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Short Communication
Studies on strain specificity of *Frankia* in *Alnus nepalensis*

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ABSTRACT

To identify an effective and compatible *Frankia* strain for *Alnus nepalensis*, experiments were carried out with four different strains. *Alnus* was infected by all the four *Frankia* strains as was evidenced by the higher growth parameters over the uninoculated control. The strain AVC - II performed better in terms of infectivity and productivity of *Alnus* when compared with other strains tested. The study clearly indicated the possibility of exploiting the host - endophyte specificity in *Alnus* for higher productivity through effective symbiosis.

Key words: *Alnus nepalensis*, *Frankia*, inoculation.

Nepalese alder (*Alnus nepalensis*) belonging to the family Betulaceae is one of 15 genera of trees that fix nitrogen but are not in legume family. *Alnus* occurs throughout the Himalaya hills at 500 - 3000 m elevation from Pakistan through Nepal, Bhutan and Burma to southwest China. It is found naturally in subtropical mountains with an average rainfall of 500 - 2500 mm and a 4 - 8 month dry season. *Alnus* forms a symbiosis with nitrogen fixing actinomycetes of the genus *Frankia*. *Frankia* are known to infect some 165 plant species distributed among eight orders of plants (Bond 1983). *Frankia* - *Alnus* symbiosis increase the nitrogen content of soil by about 61.5 to 157 kg N ha^{-1} $year^{-1}$ which indicates the importance of this association in the overall nitrogen economy of the soil (Subba Rao 1989).

Low nitrogen fixing *Frankia* strains can also infect the host, which suggests the need for inoculation of the *Alnus* roots with right endophyte before field planting. Efficient *Frankia* strains must be able to compete with wild strains to nodulate the host plant, resulting in a highly efficient association in the field. The right host-strain specificity should be assessed to find out the best combination for cultural use. Hence the experiments were conducted to identify an efficient *Frankia* strain which could establish good nodulation for higher productivity in *Alnus nepalensis*.

The experiments consisted of three *Frankia* strains and a nodule suspension of *Alnus nepalensis* besides an uninoculated control. The nodule suspension was prepared using active healthy nodules collected from natural stands. They were packed in plastic bags on ice and stored under - 10°C. Nodules were washed with sterile water and surface sterilized with 30 % hydrogen peroxide for 5 minutes under aseptic conditions. The suspension was prepared by crushing nodules using mortar and pestle (30 g nodule : 100 ml sterile distilled water). The suspension thus obtained was used for inoculation.

The *Frankia* strains viz., *Frankia* 53024, *Frankia* H - 43 and *Frankia* AVC - II were obtained as pure cultures in the liquid broth from the Department of Botany, University of Glasgow, Glasgow, United Kingdom. Aseptically grown, one year old *Alnus* seedlings were dipped in the pure cultures of *Frankia* nodule suspension for 30 minutes and planted in the field at Horticultural Research Station farm, Udhagamandalam, The Nilgiri Hills. The experiments were laid out in a randomized block design with five treatments and four replications. Six months after inoculation, the effect of each treatment was evaluated for plant growth characters viz., root length, shoot length, plant biomass and total chlorophyll content. Infectivity of each strain was examined in terms of nodule number, size, dry weight and nodule nitrogenase activity by acetylene ethylene assay (Hardy *et al.* 1968).

Results indicated that *Alnus* was infected by all the strains tested, as was evidenced by the higher growth parameters when compared with the uninoculated control (Table 1). Among the inoculated treatments, higher productivity was observed in *Alnus* plants inoculated with *Frankia* AVC II, recording higher shoot length, root length, dry matter production and chlorophyll content followed by *Frankia* H 43 and *Frankia* 53024 strains. Compared with the uninoculated control, all

Table 1. Effect of inoculation of *Alnus nepalensis* with Frankia strains on plant growth and chlorophyll content.

Treatment	Shoot length (cm)	Root length (cm)	Total chlorophyll (mg g^{-1} FW)	Plant dry weight (g)
1 Nodule suspension of *Alnus nepalensis*	38.00	13.56	2.40	4.62
2.Frankia 53024	49.65	17.56	2.65	5.92
3. Frankia H - 43	51.08	19.00	2.81	6.12
4. Frankia AVC - II	64.00	25 00	3.96	8.71
5 Control	23.00	11.50	2.18	3.10
LSD (0.05%)	4.004	1.033	0.306	0.366

FW Fresh weight

Table 2. Infectivity of *Frankia* strains on *Alnus nepalensis*.

Treatment	Nodule number	Nodule diameter (cm)	Nodule dry weight (mg)	Nodule nitrogenase activity (u mol $C_2H_4 g^{-1} hr^{-1}$)
1 Nodule suspension of Alnus nepalensis	6.14	0.34	2.60	0.34
2. Frankia 53024	8.62	0.46	3.21	1.59
3. Frankia H - 43	9.81	0 52	3.62	1.62
4. Frankia AVC - II	14.62	0.74	4.50	2.21
5 Control	3.38	0.21	0.51	0.21
LSD (0.05%)	0.472	0.909	0.344	0.237

treatments with *Frankia* inoculations helped in higher biomass built-up in *Alnus* (Simon *et al.* 1985). The infectivity assay (Table 2) also showed a similar trend: higher nodule number and nitrogenase activity when inoculated with *Frankia* AVC - II strain. The higher performance of the *Frankia* pure culture might be attributed to the high quality inoculum, high concentration of infective cells and long term viability conferred by spores (Van Dijk 1978).

The other two *Frankia* strains viz., *Frankia* 53024 and *Frankia* H 43 were similar in their performance, followed by the nodule suspension. This kind of infectivity variation among the *Frankia* strains confirmed the heterogenic infectivity of *Frankia* (Wheeler *et al.* 1991). The poor performance of these two *Frankia* strains compared to *Frankia* AVC - II may possibly be due to the lower proportion of high infective *Frankia* cells (Van Dijk and Slumier Stolk 1990) and possession of partial compatible *Frankia* strains (Torrey 1990). Nodules obtained under field conditions may contain more than one strain (Nesme *et al.*, 1985) and this might be the reason for the lower infectivity of the nodule suspension.

The results showed the infectivity variation among the *Frankia* strains tested. However, all the strains ultimately helped in higher biomass built-up, nodule number and nitrogenase activity of *Alnus*. Therefore the results suggest the necessity for selecting an optimal combination of a host genotype-endophyte strain for increased productivity. *Frankia* strain AVC - II was found to be the best symbiont for *Alnus nepalensis* in this experiment.

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