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A computational method to assist evaluation of root length of soybean based on digital image processing

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Abstract

The evaluation of the root system is important for better understanding the effects of nutrient management on soil and plant nutrition. However, root system studies and culture are slow and show low accuracy. In this context, digital image processing may be an alternative. The objective of this research was to develop a computational method to assist evaluation of the soybean root growth. Initially, the free and open access software, available at: http://rm.deinfo.uepg.br/, was developed in Java platform with the OpenCV library supply through the plug-in JavaCV. To evaluate the software, copper wires with 10 mm, 20 mm, and 50 mm of length manually measured using callipers. They were scanned with a resolution of 300 dpi and then images were loaded in the software. Variation coefficients between 0.01 and 2.99 % were obtained. Subsequently, the samples of soybean roots were scanned and the results of developed software and Safira software were correlated with those from the line-intersect method. The determination coefficients (R^2 = 0.999) of the developed software, on average, were better than those obtained with Safira software (R^2 = 0.733), when comparing with the line-intersect method. Therefore, the proposed method was accurate for length measurements of soybean roots.

Keywords: *Glycine max L.* (Merr.), image analysis, root evaluation, soil-plant relationship, software.
Abbreviations: DIP_ digital image processing, RGB_red, green, blue, JPEG_ Joint Photographic Experts Group, JDK_Java Development Kit, JNI_Java Native Interface, OpenCV_Open Source Computer Vision Library, CV_coefficients of variation, R^2_determination coefficient.

Introduction

The evaluation of plant root systems is important to understand the effects of soil nutrient management and plant nutrition (Name et al., 2016). However, this evaluation is complex, tiring and encourages sampling and measurement error (Böhm, 1979). The most important parameter to evaluate the root functions is the root length (Gaiser et al., 2013). One of the classic ways to measure root length is the line-intersect method (Newman, 1966). This method is based on mathematical relationship between the length of root segments and the number of intersections in randomly oriented straight lines. Tennant (1975) have adapted this relationship to quantify root length using a grid system, and the changes have been considered as the main method to study root length (Bouma et al., 2000). It has been used in recent studies (Basirat et al., 2011). The Tennant method is useful for measuring root properties; however, if a sample has a large size, the measurement can take a long time.

In order to improve the efficiency of root analysis, the computer programs using digital image processing (DIP) were developed to determine the root properties of the scanned images (Lebowitz, 1988; Bauhus and Messier, 1999; Kimura et al., 1999). Kimura et al., (1999) developed a thinning algorithm that calculates the root length of rice roots (*Oryza sativa* L.) by counting the number of pairs of orthogonally and diagonally connected pixels. Automated systems that calculate root length from images are the commercial software Delta-T (Delta-T Devices, 2018) and WinRHIZO™ (Arsenault et al., 1995; Wang and Zhang, 2009).
Public-domain software based on image processing has been developed and used for research, such as the Rootedge (Kaspar and Ewing, 1997). Himmelbauer et al. (2004) used this tool to estimate the root length of wheat (*Triticum durum* Desf.) and barley (*Hordeum vulgare* L.) crops and compared the obtained values with WinRHIZO™, obtaining low CV's in both

programs (0.3 % to 3.4 %). In this context, two other software that estimate root length are ImageJ (ImageJ, 2018) and IJ_Rhizo (Pierret et al., 2013). The ImageJ is a public-domain software and was developed in Java, a programming language that allows the program code to run in a cross-platform computing environment. In agronomic studies, ImageJ has been used in comparison with WinRHIZO™ for root length estimation (Tajima and Kato, 2011, 2013) and used to analyze the distribution characteristics of soil pore (Wang et al., 2017). From images of root samples, Pierret et al. (2013) compared the performance of IJ_Rhizo and WinRHIZO™ using correlation analysis and verified that the estimated lengths were linearly correlated.

In Brazil, Embrapa (Brazilian Enterprise for Agricultural Research) also developed computational systems to assist in the evaluation of root systems: SIARCS® (Jorge and Crestana, 1996) and Safira (Safira, 2018), developed in the Java language. Costa et al. (2014) used samples of roots of banana to compare the root lengths achieved by ImageJ, Safira and the reference method (Tennant). The determination coefficient between the reference method and each software was calculated resulting in R^2 = 0.514 and 0.453, using ImageJ and Safira, respectively. Mattioni et al. (2017) also used Safira to evaluate the canola root system (*Brassica napus* L.), comparing the root growth and the productivity components of hybrid grains of conventional and herbicide tolerant canola in different localities.

The cost of software licenses for root image processing may be high for researchers developing studies on plant root systems. Furthermore, the public-domain software measures the root length can generate different results for root length. This study presents a computational method developed in open source software to evaluate the length of the root samples, comparing the soybean root lengths obtained with the proposed method, using the line-intersect method modified by Tennant as reference and with the software computational system Safira.

Results and discussion

Application of software in image samples

The Figs. 1a and 1b show the scanned copper wire images with 300 dpi. This image corresponds to copper wire of 10 mm length and 0.12 mm diameter, respectively, before and after the use of a manual threshold value.

The Fig. 2a show the scanned soybean root sample image and, after application of the threshold value, results in Fig. 2b. The threshold value for estimated root length can vary widely and, because of this, most image analysis software uses manual threshold (Kaspar and Ewing, 1997; Kimura et al., 1999; Kano et al., 2011; Costa et al., 2014). However, it was difficult to determine the proper threshold value manually, because of the continuous change in estimates of root length with changing threshold values (Tajima and Kato, 2011).

The root sample images from the 10-20 cm soil layer, before and after the use of the threshold value are shown in Figs. 2a and 2b, respectively. The Fig. 2c shows the result of the multiscale thinning algorithm “skeletonization” applied to Fig. 2b. We chose the background as black and the roots as white for visual aid. As expected, the thinning algorithm for the roots shown in Fig. 2b often displays the medial axis of the roots (Luppe et al., 2003), which is equivalent to the root length (Lebowitz, 1988; Kimura et al., 1999).

Validation and test with copper wires

The use of objects that simulate roots to evaluate methods by image analysis are common, such as hair (Newman, 1966) and wire (Bauhus and Messier, 1999; Kimura et al., 1999; Kimura and Yamasaki, 2001, 2003; Zobel, 2008). For evaluating the proposed method, we analyzed wires with three different lengths 10, 20 and 50 mm. The method was tested against rotation dependence and possible position of each objects as practiced in the literature (Zobel, 2008). Estimated length values for 10 mm, 20 mm and 50 mm, and also the average length values, were obtained for each of reoriented images between 0º and 345º at intervals of 15º (Table 1). We can observe that some angles of rotation affected the results.

In the estimation of 10 mm, CV values ranged from 0.00 to 15.01 %, the highest for the angle of 345º (data not shown). The measurements of 20 mm wire at 255º angle, presented the highest CV with 18.02 % and the lowest value was 0.40 % (data not shown). Also, the changes were observed in 10 mm length at 255º angle and in the length of 50 mm (0.31 to 14.65 %) (data not shown). Our method presented similar data like that of measured, but somewhat a deviation from expected and somewhat irregular was observed. However, it was very accurate in 57 % of the total estimations, with CV < 5 % (data not presented). These differences at each angle were also reported in Zobel (2008). Therefore, the concept of average values considered all angles from 0 to 345º.

The length measurements were performed with the calliper. The average of our method and the coefficients of variation are shown (Table 2). The coefficients of variation were between 0.01 and 2.99 % ($p < 0.01$). The values of coefficients of variation were considerably low. Therefore, for methods of image processing with resolution images of 300 dpi, studies presented CV values between 0.15 % and 2.86 % (Kimura et al., 1999). The method using the average presented data similar to measured, especially lengths with 20 mm (16 mm diameter) and 50 mm (12 mm diameter).

Test with soybean roots

The relationships among length values estimated with our method and the estimated values for the root lengths achieved by line-intersect method, separated by soil layers (0-10 cm, 10-20 cm and 0-20 cm) in the left column are shown in Fig. 3. Also, the relationships between the estimated root lengths using Safira system and using the line-intersect method separated by soil layers are shown in the right column (Fig. 3).

Utilizing the line-intersect method as a reference, the results of our method and Safira were compared in all layers (Fig. 3). According to Franzblau (1958), who considered classification of the correlation coefficients, the determination of coefficient for root length in the 0-10 cm soil layer (Fig. 3a) between the reference method (line-intersect) and our method (R^2 = 0.998) was very strong. The determination coefficient between the reference method and Safira for root length in the same layer was strong (R^2 = 0.520) (Fig. 3b). For the 10-20 cm soil layers, it was also achieved a very strong determination of coefficient between the line-intersect method and our method (R^2 = 0.997) (Fig. 3c), and a strong correlation between the line-intersect method and Safira (R^2 = 0.377) (Fig. 3d). Therefore, very strong coefficients of determination R^2 = 0.999, for the 0-20 cm soil layers, were also obtained with our method (Fig. 3e) and Safira R^2 = 0.733 (Fig. 3f).

The coefficients of determination values were quite different from those obtained from Safira. However, the determination of coefficient R^2 = 0.733 is better than the low values of determination coefficient (R^2 = 0.514 and 0.453), in root length measures of banana (Costa et al., 2014). This indicates that our method explains root length better than Safira. Studies with similar values of determination coefficients (R^2 = 0.972 and 0.990) were presented by Tajima and Kato (2013), and Pierret et al. (2013), respectively.

Strong coefficient of determination indicates proportional value between measures with the two images processing software (Our Method and Safira) and the reference method (line-intersect). In 0-20 cm soil layers, where these values are strong, we can concluded that reference method shows an increase in root length compared to the tested software (Our Method and Safira). Overall, the software developed to facilitate root studies using image processing are positively correlated with the conventional method (Costa et al., 2014). In studies using image processing, which compared WinRHIZO™ with the Tennant method, the morphological root parameters of winter wheat generated by these two methods were positively correlated, but significantly different (Wang and Zhang, 2009). Pierret et al. (2013) correlated the lengths determined by WinRHIZO™ in the length of Tennant mode and demonstrated the estimated length differs from IJ_Rhizo.

Analysis of root length data showed that our method did not differ from the reference method ($p < 0.0001$). On the other hand, the root length determined by Safira differed from the line-intersect method ($p = 0.0024$) in the soil layer 0-10 cm ($p = 0.0149$), and in the 10-20 cm layer. However, in the 0-20cm soil layer, root length data for Safira and our method ($p < 0.0001$) did not differ from the reference method. Considering that our method did not differ from the reference method in root length in all soil layers, it is probable that this method of image processing quantifies the root length with values close to the real ones. The higher number of roots found in the 0-10 cm layers may have led to a measurement error, resulting in different values of the reference method for Safira ($p = 0.0024$) but not for our method.

The variation coefficients among the lengths estimated by our method and Safira are shown (Table 3). The reference length values are those achieved using line-intersect method proposed by Tennant.

Analyzing the coefficients of variation, values between 0.10 and 2.10 % were obtained by our method (Table 3). The CV's obtained were smaller than the commonly reported in the literature, near 5.0 % (Newman, 1966; Tennant, 1975). However, the coefficients obtained by Safira were in a greater range from 5.11 % to 49.45 %, the latter for the soil layer 10-20 cm. This indicates that for a smaller root amount, our method explains the determination root length better than Safira. On the other hand, our method also presented the highest coefficient value for the same layer (sample 11), demonstrating the difficulty in estimation of the length of smaller roots for both methods of image processing (Table 3).

Kimura and Yamasaki (2001, 2003) reported CV values of 2.29 % and 2.24 % for the sum of the length values of the primary, and secondary roots of rice, respectively, in images with 300 dpi. Moreover, coefficients between 0.3 % and 3.4 % were reported by Himmelbauer et al. (2004) when they compared methods. Our results are similar to those reported by these authors; however, it was underestimated in relation to that reported by Kimura et al. (1999), obtaining CV of 1.78 %. On the other hand, in this study, the minimum CV for Safira is 5.11 %, a value slightly higher than the values obtained by Newman (1966) and Tennant (1975).

Costa et al. (2014) mentioned that there are problems of root overlap, especially when the sample has a higher amount of root (soil layer 0-10 cm). This may have occurred because the density of the soybean roots is higher in the surface layer (0-10 cm), composing approximately 70 % of the total (Merten and Mielniczuk, 1991). It was also verified that the root length values (Table 3) estimated by our method were lower than those of the line-intersect, in 56.6 % of the samples (data not shown). In the studies that compared the image processing software with the line-intersect method, authors reported a trend to overestimate the root length of the last one (Bauhus and Messier, 1999; Wang and Zhang, 2009; Pierret et al., 2013). On the other hand, 90 % (Table 3) of the samples estimated by Safira were overestimated in relation to the line-intersect (data not shown). The highest values of root length obtained with Safira may be related to the higher operational difficulty. Failures in the thresholding procedure of images may cause overestimation of root length.

An image processing software should be insensitive to the preferential orientation of samples, generate results without systematic errors and be adjustable for root overlap in the samples (Kimura et al., 1999). In this context, both programs used in this study have limitations. It should be noted that our method and Safira are not prepared to solve root overlapping problems, unlike WinRHIZO™ that can detect and make corrections to areas of root overlap (Wang and Zhang, 2009). However, the overestimation of the results was greater with Safira, especially regarding the measurements of the soil layers (10-20 cm). An important aspect is that our method, like other systems developed for root studies (Tajima and Kato, 2011; Tajima and Kato, 2013; Pierret et al., 2013), is a public-domain software and offers new opportunities for researchers to carry out operations measuring digitized images of root samples.

Table 1. Estimated lengths (10 mm, 20 mm and 50 mm) for the images of re-oriented wires (n = 144).

	Diameter 0.12 mm	Diameter 0.16 mm	Diameter 0.12 mm	Diameter 0.16 mm	Diameter 0.12 mm	Diameter 0.16 mm
	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)
0	8.52	9.84	17.83	17.04	50.94	50.75
15	10.00	10.89	22.37	18.10	51.63	55.34
30	9.10	10.63	22.76	18.87	48.74	56.78
45	9.00	10.00	21.12	19.33	48.48	50.83
60	9.47	10.56	19.30	18.58	48.51	47.04
75	9.11	8.38	17.67	18.60	40.96	38.81
90	8.30	9.94	17.92	18.77	48.36	46.69
105	9.94	10.04	22.03	20.18	54.11	52.95
120	9.29	10.84	24.57	21.86	58.04	58.61
135	9.85	11.45	22.69	20.28	54.62	54.18
150	9.79	10.50	19.30	19.84	49.69	50.97
165	9.64	7.96	18.22	17.56	41.74	37.80
180	8.68	9.72	20.27	17.85	49.48	51.03
195	9.22	10.79	24.67	21.62	61.24	59.73
210	9.60	12.53	23.17	21.95	57.82	62.27
225	10.47	10.20	24.38	23.29	54.22	56.23
240	11.67	10.72	19.65	19.15	49.56	51.59
255	10.28	8.18	15.91	17.05	41.70	37.22
270	8.74	10.47	20.62	18.04	47.89	52.29
285	9.12	11.30	24.94	23.28	58.23	64.65
300	11.03	10.90	28.79	22.65	60.96	60.49
315	11.15	11.05	25.03	25.36	54.73	61.96
330	11.79	10.69	18.58	20.94	48.89	49.24
345	9.21	7.39	17.81	17.55	43.63	37.24
Average	9.71	10.20	21.23	20.00	51.01	51.86

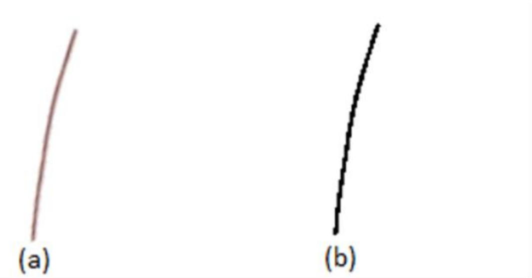

Fig 1. Images of a copper wire with 10 mm length and 0.12 mm diameter: (a) digitized, and (b) after the use of a threshold value.

Table 2. Measured lengths (calliper) and average of estimated lengths (10 mm, 20 mm and 50 mm) for the images of copper wire 0.12 and 0.16 mm in diameter (n = 144).

ID	Length 0.12mm (mm)		CV (%)	Length 0.16mm (mm)		CV (%)
	Measured	Estimated*		Measured	Estimated*	
1	10.00	9.71	1.48	10.00	10.20	1.03
2	20.00	21.23	2.99	20.00	20.00	0.01
3	50.00	51.01	1.00	50.00	51.86	1.83

ID: identifier for each sample; CV: coefficient of variation. *There was not significant difference ($p < 0.01$) in the average length between the two methods.

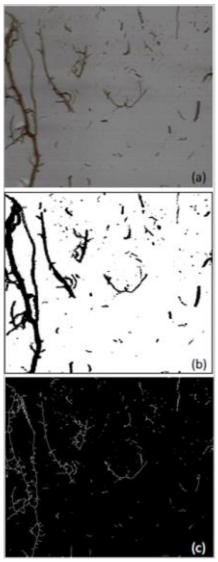

Fig 2. Image changes for root length calculation: (a) original image provided by scanner; (b) binary image obtained after threshold processing; and (c) thinned image obtained after the use of multi-scale thinning algorithm.

Table 3. Measured (Tennant), estimated (our method), and acquired (Safira) lengths of soybean root samples (n = 30).

Sample	Tennant	Estimated		Safira	
	Length (cm)	Length (cm)	CV (%)	Length (cm)	CV (%)
		Soil layer 0-10 cm			
1	382	373.79	1.07	469.18	10.26
2	635	644.34	0.74	704.92	5.23
3	645	638.41	0.52	813.24	11.53
4	672	663.55	0.62	1106.43	24.44
5	733	731.54	0.10	1006.46	15.71
6	738	740.53	0.19	838.40	6.38
7	699	704.63	0.38	955.93	15.51
8	566	558.40	0.72	852.39	20.15
9	665	661.23	0.26	993.28	19.82
10	442	444.51	0.33	530.09	9.11
11	795	782.78	0.78	340.66	40.01
12	547	544.61	0.21	605.73	5.11
13	812	820.81	0.56	940.86	7.37
14	570	571.30	0.15	955.23	25.29
15	1263	1306.04	1.69	1619.30	12.38
Total	10162	10186.47		12732.07	
Average	677	679.10		848.81	
		Soil layer 10-20 cm			
1	381	375.56	0.73	461.32	9.53
2	300	302.21	0.35	359.85	9.05
3	321	327.10	1.01	477.36	19.65
4	214	208.07	1.34	245.83	6.99
5	160	162.92	0.82	193.15	9.30
6	340	334.54	0.84	160.60	35.86
7	254	252.10	0.33	295.92	7.67
8	343	336.50	0.89	449.01	13.45
9	288	284.52	0.53	850.17	49.45
10	193	198.03	1.21	239.03	10.58
11	174	181.91	2.10	129.79	14.67
12	375	373.74	0.14	506.96	14.99
13	110	107.08	1.34	127.60	7.41
14	347	349.18	0.27	407.93	8.03
15	361	366.15	0.76	509.00	17.06
Total	4160	4147.81		5413.55	
Average	277	276.52		360.90	

CV: coefficient of variation.

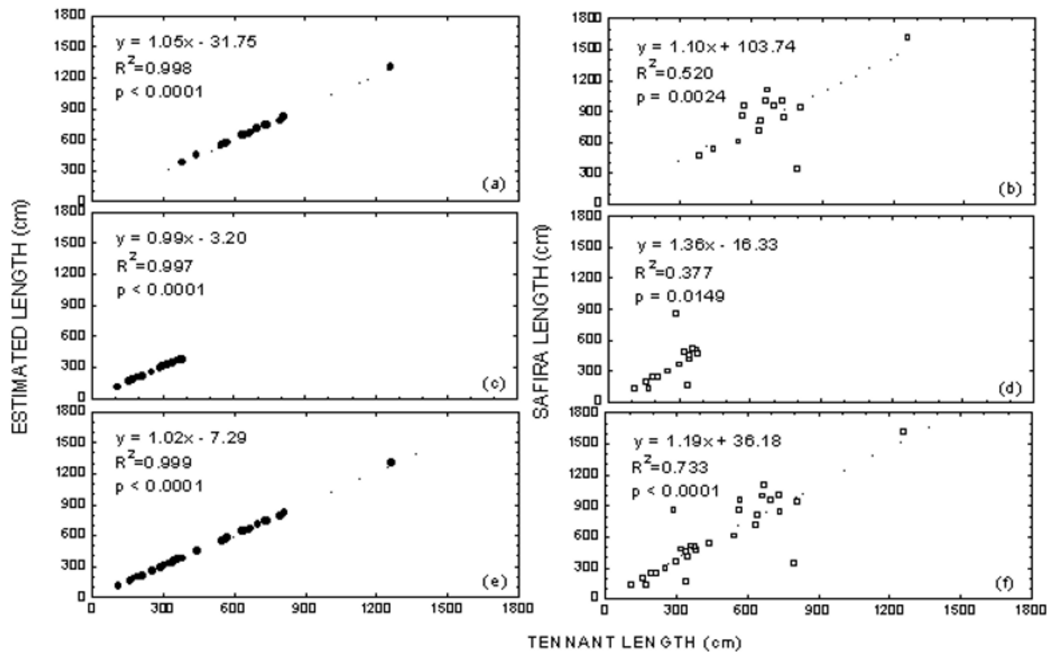

Fig 3. Relationship between estimated (our method) and measured (line-intersect method) root length values, left column; Safira and line-intersect root length values, right column, separated respectively by soil layers: (a) and (b) 0-10 cm; (c) and (d) 10-20 cm; (e) and (f) 0-20 cm.

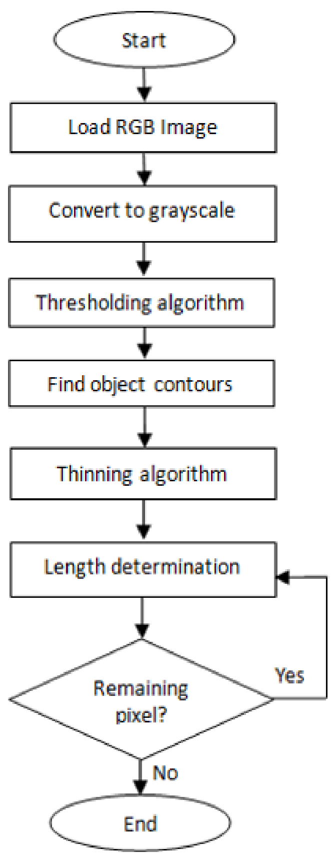

Fig 4. Operational steps of the proposed method.

Materials and methods

Copper wire and plant materials samples

Two types of samples were used for length measurements; copper wires and soybean root. Copper wires (0.12 mm and 0.16 mm diameter) were cut into segments of 10 mm, 20 mm and 50 mm. The wire lengths were measured with a calliper (resolution 0.05 mm). Soybean root samples were collected from a field in Palmeira city, Paraná State, Brazil [S25 º 24'37. 8'' W49 ° 58'22. 8'' (± 3 m), average elevation of 900 m], in a no-tillage system where lime was applied in 1998 to correct soil acidity. Soybeans (cultivar RR Turbo BMX) were sown on December 15, 2012. When the soybeans were in the R5 stage, 4 replicate soil samples were collected from the 0-10 cm and 10-20 cm soil layers (2 in the line and 2 in the rows), with a sample volume of approximately 785.40 cm^3, resulting in 30 samples. The soil in the experimental area was a typic Dystrophic Tb Haplic Cambisol (CXbd). The samples were placed in plastic bags and taken to the laboratory to separate the roots from the soil. Subsequently, the roots were placed in plastic containers with 70 % alcohol and stored at 5 ºC ± 2ºC to maintain sample integrity until scanning, image processing and manual counting.

Image acquisition

For image acquisition, two scanners were used, one for collecting wire copper images and the other for root images because the copper wires and roots were in different places. In order to obtain true colour RGB (red, green and blue) images for copper wires, we used a multifunctional Epson Stylus® TX115 in a professional mode, with a resolution of 300 dpi (0.085 mm $pixel^{-1}$). The image dimensions were 733 x 700 pixels. The digital images were stored in JPEG format (Joint Photographic Experts Group). For the root images, we used a multifunctional and all-in-one printer Lexmark® X4690. The roots were spread on a rectangular glass tray (27.0 x 18.0 x 1.5 cm), directly on a flatbed scanner, with a thin layer of water (2-4 mm) to separate the roots arranged on the tray and to avoid root overlap (Kimura et al., 1999; Kimura and Yamasaki, 2001, 2003). To avoid shadows from the chinks due to the height of the glass tray in relation to the scanner, the tray was surrounded with a border (height 1.5 cm) made of Styrofoam. True colour RGB images of root samples were also acquired with a resolution of 300 dpi. The digital images were stored in JPEG format with dimensions of 2000 x 3050 pixels.

Computational method

The Fig. 4 shows the proposed method steps. For method development, we used the same presented by Name et al., (2016), such as: the free platforms Netbeans IDE 6.9.1 and Oracle JDK 1.6.0_20, OpenCV version 2.4.0, and JavaCV 0.1. The computational method was developed using an Intel ® Core ™ i5-3470 3.20 GHz processor, 4.0 GB of RAM and Windows 7 Operating System (64-bit). The OpenCV library was developed by Intel® and is usually applied to digital image processing and computational vision. This library was written in the C/C++ language and currently supports Python, Ruby, Matlab and other languages (Bradski and Kaehler, 2013). Additionally, it was necessary to use the JavaCV plug-in (Audet, 2018) to have access, via JNI (Java Native Interface), to the functions of OpenCV library by the code in Java language and its implementation. The plug-in implements various existing functions in OpenCV, which are accessed via JNI. After initializing the method, we chose the image to be analysed, which was then stored in memory. Then, each stored image was transformed to an 8-bit grayscale image. Next, a threshold algorithm was applied to the image, resulting in a binary image, the objects of which to be analysed (root samples) were assigned a predetermined pixel value, and the background received another value for its pixels. The choice of a threshold value is critical and influences the final results or the length value (Tajima and Kato, 2011). As observed by Kaspar and Ewing (1997); Kimura et al. (1999); Kimura and Yamasaki (2001); Tajima and Kato (2011); Kano et al. (2011), most analyses and evaluation methods employ a manual threshold. Consequently, to have a better feedback of the image to be processed, we allowed the user to choose the threshold value, i.e., the developed method employs a manual threshold. With the roots located in image, the edge detection method from the OpenCV library was applied to distinguish and label the objects (root segments). Then, each of labelled objects was skeletonized with a multi-scale thinning algorithm (Luppe et al., 2003). This method thins the objects in images by successive deletions of the edge of each object. Finally, only the medial axis of the object, approximately 1 pixel wide, remains (Lebowitz, 1988; Kimura et al., 1999). Once the medial axis of the object (skeleton) was obtained, the Euclidean distance *dist* between the two pixels p_{i-1} (xp_{i-1}, yp_{i-1}) and p_i (xp_i, yp_i) was calculated via equation (1). At a minimum, a couple of pixels (points) were necessary to calculate *dist*. For only one pixel, the *dist* value was determined as the pixel size.

$$dist(p_{i-1}, p_i) = \sqrt{(xp_{i-1} - xp_i)^2 + (yp_{i-1} - yp_i)^2} \quad (1)$$

Subsequently, an iteration routine for each object to add every *dist* for the object was generated. This sum was multiplied by a scale, *K*, equation 2, where *scale*, to obtain the image metadata, for distances in centimetres, is given by equation 3 and *dpi* is the image resolution in dots per inch (Name et al., 2014; Maruyama et al., 2018).

$$K = scale_{cm} \times \sum_{i=1}^{n} dist(p_{i-1}, p_i) \quad (2)$$

$$scale_{cm} = \frac{2.54}{dpi} \quad (3)$$

Once the iteration process was complete, the method was finished and the results were displayed by the software. The developed software is free and open access for research purposes. Available at: http://rm.deinfo.uepg.br/

Evaluation of the developed computational method

The choice of a threshold value was dependent on the object analysed. In the case of copper wire images, where there was only one object in image, the threshold value was changed until the object was fully revealed, but a change of its shape was avoided. For images of root samples, the threshold value was gradually modified to resolve most fine roots, but without allowing the images of the thicker roots to change shape.

The procedure was applied to our method with Safira for the same root image, to compare the results. Additionally, to compare the lengths of wires obtained with our method, we used length measurements obtained with callipers. For root sample lengths, the length values obtained with the two computational methods were compared, as reference to the line-intersect method proposed by Tennant. For the root counting process, we used a transparent glass tray (27.0 x 18.0 x 1.5 cm), a transparency with grids (1.0 x 1.0 cm), a manual counting device and an overhead projector. Root samples were spread in the glass tray, containing a 2-4 mm water layer. The transparency was fixed on the bottom of the tray and placed on the overhead projector to reproduce the roots on the wall. Subsequently, each root that intercepted the vertical and horizontal lines were counted. The roots that cross the lines and root tips touching the lines were computed. The counting of intercepts was converted to root length using equation 4, proposed by Tennant (1975):

$$L_{cm} = {}^{11}\!/_{14} \times N \times G \quad (4)$$

Where, L = length, N = number of intercepts and G = Grid. In our case, the grid value is 1.0.

Statistical analysis

The software used for statistical analysis was Origin® 6.1, version 6.1052. To validate and test our method, we choose an image of each length (10 mm, 20 mm and 50 mm) to be redirected between 0° and 345° at intervals of 15°. Because the copper wires had two different diameters (0.12-0.16 mm), we obtained 144 images (06 digitalized and 138 reoriented), in other words, 24 images for each copper wire length of a specific diameter. The results were correlated using coefficients of variation (CV). Subsequently, the method was also applied to images of soybean root samples. To evaluate the method, the lengths were compared to those obtained from the line-intersect method (grid of 1.0 x 1.0 cm) (Tennant, 1975) and Safira (Safira, 2018). The results for the three methods were correlated using coefficients of variation (CV) and determination coefficients (R^2).

Conclusion

This study presented a public domain computational method, developed in Java, with the support of the OpenCV library and the JavaCV plug-in, which are free platforms. Our method generated accurate data for the root length that do not differ from those of the reference method, regardless of the soil layer analyzed. Safira generates root length data that differed from the reference method, especially in samples with smaller amounts of roots. The results of soybean root length obtained with our method are better than the lengths evaluated with Safira. However, we recommend it for studies of roots of other cultures.

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