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Trichoderma strains application in promoting rooting of *Schlumbergera* cactus

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Abstract

The aim of this work is to evaluate and deepen knowledge on the use of *Trichoderma* to stimulate the vegetative growth and rooting of plants. In this case, a trial was carried out on cuttings of *Schlumbergera*, a genus of cactus that is very important from an ornamental point of view but that presents numerous difficulties in the process of rooting in substrate, where cuttings often rot due to excess humidity or die due to the attack of pathogenic fungi. The trial started in March 2021 and lasted for ten months in the CREA greenhouses in Pescia, Italy. The experimental trial showed a significant effect on the rooting of *Schlumbergera* cuttings and on all agronomic parameters assessed on the plants in cultivation, following treatment in the growing medium with *Trichoderma* spp. The cuttings treated with the fungi in fact showed an increase in root weight and length, vegetative weight, number of new shoots and flowers, floral life, and a significant reduction in mortality of the treated cuttings. These results show how *Trichoderma*, in addition to its activity as an antagonist of pathogenic fungi, can increase the rooting and growth of plants and the production and duration of flowers, particularly on plant species where there is often little information. This experimentation may be of particular interest for cactus and succulent growers, since there is no research in the literature on biological propagation methods for these plants.

Keywords: Beneficial microorganisms; Cactus plants; Microbial biofertilizers; Ornamental plants; Roots stimulation

1. Introduction

Trichoderma is a genus of fungi, initially described by Christian Hendrik Persoon in 1794, belonging to the family *Hypocreaceae* and found in soils at all latitudes. Many species of *Trichoderma* are opportunistic and avirulent symbionts capable of establishing an endophytic mutualistic relationship with different plants [1,2]. Several strains of *Trichoderma* find use as biocontrol agents against fungal diseases of plants. Mechanisms of action include antibiosis, parasitism, plant resistance induction, and competition [3,4]. The main species used as biocontrol agents are *Trichoderma asperellum*, *T. harzianum*, *T. viride*, and *T. hamatum*. Biocontrol agents generally grow on the root surface, thus acting especially on diseases affecting this organ, but can be effective against foliar diseases as well [5,6]. *Trichoderma* spp. produce volatile antibiotics, active against bacteria and fungi, or both [7]. Nonvolatile, water-soluble compounds include trichodermina, suzukacillin, and alameticin [8]. The primary volatile antibiotic, which imparts the coconut odour to many *Trichoderma* strains, is 6-pentyl-a-pyron (6-PAP) [9]. *Trichoderma* spp. are known for creating mutualistic relationships with plants. Some *Trichoderma* strains, described as rhizosphere competent and selectively used for commercial development, can cause an asymptomatic infection of roots, where the fungus colonization is limited to the outer cortical regions. In many cases, the use of *Trichoderma* spp. has resulted in improved vegetative and roots growth of some vegetable and ornamental species [10] and affects photosynthesis, respiration, and nutrient uptake of plants [11-14]. Some *Trichoderma* strains, described as rhizosphere competent and selectively used for commercial development [12], can

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cause an asymptomatic infection of roots, where the fungus colonization is limited to the outer cortical regions. These fungi behave as endophytes, colonizing the root epidermis and outer cortical layers and release bioactive molecules. At the same time, the transcriptome and proteoma of plants are substantially altered. This intimate interaction with the plant provides a number of benefits only recently recognized for their variety and importance. Some of these ones include increased resistance of the plant to various biotic stresses through induced or acquired systemic resistance and to abiotic stresses such as water deficit/excess, high salinity, and extreme temperature. Also relevant is the enhanced nitrogen use efficiency by improved mechanisms of nitrogen reduction and assimilation along with reduced overexpression of stress genes or accumulation of toxic compounds during plant response to pathogen [15]. An additional benefit for consumers comes from an increased content of antioxidants in the fruit from plants treated by selected *Trichoderma* strains [16]. Moreover, it was also observed that the fertility of soils treated with some *Trichoderma* strains could be significantly improved beyond disease control, which increased the attractiveness of these fungi for general use in crop production. The effect could be particularly strong in terms of root growth promotion, even though it has been not unusual to detect an increase in stem length and thickness, leaf area, chlorophyll content, and yield (size and/or number of flowers or fruits) [3,17,18]. It is known that *Trichoderma*, as an endophyte, can colonize plant roots and release bioactive molecules [19,20]. This intimate interaction can result in several benefits (only recently recognized) that include:

- Plant resistance to various biotic and abiotic stresses, such as excess or stress due to lack of water, high salinity or extreme temperatures;
- Improved nitrogen uptake and accumulation of toxic substances as a response to pathogen attack [21-24].

1.1. Agamic propagation of cacti and succulents

Agamic reproduction is the technique whereby, with appropriate measures and under certain conditions, a part of a plant produces root to give birth to a new plant through a regeneration process that exactly reproduces the plant from which it came. Cuttings should generally be kept dry until roots appear and depending on the species. Root development can be expected within a few weeks, but those that are difficult to root may take many months. In cacti, this technique is not very simple because the root regeneration process is often very slow and during the rooting process fungi can develop in the soil of various species due to excess humidity or the type of substrate, which can often delay root development or even lead to the death of the plants [25,26]. Many species of cacti and succulents, such as *Aloe* sp., *Agave* sp., *Echinocereus* sp., *Gasteria* sp., *Haworthia* sp., *Sempervivum* sp., *Sansevieria* sp. and *Sedum* sp., can abundantly emit already rooted basal shoots that, once separated from the mother plant, can be potted up to quickly obtain new plants [27].

Cactaceae propagation has been extensively studied in the literature, but in vivo acclimatization of the seedlings is rarely reported [28-30], which has proven critical in some cases.). In fact, in the *Schlumbergera* species, a cactus of commercial interest due to its splendid flowering especially in the winter period, the rooting of the plants is very difficult or and very long and the cuttings are often subjected to rotting due to excess humidity, which compromises their quality [31]. Even the use of hormonal substances often does not bring much benefit and in many cases it leads to the drying up of the vegetative part. Various soils and hormones have been tested for the propagation of cacti, but only a few have proved useful for multi-species propagation [32]. Johnson and Emino [33] suggest, however, that each cactus species may require a unique hormone combination.

Objectives

The aim of this work is to evaluate and deepen knowledge on the use of *Trichoderma* for the root stimulation of plants, particularly on *Schlumbergera* (**Figure 1**), a cactus of commercial interest, on which there are no experiments using this fungus. In the literature the application of different species of *Trichoderma* sp. has resulted in improved rooting and plant growth, which was also evaluated on a plant species that is difficult to propagate.

Figure 1 *Schlumbergera russelliana* rooted cuttings in CREA Vegetable and Ornamental Crops greenhouses

2. Material and methods

The experiments, which started in March 2021, were carried out on unheated benches in the greenhouses of the CREA-OF in Pescia (Pt), Tuscany, Italy (43°54′N 10°41′E) on *Schlumbergera russelliana* cuttings. The cuttings were placed in ø 20 cm pots; 30 per thesis, divided into three replicas of 10 cuttings each. First, all cuttings were fertilized with a slow-release fertilizer (1 kg m^3 Osmocote Pro®, 9-12 months containing 190 g/kg N, 39 g/kg P, 83 g/kg K) added to the growing medium at the time of transplanting. Two commercial products TricorrP5 and Piranha liquid characterised by various species of *Trichoderma* spp. were used for experimentation. This was done to make it easier for growers who would later want to reuse these products. *Trichoderma* inoculum was prepared as follows: an aqueous conidium suspension ($1x10^6$ conidia mL^{-1}, number of total conidia per ml) of *Trichoderma*-based product was inoculated on authoclaved organic biomass (50% gran and 50% grain) and fermented for one week at 24°C, 12h/12h light/darkness. Finally, the fermented biomass was added to a commercial substrate for cutting growing at the final concentration of 10% (w/w).

The experimental groups were:

- Group control (CTRL) (peat 50% + pumice 50%), irrigated with water and substrate previously fertilized. In the control treatment, it was only wetted with water and previously fertilized;
- Group control1 (CTRL1) (peat 50% + pumice 50%), irrigated with water and substrate previously fertilized; the cuttings were treated with rooting hormones (Clonex - Root Growth Hormone Gel, 4-indol-3-yl butyric acid, 3g/l). In this experimental thesis, what growers normally do when rooting *Schlumbergera* sp. cuttings was simulated using rooting hormones;
- Group with *Trichoderma*1 (TR1) (peat 50% + pumice 50%) irrigated with water and previously fertilised substrate. The substrate was inoculated with the product TricorrP5 before inserting the cuttings, containing (*Trichoderma* hamatum, *Trichoderma* harzianum, *Trichoderma* koningi, *Trichoderma* longibrachiatum, *Trichoderma* reesei; 1x1011 cfu/Kg) for every 5 litres of growing medium. The product TricorrP5 was supplied by the Nutrient Company, Rockdale, UK;
- Group with *Trichoderma*2 (TR2) (peat 50% + pumice 50%) irrigated with water and previously fertilised substrate The substrate was inoculated with the product Piranha liquid, from the company Nutrient Advance, which contained the following strains (*Trichoderma* koningi, *Trichoderma* viride, *Trichoderma* harzianum; 1x1012 cfu/l); 5 kg of cultivation substrate was wetted with 500 ml of the product;

The plants were watered two times a week and grown for 10 months. The irrigation was activated by a timer, whose program was adjusted weekly according to climatic conditions and the leaching fraction. The climatic conditions were measured by a weather station placed inside the greenhouse, which provided data every hour, while the leaching fraction was retrieved weekly by means of a tank placed under the pots and measured appropriately. On December 15, 2021, rooted plant number, roots weight, roots length, vegetative growth, new shoot growth, flower number, flower life, and plant dead number were evaluated. For vegetative and root weights a precision balance was used, for root length and plant height an electronic measuring instrument was used. To assess the number of flowers, the number of flowers and new shoots produced was counted on each plant from the start of the experiment to its conclusion. For the flowering duration, the days from the time of flower opening until the senescence or browning of the petals were counted. For dead plants, all those lost during the trial were also counted.

2.1. Statistics

The experiment was done in a randomized complete block design. To analyze the collected data, one-way ANOVA was used through GLM univariate procedure, to assess significant ($P \leq 0.05$, 0.01, and 0.001) differences among treatments. Then, mean values were separated by LSD multiple-range test ($P = 0.05$). The programs Costat (version 6.451) and Excel (Office 2010) supported graphics and statistics.

3. Results and discussion

The experimental trial at the greenhouses of CREA-OF in Pescia showed a significant improvement in agronomic parameters analyzed on rooted *Schlumbergera russelliana* cuttings treated with *Trichoderma* spp. In particular, there was a significant improvement in the number of rooted cuttings, roots weight and length, vegetative weight, number of new shoots and flowers, floral life and a significant reduction in plant mortality treated with *Trichoderma* spp.

In (**Table 1**), there was a significant increase in the number of rooted cutting in TR1 25% compared to TR2 with 22%, CTRL1 with 16% and CTRL 12%. In terms of root weight, TR1 was the best thesis with 56.64 g, compared to 53.80 g TR2, 51.02 g CTRL1 and 48.86 g CTRL (**Figure 2**). In addition, there had been a significant increase in root length in the thesis TR1 with 16.83 cm when compared to 15.31 cm in TR2 and 13.89 cm and 13.36 cm in CTRL1 and CTRL, respectively (**Figure 3**). For vegetative weight, these TR1 and TR2 were the best with 65.65 g and 63.59 g, respectively, compared to CTRL and CTRL1 with 59.87 g and 59.63 g (**Figure 4**).

In (**Table 2**) there was more new shoot growth in TR1 with 14.24 g, compared to 13.21 g TR2, 12.77 g CTRL1 and 12.34 g CTRL (**Figure 5**). There was also an increase in the number of flowers in TR1 with 9.00%, compared with 7.00% TR2 and 6.00% and 6.00% in CTRL and CTRL1, respectively. The TR1 thesis also prolonged plant flower life by 5.84 days, compared with 5.21 days TR2, 4.24 days CTRL and 3.84 days CTRL1. There was a significant reduction in plant mortality treated with TR1 and TR2 with 0.00% in contrast to CTRL1 and CTRL, where it was significantly higher, with 3.00% dead plants, respectively.

Table 1 *Trichoderma* spp. effect on *Schlumbergera russelliana* plant's growth

Groups	RN (%)	RW (g)	RL (cm)	VG (g)
CTRL	12.00	48.86 d	13.36 c	59.87 b
CTRL1	16.00	51.02 c	13.89 c	59.63 b
TR1	25.00	56.64 a	16.83 a	65.65 a
TR2	22.00	53.80 b	15.31 b	63.59 a
ANOVA	-	***	***	***

One-way ANOVA; n.s. – non significant; *,**,*** – significant at $P \leq 0.05$, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test ($P = 0.05$); The data represent the average of 30 plants divided into 10 plants for 3 replications each. Legend: (CTRL): control; (CTRL1): rooting hormones; (TR1): *Trichoderma* – TNC TricorrP5; (TR2): *Trichoderma* – Piranha liquid; RN: Rooted plants %; RW: Roots weight; RL: Roots length; VG: Vegetative growth

Table 2 *Trichoderma* spp. effect on roots growth of *Schlumbergera russelliana* plants

Groups	SG (g)	FN (%)	FL (days)	PD (%)
CTRL	12.34 c	6.00	4.24 c	3.00
CTRL1	12.77 bc	6.00	3.84 c	3.00
TR1	14.24 a	9.00	5.84 a	0.00
TR2	13.21 b	7.00	5.21 b	0.00
ANOVA	***	-	***	-

One-way ANOVA; n.s. – non significant; *,**,*** – significant at P ≤ 0.05, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test (P = 0.05); The data represent the average of 30 plants divided into 10 plants for 3 replications each. Legend: (CTRL): control; (CTRL1): rooting hormones; (TR1): *Trichoderma* – TNC TricorrP5; (TR2): *Trichoderma* – Piranha liquid; SG: New shoot growth; FN: Flowers %; FL: Flowers life; PD: Plants dead %.

The rhizosphere is always subjected to continuous changes in equilibrium, linked, for example, to root movement in the soil, which alters the distribution of nutrients and water and can consequently influence the activity of rhizospheric microorganisms [34]. Microorganisms can interact with the root surface, which is covered with a mucigel consisting of polysaccharides and mucopolysaccharides, facilitating root movement in the soil [35,36]. Saprophytic, symbiotic, pathogenic or antagonistic microorganisms and nematodes that invade root tissues or colonise surfaces can be observed on the mucigel [37,38]. The colonisation of roots by beneficial microorganisms is essential to:

- Controlling diseases caused by pathogenic microorganisms [39];
- Promoting a plant's growth through improved nutrient uptake or hormone production, as can be evidenced in this experiment or others conducted on horticultural or ornamental plants [26];
- Biofertilise the soil by increasing the availability of nutrients such as nitrogen, phosphate and micronutrients [27];
- Bioremediate the soil by removing toxic products by degrading or accumulating them [27].

The fungal mass in the soil is abundant and their role in the biology of the rhizosphere is, in some ways,more important than that of bacteria [9,14]. Many fungi live exclusively in the soil, where they colonise plant tissue fragments, interact with plant roots and may interact with other fungi, bacteria, plants or soil elements [19,20,23,24]. The relation that fungi develop with plants and other microorganisms can be neutral, antagonistic or beneficial [40,41]. *Trichoderma* species are a common saprophytic fungi found in agricultural soils, grasslands, forests, desert soils, salt marshes, decaying plant materials and extreme climates [13,42]. Their nutritional requirements are minimal, they proliferate when they find suitable material and produce numerous spores. They resist many xenobiotic compounds [35,43]. They produce numerous enzymes capable of degrading various polymers, such as chitin and cellulose, the constituents of which can be used for the growth of other microorganisms [26,44,45]. In addition to being a biocontrol agent, *Trichoderma* is considered a growth promoter of horticultural and floricultural species [46,47]. As revealead in this experiment on *Schlumbergera russelliana*, the use of *Trichoderma* spp. in the cultivation medium can ensure the promotion of root development, even in species that are notoriously difficult to propagate, as well as improve growth, flower production and increased resistance to mortality usually caused by biotic factors such as pathogenic fungi. This issue has already been found for other horticultural and ornamental species [19,24,48,49]. Indeed, several horticultural species have been shown to benefit from *Trichoderma* species by increasing their growth, improving root structure, enhancing seed viability, improving photosynthesis, flowering and yield quality, among others [50]. In almost all stages of plant growth and development, phytohormones and phytoregulators are the most important stimuli. Many *Trichoderma* species from the rhizosphere can colonise the root surfaces of both monocotyledonous and dicotyledonous plants, causing significant metabolic changes [51]. Another aspect of this research work was related to the observation on interaction of different *Trichoderma* consortia with plants, so that we could highlight which strains perform best and be able to use them in combination.

Various tests have shown how *Trichoderma* can influence plant root development and increase root hairs by colonising the soil in the rhizosphere, as well as occupying root surfaces and stimulating root growth [52,53]. It was observed in different experiments that *Trichoderma* colonises roots mainly at the level of the root hairs and in the elongation zone. More recent studies have confirmed that these fungi can also colonise the root edge cellular zone (RBC). In this research work, the presence of *Trichoderma* played a crucial role in improving plant growth, an aspect that is known to be linked to the production of vitamins or increased solubility of nutrients contained in the rhizosphere (phosphates, Fe^{3+} Cu^{2+}, Mn^{4+}, ZnO), which results in better development and yield increases [54,55]. In many cases, especially on ornamental species, the application of *Trichoderma* can lead to an increase in flower production and the content of valuable compounds in the fruit, as it has been found in some works [56]. However, few studies highlight the benefits of *Trichoderma* on succulent plants and *Cactaceae* [19,57], so the effects of these fungi on these types of ornamental plants, which usually come from other continents, need to be further investigated.

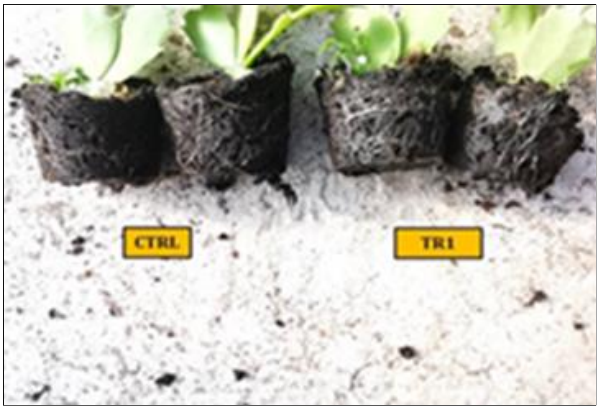

Figure 2 Roots growth in plants treated with *Trichoderma spp.*. Effect of TricorrP5 (TR1) treatment on root development compared to the untreated control (CTRL)

Figure 3 Detail of roots and root hairs in plants treated with *Trichoderma spp.*. Increased root development in terms of size and root hairs with the TricorrP5 treatment (Tr1) compared to the untreated control (CTRL)

Figure 4 Flowering plants on TricorrP5 thesis (TR1) compared to the non-flowering control thesis (CTRL).

Figure 5 Increased number of new shoots in the TricorrP5 thesis (TR1) compared to the untreated control (CTRL)

4. Conclusion

Trichoderma applications can increase biomass, leaf area, photosynthetic activity, drought tolerance and pathogen resistance independently of mycoparasitic and antibiotic activity. Detailed knowledge of *Trichoderma* mechanisms against plants and pathogens can significantly increase the effectiveness of their action. *Trichoderma* utilises a number of complex mechanisms to promote plant development, both direct and indirect biocontrol activities against pathogenic microorganisms (fungi, bacteria, insects and nematodes). Nursery treatments with *Trichoderma* formulations improve the quality of the resulting seedlings, as they promote and accelerate the production of more homogeneous and abundant root masses. This is of interest to farmers, especially in the planting phase, where plant mortality rates are often very high, particularly in cacti and succulents that are sensitive to the type of growing medium. The application of *Trichoderma* to the growing medium can also improve root and vegetative development of the plants, as well as the aesthetic quality by increasing flower production and resistance to water and temperature stresses. These ones are, aspects that are still little considered, but which play a crucial role in the cultivation of cacti and succulents and on which this research on *Schlumbergera* aims to shed light.

Compliance with ethical standards

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Disclosure of conflict of interest

The author declares no conflict of interest.

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