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Study of root *para*-nodules formation in wheat (*Triticum durum*) inoculated with *Frankia* strain CcI3 and treated with 2, 4-dichlorophenoxyacetate (2, 4-D)

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***Frankia* strains can induce N_2-fixing root nodules on certain non-leguminous plants. It is known that exogenous application of 2,4-dichlorophenoxyacetate (2,4-D) affects root morphology. In this work, wheat roots were treated with 2,4-D and inoculated with the actinomycete *Frankia*. Wheat plants grew in a growth chamber with hydroponic medium. Binocular observation revealed that para-nodules were formed when wheat roots were inoculated with *Frankia* and the root length was enhanced. When the inoculation with *Frankia* was combined to 2,4-D treatment, the para-nodules formed were bigger and more numerous, while the root length was shortened.**

Key words: *Frankia*, wheat, roots, *para*-nodules, 2,4-dichlorophenoxyacetate.

INTRODUCTION

Actinomycetes are found in soil, rhizosphere, ponds and lake sediments. They are a large group of bacteria that can produce secondary metabolites which have important applications in pharmacy, medicine and agriculture (Suttiviriya et al., 2008). Actinorhizal symbioses are mutual interactions between plants, such as *Casuarina glauca* and *Alnus glutinosa*, and the soil bacteria *Frankia* that lead to the formation of root nodules (Perrine-Walker et al., 2010), which are specialized organs for assimilation of the N derived from fixation by the symbiotic bacteria (Berry et al., 2004). Little is known about the signals exchanged between the two partners during the establishment of actinorhizal symbioses (Perrine-Walker et al., 2010).

Synthetic auxins exogenously applied stimulated lateral root primordium initiation on *Alnus* roots, an important step in nodule formation, and thus auxins may play a role in the actinorhizal nodulation process (Berry et al., 1989). It is known that nodule like structures, could be induced by 2,4-D on plant roots and that they could be colonized by microorganisms (Zeman et al., 1992). Little studies were realized about the interaction between *Frankia* and wheat. Soil with *Frankia* had significant effects on canola shoot length, plant dry weight, number of leaves, and total N, but not on wheat shoot length, fresh and dry weight of shoots and ears, dry weight of roots and total N (Cusato and Tortosa, 2000). In this work, para-nodules were induced using 2,4-D and inoculation with *Frankia* and an eventual growth promoting on root system was elucidated.

MATERIALS AND METHODS

Plant host

Seeds of wheat (Triticum durum) were surface-sterilized in 70% ethanol for 20 s, followed by 30 min in a 30% solution of hypochlorite. After various washings with sterile distilled water, the

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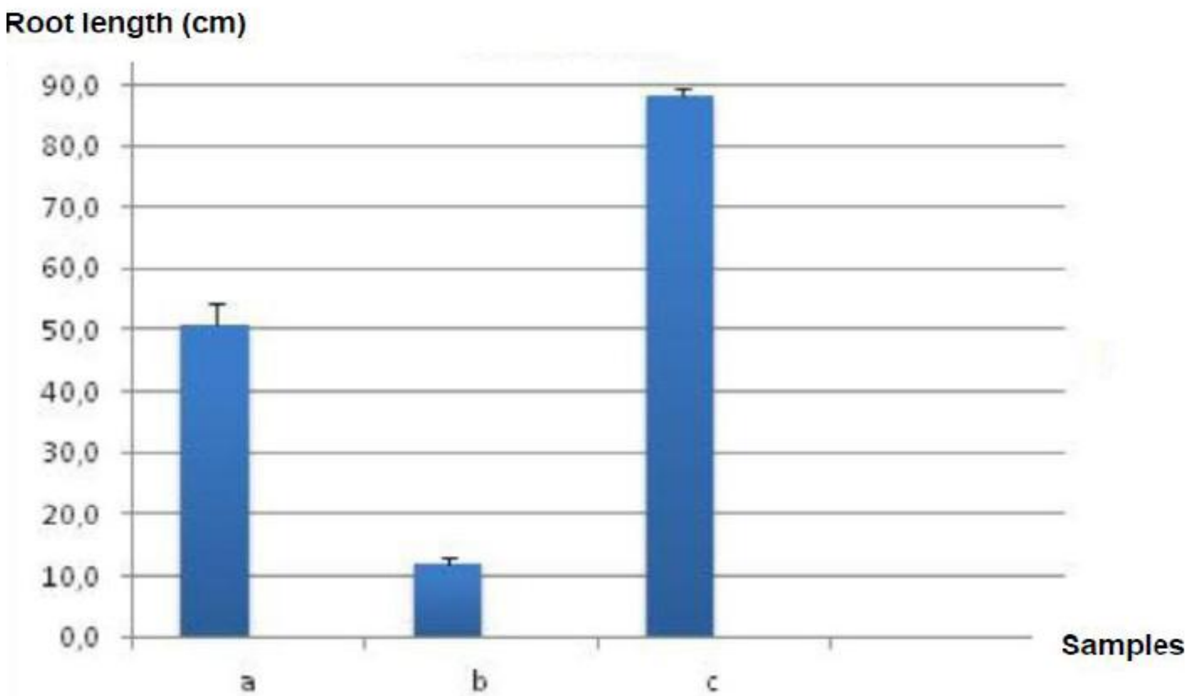

Figure 1. Root length difference (cm). **a.** control. **b.** inoculated root with Frankia and treatment by 2,4-D. **c.** inoculated root with Frankia.

seeds were germinated in the dark under sterile conditions on moist filter paper in Petri dishes (Saatovich, 2006). The germinating seeds were then placed in 1 L bottles (sterilized by UV) containing sterile hydroponic solution and grown until one week old before inoculation with Frankia. 0.4 mg/l of 2,4-D was added to hydroponic solution for para-nodule induction.

Bacterial material

20 ml of Frankia strain CcI3 culture (isolated from Casuarina glauca) were centrifuged at 5000 rpm for 15 min. The supernatant was eliminated; 5 ml of sterile distilled water were added to the residuum to reduce Frankia hyphae, using sterile syringe. 15 ml of sterile distilled water were added to the suspension and used for inoculation. Seedlings of wheat were grown for three months.

RESULTS AND DISCUSSION

Effects of 2, 4-D and *Frankia* on root system

Inoculation with *Frankia* alone increased the root length. When combined with 2,4-D treatment, the root length was considerably shortened (Figure 1), and the lateral ones were hardly absent. These results are in accordance with those of El-Shahed and Abdel-Wahab (2006), who established that different 2,4-D concentrations in combination with *Nostoc rivulare* decrease root length in wheat, maize and rice, while the simple application of *N. rivulare* significantly enhances shoot and root weight as well as plant height as we observed in this work with *Frankia*. Fischer et al. (2000), showed that wheat plants are treated with 2,4-D (0.4 μg /ml) and inoculated with *Azospirillum brasilense* present shorter roots.

Seedlings of wheat (*Triticum durum*) grew, during 3 months, in bottles containing hydroponic solution which is composed of 0.4 mg/l of 2,4-D and 20 ml of suspended *Frankia* strain CcI3 (isolated from *Casuarina glauca*). A represent control, B as inoculated root with *Frankia* and treatment by 2,4-D and C as inoculated root with *Frankia*. The significant elongation of wheat root only inoculated with *Frankia* due to the root oxygenation in bottles which was reduced, in comparison with some actinorhizal genera where the length of nodule roots is inversely correlated with the external oxygen tension (Pawlowski and Demchenko, 2012).

A pink coloration was observed on certain inoculated root parts. These pink zones were probably colonized by *Frankia* and the coloration was related to hemoglobin protein. Pawlowski and Demchenko (2012) noted that the promoters of soybean leghemoglobin and of the symbiotic hemoglobin from *C. glauca* are both specifically active in cells that stably accommodate the microsymbiont.

Effects of 2,4-D and *Frankia* on *para*-nodulation

Para-nodulation was more pronounced when inoculation was combined with 2,4-D, in terms of number and size, and the distribution of *para*-nodules was irregular but observed along the root length. The *para*-nodules resulting from the simple inoculation with *Frankia* were smaller, less numerous, had an irregular distribution and presented

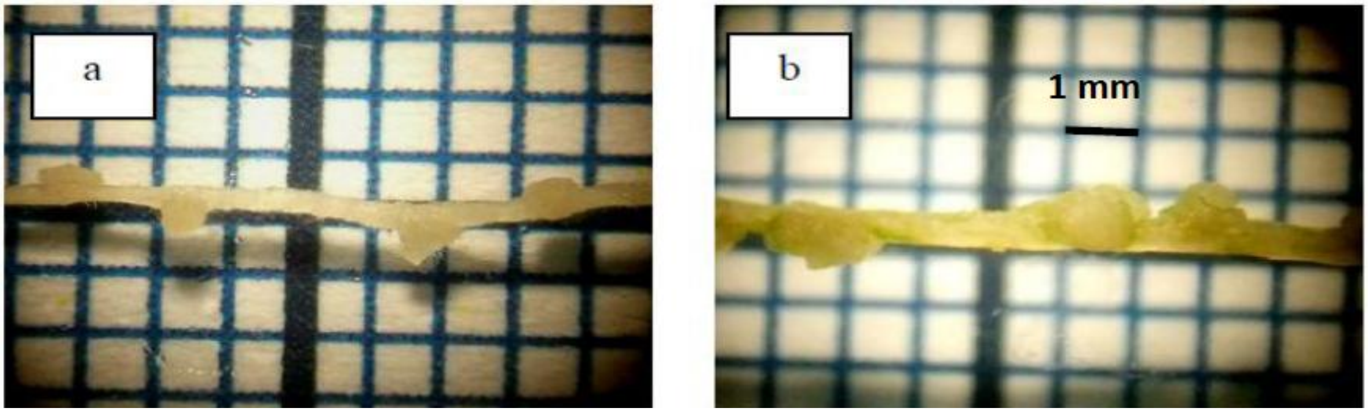

Figure 2. Binocular observation of para-nodules. **a.** Para-nodules induced by the inoculation with Frankia. **b.** Para-nodules induced by the combination between Frankia and 2,4-D.

several shapes (Figure 2). *Para*-nodule formation through 2,4-D treatment is a physiological process independent from bacterial action (Francisco and Akao, 1993). It is strongly possible that *para*-nodules obtained in our study without adding 2,4-D were resulting from the *Frankia* colonization, comparing to Saatovich (2006) who obtained *para*-nodules without the addition of phytohormones on the roots of wheat plants inoculated with *Azospirillum*. Biabani et al. (2012) reported that the colonization of *para*-nodules is more extensive than that of lateral root emergence sites in plants which are not treated with 2,4-D as we found in the present study.

Actinorhizal nodules have an indeterminate growth pattern, which generally consist of numerous conical-shaped lobes and may or may not exhibit nodular roots. The *Rhizobium*-induced nodule can be spherical, cylindrical or collar-shaped according to the patterns of plant cell division and growth of cortical cells as reported by Diouf et al. (2003), while in this study other shapes were obtained in addition to those described by Diouf et al. (2003) and some shapes exhibited root that emerged from the nodules and 2,4-D induced *para*-nodules.

Isolation of *Frankia* from wheat root and nodules

After an isolation experiment, by disinfecting the nodules with 30% of H_2O_2 (Mansour et al., 1990), pleasuring them, then incubating these ones in liquid DPM medium at 30°C (Gtari et al., 2004) for 5 to 9 weeks, it was determined that *Frankia* colonized root and both *para*-nodules obtained by the combination of 2,4-D and *Frankia*, and those obtained only by inoculation with *Frankia*. The quantity of *Frankia* filaments was higher in *para*-nodules obtained when 2,4-D was combined with *Frankia*.

In this study we found that, for the first time, that the actinomycete *Frankia* stimulated the root growth and induced the *para*-nodulation of wheat root. We suggest that *Frankia* may be regarded as Plant Growth Promoting Bacteria and may be used as biofertilizer. These results indicate that *Frankia* has the potential to be applied in natural conditions to increase the root length of wheat plants.

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