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A RESUME OF THE NODULATION SURVEYS AMONG WILD LEGUMES

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ABSTRACT

A summary of the results of nodulation surveys from 20 countries viz., Australia, Argentina, Brazil, Bulgaria, Colombia, French Guiana, Hong Kong, India, Indonesia, N. America, N. Ireland, Pakistan, Puerto Rico, Philippines, Singapore, S. Africa, Trinidad, Venezuela, W. Africa and Zimbabwe is presented. The results of nodulation surveys indicate that wild legumes, both herbs and trees are frequently nodulated in their natural environments. The results also show that new records of nodulation are being endorsed in the inventory of family *Leguminosae* especially from arid and semiarid areas of the world to which most of the above countries belong. Significance of nodulation surveys has been discussed with particular reference to their role in exploring new nitrogen-fixing associations and discovery of new nodulated species in *Leguminosae*.

KEYWORDS: Nodulation, Symbiosis, Wound infection, Ecosystem, *Burkholderia*, Bacteroid.

INTRODUCTION

Leguminosae is the third largest family of Angiosperms with 16000 – 19000 species distributed in about 750 genera (Allen and Allen, 1981). Nodulated legumes are found in all habitats in nature with the exception of open seas Sprent *et al*., (2017). The economic utilization of legumes is ranked only next to grasses (Howieson *et al*., 2008). Numerous leguminous species serve as human food, forage for cattle, timber production, medicinal, ornamental and in many other beneficial uses (Allen and Allen, 1981; Howieson *et al*., 2008, Sprent, 2009). Majority of naturally growing or 'wild' legume trees and herbs form nitrogen-fixing symbiosis with rhizobia and other non-rhizobial genera (Faria *et al*., 2010; Mahmood and Athar, 2018). Through their N-fixing ability legumes are helpful in maintaining N-balance of the soil in arid and semi-arid areas of the world. Majority of wild legumes thriving in arid regions have capability to nodulate under extreme environmental conditions (Gehlot *et al*., 2012). Thus they play a key role in ecological rehabilitation of degraded and denuded lands (Jha *et al*., 1987; Brockwell *et al*., 1995; Mahmood, 1999; Zahran, 2009). In the present paper results of nodulation surveys carried out in different countries have been compiled and importance of nodulation surveys has been discussed.

Nodulation surveys among wild legumes

In order to estimate the extent of nodulation in leguminous plants nodulation surveys have been carried out world over from time to time. The pioneer work in this field is that of Allen and Allen (1947), who published a review report based on the results of nodulation surveys among wild legumes growing in the natural ecosystems of Hawaii and Wisconsin States of North America. According to the review report 964 legumes were examined for nodules which formed about 10 percent of about 10000 legume species estimated for *Leguminosae* world-wide. Out of 964 species, 887 species were found nodulated. The percentage of nodulated and non-nodulated legumes was found 90 percent and 10 percent respectively. Publication of this report arose considerable interest in the mind of many rhizobiologists and botanists in different countries. As a result several surveys were carried out on nodulation among wild or naturally occurring legumes from geographically diverse areas of the world. For instance Mostret (1955) examined nodulation in 21 South African legumes, 14 of them were found nodulated. Bowen (1956) examined 101 legume genera for nodulation in Queensland (Australia), of which 40 genera had no proior record at all of nodulation. Pate (1961) described perennial nodules on the roots of 29 legumes growing naturally in Northern Ireland. Lange (1959) reported nodulation on the roots of 137 legumes growing in Western Australia. Out of 137 species 42 species belonged to *Mimosoideae* and 95 species belonged to *Papilionoideae* that were distributed in 24 genera including 8 previously unlisted genera. All 137 species were claimed as new records of nodulation. Allen and Allen (1961) reviewed the scope of nodulation in *Leguminosae*. Out of 13000 estimated species, 1285 legumes (about 10%), had been examined for nodulation. They reported that nodules occur on the roots of only 23 percent of the plants examined in the family *Caesalpinioideae* and about 90 percent of the *Mimosoideae* and *Papilionoideae*. It is interesting to note that in both the survey reports published by Allen and Allen in 1947 and 1961 respectively, the percentage of leguminous species examined for nodulation did not exceed 10 percent.

Grobbelaar *et al*., (1964) observed nodulation in 63 leguminous species, of which 44 were new records of nodulation. A survey of tropical legumes in Trinidad carried out by De' Souza (1966) showed that 64 of 79 legumes were nodulated. Grobbelaar *et al*. (967) examined 246 legume species for nodulation of which 123 were new records.

Norris (1969) observed nodulation status of 54 rain forest tree legumes in Amazonia and Guiyana. Nodulation was observed for the first time on the roots of 14 species, distributed in 9 genera including 3 species of *Swartzia*. Rothchild (1970) observed nodulation behavior of subtropical legume flora of Argentina. Barrious and Gonzalez (1971) surveyed root nodulation in 127 legumes growing in Venezuela, 18 of them were found non-nodulated. Dubey *et al*. (1972) recorded nodulation in 13 previously unreported legumes of Puerto Rico. Hely and Ofar (1972) reported 33 wild legumes growing in the northern Negev region of Israel, that were all nodulated. Grobbelaar and Clark (1972) examined nodulation in 215 legume species in South Africa, of which 207 species were new records. Grobbelaar and Clark (1974) observed nodulation in 213 species belonging to South Africa, 76 of them were recorded as nodule bearing for the first time. Corby (1974) surveyed 539 legume species growing in Rhodesia (now Zimbabwe) for nodulation. Twenty three genera and some 340 species were new to the world index of nodulation. Saono *et al*. (1975) reported nodulation in 13 edible legumes of Indonesia. Grobbelaar and Clark (1975) reported nodules on the roots of 184 legume species indigenous to South Africa, one hundred and forty one species were new records of nodulation. Iyer (1976) reported nodulation in 11 genera of *Papilionaceae* from Venezuela. Allen and Allen (1976) presented the nodulation profile of the genus *Cassia* in the world. Fifty nine species were examined, 50 were found non-nodulated, 5 nodulated and 4 were found disputed. Lim and Ng (1977) surveyed 35 legumes species from Singapore, of which 25 belong to *Papilionoideae*, 7 to *Mimosoideae* and 3 to *Caesalpinioideae*. Nodules were found on the roots of all species except 2 species of *Caesalpinioideae*. Lim (1977) examined a total of 68 legumes species from Singapore, comprising 27 members of *Caesalpinioideae*, 13 of *Minosoideae*, 27 of *Papilionoideae* and 1 of Swartzoideae. Slightly more than half the species examined (37) did not have nodules. Grobbelaar and Van Rooven (1979) surveyed nodulation on the roots of naturally growing legumes in South Africa. Eighty two species were investigated, of which 79 were new records. Allen and Allen (1981) in their classical work have compiled global nodulation data of the legumes of the world. The data revealed that only 15% species had been examined. At species level the frequency of nodulation in *Caesalpinioideae*, *Mimosoideae* and *Papilionoideae* was found 20, 90 and 98%, respectively. The global data on nodulation in tree legumes has been compiled by Halliday and Nakao (1982).

Grobbelaar *et al*. (1983) surveyed 90 South African legumes for nodulation, 59 out of them were new records of nodulation. Faria *et al*. (1984) examined nodulation in tree legumes from South East Brazil. Six new genera and 55 new species were identified. Fifty eight new records of non-nodulating species were also reported. All have potential importance in reforestation. Nimbalker *et al*. (1986) described the nodulation status of legumes from India. Eighty six species were examined. Seventy eight species were found nodulated, of which 19 were new records of nodulation. Kretschmer *et al*. (1987) have compiled a list of tropical forage legumes from Southern Mexico. Faria *et al*. (1987) reported nodulation in 85 species of tree legumes from South East Brazil. Six species of *Caesalpinioideae*, 23 of *Mimosoideae* and 27 of *Papilionoideae* were new additions to the world index of nodulation. Two new nodulated genera were also reported. Barnet (1988) summarized the reports of nodulation among Australian native legumes: 52 genera are listed, 49 from the *Papilionoideae*, 3 from the *Mimosoideae* (*Acacia, Albizia, Neptunia*) and none from *Caesalpinioideae*.

The second global data on nodulation in Leguminosae published by Faria *et al*. (1989) indicated that approximately 3395 species (approx. 20%) of legumes had been examined. Frequency of nodulation observed in *Caesalpinioideae*, *Mimosoideae* and *Papilionoideae* was 23, 90 and 97%, respectively. Faria *et al.* (1989) registered an increase of 5% in the number of species examined for nodulation over the first global data compiled by Allen and Allen (1981).

Since the publication of second global review on nodulation (Faria *et al*., 1989) many more genera and species have been examined for nodulation. Wester and Hogberg (1989) reported nodules on the roots of 7 tree legumes from Africa. Nodulation was reported for the first time in 2 species of *Mimosoideae*. Moreira *et al*. (1992) examined nodulation status of 172 legume species from different ecosystems within the Amazon of Brazil. Occurrence of nodulation in 8 genera and 98 species was reported for the first time. Margarita Sicardi de Mallorca and Maria Lusia Izaguirre Mayoral (1993) observed nodulation in 36 legumes collected from tropical Savanna in Venezuela during a rainy season, 34 out of 36 were found nodulated. Subramanium and Babu (1994) examined 50 leguminous species from subtropical Himalayan ecosystems in India, 4 of them were new records. Aguilar *et al*. (1994) examined 141 species of legumes for nodulation from the Philippines that were distributed in 18 tribes and 70 genera. Faria *et al*. (1994) screened roots of 120 leguminous plants for nodules from four states of Brazil. Nine genera stood out as new records. At species level 79 species were nodulated, of which 48 were new records. Athar (1996) surveyed 66 legumes for nodulation from Sacramento valley California. Twelve species were reported as nodule bearing for the first time. Athar (1997) examined 60 leguminous species from Cache valley and North Utah, 7 species were claimed as new records of nodulation. Faria and Lima (1998) examined 131 species for nodules from seven regions falling within Amazonian forest of Brazil. Four genera and 45 species were new records of nodulation. Roggy and Prevost (1999) studied the nodulation status of 62 taxa of *Leguminosae* in a rain forest in French Guiana. The results showed that 60% of the species were nodulated. The percentage of nodulation in *Caesalpinioideae*. *Mimosoideae* and *Papilionoideae* was 51, 71 and 77%, respectively. Athar and Harding (2000) surveyed 44 legumes growing in the Tahoe Basin California, of which 9 species were not described as nodule bearing before. Diabate *et al*. (2005) reported for the first time, the nodulation ability of 31 species and 4 genera of leguminous trees in the West African tropical rainforest. Nodulation in one genus was confirmed while nodulation in the other three genera needs confirmation (Sprent, 2005).

Ng and Hau (2009) observed 28 species of legumes distributed in 16 genera, of which 20 species were found nodulated and 8 non-nodulated. Five species out of 20 were new to the world’s nodulation index. dos Reis Junior (2010) recorded 13 new nodule bearing species of *Mimosa* from Cerrado and Cattinga biomes of Brazil. Faria *et al*. (2010) surveyed nodulation in 199 leguminous species from the Amazonian forest of Porto Trombetas (Brazil) of which 137 species were found nodulated. Thirty two species including 3 from *Caesalpinioideae*, 19 from *Mimosoideae* and 10 from *Papilionoideae* were found as new records of nodulation. One new genus *Cymbosema* was recorded as nodule bearing for the first time. Gehlot *et al*. (2012) observed nodulation in 35 legumes from Thar region of India. Nodulation was observed in all members of the *Papilionoideae* and *Mimosoideae* but only one species of *Caesalpinioideae* i.e., *Chamaecristra primula*. Nodulation of *Acacia jacquemontii* was a new report of nodulation. *Rhynchosia aurea* and *Tephrosia faliciformis* were new reports of nodulation in the *Papilionoideae*.

Athar and Vasileva (2013) examined the nodulating ability of 111 Papilinioid legumes from Bulgaria. Nodulation was observed in 104 species, of which 5 species were new records of nodulation.

Mahmood and Athar (2018) described nodulation status of 225 legume species distributed in 75 genera that were native to Pakistan. Out of 225 species examined, 28 belong to *Caesalpinioideae*, 29 belong to *Mimosoideae* and 168 belong to *Papilionoideae*. The percentage of nodulation in *Caesalpinioideae*, *Mimosoideae*, *Papilionoideae* was 0, 96, 99%, respectively.

Significance of nodulation surveys

1. Nodulation surveys are the sources of increasing the database of nodulated and non-nodulated legume species throughout the world. As a result about 57% genera have been examined for nodules, that include 3395 species (20%) of the Leguminosae (Faria *et al*., 1989).

2. Nodulation surveys provide a direct means to study the legume rhizobia symbiosis in natural ecosystems. There is an increasing interest in exploring nodulated legumes because of their great potential in agrosystems and ecological rehabilitation of degreaded soils (Brockwell *et al*., 1995; Mahmood, 1999; Zahran, 2009).

3. Nodulation surveys provide a means of exploring new legume-rhizobia associations in Leguminosae. A few examples are cited below:

(a) *Parasponia – Rhizobium* symbiosis: This association was discovered by Trinick (1979) where *Rhizobium* was found forming nitrogen fixing nodules on the roots of *Parasponia* a non-legume belonging to family *Ulmaceae*. The microsymbiont was later identified as *Bradyrhizobium* spp. (Parker, 2015).

(b) *Beta protobacteria* can form nitrogen fixing nodules on roots of legumes: The discovery that bacteria belonging to the group of *Beta protobacteria* form nitrogen fixing nodules on the roots of leguminous plants including both herbs and trees has great significance. *Mimosa* spp., (Mimosoideae) nodulated by species of *Burkholderia* and *Cuprivedus* has been reported from various countries (Sprent and Geholt, 2010; Zahran, 2009). The organisms that are symbiotic with legumes include isolates from the genera *Blastobacter, Burkholderia, Devosia, Einsfer, Methylobacterium, Ochrobacterium, Phyllobacterium* and *Ralstonia* (Graham, 2008).

(c) *Tribulus* and *Newmania karachiensis* symbiosis: In this case the cells of the cyanobacterium *N. karachiensis* were found living inside the cortical tissue of the *Tribulus* root nodule. This is the first instance where a *Cyanobocterium* was found living as an endophyte inside the nodule of an *Angiosperm*. The nitrogen fixing ability of the *Cyanobacterium* has not been established as yet.

(d) In addition the anatomical studies of the nodules collected during nodulation surveys have led to discoveries in the infection process of root by the rhizobia. Although root hair infection that occurs in 90% cases of legumes (Dart, 1977), is still the most prevalent mode of infection, yet other modes of infection such as crack or wound infection have been discovered. *Arachis hypogea* (Chandler, 1978) and *Stylosanthes* (Chandler *et al*., 1982) are examples of wound infection. In both cases rhizobia gain entry through spaces in the epidermal cells of the host at the point of lateral roots emergence. A slightly different type of crack or wound infection has been described in roots of *Mimosa scabrella* Benth., where rhizobia enter into epidermal cells of the root between epidermal cell walls (Faria *et al*., 1988). Microbiological studies revealed that infected cell of *Andira* spp., was packed with infection threads in which rhizobia were retained (Faria *et al*., 1986).

(e) Nodule shapes and tribal classification of *Leguminosae*: Nodulation surveys were instrumental in the study of nodule shapes of more than 400 wild legumes indigenous to Zimbabwe (Corby, 1971). Nodule shape was found related to the tribal classification of host (Corby, 1971). Characteristic nodule shapes of the *Papilionoiceous* tribes *Galegeae*, *Genisteae*, *Hedysareae* and *Phaseoleae* and of the subfamilies *Mimosoideae* and *Caesalpinioideae* have been described.

CONCLUSIONS

The prime objective of nodulation surveys is to explore new nodulating species in *Leguminosae*. Quantitative nitrogen estimation of new nodulated species is essential. High N-fixing plants particularly those that grow naturally under arid and semi-arid climates of tropical and sub-tropical regions should be identified. Rhizobia should be isolated from their nodules and stored in rhizobial banks such as is being practiced in Brazil, under the guidance of Dr. S.M. de Faria. Genetic diversity of bacterial strains should be studied. High N-fixing strains may be used in cross-inoculation experiments with agricultural species to improve productivity. Molecular methods, instead of conventional method of searching new legume *Rhizobium* associations may be adopted. Molecular technology may be employed to develop genetically engineered plants with high N-fixing potential.

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