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Short Communication

Effect of Different Hormone Concentrations in *In vitro* Seed Germination of Nepalese Alder (*Alnus nepalensis*)

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Actinorhizal root nodule formation involves the symbiotic association of an actinomycete *Frankia* and roots of dicotyledonous plants belonging to eight plant families and 24 genera [1, 2]. The host plants are called "actinorhizal plants" and its root nodules "actinorhizal root nodules". The term "actinorhizal" became the preferred designation for those taxa that form a nitrogen-fixing association with members of the Actinomycetes. Likewise, nodules found on roots were called "actinorhizal" nodules to distinguish them from legume nodules [3]. *Alnus nepalensis* is an important nitrogen fixing plant belonging to the family *Betulaceae*. *Alnus* can be found in glacial, forest and alpine ecosystems. Actinorrhizal plants have current and potential applications in reforestation and soil improvement, timber and pulp production, and acting as nurse and fuel wood plants [4, 5]. Globally they have the potential for integrating into schemes for addressing issues like pyrodenitrification and reforestation.

The seed germination of *Alnus* and its survival in both *in vitro* and *in vivo* is a serious challenge to scientists and plant growers as the germination process is very slow. The present investigation was carried out to detect the effects of different media and plant growth regulators in the process of seed germination in *Alnus*.

MATERIALS AND METHODS

Mature seeds of *Alnus* were collected from the healthy plants of Darjeeling hills. They were stored under dry and air tight conditions. Two treatments of seeds were — overnight soaked with aerated water and non-soaked using three different media, woody plant medium (WPM, Hi-Media, Cat#PT105) (pH-5.6), Murashige & Skooge medium (MS) (Hi-Media, Cat#PT0018) (pH-5.6) and Hogland solution (pH-7) [6] in half and full strength. The 1 ml of media was poured in each culture tube carrying a strip of filter paper and was autoclaved at 121ºC for 20 min at 1.08 kg/ cm^2 pressure. The seeds before inoculation were surface sterilized with mercuric chloride for 5 min and washed thoroughly with sterile distilled water. A few *Alnus* seeds were placed on filter paper in culture tube and incubated at 25º±1ºC under white fluorescent light. For the study of effects of hormones on seed germination, the media were separately supplemented with 1-5 mg/litre NAA and IBA. Sterilized seeds were also placed on sterile vermicompost for germination. Double layer culture media were prepared by pouring Hogland medium (0.4% agar) on MS medium (1.2% agar), WPM (1.2% agar) and Hogland medium (1.2% agar) supplemented with hormones as described earlier.

RESULTS AND DISCUSSION

The seeds germinated within 4 weeks. The treated and untreated conditions, media nutrients and contamination of fungus do not have any effect on the duration of seed germination. Contaminations were reported in mineral rich medium under both treated and untreated conditions. The percentage of germination varied with the media used (Fig. 1). Maximum germination was observed in Hogland media for

both treated and untreated seeds, whereas minimum germination in ½ MS for treated and ½ WPM for untreated seeds. The germination was probably inhibited by high nutrient concentrations. For treated seeds, maximum (80%) survival rate was reported in Hogland media, whereas minimum (0%) survival rate was reported in WPM. For untreated seeds maximum (100%) survival rate was reported in Hogland, whereas minimum (20%) survival rate was reported in half strength WPM. Moderate germination and survival rate with low contamination were reported when water was used as germination medium.

The hormones had profound but variable effects on seed germination and rooting of *Alnus* (Fig. 2). The IBA and NAA+IBA were found to be more effective than NAA in terms of germination percentage and rooting. Moderate germination percentages (above 50%) were observed in the media supplemented with IBA (@ 1 & 2 mg/litre) and NAA+IBA (@ 1+1 & 2+2 mg/litre). Maximum (90%) germination was observed with the application of IBA (1 mg/litre) and NAA+IBA (@1+1 mg/litre). The germination percentage increased with the increase of NAA concentration, declined with the increase of IBA and NAA+IBA concentrations. Good rooting and its growth were observed in the media supplemented with IBA (@ 3, 4 & 5 mg/litre) and NAA+IBA (@1+1 mg/litre). The maximum rooting and its growth was recorded in the medium supplemented with IBA (@ 4 mg/litre). In the medium supplemented with NAA+IBA (@2+2 mg/litre) rooting was poor but the roots showed good growth.

In the study of seed germination on sterilized vermicompost, good germination rate was reported within 10 days but seedlings did not survive. The poor seedling survival was due to poor root development. In double layer culture experiment, both germination percentage and survival rate were very low although media contained required hormones as described in earlier experiments.

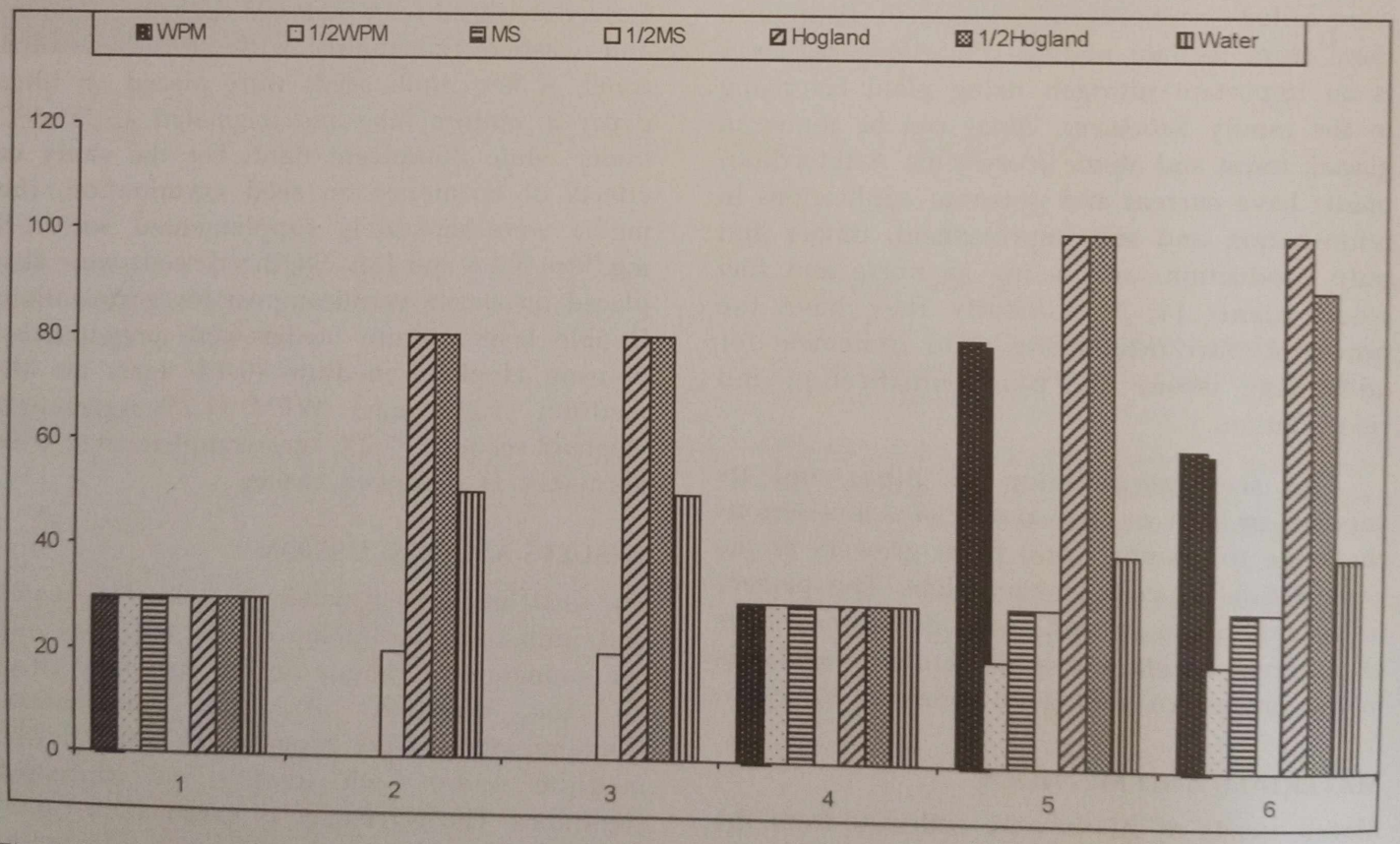

Fig. 1. Generation time (G time), germination percentage and survival rate of treated and untreated *Alnus nepalensis* seeds in different media

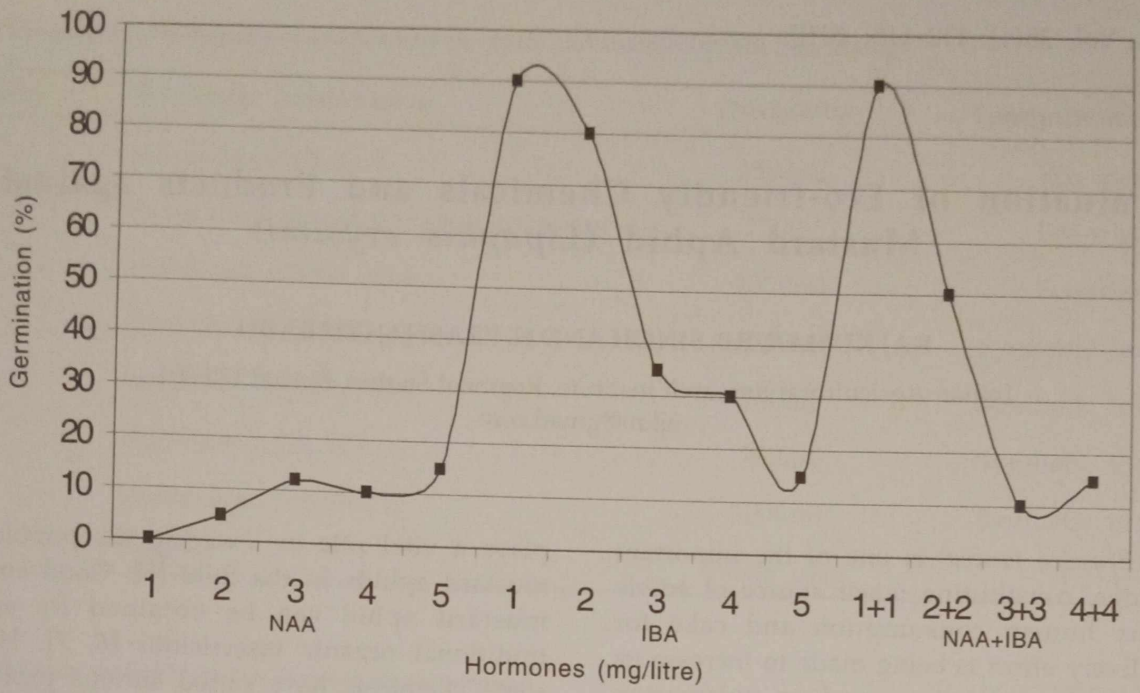

Fig. 2. Germination percentage of *Alnus nepalensis* seeds in different hormone concentrations

Present experiments showed that germination of *Alnus nepalensis* seeds had specific hormonal requirements. These hormonal requirements are mostly fulfilled by mycorrhizal or associative fungi. In vermi-compost the *Alnus* seedlings did not survive though there were plenty of nutrients. The nutrients were not absorbed due to poor root development, which require external hormonal supply. *Alnus nepalensis*, thus essentially requires good root growth in initial stage for proper association with fungi. At maturity, it is known to make another association for survival, with an actinomycetes bacteria *Frankia*.

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