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Effects of fungicide treatment, N-fertilisation and harvest date on arabinoxylan, endoxylanase activity and endoxylanase inhibitor levels in wheat kernels

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Abstract

Fungicide treatment had a significant impact on endoxylanase activity and XIP levels, but did not affect arabinoxylan (AX) and TAXI levels. The different response of TAXI and XIP type inhibitors to fungicide treatment is interesting. N-fertilisation did not affect AX levels, but significantly increased TAXI and XIP type inhibitor levels. Wheat-associated microbial endoxylanase activity levels were also affected by nitrogen supply, but levels of the endogenous enzyme did not change, except when sprouting occurred. The weather conditions before harvest had no influence on total AX (TOT-AX) and inhibitor levels, but had a large impact on both microbial and endogenous endoxylanase activity and water extractable AX (WE-AX) levels. Under most conditions, endoxylanase activity levels were related to those of α-amylases, liquefaction numbers (LN) and specific weights. WE-AX levels were often weakly but significantly correlated with endoxylanase activity levels, indicating that it is possible that part of the WE-AX in wheat originates from AX degradation by endoxylanases in the field. These results clearly indicate that agronomic circumstances significantly affect the levels of AX, endoxylanases and their inhibitors in wheat, and consequently could affect wheat quality.

Keywords: Wheat; Arabinoxylan; Endoxylanase; TAXI; XIP; Fungicide; N-fertilisation; Harvest date

1. Introduction

Wheat characteristics not only depend on genetic and climatological circumstances as described in Dornez et al. (2007), but also on agronomic conditions during wheat production. Agronomic inputs such as fungicide treatment and N-fertilisation have a major significant effect on the developing wheat during kernel filling and consequently strongly determine wheat quantity and quality characteristics (Dupont and Altenbach, 2003).

Fungicides are applied to avoid disease related yield losses and augment grain yields by prolonging grain filling and increasing kernel weights (Bertelsen et al., 2001;

Abbreviations: a.i., active ingredient; AX, arabinoxylan; AZCL-AM, azurine-cross-linked-amylose; AZCL-AX, azurine-cross-linked-arabinoxylan; GHF, glycoside hydrolase family; GS, growing stage; HFN, hagberg falling number; LN, liquefaction number; NSP, non-starch polysaccharide; TAXI, *Triticum aestivum* xylanase inhibitor; TKW, thousand kernel weight; TOT-AX, total arabinoxylan; WE-AX, water extractable arabinoxylan; XIP, WU-AX, water unextractable arabinoxylan; XIP, xylanase inhibiting protein

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Dimmock and Gooding, 2002; Ruske et al., 2003). As endoxylanases associated with wheat kernels to a large degree originate from microorganisms populating the outer wheat kernel layers (Dornez et al., 2006a), it can reasonably be assumed that those microbial endoxylanase activities would be higher when fungicide treatments are omitted. Furthermore, fungicide treatment may also affect endoxylanase inhibitor levels in wheat, as these are often thought to be involved in plant defence (Bellincampi et al., 2004). Indeed, it is unlikely that endoxylanase inhibitors play a regulatory role in plants as they are not able to inhibit endogenous endoxylanases and only inactivate fungal and bacterial endoxylanases. Endoxylanases of pathogenic microorganisms like *Fusarium graminearum* (Beliën et al., 2005) and *Botrytis cinerea* (Brutus et al., 2005), for example, were shown to be inactivated by wheat endoxylanase inhibitors. In this context, Igawa et al. (2004) reported that the expression of the major XIP (xylanase inhibiting protein) gene, *Xip-I*, is strongly induced in leaves infected by the powdery mildew fungus *Erysiphe graminis* and by wounding. In the case of TAXI (*Triticum aestivum* xylanase inhibitor), the *Taxi-I* gene does not respond to such stress signals and was considered to be a basal preexisting defence gene against fungal pathogens during seed development and germination. However, other TAXI genes such as *Taxi-III* and *Taxi-IV*, are pathogen inducible (Igawa et al., 2005). These findings might imply that omitting fungicides might increase significantly the levels of endoxylanase inhibitors in wheat.

N-fertilisation is one of the most investigated factors in wheat production. Although total albumin and globulin protein levels tend to be insensitive to applied N-fertilisation, no studies have determined whether this is true for individual albumin and globulin components such as endoxylanases and endoxylanase inhibitors (Dupont and Altenbach, 2003). Furthermore, Coles et al. (1997) already found some evidence that N-fertilisation in combination with a plentiful water supply can cause an increase in the levels of AX in wheat. Bodson et al. (2001) showed that changing the timing of nitrogen application, with dose reduction during tillering and transferring this amount to flag leaf application, not only increased grain protein contents, but also decreased disease risks, suggesting that N-fertilisation might impact microbial endoxylanase activity levels in wheat.

Besides fungicide treatment and N-fertilisation, the weather conditions before harvest may also affect wheat composition. Harvesting of wheat ideally takes place when the wheat kernel is sufficiently dry. However, in some years, wheat has to be harvested under wet conditions. This can lead to strong microbial contamination and/or preharvest sprouting. Based on the large influence of weather conditions at harvest on α-amylase activity levels and hence on Hagberg falling numbers (HFN), it can reasonably be expected that endoxylanase activity levels also change with harvest date.

The purpose of the present study was to examine whether the above described agronomic conditions (N-fertilisation, fungicide treatment and harvest date) have an impact on the AX, endoxylanase activity and endoxylanase inhibitor levels in wheat. The obtained partial correlation coefficients may help to identify which components are similarly affected by changing agronomic conditions.

2. Materials and methods

2.1. Wheat samples

The wheats were grown at an experimental site (Lonzée, Gembloux, Belgium) in three successive growing periods (2001/02, 2002/03 and 2003/04). Each sample was grown in four different plots of 16 m^2 on the same field in a fully randomised block design. Wheat kernels from the different plots were mixed to reduce location effects. Unless specified otherwise, wheat samples were sown in October and two fungicide treatments were applied at the flag leaf and the ear emergence stage [growing stages (GS) 37 and 59, respectively] (Zadocks et al., 1974). In trials in which N-fertilisation was not the factor studied, N-fertilisation (175 kg N/ha in 2001/02, 185 kg N/ha in 2002/03 and 185 kg N/ha in 2003/04) was given in three split applications, the first during tillering (GS 20–25), the second at the beginning of stem elongation (GS 30) and the third at flag leaf emergence (GS 37–39), in liquid form for the first two partial applications and in solid from the third partial application. Weed and insect control were achieved in all trials by applying appropriate herbicides and insecticides. Lodging was limited by using chlormequat chloride as growth regulator. Treatments that were not investigated in the trials were applied at the same time and in equal doses to make comparison between the samples possible.

2.1.1. Trial 1

In a first trial, the effect of fungicide treatment was examined for two wheat varieties by comparing wheat samples grown with two fungicide treatments applied at the flag leaf stage and the ear emergence stage, respectively, and wheat samples grown without fungicide treatments. As apparent endoxylanase activities were previously reported to be possibly related to the ear disease sensitivity of the wheat variety (Dornez et al., 2006b), a wheat variety that is very sensitive to ear diseases, Meunier, and a less sensitive one, Mercury were used in this trial. The fungicides were invariably made up of a triazole–strobilurin combination. Triazole fungicides inhibit sterol biosynthesis, thereby impairing the membrane production of fungi and work curative. Strobilurin fungicides, in contrast, exert their fungicidal action by blocking electron transport in the mitochondrial respiratory chain in fungi and work in a preventative rather than a curative way (Bertelsen et al., 2001). The fungicides were applied at the manufacturers' recommended field rates. In 2002, 1.0 l/ha epoxyconazol