



## **Growth and vegetative development of cuttings from different Red Flower Passion Fruit (*Passiflora trintae* Sacco) genotype populations in different substrates**

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**ABSTRACT:** Optimizing vegetative passionfruit propagation and assessing substrate effects have been explored in several studies. However, assessments concerning wild *Passiflora* genus species focusing on plant conservation are still scarce. In this sense, this study aimed to evaluate the vegetative growth of 54 genotypes from the cuttings of two passion 'red flower' (*Passiflora trintae* Sacco) at the farthest points of the city of Vitória da Conquista, Bahia. The cuttings were subjected to the three different substrates: *i*) soil with manure, *ii*) washed sand, and *iii*) vermiculite. All experiments were carried out under greenhouse conditions in the Southwest area of the state of Bahia, Brazil. The study followed a completely randomized design, in which genotypes from two plant populations were evaluated at three distinct time points (30, 60, and 90 days) based on five variables related to survival and vegetative growth. Vermiculite resulted in the highest cutting survival rates across all three evaluation periods, consistently outperforming other assessed variables. Additionally, this study identified superior genotypes with enhanced rooting and vegetative vigor potential.

**Keywords:** *Passiflora* ssp., vegetative propagation, cutting, ornamental breeding, survival rate

## **Crescimento e desenvolvimento vegetativo de estacas de genótipos de diferentes populações de maracujazeiro da flor vermelha (*Passiflora trintae* Sacco) em diferentes substratos**

**RESUMO:** O uso de substratos alternativos, que possibilitam a formação de mudas de maracujá, obtidas vegetativamente, com qualidade e com custo reduzido é objeto de muitos estudos. Contudo, em se tratando de maracujazeiros silvestres, cujo foco é a conservação de recursos genéticos, poucos são os trabalhos relatados. Neste contexto, o presente trabalho teve como objetivo avaliar o crescimento vegetativo de duas populações do maracujazeiro 'de flor vermelha' (*Passiflora trintae* Sacco) obtidas por estquia em três diferentes substratos. Estacas de *P. trintae* foram coletadas de 54 genótipos distribuídos em duas populações situadas em pontos extremos da cidade de Vitória da Conquista - Bahia. As estacas foram submetidas aos seguintes substratos: *i*-solo com esterco, *ii*-areia lavada e *iii*-vermiculita. As avaliações foram realizadas em três épocas, 30, 60 e 90 dias quanto a cinco variáveis. Os resultados mostraram que os substratos testados influenciaram no desenvolvimento das estacas de *P. trintae*. A vermiculita foi o substrato que apresentou as maiores porcentagens de sobrevivência de estacas para as três épocas de avaliação, além de, no geral, ter uma melhor performance para as variáveis em estudo. Adicionalmente, foi possível selecionar genótipos considerados superiores por apresentar maior potencial para o enraizamento e vigor vegetativo.

**Palavras-chave:** *Passiflora* ssp., propagação vegetativa, estquia, melhoramento genético, sobrevivência

## **INTRODUCTION**

The *Passifloraceae* family is native to tropical and subtropical regions. The *Passiflora* genus is both numerically and economically the most important genus in this family, consisting in around 600 species (Feuillet and MacDougal, 2007). Brazil is considered the diversity center of species belonging to this genus, presenting significant genetic variability and, thus, offering the potential to be explored regarding different genetic genus improvement aspects (Feuillet and MacDougal, 2007; Mezzonato-Pires *et al.*, 2021).

Most *Passiflora* genus studies have focused on cultivated species, such as the 'yellow' passionfruit (*Passiflora edulis*) (Silva and Souza, 2020; Ma *et al.*,

2021). The cultivation of this species yields good economic returns in Brazil, the world's largest passionfruit producer (Machado *et al.*, 2023; Pereira *et al.*, 2023). However, some wild species displaying agronomic potential are also available and should be assessed both as a source of genetic resources and commercial features (Cerqueira-Silva *et al.*, 2010; Costa *et al.*, 2021; Correia *et al.*, 2022).

The ornamental potential of *Passiflora* flowers is due to their striking beauty, unique shapes and vibrant colors (Nóbrega *et al.*, 2017; Santos *et al.*, 2023). In this sense, the potential to create interspecific hybrids is noted due to a variety of native species and wild

types, which could, in turn, offer significant agronomic benefits, such as adaptation to different soil and climate conditions, pest and disease resistance and increased flower color varieties (Ocampo *et al.*, 2016; Correia *et al.*, 2022). This is the case of the 'red-flowered' passion fruit (*Passiflora trintae* Sacco), which presents a restricted geographic distribution in Brazil, covering the northern regions of the state of Minas Gerais and southwestern Bahia (Lemos-Filho *et al.*, 2023). This species occurs in regions subject to anthropogenic changes and is under significant habitat reduction risks due to pasture development, indiscriminate vegetation burning, civil construction and plant biomass removal for energy production (Cerqueira-Silva *et al.*, 2010; Porto *et al.*, 2025).

Knowledge concerning biological and ecological characteristics, as well as genetic characterizations, are important data for the conservation of species variability (Cerqueira-Silva *et al.*, 2010; Rodríguez *et al.*, 2019). In this sense, species with reduced distribution ranges or on the verge of extinction deserve special attention. The cutting technique, in particular, can be useful in the propagation of species threatened in their natural habitat, and in the selection of genotypes of agronomic interest (Trigiano *et al.*, 2021).

The main difficulties concerning vegetative passionfruit propagation are associated to certain factors, such as temperature, humidity, and light intensity, all of which play critical roles in cutting rooting and growth processes (Faleiro *et al.*, 2019; Veimrober-Júnior *et al.*, 2022). Moreover, physiological mother plant states and cutting ages can affect success rates, with younger, more vigorous plants producing better results (Santos *et al.*, 2016). When compared to other species, however, studies focused on the development of 'red-flowered' passion fruit seedlings through the cutting approach are scarce. To obtain plants with the desired quality standards, viable propagation methods should be understood (Faleiro *et al.*, 2019). One of the main factors to consider for successful seedling production is the use of adequate substrates with ideal physicochemical characteristics regarding plant rooting and initial development (Antunes *et al.*, 2021; Shahirah Shahbani *et al.*, 2022). Substrates can be formed by raw material of animal, organic or synthetic origin, comprising either a single material or different materials presented as mixtures (Aires *et al.*, 2020).

Although several organic substrates are typically tested, no standard or consensus regarding the best substrate to produce specific passionfruit species seedlings are available to date (Silva *et al.*, 2023), even though suitable substrates and/or their best mixtures may contribute to superior quality seedlings.

In this context, this study aimed to evaluate the vegetative growth of two 'red flower' passion fruit populations obtained by cutting in three different soil substrates, namely, manure, washed sand and vermiculite.

## MATERIAL AND METHODS

All experiments were conducted in a greenhouse covered with a nylon screen, maintaining approximately 60% relative humidity and reducing light intensity by 50%, in Vitória da Conquista, Bahia, Brazil. Stem cuttings were collected from 54 *P. trintae* genotypes, distributed across two populations of 27 genotypes each, originating from distinct regions of the municipality. The first population was sampled in the southern sector, predominantly a vine forest area representing a transitional biome between Caatinga and Cerrado, characterized by dense vegetation with abundant lianas, epiphytes, and mosses (14°55'24.4"S, 40°50'35.7"W). The second population was located in the northeastern sector, within the Caatinga domain, marked by sparse vegetation and frequent water deficit conditions (14°52'32.8"S, 40°48'26.3"W).

The median portions of the plant branches were collected, taken to the greenhouse, and sectioned, discarding only the apical part, reducing the branches to 15 cm-cuttings containing between two and three nodes. Two leaves reduced to half their original size were kept in each cutting and leaf axil tendrils were eliminated. A bevel cut was made at the base of each cutting to induce scar tissue for root growth stimulation. All branches were maintained with their bases immersed in water during the 3-hour interval prior to cutting sampling and preparation. Following preparation, the cuttings were individually placed in black polyethylene bags measuring 15 x 10 cm filled with the test substrates.

The substrates comprised (*i*) washed sand, basically composed of silicon oxide (SiO<sub>2</sub>); (*ii*) vermiculite, a micaceous mineral (hydrated aluminum, magnesium and iron silicate) that, after undergoing an industrial thermal process, becomes a light material, with neutral pH and low electrical conductivity, widely used to compose substrate formulations, due to improved water and nutrient retention capacity; and, finally, (*iii*) a soil and tanned cattle manure mixture at a 1:1 ratio (Souza, 2001). The soil was made up of organic matter, rendering it a dark color, while also being airy and allowing for good water and nutrient circulation. Water was supplied twice daily to ensure the maximum water retention capacity of each substrate, maintaining all at field capacity.

Weed control was carried out manually to avoid interference in cutting development. Additionally, as no knowledge concerning the rooting ability of the studied species *per se* is available, no phytoregulators were applied, to better understand passionfruit cutting development.

A completely randomized experimental design composed of 10 repetitions under a 2 x 3 factorial scheme (two populations of 27 genotypes and three substrates) was applied, evaluated at three time points, namely 30, 60, and 90 days after grafting. Cuttings were evaluated regarding the following variables: Survival rate [SR, expressed as a percentage (%)], shoot number (NS), root length (LS), number of leaves (NL) and leaf length (LL; measured, in cm, from the base of the petiole to the apex of the leaf blade). All metric determinations were carried out using a Starrett® digital caliper.

The data for all five variables were previously subjected to  $\sqrt{x} + 0.5$  transformation prior to an Analysis of Variance (ANOVA), using the Assistat® software version 7.7. The Shapiro-Wilk test was used to verify data distribution ( $\alpha = 0.05$ ). As some of the data were non-normally distributed, the datasets were subjected to both parametric and non-parametric

analyses of variance ( $\alpha = 0.05$ ), depending on the case, to assess differential substrate performance, evaluation times and interpopulation genetic variability. Parametric and non-parametric ANOVAs, when significant, were followed by parametric (*t* test or Scott & Knott test) and non-parametric (Mann-Whitney) means comparison tests, both set at  $\alpha = 0.05$ .

## RESULTS AND DISCUSSION

The analyzed variables were not uniform regarding variance, presenting non-normal residual distributions. Thus, data transformation was performed to provide the necessary conditions for the analysis of variance and means tests. The analysis of variance indicated a significant effect between different substrates concerning the survival rate variable (*p-value* < 0.01). The highest survival percentages, regardless of passionfruit population, were observed for the vermiculite substrate, ranging between 39.96% and 57% 90 days after planting (Table 1).

**Table 1** - Comparisons between survival rate, number of shoots, shoot length, number of leaves and leaf length means for the three evaluation periods and substrates (sand, soil and manure and vermiculite).

Evaluation	Survival Rate		Number of shoots		Shoot length		Number of leaves		Leaf length	
	Pop 1	Pop 2	Pop 1	Pop 2	Pop 1	Pop 2	Pop 1	Pop 2	Pop 1	Pop 2
<b>(30 days)</b>	<b>Mean</b>	<b>Mean</b>	<b>Mean</b>	<b>Mean</b>	<b>Mean</b>	<b>Mean</b>	<b>Mean</b>	<b>Mean</b>	<b>Mean</b>	<b>Mean</b>
<b>Sand</b>	100 aA	100 aA	0.17 aB	0.037 aB	0.57 aB	0.02 aB	0.14 bA	0.18 aA	1.94 aA	1.08 aA
<b>Soil and manure</b>	96.30 aA	96.30 aA	1.07 aA	0.33 bA	0.68 aA	0.17 bA	0 aB	0 aB	0 aA	0 aA
<b>Vermiculite</b>	100 aA	100 aA	0.25 aB	0.11 aA	0.32 aB	0.16 aB	0.18 aA	0.11 aA	1.45 aA	1.90 aA
<b>(60 days)</b>	<b>Mean</b>	<b>Mean</b>	<b>Mean</b>	<b>Mean</b>	<b>Mean</b>	<b>Mean</b>	<b>Mean</b>	<b>Mean</b>	<b>Mean</b>	<b>Mean</b>
<b>Sand</b>	80.12 aB	57.29 aB	1.07 aB	0.26 bA	0.53 aB	0.31 aB	0.30 aA	0.22 aB	1.18 aA	1.76 aB
<b>Soil and manure</b>	22.16 aC	23.30 aC	0.78 aA	0.33 aA	0.39 aA	0.41 aA	0 bB	0.11 aB	0 bA	1.54 aA
<b>Vermiculite</b>	99.07 aA	90.11 aA	1.33 aAB	0.55 aA	1.49 aB	0.44 bA	0.44 aA	0.37 aA	2.46 aA	0.41 aB
<b>(90 days)</b>	<b>Mean</b>	<b>Mean</b>	<b>Mean</b>	<b>Mean</b>	<b>Mean</b>	<b>Mean</b>	<b>Mean</b>	<b>Mean</b>	<b>Mean</b>	<b>Mean</b>
<b>Sand</b>	52.80 aA	35.46 bB	1.07 aAB	0.57 bA	1.73 aB	0.38 bB	0.48 aA	0.11 aB	2.09 aB	1.63 bB
<b>Soil and manure</b>	10 aB	17.30 aC	0.33 aB	0.31 aB	0.42 aA	0.32 aA	0.04 bB	0.44 aA	1.10 aA	2.36 aA
<b>Vermiculite</b>	39.96 aA	57 aA	1.15 aA	0.44 aB	0.65 aAB	0.39 aAB	0.66 aA	0.04 bB	4.55 aAB	0.74 bA

Means followed by the same letters, lowercase and capital, do not differ statistically between populations and substrates, respectively, within each evaluation period by the Scott-Knott test at 5% significance level.

Increased number of leaves and leaf lengths were observed for the two 'red-flowered' passion fruit genotype populations during the three evaluation periods, regardless of substrate. These variables demonstrate the vegetative development of shoots, responsible for increasing cutting success. The buds located at the base of the cutting petioles give rise to the budding process, subsequently intensifying leaf emergence. The presence of shoots and leaves are

decisive in the rooting of some passionflower species cuttings (Braga *et al.*, 2006).

Although sand exhibited a reasonable performance in terms of *P. trintae* cutting development, the statistical results do not indicate it

as ideal for the evaluated passionfruit population genotypes, even though good aeration and drainage capacity are noted. This may be due to nutrient scarcity, as this substrate presents a lower moisture retention capacity and negatively influences soil-

plant-atmosphere system water movement by excessively draining water, leaving the upper part of the seedlings dry (Carvalho *et al.*, 2013; Cavalcante *et al.*, 2020).

Water substrate availability is critical for cutting survival and consequent seedling formation from vegetative or seed propagations (Rock *et al.*, 2022). Water loss is one of the main causes of cutting death prior to root formation, as the cutting tissue cells must be turgid for cell division to occur. The rooting phase in *Passiflora edulis*, the most studied and cultivated passionfruit species, typically lasts about 30 days (Valle *et al.*, 2018). However, the decreased cutting survival rates observed between 30 and 90 days suggest that the rooting process for certain genotypes may extend beyond 90 days. Therefore, the chance of cutting water losses is very high, whether through leaves or developing shoots, considering the lack of roots at this stage. This situation is worse concerning species that require long periods of time to form roots and when cuttings containing leaves and/or of a herbaceous consistency are used (Vilasboa, 2024). In a study on *Erythrina crista-galli*, the use of vermiculite led to the greatest root lengths during different cutting collection times, which the authors attributed to its high porosity and good moisture retention capacity (Bisognin *et al.*, 2021).

The use of the soil and manure substrate, on the other hand, led to the lowest survival rates, number of leaves and leaf length values in the three evaluation periods compared to the other substrates (Table 1), possibly due to the use of organic matter as fertilizer. The employed manure was probably not well tanned at the time of use and may have undergone fermentation caused by the moisture from irrigation, leading to unfeasible cutting survival rates due to the action of gases during fermentation. Tanned manure may not comprise a balanced fertilizer for many soils, due to low phosphorus contents in relation to nitrogen and potassium (Prado *et al.*, 2008; Silva, da *et al.*, 2020). Phosphorus is considered the main nutrient in basic fertilizers, comprising a macronutrient with little soil mobility, although it presents plant tissue mobility and is concentrated in active growth areas. Phosphorus movement from soil to plants takes place by diffusion, and a good supply of phosphorus during plant growth phases leads to efficient vegetative cutting development (Prado *et al.*, 2008).

Braga *et al.* (2006) tested different substrates when studying three wild *Passiflora* species, reporting that substrate pH values can change depending on manure concentration. Very low or very high pH values reduce the availability of phosphorus and other nutrients, as soil pH is mainly responsible for nutrient soil availability. Phosphorus deficiency issues in soils take place because phosphates, such as iron or

aluminum phosphates, can become insoluble and unusable for plants.

In addition to macronutrient soil deficiency and manure substrate issues, some Cerrado fruit species originating from low fertility lands do not respond to increased substrate fertility leading to low substrate performance, which may be harmful to seedling development (Oliveira *et al.*, 2023).

In contrast, vermiculite presented the highest cutting survival percentages, number of leaves and shoot lengths in the three evaluation times (Table 1), while also promoting better vegetative development compared to the other substrates regarding the analyzed variables at 60 and 90 days after planting (Table 1). Vermiculite belongs to the phyllosilicate group of minerals, basically composed of iron, magnesium and aluminum silicate. It displays a high-water retention capacity and good aeration space, which may be responsible for the better performance observed herein. This substrate is also sterile, lacking soil pathogens, thus favoring seedling development (Oliveira *et al.*, 2021).

Regarding interpopulation comparisons, significant effects for some variables were noted. Shoot number (*p*-value < 0.01) and length (*p*-value < 0.01), for example, were both higher in population 1 compared to population 2, with the former displaying superior average results for both variables. Regarding the other three variables, the average results of population 1 were consistently higher than those of population 2, albeit non-significantly (*p*-value > 0.01). Although the two populations behaved statistically similarly for most variables, a progressive statistical divergence was noted throughout the evaluation periods, highlighting possible development response divergence.

In general, the substrates tested herein significantly influenced the performance of *P. trintae* cuttings. The water retention capacity of each substrate, along with their intrinsic water and nutrient cutting flow regulation characteristics, affected cutting survival and vegetative development variables.

Cloning technology via the cutting approach has been explored in plant pre-breeding phases, with genetic factors noted as potentially influencing rooting ability. Herein, the results led to the selection of superior genotypes displaying greater rooting and vegetative vigor potential. A total of 10% of the best-cloned genotypes were selected, without stratifying by population or substrate regarding the highest mean values of the presence of leaves at 90 days. The presence of leaves in cuttings is an important variable, comprising a strong rooting stimulus (Braga *et al.*, 2006; Gomes *et al.*, 2018). The most noteworthy genotypes herein were P1-09, P2-12, H2-03, P1-14, P1-16, P1-20 and P2-12 (Table 2).

**Table 2** - Leaf length means of the superior genotypes at 90 days.

	Population 1	Means	Population 2	Means
<b>Vermiculite 90 days</b>	P1-14	23.43	P2-12	20
	P1-16	24.36	-	-
	P1-20	26.8	-	-
<b>Soil and manure 90 days</b>	P1-09	29.6	P2-12	26.85
	-	-	P2-03	36.98

Studies that aim to improve seedling production techniques through the vegetative propagation of passionfruit plants are paramount, as they allow for the identification and selection of individuals with desirable multiplication and preservation characteristics (Alexandre *et al.*, 2013). The findings reported herein demonstrate the viability of passionfruit cuttings in obtaining superior genetic quality seedlings and mother plant characteristics. Furthermore, determining genetic staking capacity differences is important to characterize genetic materials for the purpose of registering or protecting cultivars.

## CONCLUSIONS

Vermiculite is the most appropriate substrate for *P. trintae* seedling production from cuttings under greenhouse conditions. The choice of substrate significantly influences cutting development in this species. Genotypes P1-09, P2-12, H2-03, P1-14, P1-16, P1-20 and P2-12 presented better vegetative development performance.

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