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ASSESSMENT OF NITROGEN FIXATION POTENTIAL IN AHIPA (*Pachyrhizus ahipa*) AND ITS EFFECT ON ROOT AND SEED YIELD

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SUMMARY

Ahipa is a legume of great interest for the production of raw materials (starch, sugar, oil and proteins) for industrial use. Its yield potential and ability to fix atmospheric N_2 in association with rhizobia makes it an attractive option for low input agriculture systems. At present, it is cultivated on a very small scale as a food crop in a few South American countries. Little information is available on symbiotic N_2 fixation in ahipa and no work has been performed on strain selection for inoculant production. Soils in southwest Europe are devoid of specific rhizobia able to nodulate on ahipa. Selecting rhizobia for symbiotic effectiveness from a collection led to the isolation of strains which provided greater shoot growth and N content under controlled conditions. In the field, inoculation at sowing with the selected strains increased significantly seed and tuberous root yield and seed protein content. The amount of N_2 fixed, estimated by ^{15}N natural abundance, reached 160–260 kg N ha^{-1}. In previous work, ahipa appeared to be a promising alternative crop for the production of industrial raw materials. The results of the present study showed a yield increase in tuberous roots and seeds when applying effective rhizobia inoculants. Furthermore, a positive soil N balance was left after its cultivation making ahipa even more interesting for sustainable farming systems.

INTRODUCTION

Ahipa or *ajipa* (*Pachyrhizus ahipa*), a South American legume species, is a potential source of raw materials with a carbohydrate-rich tuberous root and protein- and oil-rich seeds (Leidi *et al.*, 2003). Some countries in South America (Argentina, Bolivia, Peru) traditionally cultivate it for food as a monocrop or intercropped with maize (Ørting *et al.*, 1996; Sørensen, 1996). In West Africa, ahipa has been introduced jointly with other *Pachyrhizus* species to test their potential as root crops (Zanklan *et al.*, 2007). Agronomic studies performed in southwest Europe showed ahipa may be competitive in relation to traditional sources of raw materials, mostly under sustainable agriculture systems (Leidi *et al.*, 2004). The species establishes symbiosis with rhizobia, has a low requirement for pesticides and leaves N-rich harvest residues, which may be used for fodder or organic-N soil amendment (Sørensen, 1996).

Ahipa can get adequate amounts of N through symbiotic N_2 fixation under controlled conditions (Kjær, 1992). However, poor nodulation rate was reported for

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ahipa grown under natural conditions (Grum and Sørensen, 1998), in field trials in southwest Europe using a commercial inoculant (Leidi, 2001) or in subtropical northeast Argentina (Fassola *et al.*, 2007). In Mexico, ahipa was able to fix 58–80 kg N ha^{-1} with an indigenous soil population of rhizobia maintained by frequent cultivation of yambean (*P. erosus*) (Castellanos *et al.*, 1997). To our knowledge, there has been no selection of rhizobia strains for increasing N_2 fixation in ahipa. Seed inoculation with effective strains is a basic strategy to improve plant N nutrition and increase biomass production in low input farming systems (Hardarson and Atkins, 2003). N_2-fixing legume crops decrease the negative environmental impact of farming by reducing the requirement for N fertilizers and the production costs and may even enhance soil fertility (Jensen and Hauggaard-Nielsen, 2003). Improving N_2 fixation potential in legumes depends on soil fertility, the plant host and suitable nodulating bacteria (Hardarson and Atkins, 2003). Production of rhizobial inoculants is a rather long process which begins by selecting and testing strains from collections under controlled conditions and ends in field trials to determine nodulation and N_2 fixation rates (Stephen and Rask, 2000).

Field measurement of N_2 fixation is a realistic approach to the contribution made by legumes to the N balance of a system allowing the identification of constraints on legume growth and N_2-fixing capacity (Peoples and Herridge, 1990). Several methods have been proposed to assess the contribution of symbiotic N_2 fixation to plant N economy. Among ^{15}N-isotopic techniques, the natural abundance method is a precise and accurate method for estimating the contribution of symbiotic N_2 fixation (Peoples and Herridge, 1990; Unkovich and Pate, 2000). The ureide method is an alternative technique (Herridge *et al.*, 1990), which may be used in ahipa as a portion of the N_2 fixed is transported in the xylem as ureide-N (Leidi *et al.*, 1997), although it provides only a short-term measure of symbiotic performance.

The aims of the present work were therefore: i) to select efficient rhizobia for ahipa cultivars under controlled conditions, ii) to determine the crop yield response to rhizobia seed inoculation and the symbiotic N_2 fixation under field conditions; and iii) to assess the effect of cropping ahipa on soil N balance.

MATERIALS AND METHODS

Screening of Rhizobium strains

Screening of the symbiotic performance of *Rhizobium* strains of different geographical origin (Table 1), provided by M. Grum (IPGRI-CIAT, Cali, Colombia), was performed using *P. ahipa* accession AC521. Surface-disinfected and pre-germinated seedlings were transferred to sterilized Leonard jars containing N-free nutrient solution and inoculated with 1 ml of 3 day-old bacterial cultures (10^8-10^9 cells ml^{-1}). Two uninoculated controls were run: a) −N-control, irrigated with N-free nutrient solution; and b) +N-control, irrigated with nutrient solution containing 50 mM NH_4NO_3 to provide a total amount of 7.5 mM N per pot.

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Table 1. Rhizobial strains, origin and plant host species and screening of their symbiotic performance on ahipa AC521. Shoot mass, leaf N concentration, nodule mass and number of nodules per plant after 10 weeks of growth under greenhouse conditions.

Species/strain	Origin	*Pachyrhizus sp.* host	Shoot growth (g dry wt plant^{-1})	Leaf N concentration (%)	Nodule mass (mg fresh wt plant^{-1})	Nodules per plant
Bradyrhizobium/Spec 1	Commercial	*P. erosus*	1.48 (0.09)	3.05 (0.02)	82.5 (13.9)	121 (6)
Rhizobium/PAC 5	Honduras	*P. ferrugineus*	1.61 (0.36)	2.99 (0.54)	124.3 (9.6)	85 (1)
Bradyrhizobium/PAC 18	Honduras	*P. erosus*	1.77 (0.15)	2.65 (0.16)	127.0 (7.8)	276 (33)
Bradyrhizobium/PAC 40	Costa Rica	*P. erosus*	2.26 (0.17)	3.30 (0.27)	129.7 (8.4)	215 (21)
Bradyrhizobium/PAC 41	Honduras	*P. ferrugineus*	1.12 (0.05)	1.51 (0.04)	49.3 (3.0)	307 (23)
Bradyrhizobium/PAC 43	Nicaragua	*P. erosus*	1.67 (0.30)	2.38 (0.22)	118.3 (26.9)	318 (86)
Bradyrhizobium/PAC 48	Costa Rica	*P. erosus*	2.50 (0.06)	3.36 (0.11)	154.8 (8.5)	230 (55)
Bradyrhizobium/PAC 51	Honduras	*P. erosus*	2.29 (0.24)	3.45 (0.13)	130.0 (7.3)	187 (32)
Bradyrhizobium/PAC 55	Honduras	*P. ferrugineus*	2.82 (0.56)	3.54 (0.20)	155.0 (29.4)	180 (45)
Bradyrhizobium/PAC 68	Honduras	*P. erosus*	1.93 (0.29)	2.81 (0.17)	148.7 (27.1)	212 (39)
−N control	–	–	1.49 (0.07)	1.20 (0.08)	–	0
+N control	–	–	3.00 (0.91)	4.22 (0.28)	–	0
		F	2.56*	14.11***	3.43*	2.80*
		$d.f._t$, $d.f._e$	11, 24	11, 24	9, 20	9, 20

In brackets, standard error of the mean. Levels of significance for *F*: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Degrees of freedom: $d.f._t$, treatments; $d.f._e$, error.

Studies on ahipa landrace-rhizobia strain interaction

Two factorial experiments were performed under controlled conditions to determine ahipa genotype-rhizobia strain interaction. In the first, two ahipa accessions (AC102, AC521) and three rhizobia strains (Spec1, PAC48, PAC55) were used. Plants were grown until the fruiting stage. A second experiment was carried out until flowering with more ahipa landraces (AC102, AC230, AC521, AC526) and rhizobia strains (Spec1, PAC48, PAC51, PAC55). Seeds of the different ahipa landraces were inoculated with peat-based inoculants of the rhizobia strains, sown in vermiculite-containing vessels, irrigated with nutrient solution (modified Hewitt, N-free, 10× Zn concentration) and grown in an environmental chamber (day/night temperature: 28/22 °C; 14 hr light/10hr dark periods; irradiance 150 μmol m^{-2} s^{-1}; relative humidity 40/60%). Three replicates per bacterial treatment were set up for each ahipa accession and sampling time. At vegetative and flowering stages (34 and 57 days after sowing, DAS), plants were harvested to determine plant growth (fresh and dry weight), number and mass of root nodules, and plant N content. Xylem exudates were collected from root stumps during 1 hr in Eppendorf vials maintained on ice and frozen until analysis. The concentration of nitrate, ureides and amino acids was determined as previously described by Leidi *et al.* (1997).

Field trial

The field experiment was implemented from April to November 2001 at the Experimental Station Las Torres (Seville, southwest Spain) to estimate N_2-fixation potential of two ahipa accessions (AC102 and AC521) after inoculation with selected rhizobia strains (PAC48, PAC51, and PAC55). The commercial strain Spec 1 was not included in the field trial because it had provided low nodulation in a preliminary trial (Leidi, 2001). Non-inoculated controls were included. Planting was made in 7 m rows 0.50 m apart with a seed density of 10 seeds per metre on 17 April 2001. Watering was done by furrow irrigation approximately every 2 weeks. Soil samples taken before sowing at 0–30 cm depth showed the following chemical properties: pH, 8.13; $CaCO_3$, 27.4%; organic matter, 0.22%; NO_3-N, 0.06 ppm; P, 9.6 ppm; K, 196 ppm. At harvest, plant shoots, roots, pods and seeds were weighed, oven dried and milled for further chemical analyses. N concentration was determined after Kjeldahl digestion of dried samples and ammonium measured by a colorimetric reaction (phenol-hypochlorite) in a Technicon Analyzer.

The study of N_2 fixation under field conditions was carried out only on the landrace AC521. Xylem sap was collected by applying pressure (Scholander pump) to lateral branches harvested at flowering-fruiting stage. The analysis of N-solutes was performed as indicated above to determine the relative ureides-N in the xylem sap. The $^{15}N/^{14}N$ analysis was performed at the Stable Isotope Facility (Environmental Biology Group, RSBS, Australian National University) to determine N_2 fixation by natural ^{15}N abundance (Peoples and Herridge, 1990). Seeds from the field trial were used and reference values were obtained from nodulated plants grown in N-free sand

and non-nodulated plants grown in potted soil. Isotopic fractionation among plant parts was partially assessed performing $^{15}N/^{14}N$ analysis in seeds and tuberous roots.

Experimental design

A completely randomized designed with three replicates was used for the strain selection. The analyses of plant genotype and *Rhizobium* strain interactions were performed using a factorial design. The field trial was carried out in a randomized complete block design. A statistical software package (Statistix version 7.0) was used for data analysis.

RESULTS

Only nine strains obtained from the rhizobia collection formed nodules and were tested for plant growth and symbiotic parameters (Table 1). Important variations in symbiotic parameters were recorded among rhizobial treatments, with significant differences in leaf N concentration, nodule mass and number of nodules (Table 1). Three strains (PAC48, PAC51 and PAC55) were selected for further studies because they did not show significant differences in shoot growth over the N-fed control (+N treatment) and had the highest leaf N concentration among the inoculated treatments.

The first factorial experiment for the study of the ahipa-rhizobia interaction was set up with three rhizobia strains and two ahipa accessions and showed significant effects of strain on plant growth, shoot N content and ureide concentration in the xylem sap (Table 2). The xylem ureide concentration was significantly correlated with shoot dry weight and the N content in leaves and stems ($r = 0.49$, $p < 0.05$; $r = 0.48$, $p < 0.05$; $r = 0.63$, $p < 0.01$, respectively).

When four ahipa landraces and four *Rhizobium* strains were included, significant differences between ahipa landraces were observed for plant growth at the vegetative stage (34 DAS) with no significant effect of the rhizobial strains (data not shown). However, a significant effect of strains and the interaction landrace × strain was observed for the size of nodules and the plant N content at flowering stage (57 DAS) (Table 3). The interaction of rhizobial strains with the ahipa landrace significantly affected plant shoot growth. Plant shoot growth was significantly correlated with nodule mass ($r = 0.59$, $p < 0.001$, $n = 32$) and the relative abundance of ureides in the xylem sap ($r = 0.63$, $p < 0.001$, $n = 32$). Xylem composition changed significantly with time, showing an increasing concentration of ureides and a decreasing concentration of amino acids, which might be related to nodule maturation and peaking rates of N_2 fixation at flowering (data not shown).

In the field experiment, good nodulation rates were observed on root-crowns of the inoculated ahipa landraces (AC102 and AC521) and nodules were not found on the uninoculated control. At fruiting stage, leaves from non-inoculated plants showed symptoms of N-deficiency chlorosis indicating that soil N was limiting at times of high plant N requirements (seed filling).

Table 2. Effect of the inoculation with different rhizobia strains (Spec1, PAC48, PAC55) on the shoot and root growth, N content and symbiotic parameters (number of nodules, nodule mass, nodule size) in ahipa landraces (AC102, AC521) at flowering-fruiting (79 DAS) grown under controlled conditions.

Landrace	Strain	Shoot growth (g dry wt plant^{-1})	Root growth (g dry wt plant^{-1})	Nodule mass (g dry wt plant^{-1})	Shoot N content (mg shoot^{-1})	Root N content (mg root^{-1})	Ureides (μmoles ml^{-1} sap)
AC102	Spec1	2.93 (0.43)	0.49 (0.05)	0.28 (0.04)	67.2 (8.8)	15.4 (0.17)	4.62 (0.76)
	PAC48	3.59 (0.43)	0.40 (0.05)	0.37 (0.05)	78.8 (8.8)	14.5 (0.17)	4.64 (0.85)
	PAC55	4.12 (0.48)	0.41 (0.06)	0.30 (0.05)	90.9 (9.8)	14.2 (0.19)	6.34 (0.98)
AC521	Spec1	1.61 (0.48)	0.49 (0.06)	0.36 (0.05)	38.6 (9.8)	16.0 (0.19)	3.38 (0.98)
	PAC48	4.42 (0.43)	0.60 (0.05)	0.42 (0.04)	95.8 (8.7)	18.1 (0.17)	7.72 (0.98)
	PAC55	3.76 (0.48)	0.44 (0.06)	0.39 (0.05)	83.5 (9.8)	14.8 (0.19)	3.81 (0.85)
	d.f.						
Landrace	1	0.86	13.27***	3.40	0.96	12.1**	0.11
Strain	2	9.91**	2.63	1.68	9.33**	3.59*	2.81
L*S	2	3.22	9.31**	0.09	3.37	7.82**	5.34*

Standard error of the mean are shown in parentheses.
Levels of significance for *F*: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Table 3. Shoot growth, N content and symbiotic parameters (number of nodules, nodule mass, and nodule size) at flowering (57 DAS) in ahipa landraces (AC102, AC230, AC521, AC526) inoculated with different rhizobia strains (Spec1, PAC48, PAC51, PAC55).

Landrace	Strain	Shoot growth (g dry wt plant^{-1})	N content (mg shoot^{-1})	Nodules per plant	Nodule mass (g dry wt plant^{-1})	Nodule size (mg)
AC102	Spec1	2.12 (0.07)	79.1 (2.5)	39 (2)	0.49 (0.12)	12.7 (3.7)
	PAC48	1.45 (0.34)	49.1 (11.3)	37 (4)	0.27 (0.08)	6.9 (1.3)
	PAC51	2.10 (0.32)	64.5 (9.9)	73 (6)	0.42 (0.04)	5.9 (0.7)
	PAC55	2.33 (0.17)	77.8 (5.6)	64 (5)	0.47 (0.03)	7.6 (1.1)
AC230	Spec1	1.44 (0.05)	45.3 (1.6)	52 (9)	0.39 (0.04)	7.8 (0.6)
	PAC48	1.70 (0.16)	63.2 (6.0)	53 (18)	0.31 (0.09)	6.2 (0.4)
	PAC51	1.09 (0.25)	28.9 (0.9)	27 (2)	0.21 (0.03)	8.1 (1.7)
	PAC55	1.31 (0.17)	39.5 (5.2)	27 (8)	0.24 (0.04)	9.4 (1.2)
AC521	Spec1	1.57 (0.28)	48.4 (8.6)	29 (4)	0.41 (0.04)	14.3 (1.5)
	PAC48	2.12 (0.03)	82.6 (1.1)	74 (2)	0.53 (0.11)	7.2 (1.7)
	PAC51	1.50 (0.23)	42.3 (7.9)	45 (18)	0.20 (0.10)	4.1 (0.6)
	PAC55	2.13 (0.13)	69.9 (4.2)	38 (7)	0.35 (0.09)	9.1 (0.7)
AC526	Spec1	1.85 (0.19)	68.5 (7.1)	85 (6)	0.43 (0.05)	5.1 (0.2)
	PAC48	1.71 (0.07)	65.1 (2.7)	33 (4)	0.34 (0.02)	10.7 (0.8)
	PAC51	1.76 (0.18)	54.7 (5.7)	39 (7)	0.29 (0.04)	7.5 (0.4)
	PAC55	1.98 (0.21)	70.4 (7.5)	44 (7)	0.35 (0.01)	8.3 (1.1)
	d.f.					
Landrace	3	6.93***	10.35***	1.35	1.52	0.18
Strain	3	1.78	6.21**	0.48	2.26	3.12*
L*S	9	2.21*	4.26***	4.61**	1.19	2.99*

Standard error of the mean are shown in parentheses.
Levels of significance for *F*: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Inoculation with some rhizobial strains produced a significant increase in root, pod and seed yield over the non-inoculated control in some landrace and strain combinations (Table 4). The strain PAC55 provided a significant effect on root growth in AC102 but none in AC521 while PAC48 improved mainly seed yield in both ahipa accessions. The increase in crop yield provided by effective nodulation in comparison with the non-inoculated control was a consequence of greater root weight and fruit load per plant (data not shown). Apart from the effects on crop yield, rhizobia inoculation provided a significantly greater seed protein concentration than the non-inoculated controls (Table 4) although seed oil concentration was lower in the inoculated treatments.

The concentration of N in different plant parts (shoot, root, seeds) was significantly increased by seed inoculation (Table 5). The estimation of N_2 fixation by means of the natural ^{15}N abundance method showed that at least 60% of the seed nitrogen was obtained through N_2 fixation (Table 5). Seeds from uninoculated plants showed a significant *P* value (% of plant N derived from N_2 fixation) which might suggest either contamination through the irrigation water or root N transfer from inoculated plots. The estimate of N_2 fixation based in relative ureide content in the xylem sap was related to that provided by the natural ^{15}N abundance method. A preliminary assessment of plant $^{15}N/^{14}N$ fractionation ($\delta^{15}N$) across strains showed significant differences among roots (1.08 ± 0.13) and seeds (2.03 ± 0.20).

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Table 4. Effect of inoculation with different rhizobia strains on root and seed yield (in kg dry matter ha^{-1}), root sugar concentration (Brix degrees) and seed protein and oil concentration (%) of ahipa landraces AC102 and AC521.

	Treatment	Root yield	Root sugars	Seed yield	Seed protein	Seed oil
AC102	Control	3645 (284)	6.77 (0.14)	1045 (265)	22.2 (1.0)	22.4 (0.1)
	PAC48	4185 (821)	6.41 (0.14)	1425 (100)	30.3 (1.6)	18.3 (0.4)
	PAC51	4920 (606)	6.47 (0.13)	1520 (144)	32.1 (2.7)	18.1 (0.9)
	PAC55	8670 (553)	7.03 (0.14)	855 (122)	30.7 (2.7)	17.7 (1.0)
d.f.		3, 8	3, 8	3, 8	3, 8	3, 8
F		14.4**	4.4*	2.9	4.4*	9.9**
AC521	Control	2355 (551)	5.88 (0.19)	665 (37)	20.3 (0.2)	26.6 (0.4)
	PAC48	4515 (580)	5.93 (0.19)	1900 (416)	31.9 (0.8)	19.4 (0.9)
	PAC51	3510 (270)	5.90 (0.19)	1045 (82)	30.3 (0.9)	19.5 (1.5)
	PAC55	2430 (264)	5.98 (0.17)	1730 (315)	34.8 (0.3)	19.1 (2.3)
d.f.		3, 8	3, 8	3, 8	3, 8	3, 8
F		7.9**	0.06	4.8*	102.2***	6.4*

Standard error of the mean are shown in parentheses.
Levels of significance for *F*: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Table 5. Nitrogen concentration (%) in shoots, roots, seeds and estimates of N_2 fixation by natural ^{15}N abundance (*P*, proportion of plant N derived from nitrogen fixation, %) or relative ureide content of xylem sap (*XRU*, %) of ahipa landrace AC521 inoculated with different rhizobia strains harvested at 119 DAS.

Treatment	Shoot	Root	Seed	*P*	*XRU*
Control	1.39 (0.13)	0.41 (0.06)	3.42 (0.25)	35.4 (1.4)	30.8 (4.5)
PAC48	2.27 (0.07)	0.73 (0.07)	4.94 (0.27)	63.8 (3.2)	78.6 (3.8)
PAC51	2.56 (0.02)	0.52 (0.06)	4.81 (0.35)	60.5 (7.5)	80.8 (1.9)
PAC55	2.55 (0.16)	0.47 (0.07)	4.95 (0.27)	63.0 (4.2)	85.1 (2.8)
F	14.2**	2.95	8.2*	6.7*	36.6***
d.f.	3, 8	3, 8	3, 8	3, 8	3, 8

Standard error of the mean are shown in parentheses.
Levels of significance for *F*: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

DISCUSSION

Selection of effective rhizobial strains for ahipa inoculation led to significant increases in tuber and seed yield and seed protein content under field conditions. Roots, seeds and shoots from N_2-fixing plants showed significantly greater N concentration than non-inoculated controls. As result of symbiotic N_2 fixation, seed protein content in the AC521 plants was enhanced up to 45% over the non-inoculated control plants. Interaction effects among rhizobial strains and ahipa landraces were found for shoot growth, N content, number of nodules and nodule size in experiments run under controlled conditions. It may be necessary to produce inoculants for specific plant genotype-rhizobial strain associations for particular soils or management conditions.

The present results show the importance of ahipa seed inoculation with specific strains when the species is introduced in new areas. In local soils, ahipa did not form nodules and the search for nodulating strains was performed in a rhizobial collection. An essential step for improving the legume capacity for N_2 fixation is the research

leading to the selection of elite strains of rhizobia on the basis of both specificity and high effectiveness traits (Hardarson and Atkins, 2003; O´Hara *et al.*, 2002). In controlled conditions, a commercial strain provided ahipa with sufficient N for growth and development (Kjaer, 1992). However, the same strain had not performed well under field conditions (Leidi, 2001) and showed lower N_2 fixation rates than other strains under controlled conditions (Tables 2 and 3). Yield improvement after rhizobial strain selection has been shown for most legume species (O´Hara *et al.*, 2002; van Kessel and Hartley, 2000). Our results have shown that it is also the case for ahipa, in which an effective symbiosis improves tuberous root and seed yield, and seed protein and root N concentration (Table 4).

Using the natural ^{15}N abundance method, the inoculation of ahipa with selected strains provided as much as 64 % of seed N (Table 5). Roots were more enriched than seeds in N derived from N_2 fixation and presented lower $\delta^{15}N$. Differences in isotope fractionation among organs have also been found in other legumes, but the reasons remain unclear (Yoneyama *et al.*, 1986). Our observation on fractionation is just preliminary as it was performed across strains. For other legumes, it has been reported that fractionation may be affected by rhizobia strain or plant genotype (Kyei-Boahen *et al.*, 2002; Steele *et al.*, 1983). The proportion of plant N derived from N_2 fixation (*P* values) obtained in this study agree with those reported for soybean (Peoples and Herridge, 1990) and were close to the 55–69% reported for ahipa using the ^{15}N isotopic dilution method (Castellanos *et al.*, 1997).

The ureide content in the xylem sap might be overestimating N_2 fixation as the method provides only a short-term measure of symbiotic dependence and the sampling was performed at flowering/fruiting, at times when the highest rate of N_2 fixation is expected (Peoples and Herridge, 1990). In fact, the average 80% for xylem sap N derived from fixation (*XRU*) in the inoculated treatments (Table 5) was higher than the N_2 fixation estimate obtained by the natural ^{15}N abundance method (*P*). However, the *XRU* was a reliable predictor for N_2-fixing and provided good enough values considering the low cost and simplicity of analyses (Herridge *et al.*, 1990). A significant correlation was observed between *XRU* and *P* ($r = 0.99$, $p < 0.01$, $n = 4$) as reported by other authors using different legume species showing the close agreement between values of N_2 fixation estimated by the ureide content in xylem saps and ^{15}N techniques (Hansen *et al.*, 1993; Herridge *et al.*, 1990).

The total amount of N_2 fixed by inoculated ahipa in harvestable organs, calculated from root and seed dry matter yields (Table 4) and the N concentration and *P* values presented in Table 5, reached 156–260 kg N ha^{-1}. These amounts agree reasonably well with the roughly 100–260 kg N ha^{-1} calculated from the N-difference between inoculated and non-inoculated treatments. The amount of N_2 fixed by ahipa might be considered quite high according to published data on other annual legumes (LaRue and Paterson, 1981; Peoples and Herridge, 1990; Unkovich and Pate, 2000). These values agree with the amount of N_2 fixed by yambean (*P. erosus*) but are much higher than those reported for ahipa by Castellanos *et al.* (1997) who compared the two species. A reasonable explanation for the disagreement might be in the lower efficiency of soil rhizobia infecting ahipa roots, evolved after years of yambean cultivation. Such

Table 6. Gross estimation of the total N exported in seeds and roots at harvest of uninoculated (control) and inoculated ahipa landrace AC521, N recovery (total N remaining in dried shoots on the soil) and calculation of fixed N_2 using the *P* values reported in Table 5. The amount of N remaining in the soil in secondary roots or pod walls after threshing was not considered. The gross N balance was then calculated as follows: N balance = (N recovery + N_2 fixed seed + N_2 fixed root) − (seed removal + root removal).

Treatments	Seed removal (kg N ha^{-1})	Root removal (kg N ha^{-1})	Shoot N recovery (kg N ha^{-1})	N_2 fixed in seeds (kg N ha^{-1})	N_2 fixed in roots (kg N ha^{-1})	N balance (kg N ha^{-1})
Control	24	64	56	8	22	+2
PAC48	99	220	92	63	141	+23
PAC51	53	121	104	32	74	+36
PAC55	69	76	103	44	48	+50

native strains would not be as effective for ahipa as those selected and tested in our experimental conditions. Similarly, the symbiotic behavior of a commercial strain (Spec 1) was overcome by other strains in providing greater shoot growth or leaf N concentration to ahipa landraces (Tables 2 and 3).

It should be emphasized that the total amount of N_2 fixed might be much higher if considering what is left in the field. The amount of N remaining in soils after legume cropping (shoots, secondary roots, nodules) is an important contribution to soil N economy (Jensen and Hauggaard-Nielsen, 2003). Below-ground N, that is N in roots and nodules, may represent up to 50% of the total plant N (Khan *et al.*, 2002). A significant amount of biomass (rather rich in N and composed of shoots, shed leaves, empty pods) remains in the field. A gross assessment of soil N balance after cropping ahipa was calculated to ascertain its impact on N availability after harvest (Table 6). The N balance presented in Table 6 indicates that an effectively inoculated crop may leave up to 50 kg N ha^{-1} in the soil as crop residues without considering below-ground N. The resulting positive N balance is similar to values reported for other legume seeds, like groundnut and soybean but greater than those of common bean or pigeon pea (Peoples and Herridge, 1990).

In world regions with weathered soils where ahipa is being introduced as an additional source for carbohydrates and proteins for food and/or fodder (Fassola *et al.*, 2007; Zanklan *et al.*, 2007), seed inoculation is a low cost technique that might ensure tuberous-root production while improving soil fertility. In European agriculture, ahipa might find a niche for raw material production in environmentally sound farming systems or in marginal lands for low input agriculture. The productivity of available ahipa accessions is quite competitive in comparison with traditional crops (sugar beet, potato) and important yield increases might be expected following modern breeding (Leidi *et al.*, 2004).

Seed inoculation ensures availability (in number and quality) of specific rhizobia in the rhizosphere, which permits the establishment of nodules formed by the selected strain (Hardarson and Atkins, 2003). It is essential for early nodulation when no soil rhizobia are available or to displace low-efficient natural populations (van Kessel and Hartley, 2000). Inoculation of ahipa with highly effective N_2-fixing strains may certainly increase crop yield and provide a positive soil N balance after cropping.

CONCLUSIONS

It has been shown that inoculation of ahipa with selected rhizobia strains may meet crop N demand for growth and increases seed and root yield. Furthermore, the gross estimate of soil N balance indicates cropping inoculated ahipa may even improve soil N fertility. A simple method, like the ureide content in the xylem sap, may be a helpful complementary tool to assist testing new inoculants for ahipa in field conditions.

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REFERENCES

Castellanos, J. Z., Zapata, F., Badillo, V., Peña-Cabriales, J. J., Jensen, E. S. and Heredia-García, E. (1997). Symbiotic nitrogen fixation and yield of *Pachyrhizus erosus* (L.) Urban cultivars and *Pachyrhizus ahipa* (Wedd.) Parodi landraces as affected by flower pruning. *Soil Biology and Biochemistry* 29:973–981.

Fassola, H. E., Pachas, N. A., Rohatsch, P., Uset, A. and Wiss, F. (2007). *Pachyrhizus ahipa*: un cultivo alternativo para la producción de proteínas y almidones. *Informe Técnico N° 60, Publicaciones Regionales INTA, Montecarlo, Misiones, Argentina. ISSN-0327–926X*

Grum, M. and Sørensen, M. (1998). *Pachyrhizus* DC. (yam bean) symbiosis with *Rhizobium* bacteria: Genetic variation and performance. In *Proceedings 2nd International Symposium on Tuberous Legumes*, 419–429 (Eds M. Sørensen, J. E. Estrella, O. J. Hamann and S. A. Ríos Ruíz). Celaya, Mexico: MacKenzie Press, København.

Hansen, A. P., Rerkasem, B., Lordkaew, S. and Martin, P. (1993). Xylem-solute technique to measure N_2 fixation by *Phaseolus vulgaris* L.: Calibration and sources of error. *Plant and Soil* 150:223–231.

Hardarson, G. and Atkins, C. A. (2003). Optimising biological N_2 fixation by legumes in farming systems. *Plant and Soil* 252:41–44.

Herridge, D. G., Bergersen, F. J. and Peoples, M. B. (1990). Measurement of nitrogen fixation by soybean in the field using the ureide and natural ^{15}N abundance methods. *Plant Physiology* 93:708–716.

Jensen, E. S. and Hauggaard-Nielsen, H. (2003). How can increased use of biological N_2 fixation in agriculture benefit the environment? *Plant and Soil* 252:77–186.

Khan, D. F., Peoples, M. B., Chalk, P. M. and Herridge, D. F. (2002). Quantifying below-ground nitrogen of legumes. 2. A comparison of ^{15}N and non isotopic methods. *Plant and Soil* 239:277–289.

Kjær, S. (1992). Biological nitrogen fixation in *Pachyrhizus ahipa* (Wedd.) Parodi. *Annals of Botany* 70:11–17.

Kyei-Boahen, S., Slinkard, A. E. and Walley, F. L. (2002). Isotopic fractionation during N_2 fixation by chickpea. *Soil Biology and Biochemistry* 34:417–420.

LaRue, T. A. and Paterson, T. G. (1981). How much nitrogen do legumes fix? *Advances in Agronomy* 34:15–38.

Leidi, E. O. (2001). Exploring the potential of a sustainable crop as an alternative non-food source. *Final Report, AHIPA FAIR CT98–4297.*

Leidi, E. O., Sarmiento, R., Mazuelos, C. and Rodriguez-Navarro, D. N. (1997). Efecto de la fuente de nitrógeno en la distribución de asimilados y composición de savia en ajipa (*Pachyrhizus ahipa* (Wedd. Parodi). In *Nutrición Mineral de las Plantas en la Agricultura Sostenible* (Eds R. Sarmiento, E. O. Leidi and A. Troncoso), 34–40. Servicio de Publicaciones, DGIFA, CAP, Junta de Andalucía, ISBN 84-89802-09-2.

Leidi, E. O., Sarmiento, R. and Rodríguez-Navarro, D. N. (2003). Ahipa (*Pachyrhizus ahipa* [Wedd.] Parodi): an alternative legume crop for sustainable production of starch, oil and protein. *Industrial Crops and Products* 17:27–37.

Leidi, E. O., Rodríguez-Navarro, D. N., Fernández, M., Sarmiento, R., Semedo, J., Marques, N., Matos, A., Ørting, B., Sørensen, M. and Matos, M. C. (2004). Factors affecting root and seed yield in ahipa (*Pachyrhizus ahipa* (Wedd.) Parodi), a multipurpose legume crop. *European Journal of Agronomy* 20:395–403.

O´Hara, G., Yates, R. and Howieson, J. (2002). Selection of strains of root nodule bacteria to improve inoculant performance and increase legume productivity in stressful environments. In *Inoculants and Nitrogen Fixation of Legumes in Vietnam*,75–80 (Ed. D. F. Herridge). Canberra, Australia: ACIAR Proceedings, No.109.

Ørting, B., Güneberg, W. J. and Sørensen, M. (1996). Ahipa (*Pachyrhizus ahipa* (Wedd.) Parodi) in Bolivia. *Genetic Resources and Crop Evolution* 43:435–446.

Peoples, M. B. and Herridge, D. F. (1990). Nitrogen fixation by legumes in tropical and subtropical agriculture. *Advances in Agronomy* 44:155–223.

Sørensen, M. (1996). Yam bean *Pachyrhizus* DC. Promoting the conservation and use of underutilized and neglected crops 2. IPGRI, Rome.

Steele, K. W., Bonish, P. M., Daniel, R. M. and O'Hara, G. W. (1983). Effect of rhizobial strain and host plant on nitrogen isotopic fractionation in legumes. *Plant Physiology* 72:1001–1004.

Stephens, J. H. G. and Rask, H. M. (2000). Inoculant production and formulation. *Field Crops Research* 65:249–258.

Unkovich, M. J. and Pate, J. S. (2000). An appraisal of recent field measurements of symbiotic N_2 fixation by annual legumes. *Field Crops Research* 65:211–228.

Van Kessel, C. and Hartley, C. (2000). Agricultural management of grain legumes: has it led to an increase in nitrogen fixation? *Field Crops Research* 65:165–181.

Yoneyama, T., Kounosuke, F., Yoshida, T., Matsumoto, T., Kambayashi, I. and Yazaki, J. (1986). Variation in natural abundance of ^{15}N among plant parts and in $^{15}N/^{14}N$ fractionation during N_2 fixation in the legume-rhizobia symbiotic system. *Plant and Cell Physiology* 27:791–799.

Zanklan, A. S., Ahouangonou, S., Becker, H. C., Pawelzik, E. and Grüneberg, W. J. (2007). Evaluation of the storage root-forming legume yam bean (*Pachyrhizus* spp.) under West African condition. *Crop Science* 47:1934–1946.