with exogenous application of plant growth regulators, their efficiency can be multifold, possibly due to synergistic impact of microbially produced and exogenously applied plant growth regulators.

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