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Spectroscopic investigation of wheat grains (*Triticum aestivum*) infected by wheat seed gall nematodes (*Anguina tritici*)

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ABSTRACT

The present study was aimed at analyzing uninfected wheat grain (*Triticum aestivum*) and wheat seed gall nematodes (*Anguina titici*) by means of wave-dispersive X-ray fluorescence (WD-XRF), Fourier transform infrared (FTIR) spectroscopy and Diffuse reflectance spectroscopy to assess their elemental and molecular compositions. WD-XRF was used to detect and quantify the major and trace elements in the tested samples. The minerals detected and quantified in no-infection, low infection and high infection wheat samples were potassium (K), sulphur (S), phosphorus (P), chlorine (Cl), calcium (Ca), magnesium (Mg), iron (Fe), silicon (Si), and zinc (Zn). Copper (Cu) was only observed in wheat samples showing no-infection and high infection. Chromium (Cr), manganese (Mn), and nickel (Ni) were detected only in wheat samples of low infection and high infection, and it was completely absent in uninfected wheat samples. Sodium (Na) was only detected in low infection samples whereas aluminium (Al) was only found in high infection wheat samples. The concentrations of these elements in different wheat samples varied. FTIR spectroscopy was used to study the molecular compositions of the infected and uninfected wheat grain samples. Diffuse reflectance measurements of uninfected and infected wheat samples were used to identify spectral differences among wheat samples. In the present investigation a detailed comparison of these samples has been presented at the elemental and molecular levels.

1. Introduction

Wheat (*Triticum aestivum L.*) is the one of the most important grain crops and a prime component of human diet around the world. It is considered unique among cereals largely due to the fact that its grain contains distinctive chemical and physical properties. Wheat grains are the main raw materials used to produce a large variety of cereal-based foods (e.g. bread, noodles, and biscuits etc.), and as a result increasing attention is now paid to the grain quality. In addition to significant amounts of starch (about 60–70%), protein (about 10–18%) and fat (about 10–17%), wheat grains also contain minerals (4–7%), phytochemicals and vitamins which make wheat grains valuable to human health (Harold et al., 2005; Anjum et al., 2008; Šramková et al., 2009; Peng et al., 2011). Embryologicaly, wheat grain (*Triticum aestivum*) is a complex structure consisting of germ and starchy endosperm surrounded by several peripheral tissues with different structure and chemical compositions (Evers and Bechtel, 1988). The main components of the internal structure of wheat grain are endosperm (about 80–85% of dry mass) and embryo (2–3% of dry mass) (Belderok et al., 2000; Sieprawska et al., 2014). A relatively small amount of nutrients (about 0.5–1.5%) has been recorded in endosperm, in contrast to the embryos, which collect nutrients in concentrations upto approximately

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Abbreviations: WD-XRF, Wave-dispersive X-ray fluorescence analysis; ED-XRF, Energy-dispersive X-ray fluorescence; LIBS, Laser-induced breakdown spectroscopy; XPS, X-ray photon spectroscopy; ICP-OES, Inductively coupled plasma optical emission spectroscopy; ICP-MS, Inductively coupled plasma mass spectroscopy; micro-PIXE, Micro-proton-induced X-ray emission; FTIR, Fourier transform infrared spectroscopy; UV-Vis, Ultraviolet–visible; DF, Diffuse reflectance Spectroscopy

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4.5% (Belderok et al., 2000; Sieprawska et al., 2014). The elements contained in the endosperm, as well as the macromolecules, are needed for the development of the embryo in a properly functioning plant (Sieprawska et al., 2014).

The increasing interest in the mineral composition of wheat grains is associated with findings of the World Health Organization (WHO) indicating the importance of elemental content in human diet, especially in countries where cereals are the main food source (Shi et al., 2008; Sieprawska et al., 2014). Interactions between plants, nutrients, and pathogens are very complex and not completely understood. Most soils and environments where plants are cultivated contain an abundance of pathogens. On the most basic level, plants suffering nutrient stress will be less vigorous and more susceptible to a variety of diseases. In this respect, all nutrients affect plant disease. However, certain nutrients have a direct and more profound impact on plant diseases than others.

Seed gall nematode (*Anguina tritici*) was the first plant parasitic nematode reported by Turbevill Needham in 1743. It is widely thought to be the first recorded microscopic observation in which a pathogenic organism was identified as the causal agent of a plant disease (Lehman, 1979). *Anguina tritici* juveniles feed ectoparasitically on the tissues of growing points of the leaves. Later, the juveniles penetrate the flower buds at the time of flower bud initiation and start to feed endoparasitically. Finally, the nematodes convert wheat grains into galls, causing enormous yield loss (Evans et al., 1993). In India, the annual monetary loss caused by *A. tritici* ranges from 1% to 9% corresponding to a net financial loss of about 70 million rupees (Kaushal, 1998). Similarly in Turkey, this infection rates range from 1.5% to 55.2% (Elmali, 2002). Seed gall nematode has been found in major wheat growing regions of the world and is easily disseminated in seeds.

Ever since *A. tritici* was reported as a parasite of cereal crops, its morphological, biological and ecological features have been well documented (Southey, 1972; Gokte and Swarup, 1987; Evans et al., 1993). Numerous studies have described the pathogenicity of this plant's parasitic species and addressed various control strategies (Galper et al., 1991; Singh et al., 2012). Despite what is known today, information on seed gall nematode is lacking particularly at the elemental and molecular level. Seed gall nematodes has been found in major wheat growing regions of the world and is easily disseminated in seed. The adverse effect of seed gall nematode on grain yield should not be neglected and farmers have to be encouraged to monitor carefully *A. tritici* appearance and to take appropriate measures to control it.

Several emerging elemental analysis techniques like Energy-dispersive X-ray fluorescence (ED-XRF), laser-induced breakdown spectroscopy (LIBS), X-ray photon spectroscopy (XPS), inductively coupled plasma optical emission spectroscopy (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS), and micro-proton-induced X-ray emission (micro-PIXE), have been used to study the bulk elemental concentrations and elemental spatial distribution in wheat and/or wheat samples (Martelli et al., 2010, 2011; Saad et al., 2011; Paltridge et al., 2012; Pongrac et al., 2013). Spectroscopic techniques for instance, Raman spectroscopy has been demonstrated as a powerful disease diagnostic and monitoring tool (Pandey et al., 2015; Spegazzini et al., 2014). Similarly, certain molecular techniques such as FTIR and Raman Microscopy have also been used for the identification of wheat varieties and/or to analyse wheat samples (Amir et al., 2013; Gamage et al., 2014; Mills et al., 2005). Mineral contents have widely been studied in whole wheat grains and also in the different parts of the grain. However, little is known about the mineral content in wheat gall nematode (*A. tritici*) and the relevant changes in the mineral content when the wheat grains change into galls.

In recent years X-ray fluorescence analysis (XRF) has been widely used as a versatile tool for the qualitative and quantitative determination of major, minor and trace elemental analysis in geochemistry, manufacturing and forensic science (Arai, 2006; West et al., 2009; West et al., 2010). ED-XRF has also been used recently, as a tool for the analysis of Zn, Fe, and Se in whole wheat grain. XRF is based on the principle that all elements produce secondary 'fluorescent' X-rays of characteristic energy when exposed to X-rays of appropriate higher energy. The energy and intensity of the emitted X-rays can then be used to determine elemental composition. In principle, the higher the atomic weight of an element, the higher are the energy needed to induce fluorescence and the fluorescence energy. It is also easier to detect fluorescence as the atomic weight of an element increases.

In this work we study the elemental profile within the whole grain in order to determine changes in the mineral contents that may take place during the formation of galls in wheat grains. The relative changes in the mineral content and accumulation of toxic elements will ultimate influence the quality of the grain, and consequently cause gall formation. In the present work, we study the mineral contents and their changes in different wheat samples (uninfected wheat samples, low and high infection wheat samples) using wave dispersive X-Ray fluorescence (WD-XRF). FTIR spectroscopy and UV–Vis diffuse reflectance (DF) spectroscopy were also used to study the molecular composition of different kinds of wheat samples.

2. Materials and methods

2.1. Wheat grains (uninfected and infected)

The collected infected and uninfected wheat grain samples used in

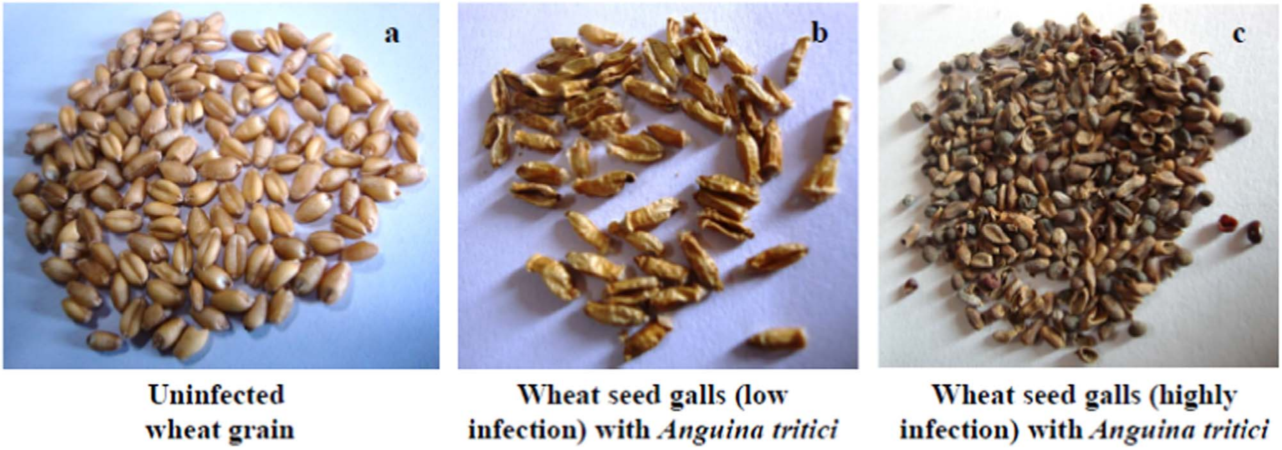

Fig. 1. Photographs of the (a) Uninfected wheat grain (b) Wheat seed galls (low infection) with *Anguina tritici* (c) Wheat seed galls (high infection) with *Anguina tritici*. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)