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RHIZOGENESIS IN BLACK RASPBERRY STEM CUTTINGS: INTERACTION BETWEEN SUBSTRATES AND LESIONS AT THE BASE OF THE CUTTINGS

Fabíola Villa[1], Luciana Sabini da Silva[1], Giordana Menegazzo da Silva[1], Daniel Fernandes da Silva[2], Jussara Carla Conti Friederich[3], Mateus Lopes Borduqui Cavalcante[1]

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ABSTRACT - The black raspberry tree has a great potential for cultivation in Brazil, as it produces large amounts of fruit, intended mainly for the processing of fine products. However, its propagation is an obstacle to the establishment of commercial plantations. Given the above, the objective of the present work was to evaluate rhizogenesis in stem cuttings, with the interaction between substrates and types of basal cuts. The experiment was conducted in a screened condition in the Institution itself. Stem cuttings were taken from mother plants in the orchard of the experimental farm. The experimental design used in the experiment was randomized blocks, consisting of a 4x2 factorial scheme, with four substrates (sand, rice husks, commercial substrate and earth) x two basal cuts (straight and bevel). After thirty days, the percentage of rooted cuttings (%), average number of roots and shoots per cutting, length of the largest root (cm) and fresh and dry biomass of the shoot and root system (g) were evaluated. Black raspberry can be propagated asexually, with cuttings without injuries at the base and rooted in gully soil or Plantmax®.
Keywords: *Rubus nivaeus* L., asexual propagation, cutting, basal cut.

RIZOGÊNESE EM ESTACAS CAULINARES DE FRAMBOESEIRA-NEGRA: INTERAÇÃO ENTRE SUBSTRATOS E LESÕES NA BASE DAS ESTACAS

RESUMO - A framboseira-negra possui um grande potencial de cultivo no Brasil, pois produz altas quantidades de frutos, destinados principalmente ao processamento de produtos finos. No entanto, sua propagação é um entrave no estabelecimento de plantios comerciais. Diante do exposto, objetivou-se com o presente trabalho, avaliar a rizogênese em estacas caulinares, com a interação entre substratos e tipos de cortes basais. O experimento foi conduzido em condição de telado na própria Instituição. Estacas caulinares foram retiradas de plantas matrizes do pomar da fazenda experimental. O delineamento experimental utilizado no experimento foi blocos casualizado, constituído de esquema fatorial 4x2, sendo quatro substratos (areia, casca de arroz, substrato comercial e terra) x dois cortes basais (reto e bisel). Após trinta dias, avaliaram-se a percentagem de estacas enraizadas (%), número médio de raízes e de brotações por estacas, comprimento da maior raiz (cm) e biomassa fresca e seca da parte aérea e do sistema radicular (g). A framboeseira-negra pode ser propagada via assexuada, com estacas sem lesões na base e enraizadas em solo de barranco ou Plantmax®.
Palavras-chaves: *Rubus nivaeus* L., propagação assexuada, estaquia, corte basal.

INTRODUCTION

The black raspberry is a shrubby fruit tree, with fine thorns on the whitish stem and classified as a species of temperate climate (SILVA et al., 2012). It is very versatile, as it adapts to Brazilian regions with a milder climate and moderate temperatures, such as the subtropics (CURI et al., 2015). The species has been standing out for its rusticity and good productivity where it is cultivated (MARCHI et al., 2018).

With flowers arranged in a terminal panicle and small-sized fruits, clustered in clusters and attractive due to their very dark purple color, these have excellent nutritional characteristics, containing more than twice the amount of anthocyanin presented by other small fruits of the same genus, such as the blackberry and red raspberry (MUNIYANDI et al., 2019).

Regarding propagation, the information found for the species in the literature is scarce. Research has been carried out using sexual and other propagation, with promising results, with asexual propagation, for the species (VILLA et al., 2018; SILVA et al., 2012), and for species of the same genus, such as *Rubus fruticosus* (TADEU et al., 2012), *R. adenotrichus* (OROZCO-RODRÍGUEZ et al., 2011) and *R. bartonianus* (WAAD and REED, 2011).

The most used methods for the asexual propagation of species of the genus *Rubus* are stem and root cuttings (VILLA et al., 2003) and micropropagation (VILLA et al., 2008). However, the black raspberry (*Rubus nivaeus*) emits few shoots from the root system, unlike the red, yellow and black raspberries. The use of herbaceous and woody stem cuttings becomes an alternative in this type of propagation,

[1]Universidade Estadual do Oeste do Paraná (Unioeste), *Campus* Marechal Cândido Rondon, Marechal Cândido Rondon, PR. Brasil. E-mail: fvilla2003@hotmail.com. *Corresponding author.
[2]Universidade Federal do Paraná (UFPR), *Campus* Curitiba, Curitiba, PR, Brasil.
[3]Universidade Norte do Paraná (UNOPAR), Campus Toledo, Toledo, PR, Brasil.

being able to take advantage of a large amount of material from pruning (MACEDO et al., 2012).

Some abiotic factors can interfere in the rhizogenesis of the cuttings, where some techniques are used, aiming to potentiate the emission of the roots. One of them is the use of substrates and types of cuts at the base of the cuttings. The substrates or mixtures play a fundamental role in the success of propagation by cuttings and must present favorable physicochemical characteristics in rhizogenesis (GOMES et al., 2015).

The practice of making lesions at the base of this can contribute to the formation of roots at the edges of these lesions (SANTORO et al., 2010). Cell division is stimulated by an increase in the respiratory rate and in the contents of auxin, carbohydrates and ethylene in the injured area. In this there is greater absorption of water and plant regulators, increasing rooting efficiency. In turn, injuries allow disruption of the physical barrier, which may even prevent the emergence of roots (FACHINELLO et al., 2005).

In some cultures, such as the case of guava, the use of wounds at the base of the cutting with exposure of the cambium, increased rooted cuttings by 15% compared to the use of cuttings without lesions (SANTORO et al., 2010). Given the above, the objective of this study was to evaluate rhizogenesis in black raspberry stem cuttings through the use of substrates and lesions at the base of the cuttings.

MATERIAL AND METHODS

The experiment was carried out between April and May/2020, in the seedling nursery of the Horticulture and Protected Cultivation Station "Professor Mário César Lopes", belonging to the State University of Western Paraná (Unioeste), *Campus* Marechal Cândido Rondon, PR. The nursery is located at the geographical coordinates of latitude 24°32'22" S, longitude 54°03'24" S and altitude of 420 m. Cuttings were taken from material derived from pruning, of matrix plants, with three years of age, kept in an orchard at the Experimental Farm of Unioeste. Subsequently, they were taken to root in screened conditions, in masonry beds, filled with different substrates. Irrigation on the beds was carried out by sprinkling and intermittently every half hour during the day and every hour at night.

The physical-chemical characterization of the substrates was carried out at the Institution's Soil Laboratory, according to the methodology described in Normative Instruction No. 17 of the Ministery of Agriculture (MAPA, 2007), obtaining the values of apparent density, water holding capacity at a tension of 10 cm (WRA 10, or microporosity), hydrogen potential and electrical conductivity. From these results, total porosity and macroporosity were obtained. The total porosity corresponds to the volume of water retained in the substrate at 0.00 hPa tension (fully saturated), while the macroporosity consists of the difference between the total porosity of the substrate and the volume of water retained at 10 hPa (CRA 10, or microporosity) (SCHMITZ et al., 2002).

The experimental design used was randomized blocks, in a 4 x 2 factorial scheme, with 4 types of substrates (medium textured sand, carbonized rice husks, PlantMax® commercial substrate and gully soil) x 2 types of lesions at the base of the cutting (horizontal and bevel), containing 4 repetitions and 15 cuttings per repetition. The different cuts at the base of the stakes were made with the aid of a clean and sterilized penknife, so as not to damage the tissues. All cuttings, before being placed on the substrates, had their bases immersed in a hydroalcoholic solution of 2000 mg L^{-1} of indolbutyric acid (IBA), for 20 sec (VILLA et al., 2018).

After sixty days, some phytotechnical factors were evaluated, such as rooted cuttings (obtained with the percentage of cuttings that took root), average number of roots (obtained by counting the roots of each cutting and average of the total sum of values), average number of shoots (obtained by counting the shoots emitted in each cutting and average of the total sum of values), length of the largest root (obtained by measuring the longitudinal length of the root system, from the stem of the plant to the end of the roots, with results expressed in cm), fresh shoot biomass (g), dry shoot biomass (g), fresh root biomass (g) and dry root biomass (g). For fresh biomass, a precision scale was used, with the values obtained containing two spaces after the decimal point. To measure the lengths, a professional digital caliper (Blackbull 5234) and a graduated millimeter ruler were used. For the dry biomass, the shoots and the root system were subjected to drying in a forced air circulation oven, at 65°C, for 72 h until constant weight, with results expressed in grams.

Data were tabulated and the Shapiro-Wilk normality test was applied, being transformed to (Y+1.0)^0.5 when necessary and later submitted to analysis of variance, with means compared by Tukey's test, at 5% probability of error. For the analysis of the results, the Sisvar statistical program was used (FERREIRA, 2011).

RESULTS AND DISCUSSION

According to the analysis of variance, there was a significant interaction between substrates and lesions at the base only for rooted cuttings (%). For the other variables, significance was observed separately for the substrate factor. In Table 1, there was a significant interaction for the rooted cuttings (%), according to the interaction between the lesions at the base and the substrates. Among the types of lesions used on black raspberry cuttings, the bevel type stood out, presenting high percentages of rooting, differing from the work carried out by Aquino et al. (2016), in canephora coffee cuttings, who found a higher percentage of rooting when a straight cut was applied at the base.

Some cuts can be performed on cuttings of plant species that have difficulty dedifferentiating meristematic cells and, consequently, a low rooting rate. The different types of cut favor the formation of calluses and roots at the edges of the lesion, where the cell division that occurs in this area is stimulated by the increase in the respiratory rate, in addition to the levels of auxin, carbohydrates and ethylene, thus increasing the efficiency of rooting. of the stake (FACHINELLO et al., 2005). Probably, black raspberry cuttings have some physical and/or anatomical barrier, caused by the presence of sclerenchyma rings at the base, which is harmful to the rooting process (BATISTA et al., 2014).

TABLE 1 - Rooted cuttings (%) of black raspberry, with injuries at the base (Inj base) and maintained in different substrates.

Inj base	Substrates			
	Medium textured sand	Carbonized rice husk	PlantMax®	Soil
Horizontal	57.64 bB	93.33 aA	68.89 bB	59.99 bB
Bevel	77.78 aA	88.88 aA	84.44 aA	93.33 aA
CV(%)	10.90			

*Lowercase letters differ from each other in the column and uppercase in the row, by Tukey's test, at 5% error probability. ns = not significant.

When studying each substrate, the carbonized rice husk was the one that was superior, regardless of the type of injury performed at the base of the cuttings. This type of substrate has low density, providing greater drainage and better aeration for the root system of the seedling, characteristics that, when combined with adequate humidity and the type of lesion at the base of the cutting, help in the formation of roots (KRATZ and WENDLING, 2016).

In Tables 2 and 3, there is significance only for the substrates in relation to the analyzed variables, regardless of the type of lesion at the base of the stakes. For the root system formed in the cuttings, the soil was superior, providing a greater number and statistically differing only from the commercial substrate. A soil-based substrate has lower porosity and higher density, reducing transport through mass flow, thus stimulating the root zone in search of water and nutrients (ZORZETO et al., 2014).

TABLE 2 - Average number of roots, length of the largest root, average number of shoots, length of the largest shoot, fresh biomass of the shoots, fresh biomass of the root system, dry biomass of the shoots and dry biomass of the root system, obtained from black raspberry cuttings, on the substrates used.

Substrates	Number of roots	Lenght of the largest root (cm)**	Number of shoots	Lenght of the largest shoot (cm)
Medium textured sand	25.90 ab	14.62 a	4.10 ab	4.98 b
Carbonized rice husk	25.73 ab	13.58 a	5.57 a	7.22 ab
PlantMax®	20.93 b	14.51 a	4.40 ab	7.63 a
Soil	30.20 a	17.22 a	3.77 b	6.51 ab
CV(%)	18.77	11.76	20.47	23.81
Substrates	**Fresh biomass of the shoots (g)**	**Fresh biomass of the root system (g)**	**Dry biomass of the shoots (g)**	**Dry biomass of the root system (g)**
Medium textured sand	6.58 a	3.99 a	4.30 a	3.19 ab
Carbonized rice husk	6.71 a	4.43 a	3.93 ab	3.09 a
PlantMax®	6.09 a	4.69 a	3.86 b	3.39 b
Soil	6.41 a	3.94 a	4.15 ab	3.15 ab
CV(%)	11.33	16.27	5.85	5.35

*Lowercase letters differ from each other in the column, by Tukey's test, at 5% error probability. **Data transformed to (Y+1,0)^0,5.

TABLE 3 - Average number of roots, length of the largest root, average number of shoots, length of the largest shoot, fresh biomass of the shoots, fresh biomass of the root system, dry biomass of the shoots and dry biomass of the root system, obtained from black raspberry cuttings, with base injuries (Inj base).

Inj base	Number of roots	Lenght of the largest root (cm)**	Number of shoots	Lenght of the largest shoot (cm)
Horizontal	26.05 ns	3.93 ns	4.37 ns	6.28 ns
Bevel	25.33 ns	4.03 ns	4.55 ns	6.88 ns
CV(%)	18.77	11.76	20.47	23.81
Inj base	**Fresh biomass of the shoots (g)**	**Fresh biomass of the root system (g)**	**Dry biomass of the shoots (g)**	**Dry biomass of the root system (g)**
Horizontal	6.50 ns	4.16 ns	4.16 ns	3.16 ns
Bevel	6.39 ns	4.37 ns	4.37 ns	3.25 ns
CV(%)	11.33	16.27	5.85	5.35

*Lowercase letters differ from each other in the column, by Tukey's test, at 5% error probability. ns = not significant. **Data transformed to (Y+1,0)^0,5.

A greater number of roots per cutting means greater absorption of water and nutrients, a determining factor for the good development of the seedling after planting. In addition, the use of a cheaper substrate reduces costs in the production of seedlings of fruit species (CARVALHO ZANÃO et al., 2016). In addition, it was visually perceptible that, in relation to substrate aggregation to the roots (clod formation), the ravine soil, being clayey, provided better aggregation than the others. This characteristic is very important in planting, bearing in mind that, for a good establishment of the seedling, it is essential that it does not suffer damage to the root system, which can be avoided or minimized, through substrates that provide good aggregation to the roots, forming a clod (KÄMPF, 2005).

Still in relation to the parameters linked to the root, although the results obtained do not demonstrate a significant difference between them, it is observed that the highest value of root length and fresh biomass of the root system was found when using the ravine soil and commercial substrate, and this result can be attributed to the greater number and length of roots formed in the cuttings. Some physical characteristics present in agricultural substrates, such as aeration (porosity), good drainage and high water retention, can facilitate root passage, helping the rooting of cuttings.

The same was observed by Paixão et al. (2017) and Yamamoto et al. (2016), where the length of the largest root also did not differ statistically when using some types of substrates, such as carbonized rice husks, vermiculite, sand, coconut fiber and commercial, in rooting cuttings of noni and blackberry plants, respectively.

As for the shoots, a greater amount formed in the cuttings can be observed with the use of carbonized rice husks and a smaller number with the ravine soil. This variation in the development of shoots in cuttings is directly related to the type of substrate used (MADRUGA et al., 2021) and also to rooting, swelling, cell division and differentiation of buds in shoots, caused by the organic reserves contained in the material. However, shoots only differentiate into leaves if adventitious root is emitted. If rooting does not occur, to supply the shoots with water and nutrients, the cuttings wither and die, due to the depletion of their reserves. The relationship between the number of shoots and rooting can also interfere with root growth points, which are a source of growth regulators, mainly cytokinin, translocated to growth points in the shoot, acting on cell multiplication (TAIZ and ZEIGER, 2017).

Also in Table 3, it can be seen that the length of the largest shoot was influenced by the substrates studied, with the highest average when the commercial substrate was used. The sand used as a substrate was not interesting for this variable, as it is considered an inert, highly porous and universal substrate, indicated for rooting cuttings of various fruit species. For the formation of shoots and growth in cuttings, the use of a given substrate does not directly interfere with these variables, but the amount of reserves present in the plant material and the hormonal balance (RISTOW et al., 2012).

Sprouting is an important variable in the study of rooting of cuttings, as the presence of shoots and leaves enables greater production of photoassimilates and auxin synthesis, which are essential factors for root emission and plant growth (CARVALHO et al., 2015). Mendonça et al. (2018) observed that, for the longest shoot length of odontonema cuttings [*Odontonema strictum* (Nees) O. Kuntze], the use of sand was more favorable, thus favoring the development of the shoot.

For the fresh biomass of shoots and root system, no statistical difference was observed for the substrates used, but superior results were found in carbonized rice husk and commercial substrate, respectively. As previously reported, these two events are distinct and their development occurs separately and is influenced by several intrinsic and extrinsic factors to the stake itself.

However, it can be inferred that the type of substrate used and its physicochemical characteristics directly interfere with the formation and biomass of the roots, where substrates with greater total porosity, such as Plantmax® and carbonized rice husks, promote greater quality of the root system and, consequently, generate seedlings with higher shoot and root dry biomass (SILVA et al., 2014). These root biomass production data are important, since adequate root development facilitates the entry of water and nutrients, which will be used mainly in the aerial part of the cuttings.

In general terms, the results obtained here prove that, regardless of the basal cut performed, black raspberry cuttings can be propagated asexually, with good root and shoot development. Future works can be carried out, with the use of other propagation methods, or even other times of removal of the cuttings from the mother plant.

CONCLUSION

Black raspberry can be propagated asexually, with cuttings without injuries at the base and rooted in gully soil or Plantmax®.

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