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Prof. Dr Snežana Šerbula

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VIABILITY ASSESSMENT OF MAIZE (*Zea mays* L.) SEEDS CONTAMINATED WITH AFLATOXIN USING FLUORESCENCE SPECTROSCOPY

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Abstract

Maize seeds are a primary source of nourishment and their viability is a critical consideration to ensure a reasonably high harvest and seed quality. In this work, we study the effect of aflatoxin induced stress on the seed viability concerning the germination, as well as the estimation on the impact on the viability using fluorescence spectroscopy. Our results showed that the contaminated seeds exhibit significant decreases in the percent of germination, even at low levels, if compared to the uncontaminated seeds ($P < 0.05$). Furthermore, the fluorescence ratio of 453/680 and 680/751, could be used for the rapid screening of the viability of the maize seeds.

Keywords: maize (*Zea mays* L.), Seed viability, aflatoxin stress, fluorescence ratios

INTRODUCTION

Aflatoxins are toxic secondary metabolites produced by the fungi, such as Aspergillus flavus. They contaminate a variety of food commodities, including wheat, maize, rice, dried fruits, and nuts [1]. Maize is one of the main energy source for animal feed amd hence, from an economical point of view, it is one of the most important crops. Maize seeds can be infested with toxigenic fungal species in the field or during storage, which can lead ultimately to a contaminating of the food, as well as the feed with mycotoxins, showing toxic effects on animals and humans [2].

The phytotoxic effect of aflatoxin is observed on the basis of the remarkable inhibitory effect on chlorophyll and carotenoid synthesis and the reduction of seed germination and seedling growth of lettuce mung, mustard, cowpea and sesame [3].

In this work we use fluorescence spectroscopy to investigate the phytotoxic effects of aflatoxin in low-stressed maize seeds, in terms of the seeds' viability.

MATERIALS AND METHODS

Plant Materials

The maize (*Zea mays* L.) seeds, both uncontaminated, as well as contaminated with aflatoxin B1(AFB1), were used in this study. The AFB1 concentration in the maize samples was 33 ppb. A total of 80 seeds were divided into two groups, with four replicates per group.

The seeds were immersed in a 0.2% sodium hypochlorite solution, after that the seeds were washed with plenty of water. The seeds are placed between the two paper filters in the Petri dishes, and 10 ml of distilled water is poured onto the upper filter paper. Incubation was performed in the laboratory, at a temperature of approximately 25° C in dark, sealed in aluminium foil bags for four days. The number of germinated seeds was noted every day (Germination was considered as the detection of the radicle breaking through the seed coat). For the preparation of the maize extracts, the seeds were homogenized with 80% cold methanol. The homogenates were kept in the shaker for one hour and then centrifuged at 10 000 x g for 5 min.

Fluorescence spectroscopy

The fluorescence spectra were recorded for both the contaminated and un-contaminated maize seeds before germination (BG), and after the fourth day of germination (compare nonviable (G0) and viable seeds (G1)). The samples' fluorescence spectra were recorded using an Fl3-221 P spectrofluorimeter (JobinYvon, Horiba, France), equipped with a 450 W Xe lamp and a photomultiplier tube. The emission spectra, ranging from 400 to 800 nm, were recorded with an excitation wavelength of 375 nm. A spectral bandwidth of 2 nm was set for both the excitation and emission slits. The fluorescence ratios 453/680 nm and 680/751 were calculated.

RESULTS AND DISCUSSION

Figure 1 shows that the aflatoxin-contaminated seeds exhibited significant decreases in the percentage of germination, compared to the uncontaminated seeds ($p<0.05$). Our results suggest that the presence of aflatoxin leads to changes in the seed metabolism, leading to a possible delay of the germination process.

The fluorescence spectra of the methanolic extracts from both contaminated and uncontaminated maize seeds, before germination (BG), as well as after the fourth day of germination, are shown for the non-viable seeds in Figure 2 a), and for viable seeds in Figure 2 b). As shown in the Figure 2, three different maxima were observed at 453 nm, 680 nm and 751 nm in all analyzed samples in the emission range between 400 nm to 800 nm. Both types of the non-viable seeds (G0) have a more pronounced fluorescence peak near 680 nm. The analyzed fluorescence peaks arising from chlorophyll fluorescence [4].

The changes in the fluorescence ratios 453/680 nm and 680/751 nm are shown in figure 3. The fluorescence in the blue and green regions is emitted by secondary metabolites (mainly plant phenols) related to plant defence [4,5]. We observed a lower ratio at 453/680 nm for the nonviable seeds that are contaminated with AFs compared to the non-contaminated maize seeds (G0X and G0Y). The opposite effects were observed for viable seeds. That could imply a difference in the seed's metabolic state under the influence of aflatoxin and that could be used as an indicator of aflatoxin stress.

On the other side, the ratio 680/751 nm, an indicator of the Chlorophyll content, was higher in the aflatoxin-stressed seed than in the non-contaminated nonviable maize seeds. This observation is in agreement with early works [6], which showed that seeds with a high chlorophyll fluorescence signal were of lower quality (viability).

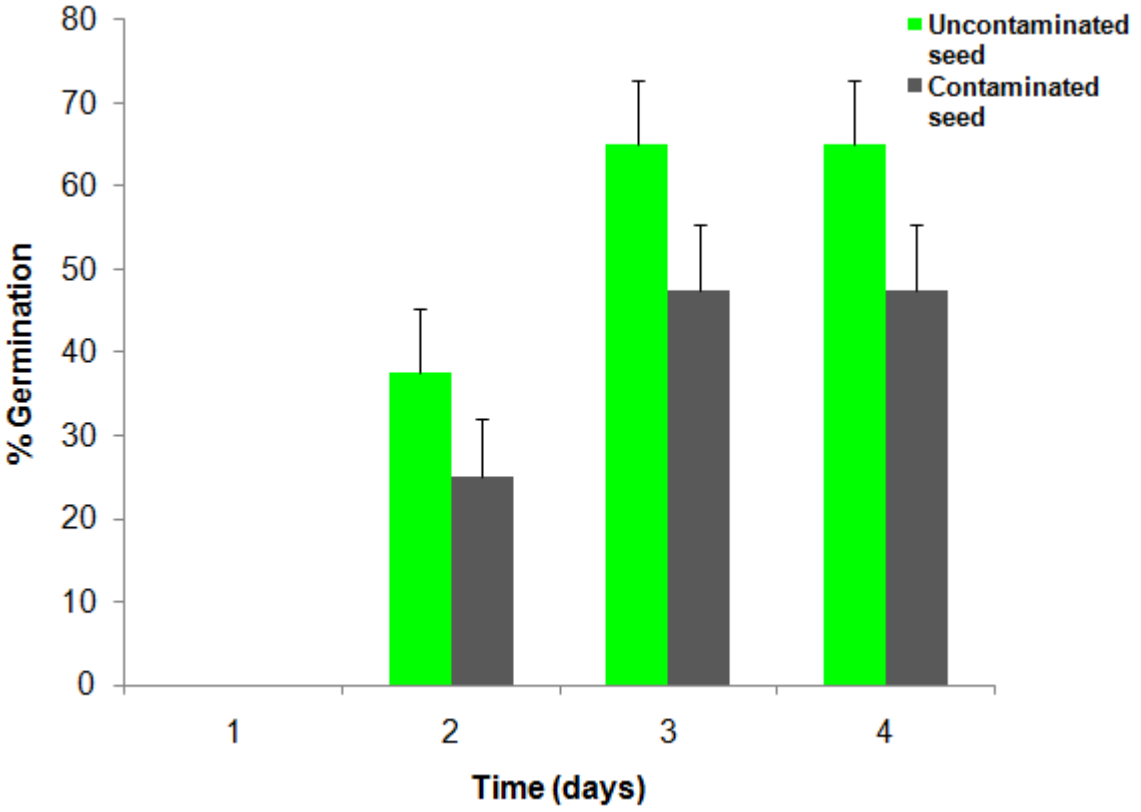

Figure 1 *Variations in germination dynamics of uncontaminated and aflatoxin-contaminated maize seeds after 1, 2, 3 and 4 days*

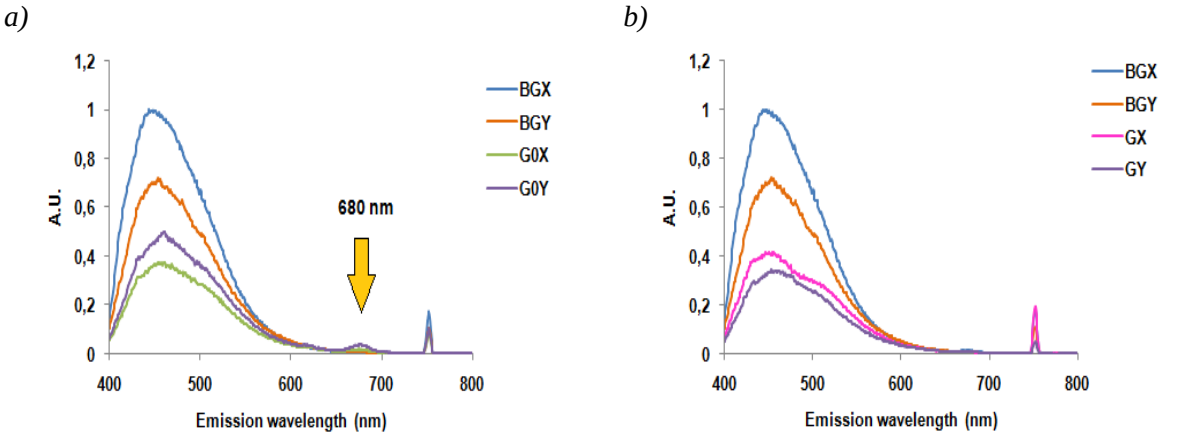

Figure 2 *Comparison fluorescence spectra of the methanolic extracts for the contaminated (Y) and uncontaminated (X) seeds before germination (BG) with a) nonviable (G0) b) viable (G1) seeds*

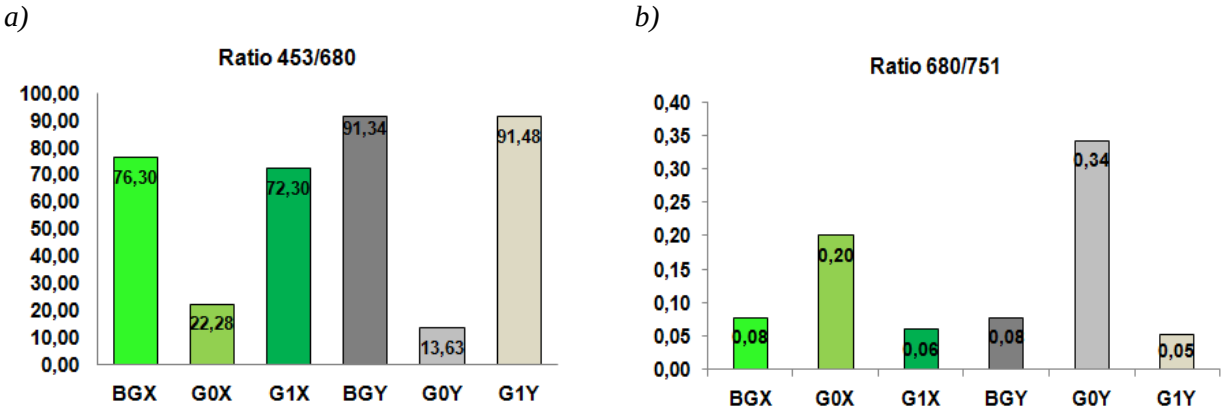

Figure 3 *Changes in the fluorescence ratios a) 453/680 nm and b) 680/751 of analyzed maize seeds*

CONCLUSION

This study shows that fluorescence spectroscopy can be a valuable tool to assess the viability of maize seeds due to aflatoxin stress, even at a low level of contamination. The observed differences in the fluorescence ratios imply that aflatoxin stress induces damage, or compromises, in the chlorophyll system of maize seeds.

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