

# Candidate gene association with summer dormancy in tall fescue

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## Abstract

Two main types of summer dormancy in tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh] are recognized, eco-dormancy and endo-dormancy. Endo-dormancy is a physiological response to environmental signals leading to slowing of metabolic activity in meristematic tissues, and most likely controlled by circadian clock genes. Therefore, it is genetically inherited and allelic variation among and between summer-dormant and non-dormant varieties is expected. The main objective of this study was to explore the association between dormancy and various candidate genes. Twenty-three genes were amplified and sequenced in two dormant and two non-dormant checks. Nucleotide variants unique to each group were converted to KASP (kompetitive allele specific PCR) markers and were tested on 52 dormant and non-dormant accessions. Five markers, from the genes *CONSTANS* and *TERMINAL FLOWER* showed significant associations ( $R^2 = 0.10$  to  $0.13$ ,  $p < 0.05$ ) with field phenotypic scores. These two genes are known to modulate meristem determinacy and growth, suggesting that meristem determinacy is probably one of the mechanisms involved in summer dormancy in tall fescue. Another five markers showed significant associations with the surrogate germination phenotype ( $R^2 = 0.13$  to  $0.20$ ,  $p < 0.05$ ). One marker originated from dormancy-associated MADS-box (DAM) gene sequence, three markers originated from auxin response factors (ARFs) sequences, and one marker was derived from Heat shock proteins (HSPs) sequences. These results confirm the implication of photoperiod and temperature in the regulation of summer dormancy. A selection index combining these markers may be valuable for the differentiation between dormant and non-dormant tall fescue genotypes.

## Background

Most Mediterranean tall fescue germplasm originating from North Africa and South Europe undergo summer dormancy, where plants stop growing and become senescent during the summer season, then resume growth in autumn when the temperatures decrease (Hopkins et al. 2009).

Summer dormancy was described as complete or incomplete, and different morphotypes of tall fescue express different levels of summer dormancy (Hand et al. 2010a)

The transition from active growth to the inactive stage requires major changes, such as increased energy storage and inhibition of meristematic activity, followed by senescence of above ground parts (Rees 1992)

Well established that the proper timing of clock outputs with the external environment confer an adaptive advantage to plants by allowing both the anticipation of the environmental transitions and the proper phasing of physiology and metabolism

Light and temperature are the main environmental cues responsible for clock synchronization with the environment

Earlier research suggested leaf senescence and the level of herbage production under full, continuous irrigation over the summer (Laude, 1953, Norton et al. 2006).

Difficult to characterize summer dormancy as it is frequently confounded with responses by which plants avoid and tolerate dehydration.

Several years of evaluation are required to sort out environmental effects.

## Objectives

Explore the association between tall fescue summer dormancy phenotypes and candidate genes involved in seasonal dormancy, flowering time, heat, and photoperiod sensing.

## Materials & Methods

### Plant material

The dormant check cultivars (AGRFA-126 and T706-1) and the non-dormant checks (Kentucky 31 and GA186) were used in the initial screening of candidate gene primers and initial screening of KASP markers. Fifty two dormant and non-dormant accessions selected based on field evaluation of 218 tall fescue PI accessions were used in the analysis of association with candidate gene allele variants.

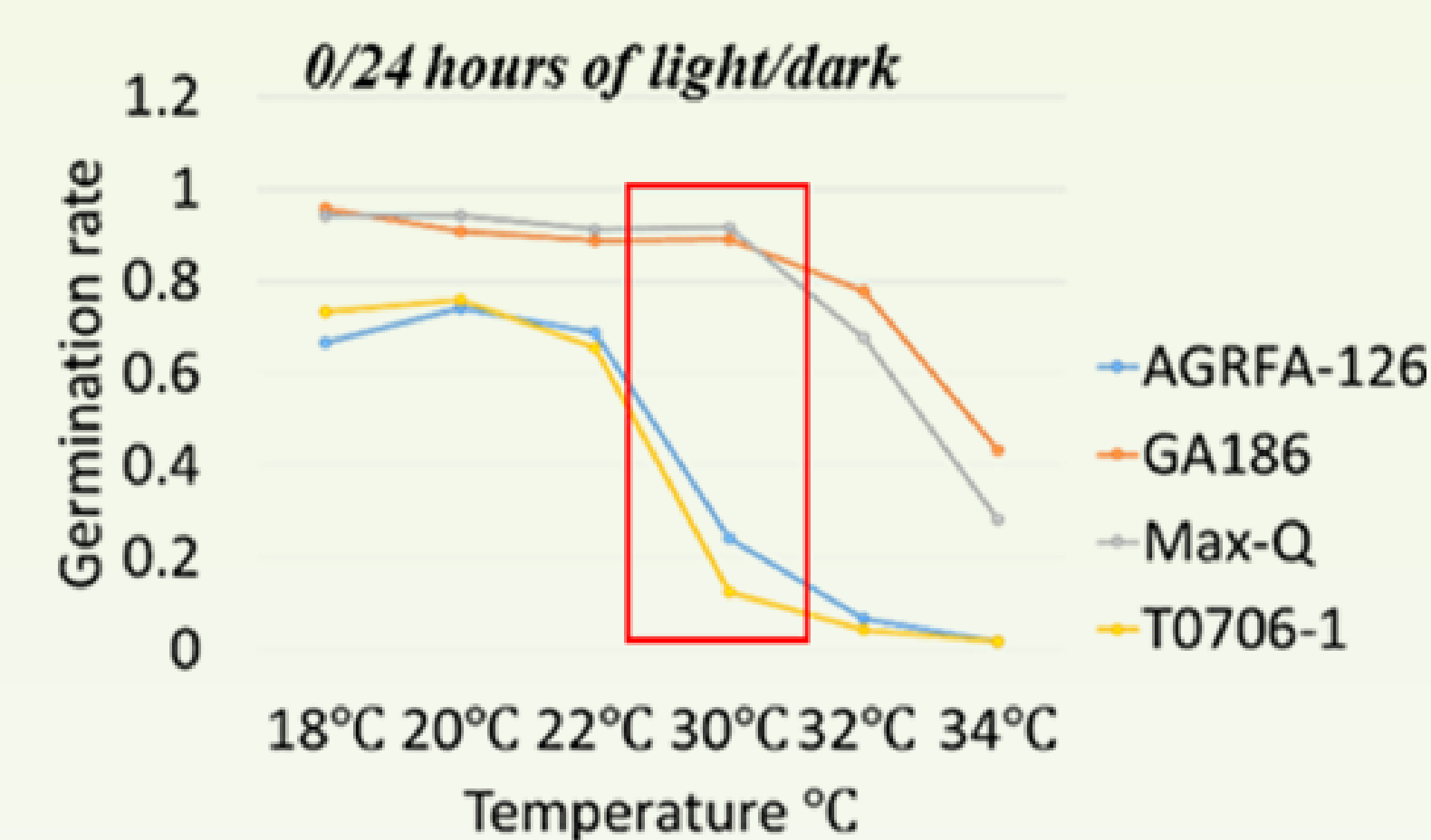
### Summer dormancy phenotype

- Field dormancy was assigned as a score combining regrowth height after clipping and senescence based on Digital Image Analysis using the software Assess version 2.0 from APS Press (American Phytopathological Society, St. Paul, MN).

Regrowth (cm)	Regrowth score	Percentage of dead plant	Senescence score
1-2.5	1	91-100	1
2.5-5	2	81-90	2
5-7.5	3	71-80	3
7.5-10	4	61-70	4
10-12.5	5	51-60	5
12.5-15	6	41-50	6
15-17.5	7	31-40	7
17.5-20	8	21-30	8
20-22.5	9	11-20	9
>22.5	10	0-10	10

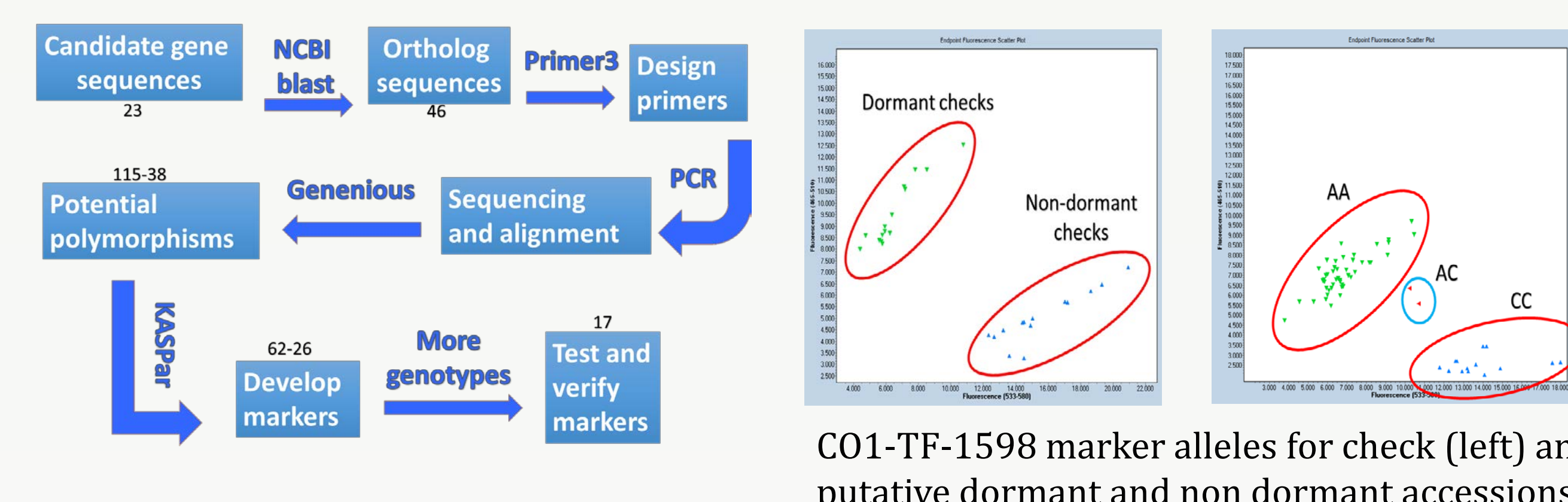


- A surrogate phenotype based on seed germination ratio at 30°C/20 °C



### Candidate genes

- 6 Flowering time and circadian clock genes (CEN, TFL1, CO, FT, FRIGIDA, Gigantea).
- 14 Dormancy and seed dormancy (DAM, BAM, GAST1 protein, NAC, viviparous-1 (VP1), SVP, HD-Zip, Per1, Phytochrome A, Phytochrome B, Phytochrome C, PHY-I interacting transcription factor, auxin response factor, and DELLA protein)
- 1 Heat tolerance: heat shock proteins
- 1 Drought inducible 22 kD protein
- 1 Biotic and abiotic stress responses: ABA stimulation MAP kinase



### Data analysis

The genotypes at each marker were assigned the number 3 for the non-dormant allele, 1 for the dormant allele and 2 if they were heterozygous. The phenotype scores were ranked in the order non-dormant to dormant. PROC GLM in SAS 9.4 was used to determine the association between dormancy scores and genotypes at each marker under the null hypothesis  $\rho = 0$ .

## Results

- Five markers showed significant association ( $p < 0.05$ ) with field summer dormancy rating scores with  $R^2 = 0.10$  to  $0.13$  (Table 1). These markers were all derived from sequences of the genes *Terminal flower*, and *Constans* (CO).
- *CONSTANS* (CO) are proteins involved in day length sensing and promoting flowering in response to long photoperiods (Böhlenius et al. 2006)
- Five markers showed significant associations with the surrogate germination phenotype ( $R^2 = 0.13$  to  $0.20$ ,  $p < 0.05$ )

KASP SNP markers	SNP alleles (D/ND) <sup>†</sup>	No. of heterozygous	Correlation with SDRS <sup>‡</sup>	R <sup>2</sup> with SDRS <sup>‡</sup>	R <sup>2</sup> with GRL score <sup>‡</sup>
CO1-TF-1598I	C/A	2	0.27	0.12*	0.01
CO1-TF-1576	T/A	4	0.27	0.11*	0.00
TFNEW-TF-15219-3	A/G	1	0.33*	0.10*	0.01
CO4-TF-1591I	C/T	0	0.33*	0.11*	0.00
CO1-TF-15180	C/G	1	0.37*	0.13*	0.06
VER-MADS-TF-1586o	G/T	8	0.16	0.00	0.20*
HEAT1-TF-15130	C/A	0	0.16	0.01	0.15*
ARF1-TF-15383	T/C	4	0.04	0.00	0.14*
ARF6-TF-1556s	G/A	16	0.16	0.02	0.16*

**Table 1.** Marker-phenotype association ( $R^2$ ) and Spearman Correlation coefficients between 17 KASP Marker genotypes and summer dormancy rating score (SDRS) from field data, and germination ratio score under 24 hours of light (GRL score) on 52 accessions and 4 checks of tall fescue.

## Conclusion

- Summer dormancy is most likely a quantitative trait controlled by several genes with small effects.
- Results of this study also confirmed the major role of photoperiod and temperature in the initiation and maintenance of summer dormancy.

## References

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