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Genotypic variability in P use efficiency for symbiotic nitrogen fixation is associated with variation of proton efflux in cowpea rhizosphere

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ABSTRACT

Vigna unguiculata sp., or cowpea, varieties vary in their adaptation to low-P soils. In order to investigate to what extent this variation may be related to P use efficiency and proton efflux by nodulated roots, three genotypes, 26-73, Danila and Melakh, inoculated with *Bradyrhizobium* sp. *Vigna* CB756 were grown in hydroaeroponic culture in a glasshouse at two levels of phosphorus supply corresponding to P sufficiency or P deficiency. After 4 weeks, individual symbiotic-plants were transferred to a reference-soil layer in a rhizotron, and harvested after 2 further weeks. Nodule and shoot biomass were less when P was deficient. The effect of P deficiency on biomass production followed the trend Danila > 26-73 > Melakh. Under P deficiency, the proton efflux for the P-efficient genotype 26-73 was 43% and 60% greater than for the P-inefficient Danila in hydroaeroponics and in soil, respectively. This increase in proton efflux was associated with an increase in nodule specific respiration that was 115% greater for Danila than for 26-73. It is concluded that the genotypic variability in P use efficiency for symbiotic nitrogen fixation is associated with a variation in nodulated-root proton efflux and respiration in cowpea rhizosphere, and that these parameters should be measured for more contrasting genotypes in order to test whether they correlate with the adaptation of N_2-dependent legumes to low-P soils.

1. Introduction

Vigna unguiculata (L.) Walp commonly called cowpea is the most widely cultivated seed-legume in tropical savanna of Africa semi-arid and arid areas. It is also cultivated in Asia, America, and the South of Europe (Ehlers and Hall, 1997). According to FAO (2001), 64% of the world production of cowpea is produced in West Africa on approximately 10 million ha, where it is essential to restore soil fertility in the traditional culture systems. However, the production of cowpea in the savanna zones, and sahelian West Africa in particular, is insufficient for the food security (Ehlers and Hall, 1997). The yields are low compared with those recorded in North America, largely because of water deficiency (Sarr et al., 2001) and low-P availability (Summerfield et al., 1974).

Phosphorus is present in small quantities in the lithosphere (0.1%), with two major forms in soils: the organic P form; the inorganic P (Pi) of which the soluble orthophosphates anions, $H_2PO_4^-$ and HPO_4^{2-} are taken up by plants. The concentration of plant-available orthophosphate in soil solution varies between 0.1 and 10 μM as a function of (i) hydrolysis of organic P, (ii) dissolution–precipitation of P-bearing minerals, and (iii) adsorption–desorption of phosphate on soil surfaces, *i.e.* Fe- and Al hydroxylate surfaces at low pH, or Fe- and Al oxides, calcium carbonate, and clay minerals at high pH (Hinsinger, 2001; Mengel and Kirkby, 2001). In common bean, soyabean, lupin and alfalfa, P deficiency has been shown (i) to reduce the number and biomass of nodules as well as their nitrogenase activity (Ribet and Drevon, 1995; Vadez et al., 1996; Qiao et al., 2007), (ii) to increase the absorption surface and density of the roots resulting in more exploration of the soil volume (Vance, 2001), and (iii) to acidify the rhizosphere by root exudates (Neumann and Römheld, 1999) and H^+ efflux (Tang et al., 2001a,b, 2004).

Some cowpea genotypes are tolerant to P deficiency and aluminium toxicity in tropical acid soils (Kolawole et al., 2000; Sanginga, 2003).

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In addition, the best adapted genotypes increased the soil P availability by about 50% after a culture-cycle (Ankomah et al., 1995; Rajput and Singh, 1996). The later was associated with an increase in symbiotic nitrogen fixation (SNF) covering 89% of the plant N requirement (Bado, 2002, unpublished) and an accumulation of 200 kg/ha N in the soil (Sanginga, 2003). However, few publications describe the physiological mechanisms by which cowpea adapts to P deficiency. In particular, the relation between the genotypic variation in P use efficiency (PUE) for the SNF and the H^+ efflux is not well documented. The aim of the present work was to investigate whether the genotypic variability in PUE for SNF among three cowpea genotypes is correlated with two rhizospheric functions of the nodulated roots, namely the proton efflux and the oxygen uptake linked with SNF, measured under P sufficiency and P deficiency at the flowering stage.

2. Materials and methods

2.1. Biological material, soil and culture conditions

From a preliminary test of 6 cowpea (*V. unguiculata* L. Walp.) genotypes (kindly supplied by IITA, Ibadan, Nigeria) among which IT82D-716 and IT86D-715 did not nodulate, we chose Danila as a traditional genotype, and Melakh and 26-73 as being adapted to water deficiency. The seeds were sterilized with 3% calcium hypochlorite for 5–7 min and rinsed by 5 washings with sterile distilled water. They were then transferred for germination on soft agar, consisting of 100 ml Bergersen solution containing 5 g mannitol and 7 g agar in 1 l of distilled water with sterilization at 120 °C for 20 min (Vincent, 1970).

After germination, the inoculation was performed by soaking 4 d-old seedlings for 30 min in a suspension of *Bradyrhizobium* sp. *Vigna* CB756 (kindly supplied by CSIRO, Canberra, Australia) containing 10^9 bacteria ml^{-1}. The inoculum was prepared from rhizobia culture preserved in tubes at 4 °C on the following 120 °C sterilized agar YEM (Yeast Extract Mannitol) medium: 900 ml distilled water; 100 ml of Bergensen concentrated solution (which is prepared with a mixture of 1 g of KCl; 0.1 g of $FeCl_3$; 0.4 g of $CaCl_2$, 4.5 g of $Na_2HPO_4 \cdot 12H_2O$ and 1 g of $MgSO_4 \cdot 7H_2O$, firstly in 100 ml of distilled water then adjusted to 1 l); 1 g Yeast extract, 10 g of mannitol and 15 g of agar (Vincent, 1970). From one of the preserved tubes, some strains are taken and put on 100 ml of liquid YEM (without agar), and maintained at 28 °C for 24 h. Seeds are, then, inoculated by maintaining them into the inoculum for few minutes.

For each P treatment (50, 100, 150, and 250 μmol $plant^{-1}$ $week^{-1}$) 20 inoculated plants were transferred into each 45-l container, 0.2 m large, 0.4 m long and 0.4 m high, for hydroaeroponic pre-culture for 28 d. Based on work of Vadez et al. (1996) P was supplied weekly in the form of KH_2PO_4 to the following nutrient solution that was changed every 2 weeks: $CaCl_2$ (1650 μM); $MgSO_4 \cdot 7H_2O$ (1000 μM); K_2SO_4 (700 μM); Fe EDDHA (8.5 μM Fe as sequestrene); H_3BO_3 (4 μM); $MnSO_4 \cdot H_2O$ (6 μM); $ZnSO_4 \cdot 7H_2O$ (1 μM); $CuSO_4 \cdot 7H_2O$ (1 μM); $Na_2MoO_4 \cdot 7H_2O$ (0.1 μM). The oxygenation of the culture solution was ensured by a permanent flow of 400 ml l^{-1} min^{-1} of compressed air. The pH was adjusted daily to a value of 6.8 with KOH (0.1 M). A supply of urea was provided with 2 mmol $plant^{-1}$ in the initial solution and 1 mmol $plant^{-1}$ at the first change of solution after two weeks, in order to optimize nodulation (Hernández and Drevon, 1991). The plants were then grown in N-free nutrient solution.

The whole experiment was carried out in a glasshouse under temperature conditions of 28/20 °C during 16/8 h day/night cycle with an additional illumination of 400 μmol photons m^{-2} s^{-1} and 70% relative humidity during the day.

2.2. Measurement of proton efflux in rhizotrons

In order to evaluate the influence of the nodulated root on the pH of the soil, 5 plants representing the mean growth in containers of each P treatment, namely P sufficiency and P deficiency, were transferred individually at 28 DAS into the rhizotron shown in Fig. 1. The soil was characterized by high cation exchange capacity (CEC), neutral pH and low content of available P, in spite of its rather large total P content (Table 1). It was sampled at a depth of 5–20 cm in Cazevieille (South of France), and was sieved (<2 mm) after removing stones and plant residues. It is classified as a fersiallitic soil, *i.e.* chromic cambisols according to FAO–UNESCO (1989). A polyamide mesh of 30 μm (Nytrel 0.2SPN, Fyltis-U.G.B., Lyon, France) separated the soil from roots without limiting the exchange of water and chemical with the nodulated roots (Hinsinger and Gilkes, 1997). Each 24 g of soil used in each rhizotron was incubated for 4 d at 20 °C. The rhizotrons were fixed vertically into 5 l buckets, with a filter paper as wick bathing in the previously described nutrient solution.

The initial pH of the soil was measured in an aqueous suspension with a soil/water 1/5 (v/v), after having calculated the water content of each sample. At harvest, a fraction of soil of each replicate was weighed then dried at 105 °C for 24 h to estimate the water content of each soil sample. The H^+ efflux, expressed in μmol $plant^{-1}$ d^{-1}, was calculated as: $QH^+ = (\beta_s \Delta H \mathrm{Ma}) t^{-1}$; where, β_s is buffer capacity of the soil in μmol OH^- g^{-1} soil unit pH; ΔH is difference between the final pH at harvest and initial pH before the culture; Ma is mass of soil used in g; t is the duration of culture in d. The soil buffering capacity was assessed by decreasing or increasing soil pH by 1 unit after addition of a solution of H_2SO_4 or KOH. According to the proton balance, the soil pH depends upon the amount of H^+ added or depleted from soil solution and the intensity of the soil buffer that depends on the contents of clay and organic matter (Conyers et al., 1995).

2.3. Measurements of proton efflux and nodulated-root O_2 uptake in serum bottle

In order to compare the growth of the plants and the H^+ efflux of the nodulated roots in rhizotron and hydroaeroponics, and to assess nodulated-root gas exchanges, more than 5 plants representing the mean growth in containers for each P treatment were transferred individually at 28 DAS in 1 l serum bottles receiving the previously described nutrient solution according to Drevon et al. (1988). To compensate for acidification of the

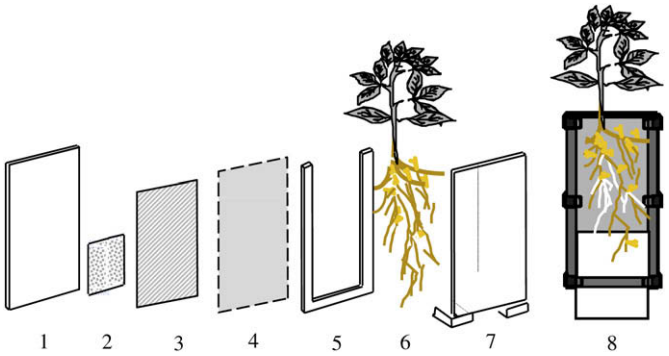

Fig. 1. Rhizotron: (1) plate of glass, (2) filter paper used as a link between the soil and the nutrient solution, (3) layer of soil, (4) polyamide of 30 μm mesh, (5) PVC block of 3 mm thickness, 20 cm length and 1 cm width, (6) cowpea, (7) plate of glass fixed vertically and (8) plant in rhizotron. The soil was calculated on the basis of apparent dry density of the soil. It was distributed homogeneously.