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Interaction between *Dalbergia sissoo* Roxb. and *Pseudomonas koreensis* AS15 Strain is Cultivar Specific

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A B S T R A C T

Shisham plantations and natural forests throughout sub Himalayas region, Indogangetic plains and Indian plateau region are facing large scale mortality. Seedling health is key to success of any tree plantation programme. Plant growth promoting rhizobacteria intimately interact with the plant roots and consequently influence soil microbial activity and seedling vigor. In present study, pot culture experiment was carried out under glass house conditions with two *Dalbergia sissoo* cultivars PS90 and PS52. The experiment was set in a completely randomized block design (CRD) and consisted of six treatments including controls with three replications in each treatment. During experiment seven day old seedlings of *D. sissoo* cultivars germinated from freshly collected seeds and subsequently treated with *Pseudomonas koreensis* AS15 strain by root dip method were planted in 1kg capacity plastic pots. Artificial inoculation of *P. koreensis* AS15 enhanced the plant vigour parameters in both *D. sissoo* cultivars PS90 and PS52; PS 90 cultivar exhibiting higher shoot fresh weight (5.8gm at 60 DAP) and shoot length (45.6cm at 60 DAP) than PS 52, shoot fresh weight (2.5gm at 60 DAP), and shoot length (40 cm at 60 DAP) in two factor analysis of variance. There was variation in soil enzyme activities with respect to cultivars. The soil planted with PS90 cultivar exhibited higher alkaline phosphatase (AP) and fluorescein di acetate hydrolysis (FDA) activities as compared to those with PS52 cultivar seedlings. The enhanced plant vigor parameters and soil enzyme activities indicated that interaction of PS90 cultivar with inoculant strain was robust. The results indicated that response of *P. koreensis* AS15 inoculation is cultivar specific and can be used to raise disease resistant *D. sissoo* seedling that also sustain transplantation shock.

Introduction

The genus *Dalbergia* comprises 300 species, of which nearly 25 species occur in India. One of the species *Dalbergia sissoo* Roxb vern. Shisham grows naturally in India and neighbouring countries Afghanistan, Pakistan, Nepal, Myanmar, Bangladesh, Malaysia and Sri lanka. In India it grows in foot hills of Himalayas upto 1000m above sea level,

Indogangetic plains central and peninsular region. It is internationally known for its priced timber and plays important role for country's economy. Additionally it is a pioneer tree species used for afforestation programmes, road side plantations, preparation of agricultural implements, its leaves are used as fodder and twigs as fuel wood. It is also used in folk medicines as remedy for gonorrhea and skin ailment. Being nitrogen fixing, *Dalbergia sissoo* adds biologically fixed nitrogen into the soil. It is well known that biological nitrogen fixation (BNF) brings more nitrogen in terrestrial ecosystem than any other natural input (Vitouset *et al.,* 2013). Nitrogen fixing trees add more than 100kg N $ha^{-1}y^{-1}$ in tropical (Binkley and Giardina., 1997), temperate (Binklay *et al.,* 1994) and boreal forests (Ruess *et al.,* 2009). For these reason it is preferred for afforestation programmes. Shisham is grown both as monoculture or mixed crop (Bisht *et al.,* 2009). Shisham is facing large scale mortality in both natural forests and plantations.

Both biotic and abiotic factors are known to be associated with shisham mortality which results in shisham decline. Soil factors are primary abiotic factors as main reason behind disease in sissoo plantation (Sah *et al.,* 1999). According to Afzal *et al.,* (2006) global warming and erratic rainfall could be one of the reasons for shisham decline. Water logged condition for a considerable period of time cause asphyxiation of the roots (Dayaram *et al.,* 2003) and hence mortality. The abiotic factors can promote the growth of pathogenic microorganism in soil and rhizosphere. These pathogenic microorganisms ultimately infect shisham trees. Almost 70% of the shisham mortality is due to soil borne pathogens. The pests and nematodes are secondary factors. Both fungi and bacteria have been isolated from diseased shisham trees. Soil borne fungal pathogen like *Fusarium solani* (Sah *et al.,* 2003), *Ganoderma lucidum* (Ahmad *et al.,* 2013) and *Phytophthora cinnamoni* (Gill *et al.,* 2001) have been isolated from diseased shisham trees. Bacteria of genera *Pseudomonas* and *Bacillus* have been reported from diseased shisham trees in Bangladesh (Valdez *et al.,* 2013).

Under this context it is of utmost importance to raise the seedlings that can withstand abiotic and biotic stresses by exploring the interaction of *D.sissoo* with beneficial microorganisms. Bacteria are ubiquitous to water, soil and plants. Of these rhizobacteria are known to be beneficial to plants through direct mechanism such as production of hormones, enzymes and metabolites (Ahemad and Khan 2012) for example indole acetic acid (IAA), ACC (1-minocyclo propane-1-carbooxylate) deaminase, hydrogen cyanide (HCN), ammonia production and dinitrogenase activity (Glick., 2012; Khan., 2005) facilitating plant uptake of macro and micronutrients from soil i.e chelation of iron through siderophore, providing plant available phosphorous through solubilization and minerlization, and indirect mechanisms such as biocontrol agents which either inhibit growth of phytopathogen or induce resistance in plants against pathogen attack. It is reported that inoculation of plant beneficial bacteria in soil or seed enhance the growth and yield in crops (Gholami *et al.,* 2009) and trees (Saheen *et al.,* 2014). There are reports that use bioinoculants during nursery establishment leads to healthy seedlings that can withstand both biotic and abiotic stress (Rincon *et al.,* 2008). Padder and co-workers (2017) reported that biomass of *D.sissoo* seedlings was enhanced upon inoculation of rhizobacteria. Rhizobacteria belonging to genera *Azotobacter*, *Arthrobacter*, *Azospirillum*, *Alcaligenes*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Pseudomonas* and *Serratia* are commonly used bioinoculants (Ahmed and Kibret, 2014). Amongst these, *Pseudomonas*

has been identified as one of the largest and most promising potential group of PGPR due to their simple nutritional requirement, robust colonization ability and substrate versatility. *Pseudomonas* inoculants significantly increased root dry weight in spring wheat (Walley and Germida, 1997) and yield in sugar beet (Cakmakc¸ *et al.*, 2001). Moreover Rahmoune *et al.*, (2017) also reported the positive impact of inoculation of *Pseudomonas* genera on Arabidopsis and Datura plants. In this study *P. koreensis* was inoculated on *D. sissoo* seedlings and its response on seedling health and soil equality was assessed. The soil enzyme activities have been suggested as a suitable indicator of soil quality. They are a measure of the soil microbial activity and therefore are strictly related to the nutrient cycles and transformation. They respond rapidly to the changes caused by both natural and anthropogenic factors (Frac, and Jezierska-Tys, 2011). Therefore in this study we evaluated how inoculation of *P. koreensis* affects soil enzymatic activities as well as overall growth of *D sissoo* seedlings.

Materials and Methods

Glass house pot trial

The pot trial was performed in three replication of each treatment. The pots were arranged in completely randomized block (CRD) design under glass house condition at AFRC (Agroforestry Research Center, G.B.Pant University of Agriculture and Technology, 28°58′N 79°25′E / 28.97°N 79.41°E) Pantnagar. The nursery trial with seven days old seedling of two disease susceptible *D.sissoo* cultivars PS90 and PS52 was carried out in 1 Kg capacity polythene bags filled with sterile autoclaved soil and vermiculite in a 2:1 ratio (w/w). Shisham seedlings were bacterized by the root dip method (Shukla, 2008). The log phase broth culture corresponding to a population of 10^8 CFU ml^{-1} of *Pseudomonas koreensis* were mixed with 1% Carboxy Methyl Cellulose (CMC) and Gum Acacia (GA) in separate flasks. Both CMC and GA were used as adhering agents. Uninoculated seedlings were kept as control. Roots of shisham seedlings at 3-5 leaf stage of seven days plants were dipped in bacterial suspension for 30 minute. Bacterized seedlings of PS 90 and PS52 were transplanted one per each pot. Plants were watered regularly through splinker system in glass house. In all, there were six treatments; T1 (PS 90 Control), T2 (CMC + *Pseudomonas koreensis*), T3 (GA + *Pseudomonas koreensis*) T4 (PS52 Control) T5 (CMC+ *Pseudomonas koreensis*) and T6 (GA + *Pseudomonas koreensis*). Plants were uprooted carefully at 30, 45 and 60 days after planting (DAP) and analyzed for plant agronomical parameters (root fresh weight, shoot fresh weight, root length, shoot length and nodule number) and soil microbiological enzyme activities.

Soil microbial enzyme activities

The soil health was monitored by estimating activities of two soil enzymes fluorescein di-acetate and alkaline phosphatase in soil from all six treatments during glasshouse trial. Fluorescein di-acetate (FDA) hydrolysis activity was evaluated according to method of Inbar *et al.*, 1991. 1 gm of fixed moist soil taken in Erlenmeyer flask was drenched with 1ml of FDA solution and 15 ml of phosphate buffer. The flasks were shaken for 20 min on rotary shaker at 25°C after which 10 ml of acetone was added for extraction. The absorbance of filtered samples was measured at 490 nm.

Alkaline phosphatase activity was assayed according to method of Tabatabai and Bremner (1969). One gram of moist soil was placed in a 50 ml Erlenmeyer flask, to which 4 ml of Modified Universal Buffer (MUB), 0.25

ml of toluene; 1 ml of p- nitrophenyl phosphate (PNPP) solution was added, swirled and incubated at 37°C. After 1 hour, 1 ml of 0.5M calcium chloride and 4 ml of 0.5M sodium hydroxide were added. Thoroughly mixed soil suspension was filtered and absorbance measured at 400nm on spectrophotometer.

Statistical analysis

The agronomical data and soil enzyme data were tested for normality and homogeneity of the variance. Two- way analysis of variance (ANOVA) was used to determine significant differences between the *D. sissoo* cultivars. In the glass house nursery trial agronomic parameters and soil enzyme activities directly depend upon inoculation of *P. koreensis* and thus served as dependent variables.

Results and Discussion

Impact of *P. koreensis* on biomass of *D. sissoo* cultivars

In both the *D. sissoo* cultivars inoculation had an overall positive effect on seedling growth. However, the performance of *Pseudomonas koreensis* inoculated seedlings was cultivar specific. In treated seedlings nodule number, root length, shoot length, shoot fresh weight and root fresh weight was higher in PS90 cultivar as compared to PS52 cultivar.

At 30 DAP the shoot length in *Pseudomonas koreensis* inoculated PS90 seedlings was 30 cm (T2= CMC + *Pseudomonas koreensis*) and 40cm (T3= GA + *Pseudomonas koreensis*) which was significantly higher than untreated control (T1= PS 90 Control) 25 cm. Similar pattern was observed at 45 and 60 DAP also. The shoot length at 45 days treatments in treated (T2, T3) and untreated (T1) was 35.4, 44.6, and 33 cm whereas at 60 DAP shoot length in treated (T4, T6) and untreated (T4) was 35.8, 39 and 34cm respectively. The similar trend was observed for shoot fresh weight, root fresh weight and root length also. In PS 52 cultivar similar variation was observed. At 30 DAP the shoot length in *Pseudomonas koreensis* inoculated PS52 seedlings was 32cm (T5= CMC+ *Pseudomonas koreensis*) and 35cm (T6= GA + *Pseudomonas koreensis*) which was significantly higher than uninoculated control i.e., T4= PS 52 control. Similar pattern was observed at 45 DAP where shoot length in treated (T4, T6) and untreated (T4) was 35.8cm, 39cm and 34cm and 60 DAP where shoot length in treated (T4, T6) and untreated (T4) shoot length was 36.4, 40 and 36cm respectively (Table 1). The similar trend was observed for shoot fresh weight, root fresh weight and root length also). Amongst the adhering agents CMC and GA both shows the positive effect on *D. sissoo* seedlings, indicating that the positive effect on seedling growth was due to inoculation of *Pseudomonas koreensis* AS15 strain only.

Impact of *P. koreensis* on soil microbiological activity

Alkaline phosphatase

Alkaline phosphatase is the measure of phosphorous recycled in the soil. Significant increase in the activity with sampling time was observed in case of *D. sissoo* cultivar PS90 as well as PS 52 cultivar. At 30 days the alkaline phosphatase activity of treated (T2, T3) and untreated (T1) seedlings of cultivars PS 90 was 31, 15.76 and 9.86 $\mu gml^{-1}hr^{-1}$ respectively.

The alkaline phosphatase activity of treated (T4, T6) and untreated (T4) PS52 seedlings was 25, 35 and 17 $\mu gml^{-1}hr^{-1}$ respectively. Similar trend was observed at 45 and 60 DAP in both the cultivars of *D. sissoo* at 45 and 60 DAP (Fig. 1).

Fig.1 Effect of inoculation of *Pseudomonas koreensis* strain AS15 inoculation on alkaline phosphatase (AP) and fluorescein diacetate (FDA) hydrolysis activities in two different *D. sissoo* cultivars PS 90 (A) and PS 52 (B) at nursery stage

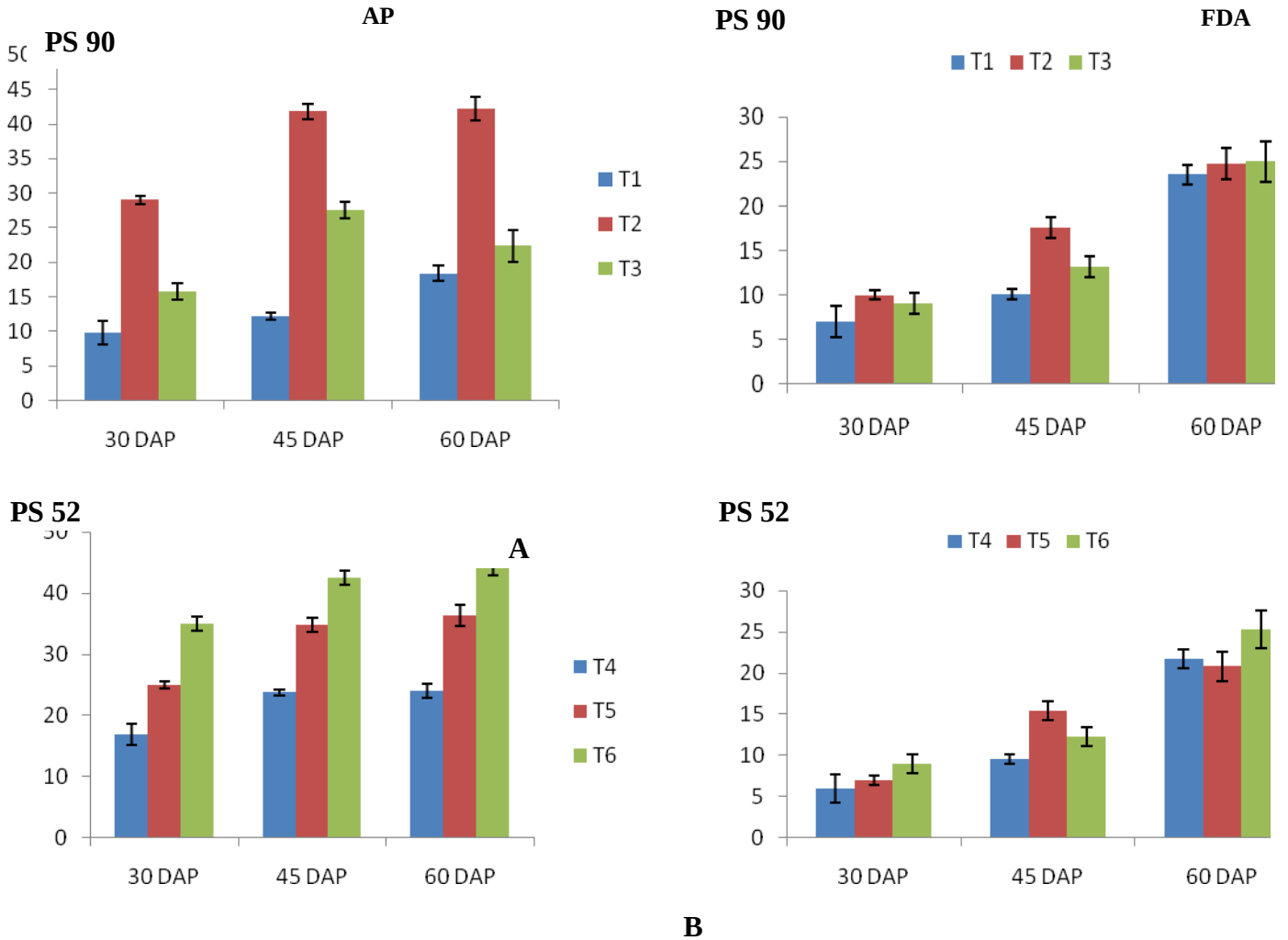

Fig.2 Correlation analysis between FDA and AP activities in two *D. sissoo* cultivars PS 52 (A) and PS 90 (B) at 45 days using R-software tool

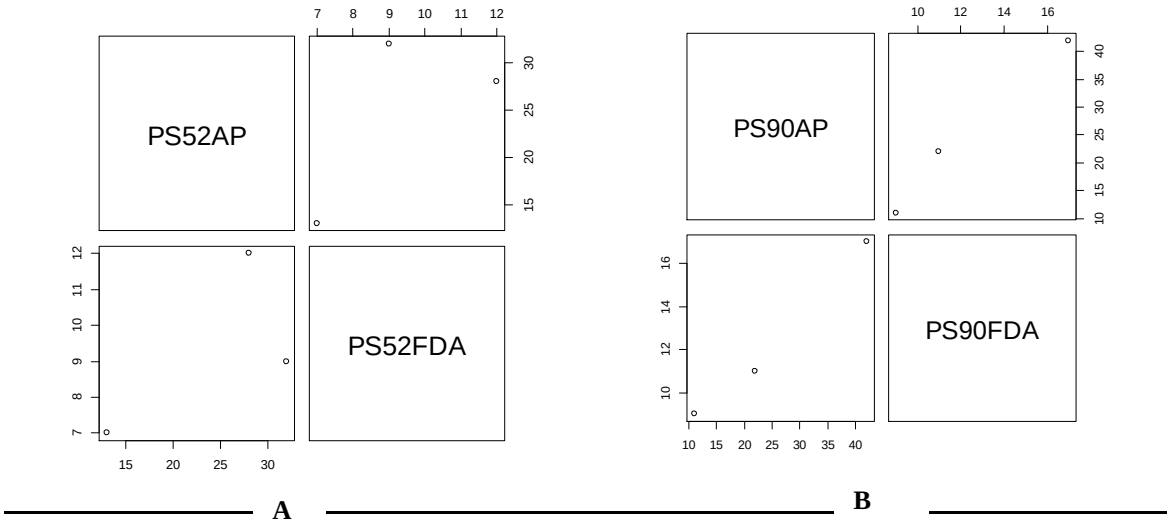

Table.1 Effect of *Pseudomonas koreensis* strain AS15 inoculation on plant vigor parameters of two *D. sissoo* cultivars PS 90 and PS 52 at nursery stage

TREATMENT	PLANT AGRONOMIC PARAMETER														
	30 DAP					**45 DAP**					**60 DAP**				
	SFW (gm)	**SL (cm)**	**RFW (gm)**	**RL (cm)**	**NODULE NO.**	**SFW (gm)**	**SL (cm)**	**RFW (gm)**	**RL (cm)**	**NODULE NO.**	**SFW (gm)**	**SL (cm)**	**RFW (gm)**	**RL (cm)**	**NODULE NO.**
T1 (control)	1.1^{A}±0.115	25^{A}±0.173	0.56^{A}±0.011	9^{A}±0.577	0	2.3^{A}±0.230	33^{A}±1.732	1.3^{A}±0.230	17.1^{A}±0.057	4±1.154	5.8^{AB}±0.461	44.4^{B}±0.23	2.8^{B}±0.057	17.6^{A}±0.346	15±2.30
T2	1.2^{A}±0.230	30^{B}±00.577	0.8^{A}±0.199	18^{C}±1.154	7±1.154	3.2^{B}±0.115	35.4^{B}±0.230	2.1^{B}±0.115	21^{B}±1.154	11±0.057	4.6^{AB}±0.173	36.2^{A}±0.461	1.4^{A}±0.1732	21.5^{B}±0.288	18±1.154
T3	1.8^{B}±1.304	40^{C}±0.173	0.9^{B}±0.057	10^{B}±1.154	0	3.3^{B}±0.173	44.6^{C}±0.115	1.4^{A}±0.173	22.4^{B}±0.230	6±0.577	5.8^{AB}±0.173	45.6^{B}±0.346	1.6^{A}±0.346	25^{C}±1.154	16±0.577
CD AT 5%	**0.22**	**1.3**	**0.24**	**0.9**		**0.44**	**1.45**	**0.6**	**1.54**		**0.77**	**1.45**	**0.6**	**1.54**	
T4 (control)	1.6^{AB}±0.305	30^{A}±1.732	0.85^{C}±0.011	16^{B}±2.30	5±1.154	2.6^{B}±0.230	34^{A}±0.577	1.4^{B}±0.155	21^{A}±2.30	12±1.154	2.5^{A}±0.288	36^{A}±0.115	1.5^{AB}±0.23	21.5^{A}±0.115	24±1.154
T5	1.8^{B}±0.057	32^{B}±1.154	0.11^{A}±0.023	15^{B}±1.154	8±1.154	2.4^{B}±0.057	35.8^{B}±0.155	1.2^{A}±0.230	23^{B}±1.154	12±2.30	2.6^{B}±0.577	36.4^{A}±0.230	1.4^{A}±0.173	24^{B}±0.115	24±1.732
T6	1.0^{A}±0.173	35^{C}±2.309	0.6^{B}±0.173	10^{A}±0.577	0	1.9^{A}±0.115	39^{C}±0.577	1.2^{A}±0	24^{B}±1.732	7±1.154	2.5^{A}±0.115	40^{B}±0.577	1.7^{B}±0.115	24.8^{C}±0.115	25±2.309
CD AT 5%	**0.6**	**1.2**	**0.06**	**1.3**		**0.3**	**1.21**	**0.01**	**1.1**		**0.06**	**1.3**	**0.2**	**0.3**	

Fluorscein di acetate hydrolysis

The amount of FDA hydrolyzed is a measurement of total soil microbial activity. FDA hydrolysis activity also varies significantly in both the treated cultivars of *D.sissoo* PS90 and PS52 as compared to their respective controls at all sampling times. At 30 days the fluroscein di acetate hydrolysis activity in rhizospheric soil of treated (T5, T6) and untreated (T4) PS90 plants were 10, 9 and 7 $\mu gml^{-1}hr^{-1}$ respectively. Whereas, fluorescein diacetate hydrolysis acticity in rhizospheric soil of treated (T5, T6) and untreated (T4) PS52 seedlings was 7, 9 and 6 $\mu gml^{-1}hr^{-1}$ respectively. The trend was similar in both *Dalbergia sissoo* cultivars at 45 and 60 DAP (Fig. 1).

The results of this study clearly showed that i) there is overall positive impact of *P. koreensis* AS15 inoculation on agronomic parameters of both the *D. sissoo* cultivars i.e., PS 90 and PS 52, and ii) inoculation of *P. koreensis* AS15 enhances soil microbiological activities e.g. AP and FDA of two *D. sissoo* cultivar PS 90 and PS 52. At 30 DAP plant agronomic parameters in treated cultivar were enhanced in treated seedlings for example, shoot length in treated PS 90 seedlings (T2, T3) and was 35.4 and 44.6 cm respectively whereas in untreated control (T1) was 25 cm. At 60 DAP shoot length in treated cultivar PS 52 seedlings (T5 and T6) was 36.4 and 40 cm respectively whereas in control (T4) 36 cm. Root length, shoot fresh weight, root fresh weight also showed the similar trend. This increase in the plant agronomic parameters may be due to inhibition of soil borne pathogen upon inoculation of *P.koreensis* AS15. The inhibition is either due to secretion of antifungal metabolites (Lee *et al.*, 2003), or competitive exclusion of pathogen through rapid colonization in the rhizospheric region (Haas and Defago, 2005). The increase in root and shoot parameters in *Pinus taeda* seedlings treated with *Pseudomonas fluorescens* have also been reported (Fernandes *et al.*, 2018). In present study AP and FDA show positive correlation analyzed with R-software tool at 45 days which signify that increase in total microbial activity increases alkaline phosphatase activity which results in the release of free phosphate for the uptake by plants. This directly enhances the plant growth (Fig. 2). There was substantial positive effect on plant growth, development and physiology upon inoculation with strains belonging to genus *Pseudomonas sp*. through a combination of direct and indirect mechanisms (Upadhyay and Srivastava, 2008). Moreover *Dalbergia latiffolia* nursery inoculated with rhizospheric bacteria exhibited enhancement in all the agronomic parameters (Soumaya *et al.*, 2017).

From the results of our investigation it is clearly evident that inoculation of *P.koreensis* AS15 clearly significantly enhanced the overall growth parameters of *D.sissoo* seedlings. However, the enhancement was cultivar specific. The impact was more in PS90 cultivar as compared to PS52. In addition to this, inoculation of *P.koreensis* AS15 strain significantly enhanced soil alkaline phosphatase and fluorescein diacetate hydrolysis activities indicating higher availability of phosphate and carbon for plant uptake. Thus bacterization of PS90 seedlings with *P.koreensis* AS15 could result in quality planting material.

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