Allelic variation at Vrn-1 and Ppd-1 loci and their effects on agronomic traits on Italian durum wheat germplasm

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Abstract

Allelic variation within genes involved in vernalization requirement *(Vrn-1)* and photoperiod sensitivity *(Ppd-1)* play a crucial role in adaptability to different wheat growing environments. Here, 193 durum wheat genotypes, covering a large historical period, were evaluated for pheno-agronomic traits (i.e., i.e., plant height, flowering time and heading date) in three different sowing dates during the 2021-22. Genetic materials were genotyped for their allelic composition at *Vrn-A1*, *Vrn-B1*, *Vrn-B3*, and *Ppd-A1* and *Ppd-B1* loci using molecular markers. Phenotypic results showed a great delay of heading date (HD) and flowering time (FT) in the landraces compared with old and modern cultivars. Furthermore, the effect of the sowing date was statistically significant since delaying in sowing decreased the number of days needed for HD and FT. At a molecular level, three different alleles were found within the *Vrn-A1*, of which *Vrn-A1b* and *Vrn-A1c* abounded in the germplasm. Conversely, a reduction of the recessive *vrnA1* was observed, since only 11%, 13% and 14% of the landraces, old and modern cultivars harbor this allele. *Vrn-B1* and *Vrn-B3* were instead monomorphic, with all individuals harboring the recessive alleles. Regarding the *Ppd* genes, 100% and 94% of landraces and old cultivars harbor the sensitive allele (*Ppd-A1b*), whereas a reduction of ~ 35% was observed within modern cultivars (65% with *Ppd-A1b* allele). The remaining modern cultivars harbor the photoperiod-insensitive allele *Ppd-A1a(GS105)*, with very few exceptions, for which the allele *Ppd-A1a(GS100)* was found. *Ppd-B1a* and *Ppd-B1b* alleles were instead detected in all accessions with similar frequencies (48% and 50%, respectively). Haplotypes analysis is ongoing to investigate the effect on phenological phases and other agronomic traits in order to select desirable genotypes for future breeding programs.

Keywords: Durum wheat, Vernalization and Photoperiod genes, Phenological stages, Agronomic traits.

Introduction

- Wheat (*Triticum aestivum* L.) is one of the most widely cultivated crops in the world.
- Durum wheat (*Triticum turgidum* L. ssp. durum) represents about 6% of the global wheat production (Royo et al., 2020).
- Italy and Spain are the first and second durum wheat producers in the Mediterranean Basin (Royo, 2005).

✓ One of the best strategies to maximize the wheat yield potential to face global climate changes is the **optimization of its phenology** (Dowla et al., 2020).

The major genetic factors influencing phenological characteristics in wheat are:

➢ **Vernalization response genes (*Vrn*):** controlling the requirement of a cold period to switch from the vegetative to the reproductive phase (Yan et al., 2004; Dubcovsky et al., 2006; Chen et al., 2013).

➢ **Photoperiod sensitivity genes (*Ppd*):** determining plant response to day length (Maccaferri et al., 2008; Wilhelm et al., 2009; Royo et al., 2016).

Objectives

1. Analyzing the allelic variations for the *Vrn* and *Ppd* genes in a panel of landraces, old and modern cultivars of durum wheat;
2. Evaluating the effect of different allelic combinations on HD and FT.

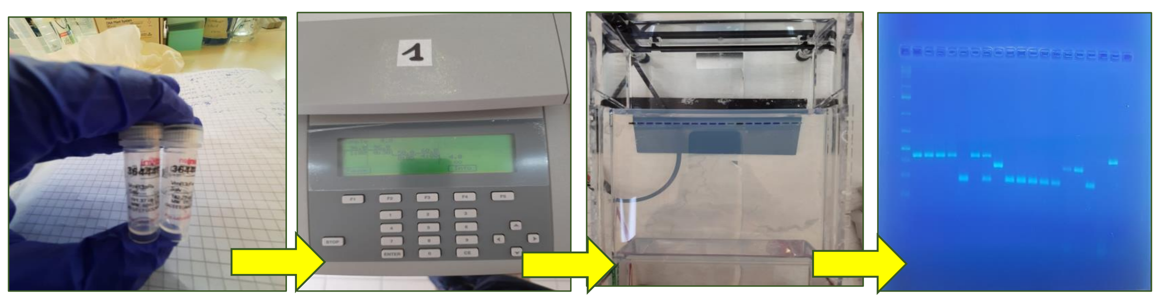
Materials & Methods

➢ A panel of 193 durum wheat genotypes available at germplasm bank of CREA-CI in Foggia:

101 modern cultivars
47 old cultivars
45 landraces

✓ DNA was extracted from leaf tissues of 14-day old seedlings by the Total DNA purification kit (Invitrogen, USA).

✓ A Qubit fluorometer (Invitrogen, USA) was used to quantify the extracted DNA.



✓ The genotypes were characterized with a set of molecular markers associated with the *Vrn-1*, *Vrn-3* and *Ppd-1* (Bentley et al., 2011; Royo et al., 2016).

✓ PCR was performed for each gene.

✓ The PCR products were separated in a 2% agarose gel in TBE buffer using GeneRuler 1 kb Plus DNA Ladder (Thermo Fisher Scientific Baltics UAB, Lithuania).

✓ Field experiments were conducted under rainfed conditions at the Research Centre for Cereal and industrial Crops (CREA-CI), Foggia, Italy during 2021-22.

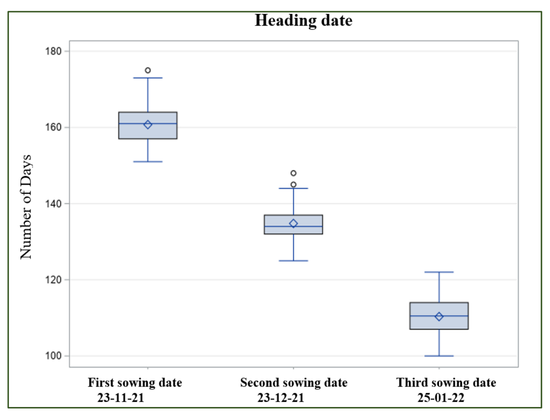
Genotypes were planted in three different sowing dates

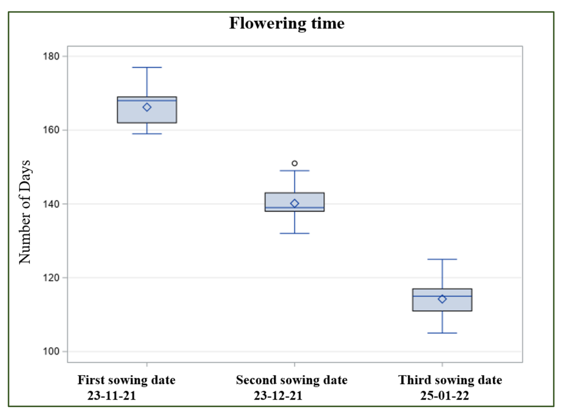
1st: 23 November 2021
2nd: 23 December 2021
3rd: 25 January 2022

✓ Flowering time and heading date were recorded according to Zadoks growth stages (Zadoks et al., 1974).

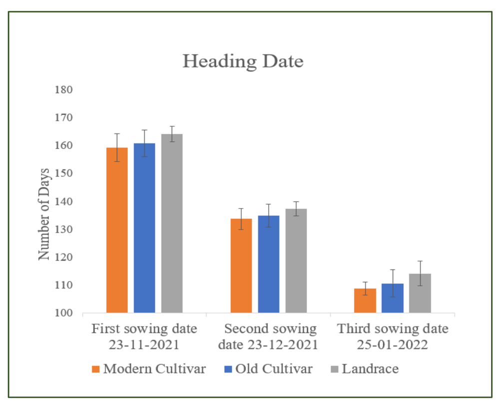
Results

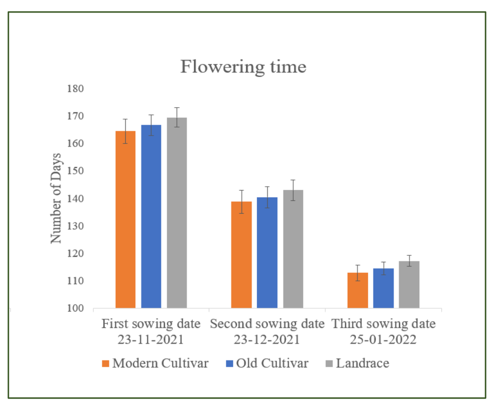
The results indicated that by delaying in sowing date, lengths of flowering time and heading date were significantly decreased.





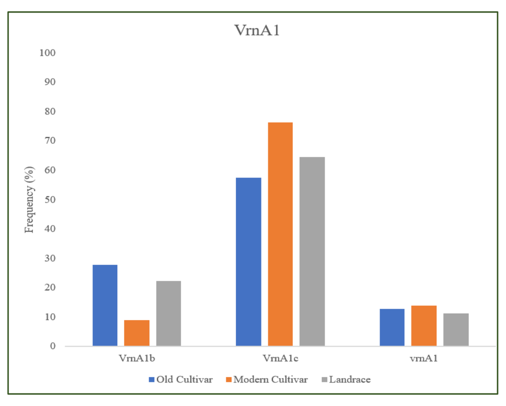
All genotypes revealed a significant reduction in flowering time and heading date by delaying in sowing date, while in all three sowing dates, landraces had the highest flowering time and heading date compared to old and modern cultivars.

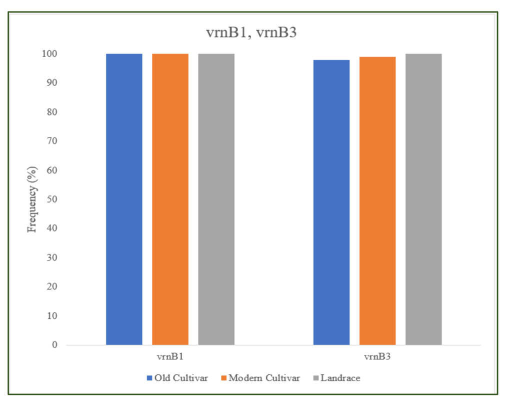




The dominant *VrnA1c* allele showed the highest frequency in the durum wheat genotypes (70%), followed by the dominant *VrnA1b* allele (16.84%) and the recessive *vrnA1* allele (13.15%).

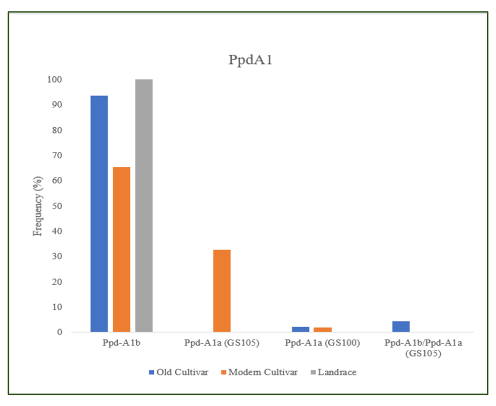
The *Vrn-B1* and *Vrn-B3* were monomorphic and all the genotypes harbored the recessive alleles (*vrnB1* and *vrnB3*).

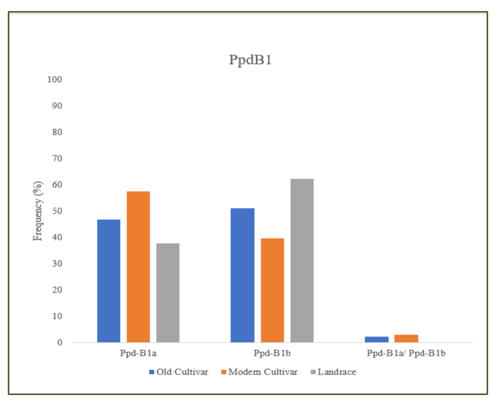




As for *Ppd-A1*, 100% of landraces and 94% of old cultivars harbor the sensitive allele (*Ppd-A1b*), whereas 65% of modern cultivars harbor *Ppd-A1b* allele.

Ppd-B1a and *Ppd-B1b* alleles were instead detected in all genotypes with similar frequencies (48% and 50%, respectively).





Types of haplotypes and their frequency in cultivars and landraces

Haplotype	Size	Number of Genotypes: OC	MC	LR	Type of haplotype: Vrn-A1	Vrn-B3	Vrn-B1	Ppd-A1	Ppd-B1	Days to heading date	Days to flowering time
1	4	0	4	0	vrnA1	vrnB3	vrnB1	Ppd-A1a (GS105)	Ppd-B1b	132.083	137.58
2	2	0	2	0	vrnA1	vrnB3	vrnB1	Ppd-A1a (GS105)	Ppd-B1a	138.000	142.00
3	8	3	2	3	vrnA1	vrnB3	vrnB1	Ppd-A1b	Ppd-B1b	138.333	142.87
4	11	3	6	2	vrnA1	vrnB3	vrnB1	Ppd-A1b	Ppd-B1a	139.485	143.94
5	16	0	16	0	VrnA1c	vrnB3	vrnB1	Ppd-A1a (GS105)	Ppd-B1b	131.271	136.40
6	8	0	8	0	VrnA1c	vrnB3	vrnB1	Ppd-A1a (GS105)	Ppd-B1a	129.875	135.00
7	1	0	1	0	VrnA1c	vrnB3	vrnB1	Ppd-A1a (GS100)	Ppd-B1b	128.667	134.33
8	1	0	1	0	VrnA1c	vrnB3	vrnB1	Ppd-A1a (GS100)	Ppd-B1a	130.667	135.67
9	44	13	13	18	VrnA1c	vrnB3	vrnB1	Ppd-A1b	Ppd-B1b	135.629	140.61
10	55	10	34	11	VrnA1c	vrnB3	vrnB1	Ppd-A1b	Ppd-B1a	134.327	139.40
11	1	0	1	0	VrnA1b	vrnB3	vrnB1	Ppd-A1a (GS105)	Ppd-B1b	156.500	146.00
12	15	6	2	7	VrnA1b	vrnB3	vrnB1	Ppd-A1b	Ppd-B1b	137.333	142.11
13	16	7	6	3	VrnA1b	vrnB3	vrnB1	Ppd-A1b	Ppd-B1a	138.917	143.48

Conclusions

Further analyses are underway to determine the impact of each haplotype not only on phenology traits but also on the yield and its components. In addition, field trials are planned for choosing the best sowing date and selecting superior genotypes well adapted to the climatic conditions of the Mediterranean basin.