The metabolomic fingerprinting and microbiological quality of winter wheat (*Triticum aestivum* L.) in different organic growing systems

DARJA MATT[1], VIACHESLAV EREMEEV, BERIT TEIN, MATI ROASTO, SIRLI PEHME, ANNE LUIK

Key words: metabolomic fingerprinting, microbiology, wheat, organic systems

Abstract

Growing system is important for soil fertility as well as for quality parameters of crops. The aim of this study was to investigate the influence of green manures as winter cover crops and their combination with composted cattle manure on microbiological quality and metabolomic fingerprinting of winter wheat in a crop rotation experiment in three organic systems. The study showed that green manures in combination with cattle manure had impact on metabolomics and significantly increased expression of metabolites of winter wheat. Also the abundance of mesophilic bacteria and the ratio of bacteria/yeasts and moulds was higher in wheat of the same growing system.

Introduction

Growing system does not only influence the soil fertility, but through that also the quality parameters of crops (Olesen et al 2009; Mäder et al 2002). In organic agriculture soil fertility is essential for harvesting high quality crop yields. Therefore, it is important to develop sustainable growing systems which have rotations with appropriate crops and cover crops. In organic farming green manures and cattle manure are used to ensure fertile and biologically active soil and to enhance biodiversity. Green manures offer supporting services, such as nutrient cycling, promotion of beneficial insects for pest control and soil formation. Also the catch crops on winter period are essential to reduce nutrient leaching (Stark and Porter 2005).

For estimation product quality depending on plant growing systems different methods are developed. In recent years food metabolomics has been used as a novel method for `fingerprinting` or for ´profiling´ food samples (Hajšlova et al 2011). `Fingerprinting` of food samples enables to perform comparative analyses aimed at detection of differences. ´Profiling´ is used for identification individual, differential sample components (both primary and secondary metabolites). Production system and interaction among the microbial population are important factors that also affect food safety and shelf life (Guerzoni et al 1996). Among the microorganisms, some moulds, yeasts, bacteria, and viruses have both desirable and undesirable roles in our food. Most bacteria, moulds, and yeasts, because of their ability to grow in foods, can potentially cause food spoilage, however mere microbial presence does not reduce the quality of food, except in the case of some pathogens (Ray 2005).

The aim of present study was to find out the influence of green manures as winter cover crops and these combined with composted cattle manure on metabolomic fingerprinting and microbiological quality of winter wheat in a crop rotation experiment in three organic systems at the Estonian University of Life Sciences in 2012.

Material and methods

The variety of winter wheat was `Olivin` and it was orginated from farming systems experiment established in 2008. In a five-field crop rotation, barley undersown with red clover, red clover, winter wheat, peas and potato were grown in succession. System Org 0, as control, follows this rotation. In System Org 1, green manures as winter cover crops were used: after winter wheat - ryegrass, after peas -winter oilseed rape and after potato - winter rye. In System Org 2 green manures plus cattle manure at 40 t/ha was applied. Thus, in both Systems Org 1 and 2 all plots had green plant cover in winter. The soil type of the area is sandy loam Stagnic Luvisol by the World Reference Base (WRB) 2006 classification (FAO 2006).

Winter wheat was analysed by ultrahigh liquid chromatography – q-Tof mass spectrometry (LC-MS) using reversed-phase LC columns and both positive and negative electrospray ionisation. Mass Profiler Software and R statistical programmes were used for PCA and ANOVA. Raw data are aligned and analyzed for a

[1] Estonian University of life Science, DARJA.MATT@EMU.EE, WWW.EMU.EE

differential effect of agricultural production system on the concentration of individual compounds in the winter wheat, and compounds of interest are identified.

Microbiological quality was analysed post harvesting (autumn 2012) and in the end of storage time (spring 2013). For the enumeration of total microorganisms (aerobic mesophilic bacteria) on winter wheat Colony-count technique at 30 ºC (EVS-EN ISO 4833:2006) and for enumeration of total yeasts and moulds Horisontal method at 25 ºC (ISO 21527-1:2008) was used. Statistical analyses were done using R programme. One-way ANOVA was applied to test the effect of farming systems on counts of total microorganisms and yeast and moulds.

Results

Metabolomic fingerprinting

Initial data analysis on one dataset with approximately 3500 detected metabolites (mass spectral features) shows that 120 compounds were differentially expressed ($p<0.05$) between the Org 0, Org 1 and Org 2. The highest number of expressed metabolites was in Org 2 ($p<0.05$). Org 1 had more expressed metabolites than Org 0, but no statistical differences occurred. PCA of winter wheat showed that it was possible to differentiate between systems Org 0, Org 1 and Org 2.

Microbiological quality

In winter wheat of all systems aerobic mesophilic bacteria was presented in bigger numbers than yeasts and moulds in both times – post harvest - in autumn and after storage period – in spring. The tendency was observed that the counts of bacteria were bigger in the end of storage than in post-harvesting period. The highest amount of aerobic mesophilic bacteria was in wheat of system Org 2 ($p<0.05$) than from other systems in post harvesting period and in spring. The counts of yeasts and moulds decreased during storage period, their smallest amounts were in wheat of Org 0 in autumn and in spring in wheat of Org 2 ($p<0.05$). In post harvesting period and in the end of storage the ratio of bacteria/yeasts and moulds was higher in wheat of Org 2 than in other systems.

Discussion

Our study showed that growing system determines the quality of wheat. In organic farming it is important to use systems that assure sufficient yields with high quality. In growing system the use of green manures as winter cover crops in combination with composted cattle manure had impact on metabolomics and significantly increased expression of metabolites of winter wheat. The studies of Zörb et al (2006) and Belleggia et al (2013) showed also that cultivation method infuences the metabolomics of wheat. Our study was also shown growing system influence on microbiological quality of winter wheat.. The abundance of mesophilic bacteria and the ratio of bacteria/yeasts and moulds was higher in winter wheat in the growing systems where green manures were combined with cattle manure. The lower number of yeasts and moulds could be explained by the ability of bacteria to suppress yeasts and moulds. Oliveira et al (2010) have shown that lettuce fertilized organically and conventionally had different bacteriological quality and content of yeasts and moulds. But several authors (Moreira et al 2003; Phillips and Harrison 2005¸ Seidler-Lozykowska et al 2008) have not found significant differences in amounts of bacteria, yeasts and moulds in spring mixture of multiple salad, thyme herb and Swiss chard from different farming systems. The investigations should be continued for the clarification of growing systems influence on the microbiological quality of product.

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