

N_2 (C_2H_4) FIXING ACTIVITY IN 17 VARIETIES OF FIELD-GROWN COWPEA
(*Vigna unguiculata* (L.) WALP.)¹ /

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Resumen

La actividad fijadora de N_2 de 17 variedades de caupi, no inoculadas, fue determinada mediante la técnica de reducción de acetileno. Todas las variedades estudiadas mostraron actividad fijadora de N_2 , con una fijación de N_2 total entre 4.3 y 0.5 Kg N fijado/ha. En algunas variedades es posible reconocer un patrón de actividad fijadora de N_2 durante el ciclo de vida de la planta. Las variaciones en la actividad fijadora en el ciclo día-noche también son consideradas en este artículo.

Introduction

Cowpeas are a widely use pulse crop in many tropical and subtropical areas (13). In Africa, because of its economic importance as a dietary staple, this species has been relatively well studied agronomically (10). Nevertheless, little information is available about its nitrogen fixing capacity outside those areas where it is normally cultivated. Due to the increased demand for high quality protein in the tropics, the International Institute of Tropical Agriculture (IITA) instituted a programme to field test cowpea varieties throughout the tropics. As part of this programme, a study on N_2 fixation by cowpea was conducted in Xalapa, Veracruz, Mexico by the Instituto Nacional de Investigaciones sobre Recursos Bióticos.

Although rhizobia inoculation is essential for many crop legumes, it has not proven effective in field trials of promiscuous tropical species, like cowpea (4). This paper presents the results of a study

carried out to determine the nitrogen fixing capacity of 17 varieties of cowpea grown in the field without inoculation.

Materials and methods

The cowpea field trials included 17 varieties of *Vigna unguiculata* produced and selected by the IITA, and *Phaseolus vulgaris* var. Negro Jamapa as a reference species. Varieties were randomly assigned to rows, 100 plants/row, in a 15 x 10 meter plot. Final planting density equaled 14 plants/m² as specified in the IITA field trial instructions. Seeds were sown in May 1979.

The soil in the experimental area is volcanic in origin, with an organic matter content of 3.5% total nitrogen content of 0.3% and 14 ppm available phosphorus. Approximately 60 kg/ha of superphosphate were applied to the experimental plot at the time of sowing. Average annual rainfall on the area is 1957 mm and average annual temperature is 18 C. Supplemental watering was necessary for only the first two weeks after planting.

Thirty days after planting and every ten days thereafter, three plants per variety were removed with care taken to extract the entire root system.

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Aerial parts were dried at 65°C for 48 hours and then weighed. Nodulated roots of each plant were placed in separate 20 ml test tubes which were then stoppered. N₂ fixing capacity of the samples was determined by the acetylene reduction technique (7), using a two hour incubation period. Nodule fresh weight was obtained immediately after C₂ H₂ reduction analysis.

Nitrogen fixed was calculated from moles of C₂H₂ reduced, using the theoretical 3:1 ratio (7). In the case of cowpea, which does not evolve H₂ during nitrogen fixation (14), the 3:1 ratio appears to accurately represent the relationship between N₂ fixation and C₂H₂ reduction. The amount of nitrogen fixed during the crop period was calculated by summing the values obtained every ten days and extrapolating between sampling periods.

In order to determine diurnal changes in N₂ fixation, the specific activity (uMoles C₂H₄ produced/g nodules/hour) of two varieties, TVx1836 and SVS-3, was measured every three hours during 36 hours. At the time of this experiment, variety TVx1836 was in the process of setting fruit, while variety SVS-3 had not yet flowered. The field and C₂H₂ reduction procedures utilized in this experiment were similar to those presented above.

Results

The reference species completed its life cycle in 100 days and yielded 2 tons/ha, which ap-

proximates typical yields for this region. The cowpea life cycle was longer, varying from 140 to 160 days according to varieties, production ranged from 124 to 2 086 kg/ha (1).

Total fixation for the varieties ranged from 0.05 to 4.30 kg N/ha, with a total nodule biomass from 0.04 to 1.5 grams per plant. This equals a N₂-fixing efficiency per variety from 0.97 to 4.09 uMoles C₂H₄/g/h.

Based on total fixation per variety, the 17 varieties can be clustered in two groups whose mean fixation values are statistically different (Table 1). Group 1 includes varieties which fixed from 1.17 to 4.31 kg N/ha, and group 2, varieties that fixed 0.91 kg N/ha or less. N₂ fixation peaked at 110 days after germination coincident with flowering and maximum plant biomass accumulation (Figure 1A). Although, in general, nodule biomass is greater at this time, some varieties exhibited maximum nodule biomass later. During the pod filling period, fixing activity and plant and nodule biomass begin to decrease until at senescence nodule biomass and nitrogen fixing activity equal 0. In contrast, specific fixing activity exhibited an opposite pattern (Figure 1B). Maximum specific fixing activity (fixation per gram of nodules per hour) occurred early in the growth cycle, preceding the plant biomass peak, and decreased until the end of the plant life cycle.

In comparison, varieties from group 2 showed no pattern in N₂-fixing activity per plant, nodule

Table 1. Mean total fixation, nodule mass, efficiency and planta biomass for the two groups of varieties.

Group (varieties)	Total ¹ fixation	Nodule mass (g)	Specific ² activity	Plant biomass (g)	Grain ³ production
1 (SVS-3; TVu201; TVu1190; TVx289; TVx1153; TVx1836; TVx11366; TVx1836E)	2.41a	0.87	2.72	87.3	1.15
2 (TVu1977; TVu4557; TVx33; TVx66; TVx337; TVx330; TVx944; TVx1193; TVx1843)	0.41b	0.16	2.30	53.1	1.60

1 Total fixation equals kgN, fixed per ha during crop cycle. Means of the group data separated by Tukeys w-procedure, 5% level

2 Specific activity equals uMoles of C₂H₄ produced per gram of nodule per hour

3 Grain production equals metric tons of grain produced per ha. Taken from Alarcón (1).

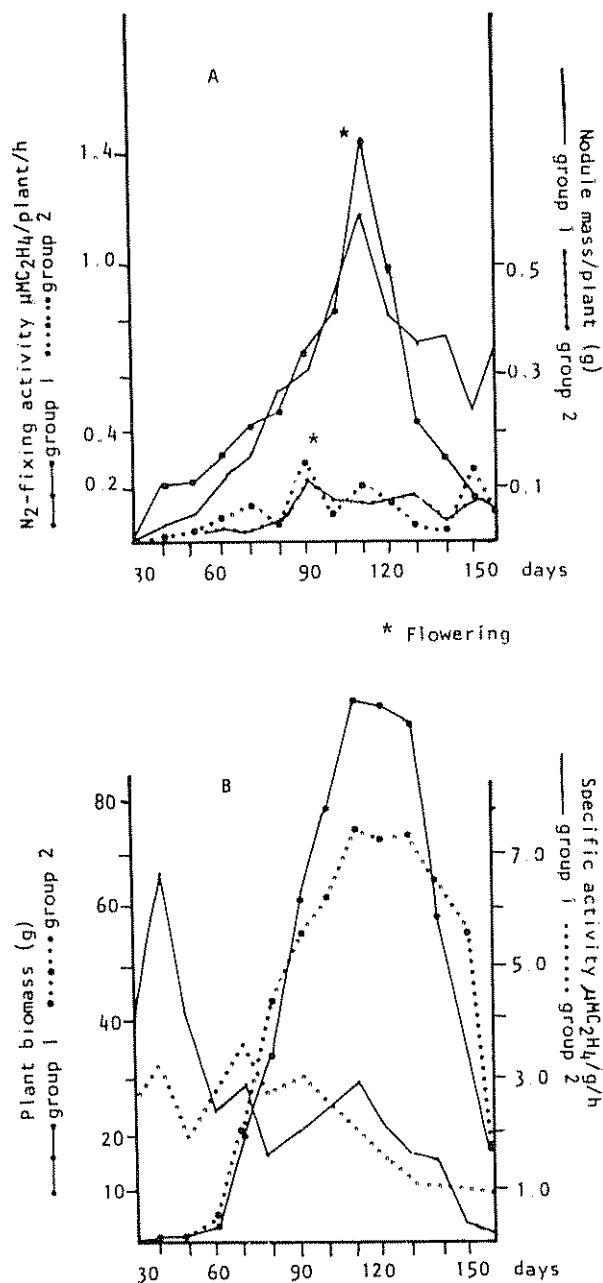


Fig. 1. Life-cycle changes in growth and N₂-fixing activity of two groups of varieties of cowpea (*Vigna unguiculata*) (A) N₂-fixing activity per plant for both groups of varieties. The flowering period is indicated for both groups. (B) Plant biomass compared to specific activity for both groups of varieties

biomass (Figure 1A) and specific fixing activity (Figure 1B). However, plant biomass, although smaller than in group 1, seems to follow a similar pattern.

Variety TVx1836 did not show a definite diurnal cycle regarding the fixing specific activity, and

variety SVS-3 showed a pattern opposite to that for atmospheric and soil temperature, and light (Figure 2). Fixing specific activity was lower during the light period and the first two hours of darkness. It then increased reaching a maximum at midnight, followed by a gradual decrease until six in the morning. From 6 am until noon, the temperature increased but activity remained constant.

Discussion

The variation in N₂-fixing activity per plant observed for varieties of group 1 was similar to that reported for other annual legumes such as soybeans, beans (5) and other varieties of cowpea (16). In the latter studies fixation values were about 50 times higher than those reported here. However, these differences may be due to the use of inoculants and/or the low nitrogen status of the soil used in the studies mentioned above.

The fact that the varieties in group 1 showed a pattern similar to that found for well nodulated legumes, suggests that N₂ fixation in these varieties is a function of the physiology of the host plant. However, the data from group 2 indicate that

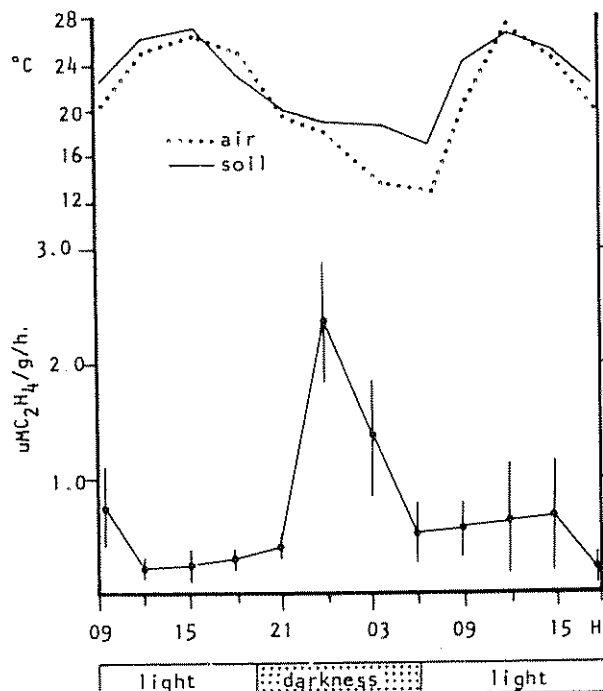


Fig 2. Diurnal changes in N₂-fixing activity of cowpea variety SVS-3. Specific activity (µMoles of C₂H₄/per gram of nodule per hour) recorded every 3 hours for 36 hours. Bars represent standard deviation of four samples.

varieties with extremely low nodulation lack any discernible pattern. This absence of pattern may be merely due to the large plant to plant variation observed or to the loss of physiological control of the nitrogen fixation process at low levels of nodulation.

The total amount of nitrogen fixed by these varieties is low compared to other values reported for cowpea, which range from 73 to 354 kg N/ha (11). Similarly, nodule biomass and N₂ fixation efficiency obtained in this study were considerably lower than values reported elsewhere: 0.52 vs. approximately 50 grams of nodules by plant and 2.56 vs. 45.0 μMoles C₂H₂/g/h of specific fixing activity (18). In spite of this, yields and nitrogen content of the cowpeas were normal (1) indicating that the bulk of the nitrogen required by the cowpea plant was absorbed from the soil.

Legumes had never been grown before in the experimental area. Therefore, the natural population of rhizobia in the soil may have been quite small. This may explain the low nodule biomass observed. In addition, it is well known that nodule formation, as well as N₂ fixing activity, are strongly reduced in the presence of high levels of combined nitrogen in the soil (15). Summerfield (17) found that 200 ppm of available nitrogen almost completely inhibited the symbiotic activity of cowpea. The total nitrogen content in the soil of the experimental area is 0.3% (1). Assuming that the available nitrogen content in tropical volcanic soils equals 13% of the total nitrogen (3), then the soil in the experimental area contained about 400 ppm available nitrogen, a level high enough to inhibit nodulation and N₂ fixation in the cowpea varieties planted according to Summerfield (16). At the same time, the high nitrogen content in the soil may be responsible for the low efficiency observed in the N₂ fixation data of this study.

The diurnal activity pattern observed for variety SVS-3 is the reverse of that reported for other legumes (6). Nevertheless, Minching and Pate (9) recently found that a pea cultivar fixed more nitrogen at night, while Ayanaba and Lawson (2) reported the same for a variety of cowpea. Whether or not in our study this diurnal pattern was constant throughout the plant development, is unknown.

The results indicate that the amount of nitrogen fixed biologically equaled only 0.04% of the total nitrogen content of the plant. At the same time, well nodulated cowpea plants can obtain 50% or more of their nitrogen requirements via biological N₂ fixation (7). In spite of these low levels of fixa-

tion, grain yields of most of the varieties were normal. Thus we conclude that in our study there was no relationship between N₂ fixation and yield, nitrogen concentration of the grain, or accumulated plant biomass.

Summary

The N₂-fixing activity of 17 non-inoculated varieties of cowpea was determined using the acetylene reduction technique. All varieties assayed exhibited N₂-fixing activity with total N₂ fixation ranging between 4.3 and 0.5 kg N fixed/ha. During the life cycle of the plant, a distinct pattern of N₂-fixing activity is discernible in some varieties. Diurnal activity variations are also considered.

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Reseña de libros

FINK, A. *Fertilizers and fertilization: Introduction and practical guide to crop fertilization*. Verlag Chemic International. Inc. Deerfield Beach, Florida, 438 p. 1982, precio \$ 51.00

El propósito de este volumen es la combinación de los fundamentos y principios de la fertilización de plantas con recomendaciones prácticas sobre la aplicación de los bonos a los principales cultivos. Aunque el contenido principal involucrado en el volumen descansa en la experiencia obtenida en

condiciones de clima templado, se introducen en el texto consideraciones referentes al trópico seco y húmedo.

El lenguaje del volumen es claro y los errores en traducción del alemán al inglés, son mínimos. El inglés del volumen es sencillo y cualquier persona con un conocimiento razonable de este idioma puede entenderlo. En general, como es de esperar de un profesor experimentado como el Dr. Fink, se ha dado gran atención a que el texto sea claro y entendible a los lectores, incluso aquellos sin profundos conocimientos en ciencias agrícolas.

En el primer capítulo se presenta una introducción al abonamiento, comentarios sobre aspectos generales de abonos su (clasificación, manera de expresar su

contenido) y los aspectos legales que reglamentan el uso de abonos. Se concluye el capítulo con un resumen de la historia del abonamiento.

El segundo capítulo se dedica a los abonos minerales que contienen un solo nutrimento. En este capítulo, uno de los más extensos del volumen, se discute en subcapítulos los abonos de N, P, K, Mg, Ca y S, sus formas y su uso práctico. La información se presenta en el contexto de la experiencia alemana con aspectos que amplían la visión en condiciones particulares, como por ejemplo para arroz inundado.

El tercer capítulo se dedica a los abonos con varios nutrimentos, a los que contienen oligoelementos y a aquellos que contienen elementos útiles pero no esenciales como Na, Si, etc. Se considera también los elementos no requeridos por las plantas pero requeridos por los animales como por ejemplo el Co. Este capítulo contiene material seco disponible en otras que tratan este tema y es muy valioso por los conceptos actualizados que presenta en el campo de los oligoelementos.

En el cuarto capítulo se estudia los materiales aplicados para mejorar el suelo y en general las condiciones para producir plantas. La primera sección analiza el encalado y los materiales utilizados en el mismo. Se estudia también los materiales para mejorar la estructura y textura del suelo y los abonos orgánicos. Esta sección es bastante corta, a pesar de la gran importancia que está adquiriendo este campo en especial para dar solución a la contaminación ambiental.

El quinto capítulo, uno de los más amplios, se dedica a estudiar el problema de la determinación de la cantidad adecuada u óptima a usar de abono. La discusión de la interacción acidez de suelos y sus causas se centra alrededor del pH del suelo, un concepto útil en condiciones templadas pero que presenta solamente un punto de vista parcial. Se estima que el concepto del Al como guía para el encalado en los trópicos, los subtrópicos y como una alternativa en condiciones templadas, merece más atención. Se estudia aquí el empleo del concepto de análisis de

suelos y plantas como guía para el abonamiento y los aspectos económicos del abonamiento, más que todo de acuerdo a las ideas de Mitscherlich. Problemas especiales sobre abonos, tales como la tecnología de la aplicación de fertilizantes en el trópico se incluyen en el capítulo 6. Se analiza aquí la preparación de mezclas de abonos, su distribución y abonamiento foliar. En este capítulo se analiza también la problemática novedosa de la interacción abonos-ambiente y el abonamiento en condiciones fuertemente limitantes de la producción como plagas, problemas climáticos y de nuevo ambientales. Se analiza también el problema de la interacción abonos-tipos de suelo y abonos-sistemas de cultivo. El capítulo concluye con una sección sobre interpretación de los resultados de experimentos de campo.

El sétimo capítulo se dedica al abonamiento de los cultivos principales con secciones sobre abonamiento de granos, de raíces y tubérculos, de oleaginosas y leguminosas de grano, cultivos industriales y de plantas para la alimentación animal como pastos. Se considera aquí el abonamiento de los cultivos para propósitos especiales como por ejemplo papas para semilla.

El capítulo octavo presenta información sobre el abonamiento de hortalizas, en silvicultura y de cultivos especiales como especies, plantas medicinales, plantas inferiores, etc. Aquí se presenta de nuevo mucha información que no está disponible a la mayoría de los lectores

El noveno capítulo discute el abonamiento y la calidad de los productos obtenidos, otro tópico actual. El volumen se concluye con una bibliografía de más de 300 referencias, en su inmensa mayoría de la literatura agrícola alemana y un buen índice de más de una docena de páginas.

Se recomienda este volumen a todas las bibliotecas agrícolas como referencia y texto.

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