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Growth-promoting microorganisms in the root stimulation of *Celtis Australis* (Bagolaro) and in the control of *Ganoderma applanatum* and *Laetiporus sulphureus*

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Abstract

Research objective: The aim of this research was to evaluate the stimulating potential of new microbial consortia obtained from the root systems of cacti and succulents in the rooting of *Celtis australis* and in the protection of certain fungal pathogens of this plant. The possible interaction between plants and substrate microorganisms in plant mortality was evaluated.

Materials and Methods: The experiments, which began in January 2024, were conducted in the CREA-OF greenhouses in Pescia, Tuscany, Italy on 2-year-old *Celtis australis* plants obtained from seed. The seedlings were potted 16, 5 plants for 3 replications for each experimental thesis, for a total of 15 plants each. After 5 months of cultivation since transplanting, the following plant and substrate parameters were analysed in June 2024: plant height, number of leaves, leaf area, vegetative weight, root volume and length, number of microorganisms in the substrate, pH of the substrate and number of dead plants for *Ganoderma applanatum* and *Laetiporus sulphureus*. In addition, the SPAD index was measured on three pinched leaves from the base to the apex of the crown of each plant.

Results and Discussion: The experiment showed that the use of microorganisms introduced into the rooting substrate of *Celtis australis* plants can significantly increase vegetative and root growth, increase plant height and the number of leaves. There was also a significant increase in leaf area, root length and chlorophyll content as demonstrated by SPAD analysis. A very interesting aspect was also the increase in microbial biomass in the treated theses, particularly in the thesis (SYB), an inoculum of microorganisms obtained from the roots of cacti and succulents. The treatments with micro-organisms in particular (SYB) resulted in a significant reduction in plant mortality caused by the pathogenic fungi *Ganoderma applanatum* and *Laetiporus sulphurous*.

Conclusions: In light of possible climate change, it is also important to evaluate new microbial selections from plants that live in extreme environments, such as cacti and succulents. Plant productivity can be maintained while reducing environmental impact and increasing resistance to biotic and abiotic stresses with microbial biofertilisers. In order to improve and speed up the growth of nursery plants, especially trees to be placed in the environment, it seems very important to develop innovative protocols to increase their rooting and vegetative growth.

Keywords: Cannabaceae; Plant growth promoting rhizobacteria; Sustainable agriculture; Bagolaro; Forest honeycomb

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1. Introduction

Celtis australis L., Urticales, Ulmaceae, is a deciduous tree native to the Mediterranean region (Southern Europe, North Africa), as well as Asia Minor, the Crimea, and the Caucasus and Iran [1]. Located between 800 and 900 meters above sea level, it appears along the Swiss border [2]. The species can be found even up to 1,150 meters above sea level on warm South Tyrolean slopes [3]. Cities in the Sub-Mediterranean region grow C. australis as an ornamental tree. The species *C. australis* is highly resistant to drought, wind, and air pollution in urban areas and can survive temperatures as low as −15 °C. In addition to preferring light, sandy soil, it prefers warm, dry limestone terrain. *C. australis* is a light-loving species. So it has a lot to do with afforestation of dry and karstic terrain [4,5]. Italian habitats include sunny, rocky slopes in the PreAlpine (possibly introduced) and Sub-Mediterranean [6]. There are many species of thermophilic trees that grow in warmer Mediterranean and SubMediterranean forests, including *Quercus pubescens* Willd., *Fraxinus ornus* L., *Pistacia terebinthus* L., and others. It grows on steep, rocky, dry karst areas, protecting the soil against erosion [7]. With a diameter of 1–2 m and an age of 1,000 years, the species *C. australis* is a large, long-lived tree with quality wood (the genus name comes from the Greek word kello, which means driven). The young shoots are slender and wiry, suitable for whips and rods, and the wood is stiff with gray colored hardwoods and yellow sapwoods. Roots are deep and strong. The leaves alternate; they are 5–12 cm long, simple, with serrated edges; the leaf surface is asymmetrical and has three stronger vessels. On young shoots, the flowers are polygamous or hermaphroditic, small, apetalic, with four–five stamens. Their fruit is round and up to 1 cm thick, with an edible wrapper [6,7]. Interestingly, according to literature data, *C. australis* has only a few diseases. Some earlier authors, such as (Potočić et al., 1983) [1], note that *C. australis* is rarely infected by fungi, such as *Laetiporus sulphureus* (Bull.) Murrill (1920) and *Ganoderma applanatum* (Pers.) Pat., which cause rot in old trees. In the monograph Insects and diseases damaging trees and shrubs of Europe [8] only one species (*Phyllonoricter millierella*) from *C. australis* is listed. It is one of the medicinal plants that have been used as a natural remedy in different countries for many diseases such as cough, colic, amenorrhea, ulcers, and stomach disorders (9-11). Many phytochemical molecules have been found to be present in the organs of C. australis, including flavonoids (12-15), terpenoids (16), and anthocyanins (17). Various biological effects can be attributed to these compounds (18-20). The pharmacological potential of this plant has been demonstrated in many studies (21,22).

1.1. Soil microbiome and its interactions

Plant microbiomes are microbial communities associated with plants [23]. They play a crucial role in plant health and adaptability to environmental factors [24]. Plants and soil microbes can interact in complex ways. Plants choose microbial partners for their growth, development, and productivity as they form the soil microbiome [25]. Plants secrete nutrients into the soil microbiota through root exudates. Only a few individual effects mutually exerted by plants and microorganisms have been characterized to date, such as nitrogen fixation by rhizobia. Plant growth-promoting bacteria (PGPB) have been extensively studied in order to develop novel strategies for improving productivity and sustainability in agriculture by understanding plant–microbe interactions and modulating the plant microbiome [26,27]. Plant productivity is currently enhanced by bacterial inoculants that contain a single strain of bacteria with a range of plant growth-promoting traits [28,29]. Through in vitro screening experiments or inoculation experiments conducted under controlled conditions, numerous characteristics of PGPB have been identified [30]. However, these characteristics are rarely tested in field conditions and related testing generally neglects the significant aspects of plant–microbe interactions [31]. Inoculants for microbial organisms can also be developed by studying microbial communities. In order to design synthetic microbial communities with predictable traits, information about individual species and possible antagonistic interactions can be used. In the plant rhizosphere, bacteria with a high potential for interaction are thought to contribute significantly to the host's characteristics. It is probable that bacteria living in the rhizosphere have coevolved with plants for a long time, and they share characteristic traits such as metabolism, biofilm formation, and others that are not common in soil, sediments, marine ecosystems, etc. [32]. Plant growth promotion and carbon, nitrogen, phosphorus, and iron cycling are among the significant functions of the soil microbiome at the soil ecosystem level [33,34]. These functions are primarily attributed to the interactions between soil microbial community members [35]. Individual soil microbiome members possess enormous genomic and metabolic capacities. Additionally, metabolic interactions at the community level can result in novel functions. Microorganisms and communities can be better understood by understanding these interactions. A native soil microbiome consists of millions of species that can interact with each other in many different ways. As no species exists in isolation, soil microbiome characteristics are heavily influenced by these interactions. Compared to single-species culture [36,37], multispecies consortia for developing inoculants are considered more promising for agricultural applications [38]. Several species coexisting within a consortium can, for example, occupy a wide range of ecological niches without antagonistic behavior towards each other [39], allowing them to colonize the plant rhizosphere more effectively [40]. Additionally, a more diverse microbial consortium may possess a greater number of plant-beneficial functions [41]. Inoculated species can alter root exudation patterns [42], produce plant-derived antimicrobials [43], or activate other plant defense mechanisms [44] to trigger plant-mediated "control" of the microbiome. Various microbial inoculants can induce relatively large shifts in

the function of the host plant microbiome [45]. Numerous reports have indicated that microbial inoculants may indirectly affect rhizosphere microbes [46,47].

1.2. Research Objectives

The aim of this research was to evaluate the stimulating potential of new microbial consortia obtained from the root systems of cacti and succulents in the rooting of *Celtis australis* and in the protection of certain fungal pathogens of this plant (**Figure 1**). The possible interaction between plants and substrate microorganisms in plant mortality was evaluated.

Figure 1 Detail of the leaves, plant and seeds of *Celtis australis*

2. Materials and methods

The experiments, which began in January 2024, were conducted in the CREA-OF greenhouses in Pescia (Pt), Tuscany, Italy (43°54′N 10°41′E) on 2-year-old *Celtis australis* plants obtained from seed. The seedlings were potted 16, 5 plants for 3 replications for each experimental thesis, for a total of 15 plants each. The experimental groups were:

- Control group (CTRL) (peat 80% + pumice 20%), fertilized and irrigated with water;
- Group with *Laminaria digitata* and *Laminaria japonica* (LAM) (peat 80% + pumice 20%), fertilized and irrigated with water;
- Group with Symbac® (SYB) micro-organisms obtained from the root systems of cacti and succulents in (peat 80% + pumice 20%), irrigated with water (*Lactobacillus* spp., *Streptomyces* spp., *Trichoderma* spp., *Bacillus* spp., *Pseudomonas* spp., *Aspergillus* spp.) (2.5 x 10^9 cfu/kg) and fertilized;
- Group with beneficial bacteria (BAC1) (peat 80% + pumice 20%) fertilized and irrigated with water, (TNC Bactorrs13: *Bacillus amyloliquefaciens*, *B. Brevis*, *B. Cirulans*, *B. Coagulans*, *B. Firmus*, *B. Halodenitrificans*, *B. Laterosporus*, *B. Licheniformis*, *B. Megaterium*, *B. Mycoides*, *B. Pasteuri*, *B. Polymyxa*, *B. Subtilis* (1.3×10^{11} cfu/kg); Mix 1.5 g (approx. 1/2 tsp) per litre of soil;
- Group with beneficial bacteria (BAC2) (peat 80% + pumice 20%) irrigated with water and previously fertilised substrate, Tarantula powder Advanced nutrients: *A. Globiformis* 25,000 cfu/ml, *B. Brevis* 2,000,000 cfu/ml, *B. Coagulans* 500,000 cfu/ml, *B. Licheniformis* 5,000,000 cfu/ml, *B. Megaterium* 500,000 cfu/ml, *B. Polymyxa* 50,000 cfu/ml, *B. Pumilis* 50,000 cfu/ml, *B. Subtilis* 1,000,000 cfu/ml, *B. Thuringiensis* 100,000 cfu/ml, *B. Thuringiensis Canadiensis* 50,000 cfu/ml, *P. Polymyxa* 300,000 cfu/ml. Mix 2gr per litre of water.

The plants during the cultivation cycle were sprayed twice a day for 1 minute. Irrigation was activated by a timer, the programme of which was adjusted weekly according to the weather conditions and the leaching fraction. All experimental theses were managed with a substrate (80% peat + 20% pumice) and appropriately fertilised with a slow-release fertiliser (3 kg m-3 Osmocote Pro®, 9-12 months with 190 g/kg N, 39 g/kg P, 83 g/kg K) mixed with the growing medium before transplanting. After 5 months of cultivation since transplanting, the following plant and substrate parameters were analysed in June 2024: plant height, number of leaves, leaf area, vegetative weight, root volume and length, number of microorganisms in the substrate, pH of the substrate and number of dead plants for *Ganoderma applanatum* and *Laetiporus sulphureus*. In addition, the SPAD index was measured on three pinched leaves from the base to the apex of the crown of each plant (a total of 90 measurements per treatment).

2.1. Analysis methods

- pH: For pH measurement, 1 kg of the substrate was taken from each plant, and 50 g of the mixture was placed in a beaker containing 100 ml of distilled water. After 2 hours, the water was filtered and analyzed [22];
- Microbial count: direct determination of total microbial count by microscopy of cells contained in a known sample volume using counting chambers (Thoma chamber). The surface of the slide is etched with a grid of squares, with the area of each square known. Determination of viable microbial load after serial decimal dilutions, spatula seeding (1 ml) and plate counting after incubation [23];
- Analytical instruments: IP67 PHmeter HI99 series - Hanna instruments; Combined test kit for soil analysis - HI3896 - Hanna instruments; Microbial diversity of culturable cells [24];

2.2. Statistics

The experiment was carried out in a randomized complete block design. Collected data were analyzed by one-way ANOVA, using GLM univariate procedure, to assess significant ($P \leq 0.05$, 0.01 and 0.001) differences among treatments. Mean values were then separated by LSD multiple-range tests ($P = 0.05$). Statistics and graphics were supported by the programs Costat (version 6.451) and Excel (Office 2010).

3. Results

The experiment showed that the use of microorganisms introduced into the rooting substrate of *Celtis australis* plants can significantly increase vegetative and root growth (**Figure 3** and **Figure 4**), increase plant height and the number of leaves. There was also a significant increase in leaf area, root length and chlorophyll content as demonstrated by SPAD analysis.

A very interesting aspect was also the increase in microbial biomass in the treated theses, particularly in the thesis (SYB), an inoculum of microorganisms obtained from the roots of cacti and succulents (**Table 2**).

The thesis (SYB) was the best for all agronomic parameters analysed, followed by the other two treatments with microbial consortia of various types; the control thesis with algae and the control thesis irrigated with water and fertilised were the worst for most agronomic parameters.

Table 2, shows that the treatment with selected microorganisms from cacti and succulents (SYB) colonised the substrate better than the other experimental theses, while no substantial differences were found for the pH of the substrate.

The treatments with micro-organisms in particular (SYB) resulted in a significant reduction in plant mortality caused by the pathogenic fungi *Ganoderma applanatum* and *Laetiporus sulphurous* (**Figure 2**).

Table 1 Evaluation of the use of selected microbial consortia from cacti and succulents on vegetative growth and roots biomass of *Celtis australis*

Groups	Plant height (n°)	Leaves number (n°)	Leaves surface area (cm^2)	Vegetative weight (g)	Roots volume (cm^3)	Roots length (cm)
CTRL	44.78 c	13.11 c	25.36 d	56.49 d	42.30 e	7.83 e
LAM	45.59 c	13.20 c	27.21 c	58.09 bc	44.07 d	8.19 d
SYB	53.37 a	18.40 a	33.77 a	64.66 a	48.77 a	11.33 a
BAC1	47.07 b	16.61 b	28.32 b	58.83 b	46.37 b	8.73 c
BAC2	47.32 b	16.00 b	27.54 b	57.95 c	45.56 c	9.22 b
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). Legend: (CTRL) control; (LAM) *Laminaria digitata* + *Laminaria japonica;* (SYB) Symbac® ;(BAC1) TNC Bactorrs13;(BAC2) Tarantula powder Advanced nutrients

Table 2 Evaluation of the use of selected microbial consortia from cacti and succulents on the microbial biomass of the growing medium and physiological analysis of *Celtis australis*

Groups	Substrate total bacteria (Log CFU/g soil)	pH substrate	Spad
CTRL	2.26 e	6.67 a	28.73 d
LAM	2.61 d	6.60 a	29.18 c
SYB	4.37 a	6.68 a	34.61 a
BAC1	3.27 c	6.52 a	30.33 b
BAC2	3.74 b	6.54 a	30.48 b
ANOVA	***	ns	***

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).Legend: (CTRL) control; (LAM) *Laminaria digitata* + *Laminaria japonica;* (SYB) Symbac® ;(BAC1) TNC Bactorrs13;(BAC2) Tarantula powder Advanced nutrients

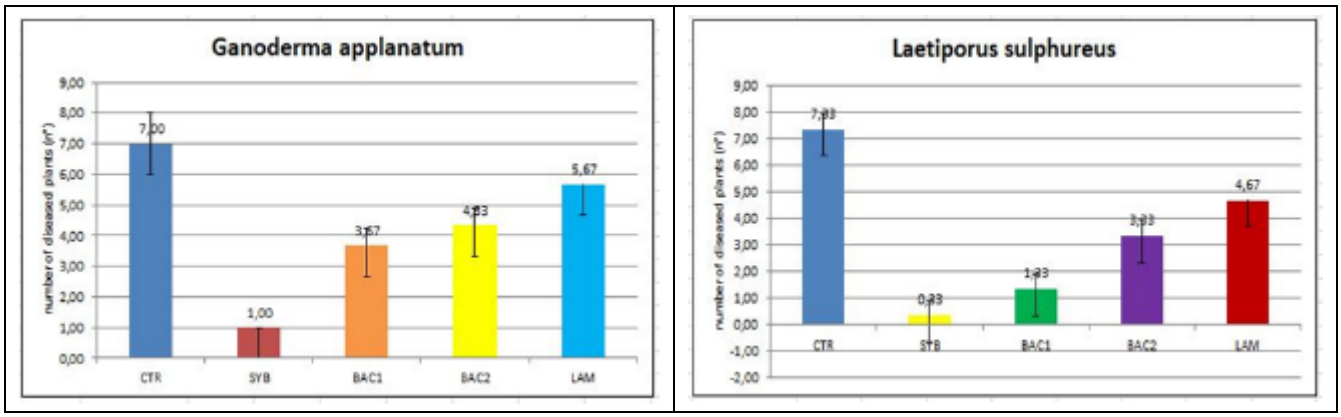

Figure 2 Effect of microorganisms and algae treatments on the control of *Ganoderma applanatum* and *Laetiporus sulphureus*

Figure 3 Comparison of the Symbac® (SYM) and the algae-based control (LAM) thesis in the vegetative growth of *Celtis australis*

Figure 4 Comparison of the Symbac® (SYM) and the algae-based control (LAM) thesis in the roots growth of *Celtis australis*

4. Discussion

An inoculant containing microorganisms from a plant's root zone or roots is referred to as a microbial inoculant. In addition to promoting seed germination and plant growth, they improve plant growth by up to 40% by colonizing the rhizospheres or roots of plants. Soil fertility and plant productivity have been improved by microorganisms [25,26] by increasing nutrient solubilization and root accessibility. Additionally, Rhizobacteria have biocontrol capabilities, which means they can control pests and diseases and promote plant growth [48,49]. As a result of plant growth-promoting rhizobacteria (PGPR), root development is improved, plant and flower life is prolonged, harmful substances are degraded, and young plants are more resistant to biotic and abiotic stress [50-52]. Due to their slow colonization of surfaces and ability to multiply independently over time, microbial inoculants can often be reduced over time [53,54]. Several microorganisms commonly used as biofertilizers have been shown to fix nitrogen and solubilize phosphate. Plants produce many phytohormones when stimulated by bacteria, many of which are used as fertilizers. Growth-promoting components, including indole-acetic acid (IAA), amino acids, and vitamins, can benefit plants [34]. In addition to supplying nutrients to plants (nitrogen, phosphorous, potassium, and essential minerals), PGPRs also produce plant hormones. By reducing the inhibitory effects of pathogens on growth and development, PGPRs can indirectly increase plant growth as biocontrol agents, environmental protectors, and root colonizers [55,56]. Indirectly, PGPRs enable sustainable soil fertility and plant growth by using a sustainable and ecological approach. Through PGPRs, agrochemicals, such as fertilizers and pesticides, can be reduced, soil fertility can be improved, antibiotics can be produced, HCNs can be produced, siderophores can be synthesized, and hydrolytic enzymes can be produced. A significant improvement in the rooting, survival and growth of rose cuttings during the nursery phase was found when microorganisms from the rhizosphere were used [57-59]. Despite the fact that there are no references in the literature, the use of microorganisms selected from succulent and cactus roots for plant stimulation and rutting appears to be an important study. It is possible to use microorganisms from extreme environments to help plants living in our latitudes adapt to climate change. In this experiment, the application of microbial consortia selected from plants living in extreme environments resulted in an improvement in the growth of *Celtis australis* plants, both in the vegetative and root systems. This is a very interesting aspect especially for those nursery species, especially trees, that are notoriously slow to grow. The trial also confirmed how the use of microbial consortia can increase plant resistance to biotic stresses, particularly in this case fungal. Aspects also found in other experimental trials on other plant species.

5. Conclusion

The growth of bacteria is certainly influenced by soil and growing media properties, as well as organic matter and phosphorous content. For sustainable agriculture to be accomplished, plant growth must be improved through bacterial activity. The composition of biofertilisers is crucial to maximizing their potential. In an ecosystem, microbes play a key role in the recycling of nutrients. They interact synergistically. It is important to determine whether the microorganisms that are to be cultivated are actually functional on the plant. In light of possible climate change, it is also important to evaluate new microbial selections from plants that live in extreme environments, such as cacti and succulents. Plant productivity can be maintained while reducing environmental impact and increasing resistance to biotic and abiotic stresses with microbial biofertilisers. In order to improve and speed up the growth of nursery plants, especially trees to be placed in the environment, it seems very important to develop innovative protocols to increase their rooting and vegetative development. Species that are notoriously slow to grow but represent an important heritage of biodiversity and history.

Compliance with ethical standards

Acknowledgements

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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