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Seasonal Patterns of Nitrogen-Fixing Ability (C_2H_2 Reduction) and Hydrogen Uptake by Actinorhizal Nodules of *Casuarina equisetifolia* Linn.

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ABSTRACT : *Casuarina equisetifolia* is an actinorhizal tree being introduced in agroforestry systems of Pakistan. A study was conducted to observe the seasonal changes in nodular nitrogenase activity (C_2H_2 reduction) and uptake hydrogenase activity of *C. equisetifolia.* Nodules showed significant ($P \leq 0.05$) seasonal variation in the nitrogenase and hydrogenase activities which were highest during June (13.52 μmoles C_2H_4 . g^{-1} fresh nodule wt. hr^{-1} and 2.55 μmoles H_2 consumed g^{-1} fresh nodule wt. hr^{-1}, respectively) and lowest during December (0.55 μmoles C_2H_4 . g^{-1} fresh nodule wt. hr^{-1} and 0.23 μmoles H_2 consumed g^{-1} fresh nodule wt. hr^{-1}, respectively). This study has many important and applied ecological implications in the management of horticulture and agroforestry systems of the tropics.

KEY WORDS : ***Casuarina*, Nitrogenase, Hydrogenase, N- fixation.**

INTRODUCTION

Many of the actinorhizal plants are of great ecological significance due to their potential contribution in reforesting endangered sites, protecting crops, rehabilitating marginal or wasted lands (Reddell *et al.*, 1991). Casuarinaceae is the best example and comprises the species which achieve these objectives (Dommergues and Girgis, 1994). *Casuarina* species are tolerant to salinity and water-logging, and are also suitable for plantations in arid as well as in saline and water-logged conditions (El-Lakany, 1994). Large plantations of Casuarinaceae are being introduced in agroforestry systems of Pakistan. Relatively high cellulose and low lignin contents in the wood of *Casuarina equisetifolia* make it a suitable alternative for use in pulp and paper industry of Pakistan (Mahmood, 1993). Some of the introduced species of *Casuarina* are nodulated in the plains of Punjab and Sindh. Athar and Mahmood (1983) described actinorhizal nodules in *C. equisetifolia* Linn. Iqbal *et al.* (1986) isolated five *Frankia* strains from actinorhizal nodules of *C. glauca* from Pakistan. In another study, Iqbal *et al.* (1993) made preliminary observations on nodule morphology

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and nitrogen-fixing ability of nodulated *C. glauca*. Seasonal variations in nitrogen-fixing ability of various actinorhizal species have been reported (Tripp *et al.*, 1979; Schwintzer *et al.*, 1982; Pizelle, 1984; Hafeez *et al.*, 1984; Zitzer and Dawson, 1989; Mirza *et al.*, 1994). However, similar studies in *Casuarina* spp. are sporadic (Bond and Mackintosh, 1975) or lacking details (Iqbal *et al.*, 1993). The present study describes the seasonal variations in nitrogen-fixing ability (C_2H_2 reduction) and hydrogen uptake by the root nodules of *C. equisetifolia* growing under field conditions.

MATERIALS AND METHODS

Collection of material

Root systems of *C. equisetifolia* plants growing in Islamabad, were excavated and examined for the presence of nodules. The study area comprised of roadside plantations in various sectors of Islamabad (33° 42' N, 73° 7' E; elevation approximately 518 m). The soil in this area is alfisol. The annual precipitation ranges from 500 to 700 mm. Most rainfall is received in March and April. Most observations were made from the plantations near National Park Road and C. D. A. Nursery. Collections were made every month from March to February at about noon time. Nodules were collected from 15 cm of the surface soil. After removing adhering soil particles, five randomly selected nodule samples were immediately subjected to enzymatic assays. Statistical differences were evaluated by analysis of variance procedures, and means were compared with the LSD multiple mean comparison test (Steele and Torrie, 1980).

Nitrogenase activity

Nitrogenase activity of *C. equisetifolia* nodules was determined by acetylene reduction assay (Hardy *et al.*, 1973). Freshly collected nodules were incubated in 23.5 mL test tubes with air and 10% acetylene at ambient soil temperature. Ethylene production was measured by collecting one mL of gas samples after one hour of incubation and analyzed on gas chromatograph on FID system (Hitachi, Model 163). Carrier gas (N_2) was 40 mL min^{-1}. Porapak R packed in loop steel column, 1.5 m long and 3 mm in ID was used for separation.

Hydrogenase activity

Hydrogenase activity of *C. equisetifolia* nodules was measured by the method described by Roelofsen and Akkermans (1979). The nodules were incubated in 1% hydrogen in 13.5 mL air tight vials with rubber stoppers. After preincubation for 10 minutes, the gas samples were collected at different time intervals to determine hydrogen uptake by the nodules using gas chromatograph (Hitachi, Model 163) on TCD system.

RESULTS AND DISCUSSION

Root nodules of *C. equisetifolia* showed significant variation ($P \leq 0.05$) in nitrogenase activity during different seasons of the year (Table 1). The lowest acetylene reduction activity (0.55 μmoles C_2H_4 . g^{-1} fresh nodule wt. hr^{-1}) was recorded during December and highest in June (13.52 μmoles C_2H_4 . g^{-1} fresh nodule wt. hr^{1}). These results corroborate with the observations of other workers on the seasonal fluctuations in nitrogenase activity by actinorhizal plants (Tripp *et al.*, 1979; Zitzer and Dawson, 1989; Iqbal *et al.*, 1993; Mirza *et al.*, 1994).

Table 1. Seasonal variations in nitrogenase activity and uptake hydrogenase activity by actinorhizal nodules of *Casuarina equisetifolia.*

Date	Temperature (°C)		μmoles $C_2H_4 \cdot g^{-1}$ fresh nodule wt. hr^{-1}	μmoles H_2 consumed g^{-1} fresh nodule wt. hr^{-1}
	Air	Soil		
January 27	15.6	15.0	0.75 a	0.34 a
February 24	21.4	20.9	3.03 bc	0.65 b
March 20	28.9	25.5	3.28 c	0.89 b
April 16	33.2	30.8	8.61 e	1.55 c
May 14	38.5	36.3	11.17 g	2.09 d
June 11	39.3	37.1	13.52 h	2.55 e
July 10	35.8	32.3	10.34 f	1.82 cd
August 7	33.9	31.1	8.55 e	1.69 c
September 3	32.7	29.4	6.65 d	1.35 c
October 11	29.3	26.0	3.38 bc	0.96 b
November 19	20.7	21.5	2.95 b	0.68 b
December 23	10.1	9.8	0.55 a	0.23 a

Values in the column followed by the same letter are not significantly different at $P \leq 0.05$. Each value represents mean of five determinations.

The temperature showed marked effect on the nitrogenase activity. The nitrogenase activity of *C. equisetifolia* nodules was low during cold winter. However, with the increase of seasonal temperature and rainfall and subsequent appearance of new leaves during March and April, the nitrogenase activity increased progressively. Maximum nitrogenase activity was recorded in June at temperature of 37°C. Effect of temperature on the nitrogenase activity of actinorhizal nodules has been investigated by Waughman (1977), Reddell *et al.* (1985), Zitzer and Dawson (1989) and Mirza *et al.* (1994).

Since ambient temperature in the field varied depending on the season, it was desirable to examine the effect of incubation temperature on the acetylene reduction. Present study revealed that with the increase in the temperature, the enzyme activity increases, and is maximum at 37°C beyond which it showed significant decline. Reddell *et al.* (1985) observed that decreasing soil temperature below 25°C markedly decreased plant growth that were reliant on symbiotically fixed nitrogen whereas effects on the growth of the plants supplied with mineral nitrogen were much smaller. Waughman (1977) found nodules of *Myrica gale*, *Alnus glutinosa* and *Hippophae rhamnoides* to have optimum acetylene reduction activities at 20 to 25°C, whereas the optimum for the *C. equisetifolia* nodules was 35°C. Bond and Mackintosh (1975) demonstrated that nitrogen fixation by detached nodules of *C. cunninghamiana* increased with temperature up to 40°C, but they indicated that factors such as supply of photosynthate may modify this response. However, Wheeler and

McLaughlin (1979) pointed out that it was not clear if there is any relevance of short term studies with detached nodules to the behavior of the plants under natural conditions.

There may also be seasonal changes in the hydrogenase activities of the nitrogenase enzyme complex (Roelofsen and Akkermans, 1979; Hafeez *et al.*, 1984; Mirza *et al.*, 1987, 1994). Most *Frankia* species examined have uptake hydrogenase capable of oxidizing H_2 so that the energy is available for N_2 reduction and is not lost to proton reduction, measured as H_2 evolution. Actinorhizal nodules that evolve no hydrogen during nitrogen fixation are 100% efficient in recycling hydrogen (Zitzer and Dawson, 1989). It has been shown that there are seasonal differences in patterns of nitrogenase activity and hydrogenase activity, suggesting that these activities are not controlled by the same regulatory system (Hafeez *et al.*, 1984; Sellstedt *et al.*, 1986). The rate of hydrogen uptake by *C. equisetifolia* nodules showed fluctuations during different seasons of the year (Table 1). Hydrogenase activity was lowest during December (0.23 μmoles H_2 consumed g^{-1} fresh nodule wt. hr^{-1}) and highest during June (2.55 μmoles H_2 consumed g^{-1} fresh nodule wt. hr^{-1}). These results differ from Hafeez *et al.* (1984), who observed that *Datisca cannabina* nodules exhibited almost constant hydrogenase activity during winter and summer. However, these results are in agreement with the findings of Mirza *et al.* (1987, 1994), who reported seasonal fluctuations in hydrogenase activity of *Coriaria nepalensis* nodules.

The values for acetylene reduction provide relative indices of seasonal pattern of nitrogen-fixing ability of actinorhizal nodules of *C. equisetifolia*. These results, however, indicate that maximum nitrogenase activity of the nodules coincides with the period of vigorous plant growth suggesting that during fast growing period increased nitrogen requirement of the host plant are met by the elevated nitrogen fixation rates by the endophyte. This study has important applied and ecological implications in horticulture and agroforestry systems. As these plants are dependent on nitrogen fixation for survival and growth under natural conditions, these results may help in selecting highly effective *Casuarina* species-*Frankia* strain combinations for use in *Casuarina* plantations.

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木麻黄根瘤季節性固氮及氫氣攝取活性變化之研究

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摘　　要

木麻黃係巴基斯坦引進的農林樹種之一種。本研究主要是觀測於季節不同時，其固氮作用及氫氣攝取的能力變化狀況。根瘤於六月份時上述兩種活性達到最高，而於十二月份時活性最低。本研究對於熱帶農林或園藝的管理於應用生態學上有多方面的重要性與參考價值。

關鍵詞：木麻黃，固氮酶，氫酶，固氮作用。

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