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Evaluating the effect of grafting methods and rootstocks through the analysis of chlorophyll *a* fluorescence

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Abstract

This study evaluated the side-cleft grafting in native species of the genus *Piper* (*Piper aduncum* L., *Piper hispidum* Sw., and *Piper tuberculatum* Jacq.) with BRS Kottanadan pepper cultivar as rootstocks (Experiment 1). The top-cleft grafting was evaluated in native species *Piper arboreum* Aubl., *P. aduncum*, and *P. tuberculatum* with Balankotta pepper cultivar as graft using black pepper cv. Bragantina (Experiment 2). Black pepper cv. Bragantina autograft was considered as control. The rootstock *P. tuberculatum* showed initial incompatibility with the cv. Bragantina, with survival of about 34.8% (side) and 62.5% (top) and total inhibition of graft shooting. The side and top graft on *P. aduncum* resulted in 84.0 and 47.5% of survival, respectively. The JIP-test parameters indicated better photochemical efficiency in the species grafted using the method of side-cleft grafting. Finally, the initial compatibility was associated with greater survival, shooting, and better energy flow through the electron transport chain.

Keywords: grafting; JIP test; pepper; propagation.

Introduction

Brazil is the third largest producer of black pepper according to data from the Food and Agriculture Organization of the United Nations (FAO 2020), with a production of 114,749 t of the spice in 2020. Sixty percent of the production is found in the southeast region, where the Brazilian state of Espírito Santo is the largest producer, with 60,425 t of pepper harvested in 2018 (IBGE 2018). One of the main obstacles that affect the cultivation of black

Highlights

- Compatibility was associated with better energy flow through the photosynthetic electron transport chain
- Lateral grafting was the most suitable for propagation between interspecific rootstocks
- Among the wild species, *Piper aduncum* better tolerated the stress of grafting

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Abbreviations: ABS/RC – energy absorption flux; DI_0/RC – dissipated energy flux per reaction center; ET_0/RC – photosynthetic electron transport flux per reaction center; F_0 – minimum fluorescence yield of the dark-adapted state; F_m – maximum fluorescence; $PI_{(ABS)}$ – photosynthetic efficiency performance index; RC/CS_m – density of reaction center per maximum cross-section; TR_0/RC – trapped energy flux; φD_0 – quantum yield of energy dissipation; φP_0 – maximum quantum yield of primary photochemistry.

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pepper is caused by biotic factors, such as soil diseases (fusariosis, nematodes, and *Phytophthora*), and abiotic factors (salinity, drought, and flooding) (Karmawati *et al*. 2020) also stand out.

The effects of biotic/abiotic factors are imperative in the production of various crops (Rouphael *et al*. 2018). Grafting is an effective and sustainable alternative to provide resistance to soil diseases, tolerance to abiotic stress, and increase the productivity of cultivars (Dash *et al*. 2021). Black pepper grafting on wild *Piper* species is a seedling production practice that has been spread in several countries to overcome the negative effects of biotic and abiotic stresses (Vanaja *et al*. 2007).

The major disease of black pepper in Brazil is fusariosis, caused by the fungus *Fusarium solani* f. sp. *piperis* which attacks the root system and causes the death of plants (Rana *et al*. 2017). Controlling this disease requires the extensive use of fungicides (Amira *et al*. 2017), as the low genetic variability and susceptibility of cultivars among pepper genotypes prevent the genetic control of fusariosis (Barata *et al*. 2021). Grafting in resistant wild species is the most viable strategy to control fusariosis in black pepper (Albuquerque *et al*. 2001, Chinnapappa *et al*. 2019). Amazonian *Piper* species (*Piper colubrinum* Link., *Piper aduncum* Linn., *Piper tuberculatum* Jacq., *Piper hispidinervum* C. D. C., and *Piper hispidum* Sw.) infected with *F. solani* f. sp. *piperis* have been identified as resistant to the fungus (Albuquerque *et al*. 2001). Among these species, *P. colubrinum* has been tested most as a rootstock for black pepper (Vanaja *et al*. 2007, Lakshmana *et al.* 2016, Aarthi and Kumar 2019, Anggraini *et al*. 2021). Among the grafts, most studies evaluate the cultivar Panniyur-1, registered in Brazil as cv. Bragantina (Schmildt *et al*. 2018). The grafting of this cultivar in wild species is possible; however, further studies are needed to evaluate other black pepper cultivars and wild species as rootstocks.

Besides the interaction between graft and rootstock, the method used, and the date of grafting determine the survival of the seedlings. The most used method for grafting cv. Panniyur-1 on *P. colubrinum* is top-cleft grafting, with a survival of 81.6% (Nguyen *et al*. 2020), 76.2% (Chinnapappa *et al*. 2019), 86.0% (Lakshmana *et al*. 2016), and ranging from 25 to 100% depending on the month (Vanaja *et al*. 2007). Aarthi and Kumar (2019) evaluated the 'stenting' grafting method, in which graft union and adventitious root formation occur simultaneously, with 41.1% settling of cv. Panniyur-1 grafted onto *P. hymenophyllum*. In Brazil, the method of side-cleft grafting was evaluated using the cultivars Panniyur-1 and Kottanadan as the graft. Rootstocks of *P. hispidum* and *P. aduncum* setting was approximately 35.0% (Crasque *et al*. 2021).

Selecting the wrong technique and an incompatible rootstock may cause graft abortion, which can be seen after grafting (Barreto *et al*. 2017). Poor binding between scion and stock can lead to plant death within three to five years (Baron *et al*. 2019). Studies conducted by Albuquerque (1968), Alconero *et al*. (1972), and Barriga (1982) showed that the genotypes of black pepper grafted on *P. colubrinum* are not entirely compatible, as, after four years, the plants died in the field.

Using sensitive and nondestructive tools to identify early signs of graft and rootstock incompatibility is important. The high production of nonenzymatic antioxidant activity through the accumulation of carotenoids is identified in incompatible grafts to protect and improve the photosynthetic process (Kiran *et al*. 2019). Chlorophyll-related parameters are considered important tools for the determination of the impact of various environmental stresses that influence the photosynthetic apparatus (Ghassemi-Golezani and Lotfi 2015). Therefore, chlorophyll *a* fluorescence parameters can be used as reliable markers to assess the health conditions of grafted plants. Thus, the objective of this work was to evaluate the effect of grafting methods and rootstocks through the analysis of chlorophyll *a* fluorescence, aiming to highlight the possibility of using it as an early incompatibility marker.

Materials and methods

Growth conditions and rootstock preparation: The tests were conducted on the experimental farm owned by Capixaba Institute of Research, Technical Assistance and Rural Extension (INCAPER), located at 19°25'0.1"S and 40°4'35.3"W, in the municipality of Linhares, Northern Espirito Santo state. Over the experimental periods, climate data were obtained at the automatic-weather station of INCAPER.

The first stage consisted of the production of seedlings of the plants used as rootstocks, using propagules of stock plants from the *Active Germplasm Bank* (BAG) of INCAPER. This step was carried out between June 2018 and June 2019 and the average values of maximum, minimum, and average temperature, rainfall, and relative humidity, recorded during this period were respectively 30.24°C, 21.28°C, 24.75°C, 2.51 mm, and 78%.

The seedlings of native species were obtained following Dousseau *et al*. (2011) with modifications, where pre-germination was carried out in a BOD-type chamber. In addition, after 40 d, the seedlings were transferred to 200-cell styrofoam trays, containing commercial organic substrate (*Bioplant*®). They were then maintained for 60 d in a nursery under 50% shading obtained with standard black screens, and then were transplanted into tubes with a capacity of 280 cm^3 filled with commercial organic substrate, fertilized with 2 g of slow-release fertilizer for 3 to 4 months (*Osmocote*®), and remained in the nursery with shade (50%). Grafting was performed 234 d after sowing.

Black pepper seedlings were produced as described in Ambrozim *et al*. (2017) with adaptations. Herbaceous cuttings with one node and two leaves were used, collected from the middle region of the herbaceous branch. The cuttings were treated with a solution containing the fungicide *Carbendazin* (the main active ingredient) for 5 min. After this treatment, the bases of the cuttings were immersed in a solution of growth regulator, 400 mg indolebutyric acid L^{-1} for 12 h. The cuttings were planted in

280 cm^3 tubes filled with the commercial organic substrate (*Bioplant*®) fertilized with 2 g of slow-release fertilizer for 3 to 4 months (*Osmocote*®).

Experiment execution: When the seedlings presented the grafting pattern, between 4 and 5 mm of stem diameter, two tests were set to evaluate the grafting methods between cv. Bragantina pepper plant and the intra and interspecific rootstocks. The graft was obtained from cuttings collected from 2-year-old stock plants in a commercial crop in the municipality of Linhares, state of Espírito Santo, located at 19°24'51"S, 40°1'37"W. The orthotropic branches collected were kept for 24 h in moisture burlap bags. The grafts were prepared with two leafless nodes and the base was prepared in a double wedge shape, measuring 3 cm.

Experiment 1 was carried out in a greenhouse from February to June 2019, using the side-cleft grafting method, in which three native species of the genus *Piper* (*Piper aduncum* L., *Piper hispidum* Sw., and *Piper tuberculatum* Jacq.) and the black pepper cultivar cv. BRS Kottanadan were evaluated. The mean values of the recorded maximum, minimum, and average temperature, rainfall, and relative humidity were 32.05°C, 23.01°C, 26.36°C, 2.12 mm, and 75%, respectively.

In experiment 1, which used the side-cleft grafting method, the rootstock was prepared by making a 3-cm lateral cut at the height of the fourth node, starting from the seedling neck. After inserting the graft in the rootstock, wrapping was performed using no. 18 rubber elastic. After that, the fungicide *Carbendazin* (active ingredient) was sprayed and the graft was covered with transparent plastics measuring 6 × 23 cm, tied just below the grafting point. Between 28 and 30 d as the first open leaf appeared, the plastic bags were removed and after 15 d the aerial part of the rootstock was decapitated.

Experiment 2 was conducted from April to August 2019 using the top-cleft grafting method in which three native species of the genus *Piper* (*P. arboreum* Aubl., *P. aduncum*, *P. tuberculatum*) and cv. Balankotta were evaluated. In both tests, the homograft (grafting cv. Bragantina on itself) was used as a control. The mean values of maximum, minimum, and average temperature, rainfall, and relative humidity recorded were 28.79°C, 19.71°C, 23.25°C, 1.79 mm, and 78%, respectively.

In experiment 2, the cleft-grating method was performed as completely described in Albuquerque (1968), with adaptations. The rootstocks were decapitated just below the fourth node, in which a perpendicular cleft was made, with a depth of 3 cm, and after inserting the graft, wrapping was performed using a no. 18 rubber elastic. Spraying was carried out using *Carbendazin* and the graft was covered with transparent plastic bags of 6 × 23 cm, being wrapped just below the graft, until obtaining the leaf, when the plastic bags were removed.

After grafting, all plants were kept in a nursery with a 50% shading screen with sprinkler irrigation four times a day, at 7:30, 11:00, 12:30, and 3:00 h, for 25 min. Also, fungicide was applied when necessary.

When the seedlings reached the commercial standard (about three months after grafting), the survival, considering the total dryness of the graft, the shooting, considering the permanence of the shoot in the graft, and chlorophyll *a* fluorescence transients were evaluated. In experiment 1, the percentage of survival and shooting was obtained at 95 d after grafting and the chlorophyll *a* fluorescence was evaluated at 98 d. In experiment 2, survival and shooting were evaluated at 88 d and chlorophyll *a* fluorescence at 102 d.

Chlorophyll *a* fluorescence was evaluated using a portable fluorometer (*Handy-PEA, Hansatech*, UK). The expanded apical leaves were dark-adapted using appropriate leaf clips (*Hansatech*, UK) for 30 min, a period for complete oxidation of the photosystem. After that, a saturating flash of light of 3,000 μmol(photon) m^{-2} s^{-1} was emitted for one second, and the readings were taken between 8:00 and 9:00 h. From the fluorescence intensities, the parameters of the JIP-test, described in the Appendix (Strasser and Strasser 1995) were calculated using the *Biolyzer* software (Bioenergetics Laboratory, University of Geneva, Switzerland).

Statistical analysis: The experimental design adopted in both experiments was the randomized blocks, with four replications of 25 seedlings per plot. Statistical analyses were performed using the *SISVAR* statistical program *version 4.3* (Ferreira 2011). The means were submitted to the *Scott-Knott* cluster test at a significance level of 5%.

A joint analysis of the two methods was performed to evaluate the relationship between survival and sprouting of rootstocks (*P. aduncum*, *P. tuberculatum*, and cv. Bragantina). The joint fluorescence analysis was based on a simple 2 × 2 factorial, with two rootstocks (*P. aduncum* and cv. Bragantina) and two grafting methods. To analyze the relationships between chlorophyll *a* fluorescence, survival, and shooting in the different grafting methods, *Pearson*'s correlation coefficient was obtained, using the means of the treatments, with the aid of the *R* software (*R Studio 4.2.1*).

Results

Survival and shooting parameters: In the side-cleft grafting method (Fig. 1*A*,*B*), the rootstock *P. tuberculatum* showed a lower survival percentage (34.8%) and total inhibition of graft sprouting (Fig. 1*B*), it was not possible to assess chlorophyll *a* fluorescence, which did not differ from each other (Fig. 1*A*,*B*).

In top-cleft grafting (Fig. 1*C*), all the evaluated rootstocks differed from the homograft, with the highest percentage of survival obtained with cv. Balankotta (92.5%), followed by cv. Bragantina (73.8%), and the wild species *P. arboreum* (45.0%), *P. aduncum* (47.5%), and *P. tuberculatum* (62.5%), which did not differ from each other. *P. tuberculatum* also did not produce enough shooting to assess chlorophyll *a* fluorescence (Fig. 1*D*). Cv. Balankotta provided the highest shooting percentage (68.8%), followed by cv. Bragantina (55.0%) and the wild

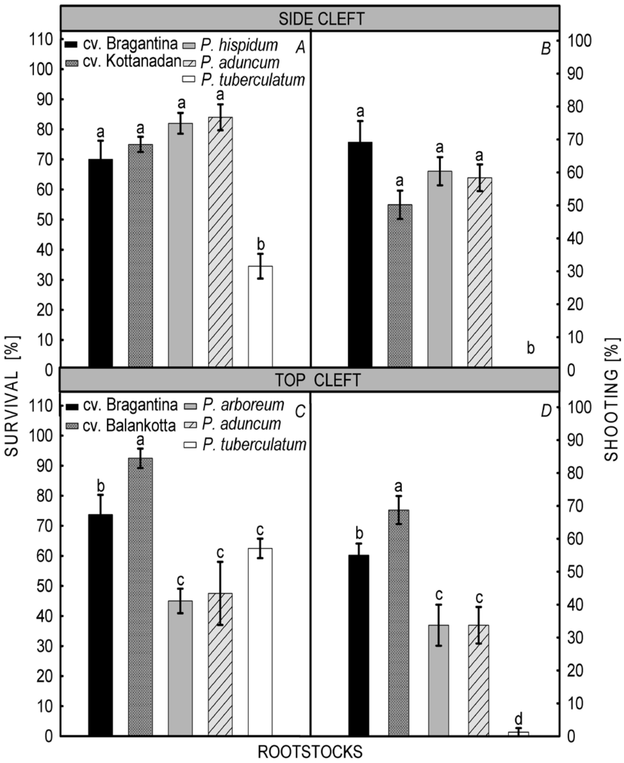

Fig. 1. Percentage of survival and shooting of black pepper cv. Bragantina on different rootstocks in the side-cleft (*A*,*B*) and top-cleft (*C*,*D*) grafting methods. Means followed by *the same letter* do not differ from each other by the *Scott-Knott* test at the 5% probability level. *The bar* corresponds to the standard error of the mean of four repetitions.

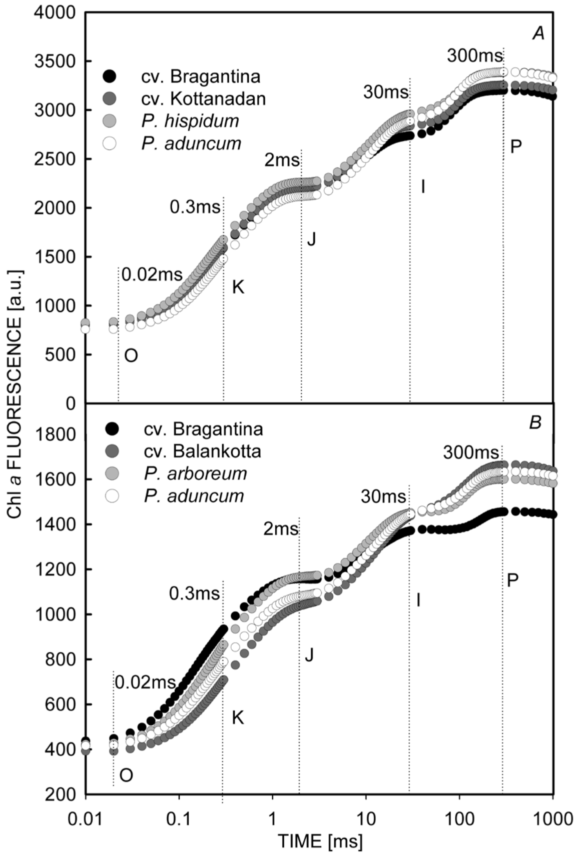

Fig. 2. Polyphasic curve of chlorophyll *a* fluorescence emission from the *Piper nigrum* graft on different rootstocks. (*A*) Side-cleft grafting and (*B*) top-cleft grafting methods.

species, *P. arboreum* (33.8%) and *P. aduncum* (33.8%), did not differ from each other (Fig. 1*D*).

OJIP curves and JIP-test parameters: All rootstocks in the two assessed grafting methods remained photosynthetically active, and showed a typical polyphasic increase in OJIP transients, except for *P. tuberculatum* which was not evaluated (Fig. 2). In the side-cleft grafting method, no increase was observed in F_0 and F_m, compared to the cv. Bragantina homograft (Fig. 2*A*, Table 1). In top-grafting, no changes were observed for F_0. However, an increase was observed in F_m in all treatments compared to the homograft (Fig. 2*B*).

Significant differences between the rootstocks in side-cleft grafting were not observed for any of the technical parameters obtained in the JIP-test (Table 1). In the top-cleft grafting, significant differences were observed for most of chlorophyll *a* fluorescence parameters, except for the parameters F_0, F_m, and photosynthetic electron transport flux per reaction center (ET_0/RC).

Higher values of absorption per active reaction center (ABS/RC), dissipation (DI_0/RC), and quantum yield of energy dissipation (φD_0) were observed for the homograft (Table 1). The photochemical quantum yield (φP_0) was higher for grafting in wild species and cv. Balankotta than for the homograft. For the trapped energy (TR_0/RC), lower values were obtained for wild species. The reaction center density per maximum cross-section (RC/CS_m) was the highest for *P. aduncum* and *P. arboreum*, followed by cv. Balankotta and cv. Bragantina. Higher values of $PI_{(ABS)}$ were observed for the wild species *P. arboreum* and *P. aduncum* (Table 1).

Fluorescence, survival, and shooting parameters: The types of the assessed rootstocks and the grafting methods influenced the survival and shooting of the grafted seedlings (Table 2). The homograft provided the highest survival percentage and sprouting among the species and in the grafting methods (Table 2). Black pepper grafting on *P. aduncum* and cv. Bragantina rootstocks had the highest survival percentage (70 and 84%) and shooting (69 and 58%) in the side-cleft grafting method. In top-cleft grafting, *P. aduncum* had the lowest survival value (48%), however, *P. tuberculatum* showed the lowest shooting percentage (1%).

Significant interactions were observed between grafting methods and rootstock combinations only for the trapped flux parameter TR_0/RC (Table 3). The cultivar Bragantina showed the highest TR_0/RC value in the top-cleft grafting method, while *P. aduncum* did not differ between the methods. When comparing species in the method, TR_0/RC for *P. aduncum* did not differ from

Table 1. JIP-test analysis of cv. Bragantina black pepper grafted on different rootstocks using the side-cleft and top-cleft grafting methods. F_0 – minimum fluorescence yield of the dark-adapted state, F_m – maximum fluorescence, ABS/RC – energy absorption flux, TR_0/RC – trapped energy flux, ET_0/RC – photosynthetic electron transport flux per reaction center, DI_0/RC – dissipation of absorbed energy per reaction center, RC/CS_m – reaction center density per maximum cross-section, φP_0 – maximum quantum yield of primary photochemistry, φD_0 – quantum yield of energy dissipation, $PI_{(ABS)}$ – photosynthetic efficiency performance index. Means followed by *the same letter* in the column do not differ significantly from each other by the *Scott-Knott* cluster test at 5% probability.

Rootstocks	F_0	F_m	ABS/RC	TR_0/RC	ET_0/RC	DI_0/RC	RC/CS_m	φP_0	φD_0	$PI_{(ABS)}$
Side-cleft grafting										
cv. Bragantina	743.63[a]	3,202.00[a]	3.00[a]	2.10[a]	0.87[a]	0.90[a]	1,153.20[a]	0.71[a]	0.29[a]	9.03[a]
cv. Kottanadan	717.75[a]	3,253.50[a]	2.92[a]	2.11[a]	0.91[a]	0.82[a]	1,125.17[a]	0.72[a]	0.28[a]	8.46[a]
P. hispidum	742.50[a]	3,386.88[a]	3.09[a]	2.22[a]	1.00[a]	0.87[a]	1,136.47[a]	0.72[a]	0.28[a]	8.69[a]
P. aduncum	678.88[a]	3,388.63[a]	2.66[a]	2.00[a]	0.99[a]	0.66[a]	1,285.52[a]	0.75[a]	0.25[a]	12.00[a]
CV [%]	8.92	9.38	16.28	12.64	7.78	26.99	15.72	4.10	10.96	47.00
Top-cleft grafting										
cv. Bragantina	413.50[a]	1,457.00[a]	4.18[a]	2.61[a]	0.82[a]	1.57[a]	363.20[c]	0.64[b]	0.36[a]	2.37[b]
cv. Balankotta	386.25[a]	1,600.88[a]	3.27[b]	2.25[a]	0.87[a]	1.02[b]	505.58[b]	0.70[a]	0.30[b]	5.01[b]
P. arboreum	356.33[a]	1,658.33[a]	2.64[b]	1.95[b]	0.92[a]	0.69[b]	640.09[a]	0.74[a]	0.26[b]	11.53[a]
P. aduncum	359.50[a]	1,663.38[a]	2.50[b]	1.84[b]	0.96[a]	0.66[b]	685.51[a]	0.74[a]	0.26[b]	12.90[a]
CV [%]	1.06	9.40	16.44	10.41	17.02	33.06	15.96	5.58	13.26	28.58

Table 2. Percentage of setting and shoots of black pepper cv. Bragantina grafted on different rootstocks using the side-cleft grafting method (Side cleft) and the top-cleft rafting method (Top cleft). Means followed by *the same letter* do not differ significantly from each other by the *Scott-Knott* cluster test at 5% probability. *The uppercase letter* in the row compares the grafting methods for each rootstock and *the lowercase letters* in the column compare rootstocks in each method.

Rootstocks	Survival [%]		Shooting [%]	
	Side cleft	Top cleft	Side cleft	Top cleft
cv. Bragantina	70.00[Aa]	73.75[Aa]	69.25[Aa]	55.00[Ba]
P. aduncum	84.00[Aa]	47.50[Bb]	58.25[Aa]	33.75[Bb]
P. tuberculatum	34.75[Bb]	62.50[Aa]	0.00[Ab]	1.25[Ac]

cv. Bragantina in the side-cleft grafting method, and for the top-cleft grafting method, *P. aduncum* showed a lower value.

The side-cleft grafting method showed higher values for F_0, F_m, and RC/CS_m when compared to the top-cleft method and did not differ for the parameter ET_0/RC (Table 3). The parameters ABS/RC, DI_0/RC, and φD_0 were influenced by the species, with higher values obtained for cv. Bragantina. *P. aduncum* showed higher values for the parameters φP_0 and $PI_{(ABS)}$ (Table 3).

The information obtained from this experiment was submitted to the estimation of *Pearson*'s correlation coefficients (*r*) at a significance level of 5% probability. Positive and negative relationships were observed between the variables analyzed in the grafting experiment (Fig. 3). Survival was positively correlated with shooting. The F_0 and F_m positively correlated with RC/CS_m. Also, φP_0 was positively correlated with ET_0/RC and $PI_{(ABS)}$. The φP_0 was negatively correlated with ABS/RC, TR_0/RC, and DI_0/RC. ABS/RC was positively correlated with TR_0/RC and with DI_0/RC. The φD_0 positively correlated with ABS/RC, TR_0/RC, and DI_0/RC and negatively with φP_0, ET_0/RC, and $PI_{(ABS)}$. DI_0/RC had a positive correlation with φD_0, ABS/RC, and TR_0/RC and also had an inverse correlation with ET_0/RC, φP_0, and $PI_{(ABS)}$. The $PI_{(ABS)}$ had a strong correlation with φP_0 and ET_0/RC.

Discussion

Grafting is successful when the plant shows a perfect junction, caused by the uniformity of the material in terms of diameter, and adequate welding of the tissues in the graft region (Roncatto *et al.* 2011). Thus, when grafting was performed using a side grafting in a side cleft, a successful setting was observed (Fig. 1, Table 2). The grafting of *P. arboreum* was tested on another species of the genus *Piper*, *P. wallichii* (Miq.) Hand.-Mazz., however, was considered incompatible, as it did not show a good establishment between graft and rootstock (John *et al.* 2020). Initial compatibility has also been reported for *P. tuberculatum* (Crasque *et al.* 2021). Initial compatibility is very important for the successful production of grafted seedlings.

As the two experiments in this article were carried out at different times, the results demonstrated here may have been influenced by variations in temperature, humidity, and precipitation, which may have favored survival or

Table 3. JIP-test analysis of cv. Bragantina black pepper grafted on cv. Bragantina and *Piper aduncum* rootstocks in the side-cleft grafting method and top-cleft grafting method. Means followed by *the same uppercase letter* in the row or *lowercase letter* in the column do not differ significantly from each other, by the *Scott-Knott* cluster test at 5% probability. Side-cleft grafting method (Side cleft), top-cleft grafting method (Top cleft).

Rootstocks	Side cleft	Top cleft	Means
F_0			
cv. Bragantina	743.63	413.5	
P. aduncum	678.88	359.5	
Means	711.25^a	386.5^b	
F_m			
cv. Bragantina	3,202.00	1,457.00	
P. aduncum	3,388.63	1,663.37	
Means	3,295.31^a	1,560.18^b	
ABS/RC			
cv. Bragantina	3.00	4.18	3.59^A
P. aduncum	2.66	2.50	2.58^B
TR_0/RC			
cv. Bragantina	2.10^{Ba}	2.60^{Aa}	
P. aduncum	2.00^{Aa}	1.84^{Ab}	
ET_0/RC			
cv. Bragantina	0.87	0.81	
P. aduncum	0.99	0.91	
DI_0/RC			
cv. Bragantina	0.90	1.57	1.24^A
P. aduncum	0.66	0.66	0.66^B
RC/CS_m			
cv. Bragantina	1,153.20	363.20	
P. aduncum	1,285.52	685.50	
Means	1,219.36^a	524.35^b	
φP_0			
cv. Bragantina	0.71	0.64	0.67^B
P. aduncum	0.75	0.74	0.74^A
φD_0			
cv. Bragantina	0.29	0.36	0.33^A
P. aduncum	0.25	0.25	0.25^B
$PI_{(ABS)}$			
cv. Bragantina	9.03	2.37	5.70^B
P. aduncum	12.00	12.90	12.45^A

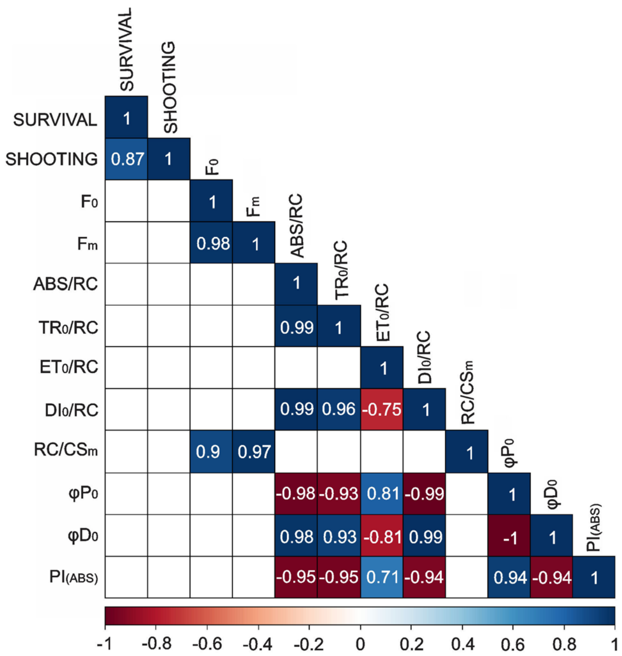

Fig. 3. *Pearson*'s correlation matrix between chlorophyll *a* fluorescence parameters and survival and shooting of side-cleft and top-cleft grafting methods. Survival – survival percentage, Shooting – shooting percentage, F_0 – minimum fluorescence yield of the dark-adapted state, F_m – maximum fluorescence, ABS/RC – energy absorption flux, TR_0/RC – trapped energy flux, ET_0/RC – photosynthetic electron transport flux per reaction center, DI_0/RC – dissipated energy flux per reaction center, RC/CS_m – density of reaction center per maximum cross-section, φP_0 – maximum quantum yield of primary photochemistry, φD_0 – quantum yield of energy dissipation, $PI_{(ABS)}$ – photosynthetic efficiency performance index.

mortality depending on the method. However, it is of interest to provide an insight into the functioning of grafted plants using different intra and interspecific rootstocks and different methods.

In the literature, studies reported initial success in black pepper grafting. Lakshmana *et al*. (2016) used the top-cleft grafting method for grafting cv. Panniyur-1 species on *P. colubrinum* and observed 86.0% success of plants grafted from orthotropic branches and 95.0% of plants grafted from plagiotropic branches. Thus, the choice of the branch also interferes with the success of the graft.

Chinnapappa *et al*. (2019) investigated grafting with two-node cuttings, as in the present study, and the highest rate of setting was in the grafting of Karimunda and *P. colubrinum*, with the highest graft success of 81.0%, followed by 76.0% for cv. Panniyur-1 grafted onto *P. colubrinum* rootstock, with the lowest success rate of 39.0% observed on cv. Panniyur-1 grafted onto IISR Sakthi rootstock at 90 d after grafting. Nguyen *et al*. (2020) observed 82.0% of *P. nigrum* graft survival on *P. colubrinum*. Crasque *et al*. (2021) considered *P. aduncum* grafted on cv. Bragantina compatible, as in the present study, due to the lower impediment of carbohydrate flow, aerial part formation, and survival.

The species *P. colubrinum* is the main rootstock used and the plants grafted on *P. colubrinum* may exhibit a maximum setting of 95.0%, being mainly influenced by the month or season of grafting; the best time for pepper grafting is in late winter/early spring (Vanaja *et al*. 2007), with a better setting and shooting release.

The successful grafting was significantly influenced by the species. The method also influenced the setting, and the wild species did not differ from cultivars in experiment 1 but in experiment 2, they had the lowest values of survival and shooting. De Paiva *et al*. (2015) also observed higher shooting values of pomegranate seedlings for the side-cleft method than for the top-cleft method. It is believed that this difference, which was observed in the present study, is attributed to the species and the type of technique used.

The behavior of the photosynthetic apparatus of the grafts through the fluorescence of chlorophyll *a* in the OJIP showed an increase in the magnitude of F_0 to F_m, with well-defined transient midpoints, indicating that the samples were photosynthetically active in all treatments (Fig. 2).

Regarding the parameters of the JIP-test, the absence of a significant difference in experiment 1, for F_0 (Table 1) in the different treatments, shows that the electron flux from the reaction center to the Q_A was maintained for all treatments, keeping a balanced energy retention flow (Fracheboud *et al*. 2004). The energy flows ABS/RC, TR_0/RC, ET_0/RC, and DI_0/RC did not show significant differences. Thus, the high efficiency of excitation energy conversion was preserved.

Biotic and abiotic stresses may reduce the quantum efficiency of PSII photochemistry (Guo *et al*. 2019). Higher φP_0 values imply a higher quantum yield on the donor and acceptor sides of the PSII. However, in the side-cleft grafting method, *P. aduncum* presented φP_0 values of 0.75, a result of the functionality of the photosynthetic apparatus, indicating that these rootstocks were stress-free, as stress-free plants present optimal values of φP_0 between 0.75 and 0.85 for most species (Bolhàr-Nordenkampf *et al*. 1989). The absence of significant differences for φP_0 in the side-cleft grafting method is reflected in a higher value of $PI_{(ABS)}$ (da Silva *et al.* 2016).

In experiment 2, no significant difference was observed in F_0 and F_m in the rootstocks compared to the control (Table 1), indicating an efficient flow of electron transport in the PSII donor, with greater energy utilization and less nonphotochemical dissipation (Kalaji *et al*. 2014).

Regarding the flow parameters per reaction center (RC), grafting on the *P. aduncum* and *P. arboreum* rootstocks showed a lower TR_0/RC value, which corresponds to an increase in active RCs (Meng *et al.* 2016). However, the lowest observed values of absorbed and trapped energy did not result in a decrease in the electron transport energy (ET_0/RC). The capture and transfer of energy from the antennas to the reaction centers led to high electron-transport efficiency (ET_0/RC) of the PSII, followed by a low energy dissipation (DI_0/RC) in the cv. Blankotta rootstocks. *P. arboreum* and *P. aduncum* (Dai *et al*. 2017), therefore, maintained their functional capacity for energy absorption and dissipation. These responses in PSII functionality were also confirmed by the increase in active RCs in *P. aduncum* and *P. arboreum* (Ferrante and Maggiore 2007).

The parameters of φP_0 and φD_0 increased and decreased, respectively, in wild species, indicating efficiency in the transport dynamics and use of excitation energy (Zhuo *et al.* 2017). The increase in φP_0 is indicative that a large part of the photons absorbed by the antenna's PSII Chl was converted into chemical energy (Kalaji *et al*. 2014). This result is supported by the increase in the value of $PI_{(ABS)}$. The performance index $PI_{(ABS)}$ has been considered a sensitive parameter in the detection and quantification of stress in plants (Oukarroum *et al*. 2007). Therefore, top-cleft grafting did not harm the PSII of the wild species rootstocks, as they had a higher $PI_{(ABS)}$ value compared to the control, with better photosynthetic capacity (Zhuo *et al*. 2017).

The results of the joint analysis of variance for the parameters of the JIP-test (Table 3) show that there was no interaction between the grafting methods and the compared species *P. aduncum* and cv. Bragantina for most of the variables. Values F_0 and F_m were higher in side-cleft grafting and decreased in top-cleft grafting. F_0 can increase if the PSII reaction centers have been affected or if the excitation energy transfer from the antenna complex to the reaction center is impaired (Baker and Rosenqvist 2004). However, a decrease in F_m can be detected when plants are under stress conditions (Li *et al*. 2012, Rattan *et al*. 2012).

P. aduncum had a lower TR_0/RC value when compared to cv. Bragantina in the top-cleft grafting method which may be explained by greater activation of RCs (Meng *et al.* 2016). The different grafting methods maintained the functionality of the electron transport chain from the donor side of the PSII to the reduction of the PSI end receptors, as there were no significant differences in the electron transport flux parameter (ET_0/RC) for cv. Bragantina and *P. aduncum*. *P. aduncum* showed lower energy dissipation flux, and higher RC/CS_m value in the side-cleft grafting method, consequently, a greater energy use (Yusuf *et al*. 2010). *P. aduncum* showed better results than cv. Bragantina regarding the parameters $PI_{(ABS)}$ and φP_0.

The correlations showed that the PSII had high use of energy, whereas the ET_0/RC was positively correlated with $PI_{(ABS)}$. This same linear growth behavior as a function of the energy transport flow can be observed in the experiment carried out by Oukarroum *et al*. (2007), who studied barley (*Hordeum vulgare* L.) in drought conditions. It can also be seen that the performance index response depends on the ABS/RC level. The fact that the energy absorption flux was correlated with φD_0 suggests that this was provided by the protection ability of the plant as it receives a large amount of light energy in photosynthesis, and it can dissipate it.

Conclusions: The species *P. tuberculatum* showed initial incompatibility regardless of the methods. Side-cleft grafting is more suitable for wild species, as it resulted in greater survival, shooting, and activity of electron transport of both PSI and II. Furthermore, the wild species had a greater ability to conserve and use light energy for chemical energy, with higher values of φP_0 and $PI_{(ABS)}$. For *P. aduncum*, the side-cleft grafting method is more suitable, as it maintained greater functionality of the photosynthetic apparatus and survival. In top-cleft grafting, greater survival and shooting of cv. Balankotta was associated with better performance of flow and electron transport and dissipation. However, for the top-cleft grafting method evaluated, the PSII reaction centers were compromised, since the F_0 values were higher in the side-cleft grafting.

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Appendix. Abbreviations of the parameters, formulas, and description of the data derived from the chlorophyll *a* fluorescence transients. For review *see* Strasser *et al.* (2004).

Fluorescence parameter	Description
Extracted fluorescence parameters	
F_0	Initial fluorescence
F_m	Maximum fluorescence
F_t	Fluorescence at time t after the start of actinic illumination
$F_{20\,\mu s}$	Minimum fluorescence signal measured at 20 µs (corresponds to F_0)
$F_{300\,\mu s}$	Fluorescence intensity at 300 µs
$F_J = F_{2\,ms}$	Fluorescence intensity at 2 ms
$F_I = F_{30\,ms}$	Fluorescence intensity at 30 ms
$F_P = F_{300\,ms} \approx F_m$	Fluorescence intensity at 300 ms
Calculated parameters	
$F_0 \cong F_{50\mu s}$ or $\cong F_{20\mu s}$	Minimal fluorescence, when all PSII RCs are open (at t = 0)
$F_m = F_P$	Maximal fluorescence, when all PSII RCs are closed
$ABS/RC = (M_0/V_J)/\varphi P_0$	Absorption flux per RC
$TR_0/RC = M_0/V_J$	Trapped energy flux per RC (at t = 0)
$ET_0/RC = (M_0/V_J) \times \psi E_0$	Electron transport flux per RC (at t = 0)
$DI_0/RC = ABS/RC - TR_0/RC$	Dissipated energy flux per RC (at t = 0)
$ABS/CS_m = F_m$	Absorption flux per CS, approximated by F_m
$\varphi P_0 = TR_0/ABS = [1 - (F_0/F_m)] = F_V/F_m$	Maximum quantum yield of primary photochemistry at (t = 0)
$\varphi D_0 = 1 - \varphi P_0 = (F_0/F_m)$	Quantum yield of energy dissipation (at t = 0)
$PI_{(ABS)} = (RC/ABS) \times [\varphi P_0/(1 - \varphi P_0)] \times [\psi E_0/(1 - \psi E_0)]$	Performance index on absorption basis