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Earliness, yield, and yield components of bread wheat under well-watered and rain-fed conditions: the role of the *Ppd-D1a* gene

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Abstract

Objective: Earliness is a critical trait for wheat grown under conditions of end-season heat and drought stress. Heading time is influenced by three groups of genes, including photoperiod (*Ppd)*, vernalization (*Vrn*), and earliness *per se* (*Eps)*. *Ppd-D1* is an important tool for marker-assisted selection and backcrossing programs. Although the effect of *Ppd-D1a* on earliness is well-documented, its impact on yield, yield components, and other key agronomic traits remains a subject of debate. In this study, near-isogenic lines for *Ppd-D1a* were developed in two genetic backgrounds: Roshan and Kalheydari cultivars. The primary aim was to examine the effect of *Ppd-D1a* on earliness, yield, and yield components.

Methods: Isogenic lines from the Kalheydari and Roshan cultivars were evaluated under both well-watered and rain-fed conditions in two distinct locations of Kerman and Sepidan, Iran, during two successive growing seasons (2020-2022). At each location, the experimental design was a randomized complete block design with four replications. Then, several agronomic characteristics such as days to heading, days to maturity, grain filling period, plant height, peduncle length, grain yield, spike number per square meter, grain number per spike, 1000-grain weight, and spike length, were measured.

Results: When compared to the *Ppd-D1b* allele, which is photoperiod-sensitive, the *Ppd-D1a* allele, which is photoperiod-insensitive, reduced days to heading and maturity by 5.14 and 7.53 days, respectively. The results also indicated that *Ppd-D1a* led to a 14% decrease in grain number per spike, while it increased 1000-grain weight by 17% and grain yield by 13% under rain-fed conditions. However, the effects of *Ppd-D1a* differed significantly under well-watered conditions, where it decreased 1000-grain weight by 18% but increased grain number per spike by 10%, with no significant effect on the grain yield.

Conclusion: These findings suggest that the impact of *Ppd-D1a* on yield and yield components is strongly influenced by the specific environmental conditions in which the wheat is cultivated.

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Introduction

Wheat (*Triticum aestivum* L.) exhibits considerable genetic diversity for several characteristics, allowing its cultivars to adapt to a wide range of climatic conditions (Derakhshani *et al*. 2013). This broad genetic diversity, particularly in terms of heading and ripening time, is a key factor in this adaptability (Trethowan *et al.* 2006; Kulkarni *et al.* 2017; Dragovich *et al*. 2021). Water-deficit stress frequently limits wheat performance, posing a significant challenge for both farmers and plant breeders (Kramer 1980; Safari *et al*. 2018). On the other hand, the adaptation of different genotypes to environmental conditions is linked to the synchronization of heading time with the growing environment. Consequently, optimal heading time will lead to increased yield compared to early or delayed time (Kamran *et al.* 2014).

As a classic adaptation, drought escape provides plants with the opportunity to complete their life cycle before drought stress occurs (Levitt *et al.* 1980; Shavrukov *et al.* 2017; Du *et al.* 2018). Early maturity is essential, especially in regions experiencing late-season drought. By maturing earlier, the cultivars can escape terminal drought stress, which contributes to higher grain yield. In addition, early heading cultivars demonstrated higher grain yield not only under drought stress but also in well-watered conditions (Regan *et al.* 1997; Iqbal *et al.* 2007; Nitcher *et al.* 2014; Zheng *et al.* 2015; C. Zheng *et al.* 2016). Nevertheless, there are reports indicating a negative impact of earliness on grain yield under well-watered conditions (Turner 1986; Radhika and Thind 2014).

Two main pathways, namely vernalization (*Vrn*) and photoperiod (*Ppd*), play a dominant role in determining the complex trait of flowering time (Herndl *et al.* 2008; Kamran *et al.* 2014). The genes regulating these traits are also influenced by environmental factors, including cold, heat, and day length (Law and worland 1977; Gororo *et al.* 2001; Kamran *et al.* 2014). With regard to the *Ppd* pathway, the dominant alleles, including *Ppd-A1a, Ppd-B1a*, and *Ppd-D1a*, govern insensitivity to photoperiod (Law *et al.* 1978). The *Ppd-D1*a allele has, generally, the largest effect, followed by the *Ppd-B1a* and *Ppd-A1a* alleles (Scarth and Law 1983). Additionally, *Ppd-D1a*, which was originally

from an old Japanese cultivar named Akakomugi (Strampelli 1932; Worland 1999) is regarded as the primary source of photoperiod insensitivity in bread wheat (Worland 1999; Beales *et al.* 2007). *Ppd-D1* is a valuable candidate for use in marker-assisted selection or backcrossing (Langer *et al.* 2014). The deletion of 2089 base pairs in the *Ppd-D1* locus resulted in the creation of the *Ppd-D1a* allele, which exhibits insensitivity to photoperiod (Beales *et al.* 2007; Guo *et al.* 2010). This gene is the most important factor influencing the flowering time of European winter bread wheat cultivars, accounting for more than half of the genetic diversity for this trait. In contrast, *Ppd-B1* only explains 3.2% of the genetic variation of flowering, contributing to a smaller proportion of the overall genetic diversity (Langer *et al.* 2014).

According to Fait and Balashova (2022), among 232 diverse winter wheat cultivars, the most common *Ppd* allele was *Ppd-D1a*, found in 81% of the samples. Its prevalence varied among cultivars, reaching 10% in those from the United States and 92% in Ukrainian cultivars. Notably, no genetic diversity was observed for the *Ppd-A1* allele. *Ppd-D1a* allele showed a very low frequency of 2.6% in wheat landraces; however, its prevalence dramatically increased after the "Green Revolution" (Dragovich *et al.* 2021). In other research, Shcherban *et al.* (2015) reported that variation in the photoperiod gene (*Ppd*-1) offers opportunities to adjust heading time and maximize grain yield. Several studies have indicated that the *Ppd-D1a* allele reduces the number of days to heading (Beales *et al.* 2007; Bentley *et al.* 2013; Wilhelm *et al.* 2013; Langer *et al.* 2014; Shcherban *et al.* 2015; Chen *et al.* 2018; Fait and Balashova 2022), grain number per spike (Kroupin *et al.* 2020), and plant height (Wilhelm *et al.* 2013; Chen *et al.* 2018; Kroupin *et al.* 2020). Similarly, it has been reported that early heading reduces plant height, tillering, and spike number in wheat (Wolde *et al.* 2019). The insensitive allele of the photoperiod gene (*Ppd-D1a*) has been shown to affect grain yield within a range of +3 to -5% (Liu *et al.* 2020), whereas according to Chen *et al.* (2018), this allele increased grain yield by 8.2%. *Ppd-D1a* also increased the 1000-grain weight by 10-16.9% as indicated by Chen *et al.* (2018) and Liu *et al.* (2020); however, Kroupin *et al.* (2020) reported an adverse effect of *Ppd-D1a*. Additionally, *Ppd-D1a* was found to reduce grain number per spike by 6.5- 10% (Chen *et al.* 2018; Kroupin *et al.* 2020; Liu *et al.* 2020) and slightly reduces plant height (Kroupin *et al.* 2020).

Inconsistent results have been reported regarding the *Ppd-D1a* allele's effects on earliness, yield, and yield components. Therefore, this study developed isogenic lines for *Ppd-D1a* within two distinct genetic backgrounds to precisely determine the allele's impact on bread wheat's earliness, yield, and yield components under both rain-fed and well-watered conditions.

Materials and Methods

Plant material (development of isogenic lines)

In the previous work, among 40 evaluated bread wheat genotypes, Kalheydari and Roshan were identified as the most drought-tolerant cultivars (Abdolshahi *et al.* 2013). Despite possessing several desirable traits, these varieties exhibit moderately late heading. To overcome this limitation, early heading was introgressed from Excalibur, an early-heading Australian cultivar, into both Kalheydari and Roshan varieties using backcrossing. This breeding approach resulted in the development of the second generation of the third backcross (BC_3F_2) (Dorrani-Nejad *et al.* 2022). In the present study, the earliest heading BC_3F_2 plant from each population was crossed with its respective recurrent parent (Roshan and Kalheydari) in 2018. Using DNA marker selection, heterozygote genotypes (*Ppd-D1a/Ppd-D1b*) were identified and backcrossed with the recurrent parents to develop the BC_5F_1 generation in 2019. BC_5F_2 generation was then advanced to the BC_5F_5 generations in the glasshouse during the 2019-2020 period using speed breeding techniques (Watson *et al.* 2018) to achieve multiple generations per year. In each generation, immediately after harvest, seeds were subjected to a 120-hour incubation period at 29 °C, followed by a 72-hour treatment with 0.5 ppm gibberellic acid at 4 °C. Additionally, in each generation, heterozygote genotypes (*Ppd-D1a/Ppd-D1b*) were selected using molecular marker analysis. Following five backcrosses and four selfing generations, lines harboring different *Ppd-D1* alleles were developed. Homozygote genotypes (*Ppd-D1a/Ppd-D1a* and *Ppd-D1b/Ppd-D1b*) were then selected in the BC_5F_5 generation to establish isogenic lines for *Ppd-D1*.

DNA extraction and polymerase chain reaction (PCR)

Freshly collected leaves were frozen in liquid nitrogen and subsequently stored at –80 ºC. Genomic DNA extraction was performed using the cetyltrimethylammonium bromide (CTAB) method as described by Zhang *et al.* (1998). Polymerase chain reaction (PCR) primers from Beales *et al.* (2007) were utilized to identify sensitive and insensitive alleles at the *Ppd-D1* locus (Table 1). The PCR amplification was carried out using a DNA gradient thermal cycler (Biometra Tone, Analytik Jena, Germany). The reaction mixture, with a total volume of 20 µl, consisted of 1 µl of genomic template DNA, resulting in a final concentration of 50 ng, and 1 µl of each primer, yielding a final concentration of 0.5 pmol. The mixture also included 10 µl of 2X Taq PreMix (Master Mix, Cat: C101081), which contained Pars Tous Taq DNA polymerase, reaction buffer, a mixture of dNTPs, protein stabilizer, and additional components to facilitate electrophoresis, including a 2X loading dye provided by Dana Zist Company, Iran. To complete the mixture, 7 µl of PCR-grade H_2O was added.

The thermal cycling process began with an initial denaturation at 94 °C for 3 minutes, followed by 35 cycles comprising denaturation at 94 °C for 30 seconds, annealing at 57 °C (*Ppd-D1*_F/R1) and 59 °C (*Ppd-D1*_F/R2) for 30 seconds, and extension at 72 °C for 45 seconds. A final extension was performed at 72 °C for 5 min. Subsequently, the PCR products were separated by electrophoresis on a 1% agarose gel using 0.5x TBE buffer, stained with DNA Green Viewer, and visualized under ultraviolet light using a Vilber Gel Doc (Quantum 4).

Table 1. PCR markers, primer sequence, target allele(s), product size, and annealing for amplification of *Ppd-A1*, *Ppd-B1*, and *Ppd-D1* genes.

PCR marker	Primer name	Primer sequence (5′→3′)	Target allele(s)	Product size (bp)	Annealing temp. (°C)	Reference
Ppd-A1 marker	TaPpd-A1F1	CGTACTCCCTCCGTTTCTTT	-	-	-	Nishida *et al.* 2012
	TaPpd-A1R3	AATTTACGGGGACCAAATACC	*Ppd-A1a*	388	65	
	TaPpd-A1R2	GTTGGGGTCGTTTGGTGGTG	*Ppd-A1b*	299	64	
Ppd-B1 marker	Ppd-B1_F	ACACTAGGGCTGGTCGAAGA	-	-	-	
	Ppd-B1_R1	CCGAGCCAGTGCAAATTAAC	*Ppd-B1a*	1600	55	
	Ppd-B1_R1	CCGAGCCAGTGCAAATTAAC	*Ppd-B1b*	1292	55	
Ppd-D1 marker	Ppd-D1_F	ACGCCTCCCACTACACTG	-	-	-	Beales *et al.* 2007
	Ppd-D1_R1	GTTGGTTCAAACAGAGAGC	*Ppd-D1b*	414	57	
	Ppd-D1_R2	CACTGGTGGTAGCTGAGATT	*Ppd-D1a*	288	59	

Field experiments

Isogenic lines from the Kalheydari and Roshan cultivars were evaluated in an experiment conducted during the 2020-2021 cropping season. The experiment was designed as a randomized complete block design with four replications. This study took place under varying rainfall conditions at two distinct locations in Iran: Kerman (29.23° N, 56.59° E; long-term average rainfall: 102 mm/year) and Sepidan (30.25° N, 51.89° E; average annual rainfall: 800 mm) (Figure 1). An additional experiment was performed at a second site in Kerman (30.28° N, 57.03° E; average annual rainfall: 140 mm) during the 2021-2022 cropping season, under both rain-fed and well-watered conditions. The experimental plots were 20 square meters in size, with a seeding density of 300 seeds per square meter. The measured traits included: days to heading, days to maturity, grain filling period (defined as the number of days from heading to physiological maturity), plant height, peduncle length, grain yield, spike number per square meter, grain number per spike, 1000-grain weight, and spike length. Grain yield was measured by harvesting the entire plots after removing border rows. However, the experiment at

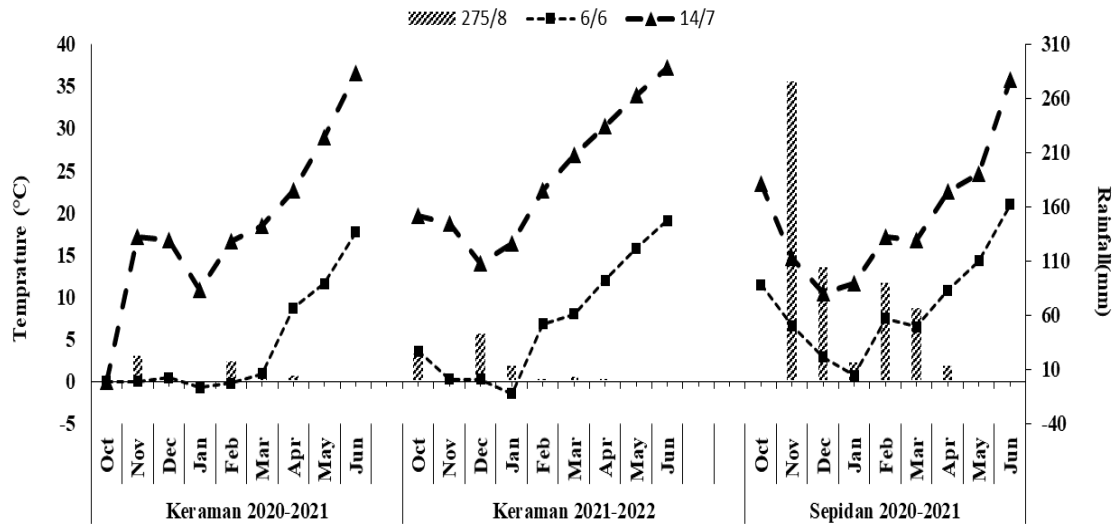

Figure 1. Monthly rainfall and temperature (maximum and minimum) from planting (October) to harvest (June) at Kerman (2020-21 and 2021-22) and Sepidan (2020-21).

Kerman under rain-fed conditions was not harvested due to rodent attack. For the remaining plots, 10 plants were randomly selected to measure plant height, peduncle length, and spike length. Grain number per spike and 1000-grain weight were measured from 200 randomly selected spikes per plot. Considering the association between grain yield and its components (Rawson 1970), the spike number per m^2 was calculated as follows:

$$Spike\ number\ per\ m^2 = \frac{Grain\ yield(g/m^2)}{Grain\ number\ per\ spike \times single\ grain\ weight\ (g)}$$

Statistical analyses

After analysis of variance, means were compared using Duncan's multiple range test at a 5% significance level, implemented in SAS V9.1 (SAS Institute Inc. 2004). In this analysis, replication was treated as a random effect and isogenic lines as a fixed effect.

Results

The recurrent parents, Roshan and Kalheydari, together with the donor parent, Excalibur, showed no genetic variation at the *Ppd-A1* and *Ppd-B1* loci, as all carried photoperiod-sensitive alleles, specifically *Ppd-A1b* and *Ppd-B1b*. The expected sizes of the PCR products were 299 and 1292 bp for *Ppd-A1b* and *Ppd-B1b*, respectively (Figure 2). Furthermore, PCR amplification of the expected product sizes, 288 bp for *Ppd-D1a* and 414 bp for *Ppd-D1b*, was successfully achieved across all the genotypes evaluated (Figure 3).

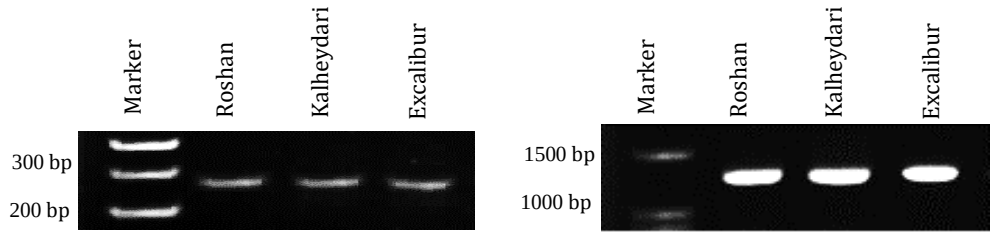

Figure 2. The expected sizes of PCR products for recurrent parents (Roshan and Kalheydari) and donor parent (Excalibur): (a) *Ppd-B1b* (299 bp) and (b) *Ppd-A1b* (1292 bp).

Ppd-D1a enhanced earliness

The *Ppd-D1a* allele, known for its photoperiod insensitivity, significantly enhanced early heading under rain-fed and well-watered conditions. In the Roshan genetic background, *Ppd-D1a* reduced the days to heading by 4.35 days under rain-fed conditions and by 4.0 days under well-watered conditions. In the Kalheydari background, the reductions were even greater, with *Ppd-D1a* decreasing the days to heading by 5.55 days in rain-fed conditions and by 7 days in well-watered conditions (Figure 4). On average, the *Ppd-D1a* allele shortened the time to heading by 4.3 days in Roshan and by 6.0 days in Kalheydari. These effects also extended to the days to maturity, where *Ppd-D1a* resulted in a reduction of 7.0 days in Roshan and 8.0 days in Kalheydari (Table 2). While *Ppd-D1a* consistently increased earliness in both cultivars, the impact was more pronounced in Kalheydari (Figure 4 and Table 2).

Ppd-D1a improved grain yield under rain-fed conditions

In the rain-fed conditions, the photoperiod-insensitive allele *Ppd-D1a* significantly enhanced grain yield with an increase of 96 kg/ha in the Roshan genetic background and 99 kg/ha in the Kalheydari genetic background. However, no significant differences were observed in grain yield between the isogenic lines derived from both genetic backgrounds under well-watered conditions (Figure 5). These findings highlight the importance of the *Ppd-D1a* gene for breeding programs focused on rain-fed regions. Under high rainfall conditions, despite insignificant yield differences among isogenic lines, the reduction of the vegetative phase (earliness) is nevertheless considered a critical trait, as it enhances resource use efficiency.

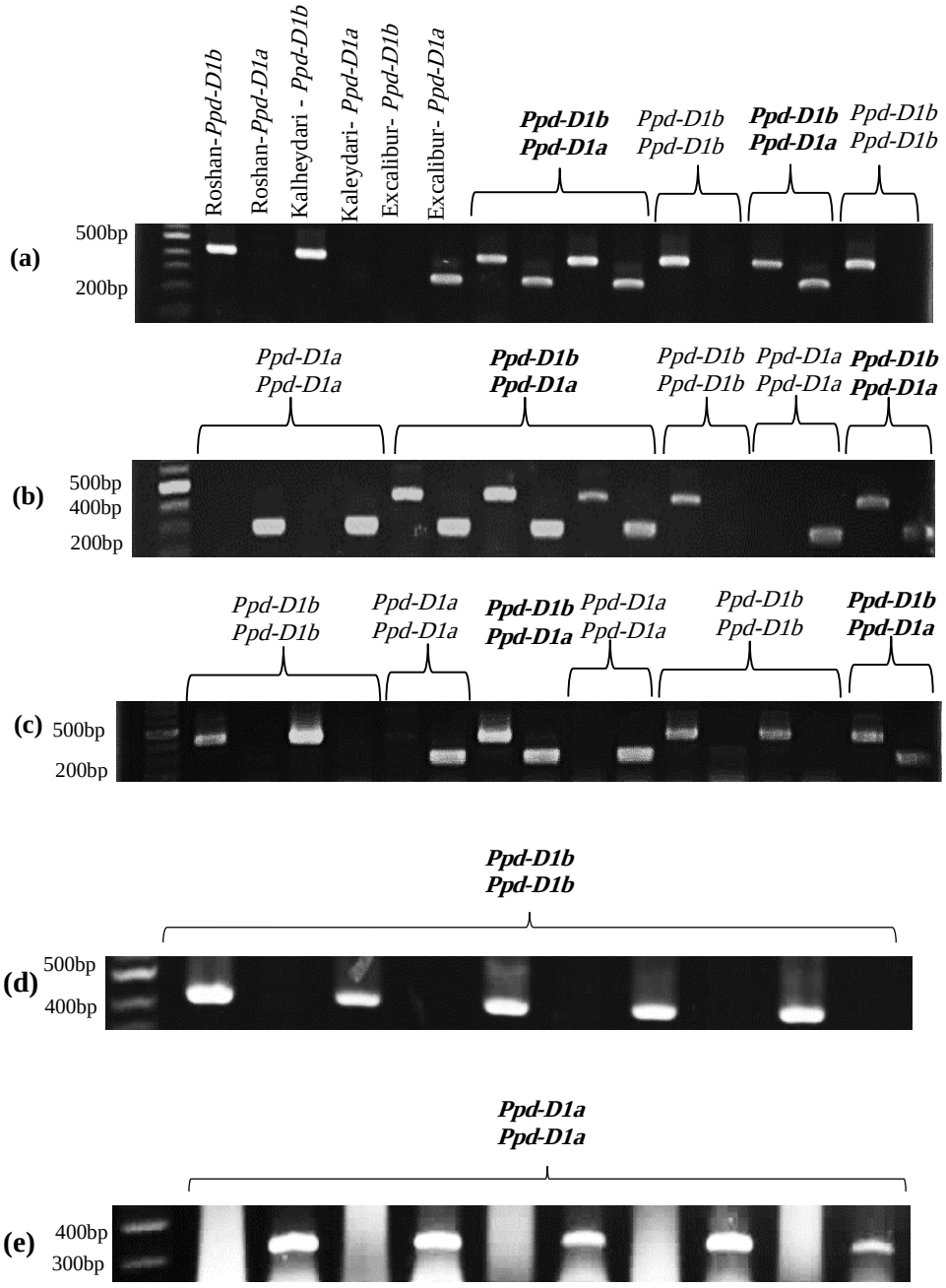

Figure 3. PCR amplification using genome specific primers for *Ppd-B1* and *Ppd-D1*. Amplification of PCR products of the sizes 414 and 288 bp, primed by the combination of the forward primer (*Ppd-D1_F*) with first reverse (*Ppd-D1_R1*) and second reverse (*Ppd-D1_R2*) primers to show the Ppd-D1b and Ppd-D1a alleles, respectively (a) Roshan BC_3F_2, (b) Roshan BC_5F_2, c) Kalheydari BC_5F_2. Homozygote genotypes including *Ppd-D1b/Ppd-D1b* and *Ppd-D1a/Ppd-D1a* for isogenic lines (d and e) Roshan BC_5F_5.

Effect of Ppd-D1a on yield components

The photoperiod-insensitive allele *Ppd-D1a* significantly decreased grain number per spike in both genetic backgrounds under rain-fed conditions in Sepidan. However, it did not have a significant

effect on this trait under rain-fed conditions in Kerman. Specifically, the *Ppd-D1a* allele demonstrated contrasting effects under different water regimes. Under Sepidan (rain-fed) conditions, it reduced the grain number per spike by 3.39 in the Roshan background and by 4.96 in the Kalheydari background. Conversely, in well-watered conditions, this allele exhibited an opposite influence; while it decreased grain number per spike under rain-fed Sepidan conditions, it notably increased this trait when well-

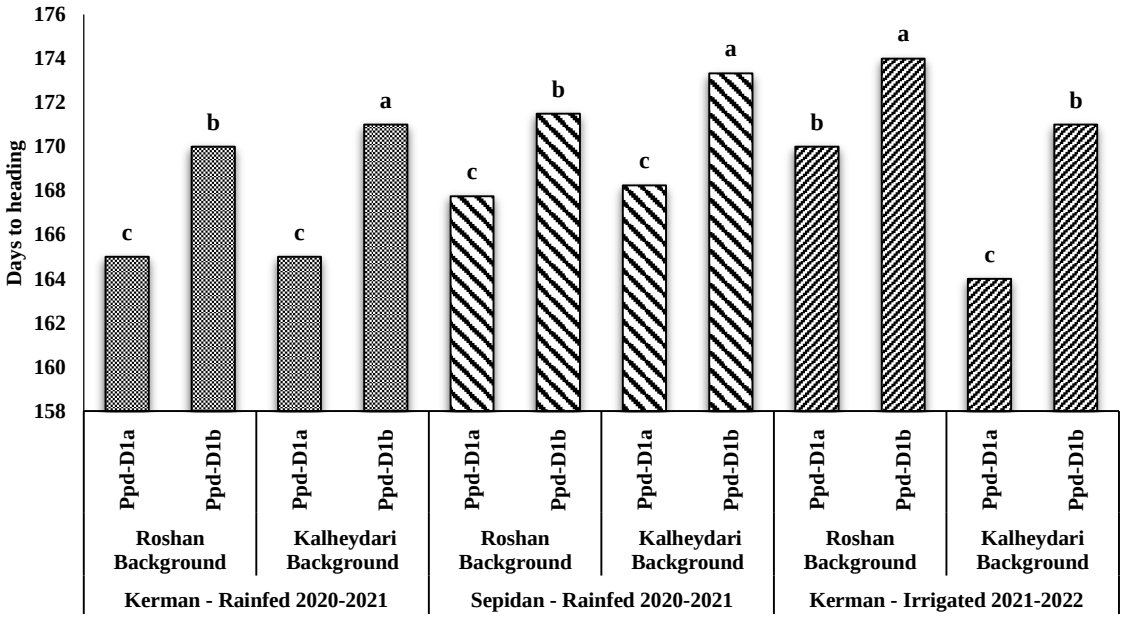

Figure 4. Days to heading of isogenic lines in two cultivars (Roshan and Kalheydari) under well-watered and rain-fed conditions of Kerman (2020-21 and 2021-22) and Sepidan (2020-21).

Table 2. Mean comparison of isogenic lines for morphological traits under well-watered and rain-fed conditions at Kerman, Iran.

Genetic background	*Ppd* allele	Plant height (cm)	Peduncle length (cm)	Spike length (cm)	Days to ripening
Kerman Rain-fed 2020-2021					
Roshan	*Ppd-D1a*	60.650^{a}	27.875^{a}	9.325^{a}	200.017^{c}
	Ppd-D1b	65.550^{a}	27.800^{a}	9.925^{a}	206.101^{a}
Kalheydari	*Ppd-D1a*	50.975^{b}	21.450^{b}	6.825^{b}	191.010^{d}
	Ppd-D1b	50.433^{b}	22.000^{b}	6.233^{b}	204.052^{b}
Kerman Well - watered 2021-2022					
Roshan	*Ppd-D1a*	119.000^{ab}	46.525^{b}	10.975^{a}	194.500^{c}
	Ppd-D1b	127.200^{a}	51.025^{a}	11.550^{a}	202.500^{a}
Kalheydari	*Ppd-D1a*	113.600^{b}	41.475^{c}	11.550^{a}	193.750^{c}
	Ppd-D1b	118.075^{ab}	44.250^{bc}	12.725^{a}	196.750^{b}

watered. Furthermore, *Ppd-D1a* significantly boosted the number of grains per spike by 4.58 in the Roshan background, yet had no significant impact on this trait in the Kalheydari background (Figure 6).

The 1000-grain weight also experienced varied responses. Under rain-fed conditions, the *Ppd-D1a* allele improved 1000-grain weight by 4.20 grams in Roshan and by 6.05 grams in Kalheydari. However, under well-watered conditions, it resulted in a considerable reduction, decreasing 1000-grain weight by 9.58 grams in Roshan and 13.50 grams in Kalheydari (Figure 7). Additionally, *Ppd-D1a* significantly enhanced spike number per square meter in Kalheydari under well-watered conditions, contrasting with its lack of effect on this trait in either genetic background under rain-fed conditions (Figure 8).

Effect of Ppd-D1a on plant height, peduncle, and spike length

The *Ppd-D1* allele did not affect plant height or spike length in either genetic background, regardless of rainfall conditions. Isogenic lines with and without the *Ppd-D1a* allele exhibited the same peduncle length under rain-fed conditions. However, under well-watered conditions, the *Ppd-D1a* allele reduced the peduncle length in Roshan. In contrast, the *Ppd-D1a* did not affect the peduncle length of Kalheydari, indicating an interaction between genetic backgrounds and *Ppd-D1* alleles (Table 2).

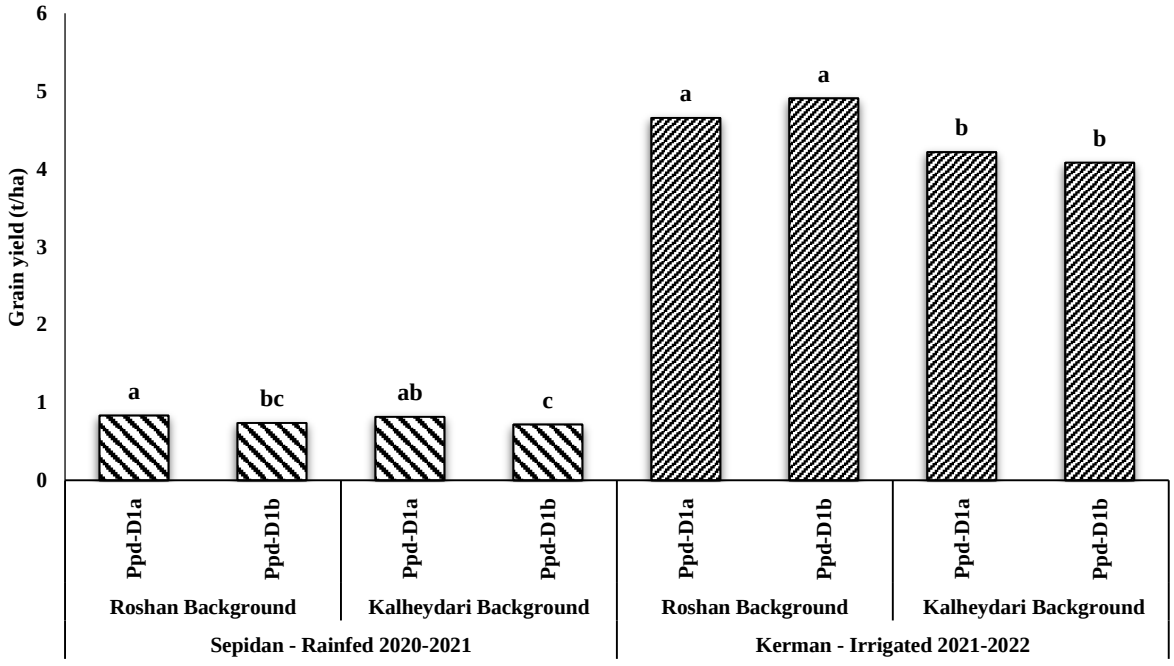

Figure 5. Grain yield of isogenic lines in two cultivars (Roshan and Kalheydari) under well-watered and rain-fed conditions of Kerman (2020-21 and 2021-22) and Sepidan (2020-21).

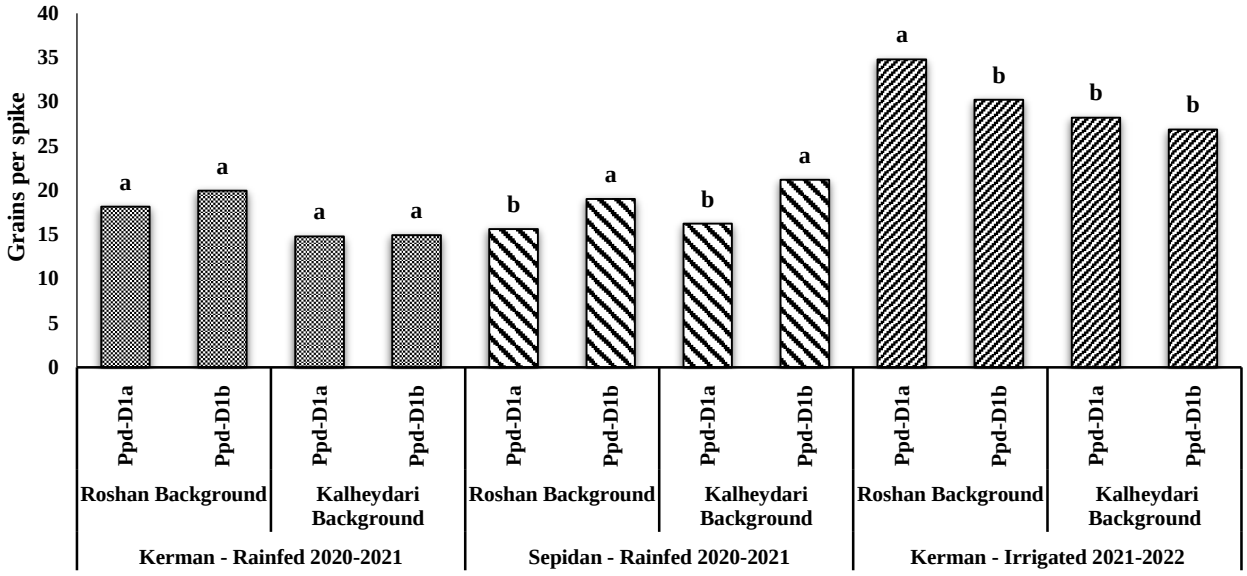

Figure 6. Grains per spike of isogenic lines in two cultivars (Roshan and Kalheydari) under well-watered and rain-fed conditions of Kerman (2020-21 and 2021-22) and Sepidan (2020-21).

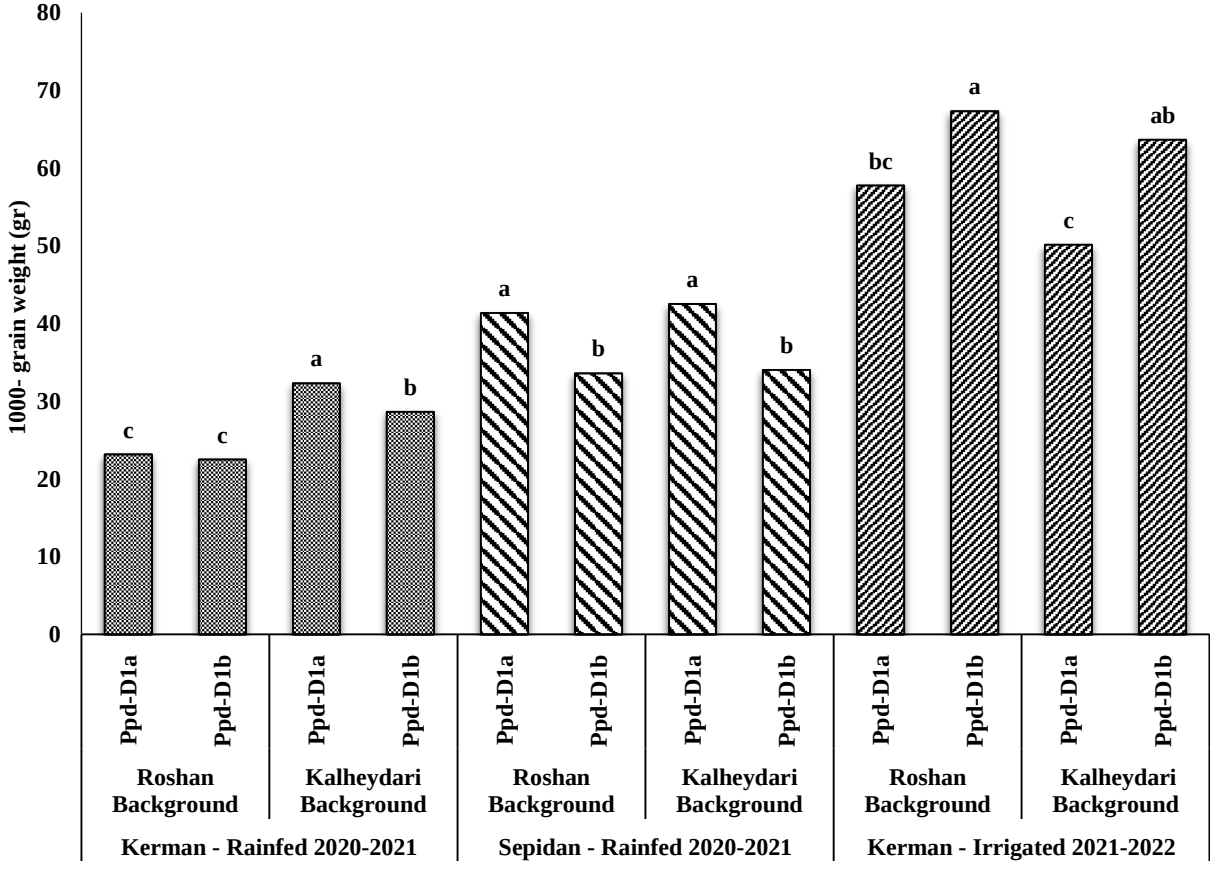

Figure 7. 1000-grain weight of isogenic lines in two cultivars (Roshan and Kalheydari) under well-watered and rain-fed conditions of Kerman (2020-21 and 2021-22) and Sepidan (2020-21).

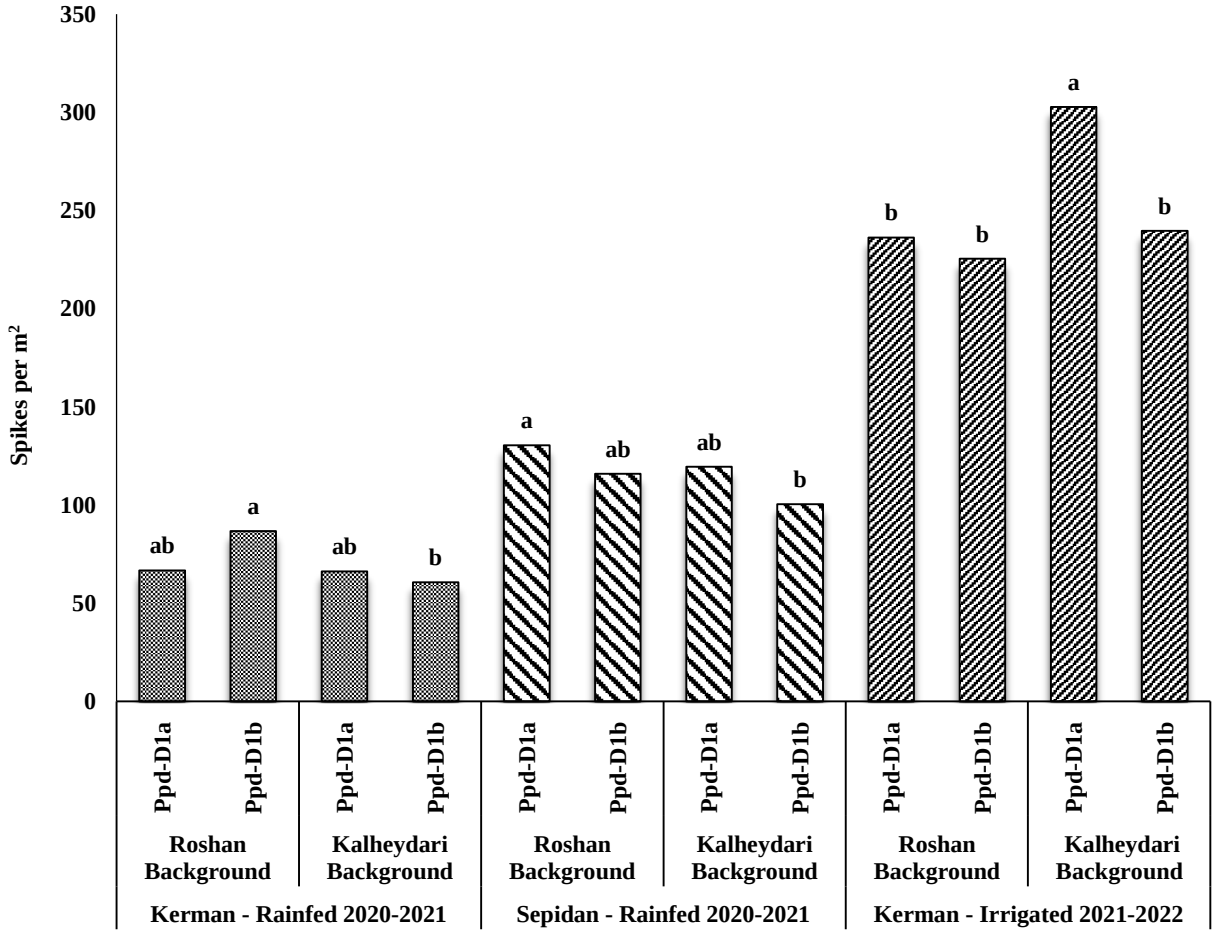

Figure 8. Spikes number per m^2 of isogenic lines in two cultivars (Roshan and Kalheydari) under well-watered and rain-fed conditions of Kerman (2020-21 and 2021-22) and Sepidan (2020-21).

Discussion

Effect of Ppd-D1a on earliness

Drought stress presents a significant abiotic challenge to wheat cultivation, given that this staple crop frequently encounters drought conditions throughout its developmental stages (Kulkarni *et al*. 2017; Movahhedi Dehnavi *et al*. 2017). Among these stages, both pollination and grain filling are particularly vulnerable to water stress, as these periods involve the crucial translocation of assimilates from vegetative tissues (leaves and stem) to the developing grains (Shavrukov *et al*. 2017). Consequently, drought escape emerges as a vital adaptive strategy. This mechanism facilitates the completion of the wheat life cycle prior to the onset of severe drought, thereby effectively alleviating the detrimental impacts of late-season water deficits (Hill and Li, 2016; Shavrukov *et al*. 2017). Over the past century, wheat breeding programs have successfully advanced earliness by 10 to 13 days (Isidro *et al.* 2011; Shavrukov *et al.* 2017). Earliness, often measured by heading date, is primarily controlled by three groups of genes: *Ppd*, *Vrn,* and *Eps* (Seki *et al.* 2013; Kamran *et al.* 2014; Langer *et al.* 2014; Zikhali *et al.* 2014). Notably, the *Ppd-D1* gene accounts for 58% of genetic diversity in

heading date among European winter wheat cultivars (Langer *et al.* 2014), making it a significant tool for marker-assisted selection and backcrossing (Langer *et al.* 2014).

The *Ppd-D1* gene belongs to the pseudo-response regulator (PRR) gene family. The dominant allele, *Ppd-D1a*, promotes earliness in wheat, contrasting with the recessive allele, *Ppd-D1b* (González *et al.* 2005; Wilhelm *et al.* 2013; Chen *et al.* 2018). In extensive research, *Ppd-D1a* has consistently demonstrated a positive and significant impact on earliness across diverse environmental conditions (Beales *et al.* 2007; Bentley *et al.* 2013; Wilhelm *et al.* 2013; Langer *et al.* 2014; Shcherban *et al.* 2015; Chen *et al.* 2018; Fait and Balashova 2022).

In this study, isogenic lines for *Ppd-D1* were developed within two genetic backgrounds, Roshan and Kalheydari, to precisely evaluate the allele's effect on earliness, yield, and yield components. Data collected across all experimental conditions demonstrated that *Ppd-D1a* resulted in an increase in days to heading and maturity. Although the *Ppd-D1a* allele had a positive effect on earliness in both genetic backgrounds and in all environments, there was an interaction between genetic backgrounds and the *Ppd-D1a* alleles for earliness. This suggests that the genetic backgrounds influence the magnitude of the response to selection. Kroupin *et al.* (2020) reported that the *Ppd-D1a* allele improved heading time by 1 to 7 days, depending on environmental and genetic backgrounds. Furthermore, Seki *et al.* (2011) found that the *Ppd-D1a* allele reduced days to heading by 10.3 days in the Japanese wheat cultivars.

Effect of Ppd-D1a on grain yield and yield components

This investigation revealed that under rain-fed conditions, the *Ppd-D1a* allele notably enhanced grain yield in the Roshan and Kalheydari genetic backgrounds. Conversely, no significant impact on grain yield was observed under well-watered conditions, highlighting a strong dependence of the allele's effect on the specific environmental context. For instance, while *Ppd-D1a* was linked to a yield reduction of 1.8% in the UK, it correlated with yield increases of 7.7% in Germany and 33% in Yugoslavia (Worland *et al.* 1998). Moreover, the *Ppd-D1a* allele influenced grain yield by a range of +3 to -5% under greenhouse conditions (Liu *et al.* 2020), and by 8.2 under field conditions in China (Chen *et al.* 2018). In the present study, the *Ppd-D1a* allele reduced heading time. Consequently, grain yield increased under rain-fed conditions due to the presence of *Ppd-D1a*, enabling early genotypes to escape late-season drought stress. These findings underscore the importance of selecting for the *Ppd-D1a* allele, a strategy to enhance grain yield under rain-fed conditions.

Under rain-fed conditions, the grain number per spike decreased in Roshan and Kalheydari. Conversely, 1000-grain weight increased due to the presence of the *Ppd-D1a* allele. However, under

well-watered conditions, the grain number per spike increased, while the 1000-grain weight decreased in Roshan and Kalheydari genetic backgrounds carrying the *Ppd-D1a* allele, respectively. These results demonstrate that the effect of *Ppd-D1a* on grain yield and its components is highly dependent on the target environment. Previous studies have shown that early-heading genotypes tend to produce higher grain yield under both drought stress (Nitcher *et al.* 2014; Khanna-Chopra and Singh 2015; Mondal *et al.* 2016; Shavrukov *et al.* 2017; Dorrani-Nejad *et al.* 2022) and well-watered (Zheng *et al.* 2016) conditions. However, early heading can also negatively impact grain yield under well-watered conditions (Turner 1986; Radhika Thind 2014; Dorrani-Nejad *et al.* 2022).

Marker-assisted selection vs phenotypic selection for Ppd-D1a

In this study, selecting for the *Ppd-D1a* allele indirectly advanced heading time in Roshan and Kalheydari. In contrast, a separate study using a backcross breeding approach, focused on direct phenotypic selection for early heading within the same genetic backgrounds, achieved a greater reduction in heading time in Roshan and Kalheydari (Dorrani-Nejad *et al.* 2022). These indicated that phenotypic selection for early heading yields a greater response than marker-assisted selection for *Ppd-D1a*. However, in speed breeding programs under greenhouse conditions (Watson *et al.* 2018), marker-assisted selection for *Ppd-D1a* could potentially achieve a higher response to selection per year compared to phenotypic selection for early heading under field conditions, where up to six generations per year are feasible. Furthermore, we hypothesize that marker-assisted selection for a combination of *Ppd* and *Vrn* genes, including the *Ppd-D1a* allele, would likely lead to a greater genetic gain for earliness.

Conclusion

In this study, we developed isogenic lines for *Ppd-D1* in two bread wheat genetic backgrounds to precisely investigate the effect of the *Ppd-D1a* allele on key agronomic traits across varying water regimes. The *Ppd-D1a* allele reduced days to heading and maturity by 5.14 and 7.53 days, respectively. A significant interaction was observed between genetic background and the *Ppd-D1* alleles; however, the earliness conferred by *Ppd-D1a* remained consistent across different environmental conditions. Under rain-fed conditions, *Ppd-D1a* increased 1000-grain weight by 17% and decreased the number of grains per spike by14%, which resulted in a 13% increase in the grain yield. Conversely, under well-watered conditions, *Ppd-D1a* decreased 1000-grain weight by 18% and increased grain number per spike by 10% with no significant impact on the grain yield. These results demonstrate that the effect of *Ppd-D1a* on yield and yield components is highly dependent on the

target environment. The significant positive effect of *Ppd-D1a* on grain yield under rain-fed conditions is likely due to the five-day advancement in heading time, enabling drought escape. Given the availability of specific markers for *Ppd-D1*, we recommend using marker-assisted selection or backcrossing in wheat breeding programs to incorporate the *Ppd-D1a* allele into new wheat cultivars intended for cultivation in the end-season drought-prone environments. Although *Ppd-D1a* showed no significant effect on grain yield under well-watered conditions, marker-assisted selection or backcrossing for *Ppd-D1a*, which shortens the wheat life cycle, remains a desirable trait. This trait is particularly valuable even under well-watered conditions, as it can help conserve substantial amounts of water in regions with limited water resources.

Conflict of Interest

The authors declare that they have no competing interests with any individuals or organizations concerning this article.

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