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Non-invasive quantitative monitoring of adventitious rooting of rose cuttings stimulated by laser wounding

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Abstract

The study of laser wounding in rose cuttings has helped to identify relevant aspects that wounded regions have to bring a positive effect on root stimulation. Nevertheless, a detailed understanding of the dynamic effects of laser wounds during rooting is not possible by traditional methods. To analyze the morphological development of rooting exposed to laser treatments, a hydroponic propagation chamber was constructed that included an internal monitoring system. Through this system, time-lapse videos recorded the entire adventitious rooting process. Results revealed how wounding influences spatial positioning of adventitious roots, depending on factors such as laser pattern, tissue removal, and the location of buds and leaves. A comparison of root distribution in terms of percentage, performed with both, the image analysis and the manual method, showed the same root growth trend with slight differences in the percentages obtained by both methods.

1. Introduction

Recent studies based on laser wound treatments at the base of rose cuttings revealed specific parameters that wounds must meet for a positive response in adventitious root development. Critical among these parameters are the level of tissue exposure and the distribution of wounds, which were found to be vital in modifying the growth of adventitious roots at the base of the cuttings (Morales-Orellana et al. 2022). As a result, the application of different levels of penetration and marking patterns have led to the formation of complex root systems with acrobasal features, and therefore, a large diversity of spatial root distribution has to be compared with cuttings showing basal rooting. Due to the architecture of the root systems obtained, the implementation of a new non-invasive evaluation method is necessary for a more precise monitoring of the stimulation of adventitious roots which is not possible by traditional methods.

Unlike invasive phenotyping methods that often involve the destruction of plants, new phenotyping techniques do not only allow the simultaneous measurement of multiple traits, but also ensure the longevity of cuttings without causing harm to plant viability (Saoirse et al. 2020). Consequently, completely non-invasive root monitoring systems have been developed, utilizing technologies such as magnetic resonance imaging, computed tomography, multispectral instruments, or cameras (Wasson et al. 2020). In the case of RGB cameras, root phenotyping relies on the analysis of individual or multiple images to detect changes in the plant rhizosphere. For laser-treated rose cuttings, the development of a non-invasive monitoring system operating in hydroponic conditions has not been reported thus far, even more considering that the system should be capable of evaluating different samples of cuttings simultaneously. This system could open up the possibility of obtaining real-time growth data, a process that could be implemented, for example, in improvement operations such as selecting rose species with favorable rooting properties or in research areas focusing on root phenotyping. Therefore, the objective of this article is to design a non-invasive system for monitoring adventitious roots of laser-treated rose cuttings to analyze the dynamic of adventitious rooting by time lapse videos, and to test an additional algorithm to quantify the root distribution in percentage based on image analysis from the photos obtained.

2. Data, Methods and Approach

Laser treatment setup

Leafy single-node stem cuttings with a diameter of 3.5 mm from *Rosa hybrida* cv. 'Beluga' were chosen and divided into eight wound treatments (table 1). The laser wounding treatments were performed using a continuous wave CO2 Laser (Pro 600, BRM, Netherlands) with the assistance of a rotary axis (CNC 80 mm 3 Chuck, Ly. Group, China). Each cutting was placed on the rotary axis, maintaining a focal distance of 50.8 mm from the cutting to the laser lens (20 mm ZnSe, Cloudray, USA). The laser pattern was positioned on the same side as the axillary bud, approximately 10 mm below the leaf petiole. In case of the strip pattern, one strip was marked on the side of the axillary bud and the other on the opposite side. In case of the ring pattern, the wounds surrounded completely the stem. Three different energy levels (2.5 J cm^{-2}, 3.8 J cm^{-2}, and 5.9 J cm^{-2}) were applied, resulting in bark penetrations of approximately 0%, 50%, and 70%, respectively.

In addition to laser treatments, a manual wound treatment done by a blade was performed to simulate the empirical wound method. Each treatment comprised 30 replicates; two samples from each treatment were tested in the non-invasive monitoring system, while the others remained under aeroponic conditions in the presence of a 1 ppm Indole-3-butyric acid (IBA) as a root stimulator. Furthermore, 16 rose cuttings of *Rosa canina* 'Pfänder' were assessed in the monitoring system to explore potential root morphological differences between rose genotypes.

Table 1: Different wound treatments applied to the stem side close to the base of leafy single-node rose cuttings.

Treatment	**Penetration level**	**Laser energy applied ($\mathrm{J\ cm^{-2}}$)**	**Bark penetration (%)**
Control	-	-	-
Two strips laser pattern (total wounded area: 51 mm^2)	Superficial	2.5	0
	Moderate	3.8	40 - 60
	Deep	5.9	60 - 80
Two rings laser pattern (total wounded area: 51 mm^2)	Superficial	2.5	0
	Moderate	3.8	40 - 60
	Deep	5.9	60 - 80
Manually wounded (blade)	Variable	0.0	0 - 50

Monitoring system design

The non-invasive monitoring system for rose cuttings was based on a photo box composing of a hydroponic propagation chamber that was periodically monitored by an electronic system through a transparent PMMA plate 4 mm thick. Within the propagation chamber the bases of 16 cuttings remained submerged in a propagation medium, 8 cuttings in water and 8 cuttings in a 1 ppm IBA solution, with permanent aeration by bubble diffusers and an air compressor (ACO-208, HAILEA, China). The monitoring system included LED lighting (IP65, Lumonic, China) in conjunction with an RGB camera (Brio 4K, Logitech, Switzerland) mounted on a 120 cm long aluminum conveyor belt moved by a step motor (NEMA 17 0.9°, PrimoPal, China) and controlled by a microcontroller (Uno R3, Arduino, Italy) as depicted in figure 1. Finally, the entire photo box was covered by a dark cover of polyethylene foam to simulate dark conditions at the cutting base and to ensure proper root development.

Monitoring system control

The non-invasive monitoring system was operated synchronously using an algorithm in two main phases. First, during the cultivation phase of the cuttings, air diffusers and the compressor ran continuously in dark conditions. Second, in the analysis phase, the electronic system collected data at 30-minute intervals. To initiate data collection, the air compressor was deactivated, and LED lighting automatically turned on. Subsequently, the camera was positioned in front of the first cutting, capturing an image before moving to the next cutting after taking 200 micro steps, equivalent to approximately 65 mm. This loop was repeated 16 times until the development of all the cuttings was recorded, and the camera returned to the starting position. Following this, the lighting turned off, and the compressor resumed operation. The rooting time of the cuttings in the system lasted 28 days, during which changes from the day after the laser treatment on were recorded.

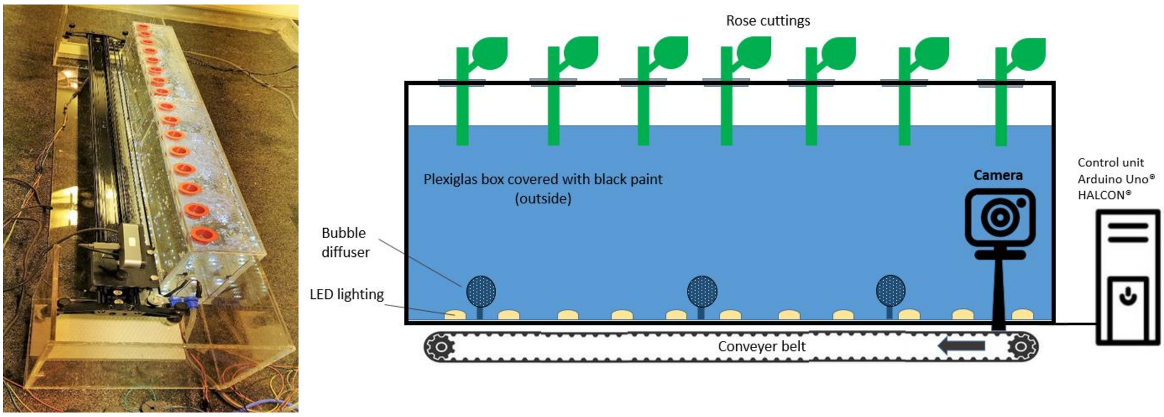

Figure 1: Non-invasive monitoring system for rose cuttings without external cover (left) and sketch of the electronic monitoring system under hydroponic conditions (right).

Phenotyping and image analysis

Pictures obtained by the system were studied individually and in form of time lapse videos. For video analysis, the system recorded callus and root emergence times, along with a comparison of growth dynamics and elongation between cuttings in the presence and absence of auxin in the culture medium. In terms of individual image analysis, the HALCON software conducted a root distribution analysis based on percentages using an additional algorithm.

The algorithm was tested with samples from each treatment at the end of the 28-day rooting time as depicted in figure 2. The process started with the positioning of the axillary bud of the cutting on the left side of each photo (fig. 2a). Later, the program proceeded to choose the best color space and color channel. At this stage, color space transformations (hsv, i1,i2,i3, cielab), image rotation, testing color channels with Otsu binarization and a selection of the best color channel based on test images were carried out (fig. 2b). Next, the algorithm proceeded to modeling the stems by ellipses to find the largest innermost rectangle that represents the stem section (fig. 2c). This stage involved determining semi-axes, centers of gravity of the ellipses, and eroding ellipses on 66% of the same area with the ellipse mask. Subsequently, Hough transformation and compaction on the central axis of the stem were performed to set stem boundaries by calculating parallel outlines in the stem region (fig. 2d). Finally, pixel quantification to the left (bud side) and right (opposite side) of the central axis of the stem was determined. In this phase, roots located to the left and right of the central axis were identified (fig. 2e), and the areas on each side were calculated and presented as a percentage using Roberts and Sobel filters (fig. 2f). Finally, the data from the evaluation of root distribution in percentage by image analysis were compared with the evaluation of the samples done manually.

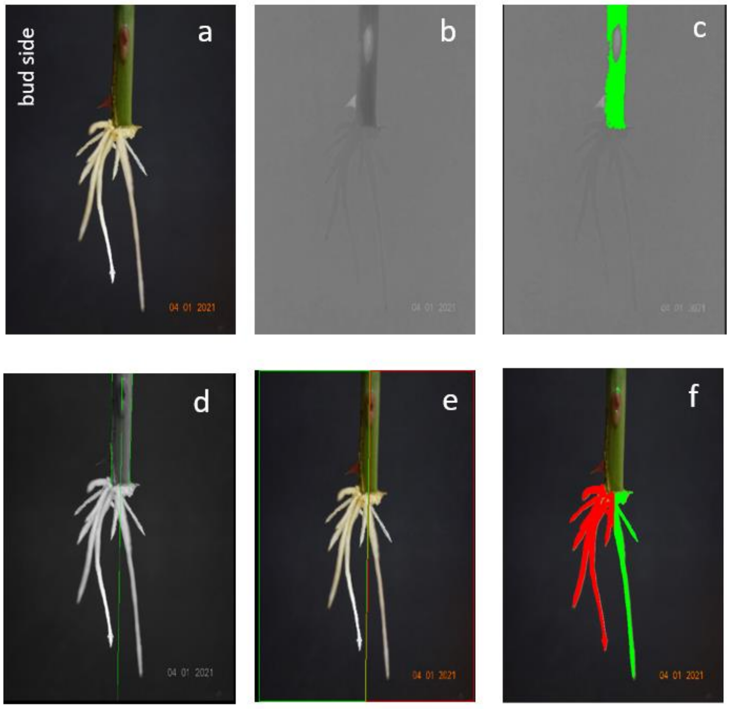

Figure 2: Image analysis process done by HALCON software to evaluate the root distribution in percentage regarding bud position from single images. Red and green color represent the percentage of roots on the bud side and its opposite side, respectively.

3. Results and Discussion

Root distribution analysis by computer image analysis

Image analysis of the adventitious root distribution, presented as percentage, revealed a clear trend of higher root growth on the side of the axillary bud, ranging between 55 % and 75 % in all treatments (fig. 3a). However, while adventitious roots exhibited a location preference, these values varied depending on the treatment applied. The most effective treatment resulted in an average of 0.6 g of roots per rooted cutting, corresponding to the strip pattern with moderate tissue exposure in comparison to the control with an average of 0.31 g of roots per rooted cutting. A manual evaluation of the root percentage over 28 days demonstrated the effectiveness of the image-based quantification method. The comparison between methods showed how the root distribution obtained by image analysis and manually presented the same distribution trend, thus, prioritizing growth on one side of the cutting, but with slight differences in the values obtained between 5% and 11%. To assess the alignment between values obtained through the software and the manual method, a linear regression was conducted, resulting in a coefficient of determination (R^2) of 0.82 (fig. 3b). This value could be attributed to the lack of information concerning the opposite side of the cuttings, the side that was behind the camera, making it impossible to quantify. Moreover, differences in percentages could also be attributed to the overlapping of roots during the elongation phase, which was not analyzed by the algorithm. However, the evaluation of root distribution through images was not significantly compromised, as it might be, for example, in the determination of fresh mass or root length from a single lateral image.

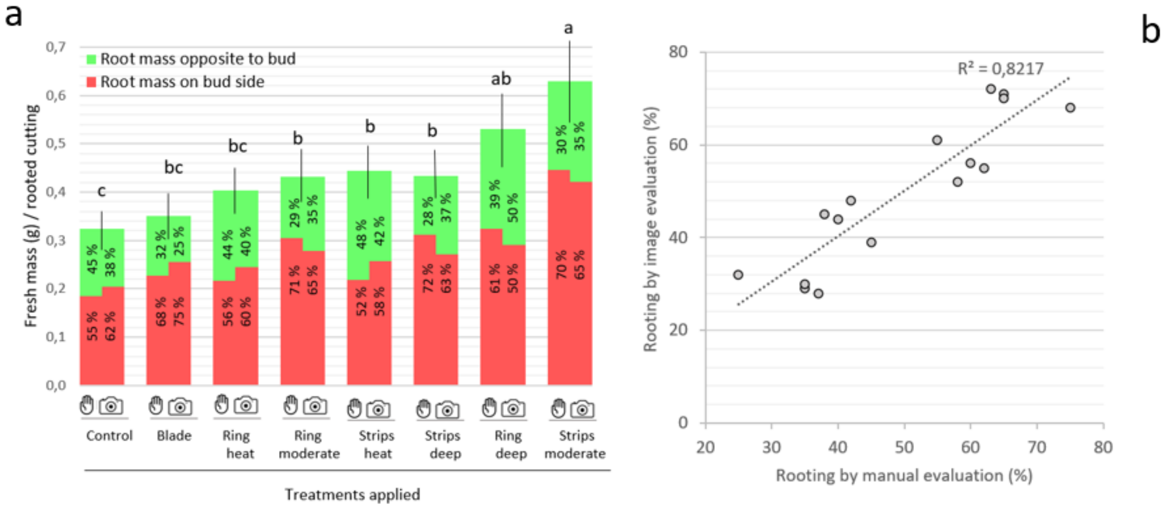

Figure 3: Comparison of root mass among wound treatments after 28 days of culture for *Rosa hybrida* cv. 'Beluga' cuttings (n = 28). Letters denote significantly different levels (Tukey test, $p \leq 0.05$). a) Evaluation of root distribution as a percentage considering bud location, comparing manual evaluation (hand icon) and image analysis (camera icon). b) Linear regression and coefficient of determination (R^2) based on root distribution percentages obtained from manual and image evaluations.

Dynamics of adventitious root development in lasered rose cuttings

The videos obtained from the system show great morphological differences in rooting between the cuttings of *Rosa canina* 'Pfänder' and *Rosa hybrida* cv. 'Beluga', characterized by having a low and high rooting capacity, respectively. The first visible changes were observed 48 hours after planting in the base regions of the cuttings as well as the wounded areas, a process that continued until the end of evaluation (see here for the video: Video 1). Interestingly, the initial emergence of adventitious roots in *Rosa hybrida* cv. 'Beluga' occurred 10 days after planting in all cuttings, irrespective of the treatment or the presence of exogenous auxins. In contrast, for *Rosa canina* 'Pfänder' samples, the first appearance of roots was recorded after 14 days, and it was not successful in all cuttings. Two types of rooting were identified: basal and acrobasal. Basal rooting was characterized by 7 to 10 adventitious roots for *Rosa hybrida* cv. 'Beluga' in the absence of IBA (see here for the resulting video: Video 2) and between 1 to 2 roots for *Rosa canina* 'Pfänder' under the same conditions (see here for the resulting video: Video 3). Basal rooting was common in all cases for *Rosa canina* 'Pfänder', while for *Rosa hybrida* cv. 'Beluga', it occurred exclusively in cuttings propagated without IBA. Acrobasal rooting was exclusively observed for *Rosa hybrida* cv. 'Beluga' in presence of exogenous auxin plus wounded tissue. Under this scenario, root systems were characterized by a high number of adventitious roots, approximately 30 to 40 roots per rooted cutting, but a length between 4 and 6 cm per root (see here for the resulting video: Video 4). Finally, even in the presence of a root stimulator, no emergence of adventitious roots occurred in some cuttings of *Rosa canina* ‘Pfänder’ (see here for the resulting video: Video 5).

4. Conclusions

The present non-invasive monitoring system for rose cuttings provided information of the whole dynamics of adventitious rooting in presence of wounded tissue by image analysis. The phenotypic analysis produced a large amount of data which described in detail the different phases of root development and allowed the identification of a large variation in root morphology. By monitoring rose cuttings, it was possible to study the development of callus in the early stages of rooting of the cuttings, as sign of cell repair, as well as root emergence and its elongation after several days of planting from a non-invasive perspective, a process that has not been reported in this way so far. Likewise, the comparison of data obtained by image analysis and by the manual method indicated similar results suggesting the potential of image analysis as an alternative tool for root evaluation. Although the current system assessed the distribution in percentage of roots as a practical application, the image analysis opens up the possibility to quantify further parameters such as the number of roots, length and root mass based on specific algorithms. Based on these results, the non-invasive system offers a new method to quantify the development of roots on cuttings of various species during their different stages of development, a process that is still unknown for many woody plants.

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Supplemental Information

https://www.dgg-online.org/proceedings/vol-11-2023/dgg-pr-11-05-rm-2023_Video1.mp4

https://www.dgg-online.org/proceedings/vol-11-2023/dgg-pr-11-05-rm-2023_Video2.mp4

https://www.dgg-online.org/proceedings/vol-11-2023/dgg-pr-11-05-rm-2023_Video3.mp4

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