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# Black pepper grafting in *Piper* wild species

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**ABSTRACT:** *Fusarium* is the main disease of black pepper (*Piper nigrum* L.) in Brazil, and grafting using resistant rootstocks can be a sustainable management strategy. Several wild *Piper* species have been considered resistant to infection. However, further studies on the grafting process are needed to generate a safe technical recommendation for farmers. This research was carried out with the objective of evaluating the initial compatibility of grafting through the interaction between two techniques (top cleft and side cleft) and six rootstocks. The *P. nigrum* 'Bragantina' was grafted onto four wild *Piper* species (*Piper aduncum* Link, *Piper tuberculatum* Jacq., *Piper marginatum* Jacq., and *Piper hispidum* Kunth) and two black pepper cultivars ('Kottanadan Broto Branco' and 'Bragantina'-homograft). *P. tuberculatum* and *P. marginatum* were incompatible with *P. nigrum* 'Bragantina' in the two grafting techniques. There was total suppression of leaf development with incompatible rootstocks inside cleft grafting. The wild rootstocks had twice as many shoots at the base of the seedling than the cultivars in the top cleft grafting. Side cleft grafting was able to suppress by half the shoots at the base of the rootstock, while the aerial part was maintained. Top cleft grafting provided greater survival, shooting, and leaf emission, which was more evident when using *P. aduncum* species as rootstock, reaching practically twice the shooting (59.3%) in comparison with the side grafting (30.6%). Therefore, the top method and the use of *P. aduncum* as rootstock for the production of grafted black pepper seedlings is recommended.
**KEY WORDS:** *Piper aduncum* Link, *Piper nigrum* L., side cleft grafting, top cleft grafting.

## INTRODUCTION

Black pepper (*Piper nigrum* L., Piperaceae) is the most consumed spice in the world, and Brazil is the second-largest producer in the world. Moreover, it ranks seventh place in productivity (FAO 2020). Fusariosis, the main crop disease in Brazil, has reduced crop productivity and shelf life, as it attacks the root system, causing plant death (Rana et al. 2017). The Iaçará, Kottanadan and Singapore cultivars were characterized as highly resistant, while 'Bragantina' has medium resistance (Barata et al. 2021). These resistant cultivars are not grown commercially, while 'Bragantina' (internationally known as 'Panniyur 1') is the most widely planted one in Brazil. In Espírito Santo, the largest Brazilian producer, a cultivar popularly known as 'Kottanadan Broto Branco' emerged. Although in recent years the planting of this cultivar has expanded throughout the state, studies on it are scarce, in addition to not having registration in the national cultivar registration system of the Ministry of Agriculture, Livestock and Supply.

Grafting black pepper on wild species resistant to fusariosis can be considered a strategy for cultivation in contaminated areas. The wild species *Piper aduncum* L., *Piper colubrinum* Link, *Piper hispidum* Sw., and *Piper tuberculatum* Jacq. have been identified as resistant to fusariosis (Albuquerque et al. 2001), but compatibility with black pepper has been little studied. Grafting on resistant rootstocks began in Brazil with Albuquerque (1968), who showed the compatibility between *P. colubrinum* and black pepper using full cleft grafting (top cleft). This study was replicated in India and in Vietnam (Mathew and Rema 2000, Vanaja et al. 2007, Chinnapappa et al. 2018). *P. colubrinum* is a species native to the Amazon and is not naturally found in Espírito Santo. Therefore, studies should be expanded to evaluate other species as rootstocks.

The grafting technique has also been little studied in the production of black pepper seedlings grafted onto wild species. Although the top cleft predominates in studies with black pepper, Crasque et al. (2021) used for the first-time lateral grafting in full slit (side-cleft) in black pepper 'Bragantina' and identified *P. aduncum* and *P. hispidum* as compatible, while *P. tuberculatum* was incompatible for this grafting method. In top cleft, the rootstock is decapitated for insertion of the graft, while in lateral grafting the graft is inserted in a lateral incision that does not exceed half the diameter of the stem, maintaining the aerial part of the rootstock (Hartmann et al. 1997). The largest leaf area due to maintenance of the aerial part increases the auxins and photosynthesis, which, according to Qureshi et al. (2022), correlates positively with the seedling development.

Thus, it is hypothesized that top cleft grafting could increase the survival of compatible rootstocks and improve the development of seedlings. In addition, the decapitation of the aerial part of the side-cleft could force union between graft-rootstock, including what is considered incompatible. For this reason, the objective of this work was to investigate the compatibility and development of grafted seedlings of 'Bragantina' black pepper, using two slit grafting techniques (top and side) and six rootstocks. The rootstocks considered compatible (*P. aduncum* and *P. hispidum*) were selected, as well as the incompatible *P. tuberculatum*. 'Bragantina' was also used as a rootstock since homograft (grafting a genotype onto itself) is commonly used as a control of the grafting process (Ramírez-Jiménez et al. 2021). In addition to these, *P. marginatum* and 'Kottanadan Broto Branco' were included, never before evaluated in grafting.

## MATERIAL AND METHODS

The study was conducted in a commercial nursery for the production of black pepper seedlings, located in the municipality of São Mateus, state of Espírito do Santo, Brazil, latitude 18°41'44.59"S and longitude 40°1'19.55"W, from April to June 2021. The mean values of minimum, average and maximum temperature, accumulated rainfall, and relative humidity were 14.1, 23, and 34.8 °C, 167.6 mm, and 78.5%, respectively, obtained at the automatic weather station of the National Institute of Meteorology, in São Mateus (INMET 2021).

The experimental design was in randomized blocks, with four replications of 27 plants per plot. A 6 × 2-factorial arrangement was adopted, consisting of six rootstocks and two grafting methods. The rootstocks evaluated in the experiment were four wild species (*Piper aduncum* Link, *Piper tuberculatum* Jacq., *Piper marginatum* Jacq., and *Piper hispidum* Kunth) and two cultivars of *Piper nigrum* L. ('Kottanadan Broto Branco' and 'Bragantina'). The grafting methods denominated top cleft (TCG) and side cleft (SCG) were evaluated. In all treatments, grafts of *P. nigrum* ('Bragantina') were used as scion.

The production of seedlings of wild species lasted seven months using seeds. Seeds were collected from stock plants of the Active Germplasm Bank of the Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (INCAPER), latitude 19º25'00"S and longitude 40º04'35"W, in the municipality of Linhares, state of Espírito Santo. Germination was performed as proposed by Dousseau et al. (2011), and seedlings were transplanted as described by Dousseau et al. (2015), when they reached 5 cm, for 450-cm$^3$ tubes containing commercial organic substrate and 3 grams of slow-release fertilizer six months (NPK 15-09-12) with micronutrients, as adapted by Serrano et al. (2012). From then on, the plants were kept in a nursery covered with a 50% black shading screen, and the sides were covered with an anti-aphid screen. Irrigation was performed daily using automatic micro-sprinklers for 40 seconds every 10 minutes and a flow rate of 76 L·h$^{-1}$. The phytosanitary treatments were carried out through the application of fungicides to control anthracnose, acaricide, and insecticide to control caterpillars. Weed management was done using manual weeding.

The seedlings of the cultivars lasted six months by cloning. This experiment used stem cuttings measuring 5–6 cm, containing one node and a pair of leaves, collected from the median region of branches from stock plants grown in a clonal garden close to the nursery, according to Ambrozim et al. (2017), with adaptations. The cuttings were immersed in a fungicide solution (87.5% thiophanate-methyl) at the dose of 0.8 mL·L$^{-1}$ for 5 minutes and treated according to Secundino et al. (2018), with 4,000 mg·kg$^{-1}$ of the growth regulator indolebutyric acid, in powder. Then, they were planted in 280-cm$^3$ tubes, filled with 75% of the commercial organic substrate, added with 25% of coffee straw, fertilized with 2 grams of granulated simple superphosphate and 1 g of the slow-release fertilizer six months (NPK 16-08-12) with S, Mg, and micronutrients. They were kept in a nursery covered with a 50%-shading screen and equipped with intermittent micro-sprinkler irrigation for 60 days when they were transplanted into 450-cm$^3$ tubes, as detailed for the wild species rootstocks.

Grafting was performed in April 2021, when the rootstocks reached between 4.5 and 5.5 mm in stem diameter, measured with a caliper at 15 cm from the ground. The seedlings of the rootstocks were prepared one week before grafting by removing all the shootings from the axillary buds, as described by Aarthi and Kumar (2019). The graft was taken from 7-month-old plants grown in the same clonal garden from which the rootstocks were obtained, by selecting the orthotropic branches with a diameter similar to the rootstocks and removing the apical part and leaves. After, the branches were placed in plastic boxes, covered with moistened jute bags, taken to the grafting site, and disinfected by immersion for 5 minutes in carbendazim (500 g·L$^{-1}$) fungicidal solution (1 mL·L$^{-1}$). The graft was sectioned, maintaining two nodes and approximate size of 12 cm, making a straight cut at the apex and a double wedge-shaped bevel at the base.

For SCG, the procedures described by Crasque et al. (2021) with adaptations were used. A lateral cut of approximately 3 cm was made at a 15-cm height from the base in the rootstock, for insertion of the graft. Binding was done with a 2.5-cm wide biodegradable elastic tape, followed by disinfestation with carbendazin fungicide and covering with 6 × 25-cm transparent plastics tied with a No. 18 elastic band, just below the grafting point. In the first days after grafting, a small cut was made at the base of the plastic bags to allow the water that had accumulated inside them to drain out. After 30 days, with the emergence of the first open leaves, the plastic bags were removed, and, 15 days later, the aerial part of the rootstocks was decapitated at a height of 1 cm from the graft.

The TCG was performed according to Albuquerque (1968), with adaptations. Rootstock was decapitated at approximately 15 cm from the base, in the internode region, where a 3-cm deep vertical incision was made, and the graft was inserted, following the same procedures described for the SCG until cover removal after 30 days.

The grafting procedures can be seen in Fig. 1. The first stage is the selection and preparation of the graft (Figs. 1a and 1b). In the second stage, the grafting procedure is carried out, which depending on the technique will follow the procedures shown in Figs. 1c–1h (top cleft) and Figs. 1i–1m (side cleft). In the steps of Figs. 1c, 1d and 1i, the procedures for the preparation of the rootstock are represented. In the steps of Figs. 1e and 1j, the step of inserting the graft into the rootstock is illustrated. The tying after insertion is shown in Figs. 1f and 1k. The stage of plastic covering of the grafted region is carried out as shown in Figs. 1g and 1l. Finally, in Figs. 1h and 1m, the seedlings after all the grafting procedures are shown.

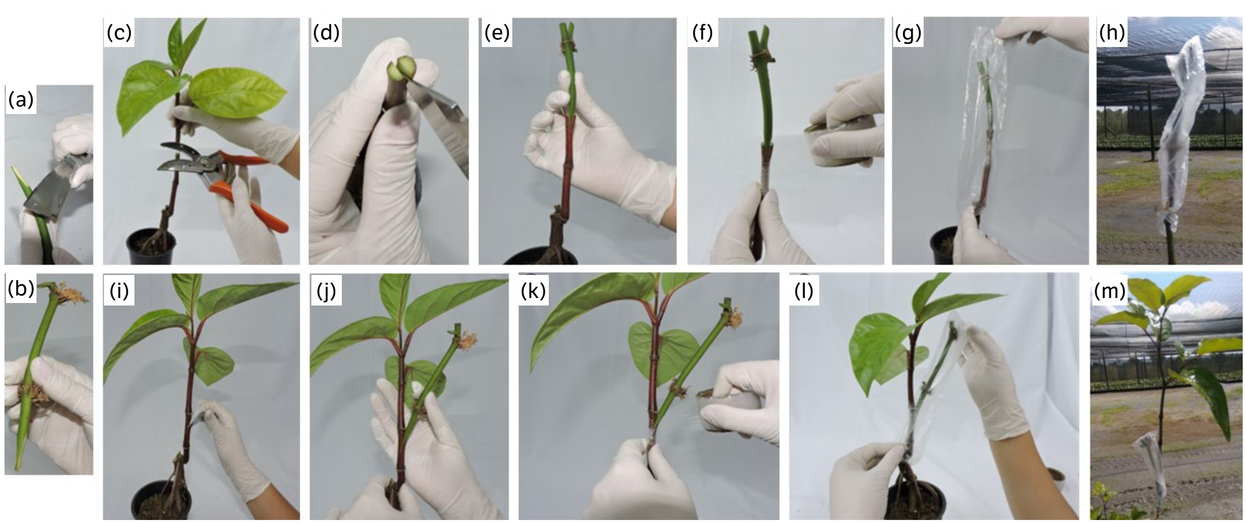


**Figure 1.** The grafting procedures starting with the (a and b) selection and preparation of the graft and the steps for (c–h) the top cleft and (i–m) side cleft.

After 30 days of grafting and fortnightly up to 90 days, evaluations were performed on the percentage of graft shooting, number of sprouts on the rootstock, and seedling survival. The percentage of graft shooting was obtained by counting the sprouts containing leaf primordia. The rootstock sprouts were counted and detached when they reached 0.5 cm. Seedlings whose grafts were dehydrated or necrotic were considered dead.

After 90 days of grafting, the seedlings were transported to the Laboratory of Plant Physiology of INCAPER, where the substrate was removed in running water, and the quality of the seedlings was quantified. The length of the rootstock and the largest sprout, stem diameter, number of sprouts, and number of leaves, as well as leaf area, were evaluated. Length measurements were obtained with a millimeter ruler. The length of the rootstock was evaluated from the rootstock base or cutting up to the sectioned part for grafting. The stem diameter was measured at the second internode of each orthotropic branch, using a digital caliper. The sprout length was determined from its insertion to the last apical bud. The leaf area was obtained using the LI-COR 3100 meter. The length of the root system was determined by the largest root. The root volume was evaluated using water displacement in a 1,000-mL beaker.

The dry biomass of the root system, rootstock, and leaves were obtained by drying in a forced air circulation oven at 70°C until constant weight, followed by weighing on a 0.01-g precision scale. The aerial part dry biomass was calculated, obtained by the sum of the rootstock dry mass with the sprout dry mass and the leaf dry mass, and the total dry biomass, by the sum of the aerial part dry biomass and dry biomass of the root system. The Dickson quality index was determined using the Dickson equation (Eq. 1) (Dickson et al. 1960):

$$DQI = TDM/[(SL/SD) + (APDM/RDM) \tag{1}$$

where: DQI: Dickson quality index; TDM: total dry biomass; SL: the largest sprout; SD: stem diameter; APDM: aerial part dry biomass; RDM: dry biomass of the root system.

The relative chlorophyll content was evaluated on a fully expanded leaf using a Portable Chlorophyll Meter, model SPAD-502, "Soil Plant Analyzer Development".

The means were submitted to data transformation using the formulas (x + 0.5)0.5, except for the percentage data, which were analyzed with their original values as they presented normal distribution. Statistical analyses were performed using the SISVAR statistical program (Ferreira 2011), performing analysis of variance and comparison of means using the Scott-Knott mean grouping test ($p<0.05$).

## RESULTS

The seedlings obtained using the wild species *P. aduncum* as rootstock and the black pepper cultivars had greater aerial part development, however, root development was superior in the wild species compared to the *P. nigrum* cultivars (Fig. 2). The TCG allowed greater compatibility expression between genotypes, except for the *P. tuberculatum* rootstock, where grafting did not develop (Fig. 2b). In the SCG, the graft did not develop in *P. tuberculatum* and *P. marginatum* (Figs. 2b and 2c).

The survival of the seedlings was progressively reduced with time after grafting, influenced by the type of rootstock and the grafting technique (Fig. 3). With the rootstock *P. aduncum*, the seedlings had greater stability in survival over time, showing higher rates during the experimental period in both grafting methods. Seedlings formed with the rootstock *P. marginatum* showed reduced survival rates in both methods. The seedlings grafted using the TCG had greater stability in survival, while in the SCG the drop was more accentuated, especially after 30 and 45 days of grafting, when the bags covering the grafts were removed, and the rootstock crowns were cut, respectively.

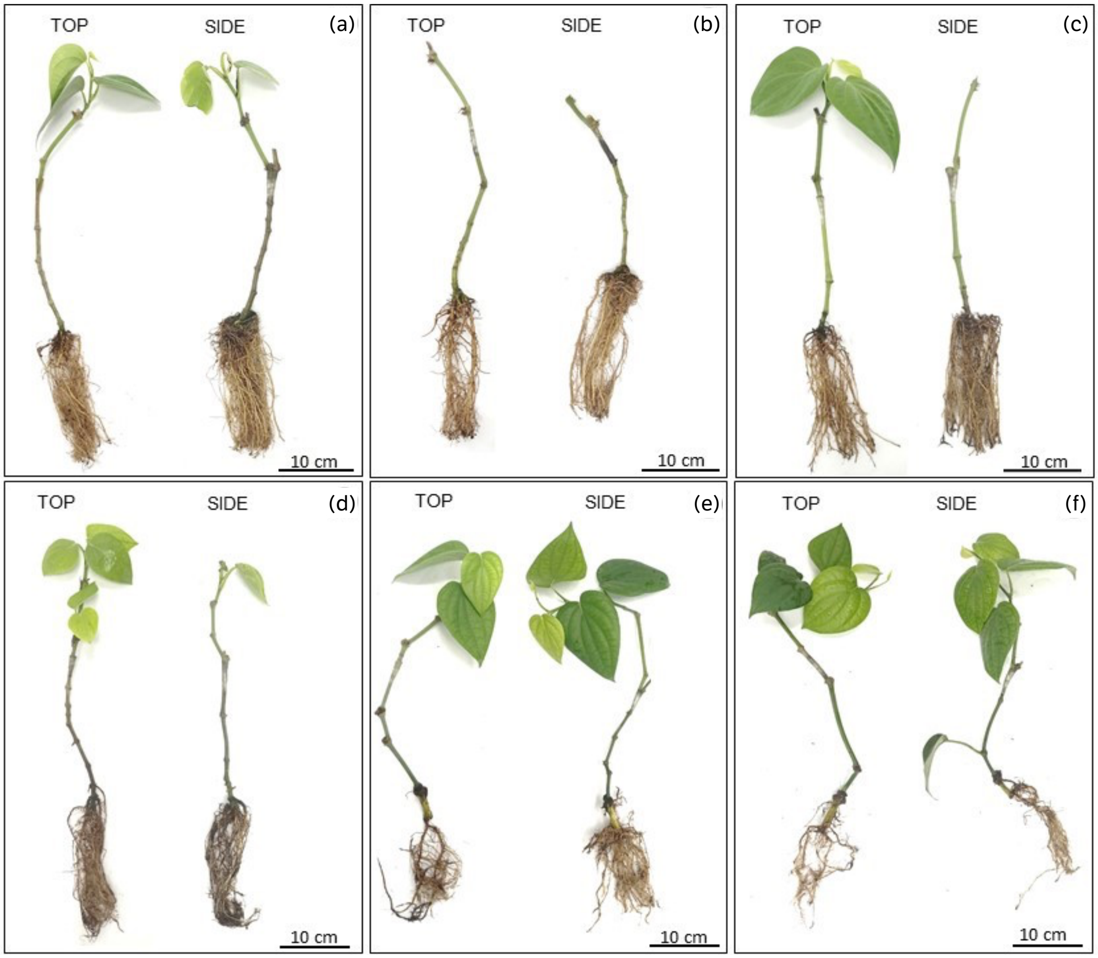


**Figure 2.** Black pepper seedlings grafted by the top and side grafting method on rootstocks: (a) *Piper aduncum*; (b) *Piper tuberculatum*; (c) *Piper marginatum*; (d) *Piper hispidum*; (e) 'Kottanadan Broto Branco'; (f) 'Bragantina' (homograft).

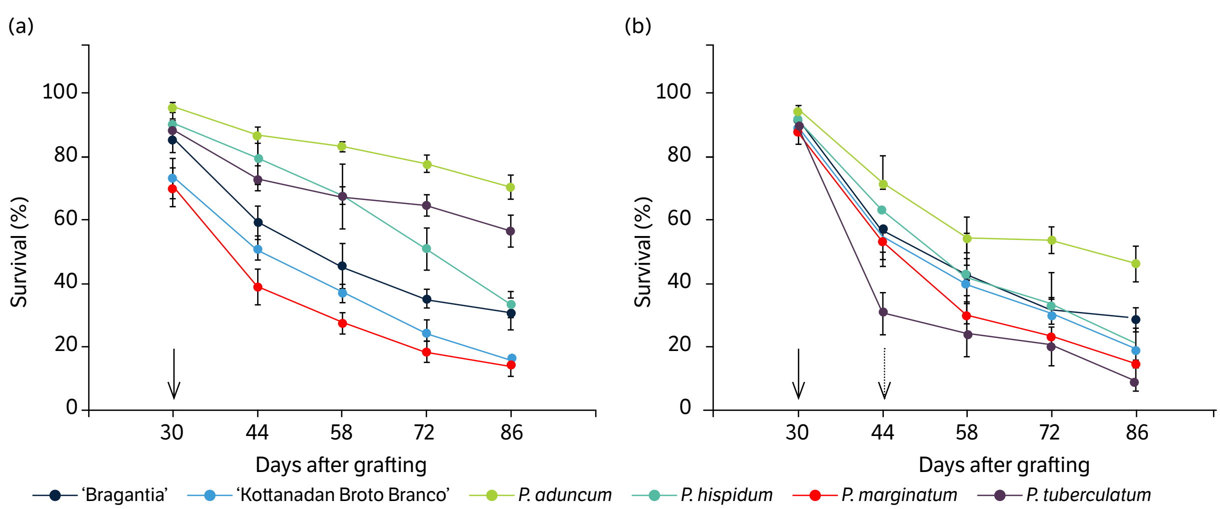


**Figure 3.** Percentage survival over time of black pepper 'Bragantina' under different rootstocks in (a) the top cleft (TCG) and (b) side cleft (SCG) grafting methods. The bar corresponds to the standard error of the mean of four repetitions. The arrows with thick lines indicate the moment of removal of the bags that covered the grafts, and the arrow with a thin line indicates the moment of cutting the crown of the rootstock in the side grafting.

The types of the assessed rootstocks and the grafting methods influenced the survival and growth of the aerial part of the grafted seedlings (Fig. 4). Black pepper grafting 'Bragantina' on *P. aduncum* rootstock provided higher survival rates in both grafting methods, but with values close to 70% on the TCG and 48% on the SCG. Although 56% of seedlings grafted on *P. tuberculatum* survived with TCG, only 11% produced shoots and 7% produced leaves (Fig. 4a). The rootstocks *P. tuberculatum* and *P. marginatum* inhibited the development of the aerial part of the seedling grafted by the side method (Fig. 4b). The two *P. nigrum* cultivars used as rootstocks also provided a lower percentage of survival when compared to *P. aduncum*.

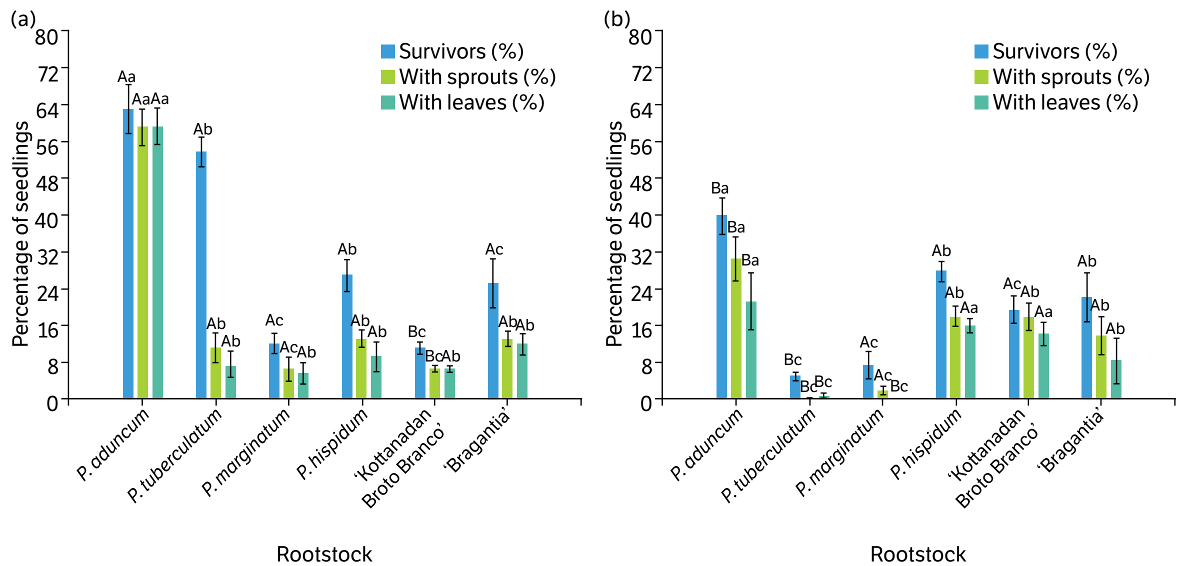


**Figure 4.** Percentage of surviving seedlings with sprouts and leaves of 'Bragantina' black pepper grafted on different rootstocks using (a) top cleft (TCG) and (b) side cleft (SCG) methods. The bar corresponds to the standard error of the mean of four repetitions. Means followed by the same letter do not differ significantly from each other by the Scott-Knott's test at the 5%-probability level. Uppercase letters compare the grafting methods for each rootstock, and lowercase letters compare the rootstocks in each method.

The rootstocks showed different vigor levels when emitting axillary sprouts in the region close to the rootstock, which was influenced by the grafting method (Fig. 5). The TCG provided higher sprout emission for all assessed genotypes, showing 67% more sprouts in relation to SCG. In the TCG, *P. hispidum* was the rootstock that sprouted the most, with the total of 107 sprouts, differing significantly from the others, followed by *P. tuberculatum* and *P. aduncum*, with averages of 94.7 and 92.5 sprouts, respectively. On the other hand, lower sprouting values were observed for *P. marginatum* and *P. nigrum* cultivars, with a lower number of sprouts emitted by 'Bragantina', with only 36 sprouts in the plot. Among seedlings submitted to SCG, the only genotype that differed significantly from the others was *P. marginatum*, which practically did not sprout.

The species used as rootstocks showed different development of the root system and the aerial part, depending on the type of graft (Fig. 6). The root volume and dry biomass of the root system of the roots were higher in the wild species and intensified by the SCG, while the development of the root system of the cultivars was lower and did not affect by the grafting technique (Figs. 6a and 6b). The root system did not differ between the grafting methods, however in the TCG *P. hispidum* was superior, while in the SCG the 'Kotadanan Broto Branco' was also outstanding (Fig. 6c). The rootstock dry mass was observed in the stem of wild species in side grafting concerning the TCG (Fig. 6d). In the SCG, the rootstocks *P. aduncum*, *P. marginatum* and 'Bragantina' presented higher dry mass, when compared to the other genotypes. In TCG, 'Bragantina' was the only genotype that differed significantly from the others in terms of dry mass, with the highest value (Fig. 6d).

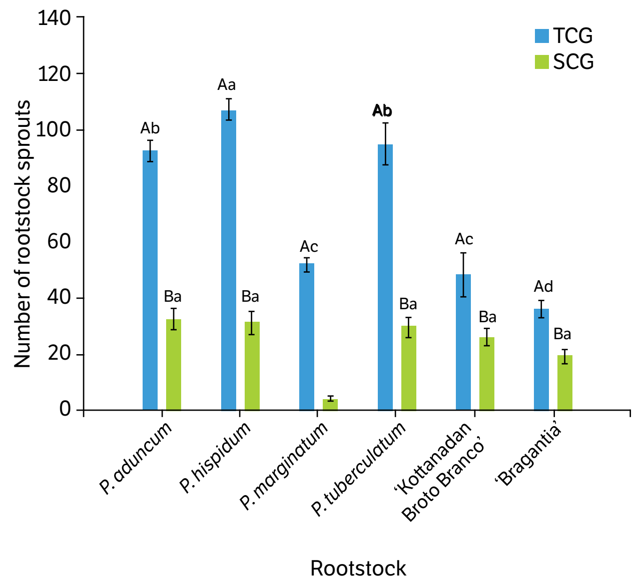


**Figure 5.** Number of rootstock sprouts at 30 days after top cleft (TCG) and side cleft (SCG). Means followed by the same upper-case letter between methods and lower case between genotypes do not differ from each other by the Scott-Knott's test at the 5%-probability level. The bar corresponds to the standard error of the mean of four repetitions.

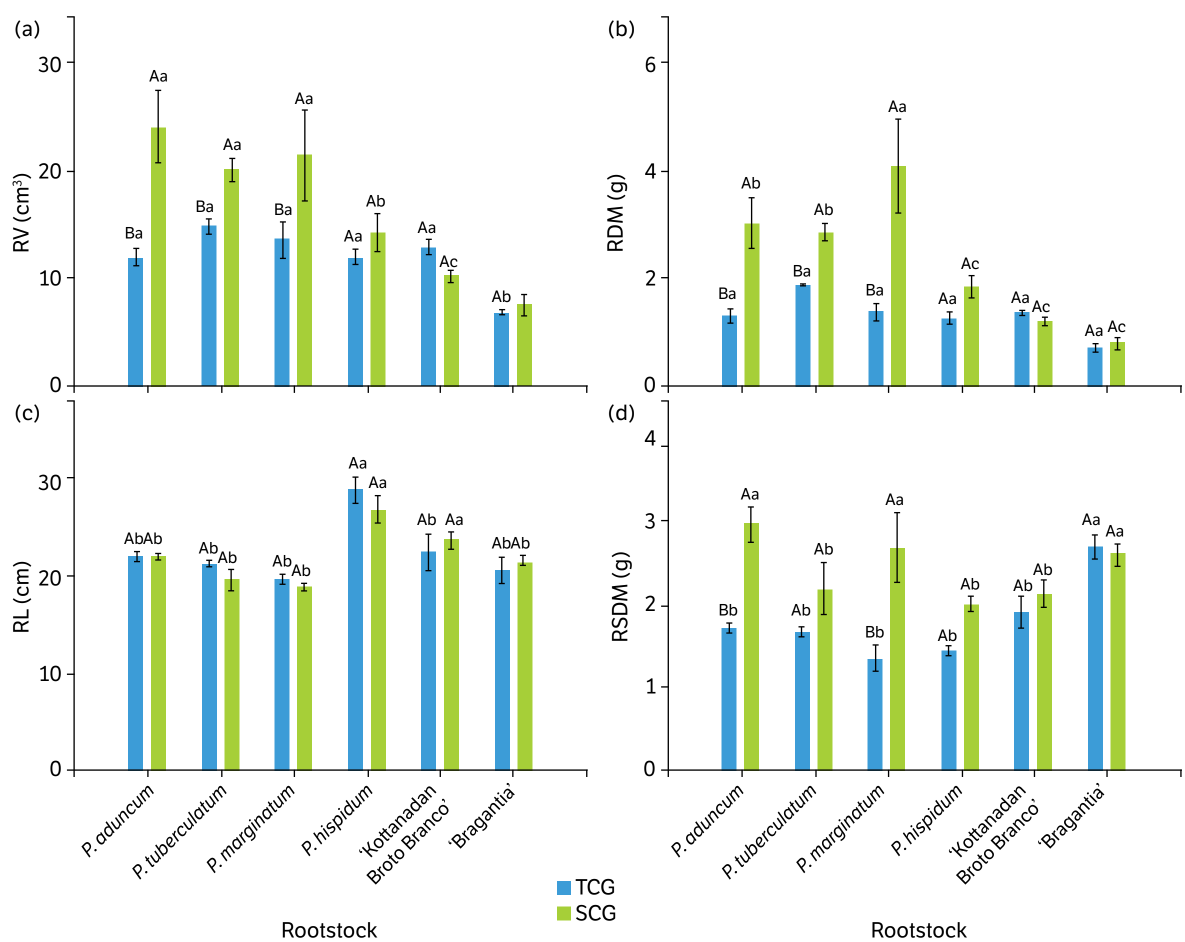


**Figure 6.** (a) Root volume (RV), (b) root dry mass (RDM), (c) root length (RL), and (d) rootstock dry mass (RSDM) of different rootstocks submitted to top cleft (TCG) and side cleft (SCG). Means followed by the same letter, uppercase letter among methods, and lowercase letter among genotypes do not differ from each other by the Scott-Knott's test at the 5%-probability level. The bar corresponds to the standard error of the mean of four repetitions.

It was not possible to detect differences between the grafting methods for the largest sprout and the dry mass of sprouts, respectively (Figs. 7a and 7b). Among the genotypes, a shorter length of the sprouts was obtained when *P. tuberculatum* was used as a rootstock in both grafting methods. The number of sprouts (Fig. 7c) and number of leaves (Fig. 7d) were higher in the wild species grafted using the TCG, in relation to the side one, however, among the cultivars, there was no influence of the grafting technique. Number of sprouts and total number of leaves were higher when *P. aduncum* was used in the TCG, while leaf development was practically inhibited in the SCG with *P. tuberculatum* and *P. marginatum*, expressed by the lowest number of leaves, leaf area and leaf dry mass (Figs. 7d–7f). In the TCG, only *P. tuberculatum* provided lower leaf area and leaf dry mass.

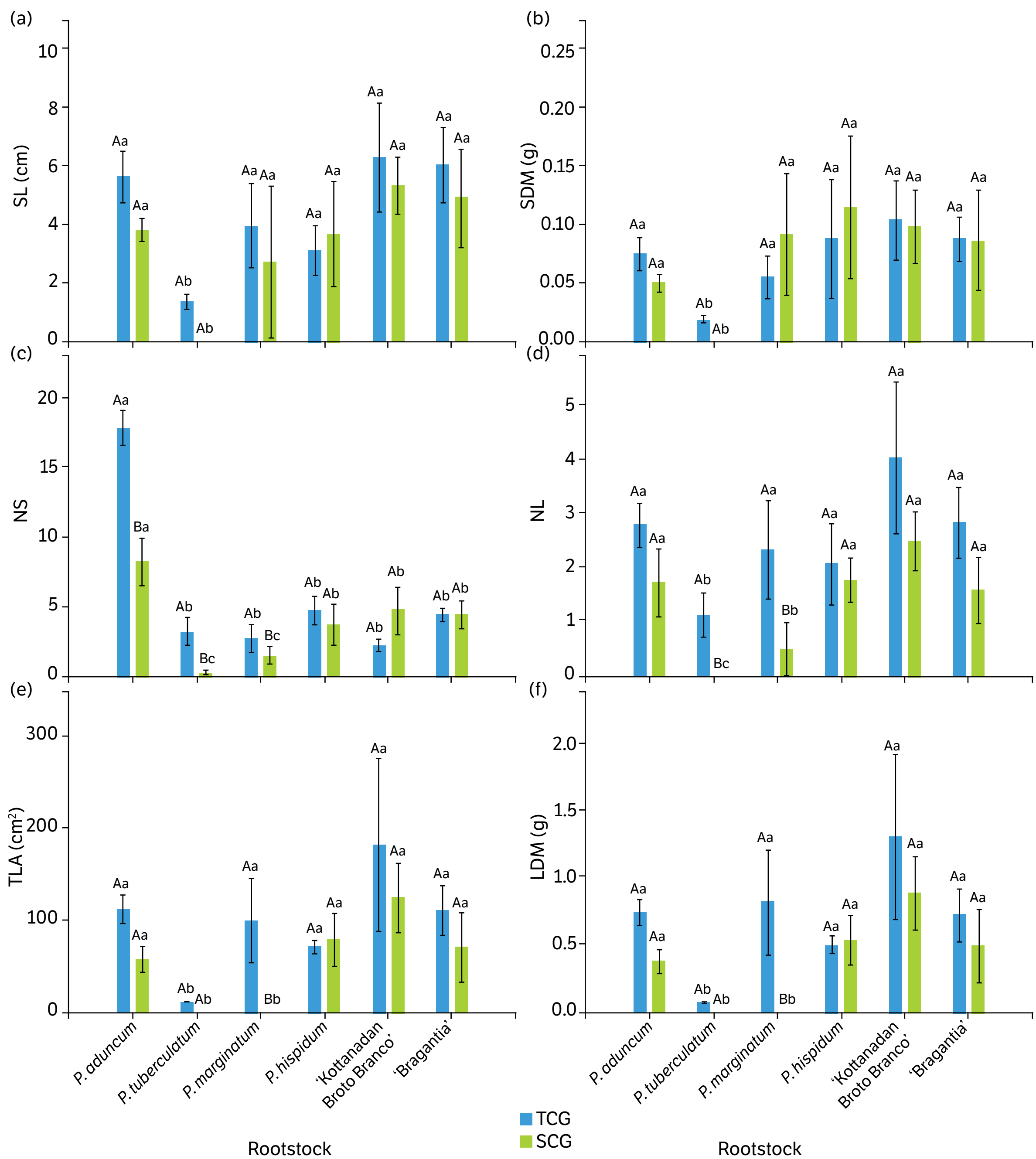


**Figure 7.** (a) Length of the sprouts (SL); (b) dry mass of sprouts (SDM); (c) total number of sprouts (NS); (d) total number of leaves (NL); (e) total leaf area (TLA); (f) leaf dry mass (LDM) of different rootstocks submitted to top cleft (TCG) and side cleft (SCG). Means followed by the same upper-case letter among methods and lower-case letters between genotypes do not differ from each other by the Scott-Knott's test at the 5%-probability level. The bar corresponds to the standard error of the mean of four repetitions.

The relative chlorophyll content was higher when using the rootstocks *P. aduncum* and *P. marginatum* in TCG, followed by 'Kottanadan Broto Branco' in both grafting methods (Fig. 8a). The seedlings grafted using SCG had higher relative chlorophyll content when grafted on *P. hispidum*, 'Kottanadan Broto Branco' and 'Bragantina'. As there was no leaf formation on the side grafts in *P. tuberculatum* and *P. marginatum*, it was not possible to evaluate the relative chlorophyll content.

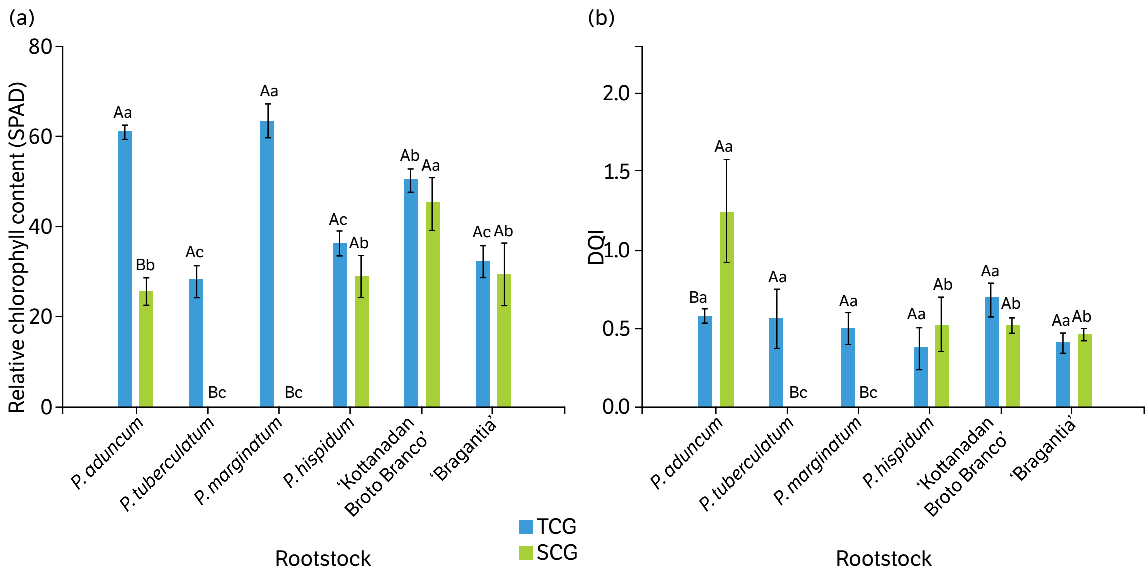


**Figure 8.** (a) Relative chlorophyll content (SPAD) and (b) Dickson quality index (DQI) of different rootstocks submitted to top cleft (TCG) and side cleft (SCG). Means followed by the same upper-case letter between methods and lower-case between genotypes do not differ from each other by the Scott-Knott's test at the 5%-probability level. The bar corresponds to the standard error of the mean of four repetitions.

The Dickson quality index was higher in the SCG in *P. aduncum*, presenting more than twice the value when compared to the other genotypes submitted to this same method. There was no significant difference between the grafting methods, except for the null results that were attributed to seedlings grafted on *P. tuberculatum* and *P. marginatum*, by the absence of leaves (Fig. 8b). Regarding the TCG, no differences were identified between the genotypes in terms of Dickson quality index.

## DISCUSSION

The superiority of grafting of the 'Bragantina' black pepper using the TCG technique in comparison to SCG was shown for wild species, especially when the rootstock *P. aduncum* was used, with almost twice as much survival, shooting, number of leaves and relative chlorophyll content. The compatibility of the *P. aduncum* rootstock has already been reported by Crasque et al. (2021) in a study of lateral grafting in which it correlated with the translocation of carbohydrates at the point of grafting.

The seedlings grafted by TCG had greater induction of axillary sprouts in the region close to the neck in all rootstocks, while in the SCG the amount produced was lower, and there were no differences between the rootstocks, except for the practically null value of *P. marginatum*. Decapitation of the rootstock in top grafting promotes the breakdown of apical dominance, which, according to Mashiguchi et al. (2021), is due to the interruption in the synthesis of auxins, and interruption, reduction of signaling for the production of strigolactones in the root, leads the cytokinins to act in the induction of axillary buds. On the other hand, by keeping the aerial part of the seedling intact for a longer time in SCG, the synthesis of auxins is maintained, which, according to Li et al. (2017), causes a greater expression of auxins over cytokinins in the rootstocks, repressing the budding of the lateral buds of the rootstock, increasing root development.

Decapitation of the rootstock in the TCG promotes the break in apical dominance caused by the interruption in the synthesis of auxins and, consequently, reduction in the signaling for the production of strigolactones in the root, leading the cytokinins to act in the induction of axillary buds (Mashiguchi et al. 2021). Higher expression of auxins over cytokinins in the rootstocks represses the shooting of lateral buds of the rootstock while increasing the grafting success and root development (Li et al. 2017).

In addition, the excess of sprouts in the rootstock leads to an increase in labor costs for the production of seedlings and the management of regrowth in the field. Therefore, it is an undesirable characteristic in the selection of compatible rootstocks. However, grafting techniques can be adjusted to reduce the incidence of shooting in the rootstock, as performed by Liu et al. (2021) for watermelon.

The SCG promoted an increase in root development in all seedlings grafted onto the rootstocks of the assessed wild species, except for *P. hispidum*, which did not differ. The induction of sprouts on the rootstock was higher in *P. hispidum*, followed by *P. aduncum* and *P. tuberculatum*, which did not differ from each other, and *P. marginatum* and 'Kottanadan Broto Branco', which did not differ from each other. The lowest value of basal sprouts was observed in the homograft, which, according to Melnyk et al. (2015), is considered a compatibility standard and serves as a control of the grafting process. However, homograft is almost not evaluated in experiments, which limits comparisons between grafting techniques.

The wild species showed greater root development compared to the assessed *P. nigrum* cultivars, a desirable characteristic in the production of black pepper seedlings and one of the major quality parameters. A well-developed root system allows for the formation of plants that are more tolerant to water and nutritional deficiencies, increasing the ability to exploit the soil and absorb water and nutrients (Francisco et al. 2015).

The TCG is the mean method used in black pepper grafting worldwide, but it had never been evaluated for the wild *Piper* species assessed in this work nor for the cultivar 'Kottanadan Broto Branco'. Most studies use the cultivar Panniyur 1 as a graft, which is registered as 'Bragantina' in Brazil (Schmildt et al. 2018), the main genotype cultivated in Brazil and worldwide. The most widespread grafting combination is black pepper 'Panniyur-1' onto *P. colubrinum*, with survival of 76.2% (Chinnapappa et al. 2018), 86% (Lakshmana et al. 2020), and ranging from 25 to 100% depending on the month (Vanaja et al. 2007).

Recently, Aarthi and Kumar (2019) evaluated another grafting method, named Stenting, in which graft union and adventitious roots formation occur simultaneously, identifying 41% of success in the grafting of 'Panniyur-1' on *Piper hymenophyllum* Miq. Experiments conducted by Nguyen et al. (2020) showed the superiority of the cleft grafting technique, in relation to Splice Graft in the production of black pepper seedlings grafted on the rootstock *P. colubrinum* in Vietnam, with 82 and 76% grafting, respectively.

The SCG was used as a grafting method for 'Bragantina' black pepper in the works by Crasque et al. (2021) and reported no differences between the rootstocks *P. aduncum, P. hispidum*, and the cultivars Kottanadan and Bragantina (autograft or homograft) in terms of survival and shooting, with mean values of 78 and 60%, respectively. These values were close to those found in this study for *P. aduncum* in the TCG, with 70% survival and 60% shooting, almost twice as much as the side one.

The grafting technique did not influence the survival and development of seedlings grafted on *P. hispidum* of the homograft. In both rootstocks, survival was close to 30%, but little more than half of that was found by Crasque et al. (2021) for the SCG. Although Crasque et al. (2021) did not observe differences in the survival of seedlings grafted on *P. hispidum*, it was observed there was a limitation in the flow of carbohydrates, with greater retention in the upper part of the grafting point.

Higher Dickson quality index values for SCG on *P. aduncum* can be attributed mainly to the greater accumulation of dry mass in the roots in relation to the aerial part of the seedlings. This index is widely used and considered as a good seedling quality indicator, as it integrates the robustness index and the balance of seedling phytomass distribution (Da Ros et al., 2018), so that, the higher the value of DQI, the better the quality of the seedling produced (Souza and Peres 2016). However, DQI values between 0.38 and 0.68 found for top grafting in the evaluated genotypes are considered good, indicating aptitude for survival when transplanted, as they tend to adapt well to field conditions, due to the greater vigor of the aerial part of seedlings. Oliveira et al. (2020), in a study on the effect of the application of indole-3-butyric acid on the growth and quality of seedlings of *P. nigrum* 'Kottanadan' propagated by cuttings found Dickson quality index = 0.476 as the highest value for the experiment.

The SCG was the best technique only for the 'Kottanadan Broto Branco', although with a value below 20% for survival and shooting and without differing from the homograft in seedling quality, except for the relative chlorophyll content.

Experiments with this cultivar are still inexistent, as, although it is widespread in Espirito Santo, it has not been registered with the Ministry of Agriculture, Livestock and Supply, a regulatory agency in Brazil.

The high mortality rates for seedlings of the Bragantina cultivar grafted on homograft and *P. hispidum*, in addition to the low value for *P. aduncum* on SCG, contrast with the superior results of Crasque et al. (2021), indicating that these results do not demonstrate a genetic incompatibility, but that external factors related to the environment affected the graft response. Studies conducted in India by Vanaja et al. (2007) evaluated eight *P. nigrum* varieties grafted monthly for one year on *P. colubrinum* rootstocks, growing these seedlings in 20 × 15-cm bags containing 800 g of a substrate (1:1:1 of barn manure, sand, and soil), under 50% shaded nursery conditions and daily irrigation. Results ranged from 25 to 100% survival, depending on the month, with the lowest value in April, one of the hottest months in the region.

Grafting development is a strong indication of a successful or a failed grafting (Aarthi and Kumar 2019). Incompatible grafts initially show similar insertion dynamics to the compatible ones, but after days or months the bond weakens and the graft are eliminated, whereas, in the compatible combinations, the graft continues to develop (Moore and Walker 1983). Although *P. hispidum* and the 'Kottanadan Broto Branco' had low grafting, the grafted seedlings developed with quality, not characterizing initial incompatibility, and strengthening the hypothesis that environmental factors may have contributed to the results.

The rootstocks *P. tuberculatum* and *P. marginatum* showed initial incompatibility with the black pepper 'Bragantina', with greater evidence in the SCG, with less than 10% shooting and inhibition of sprout growth. *P. tuberculatum* did not eliminate the graft in the TCG, and survival was 56%, but only 11% had sprouts and 7% had leaves, showing the incompatibility with the 'Bragantina' black pepper, which had already been reported by Crasque et al. (2021).

In the seedling grafted on *P. marginatum*, a yellowing followed by internode abortion immediately below the graft insertion, even after shooting, was observed, which could explain the low values of survival and aerial part development. Macêdo et al. (2020) found high concentrations of phenolic compounds in *P. marginatum*, which could explain the initial incompatibility (Pina et al. 2017). On the other hand, the seedlings grafted on *P. marginatum* that survived in the TCG had higher relative chlorophyll content, with a value similar to the rootstock *P. aduncum*.

## CONCLUSION

Under the conditions of this experiment, the TCG method pays greater survival of compatible roots and improves seedlings, although the removal of the aerial part of the change at the time of grafting stimulates greater axillary sprouts of the rootstocks.

'Kottanadan Broto Branco' is compatible with 'Bragantina', but had less survival than homograft.

The TCG of 'Bragantina' on the rootstock *P. aduncum* is recommended for the production of *P. nigrum* seedlings grafted onto wild species resistant to fusariosis.

The species *P. tuberculatum* and *P. marginatum* are incompatible for grafting with *P. nigrum* 'Bragantina' in the two grafting techniques.

## AUTHORS' CONTRIBUTION

**Conceptualization:** Arantes, L. O. and Dousseau-Arantes, S.; **Methodology:** Arantes, L. O., Dousseau-Arantes, S., Falqueto, A. R. and Ferrari, W. R.; **Investigation:** Ferrari, W. R., Cerri Neto, B., Crasque, J. and Ferreira, T. R.; **Writing – Original Draft:** Ferrari, W. R. and Dousseau-Arantes, S.; **Writing – Review and Editing:** Ferrari, W. R., Cerri Neto, B., Crasque, J., Ferreira, T. R., Souza T. C., Falqueto, A. R., Arantes, L. O. and Dousseau-Arantes, S.; **Funding Acquisition:** Arantes, L. O., Dousseau-Arantes, S. and Falqueto, A. R.; **Supervision:** Dousseau-Arantes, S.

## DATA AVAILABILITY STATEMENT

All dataset were generated and analyzed in the current study.

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