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PECTIMORF® EFFECT ON BEAN (*Phaseolus vulgaris* L.) STOMATAL INDEX

Efecto del Pectimorf® en el índice estomático de plantas de frijol (*Phaseolus vulgaris* L.)

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ABSTRACT. Plant development and growth is potentiate by Pectimorf under drought conditions, but it action mechanism is unknown. Due to, to determine the effect of Pectimorf® on stomatal distribution in bean (*Phaseolus vulgaris* L.) leaf surface was the aim of this research. C 25-9 Cuban cultivar was employed and sprayed with Pectimorf® to rate of 10 mg L^{-1}.To anatomical study, foliar epidermis was observed to optical microscope (Motic) light and photographed with a camera attached. Stomata variables were measured by morphometric ImageJ program and processed with the statistical package Stargrafic 5.0. Stomata index increase was observed in Pectimorf® treated plant and this result could be to favour plant water status. The structure of epidermal cells remained unchanged on both leaf surfaces, however, some changes in size of stomatal cells were observed in presence of Pectimorf®. More narrow and short occlusive cells on abaxial surface were exhibited on treated plants, so leaves with the most stomatal index were characterized by smallest stomata. The results obtained allowed to know that Pectimorf® causes changes in the distribution patterns and stomatal morphogenesis, which could be to favors successfully growing of bean plant in unfavorable environments.

Key words: stomatal index, oligogalacturonides, bean, *Phaseolus*

RESUMEN. Pectimorf® es un producto bioactivo que potencia el crecimiento y desarrollo de las plantas en condiciones de sequía. No obstante, se desconoce su mecanismo de acción en la planta, por lo que la presente investigación tuvo como objetivo determinar su efecto en la densidad de los estomas en la superficie foliar de plantas de frijol (*Phaseolus vulgaris* L.). Para ello se empleó el cultivar cubano Cuba C 25-9, el cual se asperjó con 10 mg L^{-1} de Pectimorf®. Para el estudio anatómico la epidermis foliar fue observada al microscopio óptico de luz (Motic) y fotografiadas con una cámara acoplada. Las variables estomáticas fueron medidas mediante el programa morfométrico ImageJ y procesadas con el paquete estadístico Stargrafic 5.0. Se demostró que Pectimorf® provoca un incremento en el índice estomático (IE) del cultivar estudiado. La estructura de las células epidérmicas permaneció inalterable en ambas superficies foliares; sin embargo el tamaño de las células estomáticas se modificó con la presencia del producto. En la superficie abaxial las células oclusivas se mostraron más estrechas y cortas, lo que evidenció que las hojas de mayor índice estomático presentaron estomas más pequeños. Los resultados permiten aseverar que Pectimorf® provoca modificaciones en los patrones de distribución y morfogénesis estomática, lo que pudiera favorece el crecimiento adecuado de las plantas de frijol en ambientes desfavorables.

Palabras clave: índice estomático, oligogalacturónidos, frijol, *Phaseolus*

INTRODUCTION

The epidermal foliar surface shows a great number of microscopic pores called stomas. The opening of such pores is controlled through the changes in size and shapes of two specialized cells called occlusive cells that flank the stomatic opening and have a characteristic structure that allows regulating the opening of the stomatic pore (1). These structures are found in all aerial parts of the plant, but they are more abundant in leaves. As the epidermis and the cuticle of aerial organs form a continuous layer and the stomas are the discontinuities where the plant performs most of the exchange of O_2, CO_2, water vapor and other gasses (1, 2).

The shape of the stomas is distinctive of the different groups of plants, being known the difference between mono and dicotiledoneous (1). The patterns

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of development and stomata structure have been dealt with in different studies (3, 4, 5), as well as the stomas response as to size, opening and density as per the CO_2 concentration (6, 7), light intensity (6, 8) water regime (9, 10) and temperature variation (11).

According to the most recent theory explaining the development and the stomatal distribution patterns, these can be formed from cells that have the necessary genetic information called satelital meristemoids that are found in the leaf epidermis (12). So this character is sensitive to change in certain growth stages of the plant as a response to certain environmental stimuli (13, 14, 15, 16, 17, 18, 19, 20) able to trigger off successive divisions of these potential cells.

For these reasons, this study looked at evaluating the effect of Pectimorf® on the stomas distribution at the foliar epidermis of bean plants (*Phaseolus vulgaris* L).

MATERIALS AND METHODS

The research was conducted by the Department of Plant Physiology and Biochemistry of the National Institute of Agricultural Sciences (INCA) and the domestic bean cultivar Cuba C 25-9 (*Phaseolus vulgaris* L.) was used. The trial included 30 plants in plastic containers of 5 liters and a compacted Red Ferralitic soil (21).

In order to guarantee an adequate biological nitrogen fixation in all plants, Azofert was applied to the seeds at planting time at the rate of 200 mL per 50 kg of seeds (22). And for the study of the effect of oligogalacturonide mixture, 15 plants were sprayed with a Pectimorf® solution at the rate of 10 mg L^{-1} at the beginning of the second vegetative stage of the crop, specifically at the time in which 50 % of the plants had deployed the first trifoliate leaf.

Foliar sampling was done in sprayed and control plants, when sheath filling started. In so doing, the third trifoliate leaf of 5 plants per treatment was selected. In order to get the foliar epidermis the scraping procedure was used (23). Sampling focused on the mid third of the leaf, between nerves and once epidermal blades were at hand they were contrasted with toluidine blue. Later on, they were photographed with a camera coupled to the optical light microscope (Motic), using a lens of 40X (IE) and 100X and an eyeglass of 10X.

A total of 20 observations (400X) were made, pertinent to 5 repetitions per treatment. The characterization of stomas (1000x) and the determination of the stomata index (IE) (400x) were done with microphotographs taken with the microscope and the use of the Morphometric Software ImageJ.

For stomas counting, the number of stomas was considered when two occlusive cells were present, and for epidermal cells, when more than 60% was present in the sampled area.

The stomatal index (IE) was estimated with the formula (24):

$$IE= [E/ (E+CE)] \times 100$$

where:
E: Number of stomas
CE: Number of epidermal cells

Means comparison was made with the Student T test for 5% of probability, using the statistical software package STATGRAPHICS Version 5.0 for Windows (25).

RESULTS AND DISCUSSION

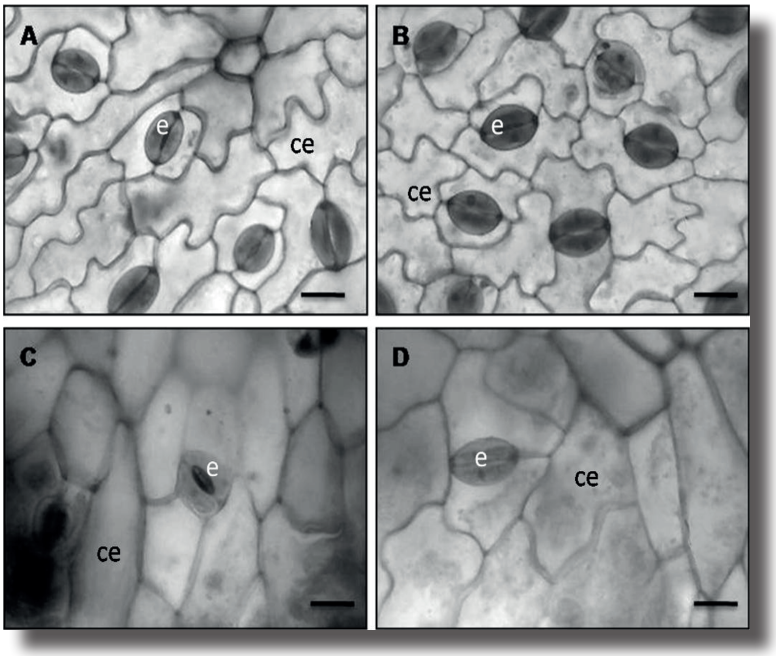
A similar epidermal structure could be seen in sprayed plants of *Phaseolus vulgaris* L. and in control plants, where paracitic stomas, randomly distributed, showed 2 attached cells in parallel regarding the occlusive ones. These characteristics coincide with the above description of common bean plants where occlusive cells showed a kidney-type shape as typical of dicotiledoneous plants (Figura 1).

In relation to their location in the leaf, stomas were seen both in the abaxial as in the adaxial surface, so it is considered an anphistomatic specie (1, 26, 27). However, a higher emergence frequency of stomas was observed on the abaxial surface (Figure 1).

This result responds to a mechanism plants have developed to avoid that sunlight falls directly over the stomas and thus avoid the water loss by transpiration.

Light is the most important stimulus to unfetter stomata movement and the response of the stomas is closely related to the reception and conversion of the light signal as a function of the metabolic activity of occlusive cells (28).

When plants are subjected to short light stimuli, stomas can regulate the transpiration by the opening and closing mechanism, however, when the exposure is at a long term, there are other implications for the plant as dehydration (29). So the lowest location of the stomas on the abaxial surface causes an increased stomata resistance of this surface directly exposed to sun radiation and thus avoids water loss.



(A, B) abaxial surface and (C, D) adaxial surface. e: stoma, ce: epidermal cell. Bar represents 60 microns. 400X

Figure 1. Microphotographies of the foliar epidermis of control plants (A,C) and sprayed plants with 10 mg L^{-1} of Pectimorf®

Table I shows how the abaxial epidermis of sprayed plants with Pectimorf® which recorded a significant increase of the number of stomas and therefore the stomata index, that is not a diagnostic value expressing the number of stomas per foliar surface in the fragments of foliar blades (30).

This result is of great importance taking into account that transpiration and the intensity of respiration in plants is directly proportional to the number of opened stomas (31), since these indicators directly influence on the regulation of the gas exchange and the photosynthetic rate of plants.

Stomata activity is one of the strategies through which plants regulate water loss and carbon uptake because stomas respond quickly to environmental changes (32). The above allows suggesting that an event that modifies the stomata density as a function of biochemical processes and photosynthesis could be an effective way to improve production in some crops. And in this regard, the results attained indicate that at least under these conditions, Pectimorf® could favor the photosynthetic capacity by influencing on the stomata development patterns of bean plants.

On the other hand, Pectimorf® works as hormonal chemical messenger that regulates growth and differentiation mechanisms in different crops accelerating the growth process in plants. So this product could have favored the symbiosis *Bradyrhizobium*-bean, as reported from the joint application of Pectimorf with Azofert and with Azofert and EcoMic in soybean (33).

It has been shown that oligogaracturonides affect the elongation induced by auxins, stimulate flower formation and root organogenesis (34, 35), as well as the thickening of cell walls of the pericycle thus favoring cell division leading to the formation of new stomas (36).

Table I. Behavior of the stomatic index in the abaxial epidermis of bean plants (*Phaseolus vulgaris* L), controls and aprayed with 10 mg L^{-1} of Pectimorf®

Treatment	Number of stomas	Number of epidermal cells	Stomatic index (mm^2)
Control	13,65 a	41,06 a	24,05 a
Pectimorf ®	15,35 b	40,56 a	27,45 b

The morphometric analysis of stomas made evident changes in their morphology. In plants sprayed with the product, both length and width of occlusive cells, significantly reduced compared to those non-sprayed (Table II); in the first ones smaller stomas were observed.

The kidney shape of the stomas seems to be an important characteristic of the plants under moisture and deep shadow conditions, but their dynamic slow behavior could lead to a problem under drought conditions (37). So it could be thought that small stomas observed in Pectimorf®-treated plants could open and close faster being able to easily increase stomatal conductance in leaves and maximize the dissemination of CO_2 during favorable conditions for photosynthesis (37). If it is considered that the transversal section, where gas exchange takes place, is determined by the stomata opening (pore opening) and the anatomical characteristics of the stomas (size and density) (38), and that consequently, the effect of environmental variables on the diffusive conductance of water vapor in the leaf consists in a long and short-term dynamics.

It is possible that Pectimorf® sprayed on the first trifoliate leaf, had favored cell division on the epidermis of subsequent leaves. Let's not forget that long-term processes refer to the expansion period of the leaves (weeks) and imply environmental effects over the stomas capacity to tune up their opening, but also their density and size (39). While short-term dynamics (seconds to hours) is mediated by adjustments in the pore opening that are reversible (28).

The relationship shown between the stomas density and the size of occlusive cells, has also been observed in plants subjected to water stress and in barley exposed to a high light intensity so these high stomata densities usually show up with small occlusive cells of plants exposed to adverse conditions, even when each step of the stomata development, since the initiation till differentiation, is highly genetically organized and regulated (40).

Nevertheless, the response of the number of stomas and the size of occlusive cells to the environmental variables can be strictly dependent on time. This is one of the reasons why the physiological mechanisms of stomatal response have not been fully studied (41, 42).

This research is the first approach to the effect of Pectimorf® on the stomatal morphogenesis of the plants and its potential to favor plant development under adverse conditions. First, it showed evidences to modify stomatal development patterns, which could minimize the water loss by transpiration. On the other hand, modifications in the size of the stomas were found, leading to an adequate relationship between stomas distribution and the size of occlusive cells.

From these results, it is a must to study those physiological indicators directly related to stomata activity, an aspect that would allow a deeper evaluation of Pectimorf® effect on the water status of the plants.

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Table II. Behavior of cell length and width on the abaxial epidermis of bean plants (*Phaseolus vulgaris* L.), control and sprayed plants with 10 mg L^{-1} of Pectimorf®

Treatments	Cell length	Cell width
Control	63,65 a	16,06 a
Pectimorf ®	55,35 b	10,56 b

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