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Factors Influencing Nitrogenase Activity (Acetylene Reduction) by Root Nodules of Groundnut, *Arachis hypogaea* L.

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ABSTRACT

Acetylene reduction assay, used to measure nitrogenase activity of legume root nodules, is influenced by environmental factors, which limit its application. The effects of some of the environmental factors on acetylene reduction by groundnut root nodules are described. The activity was nonlinear during the first hour of incubation. Assay temperature above 25 C decreased the activity. Washing the nodulated roots prior to the assay also decreased the activity. The activity was influenced by light intensity, soil moisture, and moisture content in the incubation bottle. Diurnal fluctuation with one maximum and one minimum activity period during a 24 hour cycle was observed. Nitrogenase activity was higher during the postrainy season compared to that of the rainy season. A virginia cultivar Kadiri-71 had higher nitrogenase activity than a dwarf valencia cultivar, MH 2.

Key Words; Groundnut, peanut, nitrogenase activity, light intensity, soil moisture, temperature.

The acetylene reduction technique has been widely used to measure the nitrogenase activity of nodulated plants (12). The assay is based on the quantitative relationship between reduction of acetylene to ethylene, and nitrogen to ammonia by nitrogenase. The nitrogenase activity of the nodulated plant root is determined by incubating the roots in an atmosphere containing acetylene and oxygen (usually as air) in gas tight containers and measuring the ethylene produced. Environmental parameters influencing acetylene reduction by legumes include diurnal variation (1,2,13,14,21), soil moisture and nodule water content (18,21), soil temperature (5,6), light intensity and day length (10,11). The rate of acetylene reduction depends not only on the enzymatic reduction of acetylene by nitrogenase, but also on the activity of other enzymes and cofactors, involved in electron donation and ATP generation (4). Parameters influencing the activity of these enzymes are also likely to affect *in vivo* measurement of nitrogenase, and hence restrict the use of acetylene reduction technique. This paper describes some of the factors which affect acetylene reduction by field-grown groundnut plants.

Materials and Methods

Cultivars and crop growth conditions

The groundnut cultivars used in these experiments were Kadiri 71-1 (subsp. *hypogaea* var *hypogaea*) Ah 8189, TMV 2 (subsp. *fastigiata* var *vulgaris*) and MH 2 (subsp. *fastigiata* var *fastigiata*). Ten groundnut germplasm lines i.e. NCAC 2123, Malimbana-3, NCAC 10247, NCAC 1826, Gujarat narrow leaf, NCAC 2461, Ganganjika, Kadiri 71-1, Groundnut 28, NCAC 9287, were also used to study diurnal variation in nitrogen fixation.

The data was assembled from a number of experiments to illustrate the factors which have been found to influence the activity. The experiments were conducted during 1976-80 on alfisol fields. The year and season of each experiment is given in the Figure legends. Plants were grown in four replicated plots fertilized with superphosphate (40 Kg P_2O_5/ha) on rows 75 cm apart. MH 2 plants were spaced at 10 cm apart within a row, and all other cultivars at 15 cm. The plots were not inoculated with *Rhizobium*. The plants were irrigated to field capacity every 7-10 days during the dry postrainy season, but not during the rainy season, and were regularly sprayed against insect pests.

Acetylene reduction assay

The techniques for measuring acetylene reduction were essentially as described by Dart *et al.* (7). Plants from each replication were dug from the field, the tops removed and most of the adhering soil was removed by shaking. Depending on the growth stage 4-10 nodulated roots were incubated in 350 or 700 ml glass bottles, closed with a No. 77 Suba Seal and metal lid. After removing an equivalent volume of air, acetylene was injected into these bottles to give a final concentration of 10%. Under field conditions, it was not possible to maintain a standard incubation temperature, but variations in bottle temperature were reduced by placing a wet jute bag over the bottles during incubation. In the study of the effect of incubation temperature, the bottles were held in water baths maintained at the required temperature. Unless otherwise mentioned, assays were carried out between 0930 h and 1130 h. All values expressed were obtained after deducting the ethylene values for a blank treatment without roots.

Shading effect

To study the effect of shading on acetylene reduction the plots were shaded using a muslin cloth to 40% of the day light intensity at 110 days after planting. Plants from unshaded plots were used as controls. Solar radiation in this experiment was measured by using tube solarimeter (20).

Results and Discussion

Time course of acetylene reduction

The rate of acetylene reduction varied with time, during an incubation period of 60 min. (Fig. 1). The rate decreased after the first 30 minutes for MH 2 and after 10

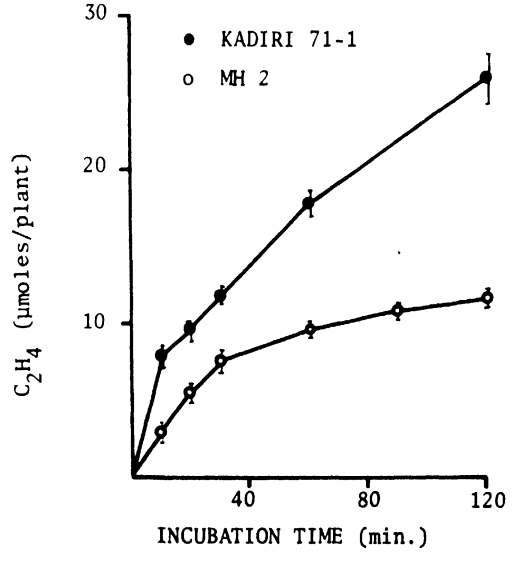

Fig. 1. Time course of acetylene reduction by groundnut root nodules. The samples were drawn at different time intervals from the same bottle and assayed for ethylene. Bars represent SE.

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minutes for Kadiri 71-1. The decline in activity could be due to reduced 0_2 concentration. Recently Minchin *et al.* (15) reported that acetylene reduction and root plus nodule respiration in white clover was curvilinear during the initial 30 min. incubation period. In lupins and soybeans the acetylene reduction was linear over the first hour if the oxygen tension was maintained (5,6,21). The shortest practical incubation time should be used for making valid treatment comparisons with groundnut roots. We have standardised an incubation period of 30 min. Under field conditions there was a 3-5 min. period between digging the plants and injecting acetylene. The change in rate of acetylene reduction with time suggets that carbohydrate reserves in the nodule may be limiting.

Incubation temperature

Nitrogenase activity was greater when the nodulated roots were incubated at 25 C than at 30 or 35 C (Table 1). The soil and air temperatures during the experiments were 25 C and 28 C, respectively. A similar situation was observed in lupins (21). The optimum incubation temperature varies between species, with tropical species having optima between 25 and 30 C and temperate species between 15 and 20 C (5,8,17).

Table 1. Effect of incubation temperature* on acetylene reduction by groundnut roots.

Incubation Temperature	µmoles C_2H_4/plant/h
25°	46
30°	34
35°	33

cv (%) = 42, SE = 3.2

*Differential temperatures were imposed by placing bottles in a waterbath for 20 min before injecting acetylene

Moisture

The rate of acetylene reduction was significantly increased when moist blotting paper discs were placed inside the bottles during the assay (Table 2). However, when the roots were washed and excess water blotted off prior to the assay, acetylene reduction was significantly decreased (Table 3). The decrease was one third for the cv TMV 2 and ten fold for cv Kadiri 71-1. Similarly, Van Straten and Schmidt (22) reported that washing lupin nodules for 30 seconds and then blotting dry reduced the activity by about 60%. Sprent (18) reported that immersion of detached soybean nodules in water decreased acetylene reduction, but the activity was restored by shaking the wet nodules in an atmosphere of oxygen. She concluded that a

Table 2. Effect of moisture content in the incubation bottle on acetylene reduction by cultivar Kadiri 71-1, 51 days after sowing.

	µmoles C_2H_4/plant/h
With wet blotting paper	31
Without blotting paper	28

CV (%) = 5; SE = 0.71

Table 3. Effect of washing groundnut roots on acetylene reduction activity.

Cultivars	µmoles C_2H_4/plant/h	
	Washed roots	Unwashed roots
Kadiri 71-1	3	32
TMV 2	10	15

CV (%) = 15, SE = 1.52

film of water on the surface of the nodule reduced the oxygen supply and nitrogenase activity.

Trinick *et al.* (21) suggested that overwatering reduced acetylene reduction activity of field grown lupins. The influence of soil moisture on nitrogenase activity in groundnut was examined at different intervals after irrigation (Fig. 2). Maximum activity was observed on the third day after the irrigation. This suggests that excess or insufficient soil moisture can decrease N_2-fixation in groundnut.

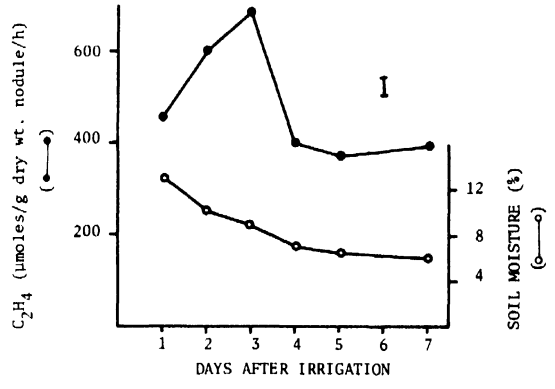

Fig. 2. Effect of soil moisture on nitrogenase activity. Kadiri 71-1 plants, were assayed on different days after an irrigation during 1979 postrainy season. Bar represents SE.

Diurnal variation and light intensity

During the postrainy season of 1976-77, we found that nitrogenase activity of 81 day old groundnut plants increased from 0600 h to a maximum at 1100 h when a cloud cover appeared and remained for the rest of the day (Fig. 3). The activity declined after mid-day and remained low

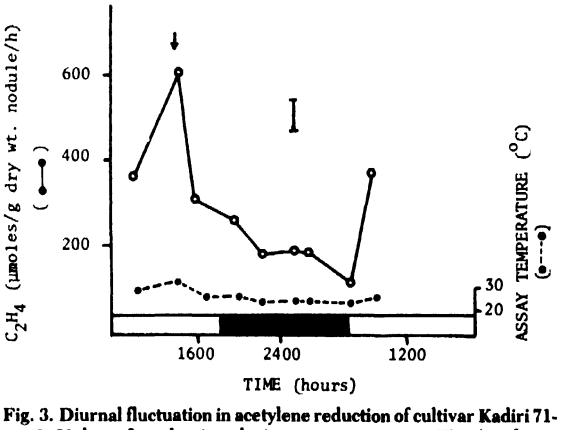

Fig. 3. Diurnal fluctuation in acetylene reduction of cultivar Kadiri 71-1, 81 days after planting, during postrainy season 1976. ▲ indicate the appearance of cloud cover. Bar represents SE.

during the night. When a similar experiment was repeated during a clear day in the postrainy 1977-78 season, the maximum activity was reached around 1000 h, and declined only after 1600 h (Fig. 4). Balandreau *et al.* (2) reported diurnal fluctuations in acetylene reduction by field grown groundnut plants, with two maxima and two minima during a cycle of 24 h. However, at ICRISAT centre only one maximum and one minimum was observed. At Ibadan, Nigeria field grown cowpeas and soybeans showed one maximum and one minimum activity during a light period (1). To study the effect of light intensity on acetylene reduction a shading experiment was conducted. Groundnut plants were shaded to approximately 40% of day light intensity at 110 days after planting. Acetylene reducing activity decreased within four hours of shading and the same trend was observed during the second day of shading (Fig. 5). Thus, light intensity, especially during cloudy days could be a limiting factor in nitrogen fixation. The role of light intensity on N_2-ase activity of soybeans and lupins is well established (3,11,20). Occasional cloudy days did not alter acetylene reduction by lupin nodules, but continuous overcast weather over a number of days did reduce the overall activity (21). Sprent and Silvester (19) reported a reduction in activity of *Lupinus arboreus* nodules as a result of long term shading treatments, but only severe shading (to 10% full day light) had a marked effect.

Nitrogen fixation by root nodules depends on the reserve energy supply in the nodules and/or on the photosynthate supply from the shoot. The lack of diurnal variation in acetylene reduction for legumes such as lupines was attributed to a plentiful supply of carbohydrate reserves in the nodules (21). Ten selected groundnut germplasm lines (with differing foliage characteristics, viz. leaf size, leaf form and amount of wrinkling, leaf area, leaf thickness, and internode length; and presumed to differ in the quantity of photosynthate and carbohydrate content in the nodule and dry matter produced per plant) were screened for nitrogenase activity from 0930 h to

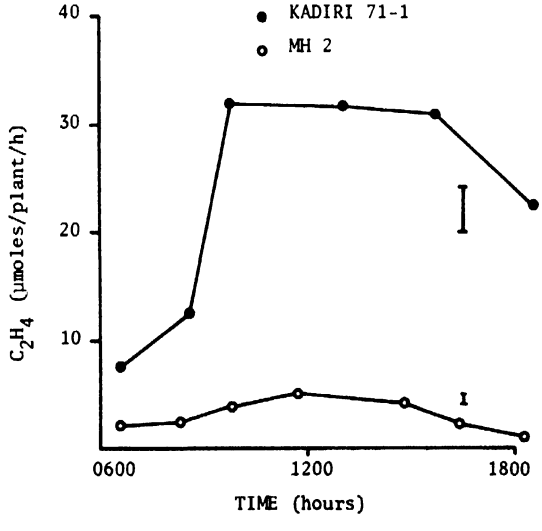

Fig. 4. Acetylene reduction by groundnut cultivars MH2 and Kadiri 71-1 at different hours of a day, during postrainy season 1977, 54 days after planting. Bars represent SE.

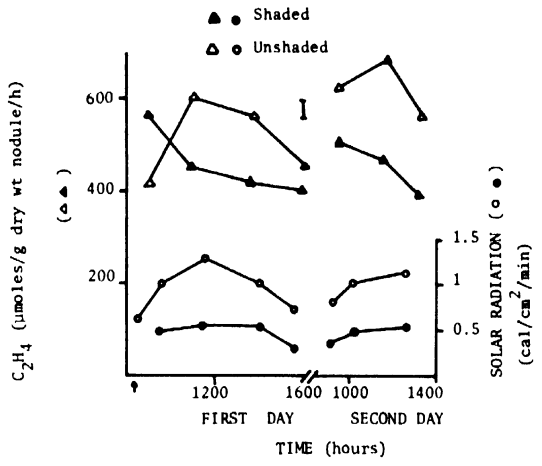

Fig. 5. Effect of shading on nitrogenase activity of groundnut. Plants grown as an irrigated crop were shaded in replicated plots at 109 days after planting. Acetylene reduction assays were carried out on the same day and on a subsequent day. ▲ indicate the start of shading treatment. Bar represents SE.

1130 h during the day and from 2130 h to 2330 h during the night in a replicated trial. The interaction between lines and time of assay was significant indicating that some lines may have less diurnal variation in nitrogen fixation (Fig. 6).

Seasonal and cultivar difference in nitrogen fixation

The seasonal variation in nitrogen fixation of two cultivars, which differed in their growth habit was examined during the 1978-79 postrainy season and in the 1979 rainy season, cv Kadiri 71-1, with running habit is a long duration cultivar producing more foliage than cv MH 2, which is a short duration bunch type. These two cvs were found to be consistently different in acetylene reduction throughout their growth cycles (Fig. 7), Kadiri 71-1 having much greater activity than MH 2. Nodulation and nitrogen fixation was well established at 20 days after planting in the rainy season. The low soil temperatures (18-25 C) prevailing during the early stage of crop growth during the postrainy season may have caused delay in nodulation and onset of nitrogen fixation. There was a severe outbreak of foliar diseases during the rainy season which caused a rapid decrease in acetylene reduction from 60 days after sowing. Nitrogenase activity during the postrainy season was found to be higher than the rainy season.

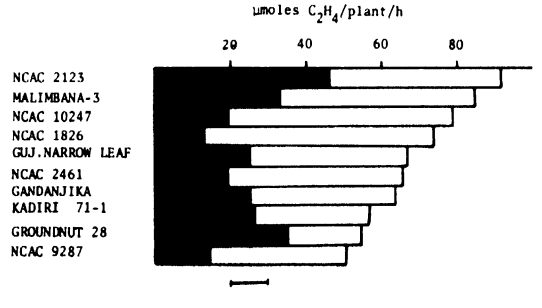

Fig. 6. Acetylene reduction of ten germplasm lines at 68 days after planting during postrainy season 1980. The histogram represents activity when assayed during day, 0930-1130 hours and the shaded area activity during night, 2130-2330 hours. Bar represents SE.

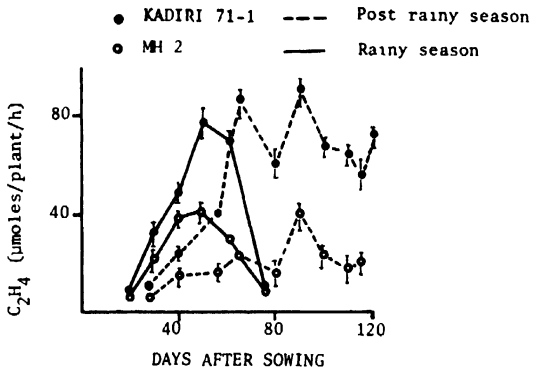

Fig. 7. Acetylene reduction activity of cultivars Kadiri 71-1 and MH2 during 1979 postrainy and 1980 rainy seasons Bars represent SE.

Host genotype differences in nitrogen fixation and nodulation in groundnut have been reported recently (9,23) It is not clear whether the cause of poor nodulation in MH 2 is related to its limited shoot growth or due to a direct genetic control of nodulation

Conclusions

There was marked diurnal and seasonal variation in acetylene reducing activity of groundnut Nitrogenase activity in groundnut was influenced by assay temperature, light intensity, moisture content in the incubation bottle, soil moisture, and growing season There were large differences between cultivars in nitrogen fixation The effects of these variables limit the value of the acetylene reduction technique for comparison between treatments on different sampling days However, it remains a useful technique for measuring nitrogenase activity between treatments on a particular sampling day provided the environmental variables concerned with the assay are controlled as carefully as possible

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