

# The effects of different *Ppd* alleles on wheat adaptability traits under conditions of eastern Croatia

Dario NOVOSELOVIĆ<sup>1</sup>, Alison BENTLEY<sup>2</sup>, Ruđer ŠIMEK<sup>1</sup>, Nicolas GOSMAN<sup>3</sup>

<sup>1</sup>Agricultural Institute Osijek, Južno predgrađe 17, 31000 Osijek, Croatia,  
(e-mail: dario.novoselovic@poljinos.hr)

<sup>2</sup>The John Bingham Laboratory, NIAB, Huntingdon Road, Cambridge CB3 0LE, United Kingdom

<sup>3</sup>Bayer Crop Science NV, Ghent, Belgium

## Abstract

Elucidating photoperiod response is important for better understanding of adaptation to specific eco-geographic conditions and manipulation for wheat breeding purposes. Using a set of wheat near-isogenic lines differing in series of photoperiod sensitive alleles on the 2 group chromosomes, under conditions of eastern Croatia, we found that the allelic effects varied from 9.2 days for date of heading, 8.7 days for date of flowering and 11.7 days for date of physiological maturity for the “strongest” allele *Ppd-D1a* vs. *Ppd-D1b* allele. *Ppd-A1* locus showed strongest effects on grain yield for both allelic variants indicating it as useful alternative source of photoperiod insensitive alleles.

Key words: wheat, adaptation, photoperiodism, *Ppd* allele, grain yield

## Introduction

Adaptation genes play a major role in plant response to environmental signals (Bentley et al., 2013b), and flowering time is one of the key adaptive traits. In temperate regions, time of flowering normally coincides with favourable climatic conditions (Trevaskis, 2010) where developmental switch from vegetative to reproductive growth is critical to enable wheat plants to flower at optimum time for pollination, seed development and dispersal as well as to adjust a wheat life cycle for maximum yields (Cockram et al., 2007). To realize optimal adaptation to local eco-geographic region, farmers and breeders have selected a complex of genes controlling vernalization requirement or winter growth habit (*VRN* genes), day-length or photoperiod sensitivity (*PPD* genes), as well as the additional smaller effects earliness per se (*Eps*) loci, which all together account for much of the genetic variation for flowering time in wheat (Distelfeld and Dubcovsky, 2009) that is controlled by at least 20 genes dispersed over the wheat genome (Sanna et al., 2014).

Wheat is a quantitative long-day plant and genetic control of photoperiod response is primarily determined by the *Ppd-A1*, *Ppd-B1* and *Ppd-D1* genes located on the homoeologous chromosomes 2A, 2B and 2D (Seki et al., 2011). Photoperiod-insensitive (PI) wheat flowers rapidly in both short and long days. This gives the advantage to early flowering wheat genotypes for resource capturing and avoidance of reduced grain filling frequently associated with late season heat or drought stress. The *Ppd-D1* is one of the most potent genes, and variation found among *Ppd-D1* haplotypes suggests that a series of alleles rather than a pair of alleles leads to continuous variation affecting not only adaptive traits but also other agronomic traits (Guo et al., 2010). According to Kiss et al. (2014) *Ppd-D1a* PI allele is carried by 57% of the cultivars, *Ppd-B1a* PI allele was carried by 22% of the genotypes. Matching the appropriate photoperiod response to eco-geographic region is of importance as illustrated by an estimated 35% yield advantage in wheat in southern European environments associated with the photo-period-insensitive *Ppd-D1a* mutation derived from Japanese cultivar Akakomugi (Worland, 1996). The objective of this paper was to assess the effects of different *Ppd*-alleles for some adaptive traits and discrepancy between early and late flowering genotypes under conditions of eastern Croatia, where climatic conditions are similar to those of southern European environments.

### Materials and methods

A field trial was set up at location Osijek (eastern Croatia) as completely randomized block design with two replications and plot size of 7.56 m<sup>2</sup>. Planting was carried out on 26<sup>th</sup> October 2012, while harvesting was on 15<sup>th</sup> July 2013. The planting density for all genotypes was the 400 kernels/m<sup>2</sup>. Agronomic practice was as typical as for local wheat technology of production.

Material included 16 wheat genotypes, out of which are six pairs of near-isogenic lines (NILs) and four cultivars (Srpanjka, Lucija, Soissons and Robigus). Six pairs of NI lines (early vs. late) include combinations from different allelic sources for earliness (Soissons, SHW173 and Timstein) into Robigus background (for details see: Bentley et al., 2013a).

“Allelic” groups were formed from genotypes possessing *Ppd-1a* alleles (“early” flowering group) and *Ppd-1b* alleles (“late” flowering group), where “early” group included nine genotypes and “late” included seven genotypes (Table 1).

Traits that were recorded and measured were:

1. Date of heading (GS 55 after Zadoks et al.) – in days from 1<sup>st</sup> April.
2. Beginning of flowering (GS 61 after Zadoks et al.) – in days from 1<sup>st</sup> April.
3. Date of physiological maturity (GS 87 after Zadoks et al.) – determined when 50 % of peduncles turned to yellow– in days from 1<sup>st</sup> April.
4. Grain filling duration: expressed in days as difference between date of physiological maturity and beginning of flowering.
5. Grain yield expressed in kg/ha and 14 % grain moisture content, after recalculation from plot yields (kg/7.56 m<sup>2</sup>).

Statistical data analysis was carried out using PROC GLM and PROC MEANS procedures in SAS/STAT(R) 9.2 software (SAS Institute, 2009).

Table 1. List of wheat NIL genotypes and respective cultivars.

Line	Flowering time group	Allele	Allele donor	Recurrent parent
R-34/RS-231-4-4	Early	<i>Ppd-D1a</i>	Soissons	Robigus
R-34/RS-231-4-3	Late	<i>Ppd-D1b</i>	Soissons	Robigus
R-35/RS-111-2-3	Early	<i>Ppd-D1a</i>	Soissons	Robigus
R-35/RS-111-2-7	Late	<i>Ppd-D1b</i>	Soissons	Robigus
ROB-173-2-A-17-7	Early	<i>Ppd-A1a</i>	SHW173	Robigus
ROB-173-2-A-17-6	Late	<i>Ppd-A1b</i>	SHW173	Robigus
ROB-173-2-A-17-1	Early	<i>Ppd-A1a</i>	SHW173	Robigus
ROB-173-16-A-10-7	Late	<i>Ppd-A1b</i>	SHW173	Robigus
Rob-Tim-Al-2-5-2	Early	<i>Ppd-B1a</i>	Timstein	Robigus
Rob-Tim-Al-2-5-1	Late	<i>Ppd-B1b</i>	Timstein	Robigus
Rob-Tim-Al-1-14-1	Early	<i>Ppd-B1a</i>	Timstein	Robigus
Rob-Tim-Al-1-13-1	Late	<i>Ppd-B1b</i>	Timstein	Robigus
Robigus	Late	<i>Ppd-B1b</i>		
Soissons	Early	<i>Ppd-D1a</i>		
Srpanjka	Early	<i>Ppd-D1a</i>		
Lucija	Early	<i>Ppd-D1a</i>		

## Results and discussion

The results of analysis of variance showed that statistically significant differences were found among different *Ppd*-alleles for all traits. The only non-significant differences were found between “early” vs. “late” groups for grain filling period and grain yield (Table 2).

Table 2. Analysis of variance among different *Ppd*-alleles and “allelic” groups (“early” and „late“ flowering group) for adaptive traits.

Sources of variation	Degrees of freedom	Mean squares (MS)				
		Date of heading	Date of flowering	Date of physiological maturity	Grain filling duration	Grain yield
Replication	1	0.0	0.0	0.13	0.13	430623.6
<i>Ppd</i> alleles	5	89.96***	83.1***	611.03***	14.06*	3065859.3*
Error	15	8.81	5.60	12.59	4.26	845807.7
“Allelic”group	1	270.0***	300.83***	316.9**	0.208 <sup>NS</sup>	3914432.4 <sup>NS</sup>
Error	15	13.79	8.78	21.0	6.08	1122760.6

\*, \*\*, \*\*\*- statistically significant at the level of probability  $p < 0.05$ ;  $0.01$  and  $0.001$ , respectively.

<sup>NS</sup> - statistically not significant at the level of probability  $p < 0.05$ .

The range of mean values for adaptive traits was 9.2 days for date of heading, 8.7 days for date of flowering and 11.7 days for date of physiological maturity (*Ppd-D1b* vs. *Ppd-D1a* allele). For grain filling duration the range was only 4.2 days (*Ppd-D1b* vs. *Ppd-B1b* allele). It suggests that the strongest effect, in terms of earliness, for dates of heading, flowering and physiological maturity had *Ppd-D1a* insensitive allele, and the weakest effect had *Ppd-D1b* sensitive allele which is in accordance with results of Seki et al. (2011) and Worland (1996). For specific homoeologous loci, the highest difference in allelic effects for adaptive traits was found for *Ppd-D1*, while smallest difference was found for *Ppd-B1* alleles. There were no significant differences between effects of *Ppd-A1a* and *Ppd-B1a* alleles on date of heading, but for date of flowering and physiological maturity statistically significant differences were found confirming the *Ppd-B1a* as „weak“ allele for earliness (Bentley et al., 2013a). For grain filling duration the differences among different *Ppd*-alleles were less pronounced. The average difference between “early” and “late” allelic group was similar for date of heading, date of flowering and date of physiological maturity (6, 6.3 and 6.5 days, respectively), whereas for grain filling duration the difference was not significant (Table 3).

In general, “early” flowering group had higher yield than “late” group suggesting the advantage of “early” over “late” alleles under conditions of eastern Croatia. More specifically, among homoeologous loci *Ppd-A1* locus had the highest grain yield (for both alleles) suggesting that some other loci are involved in the control of increased yield potential (Fig. 1). This could be a worthwhile strategy for wheat breeders in a region to introduce such alleles and combine it with omni-present *Ppd-D1a* alleles in southern European wheat germplasm to preserve or increase genetic yield potential.

Table 3. Mean values of the different Ppd-alleles and “allelic” groups (“early” and „late“) for adaptive traits.

	Date of heading <sup>1</sup>	Date of flowering <sup>1</sup>	Date of physiological maturity <sup>1</sup>	Grain filling duration <sup>2</sup>
Ppd-allele				
<i>Ppd-A1a</i>	42 <sup>A</sup>	44.2 <sup>B</sup>	89.5 <sup>CD</sup>	45.3 <sup>AB</sup>
<i>Ppd-A1b</i>	45 <sup>A</sup>	49 <sup>A</sup>	92.5 <sup>BC</sup>	43.5 <sup>B</sup>
<i>Ppd-B1a</i>	43.5 <sup>A</sup>	48 <sup>A</sup>	95 <sup>AB</sup>	47 <sup>A</sup>
<i>Ppd-B1b</i>	45.8 <sup>A</sup>	50 <sup>A</sup>	93.3 <sup>ABC</sup>	43.3 <sup>B</sup>
<i>Ppd-D1a</i>	36.8 <sup>B</sup>	41.8 <sup>B</sup>	86.3 <sup>D</sup>	44.5 <sup>AB</sup>
<i>Ppd-D1b</i>	46 <sup>A</sup>	50.5 <sup>A</sup>	98 <sup>A</sup>	47.5 <sup>A</sup>
“Allelic”group				
“Early“	39.8 <sup>A</sup>	43.8 <sup>A</sup>	89.0 <sup>A</sup>	45.3 <sup>A</sup>
“Late“	45.8 <sup>B</sup>	50.1 <sup>B</sup>	95.5 <sup>B</sup>	45.4 <sup>A</sup>
Mean value	42	46.1	91.4	45.3

<sup>1</sup>in days from 1st April.

<sup>2</sup>difference in days between date of physiological maturity and beginning of flowering.

<sup>A, B, C, D</sup> Means with the same letter are not significantly different according to Duncan’s Multiple Range Test at the level of probability  $p < 0.05$ .

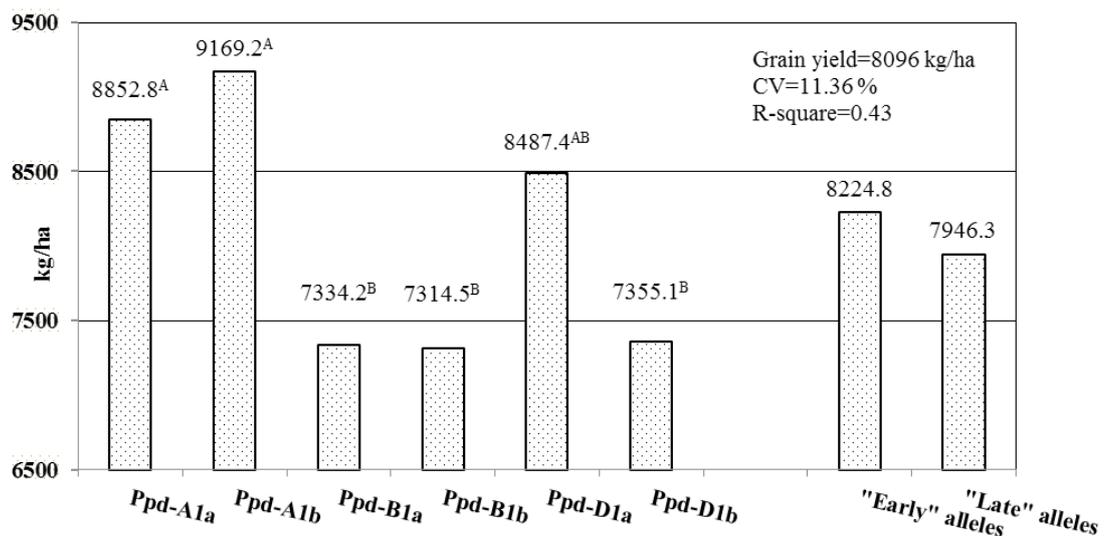


Fig. 1. Mean grain yield (kg/ha) of different Ppd-alleles and “allelic” groups.

<sup>A, B</sup> -Means with the same letter are not significantly different according to Duncan’s Multiple Range Test at the level of probability  $p < 0.05$ .

### Conclusions

The understanding of different allelic effects for photoperiodism and their ability to manipulate flowering time, along side with other flowering-time alleles, could offer the possibility to shorten or lengthen various phases in regenerative and grain filling period to refine response to specific agro-climatic conditions.

At the bottom this gives a tool to wheat breeders to adapt their elite breeding material in order to match the predicted climatic changes and to secure stable food production in the future and/or even to increase genetic yield potential.

## References

- Bentley, A.R., Horsnell, R., Werner, C.P., Turner, A.S., Rose, G.A., Bedard, C., Howell, P., Wilhelm, E.P., Mackay, I.J., Howells, R.M., Greenland, A., Laurie, D.A., Gosman, N. (2013a). Short, natural, and extended photoperiod response in BC2F4 lines of bread wheat with different Photoperiod-1 (*Ppd-1*) alleles. *Journal of Experimental Botany*, 64 (7):1783-1793.
- Bentley, A.R., Jensen, E.F., Mackay, I.J., Hönicka, H., Fladung, M., Hori, K., Yano, M., Mullet, J.E., Armestead, I.P., Hayes, C., Thorogood, D., Lovatt, A., Morris, R., Pullen, N., Mutasa-Göttgens, E., Cockram, J. (2013b). Flowering Time. In *Genomics and Breeding for Climate-Resilient Crops*, Vol. 2, C. Kole (ed). DOI 10.1007/978-3-642-37048-9\_1, Springer-Verlag Berlin Heidelberg, 1-66.
- Cockram, J., Jones, H., Leigh, F.J., O'Sullivan, D., Powel, W., Laurie, D.A., Greenland, A.J. (2007). Control of flowering time in temperate cereals: genes, domestication, and sustainable productivity. *Journal of Experimental Botany*, 58(6):1231-1244.
- Distelfeld, A., Li C., Dubcovsky, J. (2009). Regulation of flowering in temperate cereals. *Current Opinion in Plant Biology* 12:1-7.
- Guo Z., Song, Y., Zhou, R., Ren, Z., Jia, J. (2010). Discovery, evaluation and distribution of haplotypes of the wheat *Ppd-D1* gene. *New Phytologist* 186:841-851.
- Kiss, T., Balla, K., Veisz, O., Láng, L., Bedö, Z., Griffiths, S., Isaac, P., Karsai, I. (2014). Allele frequencies in the *VRN-A1*, *VRN-B1* and *VRN-D1* vernalization response and *PPD-D1* photoperiod sensitivity genes, and their effects on heading in a diverse set of wheat cultivars (*Triticum aestivum* L.). *Mol Breed* 34: 297-310.
- Sanna, G., Giunta, F., Motzo, R., Mastrangelo, A.M., De Vita, P. (2014). Genetic variation for the duration of pre-flowering development in durum wheat and its interaction with vernalization treatment and photoperiod. *Journal of Experimental Botany* doi:10.1093/jxb/eru170
- SAS Institute. (2009). *SAS/STAT(R) 9.2 User's Guide*, Second Edition. <http://support.sas.com/documentation/onlinedoc/stat/>
- Seki M., Chono, M., Matsunaka H., Fujita, M., Oda, S., Kubo, K., Kiribuchi-Otobe, C., Kojima, H., Nishida, H., Kato, K. (2011). Distribution of photoperiod-insensitive alleles *Ppd-B1a* and *Ppd-D1a* and their effect on heading time in Japanese wheat cultivars. *Breeding Science* 61:405-411.
- Trevaskis, B. (2010). The central role of the *VERNALIZATION 1* gene in the vernalization response of cereals. *Functional Plant Biology*, 37: 479-487.
- Worland, A.J. (1996). The influence of flowering time genes on environmental adaptability in European wheat. *Euphytica* 89:49-57.
- Zadoks, J.C., Chang, T.T., Konzak, C.F. (1974). A decimal code for the growth stages of cereals. *Weed Res* 14:415-421.

sa2015\_p0304