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ASSOCIATIVE NITROGEN FIXATION IN SOME GRASSES AND CEREALS GROWING AROUND FAISALABAD AREA

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ABSTRACT

Nitrogenase activity associated with the roots of five local grasses and cereals i. e. *Zea mays* L.; *Cynodon dactylon*, Pers ; *Sorghum bicolor* Pers.; *Dactyloctenium aegyptium* (L.) Beauv. and *Oryza sativa* L. was determined using acetylene reduction assay (ARA), for pre-incubated and fresh excised roots. All except *O. sativa* showed higher nitrogenase activity, both for pre-incubated and fresh roots. Among pre-incubated roots, the unwashed roots showed average nitrogenase activity in the range of 4-107 nmol g^{-1} D.W.hr^{-1}, washed roots showed in the range of 4-96 nmol g^{-1}D.W. hr^{-1} and surface sterilized roots activity which was detected in two grasses (*Zea mays* L. and *Cynodon dactylon* Pers.), was in the range of 1-9 nmol g^{-1} D.W. hr^{-1}. For fresh roots, activity was in the range of 2-29 nmol g^{-1} D.W. hr^{-1}.

INTRODUCTION

Nitrogen fixation associated with grasses is not a new concept as Lipman and Taylor (1923) reported this in wheat plant. Recent energy crises and development of sensitive and relatively easier techniques like acetylene reduction assay (Hardy *et al* , 1968) renewed the interest in this area. Considerable data have been accumulated by using acetylene reduction assay (ARA) by different workers (Dobereiner *et al.*, 1972; van Berkum and Day, 1980). Associative nitrogen fixation has also been reviewed by different authors (van Berkum and Bohlool, 1980).

The nitrogen fixing bacteria associated with grass roots have been isolated and identified (Zafar *et al.*, 1984).

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Associative Nitrogen Fixation

In this study nitrogenase activity associated with five local grasses and cereals viz. *Zea mays*; *Cynodon dactylon*; *Sorghum bicolor*; *Dactyloctenium aegyptium* and *Oryza sativa* has been reported.

MATERIALS AND METHODS

Collectious : The grass root samples were collected from different locations in NIAB fields in tne mid-afternoon. The plant roots along with soil cores were dug in a radius of 10 to 20 cm depth (Capone and Taylor, 1980). The samples were immediately brought to laboratory.

Soil Analysis : The rhizosphere soil was analyzed for some of its physicochemical characteristics. The total nitrogen was analyzed by microkjeldahl method (Bremner, 1965). For ammonium and nitrate analysis, 5 of ground soil was shaken with 50 ml of 2NKCI for 1 hr on a mechanical shaker and then filtered. For NH_4^+-N analysis, 20 ml of the aliquot of the suspension was distilled with MgO (heated at 600°C for 24 hrs) and the distillate was collected in boric acid indicator solution and contents were calculated. For NO_3^+ −N analysis, 0.20 g finely ground Devorda's alloy (E. Merck) added to the above distillation flask and redistilled. The distillate was collected as described for NH_4^+ −N. To determine the pH of the soil, a saturated soil paste was prepared in distilled water and pH was determined with pH meter. For EC_e determinations, the extract from the soil paste was taken and EC_e was determined using EC meter (WTW- LF-530).

Excised root Assay : The roots were cut into 2-3 cm long segments using sterilized foreceps and scissors. Excised root assay was done for pre-incubated and fresh roots.

a) *Assay with Pre-incubation* : The roots were divided into three portions viz. unwashed , washed (washed several times with sterile distilled water) and surface sterilized (sterilized with 0.2 $HgCl_2$ sol. for 30 sec.) roots. The assay was carried out according to the method of Dobereiner and Day (1976).

b) *Assay without Preiucubation* : Fresh excised roots were taken and subjected to ARA according to the method of van Berkum (1980).

Acetylene Reduction Assay : For estimating the amount of ethylene gas produc-

ced as a result of acetylene reduction, 100 µl gas mixture was injected into the gas chromatograph (Carlo-Erba Fractorap series 2150) consisting of a 0.75 m x 2 mm stainless steel column packed with porapak N (80-100 mesh : Water Associate., Inc USA). Nitrogen (Pakistan Oxygen Limited) was used as a carrier gas at a flow rate of 30 ml min^{-1}. The acetylene and ethylene were measured using flame ionization detecter (FID). The signal after proper amplification was recorded on Perkin Elmer 56 recorder. The quantity of C_2H_4 produced was calculated by comparing with standard ethylene in 1% Argon (Linder Technisch Gase, W. Germany). The activity was described in nmol. C_2H_4 g^{-1} D. W. hr^{-1}.

RESULTS AND DISCUSSION

Soil Analysis: It was known that high concentrations of NH_4 and NO_3 cause reduction in nitrogenase activity (van Berkum and Bohlool, 1980). The soil analysis carried out in these studies is given in Table 1. It showed that total N, NH_4^+ N_3 and pH values were almost similar for all grasses but NO_3^-- N were high in *D. aegyptium* followed by *Z. mayz* and EC_e values were higher in *Z. mayz* followed by C. *dactylon*.

Table 1. *The physicochemical characteristics of the soil amples collected from different sites in NIAB fields under various grasses and cereals*

Characteristics	*Z. mays*	*C. dactylon*	*S. bicolor*	*D. aegyptium*	*O. sativa*
pH	8.3	7.9	7.8	8.1	7.7
EC_e(mS. cm^{-1})	9.56	1.47	0.94	1.24	0.73
Total N (mg. g^{-1})	0.063	0.062	0.052	0.053	0.032
NH_4 -N (ppm)	2.8	0.7	2.8	2.8	2.8
NO_3^--N (ppm)	5.6	0.7	2.8	7.0	1.7

To determine the root associated nitrogenase activity both for pre-incubated and fresh roots, a very accurate and sensitive assay called acetylene reduction assay (ARA) of Hardy *et al.* (1968) was used to get accurate results.

ARA with Pre-incubation: The nitrogenase activity of pre-incubated roots is given in Table 2. The activity was higher i. e., in the range of 4-107 nmol g^{-1} D. W. hr^{-1} and 4-96 nmol gm ^{1} D. W. hr. ^{1} for rhizosphere (unwashed roots) and rhizoplane (washed roots), respectively but was lower i. e., in the range of 1-9 nmol g^{-1} D. W. hr^{-1} in histoplane (surface sterilized roots). The histoplane activity was detected only in two grasses i. e., *Z. mays* and *C. dactylon* but in others it was not found. It might be due to the experimental error. The histoplane activity was determined due to the reason that there were reports of endorhizosphere nature of certain microbes. The information was taken from light microscopic studies and staining the root tissues with tetrazolium (Patriquin Dobereiner, 1978). In these studies, *O. sativa* roots showed the lowest activity for pre-incubated roots.

Table 2, *Nitrogenase activity associated with preincubated roots of grasses and cereals.*

Gras ssystem	Activity in nmol g^{-1} D.W. hr^{-1}								
	Unwashed			Washed			Surface sterilized		
	Freq (%)	Ave..	Range	Freq. (%)	Ave,	Range	Freq (%)	Ave.	Range
Z. maya	34	18	9—27	67	96	6—186	23	9	4—14
C. dactylon	78	107	2—211	78	28	1—54	23	1	0—1
S. bicolor	45	20	4—36	67	4	1—7	—	—	—
D. aegyptium	23	13	2—25	23	10	2—17	—	—	—
O. sativu	23	4	3—4	34	7	5—9	—	—	—

The present results of pre-incubated roots confirmed those of workers earlier (Neves *et al.*, 1976) who found higher activity with pre-incubated roots of certain grasses.

ARA without Pre-incubation: Earlier, it was considered that pre-incubation period was necessary to detect the nitrogenase activity with fresh excised roots but van Berkum and Sloger (1981) compared the legumes and grasses for nitrogenase activity and they found that both the systems showed $C_2 H_2$ reduction

immediately after exposure to C_2H_2. They concluded that pre-incubation period was not necessary for the detection of nitrogenase activity. Similar results were obtained by De-Polli *et al.* (1982).

In these experiments, the fresh excised roots were taken for this pnrpose and subjected to assay according to the method of van Berkum (1980). The results of fresh root assay are given in Table 3. The results of fresh roots assay showed lower values than those of preincubated roots. Similar results were obtained by other workers. The *O.* sativa roots again showed lower activity than the grasses.

Table 3. *Nitrogenase activity associated with fresh roots of grasses and cereals*

	Frequency	Activity (nmol g^{-1}) D.W. hr^{-1})	
	(%)	Average	Range
Z. mays	78	28	4–51
C. dactylon	89	29	4—54
S. bicolor	56	4	2— 5
D. aegyptium	23	13	2—24
O. sativa	45	2	1— 3

Although the rates of nitrogenase activity associated with roots of grasses are lower as compared to the legumes, yet it carries considerable importance as the grasses occupy about 80% area of the earth hence contributing more nitrogen than legumes. Another important aspect peculiar to grasses is that nearly all grasses have C_4 dicarboxylic pathway of photosynthesis. They leak their photosyntoats in the form of root exudates which is a source of energy for root associated N_2-fixing microorganisms (Dobereiner *et al.*, 1972). The C_4 grasses utilize their available nitrogen very efficiently in producing dry matter which stimulates the nitrogen fixation in their rhizosphere (Black *et al.*, 1977). Hence the nitrogen fixation with grasses is helpful in food production and to meat the N-demands.

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