DIVISÃO 1 - SOLO NO ESPAÇO E NO TEMPO

Comissão 1.3 - Pedometria

PUBLIC-DOMAIN SOFTWARE FOR ROOT IMAGE ANALYSIS[(1)]

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SUMMARY

In the search for high efficiency in root studies, computational systems have been developed to analyze digital images. ImageJ and Safira are public-domain systems that may be used for image analysis of washed roots. However, differences in root properties measured using ImageJ and Safira are supposed. This study compared values of root length and surface area obtained with public-domain systems with values obtained by a reference method. Root samples were collected in a banana plantation in an area of a shallower Typic Carbonatic Haplic Cambisol (CXk), and an area of a deeper Typic Haplic Ta Eutrophic Cambisol (CXve), at six depths in five replications. Root images were digitized and the systems ImageJ and Safira used to determine root length and surface area. The line-intersect method modified by Tennant was used as reference; values of root length and surface area measured with the different systems were analyzed by Pearson's correlation coefficient and compared by the confidence interval and t-test. Both systems ImageJ and Safira had positive correlation coefficients with the reference method for root length and surface area data in CXk and CXve. The correlation coefficient ranged from 0.54 to 0.80, with lowest value observed for ImageJ in the measurement of surface area of roots sampled in CXve. The IC (95 %) revealed that root length measurements with Safira did not differ from that with the reference method in CXk (-77.3 to 244.0 mm). Regarding surface area measurements, Safira did not differ from the reference method for samples collected in CXk (-530.6 to 565.8 mm^2) as well as in CXve (-4231 to 612.1 mm^2). However, measurements with ImageJ were different from those obtained by the reference method, underestimating length and surface area in samples collected in CXk and CXve. Both ImageJ and Safira allow an identification of increases or decreases in root length and surface area. However, Safira results for root length and surface area are closer to the results obtained with the reference method.

Index terms: root length, root surface area, image processing, root system.

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(1) Research undergraduate of the second author. Funded by Fundação Cearense Apoio ao Desenvolvimento Científico e Tecnológico - Funcap. Received for publication on July 16, 2013 and approved on July 3, 2014.

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RESUMO: *SISTEMAS COMPUTACIONAIS DE DOMÍNIO PÚBLICO PARA ANÁLISE DE IMAGEM DE RAÍZES*

Procurando maior eficiência no estudo de raízes, sistemas computacionais têm sido desenvolvidos para analisar imagens digitais. O ImageJ e o Safira são sistemas de domínio público que podem ser utilizados na análise de raízes lavadas. Porém, supõe-se que há diferenças nos atributos radiculares determinados por meio do ImageJ e Safira. Este estudo foi realizado para comparar valores de comprimento e área superficial de raízes, obtidos com os dois sistemas, com os valores alcançados por meio do método de referência. Amostras de raízes foram coletadas em bananal em áreas de Cambissolo Háplico carbonático típico (CXk), mais raso, e de Cambissolo Háplico Ta eutrófico típico (CXve), mais profundo, em seis camadas e cinco repetições. As raízes foram escaneadas e os sistemas ImageJ e Safira foram utilizados para determinar comprimento e área superficial radicular. Utilizando o método da interseção modificado por Tennant como referência, valores de comprimento e área obtidos pelos sistemas foram analisados a partir do coeficiente de correlação de Pearson e comparados pelo intervalo de confiança e pelo teste t. Ambos os sistemas apresentaram coeficiente de correlação positivo com o método de referência para medidas de comprimento e área superficial em CXk e CXve. O coeficiente de correlação variou de 0,54 a 0,80, com menor valor observado para o ImageJ na determinação da área superficial de amostras coletadas no CXve. O IC (95 %) revelou que as medidas de comprimento radicular com o Safira não diferiram do método de referência no CXk (-77,3 a 244,0 mm). Quanto à área superficial, o Safira não diferiu do método de referência, tanto para amostras coletadas em CXk (-530,6 a 565,8 mm^2) como nas provenientes de CXve (-423,1 a 612,1 mm^2). Já as medidas feitas com o ImageJ diferiram do método de referência, com valores subestimados de comprimento e área superficial radicular em amostras coletadas em CXk e CXve. Os dois sistemas permitem identificar aumento ou diminuição no comprimento e na área superficial de raízes lavadas. Porém, o Safira resulta em valores de comprimento e de área superficial radicular mais próximos aos obtidos com o método de referência.

Termos de indexação: comprimento radicular, área superficial radicular, processamento de imagens, sistema radicular.

INTRODUCTION

The relevance of the root system in food production has been neglected (Herder et al., 2010). However, the evaluation of root system architecture and development is important to increase the understanding of how soils influence plant development and, consequently, food production. Scurlock & Olson (2001) point out that roots are essential components of ecosystems, since their net primary productivity (NPP) corresponds to 40 to 85 % of the total NPP.

The main reason why plant roots have been little studied is related to the methodological and analytical problems. Thus, one of the current challenges for root studies is to improve techniques and methods in the steps of sampling and property quantification (Blouin et al., 2007). Samples can be obtained from soil profiles (Böhm, 1979), monoliths (Noordwijk & Flores, 1979), nail plates, probes (Jorge & Crestana, 1996), and rhizotron (Böhm, 1979). After sampling, the next step is the quantification of root properties, which in some studies, e.g. Otto et al. (2009), is limited to root dry matter. In other studies (Imhoff et al., 2010; Mei et al., 2011; Grzesiak et al., 2013), roots were analyzed for a higher number of properties, i.e., root length, surface area, volume, diameter classes, and structure.

Root length is an important parameter to evaluate root functions and the influence of soil on them (Gaiser et al., 2013). However, to date no method for a fast and precise measurement of root length was fully established (Kimura et al., 1999). Most methods nowadays are based on the intersection principle (Newman, 1966). This principle is based on the relation between root length and the number of random intersections between lines of an underlying grid and the roots spread on a given surface. This relation was adapted for the use of a line grid, thus creating the modified line-intersect method (Tennant, 1975).

Aside from root length, root surface area is a parameter that also helps understand the soil-plant relation (Grant et al., 2012). This measurement is associated with the root diameter; thinner roots contribute to higher surface area values and form the exchange site between plant and soil for water and nutrient absorption (Waisel & Eshel, 2002). Its determination is important because some studies reported changes in root surface area related to nutrient availability in soil (Desnos, 2008; Pang et al., 2010; Mei et al., 2011). The root surface area can be calculated from data of root length and volume. The former are determined by the line-intersect method (Newman, 1966), modified by Tennant (1975). The latter can be determined based on the water displacement caused by the sample in a graduated cylinder.

The methods mentioned so far are useful to measure root properties; however, depending on the sample size and number, they can be time-consuming. To improve the efficiency of root analysis, techniques using computer programs have been developed to determine root properties from digitized images (Murphy & Smucker, 1995; Bauhus & Messier, 1999; Vamerali et al., 2003). Different systems that can be used in root image analysis have been developed outside Brazil, e.g. WinRHIZO (Arsenault et al., 1995; Wang & Zhang, 2009), Delta-T (Delta-T Devices, 2011), and DART (Lê Bot et al., 2010).

The use of many computer programs for root image analysis requires a license, which represents costs for researchers developing studies on plant root systems. However, there are some public-domain programs, which facilitate the access of users. ImageJ is an open-source, image-processing program written in Java, inspired by the NIH-image for Macintosh and developed at the National Institute of Mental Health, USA (Bailer, 2006). It has already been used for image analysis in medical (Karmonik et al., 2010) and agronomic (Perea-Flores et al., 2011) studies, with potential for use in studies on plant root systems.

The Brazilian Enterprise for Agricultural Research (Embrapa) has developed national options for image analysis, such as SIARCS (Jorge & Crestana, 1996) and Safira (Jorge & Oliveira Rodrigues, 2008). The latter is more recent than the former and, since it is open source, it can be amplified for studies on plant root systems.

Capturing images for analysis in ImageJ involves the use of scanners, cameras or video sources (Abràmoff et al., 2004; Ferreira & Rasband, 2012). In Safira, analyses can also be performed with scanned images or even digital photographs (Jorge & Silva, 2010). During the determination of image properties, ImageJ allows image treatment, followed by the process of binarization and scale setting (Ferreira & Rasband, 2012). Safira also allows image pretreatment, followed by scale setting and binarization (Jorge & Silva, 2010).

The overlapping of roots is an important source of error in image capture, which can lead to underestimated measurements (Bauhus & Messier, 1999). To date, no efficient procedure is available to overcome this problem of overlapping in the most commonly used programs for root analysis (Wang & Zhang, 2009). Thus, it is supposed that public-domain programs such as ImageJ and Safire generate different results for the studied root properties. This study aimed to compare the determination of root length and surface area by ImageJ and Safira, using the line-intersect method modified by Tennant as reference.

MATERIAL AND METHODS

Root samples were collected in a field experiment on a 24-month-old banana plantation under fertigation, located on the Apodi Plateau, in the municipality of Limoeiro do Norte, CE (5º 20' S; 38º 5' W). The climate in the region is classified as BSw'h', according to Köppen's classification, characterized as very hot and semiarid. The average annual rainfall is approximately 750 mm (DNOCS, 2009) and the natural vegetation is hyperxerophytic Caatinga (Brasil, 1973).

There were differences in the soil depth of the sampling areas associated with the microrelief. The soils sampled for this study were classified, according to SiBCS (Embrapa, 2013), as Typic Carbonatic Haplic Cambisol (CXk) and Typic Haplic Ta Eutrophic Cambisol (CXve) (Oliveira, 2012).

Soil samples with roots were obtained using a probe with internal diameter of 4.5 cm and length of 100 cm. Samplings were performed in six soil layers (0-10; 10-20; 20-30; 30-40; 40-50; and 50-60 cm) in five replications, totaling 30 samples per area. The area under the canopy of the banana trees was sampled.

The material sampled in the field was placed in plastic bags and taken to the laboratory, where roots were separated from soil. The roots were placed in plastic containers with 70 % alcohol, to maintain the sample integrity until image digitization and analysis. For each sample, one root image was obtained using a common personal computer scanner (optical resolution: 1200 × 1200 dpi). The images were prepared by spreading the roots in a transparent glass tray (25 × 35 cm) with a 3-mm water layer. Each procedure generated grayscale digital images with 672 × 1168 pixels, requiring a 40-Kb memory area. Root length and surface area were determined by the programs Safira and ImageJ. For both, the images were linearized to bring the roots to the foreground.

As a reference, the modified line-intersect method (Tennant, 1975) was used to determine root length and surface area. To this end, the following material was used: transparent glass tray, a square sheet of paper (0.5 × 0.5 cm), magnifying glass and a manual counter device. Root samples were spread in the glass tray, also containing a 3-mm water layer. The square sheet of paper was fixed at the bottom of the tray and then the intercepts between roots and the vertical and horizontal lines were counted. Only roots crossing the lines and root tips touching the lines were taken into account.

Intercepts counting was converted to root length using equation 1, according to Tennant (1975):

$$L\ (cm) = 11/14 \times NI \times SU \qquad (1)$$

where L = length; NI = number of intercepts; and SU = squared unity.

Equations 2 and 3 were used to calculate root surface area, average diameter and volume:

$$A = 2\pi RL \quad (2)$$

where A = root surface area (mm^2); R = root average radius (mm); and L = root length (mm).

$$V = (\pi R^2) \times h \quad (3)$$

where V = root volume (mm^3), measured based on the water displacement caused by root immersion in a graduated cylinder; R = root average radius (mm); and h = root length (mm).

Root length and area data obtained by the different methods were analyzed using Pearson's correlation coefficient, compared with a confidence interval of 95 % (Miller & Miller, 1984) and in pairs by the t-test. Statistical analyses were performed using software Genstat (version 11.1 VSN International Ltd).

RESULTS AND DISCUSSION

The statistical parameters used to evaluate the programs are shown in table 1. According to the criterion of Cohen (1988), considering Pearson's correlation coefficient (r) for root length data, high positive correlations between the reference method (intersection) and ImageJ (r = 0.80) and Safira (r = 0.72) for CXk were calculated. For CXve, high positive correlations were also found between the line-intersect method and ImageJ (r = 0.71) and Safira (r = 0.67).

For root surface area data, correlations in CXk between the line-intersect method and ImageJ (r = 0.80) and Safira (r = 0.75) were high and positive. In CXve, a moderate positive correlation (r = 0.54) was found when the line-intersect method was compared with ImageJ and a high positive correlation (r = 0.80) was found when compared with Safira.

High values of Pearson's correlation coefficient indicate proportionality between the measurements with the two image-analysis programs (ImageJ and Safira) and the reference method (intersect). If these values are positive, it can be concluded that, when the reference method shows increases in root length and surface area values, the tested programs (ImageJ and Safira) also do. In general, the programs developed to facilitate root studies by image analysis are positively correlated with the conventional method. Murphy & Smucker (1995) verified that the root length of alfalfa plants determined by image analysis and the line-intersect method were positively correlated with the mass of the analyzed roots. Wang & Zhang (2009), comparing the image-analysis program WinRHIZO with the Tennant method, verified that the morphological parameters of wheat roots had positive, yet significantly different correlation.

The degree of association between root properties determined with the image-analysis programs and the reference method seems to depend on characteristics of the sampling sites, which in turn influence the amount of roots per sample. Butnor et al. (2001), testing GPR (Ground-Pentrating Radar) to estimate root properties, also confirmed that the success of the method depended on the soil characteristics.

Of the soils sampled in this study, the CXve had a thicker solum than CXk (Oliveira, 2012). This greater thickness represents better conditions for root development, resulting in a greater amount of roots in the analyzed samples. It is supposed that root overlap in the CXve samples favored the lower correlation coefficients. This effect in the root surface area determined by ImageJ seemed to be more pronounced, because the correlation coefficient of this parameter was the lowest.

The confidence interval - CI (95 %) representing the "a" coefficient of the adjusted regression line is shown in table 1. Analyzing the root length data in the CXk, Safira did not differ from the reference method, because the CI included 0 (zero) values in the comparison between the methods (-77.3 to

Table 1. Statistical parameters used in the comparison of root length and surface area determined by different image-analysis programs

Method	CI(1)	SD(2)	"t"(3)	"p"(4)	r(5)
			Root Length - CXk		
Intersection × ImageJ	247.6 to 618.0 mm	97.0	4.78	<0.001	0.80
Intersection × Safira	-77.3 to 244.0 mm	345.0	1.06	0.297	0.72
			Root Length - Cxve		
Intersection × ImageJ	256.4 to 437.0 mm	80.6	7.85	<0.001	0.71
Intersection × Safira	-385.2 to -25.13 mm	476.0	-2.33	0.027	0.67
			Root surface area - CXk		
Intersection × ImageJ	1395 to 2779 mm^2	191	6.17	<0.001	0.80
Intersection × Safira	-530.6 to 565.8 mm^2	1378	0.95	0.950	0.75
			Root surface area - CXve		
Intersection × ImageJ	2001 to 3341 mm^2	429	8.15	<0.001	0.54
Intersection × Safira	-423.1 to 612.1 mm^2	1396	0.37	0.710	0.80

(1) CI: Confidence Interval (95 %); (2) SD: Standard Deviation; (3) t-test value; (4) p-value; (5) r = correlation coefficient.

244.0 mm). On the other hand, the root length determined by ImageJ differed from that of the line-intersect method ($p<0.001$). In the CXve, root length data by Safira ($p=0.027$) and ImageJ ($p<0.001$) differed from that of the reference method.

Since Safira did not differ from the reference method in root length in CXk, it is likely that this image-analysis program quantifies root length with values closer to the real ones. However, the greater amount of roots found in the CXve may have led to root overlap and higher measurement error as well, resulting in values different from those of the reference method. In a study on image-analysis programs using plant species that produce different amounts of thin roots, the root length and error of the species with highest root production were also higher (Murphy & Smucker, 1995).

Analyzing the CI (95 %) for measurements of root surface area, Safira did not differ from the reference method, for both CXk and CXve, because the CI included the 0 (zero) value in the comparison between the methods (-530.6 to 565.8 mm^2 and -423.1 to 612.1 mm^2). On the other hand, the area determined by ImageJ differed from that of the line-intersect method ($p<0.001$), for both CXk and CXve. Since the results of Safira did not differ from those of the reference method for CXk and CXve, the values of root surface area of this image-analysis program are more reliable.

Evaluating the variation of root properties determined by the two image-analysis programs, it was found that both ImageJ and Safira resulted in low determination coefficients (R^2) for length measurements in CXk and CXve samples (Figure 1). However, the coefficients obtained by ImageJ were higher ($R^2 = 0.64$ and 0.50) than by Safira ($R^2 = 0.51$ and 0.45), indicating that ImageJ explains root length better than Safira.

In terms of root surface area (Figure 2), both ImageJ and Safira resulted in low determination coefficients (R^2) for CXk and CXve samples. However, the coefficient of ImageJ for the CXk ($R^2 = 0.64$) was higher than that of Safira ($R^2 = 0.55$). This indicates that for a smaller root amount, ImageJ explains the determination of root surface area better than Safira. On the other hand, for CXve, the coefficient established by ImageJ ($R^2 = 0.28$) was lower than that by Safira ($R^2 = 0.63$), indicating that the latter explains the root surface area measurement better, especially when the sample has a greater root amount. In CXve, because of the greater root amount and, as a consequence, higher root overlap, the error was higher in the determination of this root property. However, this error was higher for ImageJ.

It was also found that root length (Figure 1) and surface area (Figure 2) values estimated by ImageJ were lower than those by Safira. Comparing the image-analysis programs with the line-intersect method, other authors reported a trend to higher root length for the latter (Bauhus & Messier, 1999; Wang & Zhang, 2009). On the other hand, root surface area varied less when estimated by the line-intersect method and image-analysis program (Wang & Zhang, 2009).

One of the factors leading to the underestimation of root properties is the quality of the analyzed images. The scanner resolution used to digitize images of washed roots must be sufficient to capture the images of the thinnest roots (Vamerali et al., 2003). Using two commercial programs to analyze the images of washed roots (Delta-T and WinRhizo), Bouma et al. (2000) found that the choice of the program is less important than the image-acquisition protocol in terms of staining period, sample density on the glass tray and image thresholding. Kimura et al. (1999) mentioned that root length determined by image analysis can be underestimated as sample size increases, since root overlap also increases. For Vamerali et al. (2003), impurities (crop residues) in the samples can overlay roots, also leading to underestimations in root property measurements.

To generate root length data closer to the real with ImageJ, methods to correct the effects of root overlap and impurities in the image need to be used. The alternative presented by Valmerali et al. (2003) is the use of a thicker water layer in the tray where roots are placed for image capture. In this thicker water, roots spread better, avoiding their overlapping with organic residues. For samples with large root amounts, subdividing the material for analysis can also contribute to minimizing the overlapping, but it makes the analytical process time-consuming. Costa et al. (2000) developed a system for the collection of homogeneous root subsamples and verified that approximately 10 % of the total root volume is enough for an accurate estimation of root length through image-analysis programs.

The lowest values of root surface area obtained with ImageJ must be related to the higher operational difficulty. To analyze surface area using ImageJ, it is necessary to outline each root on the image, which requires visual skills and motor coordination of the operator. Failures in the procedure of outlining roots cause underestimation of surface area. In other agronomic studies, such as that of Perea-Flores et al. (2011), in which ImageJ was used to estimate the surface area of round-shaped seeds, image outlining seems to be easier.

According to Kimura et al. (1999), an ideal image-analysis system should generate results without systematic errors, be valid for samples of different sizes, be adjustable for root overlap in the samples and insensitive to the preferential orientation of samples. In this sense, both systems evaluated in this study have limitations. However, the restrictions of ImageJ were greater than of Safira, especially regarding the root surface area measurements.

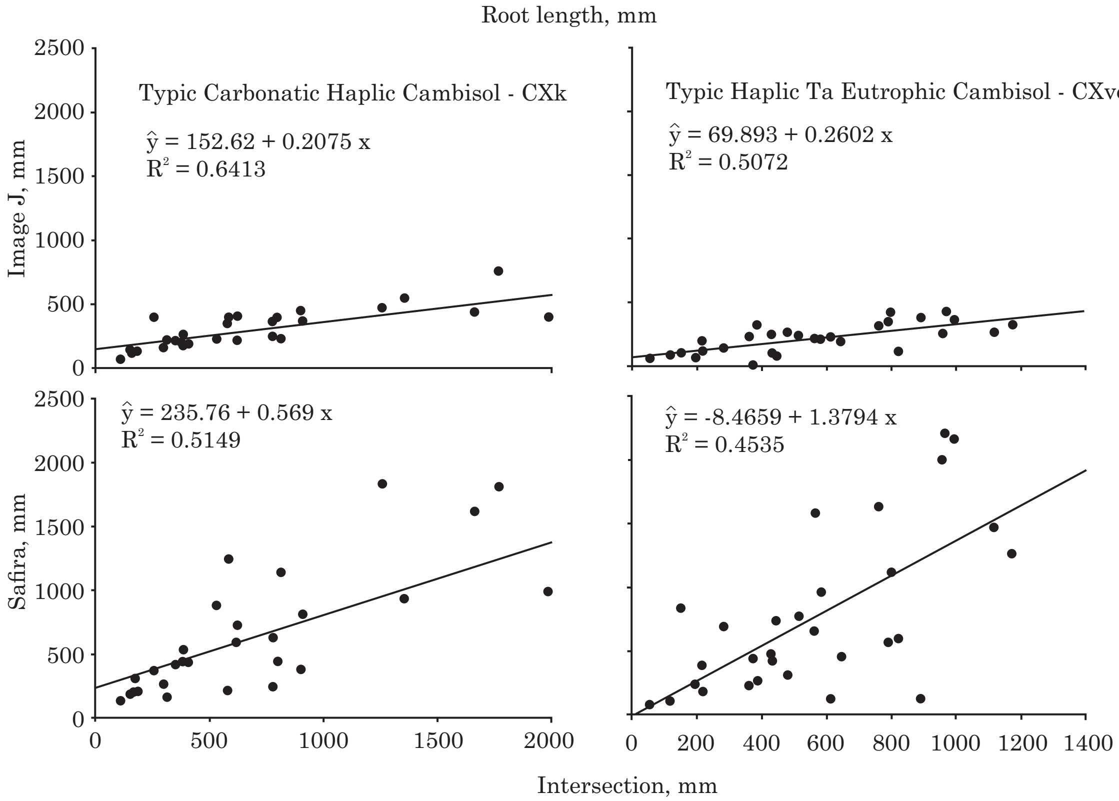

Figure 1. Relation between root length data of the line-intersect method and the programs Safira and ImageJ for the collected samples.

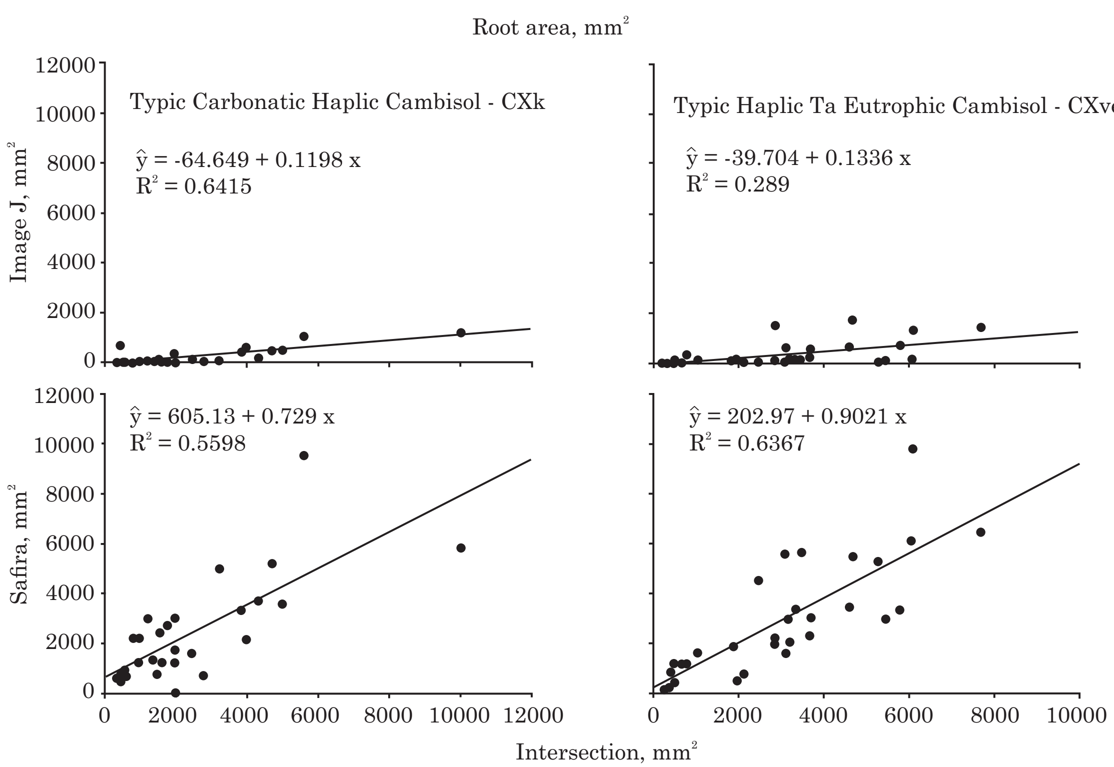

Figure 2. Relation between root area data obtained by the line-intersect method and the programs Safira and ImageJ in the collected samples.

Another important aspect is that Safira, as other systems developed for root studies (Arsenault et al., 1995), allows information detailing, quantifying root diameter classes of each sample analyzed.

CONCLUSIONS

1. Both ImageJ and Safira can identify increases or decreases in length and surface area of washed roots.

2. Safira generates root length data that do not differ from those of the reference method, especially in samples with smaller amounts of roots.

3. Safira generates root surface area values closer to those by the reference method, for both small and large samples.

ACKNOWLEDGEMENTS

We thank the Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (Funcap), for the scientific initiation scholarship of the second author; the Northeastern Bank of Brazil (BNB), for funding part of this study; and the agricultural company Agrícola Famosa, for granting access to the area for sampling.

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