

Changes in photosynthetic pigment and carbohydrate content in common bean cultivars infected by *Colletotrichum lindemuthianum*

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ABSTRACT

The aim of this study was to investigate the changes in photosynthetic pigments and carbohydrate contents on resistant and susceptible plants of *Phaseolus vulgaris* L. (cvs. Mexico 222 and Widusa) infected by *Colletotrichum lindemuthianum* race 23. The experimental design used was entirely randomized in factorial scheme, with 2 cultivars (Mexico 222 and Widusa) and 2 treatments (control and inoculated). The cultivar Widusa (susceptible) showed a significant reduction in photosynthetic pigments, and an increase in the total carbohydrates, sucrose and reducing carbohydrates, whereas the cultivar Mexico 222 (resistant) showed a significant change in the carotenoid and total carbohydrate contents.

Keywords: *Phaseolus vulgaris* L.; *Colletotrichum lindemuthianum*; chlorophyll; carbohydrate; disease

The species *Phaseolus vulgaris* L. is known as common bean, and is considered to be one of the more important leguminous in the world, because of its high protein content, and a source of carbohydrates (Vieira 2005). The exposition and consequent response of the plant to situations of abiotic stress provoked by the water, temperature and salt were previously reported (Lobato et al. 2008b, c). The behavior of the plant in relation to the application of biotic stress has been however only scantily investigated.

Anthraxnose is one of the most frequent diseases in common bean; it is caused by the fungus *Colletotrichum lindemuthianum*. The main symptoms of this disease can be found in pods and leaves with necrosis (Kimati et al. 2005). Results obtained by Lopes and Berger (2001) working with *P. vulgaris*, as well as Leon et al. (1996) and Scarpari et al. (2005) revealed changes in photosynthetic

pigments after pathogen infection in susceptible plants. The accumulation of carbohydrates under conditions of biotic and abiotic stress are described by Maust et al. (2003) and Lobato et al. (2008a). The aim of this study was to investigate the changes in photosynthetic pigment and carbohydrate contents in the cultivars Mexico 222 (resistant) and Widusa (susceptible) infected by race 23 of *Colletotrichum lindemuthianum*.

MATERIAL AND METHODS

Plant material. The pathogen *Colletotrichum lindemuthianum* race 23-resistant seeds of cv. Mexico 222 and susceptible cv. Widusa, obtained from the seed bank of the Nupagri, were grown in containers (length × width × height; 40 × 30 × 10 cm, respectively) with the substrate Plantmax in

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a greenhouse under natural day/night 12 h/12 h, temperature of 27.8°C/7.6°C and relative humidity 42%/71%, respectively. The mid-day photosynthetic active radiation (PAR) was 1860 $\mu\text{mol}/\text{m}^2/\text{s}$. Twenty seeds were placed in each container and the seedlings were thinned after the 8th day to 12 seedlings per container. Twenty-five-day-old seedlings were inoculated with fungal spores. Fungal isolate was obtained from Nupagri and was cultured at 20 \pm 2°C for 15 days for sporulation as described by Mathur et al. (1950). Plants were inoculated with suspension of spores adjusted to 1.2 \times 10⁶ spores/ml and grown in a mist chamber for 96 h at 20 \pm 2°C, relative humidity of 100% and photoperiod of 12 h.

Experimental design. The experimental design was randomized in factorial scheme, with 2 cultivars (Mexico 222 and Widusa) combined with 2 treatments (control and inoculated). The experiment was composed of 6 repetitions and 24 experimental units with 1 plant in each unit. The statistical analyses were carried out with the SAS software (SAS Institute 1996).

Chlorophyll and carotenoid estimation. Fresh 1st leaf tissue (25 mg) was homogenized in 2 ml of acetone (80%, v/v, Nuclear), centrifuged at 5000 \times g, for 10 min at 5°C. Chlorophyll and carotenoid was quantified using the equations by Lichthenthaler (1987).

Estimation of carbohydrates. Powdered oven-dried leaves (70°C for 96 h) were used for sucrose and carbohydrate estimation. Total water soluble carbohydrates were estimated as described by Dubois et al. (1956), using glucose (Sigma chemicals) as standard at 490 nm. Sucrose was extracted in methanol:chloroform:water 12:5:3 (v/v) and estimated as in Van Handel (1968), using sucrose (Sigma chemicals) as standard at 620 nm. The reducing carbohydrates were determined by deducting sucrose content from the total soluble carbohydrates.

RESULTS AND DISCUSSION

The chlorophyll content in cv. Mexico 222 showed no significant change in contrast to cv. Widusa with a significant decrease of 15.2% of control in the inoculated plants (Figure 1B). The carotenoid content was significantly reduced by 20% of control in the inoculated plants of cv. Mexico 222 compared to that of 30.5% in cv. Widusa (Figure 1A). Our results are similar to the decrease of photosynthetic pigments in *P. vulgaris* plants inoculated

with *Uromyces appendiculatus* (Lopes and Berger 2001) and with *Xanthomonas campestris* (Berova et al. 2007).

The total soluble carbohydrates in the inoculated resistant cv. Mexico 222 demonstrated a significant increase of 10.7% over the control, whereas the susceptible cv. Widusa showed a significant 26% increase (Figure 2A). Both the cultivars showed a pathogen-induced increase in the total carbohydrate content.

There was no significant change in the disaccharide – sucrose content in the infected cv. Mexico 222 (Figure 2B). On the other hand, pathogen-infested cv. Widusa (susceptible) had a significant

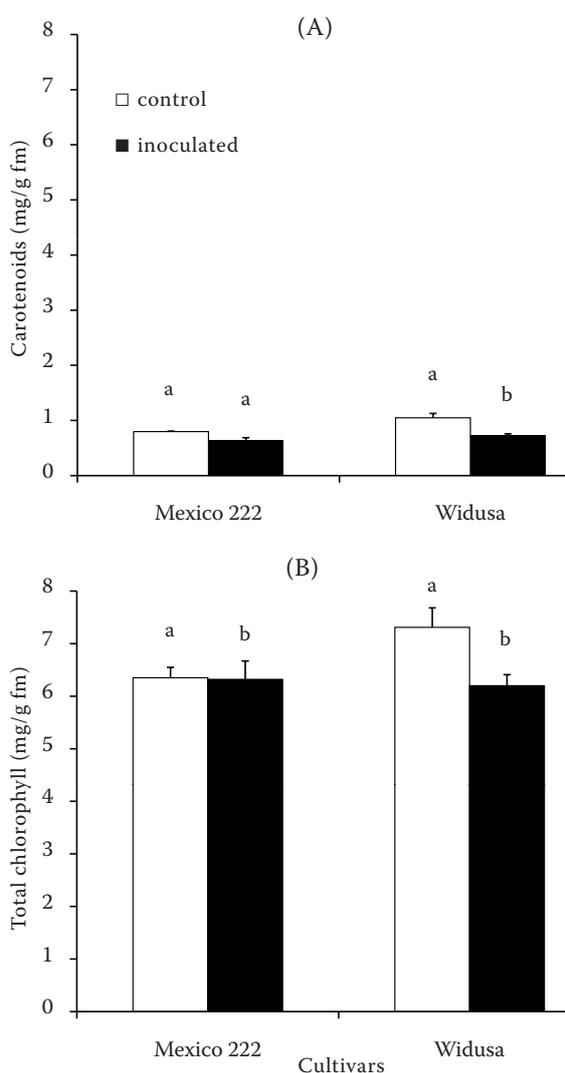


Figure 1. Changes in carotenoid (A) and chlorophyll (B) content in *Phaseolus vulgaris* L. cv. Mexico 222 and cv. Widusa inoculated with *Colletotrichum lindemuthianum* race 23. Each histogram is mean \pm SE ($n = 6$). Averages followed by the same letter do not differ among themselves in the Tukey test at 5% of probability

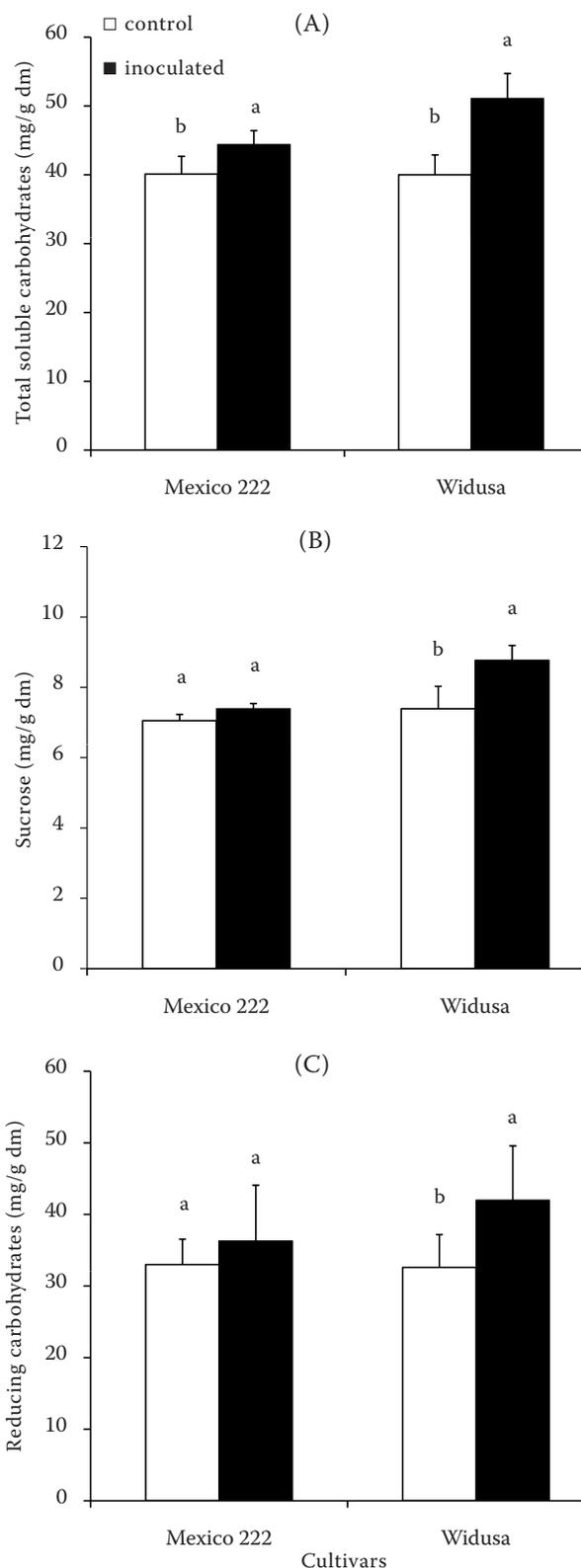


Figure 2. Total soluble carbohydrates (A), sucrose (B) and reducing carbohydrates (C) content in *Phaseolus vulgaris* L. cv. Mexico 222 and cv. Widusa inoculated with *Colletotrichum lindemuthianum* race 23. Each histogram is mean \pm SE ($n = 6$). Averages followed by the same letter do not differ among themselves in the Tukey test at 5% of probability

increase of 18.6% over control values (Figure 2B). These results reveal that unlike the total carbohydrate content, only susceptible cultivar suffers from an increase in sucrose level.

The reducing carbohydrates level, measured as total carbohydrate minus sucrose content, was 33 mg/g dm in control and 36.3 mg/g dm in inoculated cv. Mexico 222, respectively (Figure 2C). In contrast, cv. Widusa showed a significant increase of 28.8% over control values of reducing carbohydrates in the inoculated plants. These results indicate that only the susceptible cultivar showed significant changes in reducing carbohydrate content.

Reduction in photosynthates in *P. vulgaris* plants infected with *C. lindemuthianum* and *Uromyces appendiculatus* was reported by Lopes and Berger (2001). Scarpari et al. (2005) reported a reduction in the accumulation of total soluble carbohydrates in *Theobroma cacao* plants infected with *Crinipelis pernicioso*. On the other hand, in the present study both the susceptible and resistant cultivars showed an increase in total soluble carbohydrate content in the pathogen-infected plants. Arias et al. (2003) reported similar results in sunflower (*Helianthus annuus* L.) plants infected with sunflower chlorotic mottle virus. The reducing carbohydrates in the susceptible cv. Widusa showed an increase probably due to the maximization of invertase and sucrose synthase enzyme activities. Such an activity might cause a non-significant change in the sucrose content in the infected plants.

This study revealed that the Widusa cultivar when infected by race 23 of *C. lindemuthianum* suffers from reduction in photosynthetic pigments and accumulate carbohydrates. On the other hand, the resistant cv. Mexico 222 showed a significant decrease in carotenoids and an increase in total carbohydrate content when infected with the race 23 of *C. lindemuthianum*.

REFERENCES

- Arias M.C., Lenardon S., Taleisnik E. (2003): Carbon metabolism alterations in sunflower plants infected with the sunflower chlorotic mottle virus. *J. Phytopathol.*, 151: 267–273.
- Berova M., Stoeva N., Zlatev Z., Stoilova T., Chavdarov P. (2007): Physiological changes in bean (*Phaseolus vulgaris* L.) leaves, infected by the most important bean disease. *J. Cent. Eur. Agric.*, 8: 57–62.
- Dubois M., Gilles K.A., Hamilton J.K., Rebers P.A., Smith F. (1956): Colorimetric method for determina-

- tion of sugars and related substances. *Anal. Chem.*, 28: 350–356.
- Kimati H., Amorim L., Rezende J.A.M., Bergamin Filho A., Camargo L.E.A. (2005): *Phytopatology Guide: Diseases of Cultivated Plants*. Agronômica Ceres, São Paulo.
- Leon R., Santamaria L., Alpizar A., Escamilla M., Oropeza C. (1996): Physiological and biochemical changes in shoots of coconut palms affected by lethal yellowing. *New Phytol.*, 134: 227–234.
- Lichthenthaler H.K. (1987): Chlorophylls and carotenoid: pigments of photosynthetic biomembranes. *Methods Enzymol.*, 148: 350–382.
- Lobato A.K.S., Oliveira Neto C.F., Costa R.C.L., Santos Filho B.G., Cruz F.J.R., Laughinghouse IV H.D. (2008a): Biochemical and physiological behavior of *Vigna unguiculata* (L.) Walp. under water stress during the vegetative phase. *Asian J. Plant Sci.*, 7: 44–49.
- Lobato A.K.S., Oliveira Neto C.F., Santos Filho B.G., Costa R.C.L., Cruz F.J.R., Neves H.K.B., Lopes M.J.S. (2008b): Physiological and biochemical behavior in soybean (*Glycine max* cv. Sambaiba) plants under water deficit. *Aust. J. Crop Sci.*, 2: 25–32.
- Lobato A.K.S., Santos Filho B.G., Costa R.C.L., Oliveira Neto C.F., Meirelles A.C.S., Cruz F.J.R., Alves G.A.R., Neves H.K.B., Pita J.D., Lopes M.J.S., Freitas J.M.N., Monteiro B.S., Ferreira Ramos R. (2008c): Physiological and biochemical changes in soybean (*Glycine max*) plants under progressive water deficit during the vegetative phase. *Agric. J.*, 3: 327–333.
- Lopes D.B., Berger R.D. (2001): The effects of rust and anthracnose on the photosynthetic competence of diseased bean leaves. *Phytopathology*, 91: 212–220.
- Mathur R.S., Barnett H.L., Lilly V.G. (1950): Sporulation of *Colletotrichum lindemuthianum* in culture. *Phytopathology*, 40: 104–114.
- Maust B.E., Espadas F., Talavera C., Aguilar M., Santamaria J.M., Oropeza C. (2003): Changes in carbohydrate metabolism in coconut palms infected with the lethal yellowing phytoplasma. *Phytopathology*, 93: 976–981.
- SAS Institute (1996): *SAS/STAT User's Guide*, Version 6.12. SAS Institute, Cary.
- Scarpari L.M., Meinhardt L.W., Mazzafera P., Pomella A.W.V., Schiavinato M.A., Cascardo J.C.M., Pereira G.A.G. (2005): Biochemical changes during the development of witches broom: the most important disease of cocoa in Brazil caused by *Crinipelis pernicioso*. *J. Exp. Bot.*, 56: 865–877.
- Van Handel E. (1968): Direct microdetermination of sucrose. *Anal. Biochem.*, 22: 280–283.
- Vieira C. (2005): *Memory of Half Century of Study on Bean Crop*. Editora UFV, Viçosa.

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