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Attributes of wheat cultivars for late autumn sowing in genes expression and field estimates

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ABSTRACT

Due to the growing interest in Central and Eastern Europe on cropping of wheat in optional late autumn terms, called facultative, genetic research and field evaluation were taken on four spring cultivars: Tybalt (NL), Monsun (DE), Ostka Smolicka (PL) and Bombona (PL), currently being recommended by breeders. The PPD gene analyze, expression level of dehydrine genes (*WCS120* and *WDHN13*) in cooling test, and qPCR for RNA isolation and analyses of *WCS120* and *WDHN13* gene expression at the BBCH12 stage of wheat were estimated. Molecular analysis of *PPD-D1* gene confirmed the presence of photoperiod sensitive allele *ppd-D1b* in all tested genotypes. The highest level of NRE *WCS120* gene was detected in cultivars Tybalt and Bombona. Two-year field experimental study assessed the growth, development and productivity of facultative and spring crops of studied cultivars. Based on our results from field experiments and result of molecular analysis of alleles of *PPD-D1* gene, the tested genotypes can be considered as potentially facultative genotypes.

1. Introduction

Wheat (*Triticum aestivum* L.) is a worldwide agricultural crop with good adaptation to diverse environmental conditions. It can be grown in environments with varying altitudes, in areas of northern and southern hemispheres (Snape and Pankova, 2007). This adaptation is greatly influenced by flowering time, which is mainly determined by three groups of genes: vernalization response genes (*VRN*), photoperiod response genes (*PPD*) and developmental rate genes (*EPS*). These genes can modify the earliness of the genotypes by certain internal tuning of flowering processes (Kamran et al., 2014).

The *VRN* genes are responsible for vernalization request of the winter genotypes. Their function is blocking of plants ontogenesis. Unblocking of plant development occurs gradually during cold vernalization. The dominant alleles of the *VRN* genes results in the loss of this expression for spring varieties. The *VRN* genes are mainly located on homologous chromosomes 5 of wheat (VRN-A1, Vrn-B1, Vrn-D1, Vrn 2) and chromosome 7B (Vrn-B3) (Danyluk et al., 2003; Fu et al., 2005). Photoperiod response in wheat is described as sensitive when timely flowering occurs only in long days, and insensitive when flowering occurs in either long- or short-day environments. It is mainly controlled by the genes *Ppd-D1*, *Ppd-B1* and *Ppd-A1* located on the short arms of chromosomes 2D, 2B and 2A, respectively and are ranked Ppd-D1 > Ppd-B1 > Ppd-A1 in terms of their potency (Worland et al., 1998). Consequently, the dominant alleles *Ppd-D1a*, *Ppd-B1a*, and *Ppd-A1a* confer photoperiod insensitivity, whereas the recessive alleles *ppd-D1b*, *ppd-B1b*, and *ppd-A1b* confer photoperiod sensitivity (Dyck et al., 2004). Therefore, the *Ppd-D1a* photoperiod insensitivity allele is generally considered the most potent and only alleles of *Ppd-D1* gene are routinely screened to test photoperiod sensitivity of wheat genotypes (Chen et al., 2009, 2010; Milec et al., 2012; Oslovičová et al., 2014).

In spring wheat genotypes, at least one pair of spring dominant alleles of *VRN* genes (*Vrn-A1*, *Vrn-B1*, or *Vrn-D1*) and dominant allele *Ppd-D1* are present. The potential facultative wheat genotypes should differ from the spring genotype by the presence of the recessive allele *ppd-D1b* to avoid plant flowering stage during winter. The regulation of the flowering process in each individual wheat genotype is the result of complex cooperation of metabolic pathways controlled by the genes of all involved groups and are dependent upon the specific environmental conditions (Cockram et al., 2007). Winter hardiness or frost tolerance of cereals could be tested by different field or laboratory methods (Prášil et al., 2007). In

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wheat, differences in frost tolerance level have also been evaluated on dehydrine gene expression level (Ganeshan et al., 2008; Holková et al., 2009). Products of these genes, dehydrins, belong to the group COR/LEA II. These proteins are primarily synthesized in embryo tissue during seed ripening and also play an important protective role in cells of vegetative parts of plants during drought stress and cold acclimation processes (Kosová et al., 2007**).** In strictly defined temperature and light conditions, wheat genotypes with higher levels of frost tolerance showed the higher level of dehydrin genes expression than varieties with lower level of frost tolerance when already at an early stage of cold acclimation (Ganeshan et al., 2008; Holková et al., 2009). Field cropping of facultative cereal means that plants can be sown in late autumn or in spring and they only need short period of vernalization (less than two weeks at the temperature 4–6 °C). The facultative form of wheat is less resistant to frost and winter hardiness, requires a shorter period of vernalization, is faster starting vegetation in the spring, and ripens earlier than their winter forms (Stelmakh, 1998). Facultativeness is beneficial in wheat and triticale in temperate regions, where milder winters or late autumn rainfall are dominate. They are grown mainly in Central Asia, the Middle East, and Australia (Oztruk et al., 2006), as well as in Slovakia and the former Yugoslavia (Okic, 1995; Hnilicka et al., 2005). In Poland, winter wheat prevails due to higher potential yield, but spring cultivars provide better flours for baking purposes (Coboru, 2013). Since there is no clear category of "facultative cultivars" in the List of Agriculture Cultivars, the breeders may only recommend some of spring cultivars for "facultative cropping". Meanwhile, spring wheat planted in March/April is often endangered by drought if too little precipitation occurs in this period. Moisture stress negatively affects the transit of photosynthetic products to the shaping kernels. The synthesis of starch is disturbed resulting in lower grain weight and therefore decreased yield (Yildirim et al., 2013). Late autumn planting of spring wheat may foster the faster growth and development of seedlings in early spring when soil water from winter storage is more likely to be available. Shafazadeh et al. (2004) demonstrated the water stress tolerance index (STI), the index of vulnerability to water stress (called SSI), and the general tolerance (Tol) and their values of wheat grain yield should be the criterion for the suitability of facultative cultivars. Understanding the environmental regulation of yield components in cereals would benefit from a dual focus on yield-related traits per se and theirs plasticity (Sadras and Slafer 2012). The inverse relationship between heritability and plasticity described by Donovan et al. (2011) was extended by analysis of the hierarchy of the four traits, i.e. tiller number, ears number, kernels per ear and kernels size was described by Sadras and Slafer (2012), who demonstrated ranking of plasticities: tiller number per m^2 > ears number per $m^2 \approx$ kernels per ear > kernels size. This context of yield and yield's components of wheat may be also included in term of sowing and genetic variations.

The objective of this study was to determine the integrated cooperation of genetic alleles as related to the effects of sowing term (facultative vs. spring) as expressed by the phenology, yield, and yield components of various cultivars of wheat (*Triticum aestivum* L.).

The working hypothesis assumed that cultivars recommended by growers for facultative cropping have genetic attributes which signify their development and higher productivity in the field trials.

2. Methods

2.1. PPD gene analyze

DNA was isolated from 50 mg of young seedling leaves by DNeasy Plant Mini Kit (Qiagen, Hilden). The PCR with *Ppd-D1a* and *ppd-D1b* specific primers was done according Yang et al. (2009) and the PCR products were analyzed on 1.2% agarose gel stained with Midori Green DNA Stain.

2.2. Cooling test

Germinated kernels were sown into 3 pots (6 caryopses in pot) and cultivated under a light panel for 3 weeks at 12 h photoperiod and 21 °C/19 °C day/night temperatures. Subsequently, the plants at the stage of 3–4 leaves were transferred into growth chamber and maintained for 7 days at 3 °C (12 h photoperiod, 3 °C/3 °C day/night temperatures). The expression level of two dehydrine genes (*WCS120* and *WDHN13*) was evaluated in these experimental conditions of cold activation and deactivation (5 h, 22 °C).

2.3. qPCR

Samples for RNA isolation and subsequent analyses of *WCS120* and *WDHN13* gene expression were taken from the second fully developed leaf. Total RNA was isolated from 100 mg leaf discs using RNase Plant Mini Kit (Qiagen, Hilden, Germany). RNA purification was performed using Turbo DNase kit (Ambion, USA). The first cDNA chain was synthesized from 1 μg of purified total RNA using QuantiTect® Reverse Transcription kit (Qiagen, Hilden, Germany) and qPCR were performed with QuantiTect® SYBR® Green PCR Kit (Qiagen, Hilden, Germany). *WCS120* primer sequences and PCR conditions were used according Ganeshan et al. (2009) and *WDHN13* primer sequences and PCR conditions were used according modified protocols published in Holková et al. (2009).

Gene expression was evaluated as normalized relative gene expression (NRE) calculated according to Pfaffl (2001). The values of gene expression were normalized versus expression of ubiquitin gene and relative to the value of internal calibrator, i.e. value of tested gene expression of frost tolerant variety Mironovská 808 expression level of tested genes in these variety was taken as the standard 1 (100%). The results were compared within 17 randomly selected winter and spring varieties. Three independent $2 \times \pm S_E$ sample measurements were averaged.

2.4. Field experiment

2.4.1. Site description

The strict, field experiment was carried out from 2012 to 2014 as two-year. The plots were located at the Experimental Station of the University of Technology and Life Sciences in Bydgoszcz, Mochełek. Bydgoszcz is the capitol of Cuyavia-Pomerania region, geographically located 160 km from Baltic Sea (53° 12′N, 18° 01′E). Topography is a very low-lying and the climate of the region is temperate and influenced both by the Atlantic and the Asian continent. The average January temperature is −3 degrees, and in July 18° (Fig. 6).

2.4.2. Plant material and experimental design

Four wheat cultivars of different origins were selected from Polish List of Cultivars on the basis of their reported traits: Bombona, Monsun, Ostka Smolicka, and Tybalt, and all four are currently being recommended by breeders both for spring and facultative crops (Table 1). Included in the region is one of the areas with the lowest precipitation in Poland, less than 500 mm annually. The total year sum (mm) was 526 mm in 2012/2013, 486 mm 2013/2014, and 461 mm from multi year period (Fig. 7). The pre-crop for wheat was pea, planted on seed, and harvested in mid July. The soil was *Alfisols* formed in sandy loams (Soil Survey Staff, 2010). The soil cultivation before sowing consisted of plough (post harvest of pea) and tillage made with cultivator and roller (pre sowing of wheat). Phosphorus and potassium fertilization at 75 kg ha $^{-1}$ P_2O_5, and 80 kg ha^{-1} K_2O