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Original article

Effect of bioproducts combination on root growth of *Phaseolus vulgaris* L.

Efecto de la combinación de bioproductos en el crecimiento radicular de *Phaseolus vulgaris* L.

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ABSTRACT: The common bean (*Phaseolus vulgaris* L.) is one of the most important edible legumes because it is an important nutritional supplement in the diet. Its production is limited because of drastic changes in meteorological variables and soil nutrient deficiencies. An alternative to this problem could be the use of biostimulants that improve the root system development and allow plants to adapt to unfavorable environments. In the present study, the effect of two bioproducts, Azofert®-F and Pectimorf®, on the root development of common bean in early stages of vegetative growth was evaluated. Cuba Cueto-25-9-N bean seeds were treated with a mixture of the biostimulants Azofert®-F (at the recommended dose) and Pectimorf® at different concentrations (1, 5, 10, 20, 40 and 100 mg L^{-1}). At 96 hours after seed germination, the number of lateral roots, root length and root dry mass were determined. The application of biostimulants increased the number of lateral roots by more than 22 % when the mixture contained 100 mg L^{-1} of Pectimorf®. No effect of products evaluated was observed on root length and root dry mass variables. The positive effect of the biostimulants Azofert®-F and Pectimorf® on the formation of lateral roots in early stages of vegetative growth could guarantee a better utilization of soil nutrients, which contributes to better plant development and higher crop productivity.

Key words: biostimulants, bean, root system.

RESUMEN: El frijol común (*Phaseolus vulgaris* L.) es una de las leguminosas comestibles más importantes, por constituir un complemento nutricional indispensable en la alimentación. Su producción se ve limitada como consecuencia de los cambios drásticos en las variables meteorológicas y por la deficiencia de nutrientes en los suelos. Una alternativa a esta problemática podría ser el empleo de bioestimulantes que mejoren el desarrollo del sistema radical y permitan que las plantas puedan adaptarse a los ambientes desfavorables. En el presente estudio se evaluó el efecto de dos bioproductos, Azofert®-F y Pectimorf®, en el desarrollo radical de frijol común, en etapas tempranas del crecimiento vegetativo. Las semillas de frijol Cuba Cueto-25-9-N se trataron con una mezcla de los bioestimulantes Azofert®-F (a la dosis recomendada) y Pectimorf® a distintas concentraciones (1, 5, 10, 20, 40 y 100 mg L^{-1}). A las 96 horas de germinadas las semillas, se determinó el número de raíces laterales, el largo y la masa seca radical. La aplicación de los bioestimulantes incrementó el número de raíces laterales en más de un 22 %, cuando la mezcla contenía 100 mg L^{-1} de Pectimorf®. En las variables largo y masa seca de la raíz no se observó efecto de los productos evaluados. El efecto positivo de los bioestimulantes Azofert®-F y Pectimorf® en la formación de las raíces laterales en etapas tempranas del crecimiento vegetativo podría garantizar un mejor aprovechamiento de los nutrientes del suelo, lo que contribuye a un mejor desarrollo de la planta y mayor productividad del cultivo.

Palabras clave: bioestimulantes, frijol, sistema radicular.

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INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is one of the most important edible legumes because it is an indispensable nutritional supplement in the diet (1). In Cuba, it is a food of preference in the daily diet, and it is the cheapest source of protein and the highest source of income for small producers. Its production does not satisfy the consumption demand, so it is necessary to import large quantities of this grain every year. The low yield of this crop in our conditions responds to various factors such as drastic changes in meteorological variables, the presence of pests and diseases, and nutrient deficiencies in the soil (2).

A possible strategy to increase yield, both under normal growth and abiotic stress conditions, is the use of biostimulants. In bean cultivation, the combination of the microbial biostimulant Azofert®-F and a mixture of oligogalacturonides, commercially called Pectimorf®, was shown to stimulate growth as a result of increased nodulation and root system development (3). Pectimorf® rooting effect has also been shown in other crops (4,6). But it is still unknown which elements of root architecture are stimulated by the application of these bioproducts. Therefore, in this study it was considered determining the effect of Azofert®-F and Pectimorf® combination on root development of common bean at early stages of vegetative growth.

MATERIALS AND METHODS

The study was conducted at the National Institute of Agricultural Sciences, Mayabeque province, Cuba. Seeds of bean variety Cuba Cueto-25-9-N, from the seed germplasm bank of the Institute for Fundamental Research in Tropical Agriculture "Alejandro de Humboldt" (INIFAT), Cuba, were used. The commercial product Azofert®-F, with Registration No. 002/17 and patent granted by OCPI Resolution No. 556/2002 (7), based on *Rhizobium leguminosarum* CF1 strain, was used at a concentration of 1 x 10^9 CFU mL^{-1} and at a dose of 200 mL per 46.04 kg of seed. The biostimulant Pectimorf® composed of a mixture of pectic oligosaccharides (oligogalacturonides), with Registration No. RCF 017/18 and patent No. 22859/2003 (8), was used at concentrations of 1, 5, 10, 20, 20, 40 and 100 mg L^{-1}.

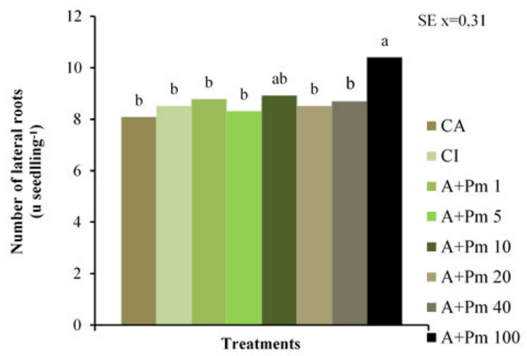
Bean seeds were surface disinfected with 1 % sodium hypochlorite for 10 minutes, followed by three rinses with distilled water (9). Then, they were covered with a mixture containing Azofert®-F and Pectimorf® at the concentrations referred to, maintaining the final proportion of 400 mL for each 46.04 kg of seed. Eight treatments were established in the trial: one in which distilled water was applied to the seeds instead of the mixture of the products (CA), in another, seeds were treated only with Azofert®-F (CI), and the rest of the treatments corresponded to the mixture of biostimulants at Pectimorf® concentrations of reported above.

Treated seeds were left to dry at room temperature for one hour. Then, 20 seeds, according to each treatment, were placed in a petri dish with a double layer of filter paper and 10 mL of distilled water was added to favor germination. The plates were placed for 96 h in a growth chamber (Wissenschaftlich Technische Werkstätten 82362 Weilheim, Austria) at 28 ºC in the dark. After that time, the number of lateral roots was determined; the radicle length, with the help of a graduated ruler of 1 mm precision; and the radicle dry mass, using an analytical balance (*Sartorius* CPA 324S, USA), after placing the samples in an oven (BINDER, USA) for 72 hours, at 75 ºC until constant weight was obtained. Three plates were placed from each treatment. The test was repeated twice.

A completely randomized design was used in the study and the data were processed by simple analysis of variance and comparison of means by Tukey's test $p<0.05$; in the statistical program SPSS, Statistics v22. Graphs were made in Microsoft Excel 2010.

RESULTS AND DISCUSSION

The application of Azofert®-F and Pectimorf®, in different concentrations, did not influence the germination process of Cuba Cueto-25-9-N bean seeds. The germination percentage was 100 % in all treatments, which demonstrated high seed viability. The mixture of biostimulants when applied at a concentration of 100 mg L^{-1} of Pectimorf® increased the number of lateral roots by 29 % compared to the treatment without the bioproducts, and by 22 % compared to the treatment inoculated only with Azofert®-F (Figure 1).



Means with equal letters do not differ significantly according to Tukey ($p<0.05$), n=60. SE x: standard error of the mean

Figure 1. Effect of Azofert®-F (A) and Pectimorf® (Pm) on the number of lateral roots formed in the radicle of Cuba Cueto-25-9-N bean seeds, evaluated at 96 hours. CA: seeds untreated with the bioproducts, CI: seeds only treated with Azofert®-F. Pm 1-100: concentrations of Pectimorf® in mg L^{-1}

In radicle length, no significant effect of biostimulants was observed in relation to the control treatments, but there were differences between treatments with the mixture of Azofert®-F and Pectimorf®, at concentrations of 5 and 100 mg L^{-1} of Pectimorf®, in favor of the latter (Figure 2A). In radicle dry mass, there were no differences between different treatments (Figure 2B).

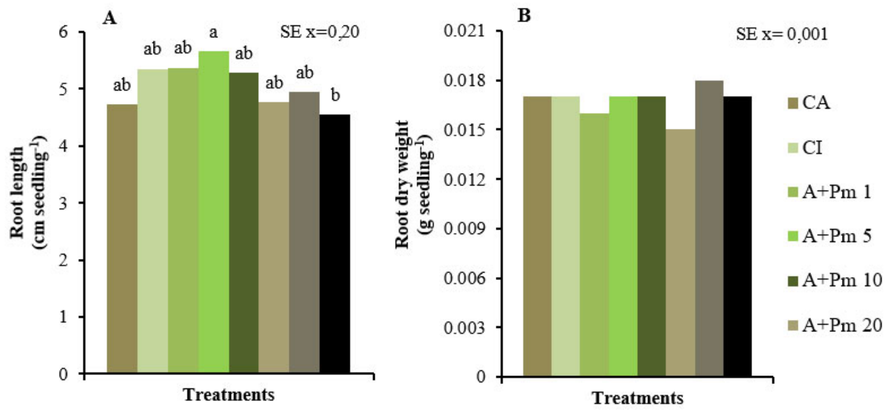


Figure 2. Effect of Azofert®-F (A) and Pectimorf® (Pm) on length (**A**) and radicle dry mass (**B**) of Cuba Cueto-25-9-N bean seeds, evaluated at 96 hours. CA: seeds untreated with the bioproducts, CI: seeds only treated with Azofert®-F. Pm 1-100: concentrations of Pectimorph® in mg L^{-1}

The biostimulant Pectimorf® also favored root system development in banana plants, cultivar 'FHIA-18', at the concentration of 2 mg L^{-1}, under *in vitro* conditions (6). This same effect was observed at higher concentrations (20 mg L^{-1}) in cuttings of guava (*Psidium guajava*) var. Enana Roja Cubana, where the rooting power of this product was manifested in the formation of adventitious roots (5).

The positive effect of the mixture of biostimulants in the formation of lateral roots could be related to the auxinic effect attributed to Pectimorf® (10,11). This phytohormone promotes cell division and differentiation and is involved in lateral root formation and primary root growth (12). Some years ago, studies in *Arabidopsis thaliana* showed that local accumulation of auxin in pericycle cells is sufficient to trigger lateral root formation and emergence from the main root (13). Currently, the mechanisms by which Pectimorf® exerts this effect are unknown, although it is considered that it could be related to the activation of a group of signals that provoke increases in the levels of this phytohormone, favoring all the processes it regulates (11,14,15).

The development of lateral roots by the application of Pectimorf® allows a greater root organization in bean plants, an effect that could guarantee a greater strength for the anchorage of the plant to the soil and favor the absorption of water and minerals, allowing a greater tolerance of plants to drought and nutrient deficit in soils (16,17). The increase in the number of lateral roots also allows greater interaction of the plant with nitrogen-fixing soil bacteria. This could guarantee a greater number of infection sites and bacteroids established in the plant tissue, favoring biological nitrogen fixation (18).

A better nutritional status of plants, as a result of increases in root development, allows greater efficiency in all metabolic processes (photosynthesis, respiration, transport of substances, biological nitrogen fixation). This leads to increases in growth, in the translocation of compounds for pod and seed formation, in yield and in bean grain quality (19,20).

The results of the present study suggest that the application of the biostimulants Azofert®-F and Pectimorf® on bean seeds at the concentration of 100 mg L^{-1} of Pectimorf® could facilitate plants to live in unfavorable environments. In a previous study, it was reported that the inoculant Azofert®-F, under conditions of water deficit, increases the yield of the varieties Cuba Cueto 25-9-R and Tomeguín 93 (21). Other studies have shown that Pectimorf® can minimize the effects caused by water stress in bean plants (22) and by salt stress in rice seedlings (23). In this sense, not only could the effect of the Pectimorf® product influence the formation of lateral roots, but it has also been observed that it has an effect on other elements of root architecture, such as the angle formed by lateral roots with the main root and the diameter and length of lateral roots (16,18). It would be interesting, in subsequent studies, to further investigate the influence of this product on the different indicators of root development.

CONCLUSIONS

The combination of Azofert®-F and Pectimorf® stimulates root development in early stages of vegetative growth in common bean. The biostimulant effect of both products was manifested in an increase in the number of lateral roots at a concentration of 100 mg L^{-1} of Pectimorf®.

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