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ROOT NODULES IN NON-LEGUMINOUS PLANTS IN WEST PAKISTAN

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Abstract

Of the 13 genera of non-leguminous dicotyledonous plants which in other regions of the world have been found to bear *Alnus*-type nodules, four genera viz., *Casuarina*, *Elaeagnus*, *Hippophae* and *Alnus* are represented in West Pakistan. In respect of this country, all these genera, except *Casuarina*, are confined to the temperate northern himalayan regions. Their root nodules are present in coralline clusters. The nodular endophyte is actinomycetal in nature. The author believes that the morphological nature and anatomy of *Alnus*-type nodules need further study.

Introduction

Although nitrogen-fixing root nodules are best known in legumes, they also occur in 13 genera of non-leguminous dicotyledons belonging to various families which are closely related (Khan, 1972). These nodules which are sometimes termed *Alnus-type* nodules since those of *Alnus* have been fully studied, form after infection of the roots by actinomycete-like organisms. Recently, Khan (1972) reported podocarp-type mycorrhizal nodules on roots of *Aesculus indica* but they have not been tested for nitrogen fixation and it seems very unlikely that they possess this faculty.

Because of the biogeochemical significance attached to plant genera bearing nodules of *Alnus*-type (Bond, 1967) the author was prompted to investigate their occurrence in West Pakistan. Of the genera which in other regions have been found to bear above type nodules, four viz., *Casuarina*, *Elaeagnus*, *Hippophae* and *Alnus*. are represented in this country. So far as the author is aware, the only previous records of the occurrence of nodules on species of these genera in this sub-continent are in respect of *Casuarina* spp. (Narasimhan, 1918; Rao, 1924; Chaudhuri, 1931; Parker, 1932) and *Alnus nitida* (Parker, 1932).

Materials and Methods

Root samples were collected from northern himalayan regions of West Pakistan (Table 1) and were studied following the previously described techniques (Khan, 1972). Those of *Casuarina cunninghamiana* were obtained from trees cultivated in Lahore.

Results

Occurrence and Distribution:

Roots of all the plants of *Casuarina cunninghamiana* Miq., *Elaeagnus umbellata* Thunb., *E. angustifolius* L., *Hippophae rhamnoides* L., and *Alnus nitida* Endl. examined,

Table I. Present distribution of the non-leguminous root-nodule bearing dicots of West Pakistan. (Values in parenthesis represent number of plants of each species examined and found nodulated).

Order, Family, Genus, Species	Present Distribution
Casuarinales	
Casuarinaceae	
Casuarina	
C. cunninghamiana Miq. (3)	Mostly Australian, cultivated in West Pakistan.
Betulales	
Betulaceae	
Alnus	
A. nitida Endl. (2)	Himalaya, 3000 — 9000ft. Along Marguzar, Madian (Swat).
Sapindales	
Hippocastanaceae	
Aesculus	
A. indica Colebr. (10)	Himalaya, 3000—10,000 ft. Murree Hills, Bahrein (Swat).
Elaeagnales	
Elaeagnaceae	
Elaeagnus	
E. umbellata Thunb. (1)	Himalaya, 3000 — 10,000 ft. Common, especially in rather dry exposed places, Madian, Bahrein, Kalam (Swat).
E. angustifolius L. (2)	Common in Quetta valley (Baluchistan).
Hippophae	
H. rhamnoides L. (3)	Himalaya, 8000 — 11,000 ft. in dry inner valley of Hazara. Along streams in Utror-Gabral (Swat).

were found to possess *Alnus-type* nodules (Fig. 1). No nodules were observed on the roots of all the ten plants of *Rhamnus pentapomica* Parker, a common shrub in Hazara, Rawalpindi and Salt Range.

It is evident from Table 1 that in respect of West Pakistan all these genera, except *Casuarina*, are confined to the temperate northern himalayan regions. They are scattered all over the world and are contemporaneous (Bond, 1967; Khan, 1972).

Morphology of Nodules:

The root nodules of *Alnus*, *Elaeagnus*, *Hippophae* and *Casuarina* species are usually present in coralline clusters, due to their perennial nature and repeated formation of new nodular lobes at the apex of the original nodule (Fig. 1).

Confirming the observations of previous authors, sections of these clusters showed that each nodular lobe possesses (a) an outer periderm surrounding the nodule, (b) a 2-5 layered hypertrophied cortex with some cells filled with the endophyte and (c) a stele bounded by tannin-filled cells of the endodermis. In some cases a group of meristematic cells, resembling a root apical meristem, is found at the apex of the nodular strand. In *Casuarina*, bifurcation of the nodular meristematic cells results in the formation of two lobes at the apex of the original nodule and the repeated repetition of the bifurcation results in the formation of a nodule cluster. Presumably the same is true for the other species.

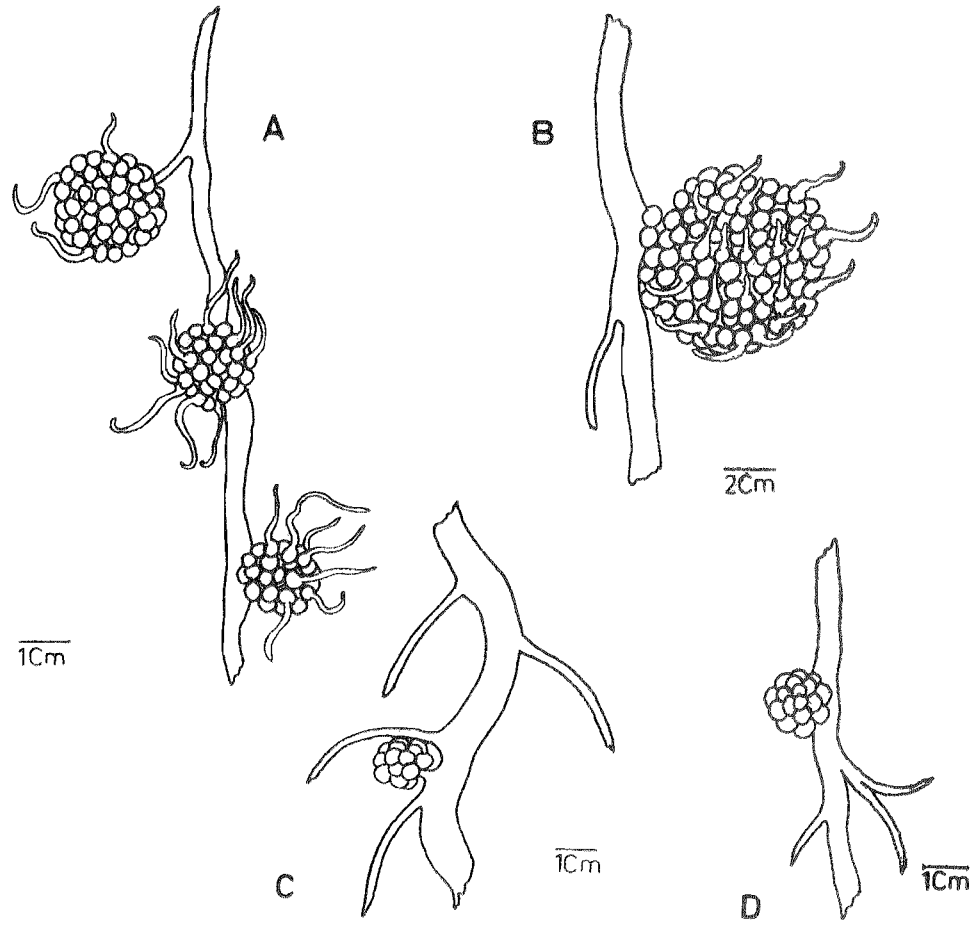
In some *Alnus* and *Casuarina* nodular clusters, the apex of each nodule lobe was found to develop into a normal root which grows upwards and which possesses a clear root cap.

Morphology of Endophyte:

The endophyte in the above type nodules was actinomycetal in nature. Under the light microscope, many homogenous and structureless vesiculous swellings on the Actinomycete like hyphae in the young nodules of these genera were present, whereas older nodules lacked them, presumably due to lysis and digestion of the endophyte by the host. Several authors have, however, shown that under electron microscope the vesicles are seen to be multicellular structures, with internal dividing walls, interpreted by some workers as sporangia (Bond, 1967).

Discussion

The present survey indicates that five out of 14 non-leguminous root nodule bearing dicots are represented in the temperate zone of Northern Himalayan regions of West Pakistan. It is suggested that these genera are survivors of an era in which conditions



EXPLANATION OF FIGURE

Root Nodules of non-leguminous dicots of West Pakistan.

A. *Casuarina cunninghamiana.* Note the development of the apices of the nodular lobes into nodular roots.

B. *Alnus nitida.* Note the nodular root development from the apices of the nodular lobes.

C. *Hippophae rhamnoides.*

D. *Elaeagnus angustifolius*

were favourable for the development of symbiotic associations and that the nodules are additional features for the retention of the endophyte (Khan, 1972). Absence of root nodules in *Rhamnus pentapomica* agrees with the observations of Rodrigues-Barrueco (1969). These observations are, however, in contrast with those of Chodat (1904) who, in an unnamed species of *Rhamnus*, reported *Alnus* -type nodules.

Although there have been many detailed physiological studies of *Alnus*-type nodules but there seems, to be no comparative morphological and anatomical studies of rootlets and nodules. Nevertheless, most authors seem to have contented to assume that the nodules are modified rootlets or lateral roots of arrested growth. The author believes that the morphological nature and the anatomy of *Alnus*-type nodules need further study, which might reveal that in their apical regions they show a closed type of structure similar to that of the nodules of podocarps and of *Aesculus indica* (Khan, 1967, 1972). A careful examination of photomicrographs by Fletcher (1955) and Lawrence et al. (1967) of nodules of *Myrica gale* and *Dryas drummondii*, respectively, rather suggests that in the apical region the endodermis completely overarches the stele, though admittedly such an appearance could result from the sections being not quite radial a situation which can easily arise because of the curving nature of the nodule lobes. In podocarps and in *Aesculus indica* the pericyclic cells underneath the overarching endodermis become meristematic and provide for the further growth of the nodule. In an illustration of a longitudinal section of a branched nodule of *Alnus glutinosa* by Bond (1963; plate 2, Fig. 3), the uninfected meristematic region at the apex of the nodular stele is probably due to the meristematic activity of the nodular pericyclic cells during the process of regeneration, rather than the apical meristem of the nodule.

Regeneration of *Alnus*-type nodules to form nodular cluster is in accord with the observations of previous workers (Bond, 1963). Similar regeneration is also common among podocarps and *Aesculus indica* (Khan, 1967, 1968, 1972; Khan and Valder, 1972) and the analogy is even more conspicuous in the case of *Saxegothaea conspicua* in which repeated division or bifurcation of the meristematic zone formed endogenously at the apex of the nodular stele gives the root its characteristic coralline, rather than beaded, appearance (Spratt, 1912). Baylis et al (1963) also noted that the regeneration of podocarp nodules is occassionally accompanied by branching so that two series of upto four nodules may surmount a single parent nodule.

The production of nodular roots from nodules in *Casuarina* is consistent with the observations of Bond (1956). In *Alnus* they are either absent (Ferguson and Bond, 1953) or rarely present (Bond, 1972, personal communications). In the present study, however, nodular roots are observed in some nodular clusters of *A. nitida*. The phenomenon of proliferation of nodules to form nodular roots in *Myrica*, *Alnus* and *Casuarina* also require further anatomical studies. Sahni (1920) reported a similar proliferation of tubercles of *Acmopyle pancheri*, a podocarp, into normal rootlets. Rootlet production from leguminous nodules of *Sesbania* and *Caragana* has also been reported (Allen and Allen, 1965). There are, however, no such reports for other podocarps, legumes and non-leguminous nodule bearing genera.

The endophyte in these nodules has been variously classified by previous observers as fungal, actinomycetal or plasmodial in nature. This discrepancy in the literature is due to the failure of isolation of any of the endophyte in pure culture, despite the exhaustive researches of Uemura (1952, 1964), Quispel (1954) and Fletcher (1955). Uemura isolated many actinomycetal strains from non-legume nodules but none of these isolates resulted in the development of nodules. Present information, however, suggests that non-leguminous root nodules in all the genera, except in *Aesculus indica*, form only after infection by actinomycetes. On the other hand the root nodules of *A. indica* are typical vesicular arbuscular mycorrhizae which develop without any fungal stimulus (Khan, 1972).

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