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Nutritional characterization of healthy and *Aphelenchoides besseyi* infected soybean leaves by laser-induced breakdown spectroscopy (LIBS)

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A R T I C L E   I N F O

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A B S T R A C T

Soybean and its derivatives are one of the most valuable and traded agricultural commodities worldwide. The major problem faced by the producers is the reduction of soybean yield due to diseases. In Brazil, the green stem and foliar retention (GSFR) was recently described as affecting soybean plants and causing concerns. Unfortunately, no effective methods of early diagnosis and treatments are known. In an attempt to better investigate the plant changes caused by GSFR infection, soybean leaves collected from healthy and sick plants of two varieties from two different places of Brazil were evaluated comparatively for their content of the three macronutrients Ca, K and Mg by laser-induced breakdown spectroscopy (LIBS). Atomic absorption spectrometry (AAS) was used as the reference technique. In general, the relative simplicity of LIBS instrumentation and the minimal sample preparation required makes it a valuable tool for agriculture application, including nutritional investigation and disease diagnosis of plant samples. The Pearson coefficients obtained for the correlation between LIBS and AAS data were close to 0.80 for the three nutrients analyzed. The results obtained by applying the Student *t*-test and Principal Component Analysis (PCA) to experimental data allowed to discern between healthy and sick plant leaves. LIBS data analyzed by the classification via regression (CVR) method associated with Partial Least Square Regression (PLSR) yielded success rates higher than 80% in class differentiation. This study demonstrates the possibility of using LIBS as a convenient analytical tool to discern between healthy and GSFR infected plants by analyzing the three macronutrient Ca, K and Mg, thus providing an early GSFR diagnostic tool.

1. Introduction

Nowadays soybean and its derivatives, including oil, animal feed, protein for human diet and biofuel, are among the most valuable and traded agricultural commodities worldwide, representing over 10% of the total value of agricultural trade [1,2]. In the last 40 years the production demand of soybean grain has increased by over 5 times mainly due to the world population growth, supply availability and maintenance of stock levels at a reasonable price [1,3]. As soybean is one of the main animal feed grain and protein meal, the increased production is related mainly to livestock production, besides manufacturing of vegetable oils [4]. Thus, to face the increasing global soybean demand it is prime to increase its yield and decrease its losses, without increase the soybean cropped areas significantly [1].

Currently, USA (31.3%), Brazil (27.6%) and Argentina (17.4%) are the main soybean producers totalizing more than 76% of all soybean production in the harvest 2014/2015 [5]. According to the Agricultural Projections to 2025 of the United States Department of Agriculture (USDA), the USA soybean exports are expected to rise, although Brazil would remain the world leader in soybeans exportation. Nowadays, soybean is cultivated in 57% of the total Brazilian cropped area, and until 2025 it is expected to achieve the greatest increase (more than 1.8%) [6] in an attempt to compensate losses due to diseases [7]. Thus, the development of new agricultural technologies able to render the production process more efficient and improve the yields is a unanimously recognized need in order to respond to the worldwide demand of increasing soybean production with no further increase of cropped areas.

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The problems encountered by the producers in the field are associated to abiotic and especially biotic constraints due to numerous pests and pathogens that cause diseases to soybean plants and may lead to their death and/or marked reduction of yield [8]. In particular, in Brazil, great production loss were ascribed to a recently described disease, i.e. the green stem and foliar retention (GSFR), a syndrome popularly known as "Soja Louca II" (mad soy II), currently ascertained to be caused by the nematode *Aphelenchoides besseyi* [9]. The main symptoms of GSFR are more evident after flowering until harvest, when stems, petioles, leaves and pods of diseased plants remain green. Younger leaves in the upper canopy usually show symptoms such as distortions, blistering, strapping and vein thickening. Stems remain moisted, immature and sometimes are twisted and fluted, with enlarged nodes. Pods are distorted, thickened, with brown corky necrosis. High levels of flower abortion are common with bud proliferation occurring in some instances [10,11]. High temperatures (average above 28 °C) and frequent rainfall in the soybean vegetative stage are the main climatic factors responsible for the GSFR. Since the 2005/2006 crop season, this disease has caused a decrease of up to 60% of soybean yield, especially in the states of Maranhão, Tocantins, Mato Grosso and Pará [12].

Unfortunately, methods able to provide early diagnosis of *Aphelenchoides besseyi* infection and related treatments are not yet available. However, a prior desiccation of soybean seedlings and a thorough effective weed control immediately after soybean emergence, i.e. during the first vegetative state of plant growth, appear to prevent the insurgence of the disease [10,11]. Actually, the identification of GSFR in the field is done by visual inspection of plants, thus the need of more information on disease progress and available tools able to struggle the disease urges the development of convincing and convenient techniques and methods able to distinguish soybean leaves from healthy and GSFR infected plants.

Laser-induced breakdown spectroscopy (LIBS) is a current analytical technique that uses an intense and short-pulsed focused laser to vaporize, atomize and excite the sample in a single step [13]. The laser-induced plasma emission allows the measurement of sample multi-elemental composition, which makes this technique fast and reliable for qualitative and quantitative analysis. The relative simplicity of LIBS instrumentation, the minimal or no sample preparation required and the possibility to be employed in the field made this technique a valuable tool to be applied in a huge variety of fields, including agriculture. In particular, LIBS showed a very promising analytical performance in investigating the qualitative and quantitative composition of macronutrients, micronutrients and contaminants in plants [14–22] and soils [23–29].

The appearance of GSFR symptoms has been possibly ascribed to the unbalance of the macro nutrients Ca, K and Mg [30], in the present paper, leaves from healthy and diseased soybean plants were analyzed comparatively by LIBS for their content of these three macro elements using atomic absorption spectrometry (AAS) as the reference technique. The Student *t*-test and the chemometric tool Principal Component Analysis (PCA) were applied to evaluate the experimental data. In order to verify the possibility of LIBS analysis of Ca, K and Mg as an early diagnostic and discrimination tool of GSFR, data were also evaluated by the Classification Via Regression (CVR) method associated to Partial Least Square Regression (PLSR).

2. Materials and methods

2.1. Leaf samples

The soybean leaves were collected from 35 healthy plants and 35 plants affected by the GSFR disease in two different fields, Xingu (Riachão city) and Parnaíba (Balsas city), located at about 180 km one from the other in Maranhão State, Brazil. The Xingu field was planted with the soybean variety M9144RR and the other with the variety TMG1188RR. Based on the different variety and field location, two separate groups were considered to minimize the variables and make the study more consistent in terms of GSFR characterization.

Fresh leaves were packed in individual paper bags and dried in a stove under air flux (Nova Ética Produtos e Equipamentos Científicos LTDA, model 400 ND v5) for 72 h at 60 °C. The dried samples were frozen and then homogenized by manual grinding to obtain particles sizes smaller than 250 μm. Finally, the leaf powders were pelletized by applying a pressure of 6 t cm^{-1} for 1 min, and pellets obtained were kept in a desiccator until analyses.

2.2. Laser-induced breakdown spectroscopy (LIBS) setup and data processing

LIBS measurements were performed by a LIBS2500 apparatus (Ocean Optics, Dunedin, USA) consisting of a Q-switched Nd:YAG laser (Quantel, Big Sky Laser Ultra50) operating in ambient atmosphere (air) at the wavelength of 1064 nm with a pulse energy of 50 mJ, a repetition

Table 1
LIBS spectral ranges presenting the most statistically significant differences between Ca, K and Mg peaks as identified by the Student's *t*-test.

Element (atomic/ionic lines)	Number of peaks	Range (nm)
Mg (ionic)	2	278.5–281.0
Ca (ionic)	2	315.0–321.0
Ca (atomic)	1	420.0–426.0
Mg (atomic)	2	517.0–518.7
K (atomic)	2	765.0–772.0
Ca (ionic)	1	850.0–858.0

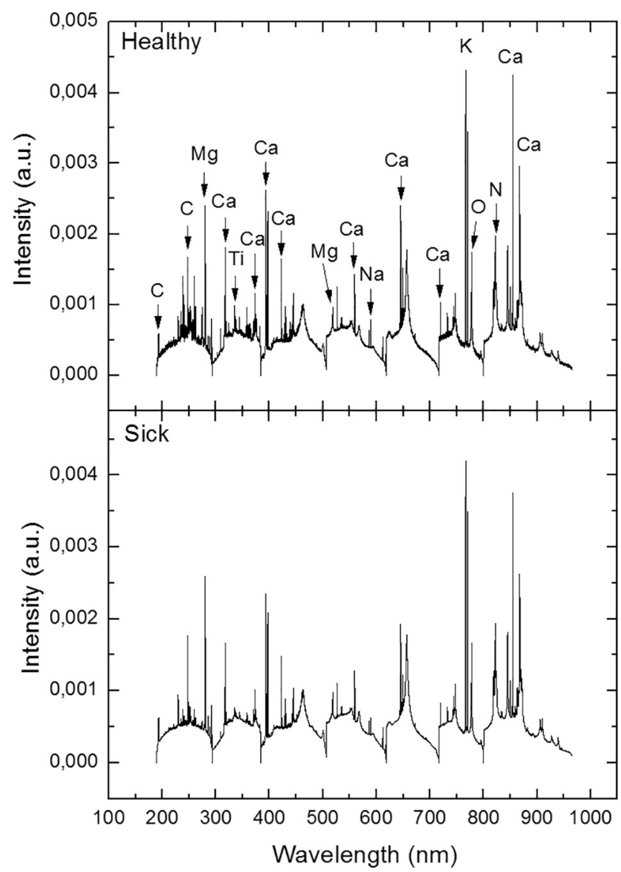

Fig. 1. Typical averaged broadband LIBS spectra of healthy and sick soybean leaves.