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PRELIMINARY INVESTIGATIONS ON INDIGENOUS STRAINS OF *RHIZOBIUM* NODULATING YAM BEAN (*Pachyrhizus erosus* Urban) IN GUADELOUPE

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ABSTRACT

Yam bean plants formed efficient nodules with indigenous strains of *Rhizobium* living in vertisols (pH 8.4), in neutral ferralitic soils (pH 6.9) but not in acid ferrallitic soils (pH 4.6). Mineral amendments imploying Urea, basic slag, lime incorporation in the acid soils overcome the pH inhibition of nodule formation. *Rhizobium* strains from the different soils were isolated and characterized. Apparent rate of growth (ARG) and rate of nodulation in gibson tubes (RNG) were determined. Their aptitude to use sugars and other compounds was assessed by API STAPH tests. The efficiency of the symbiosis of each strain of *Rhizobium*-Pachyrhizus was measured by estimating the biomass produced in gibson tubes and by determining carbohydrates, ureides and amino-compounds in the nodules of the plant.

RESUME

RESULTATS PRELIMINAIRES SUR LA SELECTION ET LA CARACTERISATION DES SOUCHES DE *Rhizobium* INDIGENES NODULANT LE DOLIQUE TUBEREUX (*Pachyrhizus erosus* Urban) EN GUADELOUPE

Le dolique tubéreux forme des nodosités efficientes avec des souches de *Rhizobium* indigènes des vertisols (pH 8) et des sols ferralitiques neutres (pH 6.9). La nodulation est inexistante dans les sols ferralitiques acides (pH 4.6). L'incorporation des amendements minéraux (urée, scories de déphosphoration, chaux) capables d'élever le pH permet de lever l'inhibition de la formation des nodosités dans ces derniers sols. Les souches de *Rhizobium* ont été sélectionnées et caractérisées. Les vitesses de croissance apparente et de nodulation en tubes Gibson ont été déterminées. Leur aptitude à utiliser les sucres et d'autres composés ont été mis en évidence

par les tests API STAPH. L'efficience de chaque souche a été estimée par la matière organique produite en tubes Gibson, les quantités de sucres solubles, d'uréides, et composés aminés des nodosités.

INTRODUCTION

Yam bean, *Pachyrhizus erosus* Urban, is one of the promising crop, interesting to be cultivated in the tropical regions as a diversification crop. It produced fleshy tuber with high yields, which can be used raw or cooked in human or animal diets. The grains which are rich in proteins contain rotenone and make them unsuitable for consumption. From 5 species actually found cultivated or wild, only one is completely insensitinve to photoperiod. As a new crop little information is available on its potentialities. So different investigations are now on the way to compare the interfertility of the five species in order to assess the creation, through interspecific breeding, of new varieties adapted to specific climatic conditions (Sorensen, 1989) after a taxonomic revision of the genus (Sorensen, 1989). Physiological and biochemical studies on growth and development were undertaken to understand the yam bean responses to the climatic factors or growth factors (Zinsou et al. 1987a, 1987b, 1989 ; Vaillant et al. 1989). As a new legume in the tropical regions yam bean must meet appropriate conditions to grow and to give the better yields. So it must meet in the soils the appropriate strains of *Rhizobium* in order to develop the highest efficient nodules. It is possible now to find in the world strain collections the right inoculum to overcome the absence of the *Rhizobium* in a soil. Although yam bean has not been grown before in Guadeloupe, its grows well, bearing nodules in ferrallitic soil with low acidity. Furthermore, nodules were present through flowering and grain maturation. Yam bean seems to behave differently compared to the other grain legumes of which nodules necrosed during pod filling, leading the shoot to empty for the benefit of the grains. In yam bean the aerial parts empty for the benefit of tuber long time after grain maturation. All this prompted us to investigate on the local strains of *Rhizobium* in order to isolate and to select those capable to induce the highest efficiency in nitrogen fixation and appropriate to match the new forthcoming cultivars.

MATERIAL AND TECHNIQUES

1 - Plant materials

Yam bean seeds (cultivar Tpe-1) have been generously supplied by D Steele from IITA and multiplied locally. For all experiments, seeds sterilised with Calcium hypochloride and Mercure chloride were pregerminated in Petri dishes and transferred either in pot or in gibson tubes for inoculation according Bonnier and Brouwers (1958). Growth studies were performed in

pots of 1 litre containing 2 plants on sterilized or non sterilized soil. In the gibson tubes plants were fed with nitrogen free Pochon-Jensen nutritive solution and harvested after 12 weeks of growth.

2 - Soil amendment.

Different mineral amendments were incorporated in Fond d'Or (FO) soil in order to raise the pH. The treatments were the following : 1) FO plus 6 % Tufa ; 2) FO plus 3 % basic slag ; 3) FO plus urea. The final pH of the treated soils at harvest of the plants were 1) 7.95 ; 2) 8.05 ; 3) 5.4.

3 - *Rhizobium* isolation and plant inoculation

The isolation of strain *Rhizobium* was carried out on yam bean nodules at different physiological stages (vegetative and reproductive), under different photoperiodic conditions (short or long days), from two types of ferrallitic soils (Fond d'or pH 4,5 : Duclos pH 6.9) and from a basic soil (Godet pH 8,4).

The isolations and purifications in Petri dishes on Yeast Extract Mannitol agar (YEM) and inoculations in Gibson tubes as described by Bonnier and Brouwers (1958) led to different strains used to evaluate the performances of the different couples Yam bean-*Rhizobium*.

4 - Determination of the strain characteristics

The apparent rate of growth (ARG) was determined on Petri dishes as the average number of days necessary to the appearance of isolated colony, after the spreading of a precedent one on yeast extract mannitol agar (YEM) Petri dishes at 29° C. The mean was determined on 5 successive spreadings in double repliacate.

The rate of nodulation in Gibson tube (RNG) was the number of days from inoculation to the appearance of root nodules.

Carbon utilization source was studied by using API STAPH tests.

The efficiency of the symbiotic couple was evaluated by determining and comparing the organic matter production of the different couples. Plants were harvested from Gibson tubes after 12 weeks of growth. The amino-

compounds were determined according to Yemm and Cocking (1955) using ninhydrin reagent and expressed in aspartic acid equivalents.

The ureids (allantoin and allantoate) were estimated as described by (young an Conway (1942).

Sucrose, fructose and glucose (total soluble carbohydrates) were extracted according to Cerning-Beroard (1975) and estimated by the enzymatic method of Bergmeyer (1979).

RESULTS

1 - Evaluation of the aptitud of the local soils to nodulate yam bean

The aptitude of the three types of soils to nodulate yam bean was evaluated in pots. The fresh matter production was represented on the fig. 1. The highest matter produced was found on Duclos soil followed by Godet soil. Growth was poor in Fond d'Or soil. Nodulation of the plants occurred well in Duclos and Godet soils whereas it was nil on Fond d'Or soil. The absence of nodulation in Fond d'Or soil caused the poor growth of the plants. As Fond d'Or soil was very acid, it was questionned whether the low pH or the absence of specific *Rhizobium* was the limiting factor.

Pot trials on Fond d'Or soil with pH modified by incorporation of different chemical amendments (urea, lime, basic slag) gave the results represented in fig. 2. The production of matter and nodules on these treated soils with pH 5.6 (urea), 7,95 (liming) and 8.05 (basic slag) was compared to that of the same soils inoculated with inoculum prepared from Duclos soil. In all cases, the rise of pH favoured growth of the aerial and ground parts. Matter production was of the same order when the pots were or not inoculated with Duclos inoculum and reached the matter production on Duclos soil used as reference. The rise of the pH caused and favoured nodulation in amended Fond d'Or soil. This revealed that *Rhizobium* strains capable to nodulate yam bean in acid soil were present but not infective under the conditions of low pH of Fond d'Or. Duclos inoculum was inoperative in Fond d'Or soil at pH 4.6.

2 - Some characteristics of selected strains

Apparent rate growth (ARG) and rate of nodulation in gibson tubes (RNG).

The determination of ARG in Petri dishes and RNG in gibson tubes shows that the local strains isolated could be divided into three types : slow growing strains ; fast growing strains and intermediate growing strains independently

Figure 1 : Yam Bean growth after 6 weeks on different soils :
Duclos pH 6,9 ; Godet pH 8,4 ; Fond d'Or pH 4,6 ;
A.P. : Aerial parts ; G.P. : Underground parts ; Nod : Nodules

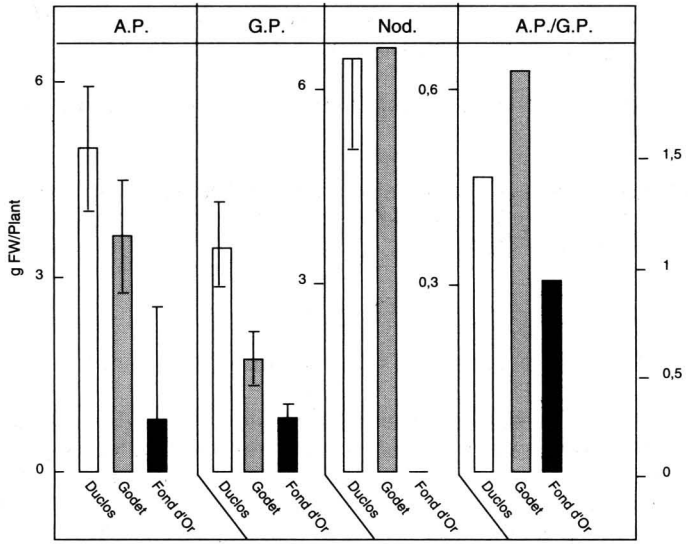


Figure 2 : Yam Bean growth after 10 weeks in Fond d'Or amended soils.
Soils pH after harvest under different treatment :
Control : 4,6 ; Urea : 5,4 ; tufa : 7,95 ; Basic slag : 8,05
Duclos as reference 6,9, the amended soils were inoculated with inoculum prepared from Duclos soils (▨)

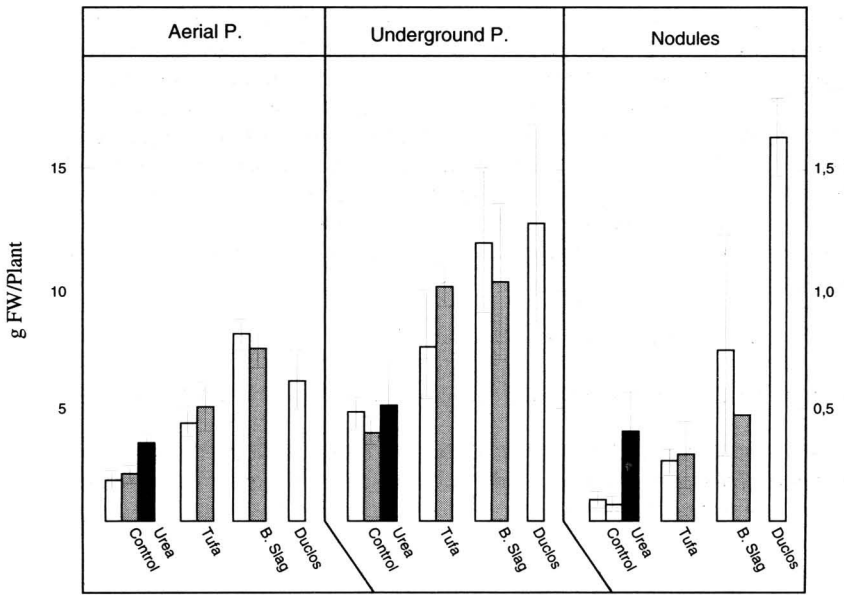
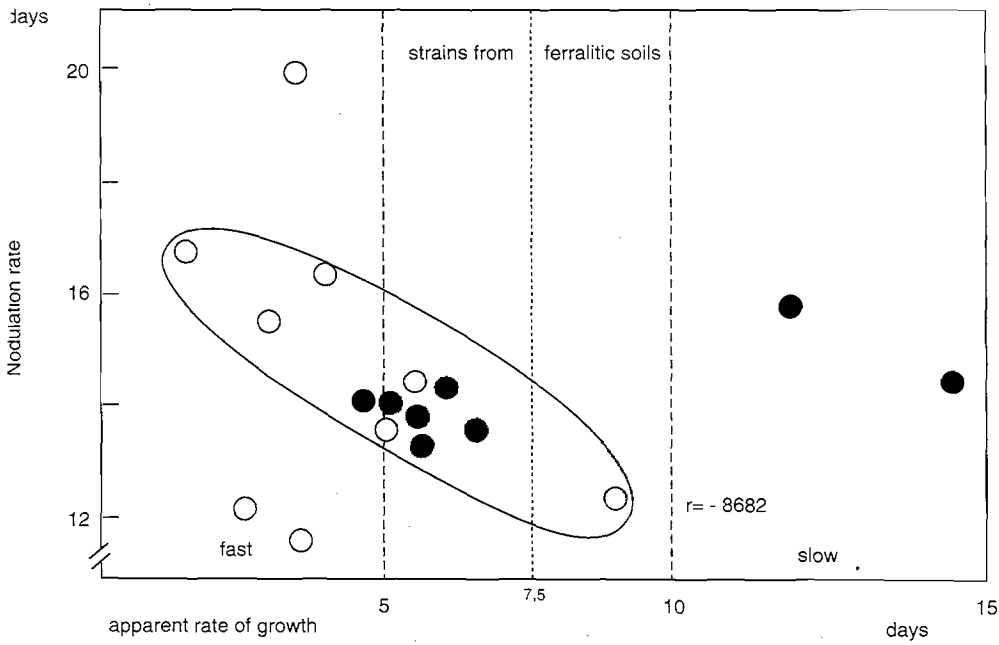


Figure 3 : Relationship between the apparent rate of growth and rate of nodulation in ibson tube of the strains isolated from ferrallitic soils of Duclos (○) and Fond d'Or (●)



on other characteristics like mucosity and pigmentation of the colonies in Petri dishes (fig. 3). The fast growing strains (with ARG < 5,5 days) consisted of 8 out of 9 isolated from neutral ferrallitic soil of Duclos, and 2 out of 8 (FOU7 and FOT2) from acid ferrallitic soil of Fond d'Or. Only 2 low growing strains with ARG > 10 days were found and they come from acid ferrallitic soil. All the strains from basic soil of Godet and the remaining strains from the other soils were considered as intermediate growing strains. The RNG were more discriminative for the strains. All the strains from ferrallitic soils (acid of neutral) showed a RNG of about 14-15 days. The RNG of the strains from basic soil was about 22 days.

API STAPH Test

In this case we tried to test the aptitud of the strains to use different carbohydrates as Carbon source, nitrate and urea as Nitrogen source. Although all the strains were not tested the first results were interesting. All the strains isolated used trehalose, mannitol raffinose, melibiose, xylose and xylitol as carbone source. Four of them (FOS6, FOT1, Dc15 and Dcl9), from ferrallitic soils, and Gb6 from Godet soil, were able to use sucrose. All of them used nitrate and urea as nitrogen sources except FOU8, FOT2 and GA5 which gave a negative reaction in presence of urea. Further investigations are needed to confirm this result.

3 - Symbiotic performance of the different strains

Growth performances of the yam bean plants

Apart from two strains isolated from acid and neutral ferrallitic soils, dry weights of the plants coupled to the *Rhizobium* strains were not different. Only FOU7 and FOT2 strains induced a very low growth of the whole plant. (Table 1). With strains isolated from basic soil low growth were observed. Only Gb7 gave a growth of the same order as that found with the acid soil strain. Generally poor growth paralleled with the little nodulation. Strains from basic soils exhibited nodulation but with a very poor production of nodules under the condition of Gibson tube medium with pH near 7, whereas all strains from ferrallitic soils showed a good formation of nodules.

Carbohydrate composition of the nodules

The study of carbohydrate composition of nodules induced by the different strains coupled to yam bean showed that the composition were different after the strains. In all cases the soluble carbohydrates are sucrose, glucose and fructose. The main sugar was sucrose with a percentage of 50 to 75 % of the toral soluble carbohydrates. The highest levels of soluble sugars and

Table 1 : Performance of symbiosis of different strains of Rhizobium isolated and coupled to yam bean . Harvest and analysis were performed after 12 weeks growth in Gibson tubes

Sites	Strains	Plant Matter gDW/p	Nodule gFW/p	Tot. sol. sugars mg/gFW	Amino-compds mg/gFW	Ureides µg/gFW
	C3	5,2	0,7±0,1	10,2	44,5±1,9	390±60
	C4	5,2	0,8±0,2	14,3	40,3±2,3	238±18
	S5	5,2	0,8±0,1	8,3	47,2±8	248±48
Fond D'Or	S6	4,8	0,8±0,1	5,3	48,4±1,8	327±9
(FO)						
	T1	5,1	0,7±0,1	2,1	130±2	478
pH 4,6	T2	2,3	0,9±0,1	2,1	19,1±2	158±28
	U7	3,7	0,7±0,1	3,3	34,2±1	270±40
	U8	5,5	0,7±0,1	3,1	35,8±1	193±17
	D3b	4,3	0,8±0,2	1,6	14,7±3	137±7
Duclos	Dc15	5,7	0,6±0,1	6,6	8,8±1	176±10
pH 6,9	Dc19	5,4	0,5±0,1	3,5	25,6±1	137±7
	DN4	4,2	0,6±0,1	7,9	39,4±1	420±30
	Ga5	5	-	5,1	28,8±2	256±30
	flb2	1,8	0,05	10,1	19±1	-
	Gb6	2,1	0,3	11,8	25±1	-
	Gb7	2,1	0,12	6,8	18,5±1	393±13

sucrose were found with FOC4 strain, followed in the decreasing order by FOC3, FOS5 and DN4. In the basic soil Gb6 and Gb2 induced the highest levels of soluble carbohydrates in the nodules. (table 1). Some strains, all isolated from ferrallitic soils (D3b, FOT1 and FOT2) induced very low concentrations in carbohydrates in the nodules.

Amino-compound and ureide composition of the nodules

The amino-compound and ureide concentrations in the nodules gave an idea of nitrogen fixation performance under the condition of Gibson tubes since no other source of nitrogen was supplied (Table 1).

In the decreasing order, the highest levels of ureides in the nodules were found with FOT1, DN4, Gb7, FOC3, FOS6. The same strains gave the highest amounts of amino-compounds ; the lowest ones with FOT2, FOU8, D3b Dcl9). FOT2, FOU8 were found to give negative reaction in presence of urea.

DISCUSSION AND CONCLUSION

All the soils studied contained *Rhizobium* strains susceptible to nodulate yam bean although their performances were different. Duclos and Godet strains expressed their infectivity and efficiency in their soil medium pH. As for Fond d'Or strains only the raise of pH met the conditions of the expression of their infectivity for yam bean. Any amendment susceptible to raise the pH is necessary for the introduction of yam bean crop in the ferrallitic acid soils of Guadeloupe. Contrary to the strains of Duclos and Fond d'Or, most of the strains of Godet induced in Gibson tube a reduced growth to the plant and of nodules although they manifested a good infectivity for the yam bean. The poor growth observed might be explained, either by the long time needed to nodulate the yam bean since the rate of nodulation in Gibson tube was about 22 days instead of 14-15 days found for the strains from ferrallitic soils, or by the neutral pH of the nutritive medium of Pochon-Jensen. These strains seemed to function better in basic pH. FOT2, isolated from nodules on plant grown in Fond d'Or soil amended to raise the pH to 8.05 behaved as Godet strains. All the strains have in commun high apparent rate of growth (slow growing strains).

All the strains of Fond d'Or soil approximately induced the same weight of nodules with yam bean but the carbohydrate contents were very different. Some nodules were rich in sugars. The levels of carbohydrate seemed to be under the control of the strains. The high concentrations of sucrose in some

nodules (induced by FOC4, FOC3, FOS5, DN4, GB2 and Gb6) might be interpreted as an accumulation of that sugar in the nodule due partly to the non utilization of sucrose as carbon source. FOT1 and Dcl9 strains which used sucrose as carbon source induced a nodule poor in sucrose. However some other nodules induceb by sucrose using strains (FOS6 and Dcl5) showed intermediate concentration of sucrose. More precise investigations using enzyme studies are needed.

Taking into account the levels of ureides, amino-compounds and the weights of nodules produced by plant FOT1 seemed to have induced the most efficient nodules, followed by DN4 and FOC4. FOT1 and FOC4 came from acid ferrallitic soil and their performance make them interesting for further investigations. In conclusion the different soils contain at least one infective and efficient strain for the nodulation of yam bean. However the acid soil need to be amended for raising the pH up to neutrality necessary to the expression of the strain performance.

Some preliminary research of our laboratory revealed a double nodulation during the cycle of yam bean one during the vegetative stage, the second at the pod setting and filling. We were questioning to know if the double nodulation was caused by one strain or two or more strain(s). From the isolated strains further investigation is on the way to give a response.

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