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PHENOLIC ACIDS AND ANTIOXIDANT ACTIVITY OF WHEAT SPECIES: A REVIEW

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Wheat (genus *Triticum*) is considered to be an important source of polyphenols, plant secondary metabolites with numerous health-promoting effects. Many phytochemicals are responsible for the high antioxidant activity of whole grain products. However, there is a lack of information about composition of phenolic acids and their concentrations in different *Triticum* species. Despite the fact that the increased consumption of whole grain cereals and whole grain-based products has been closely related to reduced risk of chronic diseases, bioactive compounds found in whole grain cereals have not achieved as much attention as the bioactive compounds in vegetables and fruits. Recent studies have revealed that the content of bioactive compounds and antioxidant capacity of whole grain cereals have been regularly undervalued in the literature, because they contain more polyphenols and other phytochemicals than was reported in the past. Phenolic acids represent a large group of bioactive compounds in cereals. These compounds play a significant role in the possible positive effects of the human diet rich in whole grain cereals, especially in wheat and provide health benefits associated with demonstrably diminished risk of chronic disease development. Ferulic acid, the primary and the most abundant phenolic acid contained in wheat grain, is mainly responsible for the antioxidant activity of wheat, particularly bran fraction. In this paper, selected phenolic compounds in wheat, their antioxidant activity and health benefits related to consumption of whole grain cereals are reviewed.

Key words: antioxidant activity, health benefits, phenolics, phytochemicals, wheat

Wheat is an important agricultural commodity and a main food all around the world. It is one of the major food grains consumed by people (Van Hung *et al.* 2009) with global wheat production forecast in 2015 at 735 million tonnes (FAO 2015). It contains important beneficial components for human nutrition. Due to the high content of natural antioxidants, wheat and wheat-based products can perfectly serve as a basis for the functional foods development designed to improve the health of millions of consumers (Vaher *et al.* 2010). Although wheat is used mainly as a source of energy, whole wheat grains are an excellent source of dietary fiber, vitamins, minerals and other bioactive phytochemicals such as antioxidant compounds (Cai *et al.* 2014). For this reason, numerous studies have been conducted to investigate the nutritional quality and health benefits of whole wheat grains.

The phenolic compounds, one of the most widely occurring groups of phytochemicals, are secondary metabolites synthesized during the plant development and in response to stress conditions (Brandolini *et al.* 2013). These compounds, most of which are present in wheat bran fractions, play a significant role in plant growth and reproduction because they provide protection against pests and pathogens and contribute to the colour and sensory characteristics of plant species. In addition to their role in plants, in human diet phenolic compounds provide many health benefits associated with diminished risk of

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chronic diseases caused by reactive oxygen species (Harborne & Williams 2000; Gani *et al.* 2012).

According to Heim *et al.* (2002), the beneficial effects of phenolic compounds have been attributed to their antioxidant activity. It depends on the structure, particularly the number and position of the hydroxyl groups and the type of substitutions on the aromatic rings (Balasundram *et al.* 2006). Phenolics have lower electron reduction potential than the oxy-gen radicals and they are excellent oxygen radical scavengers. Additionally, the phenoxyl radicals are less reactive than the oxygen radicals and they scavenge reactive oxygen intermediates without promoting further harmful oxidative reactions (Ainsworth & Gillespie 2007). This strong antioxidant activity of phenolic compounds leads to anti-inflammatory, anti-thrombotic, anti-atherogenic, cardioprotective and vasodilatory effects on human health (Quiñones *et al.* 2013).

The phenolic compounds have the structure that varies from simple molecules to complex polymers. They contain one or more aromatic rings with one or more hydroxyl groups. This crucial structural diversity has an impact on their differences in bioavailability. Simple phenolic acids cross the intestinal barrier more easily, while complex molecules are hardly absorbed (Scalbert *et al.* 2002). Cereals represent staple food of humankind and are an important source of phenolic compounds. One of the most common types of phenolic compounds in cereals are phenolic acids (Li *et al.* 2008), which are present in three forms: soluble free, soluble conjugated with mono- and polysaccharides and insoluble bound (Naczk & Shahidi 2004). According to Li *et al.* (2008), the most abundant fraction in wheat grains is the insoluble bound (77%), followed by the soluble conjugated (22%) and the soluble free (<0.5 – 1%). In the past, the phenolic content of whole grain cereals had been undervalued in the literature, because most of researches only studied the free phenolic content and not the content of bound phenolics (Adom & Liu 2002). Phytochemicals in the bound form cannot be digested by human enzymes. They could survive stomach and small intestine digestion and therefore can reach the colon. The colonic microflora may release the bound phytochemicals by fermentation processes and after absorption provide health benefits in colon or other body tissues. This can partly explain the mechanism of consumption of whole grain cereals in the prevention of colon cancer, other gastrointestinal cancers, breast cancer, prostate cancer and other chronic diseases, which is supported by many epidemiological studies (Liu 2007).

Antioxidant activity of phytochemicals in wheat

The important group of phytochemicals with small molecular weight present in wheat grains are antioxidants. Antioxidants are defined as molecules that can delay or prevent oxidative stress at low concentration and specific assay conditions. Oxidative stress has been related to cardiovascular diseases, cancer and other chronic diseases that account for a major part of deaths today (Willcox *et al.* 2004; Vaher 2010).

Wheat contains a diverse range of biologically active compounds that may contribute to its antioxidant capacity. Wheat antioxidants are mainly concentrated in bran fraction and the amount of these antioxidants depends on the grain variety (Kim *et al.* 2006). First of all, wheat bran represents a good source of phenolic acids, which significantly contribute to the total antioxidant activity of wheat (Baublis *et al.* 2000; Yu *et al.* 2003; Kosík *et al.* 2014c). Extract of wheat bran with a high concentration of phenolic acids was shown to have stronger antioxidant activity than other wheat fractions (Onyeneho & Hettiarachchy 1992). In addition, wheat bran is able to inhibit lipid oxidation catalysed by either peroxyl or iron radicals (Baublis *et al.* 2000). Most recently, Zhou *et al.* (2004) reported that wheat grain, bran and other fractions had different antioxidant activities and total phenolic contents. Their research also showed that ferulic acid, with a concentration range of 99–231 μg/kg, was the main contributor to the antioxidant activity of wheat. It has been suggested that ferulic acid could be used as a marker of wheat antioxidants. According to Lacko-Bartošová *et al.* (2013), antioxidant activity of whole grain flour was significantly higher than the white flour. Antioxidant activity of white flour (23.26%) was two times lower than antioxidant activity of whole grain flour (49.57%). The highest antioxidant activity (76.47%) was determined in wheat bran.

Phytochemicals in wheat exhibit strong antioxidant properties. They scavenge or neutralize free radicals and reduce oxidative damage to DNA, proteins and membrane lipids. Decreases in oxidative damage to cells or cell components may explain the reduction of chronic diseases, which may be caused by oxidative stress (Willcox *et al.* 2004; Kim & Kim 2016). To investigate health benefits of wheat, total phenolic contents and antioxidant capacities in wheat have been studied (Verma *et al.* 2008; Okarter *et al.* 2010). Adom and Liu (2002) reported that the antioxidant capacity of wheat was higher than that of rice or oats. Verma *et al.* (2008) found that the antioxidant activity in wheat bran was highly correlated with its free, bound and total phenolic contents. Most of the antioxidant compounds in wheat are bound and may survive digestion to reach the colon intact, where they induce an antioxidant environment (Pérez-Jiménez & Saura-Calixto 2005). Okarter (2011) found that phenolic extract from the insoluble-bound fraction of whole wheat inhibited the proliferation of human colon cancer cells *in vitro*. A major action in the protection of colon from cancer probably exert ferulic acid – the typical whole wheat phenolic acid with only 0.5–5% of absorption within the small intestine. Thus, phenolic acids in the bound form can act along the whole length of the digestive tract by trapping oxidative compounds (Fardet 2010).

Phenolic acids

Phenolic acids are derivatives of benzoic and cinnamic acids and are present in all cereals. They can be subdivided into two major groups, hydroxybenzoic acid and hydroxycinnamic acid derivatives (Figure 1). Hydroxybenzoic acid derivatives include *p*-hydroxybenzoic, protocatechuic, vannilic, syringic and gallic acids. Hydroxycinnamic acid derivatives include *p*-coumaric, caffeic, ferulic and sinapic acids (Figure 1) (Mattila *et al.* 2005; Gani *et al.* 2012). The common phenolic acids found in whole grain cereals include ferulic acid, vanillic acid, caffeic acid, syringic acid and *p*-coumaric acid (Sosulski *et al.* 1982; Liu 2007). Ferulic acid is the primary and the most abundant phenolic acid in wheat grains. Smaller concentrations of *p*-hydroxybenzoic, *o*-coumaric, *p*-coumaric, vanillic, syringic, salicylic and sinapic acids are also present in wheat (Moore *et al.* 2005; Liyana-Pathirana *et al.* 2006).

Composition, content and effects of food processing

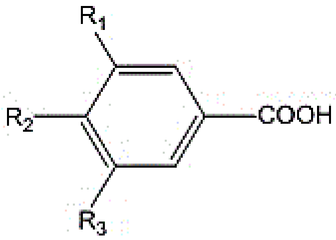
The phenolic acids reported in cereals occur in both free and bound form. Free phenolic acids are found in outer layers of the pericarp. Bound phenolic acids are esterified to cell walls and released from the cell matrix by acid or base hydrolysis (Mattila *et al.* 2005; Gani *et al.* 2012). Phenolic acids in wheat grains are present mostly in the bound form with other grain components such as saccharides – starch, cellulose, β-glucan and pentosane (Yu *et al.* 2001; Vaher *et al.* 2010). Also, food processing, such as fermentation, thermal processing, pasteurisation and freezing, contributes to the release of these bound phenolic acids (Dewanto *et al.* 2002). They can be degraded at high temperature and high pH, particularly caffeic acid (Dimberg *et al.* 2001).

Phenolic acids are predominantly present in the bran, i.e. the aleurone layer and the outermost pericarp, which are usually eliminated during milling (Bondia-Pons *et al.* 2009; Belobrajdic & Bird, 2013). As a result, whole meal flours contain higher amounts of phenolic acids than commercial wheat flours. In whole meal flours, the number of polyphenols is highly variable and is closely related to species and variety of cereals (Adom *et al.* 2005; Andersson *et al.* 2014; Kosík *et al.* 2014b). Different extraction conditions also influence the content reported in different studies (Yu *et al.* 2001).

Einkorn is a high-nutritional-value cereal with the high content of proteins, carotenoids and tocols (Hidalgo *et al.* 2006; Brandolini *et al.* 2008). However, a significant gap remains in its phenolic acid composition. Li *et al.* (2008) recorded a total phenolic acid content similar to winter, spring and durum wheats, slightly higher than spelt, but marginally lower than emmer. Serpen *et al.* (2008) measured higher values of bound ferulic acid in emmer than in einkorn. However, Abdel-Aal and Rabalski (2008) reported more phenolic acids in einkorn than in several primitive and modern wheat species. According to HEALTHGRAIN diversity screen, the large EU project, the content of phenolic acids in wheat species varied between 326 and 1171 μg/g dry matter (DM) (Li *et al.* 2008). Other studies have revealed that the total concentrations of phenolic acids are typically in the range of 200–900 μg/g DM in whole grain wheat (Belobrajdic & Bird 2013). The content of phenolic acids in spelt also showed differences between varieties and ranged from 507 to 1257 µg/g DM (Gawlik-Dziki *et al.* 2012). Some studies have concluded that the content of phenolic acids is related to genotype (Hernández *et al.* 2011; Ragaee *et al.* 2012). However, others have found that location (i.e. environmental factors) is more important (Vaher *et al.* 2010).

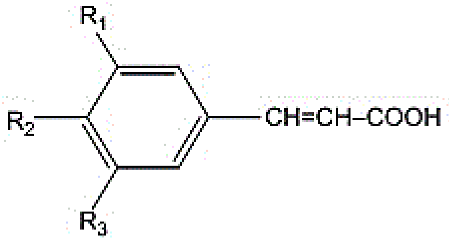
The conjugated and bound phenolic acids recorded in selected wheat species are summarise in the Table 1. In the study of Brandolini *et al.* (2013), 39 different *Triticum monococcum, T. turgidum* and *T. aestivum* accessions were evaluated. The total conjugated phenolic acids in whole meal flour ranged between 36.0 and 52.6 mg/kg DM. The total bound phenolic acids varied between 441 and 715 mg/kg DM that correspond with the results (208–964 mg/kg DM) reported by Li *et al.* (2008). Volkan *et al.* (2015) analysed 15 wheat accessions from different *Triticum* species. The phenolic acid contents in the conjugated and bound extracts varied between 28.2–70.8 mg/kg DM and 482–766 mg/kg DM, respectively. Hidalgo and Brandolini (2017) tested conjugated and bound phenolic acids content of three einkorn accessions. The total conjugated phenolic acids ranged between 33.9 and 54.9 mg/kg DM. The concentrations of total bound phenolic acids varied between 484.2 and 579.5 mg/kg DM.

a



Benzoic acid derivatives	Substitutions		
	R_1	R_2	R_3
Benzoic acid	H	H	H
p-Hydroxybenzoic acid	H	OH	H
Protocatechuic acid	H	OH	OH
Vannilic acid	CH_3O	OH	H
Syringic acid	CH_3O	OH	CH_3O
Gallic acid	OH	OH	OH

b



Cinnamic acid derivatives	Substitutions		
	R_1	R_2	R_3
Cinnamic acid	H	H	H
p-Coumaric acid	H	OH	H
Caffeic acid	OH	OH	H
Ferulic acid	CH_3O	OH	H
Sinapic acid	CH_3O	OH	CH_3O

Figure 1. Structure of common phenolic acids: (a) benzoic acid derivatives and (b) cinnamic acid derivatives (Liu 2007)

Although the content of phenolic acids depends on variety and various agricultural practices, it seems that bound phenolic acids variation is lower in different growing conditions than free and conjugated forms (Fernandez-Orozco *et al.* 2010; Belobrajdic & Bird 2013; Kosík *et al.* 2014a). Organic cultivation systems have been found to lead to a small increase in the total amount of phenolic acids, especially ferulic and *p*-coumaric acid, in spring and winter wheats, compared with conventionally grown wheat (Zuchowski *et al.* 2011). These differences were attributed to a concentration effect due to differences in the size of the grain, which was smaller in organically grown crops. It is also important to note that the study was performed on plants grown within the two cropping systems only in one year (Andersson *et al.* 2014).

Both free and bound phenolic acids are affected by processing, e.g. baking. A study performed by El-Sayed and Rabalski (2013) found that baked products contained higher amounts of free phenolic acids and lower amounts of bound ones than the whole grain wheat flour used. This is probably the result of release of bound compounds. Most of the changes occurred during baking, but no change could be seen in the dough. In other studies, baking has resulted in a reduced amount of free phenolic acids, although in those studies a fermentation process preceded the baking process, which might explain the differences in the results (Menga *et al.* 2010). The free phenolic acids also increased and actually seemed to be more stable in biscuits and muffins than in bread products. El-Sayed and Rabalski (2013) explained this by the fact that phenolic acids are less accessible to oxidation in fat products than in aqueous food systems. However, different mechanisms may occur simultaneously in the cereal products during processing, influencing the content of various phenols. Concurrently to release of bound compounds from the food matrix, phenols may be oxidised, polymerised or thermally degraded.

Bioactivity and implications for human health

Some studies have been conducted to evaluate the bioavailability of hydroxycinnamates from cereals and it has been found that esterification of ferulic acids impairs their uptake in the intestine (Belobrajdic & Bird 2013). However, colonic fermentation in the digestive tract releases phenolic compounds, which in turn may be taken up in the large intestine (Maki *et al.* 2012). Zhao and Moghadasian (2010) suggested that the bioavailability of hydroxycinnamic acids follow the order *p*-coumaric acid > ferulic acid > caffeic acid, and that the free compounds have higher bioavailability than their corresponding bound forms. The metabolism of these compounds in the colon is not fully understood, but it is supposed that they undergo reduction, dihydroxylation, demethylation and β-oxidation.

Phenolic acids have many functions in cereals. They act as stabilisers of the cell wall structure, but they may also be involved in the physical and chemical defence against various microorganisms, pests and insects. Phenolic acids have also been reported to inhibit the biosynthesis of trichothecenes of *Fusarium* fungi, which are potent human toxins (Boutigny *et al.* 2009). They are mentioned as bio-

T a b l e 1

Soluble conjugated, insoluble bound and total phenolic acids in selected wheat species [mg/kg DM]

Species	Phenolic acids		
	Soluble conjugated	Insoluble bound	Total
Einkorn[a,b,c]	34.4–63.9 43.8–54.9 52.6	482–766 534.1–579.5 584	516–831 578–634 637
Emmer[a,c]	44.3 49.9	506 546	551 596
Spelt[a,c]	39.7 42.8	538 594	578 637

[a]Volkan *et al.* (2015); [b]Hidalgo & Brandolini (2017); [c]Brandolini *et al.* (2013)
DM – dry matter

active phytochemicals promoting human health in a multidisciplinary way. They may provide health benefits because of their antioxidant properties (Thompson 1994; Bondia-Pons *et al.* 2009). Ragaee *et al.* (2012) found a high correlation between the concentration of bound phenolic acids in whole grain wheat and DPPH (diphenylpicrylhydrazyl) scavenging capacity. Phenolic acids may also inhibit LDL-cholesterol oxidation (low density lipoproteins) and oxidative damage to DNA and lipid membranes, which might otherwise lead to several pathological conditions, including cardiovascular diseases, type 2 diabetes and cancer. *In vitro* studies on phenolic acids have shown anti-inflammatory effects and anti-proliferative activities on cancer cells. Several phenolic acids have also been shown to be responsible for inhibiting cataracts of the eye lens (Chethan *et al.* 2008).

Ferulic and p-coumaric acid in cereals

Ferulic acid (trans-4-hydroxy-3-methoxycinnamic acid) is the most abundant hydroxycinnamic acid found in cereal grains. It occurs primarily in the plant seeds and leaves, mainly covalently conjugated to mono- and disaccharides, plant cell wall polysaccharides, glycoproteins, polyamines and lignin. Wheat bran is considered to be a good source of ferulic acid, which is esterified to hemicellulose of the cell walls (Dewanto *et al.* 2002). The content of ferulic acid in wheat grains is near to 0.8–2 g/kg dry weight basis that represents up to 90% of total phenolic compounds (Sosulski *et al.* 1982; Lempereur *et al.* 1997; Gani *et al.* 2012).

Ferulic acid in cereals can be found in free, soluble-conjugated and bound forms. About 90–95% of the total amount of ferulic acid represent the insoluble bound ferulic acid (Lv *et al.* 2012). The content of bound ferulic acid was significantly higher than free and soluble-conjugated ferulic acid in wheat, corn, oats and rice. The ratio of free, soluble-conjugated and bound ferulic acid in corn and wheat was 0.1:1:100. The order of total ferulic acid content among the tested grains was corn > wheat > oats > rice (Adom & Liu 2002; Boz 2015). In wheat, the insoluble dietary fraction contains 3544 μg/g of phenols, whereas the soluble fraction contains 95 μg/g. This may indicate that ferulic acid is bound to arabinoxylan but not to β-glucan, which is a more soluble polysaccharide (Caprita *et al.* 2011). The content of ferulic acid seems to be higher in soft wheat than in hard wheat (Ragaee *et al.* 2012).

Ferulic acid and other phenolic acids protect wheat kernels by providing both physical and chemical barriers through cross-linking saccharides, antioxidant activities against destructive radicals and astringency that deters insects and animals from consumption (Arnason *et al.* 1992; Liu 2007). Like several other phenolic compounds, ferulic acid exhibits antioxidant activity in response to free radicals *via* donating one hydrogen atom from its phenolic hydroxyl group (Kumar & Pruthi 2014). Beneficial effects on human health such as anti-inflammation and free radical scavenging have been demonstrated. It has been suggested that ferulic acid can treat diverse disorders including Alzheimer's disease, cardiovascular diseases, *diabetes mellitus,* cancer and skin diseases (Mancuso & Santangelo 2014; Karunaratne & Zhu 2016). Suzuki *et al.* (2007) found that ferulic acid may have positive effects on blood pressure through stimulation of nitric acid production, which induces arterial vasodilation and increases the blood flow.

p-coumaric acid (4-hydroxycinnamic acid) is a hydroxyl derivative of cinnamic acid (Garrait *et al.* 2006). It is present in the lowest amount in the centre of the kernels and in increasing amount towards the outer layers (Awika & Rooney 2004; Madhujith *et al.* 2006). This acid is another phenolic acid of great interest due to its chemoprotectant and antioxidant properties (Torres y Torres & Rosazza 2001). It has been suggested to have anti-tumour activity against human malignant tumours. There is a report revealing that *p*-coumaric acid reduces the risk of stomach cancer (Ferguson *et al.* 2005). It has also potentially protective effect against heart diseases because of its ability to decrease the resistance of LDL lipoproteins, cholesterol oxidation and lipid peroxidation (Garrait *et al.* 2006). It shows anti-mutagenesis, anti-genotoxicity and anti-microbial activities, inhibits cellular melanogenesis and plays a role in immune regulation in humans (Kiliça & Yeşiloğlub 2013).

CONCLUSIONS

Whole grain cereals may represent a significant share in intake of bioactive compounds in human diet. They are mainly present in bran fraction of cereals, which is usually eliminated by milling. Growing evidence indicates that the consumption of whole grains at regular intervals can be related to many health benefits, for example the reduced risk of cardiovascular diseases, obesity, type 2 diabetes and certain types of cancer. Bioactive compounds play an important role in plant growth, protection against pathogens and pests, affect the colour and sensory properties of plants. Despite this fact, antioxidant properties of cereals reported in the literature have been undervalued, since only unbound forms have been studied. Most of the bioactive compounds in wheat are in the insoluble bound fraction and may survive gastrointestinal digestion. They can be released from cell matrix by acid or base hydrolysis, usually by colonic microflora through fermentation. The antioxidant potential of whole grains is closely related to their phenolic content, primarily ferulic acid as the main source of antioxidant activity. Einkorn, emmer and spelt wheat belong to the non-traditional wheat species with different valuable traits and properties. Significant gap remains in their antioxidant activities, phenolic acid composition and the possible role in human diet. More knowledge is needed to prepare strong arguments for an increased consumption of whole grain cereals and whole grain-based products and to provide better information about their health benefits. For this reason, the study of bioactive compounds reviewed in this paper is constantly important issue.

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VARIABILITY OF QUANTITATIVE AND QUALITATIVE TRAITS OF COLOURED WINTER WHEAT

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The aim of research was to analyse winter wheat of different grain colour and to compare newly bred coloured genotypes from our breeding in grain yield and technological and nutritional quality. The set of seven purple, five blue and four yellow wheats of different origin, including seven newly bred genotypes from Vígľaš-Pstruša, was evaluated in the field experiments established by randomised complete block design in two replications in Piešťany, in the vegetations 2012/13 and 2013/14. In seven wheat varieties differing in grain colours (selected after two of each colour plus control red variety Ilona) anthocyanin composition was evaluated by HPLC analysis. Significant differences were between growing years and among colour groups in most analysed traits. Blue grain newly bred K 3575 699/3 showed the highest anthocyanin content (by 33.5% higher compared to blue grain registered variety Scorpion). However, blue grain genotypes showed negative agronomic traits combined with low number and grain weight per spike and high plant height. In new purple variety PS Karkulka, declared grain yield and its quality were confirmed and the highest mineral content (Fe, Zn, Cu, Mn) was found in selected set. Purple grain newly bred PS 5711 had lower anthocyanin content (by 17.7%), but in quality it was comparable to PS Karkulka. Varieties with yellow endosperm showed the highest number and weight of grains per spike, however it was significantly lower to Ilona. The breeding goal of coloured winter wheat is still to improve the grain yield as well as additional agronomics traits.

Key words: winter wheat, grain colour, grain yield, quality, HPLC, anthocyanin

In wheat breeding, in addition to obligate traits (grain yield, quality, and tolerance to biotic and abiotic stresses), new directions are aimed on increasing health promoting substances. The anthocyanins represent a new goal for wheat genetic improvement. In colour wheat anthocyanins are located either in a purple pericarp, or in blue aleurone and carotenoids in the endosperm. Renewed interest for wheat breeding with a high anthocyanins content is linked to various health benefits associated with anthocyanins from their natural sources. Studies have shown that anthocyanins have antioxidant (Reque *et al.* 2014, Ficco *et al.* 2014), anti-cancer (Fernandes *et al.* 2014), anti-obesity (Esposito *et al.* 2015, Johnson *et al.* 2016), and anti-inflammation effects (Esposito *et al.* 2014). However, it is unknown whether these health benefits are solely due to anthocyanins or the synergistic effect of diverse phytochemicals (Li *et al.* 2017).

Common wheat varieties, which are characterized by purple, blue or yellow grains are actually

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produced in small amounts, but growing interest has recently been shown in the genetic development of novel pigmented varieties (Jaafar *et al.* 2013, Martinek *et al.* 2013b). The winter wheat variety Scorpion with blue grain colour was registered in 2011 in Austria and in 2012 it was also registered in European list of cultivars (Martinek *et al.* 2013b). In 2013 in Slovakia, the first local winter wheat variety PS Karkulka with purple grain was registered (Hanková *et al.* 2014). Varieties Bona Dea and Bona Vita with yellow endosperm were bred and registered in Slovakia in 2006 and 2011, respectively. Varga *et al.* (2013) analysed the anthocyanin content of blue and purple wheat cultivars and their hybrids cultivated under Hungarian growing conditions. Jaafar *et al.* (2013) evaluated progeny from crosses between red, purple and blue grained wheat varieties selected over several cycles. The results showed that increasing anthocyanin content is possible by the combination of different genetic backgrounds for purple pericarp and blue aleurone. In fact, these unconventional varieties might be important sources of biologically active phytochemicals and as a result, they could be valuable as a raw materials for the production of functional foods (Ficco *et al.* 2016). Higher anthocyanin content of blue and purple wheat can be exploited to the anthocyanin content of bakery products if whole-meal flour or bran are used (Li *et al.* 2015). The content of yellow pigment is studied mainly in *T. durum* for producing superior pasta products (Lachman *et al.* 2017).

The aim of research was to evaluate the variability of quantitative and qualitative traits of different grain coloured winter wheat and to compare newly bred coloured genotypes originated from local breeding programmes.

MATERIAL AND METHODS

Evaluated set of winter wheats consisted of 17 varieties and genotypes (next variety) of different grain colour – seven purple samples (K 3517, K 3513, 994/3, PS Karkulka, PS 5711 from Research and Breeding Station at Vígľaš-Pstruša, Indigo (GB), Zernofialovetaja (unknown origin)), five blue (K 3575 699/3, 930/1 from Research and Breeding Station Vígľaš-Pstruša, Barevná 9, Barevná 25 (CZ), Scorpion (AT)), four yellow (Bona Dea, Bona Vita (SK), Luteus, Citronova (synonym Citrus) (DE)). As a control, local variety Ilona with standard red grain colour was used. Field experiments were established by randomized complete block design in two replications at the Research Institute in Piešťany (west part of Slovakia) in the vegetative years 2012/13 and 2013/14. Experimental unit consisted of a 1 m^2 plot. Locality is at an altitude of 163 m with a continental character of climate, long-time average annual precipitation is 608 mm and temperature 9.2°C. Soil type was Luvi-Haplic Chernozem; the locality belongs to a maize production type. In the Table 1 month precipitations and average temperatures are presented.

T a b l e 1

Precipitations and average temperature over two growing seasons (2012/13, 2013/14) at Piešťany, Slovakia

Month	10	11	12	1	2	3	4	5	6	7	Sum, mean
Normal* precipitations [mm]	40	52	46	32	33	28	40	66	72	59	468
Normal* temperature [°C]	9.7	4.2	–0.1	–2.0	0.4	4.5	9.6	14.5	17.4	18.9	7.9
2012/13											
Precipitations [mm]	63.4	15.8	26.2	37.4	68.8	70.6	9.8	40.8	102.4	3.0	438.2
Temperature [°C]	10.3	7.7	–1.1	–1.2	0.9	2.6	11.5	15.3	18.7	22.4	8.71
2013/14											
Precipitations [mm]	25.0	46.8	3.6	21.0	25.2	11.4	54.2	75.6	29.6	95.0	387.4
Temperature [°C]	12.1	6.7	2.7	3.0	3.2	8.5	11.8	15.2	19.2	21.7	10.4

*(1961–1990)

During the growing seasons standard cultivation practices were used. Within vegetations obligate traits were observed. In maturity, an average sample of 300 productive ears was collected from each plot and the number and weight of grains per ear and 1,000 grain weight (TGW) were determined. Protein, starch, gluten content and Zeleny test were assayed by the DA 7200 NIR analyser.

Extraction of anthocyanins from wheat grain bran and determination of total anthocyanins using the pH differential method were described in Žofajová *et al.* (2012).

Composition of anthocyanins, total ash and microelements (Fe, Zn, Cu, Mn) were determined in wheat varieties grown in 2013/14 (PS Karkulka, PS 5711, K 3575 699/3, Scorpion, Bona Vita, Luteus) selected after two of each colour group plus control (red) variety Ilona.

Composition of anthocyanins

The anthocyanins were extracted according to the method described by Abdel-Aal and Hucl (2003), with a modification according to Ficco *et al.* (2014). Homogenized cereal grain material (500 mg) was extracted twice by 8 ml of methanol acidified with 1.0 M HCl (85:15, v/v) at laboratory temperature for 30 minutes. Crude extracts were centrifuged at 9,000 RPM for 15 minutes. Separated supernatants were filtered through 0.45 μm cellulose syringe filters before HPLC analysis.

The Agilent 1200 Series HPLC System (Agilent Technologies, Santa Clara, CA, USA) used for analysis consists of a binary pump, the DAD SL detector, degasser, and column temperature controller. System control and data analysis were processed using the Agilent ChemStation software Rev. B.04.03 (Agilent Technologies, Santa Clara, CA, USA). The chromatographic separation was performed in Eclipse XD8-C18 column (3.5 μm, 3.0 × 100 mm) using water solution of formic acid (A; 4.5%, v/v) and methanol (B) as mobile phase at flow rate of 0.4 ml/min. The gradient program was set as follows: 0–30 min, 10–25% B; 30–40 min, 25-45% B; 40–42 min, 45–90% B; 42–45 min, 90% B. The chromatogram was monitored at a wavelength of 520 nm throughout the experiment. The column temperature was maintained at 30°C and the injection volume of each sample and standard solution was 10 μl. The HPLC mobile phase was prepared fresh daily, filtered through 0.45 μm membrane filter and then degassed before injection into the column.

Determination of total ash

For determination of total ash in wheat samples ashing furnace Carbolite AAF 1100 was used. The whole test of total ash was performed via STN ISO 2171. Each sample was prepared in duplicate.

Determination of microelements

All measurements were performed using an Agilent 4200 MP-AES with nitrogen plasma gas supplied via an Agilent 4107 Nitrogen Generator. The instrument operated in a fast sequential mode and featured a Peltier-cooled CCd detector.

Microwave digestion was used to prepare all wheat samples for total metal analysis of Cu, Zn, Mn and Fe by MP-AES. 7 mL of 65% HNO_3 and 1 mL of 30% H_2O_2 was added to 0.5 g of the sample. A preloaded method for the Milestone ETHOS 1 microwave system was used to digest the sample. Once cooled, the solution was diluted to 50 mL using ultrapure water. Each sample was prepared in triplicate and the quality of the MP-AES results was evaluated by comparing them with the values for reference material, strawberry leaf, Metranal 3.

The data were analysed by analysis of variance using Statgrafics Centurion X64.

RESULTS AND DISCUSSION

Effect of vegetative year (Table 2)

Significant differences were observed between vegetative years in all traits except grain number per ear. In seven evaluated traits (from eleven), higher average values were found in the vegetative year 2012/13 compared to subsequent one. The vegetation 2012/13 was in average by nine days exceed (not shown) and heading of varieties was by 10.9 days later, what was caused by later beginning of vegetation (average temperature in March 2013 was only 2.6°C) (Table 1). In analysed traits such as protein complex and Zeleny test higher results were observed in vegetation 2012/13 (from 13.6% for protein content to 18.9% for Zeleny test). The higher amount of precipitation (by 50.8 mm) and lower average temperature (by 1.7°C) during the growing season 2012/13 (Table 1) also positively influenced anthocyanin and ash content (increasing by 17.8% and 8.1%, respectively). Anthocyanin content is affected by environmental factors, e.g. weather conditions and soil type and quality, what was also expressed in significant interaction year × variety (not shown). Similarly, in spring wheat Abdel-Aal *et al.* (2016) found that genotype, year and location

T a b l e 2

Mean values of traits of 17 coloured winter wheat evaluated over two growing seasons (2012/13, 2013/14) at Piešťany, Slovakia

Trait	Growing season		Mean	$LSD_{0.05}$
	2012/13	2013/2014		
HEA	25.8[b]	14.9[a]	20.3	0.86
PH	101.7[a]	118.0[b]	109.9	3.07
TGW	42.4[a]	47.6[b]	45.0	1.74
GN	43.5	42.4	43.0	2.95
GW	1.85[a]	2.03[b]	1.94	0.45
PC	13.4[b]	11.8[a]	12.6	0.38
SC	61.3[a]	62.3[b]	61.8	0.34
GC	28.5[b]	24.9[a]	26.7	0.83
ZT	45.8[b]	38.5[a]	42.2	0.98
TAC	44.3[b]	37.6[a]	41.0	3.10
ASH	1.72[b]	1.59[a]	1.65	0.06

HEA – heading [number of days from May, 1]; PH – plant height [cm]; TGW – thousand grain weight [g]; GN – grain number per ear [piece]; GW – grain weight per ear [g]; PC – protein content [%]; SC – starch content [%]; GC – gluten content [%]; ZT – Zeleny test [ml]; TAC – total anthocyanin content [mg/kg]; ASH – ash content [%]
$LSD_{0.05}$ – least significant difference at the level $\alpha = 0.05$
Different letters indicate significant differences at $P < 0.05$

T a b l e 3

Descriptive statistics of traits of coloured winter wheat groups over two growing seasons (2012/13, 2013/14) at Piešťany, Slovakia

Trait	purple (n=7)		blue (n=5)		yellow (n=4)		control	$LSD_{0.05}$
	mean	range	mean	range	mean	range	mean	
HEA	19.8	5.0–26.0	23.7	5.0–37.0	18.8	3.0–35.0	9.5	4.91
PH	108.8	76.0–132.0	119.4	92.7–150.3	105.9	80.0–120.7	100.6	8.76
TGW	44.6	33.0–54.7	46.0	33.5–58.9	43.7	39.4–49.0	44.4	3.92
GN	40.9	27.4–57.0	37.6	23.8–55.2	49.3	30.6–69.5	55.2	5.02
GW	1.84	1.07–2.43	1.77	0.80–3.00	2.14	1.33–2.94	2.50	0.30
PC	12.2	10.3–13.8	13.4	10.5–17.9	12.7	11.2–15.8	12.4	0.83
SC	62.0	60.7–63.3	61.3	57.3–63.5	61.7	58.4–63.4	62.0	0.73
GC	25.9	20.5–30.2	28.4	22.8–38.4	26.9	23.6–33.7	25.7	1.88
ZT	38.8	27.0–48.0	44.3	33.5–57.0	45.3	37.5–53.0	44.8	3.30
TAC	48.2	7.8–93.4	61.0	21.6–120.2	12.7	2.87–28.8	15.5	13.02
ASH	1.74	1.58–1.93	1.57	1.43–1.78	1.69	1.571.81	1.57	0.11

Abbreviations see Table 2
$LSD_{0.05}$ – least significant difference at the level $\alpha = 0.05$

significantly influenced content and composition of anthocyanin pigments.

Higher precipitations in the period of intensive growth in the vegetation 2013/14 induced higher plant height (by 16.3 cm), grain weight and starch content (by 12.2% and 1.6%, respectively) compared to the previous vegetation.

Quantitative and qualitative traits among and within colour groups (Table 3, 4)

Significant differences were found among colour groups nearly in all evaluated traits (except heading, TGW and starch content). The earliest were varieties with yellow grains (heading – May, 18 to 19, on average), a day later purple ones and approximately 5 days later blue grain wheats. In each wheat group, high variability and varieties earlier compared to Ilona (average heading from May, 3 to 5) were found. The highest plant height had blue varieties (119.4 cm) and the lowest wheat varieties with yellow endosperm (105.9 cm). The highest number and weight of grains per spike were in varieties with yellow endosperm (49.3 pcs and 2.14 g, respectively) and the lowest results were determined for blue grain wheats (1.77 g and 37.6 pcs). Varieties with yellow endosperm showed lower weight and number of grains per ear compared to the control Ilona by 14.4% and 10.4%, respectively, and were comparable to Zeleny test. A valuable source of high grain weight per ear and TGW is the blue grain variety Scorpion (2.85 g and 57.2 g, respectively). These parameters were declared in the variety description presented by Martinek *et al.* (2013b). Variety Scorpion is suitable for baking bread, since intake of anthocyanins may play an important role in the prevention of human diseases (Bartl *et al.* 2015). Compared to control Ilona, the blue wheats showed good qualitative traits in term of protein as well as gluten content.

Our results are in agreement with other research finding and suggest that blue wheat varieties contain higher anthocyanin contents compared to purple seeds (Ficco *et al.* 2014; Žofajová *et al.* 2012 and others). The highest total anthocyanin content was observed in K 3575 699/3, what was by 33.5% higher compared to Scorpion. Purple wheats were rich in ash content, what was significantly higher compared to Ilona.

To compare grain yield traits of colour wheats (mainly purple and blue) to the red control variety Ilona we can confirm that the main objective in coloured wheat breeding is increasing the grain yield. Growing of colour wheats will depend on the grain yield and agronomic characters, comparable to commercial wheat varieties. In this direction, some progress has been achieved as reported Garg *et al.* (2016), who selected pigmented lines with commercial potential, having grain yield and thousand grain weight equivalent to the high yielding commercial cultivars. Anthocyanin rich lines adapted to local growing conditions were developed from low yielding exotic donor lines.

Table 4

Average values of selected traits of coloured winter wheat over two growing seasons (2012/13, 2013/14) at Piešťany, Slovakia

Varieties	colour	TGW	GN	GW	PC	SC	GC	ZT	TAC	ASH
PS Karkulka	purple	48.4^{ab}	39.0^{a}	1.91^{a}	12.8	61.9	26.8	42.8	44.7^{abc}	1.83^{c}
PS 5711	purple	42.1^{a}	41.1^{ab}	1.73^{a}	12.4	61.5	26.6	41.7	36.8^{ab}	1.66^{ab}
K 3575 699/3	blue	54.4^{b}	35.2^{a}	1.92^{a}	13.0	61.8	28.1	46.6	76.1^{c}	1.61^{ab}
Scorpion	blue	57.2^{b}	50.2^{bc}	2.85^{b}	13.0	61.7	26.9	42.3	57.0^{bc}	1.54^{a}
Bona Vita	yellow	41.8^{a}	40.6^{a}	1.70^{a}	14.2	59.9	29.7	46.0	17.9^{a}	1.70^{bc}
Luteus	yellow	42.8^{a}	50.6^{c}	2.16^{a}	11.7	63.0	25.0	44.5	8.6^{a}	1.67^{ab}
Mean	–	47.8	42.8	2.05	13.0	61.7	27.2	44.0	40.2	1.67
$LSD_{0.05}$	–	8.85	9.47	0.57	1.72	1.36	3.98	6.18	38.40	0.15

Abbreviations see Table 2
$LSD_{0.05}$ – least significant difference at the level $\alpha = 0.05$
Different letters within the same column of each trait indicate significant differences at $P < 0.05$

Anthocyanin composition

Anthocyanin composition in selected wheat varieties differing in grain colours was evaluated by HPLC analysis. HPLC analysis is method for detailed description of differences between analysed varieties of wheat because photometric determination of total anthocyanins is not sensitive to differentiation of varietal specificities such as composition of anthocyanins, presence of other coloured compounds and effects of non-coloured compounds interfered during spectrophotometric determination. HPLC fingerprints in Figure 1 show composition of individual anthocyanins and other compounds (serotonin, melatonin and secoisolariciresinol diglucoside). Similar fingerprints have been also presented in wheat varieties by Hosseinian *et al.* (2007). These fingerprints prove that varieties Ilona (1), Luteus (2) and Bona Vita (3) do not contain anthocyanins whereas varieties Scorpion (4), K 3575 699/3 (5) (blue) and varieties PS 5711 (6) and PS Karkulka (7) (purple) contain some compounds with elution and chromogenic properties characteristic for anthocyanins. The peak areas of all these compounds expressed by percentage of total colour is described in Table 5. From the Table 5 it is evident that the colour of studied varieties is not caused only by presence of anthocyanins. Anthocyanins caused contribution to wheat colour in the range from 0 (Bona Vita) to 96% (PS 5711) of total colour which can be determined by classic methods based on spectrophotometric analysis (Table 4). The most common anthocyanin in purple wheat is cyanidin-3-O-glucoside, followed by peonidin-3-O-glucoside, whereas delphidin-3-0-glucoside is the most abundant anthocyanin in wheat (Abdel-Aal & Hucl 2003; Escribano-Bailón *et al.* 2004). On the base of described chromatographic method and spectral properties of separated compounds, anthocyanins were eluted from chromatographic column in retention time varied from 1.74 to 34.14 minutes. Compounds eluted out of this range can be included into groups of proteins, melatonins and serotonins (Hosseinian *et al.* 2007).

Grain minerals content (Table 6)

Concentration of mineral elements including Fe and Zn in wheat grains is important for human

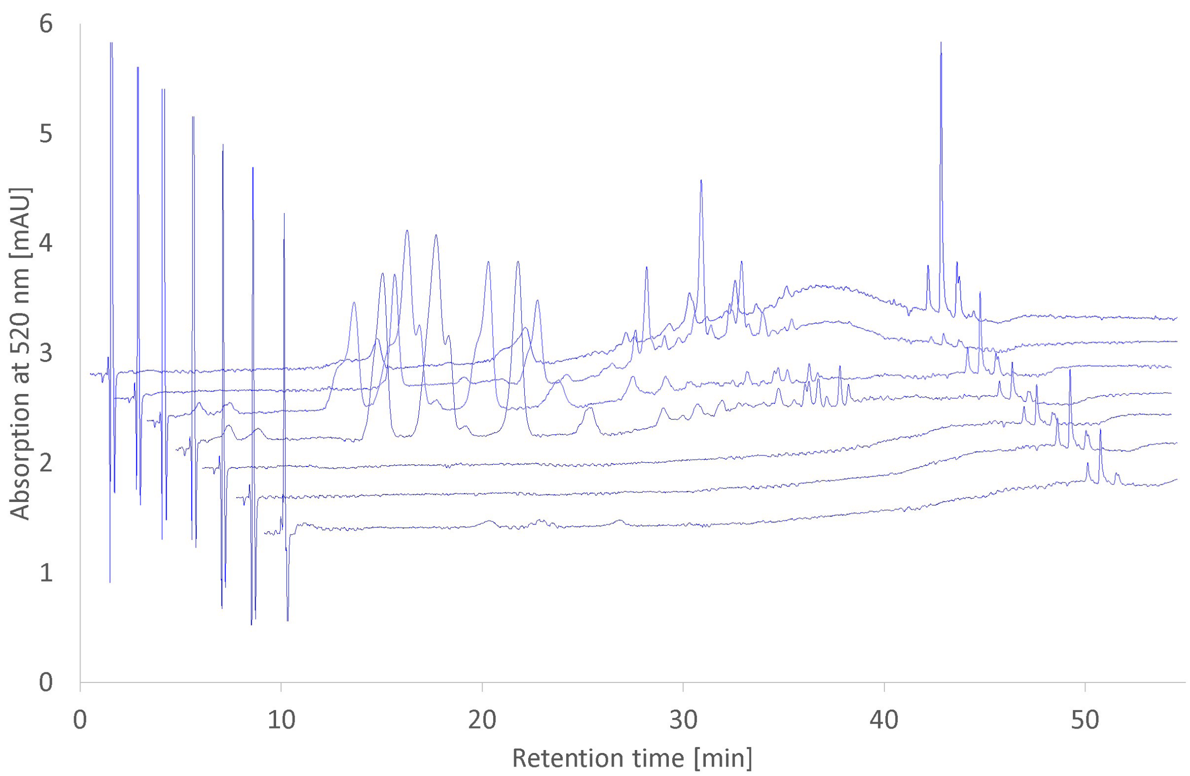

Figure 1. HPLC fingerprints of wheat extract determined at 520 nm; samples are in ascending order (upper is sample 7 and lower is sample 1) (1 – Ilona, 2 – Luteus, 3 – Bona Vita, 4 – Scorpion, 5 – K3575 699/3, 6 – PS 5711, 7 – PS Karkulka)

health. For deeper characterisation of grain coloured wheats, seven selected varieties were explored for minerals content. There were no significant differences among different colour winter wheat groups in Fe, Zn, Cu and Mn content (results not shown). The highest Fe in the grain showed the purple varieties in average (34.20 mg/kg) and the lowest varieties with yellow endosperm (32.09 mg/kg), which contrary showed the highest content of Zn and Mn (21.42 mg/kg and 29.72 mg/kg, respectively). In regard to small number of evaluated varieties and composition of file we could not confirm the results published by Ficco *et al.* (2014), who by evaluation of 76 colour wheats genotypes showed the highest content of Zn and Fe in blue grain wheat and in Cu and Mn he did not record any definite trend. On the

T a b l e 5

The contribution of anthocyanins to colour of selected wheat varieties

Peak No.	Retention time [min]	Colourful contribution of anthocyanins [%]						
		Ilona	Luteus	Bona Vita	Scorpion	K3575 699/3	PS 5711	PS Karkulka
1	1.735	0	36.4	0	4.01	0	0	0
2	2.103	0	8.21	0	0	0	0	0
3	2.325	36.53	0	0	0	0	0	0
4	3.021	9.91	0	0	1.37	1.50	0	0
5	4.534	0	0	0	0	1.50	0	0
6	10.976	0	0	0	16.63	12.73	0	0
7	13.690	0	0	0	19.57	20.82	0	0
8	14.351	4.22	0	0	9.58	0	0	0
9	14.651	2.87	0	0	0	9.94	14.73	6.61
10	14.900	0	0	0	0.65	0	0	0
11	17.775	0	0	0	17.61	1.15	0	0
12	20.992	0	0	0	2.74	17.35	8.59	0
13	21.413	0	0	0	0	0	0	0
14	23.000	0	0	0	0	3.35	10.55	0
15	24.670	0	0	0	1.46	0	0.95	0
16	25.303	0	0	0	0	0	0	0
17	26.623	0	0	0	1.37	2.42	0.94	0
18	27.068	0	0	0	0	1.96	3.92	0
19	27.974	0	0	0	1.37	0	11.17	0
20	28.692	0	0	0	1.37	0.77	1.59	0
21	29.775	0	0	0	0	0	0.88	0
22	29.851	0	0	0	0	0.76	0	0
23	30.675	0	0	0	1.86	0	20.86	0
24	31.286	0	0	0	0.73	1.73	1.84	0
25	31.888	0	0	0	2.15	0	4.29	0
26	32.242	0	0	0	2.35	1.50	9.57	0
27	32.625	0	0	0	2.73	0	0	0
28	32.831	0	0	0	1.07	1.62	1.59	0
29	33.723	0	0	0	4.01	2.19	3.06	0
30	34.141	0	0	0	1.95	0	0	0

T a b l e 6

Grain minerals concentration of coloured winter wheat genotypes evaluated during the 2013/14 growing season at Piešťany, Slovakia

Varieties	Colour	Fe [mg/kg]	Zn [mg/kg]	Cu [mg/kg]	Mn [mg/kg]
PS Karkulka	purple	37.71[b]	22.84[d]	5.04	31.23[d]
PS 5711	purple	30.71[a]	18.80[a]	3.92	22.33[a]
K 3575 699/3	blue	32.83[a]	19.53[ab]	4.08	26.01[b]
Scorpion	blue	33.52[a]	20.25[abc]	4.19	30.25[cd]
Bona Vita	yellow	32.13[a]	21.82[cd]	4.68	30.47[cd]
Luteus	yellow	31.93[a]	21.03[bcd]	4.27	28.98[c]
Ilona	control (red)	30.85[a]	21.17[bcd]	4.70	28.87[c]
Mean	–	32.81	20.78	4.41	28.31
$LSD_{0.05}$	–	2.84	2.06	1.09	1.82

$LSD_{0.05}$ – least significant difference at the level $\alpha = 0.05$
Different letters within the same column of each trait indicate significant differences at $P < 0.05$

contrary, Guo *et al.* (2013) analysed nutrient composition in seven purple lines and found out 100% higher Fe content compared to control, white grain wheat.

Varieties with purple grain possessed the highest variability in all mineral elements. The registered variety PS Karkulka showed the highest mineral content and the lowest was observed in PS 5711. In grain mineral content instead of Mn, the newly bred blue genotype K 3575 699/3 was comparable with registered blue variety Scorpion. Among varieties, there were significant differences in Fe, Zn and Mn contents. The highest coefficient of variability (12.43%) was in Cu content and differences among varieties were not significant. The lowest coefficient of variability (1.90%) was in Mn content. Control variety Ilona was comparable in Zn and Mn content to the average value for coloured wheats and its grains showed more Cu (by 7.8%) and less Fe (by 6.9%). Variability in the content of mineral elements can be used in breeding for improved mineral availability in the end products. Information about the mineral content in the evaluated genotypes are within the range that published Ficco *et al.* (2014) and Zhang *et al.* (2010).

In Slovakia, there is an interest in coloured wheat observed especially in the production of extruded cereal products (puffed bread). Our assumption is to utilize coloured wheat grains in the production of baker's ware (Rückschloss *et al.* 2011). Martinek *et al.* (2013a) noticed that it will be necessary to know the extent of natural degradation of dyes during thermal processing of the wheat grain when during Maillard reaction chemical changes occur. The coating grain layers would be used for fortification of dairy products (for example yogurt), where not only the beneficial component of pigments, but also high content of dietary fibre will be used (Rückschloss *et al.* 2011).

CONCLUSIONS

Anthocyanin content in 17 colour wheat was influenced by weather conditions of experimental years and their interaction. The higher temperature and unequal distribution of precipitation caused reduction in anthocyanin content. Blue varieties contained higher anthocyanin content compared to purple ones. The highest total anthocyanin content showed K 3575 699/3 (blue grain newly bred), what was by 33.5% higher compared to blue registered variety Scorpion. K 3575 699/3 can be utilised in breeding programmes as a source of high anthocyanin content. Blue varieties (in average) were the highest in plant height and the lowest in number and weight of grain per spike, which may be negative for their commercial cultivation. However, the highest grain weight per ear and TGW were confirmed in the variety Scorpion. The registered purple variety

PS Karkulka showed the highest mineral content (Fe, Zn, Cu, Mn). Purple bred PS 5711 not reached the variety PS Karkulka in quantitative or qualitative traits. Varieties with yellow endosperm were the most yielding, but worse compared to the control Ilona.

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MOLECULAR DETECTION OF '*CANDIDATUS* PHYTOPLASMA AUSTRALASIA' AND '*CA.* P. CYNODONTIS' IN IRAQ

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The association of phytoplasma was investigated in symptomatic tomato (*Solanum lycopersicum* L.), eggplant (*Solanum melongen* L.), mallow (*Malva* spp.) and Bermuda grass (*Cynodon dactylon* L.) plants exhibiting witches' broom and white leaf diseases, respectively. Total DNA was extracted from tomato (n=3), eggplant (n=2), mallow (n=2) and Bermuda grass (n=8) samples. Direct polymerase chain reaction (PCR) was performed using P1/P7 primer set, then PCR products were sequenced. Sequences obtained from tomato, eggplant and mallow shared 99% maximum nucleotide identity with phytoplasma belonging to subgroup 16SrII-D, and resulted therefore *'Candidatus* Phytoplasma australasia'-related. Sequences obtained from Bermuda grass showed 100% maximum nucleotide identity to 16SrXIV-A subgroup and were '*Ca*. P. cynodontis'-related. The study presents the first molecular confirmation and sequence data of presence of *'Ca.* P. australasia' and '*Ca*. P. cynodontis' in Iraq.

Key words: witches' broom, white leaf, tomato, eggplant, Bermuda grass

Phytoplasms impact a wide range of economically important plant species causing serious losses worldwide (Bertaccini & Duduk 2009). They affect the quality and quantity of products resulted from infected crops including vegetables, field crops, fruit trees and ornaments (Chaturvedi *et al.* 2010; Bertaccini *et al.* 2014; Maejima *et al.* 2014). An extra damage may occur due to the cost paid for phytoplasma indexing procedures, quarantine regulations and disease control. Phytoplasms are wall- less prokaryotic plant pathogens belonging to the class Mollicutes (IRPCM 2004). They are phloem limited and insect transmitted by *Cicadellidae* (leafhoppers), *Fulgoridae* (planthoppers), *Cercopidae* (spittlebugs or froghoppers), *Cixiidae* (Cixiid planthoppers), *Derbidae* (Derbid planthoppers), *Delphacidae* (Delphacid planthoppers) and *Psyllidae* (Psyllid bugs) (Jarausch & Weintraub 2013). Moreover phytoplasms have been found to be transmitted by dodder, grafting (Bertaccini & Duduk 2009; Bertaccini *et al.* 2014) and through seeds (Bertaccini & Duduk 2009; Calari *et al.* 2011). Taxonomically, phytoplasma strains have been classified into 116 subgroups within 34 groups based on 16S ribosomal gene restriction fragment length polymorphism (RFLP) analyses (Duduk & Bertaccini 2011; Bertaccini *et al.* 2014; Fránová *et al.* 2014).

Phytoplasmas classified in 16SrII group are often associated with typical witches` broom symptoms including virescence, phyllody, proliferation and stunting (Bertaccini *et al.* 2014), while those belonging to 16SrXIV group are usually associated with white leaf symptoms (Marcone *et al.* 2004; Salehi *et al.* 2009). Papaya yellow crinkle disease associat-

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ed phytoplasma (16SrII-D subgroup) was identified in Australia for the first time in 1996 (Gibbs *et al.* 1996). In 1998, this phytoplasma was assigned into the new taxon *'Candidatus* Phytoplasma australasia' (White *et al.* 1998). Phytoplasmas associated with white leaf diseases were reported for the first time on Bermuda grass since 1972 in Taiwan (Marcone *et al.* 1997), and were assigned into a separated taxon '*Ca. P. cynodontis*' in 2004 (Marcone *et al.* 2004). PCR technique is a powerful tool for phytoplasma characterization especially when combined with RFLP and sequence analyses (Bertaccini & Duduk 2009; Delić 2012). PCR approaches using universal primers designed to target conserved sequences (e.g. 16S rDNA) enable detection of phytoplasmas in both plants and insects (Bertaccini & Duduk 2009; Delić 2012). Then, phytoplasma sequences resulting from universal primers amplification can easily be identified up to group/sub group level using virtual RFLP digestion (Wei *et al.* 2007; Zhao *et al.* 2009).

In Iraq, phytoplasma diseases have been investigated based on biology, serology and microscopy (Al-Rawi *et al.* 2001). PCR amplification was applied to detect the association of phytoplasmas with a phyllody disease of Arabic jasmine (Al-Kuwaiti *et al.* 2015) and sesame (Mohammed *et al.* 2016). Witches' broom symptoms, suspected to be a phytoplasma disease, were observed in tomato (*Solanum lycopersicum* L.) and eggplant (*Solanum melongena* L.) in Basra province. This disease caused serious economic losses on tomato and eggplant crops to producers as most fruits failed to set. White leaf was another phytoplasma disease observed in Bermuda grass (*Cynodon dactylon* L.) in Baghdad. Two mallow (*Malva* spp.) samples exhibiting witches' broom symptoms were found in Al-Nassiriya province. This study aimed at the molecular investigation of identity of the phytoplasma associated with witches' broom and white leaf diseases in Iraq.

MATERIAL AND METHODS

Leaf samples were collected from symptomatic tomato (n=3) and eggplant (n=2), grown under tunnels at Al-Zubair region in Basra province. Mallow (n=2) samples were collected from a field in Al-Nassiriya province. Bermuda grass (n=8) samples were collected from Baghdad province. Total DNA was extracted from samples using AccuPrep® Plant DNA Extraction Kit from (Bioneer, S. Korea) following the manufacturer instructions. Direct PCR was performed using AccuPower PCR PreMix kit from (Bioneer, S. Korea) and P1/P7 primer set (Deng & Hiruki 1991; Smart *et al.* 1996). PCR reaction was prepared by adding 1µl (50 ng) of extracted DNA and 1 µl of each primer (10 picomole) to PCR Premix then reaction volume was adjusted to 20 µl. PCR amplification was preformed using 1 cycle of pre-denaturation for 2 min. at 94°C, 35 cycles of denaturation for 30 s min at 94°C, annealing for 2 min at 55C° and extension for 3 min. at 72°C. The Final extension step was set for 15 min at 72°C. PCR products were analysed by ethidium bromide gel electrophoresis using 2.5% agarose for 15 min at 125 mAmp (Sambrook & Russell 2006). PCR products of expected size were sent to (Bioneer, S. Korea) for sequencing in both directions, to generate consensus sequences. Sequences obtained were compared to equivalent GenBank sequences using MEGA BLAST analysis. Phylogenetic tree was constructed using MEGA6 software package (Tamura *et al.* 2013). Virtual RFLP was performed to resolve group/subgroup of phytoplasma sequences isolated using *i*PhyClassifier (Zhao *et al.* 2009). Phytoplasma sequences obtained were deposited in GenBank database with accession codes (KU724309), (KX008307- KX008310) and (KY284836- KY284845).

RESULTS AND DISCUSSION

During December 2015, an outbreak of witches' broom disease occurred in tomato and eggplant grown under tunnels in Al-Zubair region at Basra province in Iraq. About 80% of tomato and eggplant exhibited typical symptoms of phytoplasma disease including phyllody, proliferation and stunting (Figure 1 A-D). In the same time a white leaf disease was observed in Bermuda grass grown in public and private gardens in Baghdad (Figure 1 E-F). PCR amplification using P1/P7 phytoplasma specific primers amplified ~1.8 kb DNA fragment size (Smart *et al.* 1996) from symptomatic tomato, eggplant, mallow and Bermuda grass (Figure 2). Sequence analyses confirmed all sequences obtained from symptomatic plants were from 16S rRNA region of phytoplasma genome (Smart *et al.* 1996), when compared to equivalent GenBank sequences. Sequences from tomato, eggplant and mallow plants, infected with witches' broom disease, shared 99.8–99.9% nucleotide identities to 16SrII-D subgroup phytoplasmas from Egypt (GenBank accession number KU056919), Iran (GenBank accession numbers KP869129, JX441321, KR706443 and KJ016231), Oman (GenBank accession number AB257291), Australia (GenBank accession numbers Y10097 and JQ868446) and South Korea (GenBank accession number AB690307) (Tables 1 and 2). The sequences obtained from Bermuda grass showing white leaf disease shared 99.7–100% identities

Figure 1. Naturally infected tomato (A,B), eggplant (C, D) plants exhibiting phyllody (white arrows) and proliferation symptoms. (E) and (F) Bermuda grass exhibiting white leaf symptoms

T a b l e 1

Nucleotide sequence identities representing partial16S rDNA region of *'Candidatus* Phytoplasma australasia' and *'Ca*. P. cynodontis' (Bold letters) from Iraq (marked with *) and other GenBank strains. Sequence identities were calculated using MEGA6 software.

	Isolate/Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1.	Al-Zubair*																												
2.	Al-Zubair1*	99.1																											
3.	Al-Zubair2*	99.9	99.2																										
4.	Al-Zubair3*	99.6	99.3	99.8																									
5.	Al-Zubair4*	99.8	99	99.6	99.6																								
6.	Malva1*	98.9	98.4	99.1	99	98.9																							
7.	Malva2*	99.5	99	99.6	99.6	99.4	98.9																						
8.	(KU056919)Egypt	99.8	99.3	99.9	99.9	99.7	99.2	99.7																					
9.	(KP869129)Iran	99.8	99.3	99.9	99.9	99.7	99.2	99.7	100																				
10.	(JX441321)Iran	99.8	99.3	99.9	99.9	99.7	99.2	99.7	100	100																			
11.	(KR706443)Iran	99.8	99.3	99.9	99.9	99.7	99.2	99.7	100	100	100																		
12.	(KJ016231)Iran	99.7	99.2	99.9	99.8	99.6	99.1	99.6	99.9	99.9	99.9	99.9																	
13.	(Y10097)Australia	99.8	99.3	99.9	99.9	99.7	99.2	99.7	100	100	100	100	99.9																
14.	(AB690307)_S._Korea	99.6	99.1	99.7	99.6	99.5	98.9	99.5	99.8	99.8	99.8	99.8	99.7	99.8															
15.	(JQ868446)Australia	99.6	99.2	99.8	99.7	99.6	99	99.6	99.9	99.9	99.9	99.9	99.8	99.9	99.9														
16.	(AB257291)Oman	99.8	99.3	99.9	99.9	99.7	99.2	99.7	100	100	100	100	99.9	100	99.8	99.9													
17.	**WF1***	**90.7**	**90.2**	**90.7**	**90.7**	**90.8**	**90.2**	**90.7**	**90.8**	**90.8**	**90.8**	**90.8**	**90.7**	**90.8**	**90.9**	**90.8**	**90.8**												
18.	**WF2***	**91.1**	**90.6**	**91.1**	**91**	**91.2**	**90.5**	**91**	**91.2**	**91.2**	**91.2**	**91.2**	**91.1**	**91.2**	**91.2**	**91.2**	**91.2**	**99.4**											
19.	**WF3***	**91.4**	**90.9**	**91.4**	**91.3**	**91.4**	**90.8**	**91.3**	**91.4**	**91.4**	**91.4**	**91.4**	**91.4**	**91.4**	**91.5**	**91.4**	**91.4**	**99.2**	**99.7**										
20.	**WF4***	**91.4**	**90.9**	**91.4**	**91.3**	**91.4**	**90.8**	**91.3**	**91.4**	**91.4**	**91.4**	**91.4**	**91.4**	**91.4**	**91.5**	**91.4**	**91.4**	**99.2**	**99.7**	**100**									
21.	**WF5***	**91.2**	**90.7**	**91.2**	**91.2**	**91.3**	**90.7**	**91.2**	**91.3**	**91.3**	**91.3**	**91.3**	**91.2**	**91.3**	**91.4**	**91.3**	**91.3**	**99.4**	**99.7**	**99.9**	**99.9**								
22.	**WF6***	**91.2**	**90.7**	**91.2**	**91.1**	**91.2**	**90.6**	**91.1**	**91.2**	**91.2**	**91.2**	**91.2**	**91.2**	**91.2**	**91.3**	**91.2**	**91.2**	**99.3**	**99.6**	**99.8**	**99.8**	**99.9**							
23.	**WF7***	**90.4**	**89.9**	**90.4**	**90.3**	**90.4**	**89.8**	**90.3**	**90.4**	**90.4**	**90.4**	**90.4**	**90.4**	**90.4**	**90.5**	**90.4**	**90.4**	**98.9**	**99.1**	**98.8**	**98.8**	**98.9**	**99**						
24.	**WF8***	**91.1**	**90.6**	**91.1**	**91**	**91.2**	**90.5**	**91**	**91.2**	**91.2**	**91.2**	**91.2**	**91.1**	**91.2**	**91.2**	**91.2**	**91.2**	**99.4**	**99.9**	**99.7**	**99.7**	**99.9**	**99.8**	**99.1**					
25.	**(KF383980)Albania**	**91.4**	**90.9**	**91.4**	**91.3**	**91.4**	**90.8**	**91.3**	**91.4**	**91.4**	**91.4**	**91.4**	**91.4**	**91.4**	**91.5**	**91.4**	**91.4**	**99.2**	**99.7**	**100**	**100**	**99.9**	**99.8**	**98.8**	**99.7**				
26.	**(AJ550984)Italy**	**91.4**	**90.9**	**91.4**	**91.3**	**91.4**	**90.8**	**91.3**	**91.4**	**91.4**	**91.4**	**91.4**	**91.4**	**91.4**	**91.5**	**91.4**	**91.4**	**99.2**	**99.7**	**100**	**100**	**99.9**	**99.8**	**98.8**	**99.7**	**100**			
27.	**(AB741630)Myanmar**	**91.2**	**90.7**	**91.2**	**91.2**	**91.3**	**90.7**	**91.2**	**91.3**	**91.3**	**91.3**	**91.3**	**91.2**	**91.3**	**91.4**	**91.3**	**91.3**	**99**	**99.5**	**99.8**	**99.8**	**99.6**	**99.6**	**98.7**	**99.5**	**99.8**	**99.8**		
28.	**(AF248961)Thailand**	**91.2**	**90.7**	**91.2**	**91.1**	**91.2**	**90.6**	**91.1**	**91.2**	**91.2**	**91.2**	**91.2**	**91.2**	**91.2**	**91.3**	**91.2**	**91.2**	**98.9**	**99.4**	**99.7**	**99.7**	**99.6**	**99.5**	**98.7**	**99.4**	**99.7**	**99.7**	**99.9**	
29.	Acholeplasma palmae (L33734)	87.3	87	87.3	87.3	87.3	86.8	87.3	87.4	87.4	87.4	87.4	87.3	87.4	87.3	87.4	87.4	88.6	89	89.2	89.2	89.2	89.1	88.3	89	89.2	89.2	89.2	89.2

with 16SrXIV-A subgroup phytoplasmas detected in Bermudagrass from Albania (KF383980), Italy (AJ550984), Myanmar (AB741630) and Thailand (AF248961) (Tables 1 and 2). Phylogenetic analysis supported this grouping of all the Iraqi sequences from witches' broom diseased plants with phytoplasmas enclosed in 16SrII-D subgroup (Figure 3). Moreover in the phylogenetic tree all sequences obtained from white leaf diseased Bermuda grass and those of phytoplasmas enclosed in 16SrXIV-A subgroup groupend in a single clade (Figure 3).The two phylogenetic groups were separated from each other's and the relatedness was supported by 99% boots trap value. Data obtained from *i*PhyClassifier analyses confirmed that all sequences isolated from witches' broom diseased plants belong to 16SrII-D. The virtual RFLP patterns derived from Al-Zubair, Al-Zubair(1–4) and Malva1 16S rDNA

T a b l e 2

Phytoplasma sequences obtained in this study. Identity percentages were calculated based on ''*Candidatus* Phytoplasma australasiae'' reference strain (GenBank accession: Y10097) ''*Ca.* P. cynodontis'' reference strain (GenBank accession: AJ550984) using iPhyClassifier

GenBank acc. No.	Isolate/ sequence name	Source	Location	Group/sub group	Ident. [%]	Similarity coefficient
KU724309	Al-Zubair	tomato	Basra	16SrII-D	99.0	1.00
KX008307	Al-Zubair1	eggplant			99.3	
KX008308	Al-Zubair2				99.9	
KX008309	Al-Zubair3				99.7	
KX008310	Al-Zubair4				99.6	
KY284836	Malva1	mallow	Al-Nassiriya		98.6	
KY284837	Malva2				99.1	0.98
KY284838	WF1	Bermuda grass	Baghdad	16SrXIV-A	99.0	0.98
KY284839	WF2				99.7	0.98
KY284840	WF3				99.9	1.00
KY284841	WF4				99.9	1.00
KY284842	WF5				99.8	1.00
KY284843	WF6				99.7	1.00
KY284844	WF7				99.7	0.98
KY284845	WF8				99.6	0.98

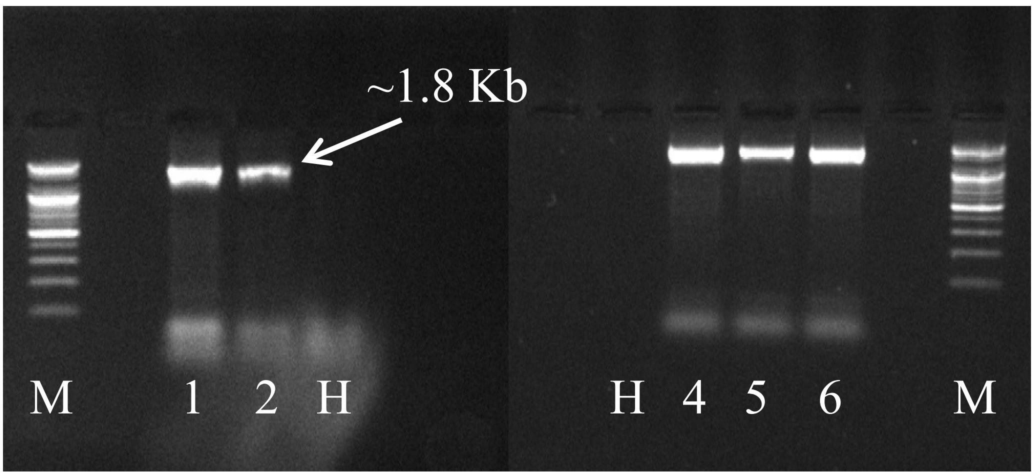

Figure 2. Gel electrophoresis pattern of ~1.8 Kb DNA fragments amplified by P1/P7 primers set. M: 100bp DNA marker, H: healthy plant, 1 & 2 lanes: witches' broom infected tomato, 4–6 lanes: white leaf infected Bermuda grass

F2n/R2 fragments were identical to the reference pattern of 16SrII-D (GenBank accession number Y10097) with similarity coefficient of 1.00 (Table 2). Malva2 shared low similarity coefficient (0.98) with 16SrII-D phytoplasmas showing a slightly different profile in virtual restriction pattern with *Mse*I. Thus, Malva2 could be a variant of 16SrII-D. *In silico* RFLP performed confirmed that all sequences obtained from white leaf diseased Bermuda grass were members of subgroup 16SrXIV-A. The virtual RFLP patterns derived from WF (1–8) 16S rDNA F2n/R2 fragment shared similarity coefficient ranging between 0.98 and 1.00 with the reference pattern of 16SrXIV-A (GenBank accession number AJ550984) (Table 2). WF(1, 2, 7 and 8) showed also a slightly different RFLP pattern when virtually cleaved with *Bst*UI. Thus, WF(1, 2, 7 and 8) may represent variants of 16SrXIV-A. Based on molecular and *in silico* approaches, the association of '*Ca.* P. australasiae', subgroup 16SrII-D with witches' broom disease has been reported in tomato and eggplant worldwide including Oman (Al-Subhi *et al.* 2011) Egypt (Omar & Foissac 2012), Iran (Salehi *et al.* 2014), and India (Singh *et al.* 2012; Yadav *et al.* 2016). Whereas, '*Ca.* P. cynodontis' subgroup 16SrXIV-A associated with Bermuda grass white leaf disease has been investigated based on molecular and *in silico* analyses in Iran (Salehi *et al.* 2009),

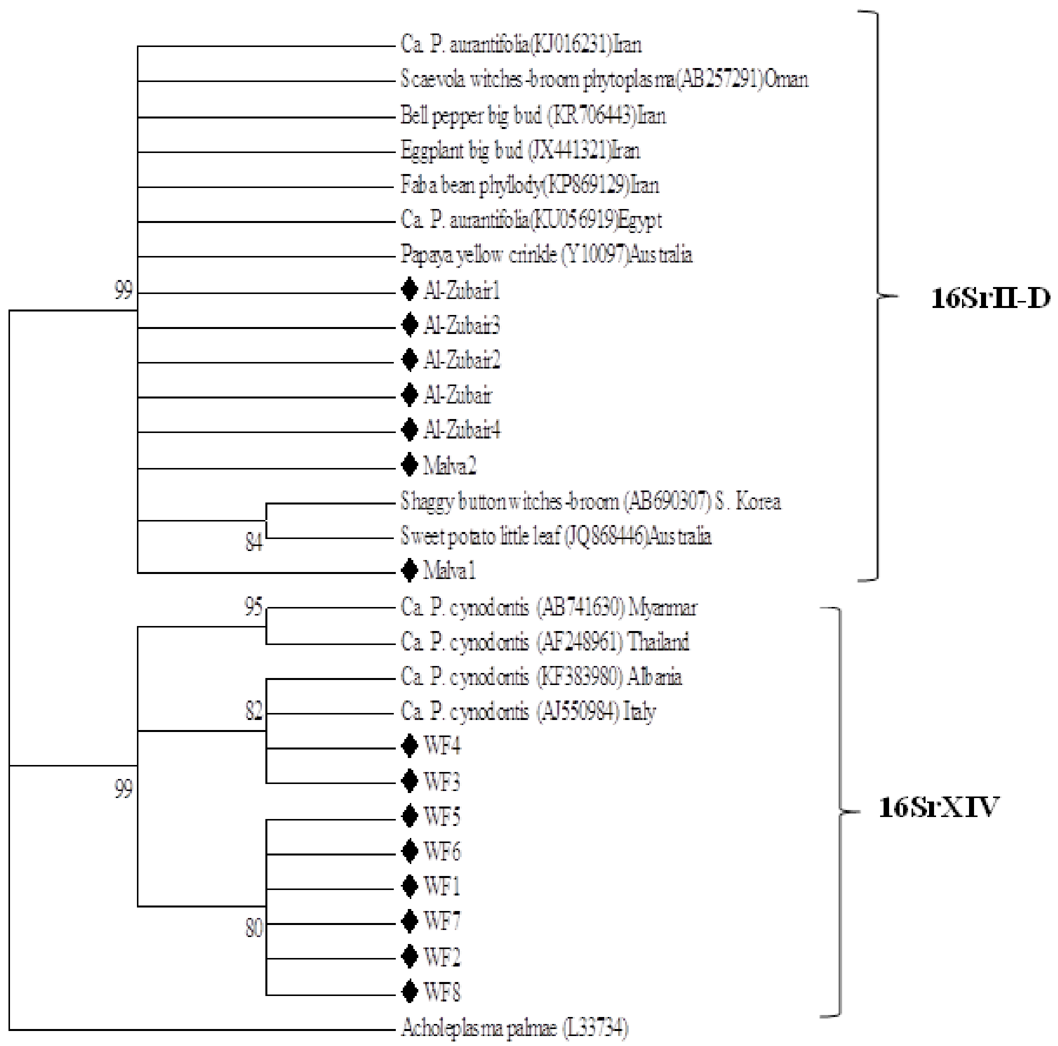

Figure 3. Neighbor-Joining phylogenetic tree of '*Candidatus* Phytoplasma australasia' from tomato, eggplant and mallow and '*Ca.* P. cynodontis' from Bermuda grass. This tree was constructed from partial 16S rDNA sequences (including 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal RNA intergenic spacer, complete sequence; and 23S ribosomal RNA gene, partial sequence) from Iraq (marked with•) and selected GenBank sequences. *Acholeplasma palmae* (L33734) used as an out group comparison. This tree was constructed by MEGA6 software.

Kenya (Obura *et al.* 2010) Italy, Albania and Serbia (Mitrović *et al.* 2015) and Saudi Arabia (Omar 2016) .

CONCLUSIONS

This study presents the first report of *'Ca.* P. australasiae' and '*Ca.* P. cynodontis' association with witches' broom and white leaf diseases in Iraq, respectively. Moreover it confirms the presence of phytoplasmas in tomato, eggplant, mallow and Bermuda grass based on molecular and *in silico* analyses since previous studies conducted (Al-Kuwaiti *et al.* 2015; Mohammed *et al.* 2016) did not present sequence information regarding phytoplasmas in Iraq. Further molecular based studies, however, are required to investigate phytoplasma diseases and their epidemiology in Iraq.

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IMPACT OF OPERATING TEMPERATURE OF GAS TRANSIT PIPELINE ON SOIL QUALITY AND PRODUCTION POTENTIAL OF CROPS

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The aim of this study is to investigate the effects of gas transit pipeline temperature on soil moisture, soil temperature and yield of harvest crops. The study area was located in the village Ivanka pri Nitre (Nitra District, Southwestern Slovakia). Soil type in the site is Orthic Brown Chernozem. Temperature of the transported gas increased the soil temperature in the range of 2.07°C to 3.4°C measured in a depth ranging from 250 mm to 350 mm above the gas lines. The temperature also reduced soil moisture by 1.27–3.18 percentiles of weight. Yield of the winter wheat grown above the gas lines was higher by 9.40% in 2004 and by 13.06% in 2006. Yield of the sunflower grown above the gas lines was higher by 8.05% in 2005. In treatment 1, organic fertilisation in a dose of 50 t/ha affected the yield of the winter wheat above the gas pipeline and the yield increased by 13.95% in 2004.

Key words: gas pipeline, soil moisture, soil temperature, sunflower, wheat, yield

Agricultural land is economically important and it is an integral part of the landscape. It is the most common type of rural cultural landscape, where soil plays a primary function. Many types of landscape are exposed to many negative impacts caused by human interventions, which reduce its ecological stability (Yakovleva 2011; Olson & Doherty 2012; Shi *et al.* 2015). Therefore, it is necessary to constantly monitor and evaluate the changes of biotic and abiotic components of the landscape. The landscape is also significantly affected by transit pipelines. It has disrupted the original landscape and affected its production ability. Installing the gas pipeline into the soil caused a soil cover destruction. The soil degradation caused by pipelines was reported by Rusanova (1997) and Soon *et al.* (2000), and its effect on the soil erosion was evaluated by Bayramov *et al.* (2013) and Shi *et al.* (2014); the destruction of soil cover and significantly different soil characteristics even after 20 years was confirmed by Gel'tser *et al.* (1990). Changes in plant species composition visible after 12 years were recorded by Walker and Koen (1995) and the impact on vegetation state was reported by Xiao *et al.* (2014). In addition to the disruption of the natural arrangement of soil profile horizons and properties, the transported gas permanently affects the soil (Széplaky *et al.* 2013), plants grown on it and probably micro- and macro-edaphon due to the increased temperature. Gas temperature at the outlet of the compressor station is usually

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40°C. It increases the temperature of the studied soil and causes changes in its moisture conditions (Gu *et al.* 2004; Wen *et al*. 2006; Penuelas *et al*. 2007; Krakauer *et al*. 2010).

The aim of this paper is to find out how the increased temperature of the transported gas affects the soil moisture and crop yields, and investigate the impact of the intensification factor (organic fertiliser) on the yield of the crops grown above the pipelines.

MATERIAL AND METHODS

The experiment was established on an agricultural land cultivated by agricultural farm PD Ivanka pri Nitre (Nitra district, SW Slovakia), on a site located close to the transit pipeline. The site is located behind the gas compressor stations in Ivanka pri Nitre, where the soil is affected by a higher temperature of the pressurized gas. The soil type is Luvic Chernozem. The disturbance of soil cover caused by the construction of transit pipeline was found in the form of Regosol (Relocatic) deposited directly above the gas pipelines (Skalský *et al*. 2002).

The crop rotation on the monitored site was as follows: 2004 winter wheat (variety Brea), 2005 sunflower (variety Pedro) and 2006 winter wheat (variety Bonita).

Plots (1.5 m × 3.0 m) were staked above five lines of the transit pipeline in two treatments:

– Treatment 1 – soil disturbed by the pipeline construction fertilised with compost-based organic fertiliser – Vitahum (200 g of Vitahum was dried for eight hours, weight of dry mass was 80.4 g and content of nutrition was: 1.9% N, 0.5% P, 0.9% K) as a growing medium in the dose of 50 t/ha, which was used as a single application on the experimental plot during the establishment of the experiment,
– Treatment 2 – soil disturbed by the pipeline construction that was not fertilised – served as the Control treatment above the pipeline.

Individual treatments were separated by a 2 m wide strip of land.

– Treatment 3 – soil undisturbed by the pipeline construction that was not fertilised – served as the Control treatment 20 m from the gas lines.

Each treatment consisted of five plots.

The aim of the soil temperature moisture measurement was to prove the existing difference between the temperature of the soil above and outside the transit gas pipeline. Soil temperature due to fluctuation of gas temperature (in shorter time intervals)

T a b l e 1

Average monthly and annual temperature [°C] during 2004, 2005 and 2006

Year	Month												Annual $\overline{x}$
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	
2004	–3.1	1.6	4.7	11.7	14.3	17.9	20.0	20.1	15.2	11.7	5.7	0.8	10.1
2005	–0.1	–2.6	2.7	11.0	15.2	18.0	20.7	19.1	16.3	10.7	4.2	0.4	9.6
2006	–4.1	–1.6	3.5	11.4	14.0	19.2	22.6	17.3	16.6	12.2	7.5	3.2	10.2

T a b l e 2

Monthly and annual amount of precipitations [mm] during 2004, 2005 and 2006

Year	Month												Annual Σ
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	
2004	55.9	31.1	52.8	36.3	36.9	93.8	33.8	19.4	36.7	45.3	45.7	26.8	514.5
2005	36.4	53.0	3.4	78.7	60.9	31.5	59.0	94.5	47.1	12.1	43.2	113.2	633.0
2006	57.4	39.0	35.2	48.1	95.6	63.9	23.7	84.0	12.7	15.3	24.4	7.8	507.1

was not evaluated. Therefore, measurements were made once a month during the crop growing season. The same aim was also observed for soil moisture.

The soil temperature was measured at a depth of 0.25–0.35 m using a digital thermometer, above each line of the pipeline as well as further away from the pipeline. Subsequently, the arithmetic mean of the measured values was calculated.

The soil samples to determine the moisture content were taken at the same time as the measurements of the soil temperature, during the growth period. The soil moisture was calculated by the gravimetric method – percentage by weight (*after* Houšková 1999). Average monthly and annual temperature, and monthly and annual amount of precipitations during 2004−2006 (Šiška & Čimo 2006; Čimo 2007) are listed in the Tables 1 and 2.

Green biomass of seven winter wheat plants was collected from individual plots (35 plants per treatment) above each gas pipeline and further from the pipeline as well (comparable number of plants) in May. The fresh weight of the plants was determined after their collection. The samples were dried at 105°C to a constant weight and their dry weight was determined. The data obtained were used to compare the difference in the weight of plants grown above the pipeline and away from the pipeline. The weight of the sunflower fresh biomass was not evaluated for technical reasons.

The wheat and sunflower yields were evaluated from one square meter in three replicates from each plot, above each line of the gas pipeline and away from the pipeline. After the final grain cleaning, the grain yield was calculated and the weight of 1,000 grains was evaluated. Arithmetic means were calculated from the obtained results.

Statistical hypotheses testing method was used to process statistical differences between the treatments, since this method offers tests of contrasts through unpaired t-tests. The testing was done on significance level $\alpha = 0.05$.

RESULTS AND DISCUSSION

The average temperature of the gas at the outlet of the compressor station in Ivanka pri Nitre ranged from 24.2°C to 40.0°C in 2004, from 24.3°C to 39.0°C in 2005 and from 19.4°C to 35.4°C in 2006 (SPP Inc. 2004–2006) depending on the amount of transported gas. The highest differences in the gas temperature at the inlet and outlet of the compressor station were 25.4°C, 19.4°C and 20.5°C, respectively during 2004–2006.

Soil temperature and soil moisture

Based on the trend of average temperatures and soil moistures during 2004–2006 (Figure 1–3), we can state that the soil temperature measured above the pipelines was always higher than the temperature measured away from the pipelines. The same results were recorded by Demo and Poláková (2012) from the Veľké Kapušany (Michalovce District, SE Slovakia) and Jablonov nad Turňou (Rožňava District, SE Slovakia).

The difference was affected mainly by the temperature of the transported gas that increased the soil temperature. This fact also had an effect on the soil moisture above the pipeline. Statistically highly significant difference was observed in 2004, when the soil temperature above the pipeline was on average 3.4°C ($P = 0.0000003 < \alpha = 0.01$) higher than the soil temperature away from the pipeline and the soil moisture was on average 1.27 percentage of weight [wt %] ($P = 0.0004 < \alpha = 0.01$) lower than the soil moisture away from the pipeline. In 2005, the temperature above the pipeline was 2.07°C higher (average value) and the moisture 0.10% lower (average value) than the value measured further from the pipeline. In both cases, we did not find any statistically significant differences. In 2006, a higher soil temperature by 2.25°C above the pipeline was recorded. It represents a highly significant difference ($P = 0.00028 < \alpha = 0.01$) compared with the soil temperature away from the pipeline. At the same time, the soil moisture was on average 3.18 wt % lower as compared with the soil moisture measured away from the pipeline. The soil temperature above the gas pipeline was higher due to the exposure of the transported gas temperature than the soil temperature in the treatment located 20 m from the pipeline. A high statistically significant difference was observed in 2004 and 2006.

Except the air temperature and precipitations, the soil temperature and moisture were affected

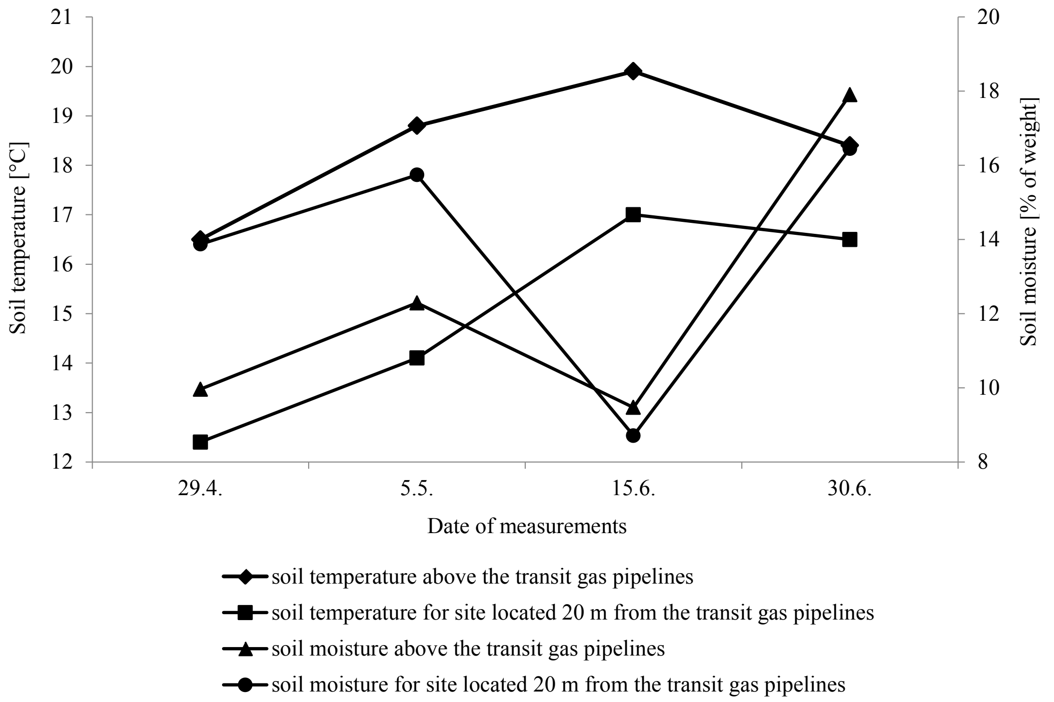

Figure 1. Trend of the average soil temperature and soil moisture, Ivanka pri Nitre during the growing period 2004

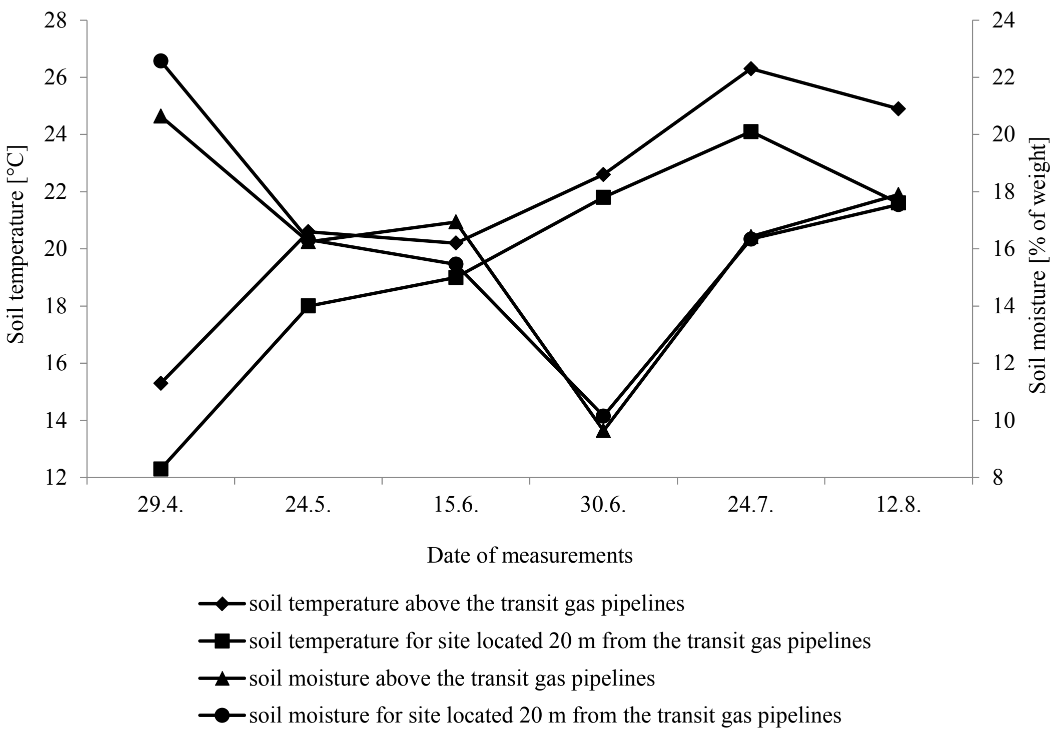

Figure 2. Trend of the average soil temperature and soil moisture, Ivanka pri Nitre during the growing period 2005

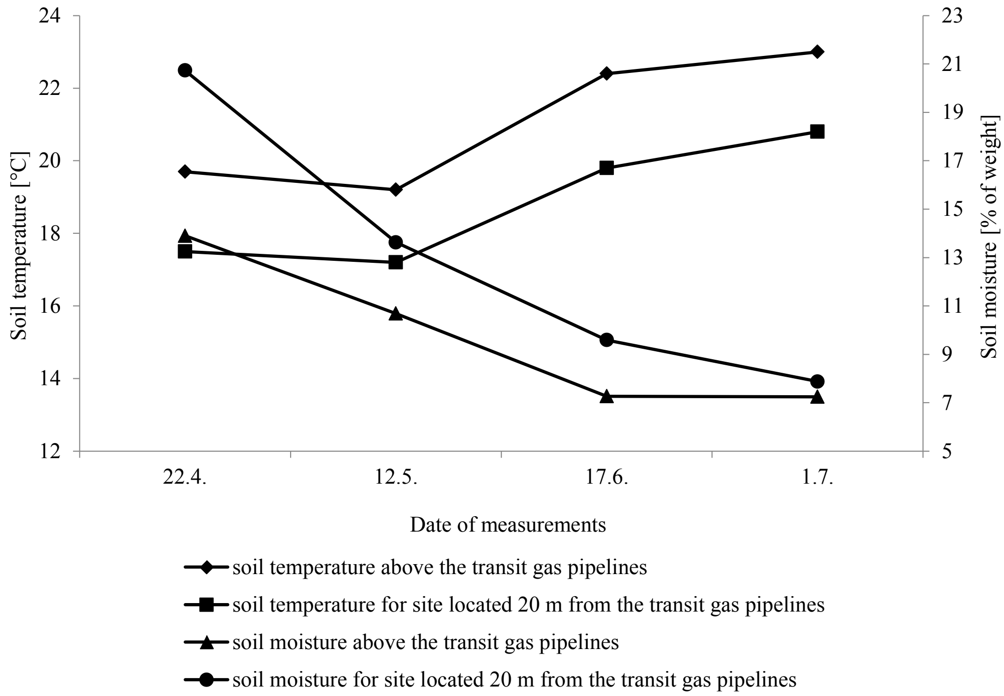

Figure 3. Trend of the average soil temperature and soil moisture, Ivanka pri Nitre during the growing period 2006

also by the temperature of the transported gas – the increased soil temperature above the pipeline led to the reduction of its moisture. A high statistically significant difference was observed in 2004.

Impact on a phytomass and yield

The higher soil temperature caused by the temperature of the transported gas, together with sufficient sources of winter moisture and nutrients, significantly influenced not only the growth and development of the crops grown directly above the pipelines, but also in the adjacent land strips. The difference in the plant heights obtained above the pipeline was visible in the early stages of the growth and lasted until the harvest. Similarly, the differences in the height of sunflower (in the beginning of flowering) ranged from 0.25 to 0.30 m and persisted until the harvest.

The height differences in the winter wheat ranged from 0.25 m to 0.30 m in April 2004 and from 0.1 m to 0.2 m in May 2006, and partially decreased until the harvest of the plants (the differences were up to 0.1 m). Evaluation of the above-ground wheat biomass provided the following results. In absolute terms, the weight of the dry matter was in both studied years higher by 33.12% (2004) and 18.12% (2006) on the soil above the pipeline. Effect of the organic fertiliser was only in the first studied year when the biomass weight was 48.93% (2004) higher compared with the control treatment 3.

Winter wheat grew faster on the soil above the pipeline. Individual growth phases, such as flowering and maturation began 10–14 days earlier as compared with the wheat growing further from the pipeline. Organic fertilisation, in the treatment 1, had no significant effect on the ears/head density of the winter wheat and sunflower stands. The number of wheat spikes per m^2 was the highest on the soil undamaged by the pipeline construction (Figure 4). However, this fact did not affect the crop yields (the weight of 1,000 grains was the lowest, Halmová, (2009)). The number of sunflower flower heads per m^2 was in all three

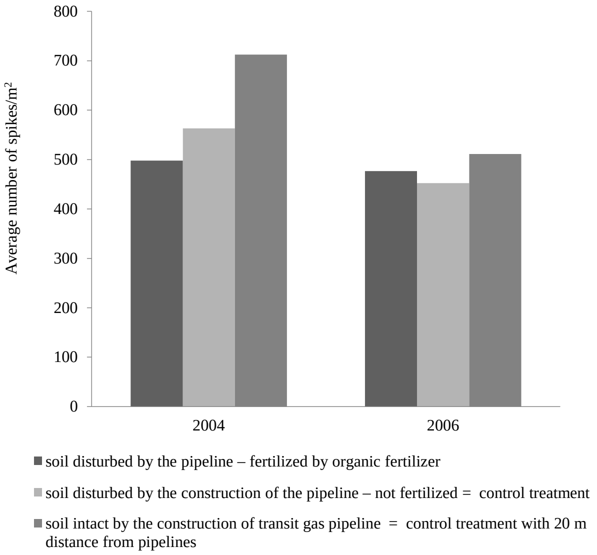

Figure 4. Average number of winter wheat spikes per m^2, Ivanka pri Nitre, 2004 and 2006

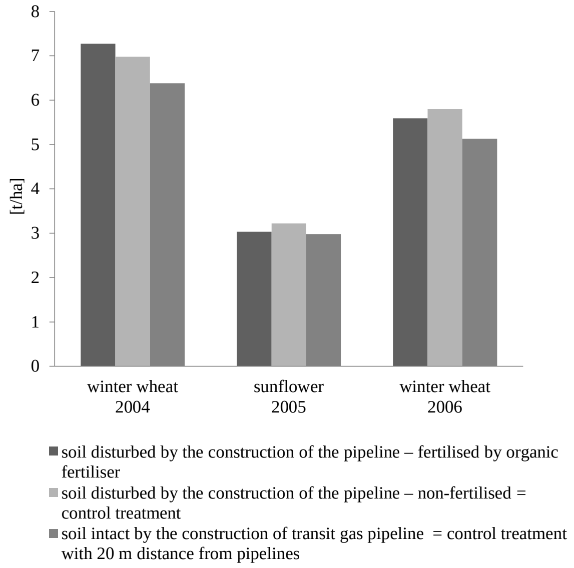

Figure 5. Crop yields trend, Ivanka pri Nitre 2004–2006

treatments comparable and ranged from 5 to 5.27 (in 2005).

The grain yields of wheat (Figure 5) were higher by 9.4% (2004) and 13.6 % (2006) on the soil disturbed and affected by the pipeline compared with the yields obtained on the soil unaffected by the pipeline. Blaško (2005) found lower wheat yields on the Cambisol in extremely dry years 2002 and 2003. The same results were recorded by Demo *et al.* (2012) from the Cabaj-Čápor (Nitra District, SW Slovakia) site that is also located behind the gas compressor station Ivanka pri Nitre.

Similarly, the sunflower yields were 8.05% higher on the soil affected by the pipeline compared with the control treatment. This corresponds to the findings of Blaško (2005).

The impact of the fertilisation was observed only in the winter wheat yields in the first experimental year.

Rainfall and its distribution during the growing period was an important factor affecting the crop yields. Taking into consideration that the constant impact of the transported gas temperature affects the temperature and moisture regime of the concerned soil, it can be assumed that the crop yields were reduced during the years with low precipitation. Another important factor influencing the crop yields except the soil type might be the persistence of soil damage caused by the pipeline construction (Halmová & Fehér 2014). Chemical properties of the soil were evaluated during the three-year research, as well. Only minimal differences in the values of the basic pedo-chemical parameters of the damaged and undamaged soils were recorded (Halmová 2009).

An increase in the yields of the crops (wheat and sunflower) grown on the soil above the pipeline was recorded in each of the studied years. It is assumed that the yield increase occurred mainly due to the combination of the three factors: the soil type, uniform distribution of rainfall (cf. Šiška & Čimo 2006; Čimo 2007) during the growing season and increased soil temperature caused by the transported gas.

CONCLUSIONS

The gas transit pipelines affect some soil characteristics as well as crop yields. These changes do not necessarily repeat regularly but depend on weather conditions, amount of transported gas and crop grown in the given year. These soils are usually drier and warmer than soils that are not affected by temperature of the transported gas.

Amount and distribution of precipitations during the growing period is a very important factor. Crop yields on the soils above the pipelines can be expected to be higher; however, they are endangered by faster crop maturation and the resulting losses. The gas transit pipeline is a linear structure with an indisputable importance for the economy, impacts caused by its construction and operation can still be seen today.

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