Notas Científicas

Method for evaluation of root hairs of common bean genotypes

Rogério Faria Vieira[1], Celestina Nhagupana Jochua[2] and Jonathan Paul Lynch[2]

[1]Empresa de Pesquisa Agropecuária de Minas Gerais, Caixa Postal 216, CEP 36571-000 Viçosa, MG, Brazil. E-mail: rfvieira@epamig.br
[2]Penn State University, Dept. of Horticulture, 102 Tyson Building, University Park, PA, 16803 USA. E-mail: cnj112@psu.edu, jpl4@psu.edu

Abstract – The objective of this work was to test a simple method for root hair evaluation of 21 common bean (*Phaseolus vulgaris*) genotypes, most of them used in breeding programs in Brazil. Hairs of basal and primary roots of 5-day old seedlings, produced on germination paper with no phosphorus addition, were visually evaluated by a rating scale after staining with 0.05% trypan blue. The method reveals variability among the genotypes, and the standard error of the mean is relatively low.

Index terms: *Phaseolus vulgaris*, rating scale, seedling, cultivar.

Método de avaliação de pêlos radicais de genótipos de feijão

Resumo – O objetivo deste trabalho foi testar um método simples para a avaliação de pêlos radicais de 21 genótipos de feijão (*Phaseolus vulgaris*), a maioria deles usada em programas de melhoramento no Brasil. Os pêlos de raízes basais e primárias de plântulas com idade de cinco dias, produzidas em papel de germinação sem adição de fósforo, foram avaliados visualmente, com o auxílio de uma escala de avaliação, depois de coloridos com "trypan blue" (0,05%). O método diferencia bem os genótipos, e o erro-padrão da média é relativamente pequeno.

Termos para indexação: *Phaseolus vulgaris*, escala de avaliação, plântula, cultivar.

The capacity of plants to absorb both water and mineral nutrients from the soil is related to the development of an extensive and well-positioned root system. Root hairs can contribute up to 67% of the total root surface area (Nielsen et al., 2001). Root hairs are subcellular extensions of the root epidermis that contribute to the acquisition of immobile nutrients such as phosphorus (Jungk, 2001). Root hair production is stimulated by the deficiency of several nutrients, including iron, zinc, manganese, and phosphorus, but, at least in *Arabidopsis*, phosphorus has a greater effect than other nutrients on root hair density and length (Bates & Lynch, 1996; Ma et al., 2001). Phosphorus mobility in soil is governed by diffusion rather than mass flow. Therefore, phosphorus uptake by root is limited by localized phosphorus depletion around the root. Root hairs extend the phosphorus depletion zone from the root epidermis, thereby increasing the rate of phosphorus uptake and the total amount of phosphorus accessible by the roots (Bates & Lynch, 2001). Other important effects of root hairs are increased root exudates such as phosphatases, organic acids and other chelating compounds that may liberate phosphorus from pools like organic phosphate esters, Fe-P, and Al-P compounds that are characteristic of many tropical soils (Marschner et al., 1987).

Common bean exhibits substantial genotypic variation in root hair length and density, ranging from abundant long hairs to virtually hairless (Yan & Lynch, 1998). Common bean genotypes with longer and denser root hairs had more total biomass under low phosphorus availability, compared to those with shorter and less dense root hairs in the same level of phosphorus availability. In addition, genotypes with longer and denser root hairs acquired more phosphorus than their counterparts (Miguel, 2004). Information about root hair production of genotypes used as source of disease resistance, in breeding programs, may be useful to genetically improve common bean root systems, as well.

For determination of root hair length and density, Miguel (2004) stained roots of 14-day old plants that

were observed under microscopy at 40x magnification. Using a comparative picture taken at the same magnification with a micrometer scale, actual root dimensions were determined. Pictures were taken using a digital camera, attached to dissecting scope, and saved using Adobe Photoshop 7.0 software. Then pictures were transferred to Scion Image 1.63 software, for root length determination at that magnification. For root hair density, a known surface area at that magnification was selected, and the number of root hairs in the selected area was determined. From these observations, actual hair length and density were calculated based on the picture magnification, micrometer scale and conversion of the selected root surface area to the unit of root surface area in square millimeters. In this way, root hair length (mm) and root hair density (number of root hair per mm^2 of root surface area) were determined for basal and lateral roots. This method is very laborious in a breeding program, in which a large number of genotypes need to be evaluated.

The objective of this research was to test a simpler screening method for root hair evaluation of common bean genotypes.

Twenty-one genotypes were evaluated in relation to root hair density and length. Seeds of 18 common bean genotypes were used. Eight of them are high yielding cultivars or lines (Ouro Negro, Diamante Negro, Valente, Talismã, Jalo MG-65, Carnaval MG, Vi 4899, and Vi 10-2-1), and ten are genotypes used for disease resistance in breeding programs (AB 136, Cornell 49-242, G 2333, Kaboon, México 54, México 309, Pi 207262, TO, TU, VC-4). The line Vi 4899 was released in 2005 as a cultivar (Pioneiro). Seeds of all these genotypes were harvested from the same place and with the same fertilization in Brazil. The genotypes DOR 364, G 19833, and Carioca were obtained from the International Center for Tropical Agriculture (CIAT). DOR 364 is known as inefficient under P-deficient conditions. Carioca is a Brazilian landrace, which is among the most widely grown tropical genotypes, perhaps because of its tolerance to infertile soils. G19833 is a landrace from Peru, which is well adapted to P-limited conditions. Jalo MG-65, Carnaval MG, G 19833, Kaboon, and México 54 belong to the Andean gene pool while the remaining genotypes belong to the Mesoamerican gene pool.

Seeds were surface-sterilized with 0.5% NaOCl for one min, rinsed thoroughly with distilled water and scarified with a razor blade. They were placed 2 cm from the top of a brown germination paper (Zhu et al., 2005), soaked in 0.5 mM $CaSO_4$, and with radicles pointing downwards. The paper was then rolled into a moderately tight cigar roll configuration, and placed in a 1 L beaker with 100 mL of 0.5 mM $CaSO_4$ at the bottom. Beakers were wrapped with cellophane plastic punctured with holes before being placed in a germination chamber at 28°C. Five days later, shoots of seedlings were eliminated, and roots of each genotype were separated in basal and primary roots. They were conserved in 25% v/v ethanol immediately after harvest. Root hairs were visually evaluated, after being stained with 0.05% trypan blue using a rating scale of 1–9 to rank the density and length, as follows: 1, no root hairs; 3, between 1 and 5 rating scale; 5, intermediate root hair density and length, as recombinant inbred lines (RILs) 28 and 32 of DOR 364 x G 19833; 7, between 5 and 9 rating scale; 9, abundant root hairs, such as RILs 13 and 53 of DOR 364 x G 19833. These RILs were selected from the research of Miguel (2004). Data were analyzed as a completely randomized design (CRD), with each treatment replicated four times. Pearson's correlation was calculated between the rating scales of root hairs, in basal roots and those in primary roots.

A preliminary test was performed comparing root hairs on 5-day old plants (new method) with those on older plants used by Miguel (2004). Six contrasting RILs (13, 28, 32, 53, 80, and 84) of DOR 364 x G 19833 for root hairs were used. Results obtained with the new method matched those achieved by Miguel (2004).

The method worked well with relatively low standard error of the mean (SE) for each genotype (Figures 1 and 2). In general, SE was lower for basal roots than for primary roots. The correlation between root hairs in basal root and root hairs in primary root was significant (r = 0.54, p = 0.0057). One important advantage of this simple method, compared to the laborious one used by Miguel (2004) is the visualization of the entire root system. The fact that density and length were considered together, in our evaluation, is pertinent, since there is a positive correlation between root hair length and density, when plants grow under low phosphorus availability (Miguel, 2004). Evaluations were made without phosphorus addition, in view of the results of Miguel (2004) that root hair length increased from 0.35 mm, in high phosphorus treatments, to 0.85 mm in low phosphorus at 35 DAP (days after planting), and that genotypes under high phosphorus availability did not differ in root hair length.

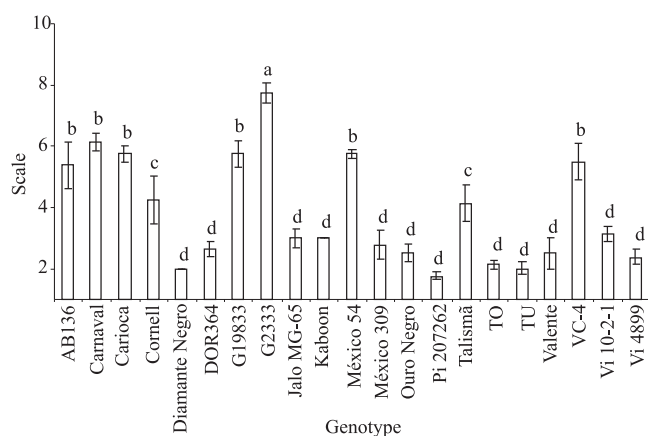The genotypes G19833, México 54, VC-4, and especially G 2333, had strong root hairs in both classes of roots (Figures 1 and 2). The genotypes AB 136, Carnaval MG, and Carioca had strong root hairs in basal root (Figure 1), but weak in primary root (Figure 2). The genotypes Jalo MG-65 and Talismã had weak root hairs in basal root, but strong in primary root. The genotypes Diamante Negro, DOR 364, Kaboon, México 309, Ouro Negro, PI 207262, TO, TU, Valente, Vi 10-2-1, and Vi 4899 had weak root hairs in both classes. According to Miguel (2004), the greater root surface area the more contact with the soil, and long root hairs make this contact to take place beyond the depletion zone, which otherwise is out of reach by roots alone. Moreover, root hairs are responsible for secretion of a variety of compounds (exudates) into rhizosphere. The greater root hair density and length of G 19833 (P efficient) compared to DOR 364 (P inefficient) was also found by Yan et al. (2004).

Figure 1. Root hairs in basal roots, 5 days after germination of 21 genotypes of common bean. Data are means±SE (n = 4). Columns with equal letters belong to homogenous groups by Scott-Knott test, at 5% of probability. Scale: 1, no root hairs; 3, between 1 and 5 rating scale; 5, intermediate root hair density and length, as RILs 28 and 32 of DOR 364 x G 19833; 7, between 5 and 9 rating scale; 9, abundant root hairs, such as RILs 13 and 53 of DOR 364 x G 19833.
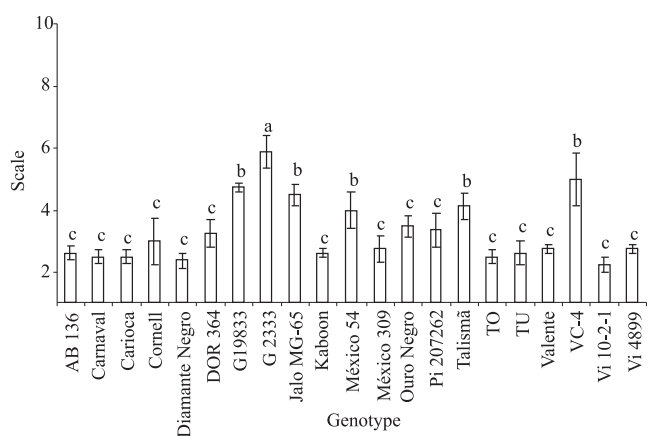
Acknowledgements

References

BATES, T.R.; LYNCH, J.P. Root hairs confer a competitive advantage under low phosphorus availability. **Plant and Soil**, v.236, p.243-250, 2001.

BATES, T.R.; LYNCH, J.P. Stimulation of root hair elongation in *Arabidopsis thaliana* by low phosphorus availability. **Plant, Cell and Environment**, v.19, p.529-538, 1996.

JUNGK, A. Root hairs and the acquisition of plant nutrients from soil. **Journal of Plant Nutrition and Soil Science**, v.164, p.121-129, 2001.

MA, Z.; BIELENBERG, D.G.; BROWN, K.M.; LYNCH, J.P. Regulation of root hair density by phosphorus availability in *Arabidopsis thaliana*. **Plant, Cell and Environment**, v.24, p.459-467, 2001.

MARSCHNER, H.; ROMHELD, V.; CAKMAK, I. Nutrient availability in the rhizosphere. **Journal of Plant Nutrition**, v.10, p.1174-1184, 1987.

MIGUEL, M.A. **Genotypic variation in root hairs and phosphorus efficiency in common bean (*Phaseolus vulgaris* L.)**. 2004. 106p. Thesis (M.Sc.) - Penn State University, University Park.

NIELSEN, K.L.; ESHEL, A.; LYNCH, J.P. The effect of phosphorus availability on the carbon economy of contrasting common bean (*Phaseolus vulgaris* L.) genotypes. **Journal for Experimental Biology**, v.52, p.329-339, 2001.

Figure 2. Root hairs in primary roots, 5 days after germination of 21 genotypes of common bean. Data are means±SE (n = 4). Columns with equal letters belong to homogenous groups by Scott-Knott test, at 5% of probability. Scale: 1, no root hairs; 3, between 1 and 5 rating scale; 5, intermediate root hair density and length, as RILs 28 and 32 of DOR 364 x G 19833; 7, between 5 and 9 rating scale; 9, abundant root hairs, such as RILs 13 and 53 of DOR 364 x G 19833.

YAN, X.; LIAO, H.; BEEBE, S.E.; BLAIR, M.W.; LYNCH, J.P. QTL mapping of root hair and acid exudation traits and their relationship to phosphorus uptake in common bean. **Plant and Soil**, v.265, p.17-29, 2004.

YAN, X.; LYNCH, J.P. Genetic variation for root hair density and length in the common bean in response to low phosphorus availability.

In: LYNCH, J.P.; DEIKMAN, J. (Ed.). **Phosphorus in plant biology**: regulatory roles in ecosystem, organismic, cellular, and molecular processes. Rockville: American Society of Plant Physiologists, 1998. p.332-334.

ZHU, J.; KAEPPLER, S.M.; LYNCH, J.P. Mapping of QTL controlling root hair length in maize (*Zea mays* L.) under phosphorus deficiency. **Plant and Soil**, v.270, p.299-310, 2005.