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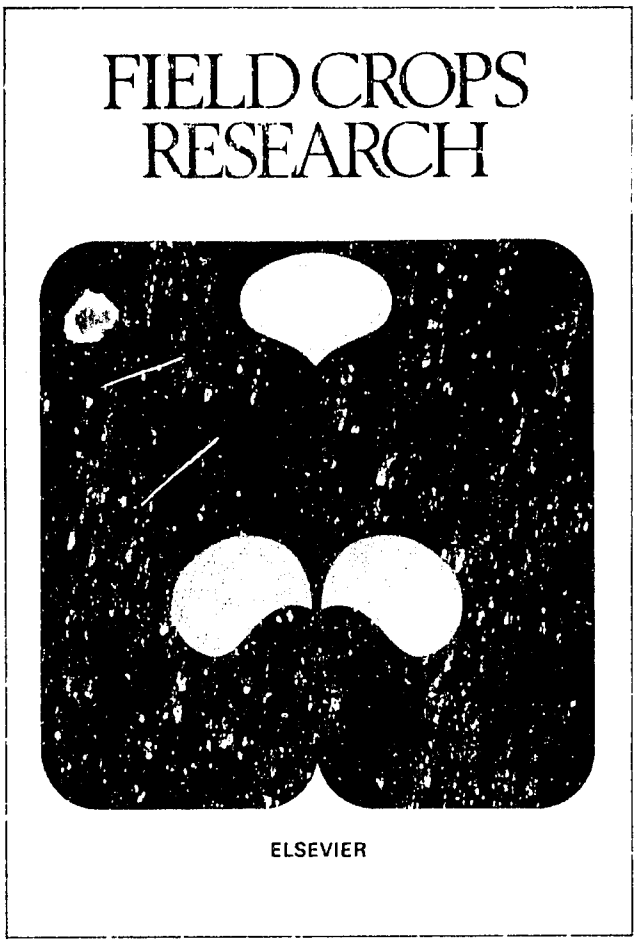

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SCREENING AND SELECTION OF PEARL MILLET FOR ROOT ASSOCIATED BACTERIAL NITROGEN FIXATION

J.H. BOUTON[1], S.L. ALBRECHT[2] and D.A. ZUBERER[3]

[1]*Agronomy Department, University of Georgia, Athens, GA 30602 (U.S.A.)*
[2]*Agronomy Physiology Laboratory, Building 164, University of Florida, Gainesville, FL 32611 (U.S.A.)*
[3]*Department of Soil and Crop Science, Texas A&M University, College Station, TX 77843 (U.S.A.)*

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ABSTRACT

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There are several reports of genotypic variability in grass species for their ability to achieve associations with N_2-fixing soil bacteria. These findings imply that plant breeding may be useful in enhancing these associations and thereby making available plant germplasms needed for agronomic and physiological studies of associative N_2-fixation in grasses.

When the N_2-fixing bacterium *Azospirillum brasilense* (strain JM 125) was used as inoculum, differences were found among selfed lines of pearl millet (*Pennisetum americanum* (L.) K. Shum.) for an ability to support bacterial acetylene reduction activity (ARA) when assayed in enclosed seedling agar tubes. These lines were selfed again or hybridized. Although line to line discrepancies were seen, the ARA of these lines and hybrids when categorized into high or low groups matched the activity of their original parental lines. Also, the selfed and hybrid groups obtained from high ARA lines supported higher numbers of bacteria and lost greater quantities of ^{14}C from their roots than did those groups obtained from low ARA lines. Two of these lines, one having high ARA and one having low ARA, were grown in soil in the greenhouse and were found to differ in their respective ability to support ARA by the soil N_2-fixing microbes. However, overall ARA of the high line was quite low unless analyzed in a hydroponic culture system at low O_2 tensions.

INTRODUCTION

Two major strategies are generally put forward to enhance direct biological N_2-fixation by grass crops. The most ambitious is to use the new and evolving genetic engineering techniques to incorporate the N_2-fixing mechanisms directly into plants. Although these procedures show potential, the biological problems are considered to be formidable. Therefore, the remaining strategy, enhancement of the productivity of existing systems,

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deserves attention since a real contribution of nitrogen has been documented over time with wheat in the Broadbalk fields at Rothamsted (Jenkinson, 1977; Witty et al., 1977).

Associative N_2-fixation shows promise for supplying nitrogen to grasses. Although rates are low in comparison to legumes, N_2-fixing associations have been shown to occur in a wide variety of species, including rice (Dommergues et al., 1973; Hirota et al., 1978); maize (Ela et al., 1982); sugarcane (Dommergues et al., 1973); wheat (Witty et al., 1977); and forage grasses (De-Polli et al., 1977). Species in the genus *Pennisetum* are also reported to support associative N_2-fixation by soil bacteria, and pearl millet, *Pennisetum americanum* (L.) K. Shum., has been shown to be a good host plant (Rao and Dart, 1981).

Differences for N_2-fixing ability as measured by acetylene reduction activity (ARA) on washed roots or intact soil plant cores occur among different cultivars of *Paspalum*, *Digitaria* (Döbereiner, 1977), and rice (Hirota et al., 1978). In maize, bacterial N_2-fixation in and on roots was increased through plant screening and selection (Ela et al., 1982). Significant differences were shown among lines of pearl millet for ability to support ARA by *Azospirillum brasilense*, strain 13t (Bouton and Brooks, 1982). These reports suggest the possibility of using plant breeding and genetic techniques to enhance the N_2-fixing productivity of these associations.

In this paper we report the results of screening of pearl millet lines for ability to support bacterial growth and N_2-fixation, and the ability of selected lines to transfer these traits to their offspring. Lines with both high and low ARA were also grown in soil and tested for their ability to support N_2-fixation. Additionally, plants from the same two lines were grown in soil and subsequently assayed in a hydroponic culture system with controlled oxygen tension.

MATERIALS AND METHODS

Screening pearl millet lines for ability to support bacterial growth and acetylene reduction

Thirty-six inbred lines were established by selfing individual plants from the Tift No. 1 S-1 population (Burton, 1978) for two generations. Twenty-two plants from each of these lines were then screened for ability to support bacterial ARA by using an enclosed seedling procedure described previously (Bouton and Brooks, 1982) with the exception that *A. brasilense*, strain JM 125, was used. Lines varying from high to low in acetylene reduction activity were identified and remnant seed of each were planted in the field where they were all selfed by randomly bagging seedheads within each line. They were also hybridized using crossing techniques outlined by Burton (1980) to achieve high ARA × high ARA, high ARA × low ARA, low ARA × high ARA, and low ARA × low ARA hybrid combinations.

These selfed lines and hybrids were next screened for ARA in the enclosed agar tubes as described above.

Bacterial growth associated with selected pearl millet selfed lines and hybrids

In this experiment, the seed of selfed lines and hybrids were sterilized by placing them in 70% ethanol for 10 min, then washing seven times with sterile distilled water followed by covering them with 2.12% sodium hypochlorite solution for 15 min. The seeds were then washed with seven changes of sterile distilled water, and allowed to imbibe the water of the last wash for at least 60 min. The sterile seeds were germinated on nutrient agar (NA). Sterile seedlings were transfered from the NA plates to seedling tubes assemblies and inoculated with nitrogen-fixing bacteria. The enclosed seedling tube assemblies consisted of larger glass test tubes (3.5 cm dia × 30 cm). The rooting medium was made using nitrogen-free Hoagland's solution, pH adjusted to 6.8—7.0, and 0.75% agar. The entire apparatus was sterilized by autoclaving. Sterile seedlings, bacteria, and tubes were manipulated in a laminar flow hood (Baker Edgeguard*) to reduce the risk of contamination. Three replicates of each line and hybrid were inoculated with 5 ml of a liquid culture of *Azospirillum brasilense* (strain JM 125-A2), kindly supplied by J.R. Milam, containing 2.210^5 CFU (colony forming units) per ml. The bacteria were grown, harvested, maintained in culture, and the inocula prepared as described earlier (Albrecht and Okon, 1980). At 14 days, the shoots were harvested, dried, and weighed. The rooting medium, including the roots, was homogenized, and the pH of the medium determined. The number of viable bacteria in the inoculum and the rooting medium were estimated by the dilution agar-plate method (Clark, 1965), and consists of preparing serial ten-fold dilutions in sterile 0.085% NaCl solution and making surface colony counts on NA plates.

Loss of ^{14}C photosynthate from selected pearl millet selfed lines and hybrids

Three replicated plants of each line and hybrid were grown in sterile elution tubes, using acid washed sand as the rooting medium. The tubes were made from a 2.25 cm dia. × 20 cm test tube, with a small (1 mm dia) hole in the bottom. Sand was obtained from Florida Rock Industries, Gainesville, FL. It was soaked in 6 *N* HCl and washed in deionized water. Before sterilization, Pasteur pipets, with cotton plugs, were placed in the sand to provide a sterile method for watering the plants during growth. The sand was moistened to 100% water holding capacity with a complete Hoagland's solution. The tube and sand were sterilized by autoclaving at 121°C for 30 min on three alternate days. Seeds were sterilized and seedlings

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and sterile elution tubes were manipulated as described above. After the seeds had been transferred to the sterile tubes they were covered with sterile parafined sand (Vincent, 1970), to keep contaminating bacteria from entering the rooting media from above. The tubes were placed in a test tube rack, and suspended over acidified 3% mercuric chloride (Vincent, 1970) to prevent bacteria entering from below. Tubes were wrapped in aluminum foil to prevent light penetration of the rooting media. The tubes were arranged in a completely randomized block design. Plants were grown in a greenhouse during the late fall. Natural daylength was used and the maximum irradiance was 1500 $\mu E\ m^{-2}\ s^{-1}$.

Temperature ranged from 23 to 34°C, mean relative humidity of the air was 60%. After 28 days the plants were labeled for 3 h with $^{14}CO_2$ by placing assemblies in an airtight plexiglas chamber. The $^{14}CO_2$ was generated in situ by adding an excess of lactic acid to $NaH^{14}CO_2$ to achieve 35 mCi inside the chamber. The amount of label in the root zone was determined 48 h later by leaching the sand with sterile distilled water. The leachate was acidified and shaken to remove any disolved CO_2, centrifuged (2500 × g 10 min, 10°C) to remove nonsoluble material, and 1 ml aliquots were counted in a Beckman LS7000 liquid scintilation counter using 10 ml of Fisher Scinti Verse II scintillation fluid. Plants were harvested, separated into roots and shoots and dried to constant weight at 100°C.

Acetylene reduction associated with pearl millet selfed lines grown in soil

Two of the selfed lines, 80-818 and 80-847, were compared in soil for an ability to support acetylene reduction by native soil N_2-fixing bacteria. Experimental units were metal tubes containing soil brought into the greenhouse from the field (tubes measured approximately 8.5 cm dia. × 20 cm). Soil was taken from an area which had grown up in grasses (mainly *Panicum* sp., *Festuca arundinacea*, *Digitaria* sp., *Cynodon dactylon*) by inserting metal tubes into the soil and removing the soil intact. There was approximately 1 kg of soil. Each of eight replicated tubes containing soil were then established to the following treatments: (1) three seedlings of line 80-818; (2) three seedlings of line 80-847; and (3) unplanted (soil alone). No nitrogen fertilizer was applied. Sampling dates for ARA were 2 weeks and 1 month after seedling emergence. Plant tops were removed only at the last sampling date. Cores were capped on both ends with rubber caps which contained a port for injecting and removing gas samples, and ARA was assayed directly from the cores after a 3.5-h incubation in 7% (v/v) acetylene.

Acetylene reduction associated with pearl millet selfed lines following exposure of roots to reduced oxygen concentration

The ARA associated with pearl millet lines 80-818 and 80-847 was determined following 24--48 h exposure of roots of intact plants to 2% oxygen

in a balance of N_2. Five replicated plants of each line were gently washed from the field soil in which they had been grown in greenhouse pots and transferred to polycarbonate cylinders (3.8 cm dia × 25 cm) fitted with threaded caps containing a stainless steel (3.2 mm O.D.) tube for delivering the gas mixture at the base of the cylinder. The stem was sealed into the top of the cylinder by mounting a slotted rubber stopper around the stem and sealing with non-toxic pourable silicone sealer (General Electric RTV-11) which provided a gas-tight seal. The plants were maintained in a nitrogen-free nutrient solution and continuously sparged with the 2% O_2 mixture which was vented just below the rubber stopper into which the plant had been sealed. To conduct the assay for ARA, the solution was slowly withdrawn (only a few milliliter remained in the bottom) into syringe so as not to draw ambient air into the cylinder and the gas supply line and exhaust vent were then sealed with pinch clamps. Acetylene was added, following evacuation of the appropriate volume of gas from the cylinder, to achieve 0.11 atm C_2H_2 in the 2% oxygen mixture. Gas samples were taken through the surgical tubing attached to the exhaust vent and analyzed for ARA by gas chromatography. After sampling, the gas flow was restored and the nutrient solution was returned to the cylinders.

The plants were incubated under fluorescent lights (200—300 $\mu E\ m^{-2}\ s^{-1}$; 14 h light 10 h dark) at 25—29°C.

RESULTS

A range of ARA was found among the pearl millet lines. In Table I, data for the five highest and five lowest ARA lines are presented. These lines showed no statistical overlap in ARA, which allows them to be placed into an ARA grouping of high or low (Table 2). When their selfed progeny

TABLE 1

Acetylene reduction activity (ARA) of pearl millet lines when grown in seedling agar tubes and inoculated with *Azospirillum brasilense*, strain JM 125

Line	ARA (nmol C_2H_4 plant^{-1} h^{-1})
80-818	17.4
80-978	17.4
80-817	16.8
80-838	14.9
80-923	13.8
80-789	5.9
80-782	4.1
80-849	3.2
80-847	3.1
80-714	1.6
LSD (5%)	6.8

TABLE 2

Acetylene reduction activity (ARA), number of bacteria (*A. brasilense*, strain JM 125) in root rhizosphere, and ^{14}C lost by roots of selfed progeny from pearl millet lines differing in ARA activity

ARA grouping[a]	Original line	Selfed progeny		
		ARA[b] (nmol C_2H_4 plant^{-1} h^{-1})	Bacterial No.[b] ($\times 10^{-8}$ plant^{-1})	^{14}C lost[b] (CPM plant^{-1})
High	80-818	22.6	14.4	6239
High	80-978	7.7	15.2	15075
High	80-817	10.8	5.4	5938
High	80-838	6.3	17.4	4979
High	80-923	13.4	0.5	22932
	$\bar{X}$	12.2	10.6	11034
Low	80-789	10.5	—	—
Low	80-782	3.0	—	—
Low	80-849	6.5	0.1	3633
Low	80-847	3.2	10.5	1304
Low	80-714	8.2	8.7	963
	$\bar{X}$	6.3	6.4	1966
LSD (5%)		8.0	6.3	5420

[a]Grouping based on data in Table 1.
[b]Data determined in separate experiments.

were analyzed, there was a general trend (see grouping means) of the high ARA lines having higher ARA, bacterial numbers, and ^{14}C loss per plant than low lines (Table 2). For individual lines, there were discrepancies seen for ARA and bacterial numbers, with high ARA lines 80-978 and 80-838 producing selfed progeny no different in ARA from all the low ARA selfed progeny lines, and high ARA line 80-923 supporting very low numbers of bacteria. Also, low ARA line 80-789 had ARA statistically similar to all high ARA lines except 80-818.

Hybrids between lines showed high ARA × high ARA combinations to give higher ARA than low ARA × low ARA combinations (Table 3). The high ARA × low ARA hybrids gave ARA equal to high ARA × high ARA hybrids and both of these hybrids were higher than the low ARA × high ARA hybrids. The low ARA × high ARA hybrids were no different in ARA from the low ARA × low ARA hybrids (Table 3).

Bacterial numbers were variable among individual hybrids and did not match ARA in many cases. However, bacterial numbers of the high ARA × high ARA hybrid group were higher than the low ARA × low ARA hybrid group (Table 3). Also, although high × low hybrid group had high ARA, it had bacterial numbers no different than low × low hybrid group.

TABLE 3

Acetylene reduction activity (ARA), number of bacteria (*A. brasilense* strain JM 125) in rhizosphere, and ^{14}C lost by roots of hybrids between pearl millet lines which originally possessed high or low ARA

Type hybrid	Parents	ARA[a] (nmol C_2H_4 plant^{-1} h^{-1})	Bacterial No.[a] (× 10^{-8} plant^{-1})	^{14}C lost[a] (CPM plant^{-1})
High × high	80-978 × 80-817	15.0	7.9	8909
	80-978 × 80-923	16.0	13.4	9767
	80-923 × 80-817	13.1	2.9	8967
	$\bar{X}$	14.7	8.1	9214
High × low	80-817 × 80-714	17.5	6.7	5467
	80-817 × 80-789	15.7	4.2	6233
	80-978 × 80-714	19.8	—	—
	$\bar{X}$	17.9	5.5	5850
Low × high	80-854 × 80-978	4.3	—	—
	80-847 × 80-923	9.6	—	—
	$\bar{X}$	7.0	—	—
Low × low	80-714 × 80-789	2.5	10.2	3259
	80-854 × 80-789	4.3	3.2	7333
	$\bar{X}$	3.4	6.7	5296
LSD (5%)		8.1	6.9	NS

[a]Data determined in separate experiments.

The amount of soluble carbon loss from the roots of these breeding lines, given as CPM of ^{14}C in the leachate/plant, indicates that the high ARA lines provide significantly more carbon to the rhizospere than the low ARA lines (Table 2). The differences are not as clear when the hybrids are compared for ^{14}C losses (Table 3). All the hybrids are statistically indistinguishable from one another. Also, no significant correlation was found between the size of the plant and the amount of ^{14}C loss by the root system.

When grown in soil cores, the activity of high ARA line 80-818 was significantly higher than that of low ARA line 80-847 (Table 4). In fact, the activity of 80-847 was not statistically different from the soil control.

The results of incubating roots of intact plants of pearl millet line 80-818 and 80-847 in 2% O_2 (approximately 0.8 ppm dissolved oxygen) are shown in Fig. 1. Acetylene reduction rates were determined after the roots had been exposed to the low pO_2 for 48 h. The ARA commenced without a significant lag period and was linear for up to 2 h. Pearl millet line 80-818 exhibited approximately twice the ARA of line 80-847 under these incubation conditions, although it should be pointed out that the higher replications of line 80-847 approached or exceeded the lower replications of line

TABLE 4

Acetylene reduction activity (ARA) of two pearl millet breeding lines when grown in metal tubes containing intact field soil

Line	ARA grouping[a]	ARA (nmol C_2H_4 core^{-1} h^{-1})
80-818 ♀	High	45.6
80-847 ♀	Low	35.3
Soil control		32.3
LSD (5%)		4.6

[a]Grouping based on data in Table 1.

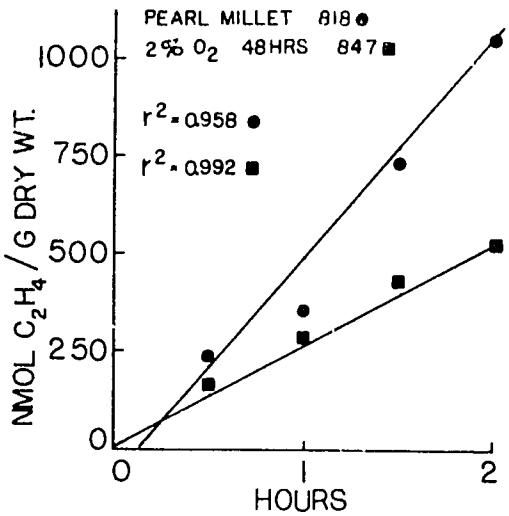

Fig. 1. Acetylene reduction activity (ARA) associated with two pearl millet lines following exposures of their roots to 2% O_2. Line 818 was selected previously as a high ARA line while line 847 was selected previously as a low ARA line. Both lines had been grown in soil before transferring to a nitrogen free nutrient solution where they were continuously sparged with 2% O_2 for 24—48 h before determining ARA.

80-818. It is clear that given favorable environmental conditions (e.g. reduced pO_2 and low levels of combined nitrogen in the "rhizosphere"), substantial ARA can be supported by these pearl millet lines and the differences in lines detected in other experiments during the study are borne out here as well.

DISCUSSION

These results confirm previous findings (Bouton and Brooks, 1982; Rao and Dart, 1981) of genotypic variation in pearl millet to form N_2-

fixing associations with soil bacteria. However, there still exists variation among individual plants within the lines. Examples are the high line 80-978 where individual plants ranged from 0—45 nmol C_2H_2 plant^{-1} h^{-1} to give an ARA mean of 17.4 and the low line 80-749 whose individual plants ranged from 0—11 nmol C_2H_2 plant^{-1} h^{-1} to give an overall ARA mean of 5.9 (Table 1). This within-line variation could have caused the overlap of mean ARA, bacterial numbers, and $^{14}CO_2$ loss among selfed progeny of high and low lines seen in Table 2, because *random* selection of plants for selfing (see Materials and Methods section) may have resulted in selection of low plants from within a high line such as 80-978 and high plants from a low line such as 80-789 to give the discrepancies. However, it is encouraging that overall (see group means) the transfer of these traits to both selfed and hybrid offspring did occur (Tables 2 and 3). This is in agreement with work in maize (Ela et al., 1982) and indicates that plant breeding techniques would be useful in promoting root-associated bacterial N_2-fixation in pearl millet. Further selection within these lines, to stabilize and possibly increase their ability, seems justified.

It was also noted that lines identified as having high and low ARA (80-818 and 80-847, respectively) in the aseptic agar screenings also possessed similiar activity when grown in soil and analyzed in situ (Table 4). However, the overall activity of the high line (80-818) represents a very small percentage of what would be found for legumes growing under similar conditions. The effect of lowering the O_2 tension to 2% did result in rates approaching that of legumes with both lines maintaining their respective differences in ARA (Fig. 1). It may be envisioned that low O_2 tensions could possibly be achieved in wet soils resulting in higher N_2-fixing activity for high lines. This, of course, still needs to be tested, as well as the ultimate question of the short term fate of the nitrogen fixed by the bacteria, before its agronomic importance is known.

The nitrogenase activity of the grouped lines and hybrids matched the numbers of bacteria. It is not known at this time if any lack of relationship between nitrogenase activity and bacterial numbers, especially for the high × low ARA groups, is due to experimental error (Table 3). Also, the amount of soluble carbon lost on a per plant basis is proportional to both the bacterial numbers and the nitrogenase of the grouped lines. This indicates that the mechanism of increased ARA activity may be the ability of high ARA plants either to fix more CO_2 or to translocate reduced carbon compounds to the rhizosphere, thereby supporting a larger population of N_2-fixing bacteria and their metabolic activity for this energy-demanding process. However, this would not be in agreement with the findings of Rennie and Larson (1981) that wheat lines which supported greater numbers of N_2-fixing *Bacillus* exuded little carbon from their roots. The mechanism for increased bacterial ARA on the roots of high ARA pearl millet lines therefore deserves further study.

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REFERENCES

Albrecht, S.L. and Okon, Y., 1980. Cultures of *Azospirillum*. Methods Enzymol., 69: 740—749.

Bouton, J.H. and Brooks, C.O., 1982. Screening pearl millet for variability in supporting bacterial acetylene reduction activity. Crop Sci., 22: 680—682.

Burton, G.W., 1978. Registration of Tift #1 S-1 pearl millet germplasm. Crop Sci., 18: 697.

Burton, G.W., 1980. Pearl millet. In: W. Fehr and H. Hadley (Editors), Hybridization of Crop Plants. Am. Soc. Agron., Madison WI, pp. 457—469.

Clark, F.E., 1965. Agar-plate method for total microbial count. In: C.A. Black, D.D. Evans, J.L. White, L.E. Ensminger and F.E. Clark (Editors), Methods of Soil Analysis, Pt. 2. Am. Soc. Agron., Madison, WI, pp. 1460—1466.

De-Polli, H., Matsui, E., Döbereiner, J. and Salati, E., 1977. Confirmation of nitrogen fixation in two tropical grasses by $^{15}N_2$ incorporation. Soil Biol. Biochem., 9: 119—123.

Döbereiner, J., 1977. Plant genotype effects on nitrogen fixation in grasses. In: A. Muhammed, R. Adsel and R.C. von Borstel (Editors), Genetic Diversity in Plants. Plenum Press, New York, pp. 325—334.

Dommergues, Y., Balandreau, J.P., Rinaudo, G. and Weinhard, P., 1973. Non symbiotic nitrogen fixation in the rhizospheres of rice, maize, and different tropical grasses. Soil Biol. Biochem., 5: 83—89.

Ela, S.W., Anderson, M.A. and Brill, W.L., 1982. Screening and selection of maize to enhance associative bacterial nitrogen fixation. Plant Physiol., 70: 1564—1567.

Hirota, Y., Fujii, T., Sano, Y. and Iyama, S., 1978. Nitrogen fixation in the rhizosphere of rice. Nature, 276: 416—417.

Jenkinson, D.S., 1977. The nitrogen economy of the Broadbalk experiments. I. Nitrogen balance in the experiments. In: Rothamsted Report for 1976, Part 2. Bartholomew Press, Dorking, pp. 103—106.

Rao, R.V. and Dart, P.J., 1981. Nitrogen fixation associated with sorghum and millet. In: P.B. Vose and A.P. Ruschel (Editors), Associative N_2-Fixation (Vol. 1). CRC Press, Boca Raton, FL, pp. 169—177.

Rennie, R.J. and Larson, R.I., 1981. Dinitrogen fixation associated with disomic chromosome substitution lines of spring wheat in the phytotron and in the field. In: P.B. Volse and A.P. Ruschel (Editors), Associative N_2-Fixation (Vol. 1). CRC Press, Boca Raton, FL, pp. 145—154.

Vincent, J.M., 1970. A Manual for The Practical Study of Root-Nodule Bacteria. Blackwell Scientific Publications, Oxford, 164 pp.

Witty, J.F., Day, J.M. and Dart, P.J., 1977. The nitrogen economy of the Broadbalk experiments. II. Biological nitrogen fixation. In: Rothamsted Report of 1976, Part 2. Bartholomew Press, Dorking, pp. 111—118.

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