MANIPULATING SUMMER DORMANCY TO IMPROVE FORAGE YIELD AND SEASONAL DISTRIBUTION OF TALL FESCUE

by

RUYUE DING

(Under the Direction of Ali Missaoui)

ABSTRACT

Tall fescue is a cool-season grass commonly used as forage. Understanding the mechanisms and genetic factors underlying summer dormancy in tall fescue can aid in a faster transfer of favorable traits across germplasm pools and speed up the process of cultivar development. In this study, we developed a potential surrogate phenotyping method for summer dormancy. The ratio of germination rate at 30°C divided by germination rate at 20°C under 24 hrs photoperiod correlates well ($r = 0.7$) with field dormancy rating. We re-sequenced 23 candidate genes that have been implicated in seasonal dormancy, in dormant and non-dormant checks, and developed 62 KASP markers based on nucleotide variants. Five SNP (Single Nucleotide polymorphism) markers from the genes CONSTANS and TERMINAL FLOWER, known to modulate meristem determinacy and growth, showed significant associations ($p<0.05$) with field summer dormancy rating scores. Another five markers derived from *dormancy-associated MADS-box* (*DAM*), auxin response factors (ARFs), and Heat shock proteins (HSPs) sequences, showed significant associations at $p<0.05$ with the surrogate germination phenotype ($R^2 = 0.13$ to 0.20). These markers need to be validated in a segregating population for potential application in marker assisted selection for summer dormancy. The relation between summer

dormancy and determinacy was tested. The data showed that summer dormant genotypes will go dormant only after flowering and setting seed. These findings need to be confirmed using a larger set of dormant and non-dormant populations.

INDEX WORDS: Tall fescue, Summer dormancy, Germination test, KASP marker, Determinacy

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BS, Michigan State University, 2013

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A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2016

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DEDICATION

This thesis is dedicated to the people who I love and love me the most in my life. To my parents, Dejun Ding and Jing Zhao, for giving me life and their unconditional love and support ever since, I know they will always be there for me. To my dear husband, Adam Adrain Merlington, for being the one in my life, loving and supporting me whenever and wherever, helping me get through every obstacle I met during my graduate school.

ACKNOWLEDGEMENTS

Above all, I am deeply grateful to Dr. Ali Missaoui, who has been very patient and helpful during my entire graduate study. I would like to thank my committee members, Dr. Zenglu Li and Dr. Brian Schwartz for all their great suggestions and guidance through my research project. I thank Dr. Scott Allen Jackson's lab for letting me use their growth chambers for the seed germination experiments. I thank Dr. Li's lab for their technical and equipment's supports. I would also like to thank Joseph Young and Kurk Lance for their help on the seed germination test. I am sincerely thankful to Franco Villegas Chirinos, and everyone from the forage and biomass breeding and genetics lab for their technical assistance in the lab, greenhouse and field. This research was supported by funds from Grasslanz Technology and The University of Georgia Research Foundation (UGARF).

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Importance of tall fescue

Tall fescue [*Festuca arundinacea* Schreb. syn. *Lolium arundinaceum* (Schreb.) Darbysh] is one of the most important forage species in the world. It is a cool-season perennial grass widely used in the United States (US), especially in the transition zone between cool-temperate and subtropical zones (Sato et al., 2012). Tall fescue is used to provide fodder for various types of livestock in the US, and is grown on more than 14 million ha (Browning, 2012; Fribourg et al., 2009). Tall fescue is the base for beef cow-calf (*Bos taurus*) production in the southeast and east-central US, providing feed for more than 8.5 million beef cows occupying near 10 million ha (Hoveland, 1993). It is also one of the major pasture species for sheep (*Ovis aries*) and horse (*Equus caballus*) production. A survey of Tennessee goat producers revealed that two-thirds of pastures used by goat producers have tall fescue (Leite-Browning et al., 2002).

Tall fescue's widespread agriculture and forage use in the US is due to its strong adaptability to various growing conditions such as, flooding, drought, and low nutrients or shallow topsoil. In addition, tall fescue has other characteristics such as, high forage and seed yields, high persistence under grazing, long grazing season, and tolerance to various biotic and abiotic stresses (Hanson, 1979). Furthermore, tall fescue can persist under low-input management systems, which makes it more beneficial than other cool-season grasses. Many part-

time beef operations in the east-central and southern US make use of these advantages, which reduces economic risk and increases their return on investment (Sleper and West, 1996).

The rapid vegetative spring growth of tall fescue makes it a good hay crop (Matches, 1979), because biomass accumulation exceeds removal by animal grazing during these months. The excessive spring growth is often harvested and stored for later use. Another major advantage of tall fescue is the ability to stockpile summer and autumn growth for later grazing in late fall and winter. Stockpiled tall fescue has less forage digestibility loss compared to other stockpiled cool-season grasses (Matches, 1979).

Tall fescue is used as a companion crop in mixed pastures with other forage species. It can be grown in combination with legumes such as white clover and red clover and exploited during spring to early summer. Legumes provide a low cost nitrogen source for tall fescue and increase animal productivity by enhancing forage quality (Fribourg et al., 1984; Matches, 1979). For beef production in the eastern and southern US, tall fescue is the major cool-season grass component of multiple-species forage systems. Subtropical grasses such as bermudagrass (*Cynodon dactylon*), bahiagrass (*Paspalum notatum*) in the southern US, switchgrass (*Panicum virgatum*) and caucasian bluestem (*Bothriochloa bladhii*) in the central and northern US are often used to supplement tall fescue's inadequate summer growth.

Water and soil conservation

Tall fescue is widely planted on slopes for soil conservation and water retention while also providing forage for livestock production (Bennett, 1979). On sloping soils of the Piedmont and West Virginia, no-till planting corn into herbicide-suppressed tall fescue sod can produce high quality grain with reduced water runoff and soil erosion (Bennett et al., 1976; Wilkinson et al.,

1987). Tall fescue is also used to recover and stabilize strip-mined soils (Pietz et al., 1989; Roberts et al., 1988) and is usually planted on land set-aside for long-term land conservation such as in the Conservation Reserve Program (Sleper and West, 1996).

Turf

Compared to other cool-season turf grasses such as Kentucky bluegrass (*Poa pratensis*) and perennial ryegrass (*Lolium perenne*), tall fescue has increased heat and drought tolerance, which makes it a good turf grass choice for the transition zone. Tall fescue is able to adapt to different soil types and environments (Sleper and West, 1996), while warm-season turf species such as bermudagrass and Japanese 1awngrass (*Zoysia japonica*.) do not perform well in this zone, due to the lack of cold tolerance. Therefore, tall fescue is increasingly becoming the major turf grass option in the transition zone. Tall fescue has minimal insect problems, which may be related to the presence of the endophyte (*Neotyphodium coenophialum* syn. *Acremonium coenophialum*) that lives in association with tall fescue (Funk et al., 1984). Reports have shown that the presence of the endophyte can act synergistically to improve long term persistence, fall recovery, and competition of tall fescue with weeds (Hurley et al., 1984). Brown patch infection, which often occurs under warm and humid conditions, is one of the major disease problems of tall fescue when it is used as a turf species in the transition zone, which often occurs under warm and humid conditions. However, when the weather becomes cooler and drier in the fall, symptoms of brown patch will disappear as the turf recovers.

Importance of seasonal dormancy

Dormancy has been defined as a temporary suspension of visible growth of any plant structure containing a meristem (Lang et al., 1987). Seasonal dormancy is an adaptive response,

which has evolved in the environment of the species origin, enabling it to survive during seasons when environmental conditions are not favorable or most threatening to plant growth (Vegis, 1964). Dormancy has been studied extensively, mostly in woody species adapted to temperate environments. The two major environmental cues that plants use to sense environmental changes are day length and temperature (Olsen, 2010; Tanino et al., 2010). Several studies showed that photoperiod has less influence on dormancy induction compared to temperature effects in some species like apple and pear. In other species where growth cessation is influenced by photoperiod, temperature effects can still dominate (Olsen, 2005). In the perennial grass *Hordeum bulbosum* L., dormancy is induced during early stages of reproductive growth when the plants experience either low temperature or short days followed by long days (Ofir and Koller, 1972; Ofir and Koller, 1974). Some reports also claimed that the combined action of long days and increased temperature could induce summer dormancy in *Poa bulbosa* L., *P. scabrella*; *Allium cepa* L., *A. sativum* L., *Ranunculus spp.*, and *Anemone coronaria* L (Ofir and Kigel, 2007).

Many warm season grass species have evolved fall/winter dormancy as an escape mechanism to protect themselves from extreme cold winters, especially in the northern latitudes. Under temperate or continental climates, low temperature is usually the major limitation for the growth of forage plants, and fall/winter dormancy are the main adaptive responses (Eagles, 1989). Related studies have been conducted on various plant species, including switchgrass (*Panicum virgatum* L.) and alfalfa (*Medicago sativa* L.).

It has been shown that winter dormancy in summer grasses can be manipulated through daylength and photoperiod. Experiments were conducted to reverse dormancy in switchgrass germplasm by exposing plants to extended photoperiod, which resulted in increases in tiller

numbers and biomass production (Van Esbroeck et al., 2004). Testing of three subtropical forage grasses including bahiagrass, bermudagrass , and star grass (*Cynodon nlemfuensis* Vanderyst) shown increased yield after extending day length to 15 h compared to yield under natural day length (Sinclair et al., 2001). These results suggest that manipulating fall dormancy by selection of non-dormant or semi-dormant germplasm would help increase yield and extended the seasonal distribution in mild environments.

In addition to warm season grasses, cool season legumes such as alfalfa, one of the most important forage crops in the US. exhibits fall-winter dormancy (Ariss and Vandemark, 2007). Fall dormancy is an adaptive strategy enabling the success of cultivation of alfalfa in climatic zones outside its natural range. It has been positively associated with winter survival (Cunningham et al., 2001), even though genetic analysis suggests that these two traits may be inherited independently (Brouwer et al., 2000; Brummer et al., 2000). The physiological mechanisms underlying fall dormancy are not well known (Leep et al., 2001).

Summer dormancy

This type of dormancy has been commonly found in some perennial grass species originating from Mediterranean climates, where summer stress is prolonged and intense. Growth cessation and dormancy during summer months help plants survive by keeping the plant meristems alive (Koller, 1969). There has been extensive research investigating dormancy in plants that experience severe winter conditions, but much less attention has been devoted to perennial herbaceous plants that undergo summer dormancy (Vegis, 1964). Recently, research has been conducted on the physiological influence of summer dormancy on different crops (Kamenetsky et al., 2003; Parsons, 2000; Vaughton and Ramsey, 2001).

Two main types of summer dormancy were described, eco-dormancy and endo-dormancy. Eco-dormancy is a response to environmental stress factors and it involves the interaction of stress response mechanisms that are not well understood (Volaire and Norton, 2006). Endo-dormancy is a physiological response to seasonal changes and is regulated by circadian clock genes in responses to environmental factors, such as temperature and photoperiod (Salome et al., 2008). The control mechanism of endo-dormancy may involve hormones, and usually associated with flowering and a slowing of metabolic activity in meristematic tissues (Volaire and Norton, 2006). Endo-dormancy, therefore, is genetically inherited, which makes it a good candidate to investigate its mechanism and make use of it in forage production. Although dehydration tolerance and summer dormancy are independent responses to the environment and could be distinguished by plant growth and recovery. Under severe drought, it is difficult to characterize the responses by which plants avoid and tolerate dehydration from those caused by physiological summer dormancy. Consequently, this type of endo-dormancy can only be tested in plants that have not been exposed to water stress.

The proposed terminology identifies two different levels of summer dormancy: (1) complete dormancy, when plants undergo full senescence and induced dehydration of leaf bases, as in orchard grass [*Dactylis* spp. (Norton et al., 2006a)]; and (2) incomplete dormancy, when plants undergo moderate levels of senescence and leaf growth is partially inhibited, as in tall fescue (Norton et al., 2006b).

Summer dormancy has been associated with greater survival after recurrent and severe summer stress in many perennial grasses, including wild grasses such as pine bluegrass [*Poa scabrella* (Laude, 1953)], bulbous bluegrass [*P. bulbosa* (Ofir and Kigel, 1999; Volaire et al., 2001), [*Hordeum bulbosum* (Ofir et al., 1967)], and some populations and ecotypes of

orchardgrass originating from Morocco and Israel (Volaire, 2002). Plant adaptation is usually considered as the interaction between environmental factors and plant growth responses. The distribution of plants is primarily determined by climatic (temperature, air movement, light, and precipitation), edaphic (soil texture and moisture) and geographic (latitude and elevation) factors (Burns and Chamblee, 1979; Good, 1931; Good, 1953).

A distribution map for the tribe *Festuceae,* representing the global distribution of tall fescue suggested that the major factors influencing tall fescue distribution are climatic and geographic (mainly altitude) factors (Hartley, 1950; Hartley, 1954). The bulk of tall fescue's growth occurs during spring, followed by a decline during summer, and regrows in the fall until early winter (Burns and Chamblee, 1979). In the southern Great Plains, hot summers coupled with severe drought adversely influence the persistence and productivity of many traditionally cultivated summer active cool-season perennial grasses including tall fescue (Malinowski et al., 2005; Nielsen-Gammon et al., 2005). Summer dormancy helps cool-season perennial grasses survive severe summers (Vegis, 1964), increases the level of persistence, and improves fall regrowth (Malinowski et al., 2005; Norton et al., 2006a; Norton et al., 2006b).

In most temperate grasses, growth ceases at temperatures exceeding 30 to 35°C (Cooper and Tainton, 1968). Such a response has been demonstrated on meadow fescue (*Festuca elatior* L.) and tall fescue (Hoveland et al., 1974; Sprague, 1943). The persistence of tall fescue also depends on soil moisture and rainfall. Although summer dormancy in tall fescue is triggered by high temperatures, dormancy will be reduced with adequate soil moisture (Burns and Chamblee, 1979). As summer dormancy is associated with water stress, it is difficult to measure the plant's response strictly to high temperatures.

Genetic control of dormancy

Research conducted to infer the evolutionary history of tall fescue morphotypes using molecular phylogenetic analysis genus showed that different morphotypes express different levels of summer dormancy (Hand et al., 2010). Understanding the genetic control of summer dormancy will help in the manipulation of this trait to improve forage production. Most complete dormancy has been found in populations with low yield potential (Volaire and Norton, 2006).

Growth cessation during dormancy implies that meristems and crowns sense the triggering signals and become inactive even in the presence of favorable growth conditions (Rohde and Bhalerao, 2007). Several biochemical studies reported differential expression levels of growth hormones during physiological dormancy with increased levels of growth inhibiting hormones like abscisic acid (Avendaño López et al., 2011) and reduced levels of growth promoting hormones like gibberellins and cytokinins (Powell, 1987). The onset of dormancy is also associated with senescence and remobilization of photosynthates and nutrients from the shoots to reserve structures such as buds and crowns. These nutrients are believed to be remobilized to support regrowth of new shoots in the spring (Millard and Neilsen, 1989; Schwartz and Amasino, 2013). Expression analysis of genes involved in carbohydrate metabolism and bud dormancy of leafy spurge (*Euphorbia esula*), showed a steep increase in up-regulation in the expression of a specific *Euphorbia esula* beta-amylase gene (Ee-BAM1) after growth induction (Chao and Serpe, 2010). Expression analysis of three dormancy-associated MADS-box genes in the leaf buds of a dormant and a less dormant pear (*Pyrus*) cultivars showed an earlier down-regulation in the expression of MADS13-1 levels of these genes in the less dormant cultivar prior to dormancy release. The transcripts differed by an insertion of 3.218 kb in the first intron of the dormant cultivar (Saito et al., 2013). A number of genes involved in the control of dormancy induction

and growth cessation were identified in genomic studies, including circadian clock regulators (Ibanez et al., 2010). Potential candidate regulators implicated in growth cessation and dormancy induction include *FLOWERING LOCUS T* (*FT*) and the *CO*/*FT* complex (Pin and Nilsson, 2012), *CEN1*/*TFL1* (Mohamed et al., 2010), as well as dormancy-associated MADS-box factors (Horvath et al., 2010). Growth cessation in aspen exposed to short photoperiod was prevented by overexpression of *FT1* and *CO* homologues of poplar [*Populus trichocarpa* (Bohlenius et al., 2006)]. It is possible that this information could be extrapolated to understand the genetic basis and the mechanisms regulating seasonal dormancy in herbacious crops.

Determinacy is an agronomically important trait associated with domestication (Tian et al., 2010). The meristems in determinate cultivars cease vegetative activity at or soon after photoperiod-induced floral initiation, in which the meristems undergo reproductive inflorescence (Bernard, 1972), similar to the dormancy phenotype. *TFL1*-like genes are highly conserved in plants and are believed to help maintain meristem indeterminacy. Danilevskaya et al. 2010 described six maize (*Zea mays*) *TFL1*-related genes, named Z*EA CENTRORADIALIS1* (*ZCN1*) to *ZCN6*. Their findings suggest that ectopic expression of the *TFL1*-like genes in maize modifies flowering time and inflorescence architecture through maintenance of the indeterminacy of the vegetative and inflorescence meristems (Danilevskaya et al., 2010). It is plausible to hypothesize that *TERMINAL FLOWER1 (TFL1*)-like genes are reasonable candidate genes that influence dormancy by influencing meristem determinacy.

Understanding the genetic basis and environmental determinants of dormancy is essential for interpreting the evolution history of seasonal dormancy and could help identify regulatory components involved in seasonal endo-dormancy. Variations in gene expression occur in crowns and buds during the onset of dormancy and suggest there are allelic variations among and

between summer dormant and non-dormant varieties (Rohde and Bhalerao, 2007; Avendaño López et al., 2011; Powell, 1987; Millard and Neilsen, 1989; Schwartz and Amasino, 2013; Chao and Serpe, 2010; Saito et al., 2013; Ibanez et al., 2010; Pin and Nilsson, 2012; Mohamed et al., 2010; Horvath et al., 2010; Bohlenius et al., 2006; Tian et al., 2010; Bernard, 1972; Danilevskaya et al., 2010). This variation may allow for the genetic mapping of quantitative trait loci (QTL) underlying the dormancy phenotype and development of molecular markers that can be used for selection of non-dormant progenies during early generations in breeding programs.

Seed dormancy and germination

Seed dormancy is defined as the inability of an intact viable seed to complete germination under favorable conditions (Hilhorst, 2007). It is a strategy to escape extreme weather conditions, which mimics the response of annual plants to avoid the harmful effects of drought as seed. In herbaceous plants, seed and bud dormancy display similar phenomena, but the mechanisms involved may be different (Dennis, 1996; Okagami, 1986).

Seed dormancy and germination are controlled by developmental and environmental cues (Bewley, 1997; Finch-Savage and Leubner-Metzger, 2006; Koornneef et al., 2002). Abscisic acid [ABA (Avendaño López et al., 2011)] is one of the hormones involved in seed dormancy and germination control (Hilhorst, 1995; Kermode, 2005). Transgenic lines over-accumulating ABA exhibited enhanced dormancy (Okamoto et al., 2006; Okamoto et al., 2010; Qin and Zeevaart, 2002; Thompson et al., 2000). This suggests that ABA prevents precocious germination by inducing dormancy. In the genus *Arabidopsis*, ABA is transported from the mother plant as it is largely synthesized in maternal tissues (Karssen et al., 1983). This suggests that ABA in the mother plant could induce dormancy of the mother plant itself.

The maternal photoperiod at the time of seed maturation can predict the seasonal conditions of newly dispersed seeds. Research has shown that maternal photoperiod influenced the rate and speed of seed germination in *Arabidopsis thaliana*, with the short-day photoperiod causing higher germination rates (Munir et al., 2001). Variation in seasonal dormancy may be strongly influenced by genes expressed in the maternal parent. Maternal control of germination can operate through the seed coat, endosperm, or maternal provisioning of resources and hormones in response to light environments experienced by the embryo (Botto et al., 1995). The evolution of seed dormancy may involve selection based on genetic variation in germination responses to the offspring environments among maternal parents, as well as among individual seeds (Donohue and Schmitt, 1998). Maternal environmental effects have been viewed as a form of adaptive phenotypic plasticity across-generations. It is reasonable to hypothesize that the performance of seed germination under a certain environment can be an indicator and predictor of the response of its mother plant under the same environmental condition. Therefore seed germination could present a potential surrogate phenotype for summer dormancy.

Phenotyping summer dormancy

To understand tall fescue summer dormancy, scientists need to be able to accurately identify and evaluate a measurable phenotype. To develop an accurate phenotypic evaluation system, plant behavior during summer dormancy of tall fescue must be considered. Summer dormancy can be defined by four criteria, one of which is considered optional: (1) reduction or cessation of leaf production and expansion; (2) senescence of mature foliage; (3) dehydration of surviving organs; and (4, optional) formation of resting organs (Volaire and Norton, 2006). Earlier research suggested leaf senescence could be used to identify summer dormant plants (Norton et al., 2006b). However, senescence may not always be a reliable indicator of summer

dormancy. The number or percent green leaves may not differentiate tall fescue genotypes accurately because loss of greenness can be a response to drought and/or heat stress rather than a sign of summer dormancy (Bhamidimarri et al., 2012). Herbage production under full, continuous irrigation condition over the summer could be another indicator of summer dormancy (Laude, 1953). Most studies in bulbous canarygrass (*Phalaris aquatica*) evaluated dormancy by measuring the growth of the buds after a midsummer rainstorm in the field (Culvenor and Boschma, 2005; Oram, 1983; Oram, 1984; Oram and Freebairn, 1984), or after being transferred into controlled environmental conditions such as lower temperature and higher soil moisture (Biddiscombe et al., 1977; Hoen, 1968). These methods are similar to those used for testing winter dormancy of tree buds (Williams et al., 1979). Nevertheless, it is difficult to replicate and accurately identify the phenotype by re-watering plants in the middle of summer since length of the dormancy period can vary largely (Volaire and Norton, 2006).

Due to the absence of an accurate reliable system to phenotype summer dormancy and the costly, time consuming field data collection methods, developing a surrogate phenotyping system or identifying QTL controlling the phenotype would be highly valuable, thus the objectives of this research is to develop a surrogate phenotyping system for summer dormancy in tall fescue and to analyze candidate genes associated with summer dormancy to develop potential molecular markers.

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CHAPTER 2

PHENOTYPING SUMMER DORMANCY IN TALL FESCUE: ESTABLISHMENT OF A SURROAGTE PHENOTYPE AND A DORMANCY RATING SYSTEM[1]

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Abstract

Tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh] is a cool-season perennial grass from two major types of germplasm, continental and Mediterranean germplasms. Most Mediterranean germplasms exhibit summer dormancy even under favorable growth conditions. Summer dormancy is believed to be associated with longer persistence and fast recovery following severe summer stress, which are important for perennial forage grasses. Phenotyping summer dormancy in the field is difficult and costly. The objective of this study is to develop a fast and low-cost surrogate phenotyping approach for summer dormancy. The environmental cues that trigger dormancy in the mother plants would act similarly on seed dormancy/germination and thus may be used as a surrogate phenotype. Germination tests were conducted under 30 combinations of photoperiod and temperature extremes with two non-dormant and two dormant check varieties in 2 growth chambers. Thirty combinations of six temperatures (18, 20, 22, 28, 30, 32, and 34°C) and five photoperiods (0/24, 12/12 14/8, 16/8 and 24/0 hrs of light/dark) were tested for their effects on germination. The results showed that temperature had a larger effect on germination than photoperiod, with higher temperatures reducing the germination of dormant checks, but not non-dormant checks. One hundred and sixty-eight accessions were phenotyped for dormancy in the field using a combined score of regrowth height and senescence based on Digital Image Analysis. Twenty six putative non-dormant and 30 dormant accessions were selected for seed germination testing to establish a correlation between seed germination and field dormancy. The results showed that the ratio of the germination rate at 30°C over the germination rate at 20°C under 24 hr photoperiod correlated well ($r = 0.7$) with field dormancy phenotypes and has potential to be used as proxy for phenotyping summer dormancy in tall fescue.

Keywords: Summer dormancy, Surrogate phenotype, Germination ratio, Senescence rating score, Regrowth height.

Introduction

Tall fescue [*Festuca arundinacea* Schreb. syn. *Lolium arundinaceum* (Schreb.) Darbysh] is an important forage species, grown on more than 14 million ha (Browning, 2012; Fribourg et al., 2009). It is a cool-season perennial grass with rapid vegetative spring growth, widely used in the USA, especially in the transition zone between cool-temperate and subtropical zones (Sato et al., 2012). Tall fescue can be grown as a companion crop in mixed pastures with warm season grasses such as bermudagrass (*Cynodon dactylon*), bahiagrass (*Paspalum notatum*) in the southern USA to provide forage in spring and fall (Matches, 1979).

Tall fescue germplasm has been classified by taxonomists into five botanical groups, from which, plant breeders defined three major pools, Continental, Mediterranean, and Rhizomatous (Hand et al., 2010). The Continental pool predominates in Northern Europe, is characterized by wide leaf blades, and is relatively winter hardy and summer active like 'Kentucky 31'(Hopkins et al., 2009). The Mediterranean germplasm originated from North Africa and was characterized by its lax leaves and incomplete summer dormancy, where plants stop growing and become senescent during the summer season and then resume growth in autumn when the temperatures decrease (Hopkins et al., 2009). Rhizomatous tall fescue, originating primarily in northwest Portugal and parts of Spain (Borrill et al., 1971), is considered a third germplasm pool that is distinguished by more widespread and longer rhizomes compared to the Continental type (Borrill et al., 1971; Jernstedt and Bouton, 1985). In comparison to Continental type, the Mediterranean and Rhizomatous germplasm both have less susceptibility to winterkill and increased winter hardiness (Diesburg and Carlson, 1983; Hopkins et al., 2009).

Extensive phenotypic and genotypic variations among the three breeding pools were documented and can be exploited to develop cultivars with desirable traits (Hand et al., 2012a;

Hand et al., 2012b; Hopkins et al., 2009). Flow cytometry analysis of different accessions showed various ploidy levels among different germplasms, with most Continental and Mediterranean germplasm being hexaploid (Cuyeu et al., 2013).

Summer dormancy frequently observed in the Mediterranean germplasm is an adaptive response that has evolved in the environment of origin of the species, enabling it to survive during the seasons when conditions are harsh (Vegis, 1964). Summer dormancy is defined as a combination of processes endogenously controlled that lead to growth cessation and senescence under non-limiting growth conditions in summer (Norton et al., 2008). The trait is believed to be associated with longer persistence and fast recovery following severe summer stress in many perennial grasses (Laude, 1953; Norton et al., 2006a; Norton et al., 2006b; Ofir and Kigel, 1999; Ofir et al., 1967; Shaimi et al., 2009a; Shaimi et al., 2009b; Volaire et al., 2001). Dormancy enables plant survival by reallocation of energy and storage in meristems to keep the plant alive and to support regrowth (Koller, 1969; Millard and Neilsen, 1989; Rees, 1992; Schwartz and Amasino, 2013). Two types of summer dormancy were described. One is eco-dormancy which involves the interaction of stress response mechanisms and is hard to dissect (Volaire and Norton, 2006). The other is endo-dormancy, which is controlled by circadian clock genes and environmental factors like temperature and photoperiod (Salome et al., 2008). It is mediated by phytohormones and usually associated with flowering (Volaire and Norton, 2006). Endo-dormancy also slow down the metabolic activity in meristems, and is genetically inherited (Volaire and Norton, 2006).

Seasonal dormancy has been investigated extensively in woody species adapted to temperate environments and exhibiting fall/winter dormancy. The two major environmental cues that plants use to sense environmental changes are day length and temperature (Olsen, 2010;

Tanino et al., 2010). A clear understanding of the mechanisms and factors controlling summer dormancy in tall fescue will facilitate the transfer of traits between germplasm pools and enable developing beneficial cultivars. The challenge in utilizing summer dormancy in tall fescue is the difficulty to accurately identify and measure the phenotype. Earlier research suggested that leaf senescence could be used to identify summer dormant plants (Norton et al., 2006b). However, senescence may not always be a reliable indicator of summer dormancy (Bhamidimarri et al., 2012). Another indicator might be herbage production, assessed by measuring tiller numbers, regrowth height, fresh weight, and dry weight under continuous irrigation over summer (Culvenor and Boschma, 2005; Laude, 1953; Oram, 1983; Oram, 1984; Oram and Freebairn, 1984). These criteria are similar to those used to characterize winter dormancy of tree buds (Williams et al., 1979). Methods relying on visual ratings of percentage of senescence to evaluate summer dormancy in the field can be problematic due to the confounding effects of heat and drought and the inability to control stress conditions in the field. It is also believed that dormancy is regulated by plant hormones in response to environmental factors, therefore the phenotype may not be consistent among populations of the same species and places of origin (Bhamidimarri et al., 2012).

The environmental conditions experienced by the mother plants may influence natural selection on seed germination, the genetic variation for germination, and even the genes associated with seed germination (Donohue, 2009). The photoperiod condition that maternal plant experiences at the time of seed maturation can predict the seasonal conditions of newly dispersed seed (Penfield et al., 2005). Variation in seasonal dormancy of the seed is possibly influenced by the genes expressed in the maternal parent. The evolution of seed dormancy may involve selection based on genetic variation in germination responses to the offspring

environments among maternal parents, as well as among individual seeds (Donohue and Schmitt, 1998). Seed dormancy is defined as the inability of intact viable seed to germinate under favorable conditions (Hilhorst, 2007). In herbaceous plants, seed dormancy bears similar characteristics to bud dormancy, but the mechanisms involved may be different (Dennis, 1996; Okagami, 1986). The physiological mechanisms underlying the shift between dormancy and germination are regulated by hormones (Hilhorst, 1995; Kermode, 2005; Koornneef et al., 2002; Kucera et al., 2005). Gibberellins (GAs) and cytokinins promote seed germination (Finch-Savage and Leubner-Metzger, 2006; Powell, 1987; Toh et al., 2008) while ABA induces and maintains dormancy (Finkelstein, 2010; Grappin et al., 2000; Ofir and Kigel, 2007; Okamoto et al., 2006; Okamoto et al., 2010; Qin and Zeevaart, 2002; Thompson et al., 2000). Maternal control of germination can operate through maternal provisioning of resources and hormones in response to light environments experienced by the embryo (Botto et al., 1995). In *Arabidopsis*, ABA is produced by the embryo when the seed is produced in the mother plant and transported from the mother plant to the seeds as it is largely synthesized in maternal tissues (Argyris et al., 2008; Corbineau et al., 2002; Karssen et al., 1983). This suggests that ABA in the mother plant could induce dormancy of the mother plant itself.

Seed dormancy and germination are controlled by developmental and environmental cues (Bewley, 1997; Finch-Savage and Leubner-Metzger, 2006; Koornneef et al., 2002). The timing of germination is strongly influenced by the interaction between genetic and environmental factors before and after seed maturity. Delay of germination1 (DOG1) was identified to be related to the variation in dormancy in *Arabidopsis thaliana* and the expression of DOG1 was correlated with increased seed dormancy (Chiang et al., 2011). Phytochrome A and B genes are also believed to have effects on the regulation of seed dormancy and germination in lettuce,

tomato and many other species (Borthwick et al., 1952; Frankland and Taylorson, 1983; Furuya, 1968; Mancinelli et al., 1966; Olsen, 2005; Sage, 1992; Shinomura, 1997; Toole et al., 1956; Toole et al., 1955). The phytochrome system played an important role in daylength sensing in woody plants, and exhibited effects on biosynthesis of plant hormones (Olsen, 2005).

It is reasonable to hypothesize that environmental cues (temperature and photoperiod) that trigger dormancy in the mother plant would act similarly on seed dormancy/germination, and that seed germination can be a reliable indicator and predictor of the response of its mother plant under the same environmental condition. Precise phenotypic dissection of summer dormancy and associated traits will greatly enhance the understanding of tall fescue dormancy mechanism. Therefore, the goals of this research were 1)to explore the potential of using seed dormancy/germination as a surrogate phenotype for summer dormancy in tall fescue; 2)to establish a reliable summer dormancy rating system in field conditions based on regrowth height and senescence; and 3)to explore if seed germination/dormancy in response to various light and temperature combinations correlate with summer field dormancy.

Materials and methods

Evaluation of Field dormancy and establishment of a summer dormancy rating system

Two hundred and eighteen tall fescue accessions, requested from the National Plant Germplasm System (NPGS), in Pullman, Washington. The seeds of each accession are from one population of different plants with unknown produced year and environment. The seeds were then planted for evaluation of field summer dormancy at the J. Phil Campbell (JPC) Research Station at Watkinsville in November 2013. The soil was a Cecil sandy loam, Fine, kaolinitic, thermic Typic Kanhapludults. The average annual precipitation is 48 in. The average high

temperature is 32ºC in July and the lowest is 11ºC in January in Watkinsville. Seed of each accession was planted in a single row with three replications. Missing entries or incomplete rows were replanted in October 2014 in the same field in Watkinsville. Nine of the 218 accessions did not germinate or survive even after the replanting, which led to a total number of 209 accessions for field dormancy evaluation. The rows were evaluated for dormancy during summers 2014 and 2015. Summer dormancy was rated as a phenotypic score of percent senescence and regrowth height after clipped by a pushing mower at residual heights of 15cm in 2014 and 10cm in 2015. The senescence scores were determined based on the percentage of dead plants in each row. Digital image analysis was also used to estimate senescence percentage in each row. Digital images were captured in JPEG (joint photographic experts group, or ".jpg") format with an image resolution of 5152 x 3864 pixels using a Canon PowerShot ELPH 150IS digital camera (Canon USA, Melville, NY). The camera was set up on a light box equipped with four incandescent light bulbs (800 lumens, Longstar) inside and on the four corners of the box to provide a consistent lighting background. Digital images of each row were captured on July 28, 2014 and August 7, 2015. The growth stage of the plants were at seed set. The images were analyzed using the software Assess version 2.0 from APS Press (American Phytopathological Society, St. Paul, MN), developed originally for disease analysis on turf grass (Horvath and Vargas, 2005) using the options "Agronomist panel" and "GC-Auto". The percentage of green area was obtained from "GC-Auto" for the selected area of interest [AOI (Figure 1.1)]. Senescence percentage was calculated as 1-"percentage of green area of AOI from 'GC-Auto'". Rating scores were assigned to each accession based on senescence percentage, where: 90-100% =1, 80-90% =2, 70-80% = 3, 60-70% = 4, 50-60% = 5, 40-50% = 6, 30-40% = 7, 20-30% = 8, 10-20% = 9, and 0-10% = 10. The lower scores indicate higher summer dormancy (dormant),

and the high scores indicate higher summer activity (non-dormant). Visual ratings of percent senescence were also taken on June 14, June 25, July 2, July 10 2014, and June 25 2015, to establish an optimal timing for measuring the phenotype. The growth stage of the plants were at seed set.

Plants were clipped to a residual height of 15cm on June 28th, 2014 and on July 8th, 2015. Regrowth heights for each accession were measured after four weeks, on July 29th, 2014 and August 7th, 2015, and scores were assigned according to the following rating system where measurements are rounded off to the nearest half: 2.5cm or below = 1, 3 -5cm = 2, 5.5 -7.5cm =3, 8 -10cm = 4, 10.5 -12.5cm = 5, 13 -15cm = 6, 15.5 -17.5cm = 7, 18 -20cm = 8, 20.5 -22.5 cm = 9, and 23 cm or above = 10. Higher scores indicate a lower level of summer dormancy (non-dormant) and the lower scores indicate higher summer dormancy (dormant). To avoid confounding effects of water deficiency, supplemental irrigation was provided on an average frequency of twice a week. Weeds were controlled by spraying herbicides for broadleaf weeds, and manually weeding was conducted for summer grasses within and between rows.

An average phenotypic score of summer dormancy was assigned to each accession, using a combined average of digital image analysis senescence percentage scores (SPS) and regrowth height scores (RHS) of 3 replications and 2 years for each accession as in the following equation:

Summer Dormancy Rating Score (SDRS) = (SPS + RHS)/2

Testing the effect of different photoperiod/temperature combinations on seed germination

An initial germination test was conducted in a growth chamber (Conviron CMP5090, Winnipeg, Canada) on a set of 214 tall fescue accessions of unknown dormancy phenotypes from the NPGS collection and 4 checks with known dormancy. Seeds from each accession were

divided in two groups. One group with 3 replications was germinated under (16/8 hrs light/dark) and the other group was tested in 24 hrs dark, under the same temperature conditions, by placing the plates in wooden boxes covered with aluminum foil. The tests were conducted at temperatures 32 and 22^{o}C, respectively. Each replication consisted of 50 seeds placed on wet filter papers (Whatman, 90mm Circles, UK) in 10 cm petri dishes. Temperature affected germination more than photoperiod in this initial test. Therefore, a more extensive test was conducted using only germplasms with known dormancy phenotypes under various combinations of photoperiod and temperature extremes to determine the conditions that correlate with summer dormancy. Continental tall fescue cultivars GA186 and Jessup MaxQ (both developed at the University of Georgia) were included as non-dormant checks. The experimental population AGRFA-126 (AgResearch USA, Asheville, NC) and T0706-1 (a Mediterranean dormant experimental population developed at the University of Georgia) were included as dormant checks. Germination tests were conducted at 3 different low temperatures (18, 20 and 22ºC), which represent the temperature range of favorable growing conditions for tall fescue, and three high temperatures (30, 32 and 34ºC), which represent the high temperature range of summer conditions. The seed were tested in each temperature in combinations with each of 5 different photoperiods: 0/24, 12/12, 14/8, 16/8, and 24/0 hours of light/dark with light intensity of 230 µMol provided by 12 fluorescent lamps (Philips, 86W, 2.43m). Thirty seeds from each check accession were also placed on wet filter papers in 10 cm petri dishes. Germination rates were counted on the 14th day from the start of the test. To prevent drying from excessive evaporation in the growth chamber at the high temperatures, the petri dishes were placed in a 54.6 cm x 27.3 cm x 2.5 cm plastic trays containing about 500 ml water. The plates were arranged in a randomized complete block design with 3 replications within the growth chamber.

Each tray was considered a block. Germination rates of dormant and non-dormant checks were compared and the differences under each temperature and photoperiod combination were analyzed. The combinations of light/temperature that showed the least difference between dormant and non-dormant accessions under low temperature and the largest difference between the two at high temperature were considered potential candidates for a surrogate phenotype. The nature potential germination rate for each accession should be 100%, however it may variate due to different production environment, year and storage conditions. The seeds of the PI accessions were requested from NPGS, produced and increased in Pullman, Washington. The seeds production environment, year, processing standards and storage condition are uncertain. In this study, the low temperatures are the favorable germination condition for tall fescue seeds, under which the germination rate of each accession should reach its highest. In comparison, the high temperature representing a summer stress factor which influences the germination rate of each accession. Thus we used a relative ratio of the germination rate at a high temperature over the germination rate at a low temperature for each accession rather than an absolute value of germination rate at on certain temperature. This ratio was calculated for each accession by equation "Germination Ratio= Germination rate at high temperature/ Germination rate at low temperature" to account for any possible factor that may have influenced the germination rate difference among different accessions due to uncertain different production environments, years and storage conditions. The ratio was adopted to standardize germination rates of different accessions and eliminate variations caused by poor germination.

Correlation between field dormancy and germination phenotype

Based on field summer dormancy rating scores of the 209 accessions, 26 accessions with high scores (near 10) were selected as potential non-dormant candidates and 30 accessions with

low scores (near 1) were selected as putative dormant candidates to test the correlation between field scores and germination ratio scores. Field visual inspection of the activity of summer growth was conducted to confirm the dormancy status. Germination tests of the 56 putative dormant and non-dormant accessions were conducted as described above at 30ºC and 20ºC, under both 24 hrs light (GRL) and 24 hrs dark (GRD). The germination rates were counted from each combination and the ratio of germination rates at 30ºC over the germination rate at 20ºC was calculated for each photoperiod. A 30ºC/20ºC germination ratio near 1 indicates non-dormant while a ratio near zero indicates summer dormant accessions. Since tall fescue is a cross pollinated species and usually maintained as heterogeneous populations, we expect some degree of variation in the ratios within the same line.

Data Analysis

Statistical analysis of field senescence and regrowth heights were performed using Proc Mixed procedure in SAS 9.4 for windows (SAS 2012), assuming a compound symmetry variance-covariance structure. Replications and Year were considered random. Other variables were considered fixed. Lsmeans for each response variable were determined. Germination data was analyzed using Proc GLM in SAS 9.4 for windows (SAS 2012). The expected mean squares and estimates of the variance components were determined using the VARCOMP procedure in SAS 9.4. Phenotypic correlations (Pearson) among senescence, regrowth height, and germination ratios were estimated on the basis of accession means. T-tests were conducted to test the significance of the difference between the average germination rates of dormant and non-dormant checks at all the combinations of temperatures and photoperiod conditions in the growth chamber. The germination ratio under 24 hours of light for each putative accession was statistically analyzed and spearman correlation coefficients were determined between

germination ratios and field dormancy scores. Spearman correlations between the mean of field dormancy rating scores and the mean of germination ratio scores were determined. Broad-sense heritability for the three phenotypes (regrowth height, senescence percentage, and germination ratio under 24 hours of light) were calculated according to Vogel et al. (1981) using the equation: $H^2 = \sigma^2_G / (\sigma^2_G + (\sigma^2_{GY}/y) + (\sigma^2_E/yr))$, Where σ^2_G = Variance of accessions/genotypes, σ^2_{GY} = variance of accession x year, σ^2_E = variance of error, y = number of years, and r = number of replications. It was also calculated for the for the germination ratio under 24 hours of light as: $H^2 = \sigma^2_G / (\sigma^2_G + (\sigma^2_E/r))$, where σ^2_G = Variance of accessions/genotypes, and σ^2_E = variance of error).

Results

Summer dormancy evaluation in the field: Regrowth height and senescence

Over two years, the average regrowth height for the 209 tall fescue accessions grown in the field ranged from 0 to 28.7cm (Table 2.1). Senescence percentage ranged from 30.3to 93.4% and was significant among accessions (Table 2.1). Regrowth height scores ranged from 1 to 10 and senescence percentage scores ranged from 1-7 (Table 2.1). Variance analysis of both plant regrowth height and senescence percentage in the field over two years showed a significant difference ($p<0.01$) among 209 accessions tested (Table 2.2). There was a significant accession by year interaction ($p <0.01$). The year effect was significant for plant regrowth height of the 209 accessions tested ($p<0.01$) between 2014 and 2015, however, there was no significant difference in senescence percentage ($p>0.05$) between the two years (Table 2.2).

Effect of photoperiod/temperature combinations on seed germination

Within each of the five different photoperiod conditions, the magnitude of differences between dormant and non-dormant checks under low temperatures (18ºC to 22ºC) were much smaller than those observed at high temperatures (30ºC to 34ºC),especially at complete dark and 24 hrs light (Table 2.3, Figure 2.2). At the photoperiods involving light/dark cycles (12/12, 14/10, 16/8), the magnitude of differences in germination between non-dormant and dormant checks were close between both low and high temperatures (Table 2.3, Figure 2.2). They ranged from 9.4% to 12.8% at 12 hrs photoperiod and lower temperature, and from 33.9% to 60.6% at the higher temperatures for the same photoperiod (Table 2.3). At 14 hrs photoperiod, the difference in germination rates between non-dormant and dormant checks ranged from 17.2% to 28.9% at the low temperature gradient and from 32.2% to 53.9% at the high temperature gradient. At the 16 hrs photoperiod, the difference in germination rates between non-dormant and dormant checks ranged from 12.2% to 28.9% at the low temperature gradient and from 28.9% to 56.7% at the high temperature gradient (Table 2.3).

Within the low temperatures and across photoperiod gradients, the differences in germination between non-dormant and dormant means ranged from 9.4% to 28.9% at 18ºC, from 10% to 24.4% at 20ºC, and from 12.8% to 32.8% at 22ºC. Within the high temperature range and across photoperiod gradients, the magnitude of differences between non-dormant and dormant check were much greater compared to low temperatures. They ranged from 28.9% to 77.2% under 30ºC, from 39.4% to 70.6% under 32ºC, from 37.8 % to 62.8% at 34ºC. Above 32ºC, the magnitude of difference was smaller, mainly because the germination of both dormant and non-dormant checks was affected by the high heat (Figure 2.3). The largest differences in germination were observed for the combinations 24 hrs light and 30ºC (71.7%) and 0 hrs light

and 30°C (77.2%). The smallest differences were observed at the combinations of 24 hrs light and 20°C (10.6%) followed by the combination of 0 hrs light and 20°C (18.9%). The ratio of germination at 30°C over at 20°C under 24 hrs photoperiod was therefore selected for validation as a surrogate phenotype to separate dormant and non-dormant tall fescue germplasms.

Validation of seed germination as a predictor of summer dormancy

Regrowth height in 56 accessions selected based on their field dormancy classification ranged from 2.2 to 26.6cm corresponding to regrowth height scores ranging from 1-10 (Table 2.4). Senescence percentage ranged from 30.3% to 84.3% corresponding to senescence scores ranging from 2 to 7. The average phenotypic scores for the 56 accessions ranged from 1.5 to 8.5 (Table 2.4). Ratio of germination rates at 30°C over at 20°C under 24 hours of light ranged from 0.1 to 1.3 corresponding to germination scores ranging from 1-10 and was significantly different between the 56 accessions [$p < 0.01$ (Table 2.4, Table 2.5)].

Correlation between field phenotypes and germination ratios, and Inheritance of field summer dormancy traits and seed germination ratio.

Spearman correlation analysis showed a significant negative correlation between regrowth height and senescence [$r = -0.71$, $p < 0.01$ (Table 2.6)]. The ratios of germination rates at 30°C over germination percent at 20°C under 24 hours of light showed a significant positive correlation with regrowth height ($r = 0.52$, $p < 0.01$), but a negative correlation with senescence ($r = -0.48$, $p < 0.01$). The average field phenotypic scores for dormancy (SDRS) showed a high correlation with the germination ratio scores ($r = 0.7$, $p < 0.01$). Broad-sense heritability for regrowth height was 0.59 (Table 2.7). Senescence percentage showed a higher heritability of 0.85, while the germination ratio showed a broad sense heritability of 0.87 (Table 2.7).

Discussion

Summer dormancy evaluation in the field: Regrowth height and senescence

To accelerate the breeding process, a less time consuming and accurate phenotyping method is needed for an efficient selection in breeding programs. The significant differences in plant regrowth height and senescence percentages ($p<0.01$) among these accessions in the field over two years indicate that the genetic variation of the two growth parameters existed in these 209 accessions tested, given the control of potential confounding factors like water shortage. The significant accession by year interaction ($p < 0.01$) appeared to be due mostly to the magnitude of year effect since the correlation between years was 0.50 for regrowth height and 0.60 for senescence. Even though soil moisture factor was uniformly maintained across the two years by providing supplemental irrigation, temperature appears to be the primary factor leading to the differences in growth between years. The year 2014 was milder with an average high temperature of 32.2°C (normal: 32.8°C) in July compared to the year 2015 where the average high temperature was 33.8°C for the same month. There was no significant difference in senescence percentage between the two years (Table 2.2), suggesting that senescence, if measured accurately might be a good indicator of summer dormancy. Some research has suggested that leaf senescence could be used to identify summer dormant plants (Norton et al., 2006b). Others postulated that it may not always be a reliable indicator since the number of green leaves and percent greenness did not differentiate tall fescue genotypes accurately and that the loss of greenness can be a response to drought and/or heat stress rather than a sign of summer dormancy (Bhamidimarri et al., 2012). There is also support for the use of regrowth height after a midsummer rain in the field as a measure of summer dormancy (Culvenor and Boschma, 2005; Oram, 1983; Oram, 1984; Oram and Freebairn, 1984). Dormancy induction has been related to

slower rates of cell division and hormone interaction with the mechanisms of cell cycle regulation (Olsen, 2005). Previous studies indicated that the herbage production under full, continuous irrigation condition over the summer (Laude, 1953) could be another indicator of summer dormancy. Studies in bulbous canarygrass (*Phalaris aquatica*) suggested quantifying dormancy by measuring growth of the buds in the field after a midsummer rainstorm (Culvenor and Boschma, 2005; Oram, 1983; Oram, 1984; Oram and Freebairn, 1984), or following transfer into controlled environment with lower temperature and higher moisture (Biddiscombe et al., 1977; Hoen, 1968).

Our field evaluation results indicated that the summer dormancy phenotype is highly variable and can be partitioned into several classes rather than either dormant or non-dormant. Therefore, establishing a rating method that accounts for the variability would be more realistic. In this research, we devised a scoring system based on plant regrowth height and digital image analysis of senescence. Digital image analysis allows a more accurate measure of greenness compared to visual ratings. The high correlation between regrowth height and senescence percentage ($r = -0.7$) suggest that the two phenotyping methods are strongly associated with summer activity and therefore it is reasonable to combine them to generate a comprehensive field phenotypic score for summer dormancy, and reduce the error associated with each individual phenotyping method.

The heritabilities of regrowth height (0.59) and senescence percentage (0.85) suggest that there is considerable genetic variation in the two traits that can form the basis of selection for either higher summer activity or dormancy. The two traits appear to be quantitative in nature, and the lower heritability of regrowth height is a possible indication of the impact of environmental variables. Tall fescue is also an outcrossing allohexaploid ($2n = 6x = 42$) with a

high level of self-incompatibility (Hopkins et al., 2009), that is usually maintained as heterogeneous populations. Therefore, some degree of variation within populations in the field and germination phenotypes is expected.

Effects of photoperiod and temperature combinations on seed germination

The difference in germination between non-dormant and dormant checks appeared to be greater across the temperature gradient (low vs high) and much smaller across the photoperiod gradient within each temperature. These results suggest that temperature may have a more pronounced effect in inducing summer dormancy in tall fescue. Photoperiod showed less influence on dormancy induction compared to temperature in other species like apples and pears (Olsen, 2005). The seed used in the study originated from different locations, therefore variation in germination is expected. The ratio of germination rate at high temperature over the germination rate at low temperature was used instead of the absolute values of germination rates to eliminate variations caused by differences in germination caused by factors such as seed production environments, processing, storage conditions, and duration of storage. Germination at the lower temperature is considered the base line and germination at the high temperature represents the deviation from the base line.

The wide range in germination ratios at 30ºC /20ºC observed between the 56 accessions tested (0.1 to 1.3) and the fairly high genetic variance component (Table 2.5) suggest that the variation of seed dormancy and germination may be strongly influenced by genetic factors. Different genotypes can exhibit variations in gene expression and protein synthesis in seeds leading to different seed germination responses (Donohue, 2009). Genes such as *DOG1* was related to the variation in dormancy in *Arabidopsis thaliana*, where low temperature during seeds maturation, increases its expression leading to higher seed dormancy (Chiang et al., 2011). The

differences in *DOG1* expression were viewed as a possible indication of the influence of geographical origins on the level of dormancy with southern area tending to produce more dormant accessions (Chiang et al., 2011). Temperatures above 30ºC seemed to affect germination of both dormant and non-dormant checks as evidenced by the mixed magnitude of differences, suggesting that 34ºC and possibly 32ºC are too high to represent a practical testing environment. Benvenuti et al. (2001) found that seed germination was more dependent on light at lower temperature. Several reports suggested that summer growth in most temperate grasses is slowed by high temperature and can be influenced by soil moisture (Burns and Chamblee, 1979; Cooper and Tainton, 1968; Hoveland et al., 1974; Sprague, 1943). The combined action of long days and increased temperature induced summer dormancy in various species like Poa bulbosa L., P. scabrella; Allium cepa L., A. sativum L., Ranunculus spp., and Anemone coronaria L (Ofir and Kigel, 2007). The results of our study were similar to those under temperatures of 30ºC, the non-dormant check accessions germinated normally while germination of the dormant checks remained very low. Germination studies in switchgrass reported low germination rate under a constant, warm temperature (30ºC) for a high-dormancy while the low-dormancy cultivar showed good germination percentage under the same temperature condition (Duclos et al., 2014).

Validation of seed germination as a predictor of summer dormancy

The environment experienced by the maternal plants may influence both seed and mother plant dormancy. Seed dormancy and germination are controlled by the interaction of developmental and environmental cues. The distribution of dormancy in the sea beet *Beta vulgaris* ssp. *maritima* was reported to have a positive correlation with annual temperatures, especially in summer (Wagmann et al., 2012). In *Arabidopsis thaliana,* maternal photoperiod

influenced seed germination rates and the speed of germination with the short-day photoperiod causing higher germination (Munir et al., 2001). The photoperiod at the time of seed maturation can predict the seasonal conditions of newly disseminated seeds. Variation in seasonal dormancy may be influenced by genes expressed in the maternal parents, or maternal provisioning of resources and hormones in response to light environments that the embryo is exposed to (Botto et al., 1995). The physiological mechanism behind the transition between dormancy and germination is known to be regulated by hormones like Abscisic acid (Koornneef et al., 2002; Kucera et al., 2005) that promotes dormancy (Hilhorst, 1995; Kermode, 2005) and Gibberellic acid that promotes germination (Finch-Savage and Leubner-Metzger, 2006; Toh et al., 2008). Abscisic acid is produced by the embryo during seed development in the mother plants, and controls dormancy and germination through seed sensitivity to endogenous concentration (Argyris et al., 2008; Corbineau et al., 2002). Studies in *Poa bulbosa* suggested that summer dormancy is possibly induced by a combined effect of increased levels of endogenous ABA, long day photoperiod, and water deficit (Ofir and Kigel, 2007). In *Arabidopsis,* ABA is mostly synthesized in maternal tissues and transported from the mother plant (Karssen et al., 1983).

In the present study, the ratios of germination percent at $30^oC/20^oC$ under 24 hours of light showed a positive correlation with regrowth height ($r= 0.52$, $p<0.01$) and a negative correlation with senescence ($r= -0.48$, $p<0.01$). The average field phenotypic scores for dormancy (SDRS) showed a high correlation with the germination ratio scores ($r= 0.7$, $p<0.01$). The high correlation between field phenotyping data and surrogate phenotyping data suggests that germination ratio under 24 hours of light can be successfully used as potential surrogate phenotyping method for summer dormancy.

Inheritance of field summer dormancy traits and seed germination ratio

The high broad-sense heritability observed for regrowth height (0.59), senescence (0.85), and germination ratio (0.87) suggest that most of the variation is due to genetic factors and also to the controlled environments compared to field conditions. Studies of summer dormancy in sea beet suggested that the trait is highly heritable and could change in the future because of climate change (Wagmann et al., 2012), inferring that the mother plants may show the same degree of dormancy. Maternal environmental effects have been viewed as a form of adaptive phenotypic plasticity across-generations. Donohue and Schmitt (1998) suggested that the evolution of seed dormancy may involve selection based on genetic variation in germination responses to the offspring environments among maternal parents, as well as among individual seeds. The high correlation between field phenotyping of summer dormancy and the surrogate phenotype based on seed germination, in addition to the high heritability, suggests that the surrogate phenotyping method can be applied in the breeding process to select for summer dormancy or summer activity. Research to determine the genetic gain from selection based on the surrogate phenotype which would require making crosses and developing structured populations which should be carried out in the future.

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Table 2.1. Least square means of regrowth height; senescence percentage and their assigned scores, along with summer dormancy rating score (SDRS) calculated as an average of regrowth score and senescence score for 209 PI accessions evaluated in the field in Watkinsville, GA in 2014 and 2015.

Accession	Origin	Regrowth height (cm) †	Regrowth score ‡	Senescence (%) †	Senescence score ‡	SDRS §
PI 610952	Tunisia	16.3	7	62.9	4	5.5
PI 249738	Greece	9.2	4	75.6	3	3.5
PI 234747	Spain	20.6	9	50.9	4	6.5
PI 598944	Italy	10.8	5	73.2	3	4.0
PI 235018	Germany	16.9	7	58.7	5	6.0
PI 251822	Italy	16.7	7	62.1	4	5.5
PI 289654	N/A	12.7	6	68.3	4	5.0
PI 598868	Morocco	11.5	5	50.1	5	5.0
PI 235244	Spain	16.8	7	69.1	4	5.5
PI 598889	Morocco	20.0	8	34.4	7	7.5
PI 234746	Spain	14.8	6	71.0	3	4.5
PI 199249	Greece	11.1	5	65.2	4	4.5
PI 598893	Morocco	17.4	7	46.4	6	6.5
PI 659606	Tunisia	14.7	6	80.8	2	4.0
PI 283312	N/A	3.0	2	85.9	2	2.0
PI 261029	United Kingdom	15.0	7	54.3	5	6.0
PI 610949	Morocco	18.9	8	40.9	6	7.0
W6 16099	Tunisia	12.6	6	66.1	4	5.0
PI 231564	Tunisia	14.7	6	58.1	5	5.5
PI 234748	Spain	7.5	3	70.4	3	3.0
PI 234718	France	11.9	5	64.8	4	4.5
PI 251383	Pakistan	11.7	5	65.0	4	4.5
PI 610933	Italy	14.3	6	68.8	4	5.0
PI 610957	Italy	16.3	7	67.7	4	5.5
PI 265359	Netherlands	16.7	7	62.3	4	5.5
PI 231553	Algeria	11.9	5	78.8	3	4.0
PI 610948	Morocco	7.6	4	67.0	4	4.0
PI 598831	Morocco	12.3	5	43.8	6	5.5
PI 610943	Tunisia	15.3	7	61.2	4	5.5
PI 251584	Former Serbia and Montenegro	13.7	6	72.4	3	4.5
PI 231555	Algeria	16.4	7	59.7	5	6.0
PI 231557	Morocco	9.8	4	62.7	4	4.0

PI 229692	Iran	0.0	1	74.5	3	2.0
PI 639781	Morocco	11.1	5	66.2	4	4.5
PI 206270	Turkey	4.0	2	75.1	3	2.5
PI 610918	Tunisia	5.7	3	69.6	4	3.5
PI 231561	Morocco	12.4	5	77.5	3	4.0
PI 632568	Italy	13.5	6	62.7	4	5.0
PI 610954	Morocco	25.2	10	39.1	7	8.5
PI 234893	Switzerland	20.5	9	55.3	5	7.0
GA 186-T	United States	26.6	10	34.4	7	8.5
PI 632587	Tunisia	24.3	10	34.6	7	8.5
PI 227446	Iran	10.0	5	72.1	3	4.0
PI 231559	Morocco	16.0	7	40.2	6	6.5
GAFAll03	United States	22.4	9	35.7	7	8.0
PI 255874	Poland	12.9	6	45.7	6	6.0
PI 204447	Turkey	11.6	5	54.3	5	5.0
PI 598917	Tunisia	18.5	8	47.7	6	7.0
PI 237516	Tunisia	14.8	6	56.4	5	5.5
PI 231558	Morocco	16.5	7	38.3	7	7.0
PI 610961	Morocco	22.3	9	37.5	7	8.0
PI 598934	Italy	22.0	9	40.0	7	8.0
PI 223369	Iran	9.0	4	74.7	3	3.5
PI 610953	Morocco	13.1	6	60.3	4	5.0
PI 598870	Morocco	14.0	6	60.1	4	5.0
PI 224974	South Africa	12.2	5	69.4	4	4.5
PI 265353	Netherlands	16.3	7	62.4	4	5.5
PI 203728	Uruguay	15.4	7	53.6	5	6.0
PI 598945	Italy	12.8	6	68.5	4	5.0
PI 610919	Tunisia	20.1	9	47.2	6	7.5
PI 632585	United States	17.6	8	50.8	5	6.5
PI 610960	Morocco	17.5	8	46.5	6	7.0
PI 229947	Iran	5.3	3	92.9	1	2.0
PI 632572	Tunisia	15.8	7	68.9	4	5.5
PI 265357	Netherlands	11.1	5	61.7	4	4.5
PI 237559	Italy	20.7	9	70.3	3	6.0
PI 598866	Morocco	17.4	7	51.6	5	6.0
PI 234882	Switzerland	18.9	8	63.6	4	6.0
PI 598826	Morocco	0.0	1	93.4	1	1.0
PI 598850	Morocco	20.8	9	55.7	5	7.0

PI 598828	Morocco	13.9	6	46.1	6	6.0
PI 598943	Italy	19.7	8	51.0	5	6.5
PI 231556	Algeria	15.6	7	50.0	5	6.0
PI 639823	Japan	13.2	6	30.8	7	6.5
PI 598948	Italy	21.0	9	38.8	7	8.0
PI 598848	Morocco	23.7	10	45.7	6	8.0
PI 229755	Iran	10.5	5	72.8	3	4.0
PI 255416	Former Serbia and Montenegro	14.2	6	51.0	5	5.5
PI 234884	Switzerland	17.1	7	67.2	4	5.5
PI 235125	Netherlands	19.1	8	38.7	7	7.5
PI 598930	Italy	17.7	8	62.7	4	6.0
PI 610946	Morocco	14.6	6	56.0	5	5.5
PI 619476	Tunisia	8.0	4	83.2	2	3.0
PI 255363	Former Serbia and Montenegro	14.8	6	63.4	4	5.0
PI 636646	Japan	16.4	7	39.3	7	7.0
PI 231560	Morocco	13.2	6	48.9	6	6.0
PI 598832	Morocco	18.0	8	52.2	5	6.5
PI 255875	Poland	17.0	7	64.3	4	5.5
PI 200339	Israel	12.2	5	70.7	3	4.0
PI 598949	Italy	17.2	7	78.8	3	5.0
PI 265358	Netherlands	16.4	7	58.2	5	6.0
PI 598852	Morocco	16.4	7	47.8	6	6.5
PI 234719	France	24.5	10	46.6	6	8.0
PI 610963	Tunisia	21.4	9	57.6	5	7.0
PI 610938	Morocco	20.5	9	60.9	4	6.5
KY 31	United States	21.8	9	35.8	7	8.0
PI 253311	Former Serbia and Montenegro	27.5	10	47.3	6	8.0
PI 639782	Spain	22.8	10	52.0	5	7.5
PI 234047	Spain	25.7	10	36.5	7	8.5
PI 632493	Tunisia	18.4	8	62.1	4	6.0
PI 231554	Algeria	0.0	1	74.9	3	2.0
PI 632567	Tunisia	13.3	6	68.6	4	5.0
PI 610951	Morocco	23.7	10	38.7	7	8.5
PI 639780	United States	21.7	9	48.4	6	7.5
PI 598924	Italy	20.5	9	53.6	5	7.0

PI 598860	Morocco	24.0	10	59.1	5	7.5
PI 598936	Italy	15.2	7	60.7	4	5.5
PI 257742	Sweden	20.6	9	37.2	7	8.0
PI 234892	Switzerland	17.1	7	62.5	4	5.5
PI 234881	Switzerland	16.8	7	35.3	7	7.0
PI 598919	Tunisia	21.0	9	58.2	5	7.0
PI 231562	Morocco	18.0	8	46.7	6	7.0
PI 235036	Sweden	3.0	2	90.1	1	1.5
PI 610921	Tunisia	14.6	6	62.6	4	5.0
PI 598851	Morocco	17.4	7	38.1	7	7.0
PI 598891	Morocco	26.7	10	51.7	5	7.5
PI 232878	Algeria	15.9	7	56.0	5	6.0
PI 174209	Turkey	4.8	2	70.5	3	2.5
Max Q	United States	21.5	9	35.0	7	8.0
W6 16094	Tunisia	14.6	6	65.7	4	5.0
PI 598829	Morocco	8.8	4	78.9	3	3.5
PI 598923	Italy	12.9	6	67.5	4	5.0
PI 610940	Morocco	18.4	8	43.6	6	7.0
PI 251122	Bosnia and Herzegovina	10.4	5	49.1	6	5.5
PI 229501	Iran	0.0	1	87.5	2	1.5
PI 237178	N/A	18.5	8	57.6	5	6.5
PI 636645	Japan	10.3	5	38.1	7	6.0
PI 598855	Morocco	16.3	7	55.5	5	6.0
W6 16206	Italy	18.1	8	49.1	6	7.0
GA-95-101T	United States	18.4	8	47.9	6	7.0
PI 232877	Algeria	15.8	7	47.6	6	6.5
PI 598858	Morocco	18.1	8	37.5	7	7.5
PI 598844	Morocco	20.4	9	45.1	6	7.5
PI 195477	South Africa	18.8	8	60.3	4	6.0
PI 198088	Morocco	2.5	2	81.3	2	2.0
W6 16065	Tunisia	15.2	7	67.0	4	5.5
PI 619480	Tunisia	14.9	6	69.2	4	5.0
PI 598918	Tunisia	8.2	4	61.9	4	4.0
PI 619481	Tunisia	11.2	5	76.0	3	4.0
PI 598941	Italy	11.5	5	64.7	4	4.5
PI 632496	Tunisia	18.8	8	57.3	5	6.5
PI 208681	Algeria	25.8	10	30.3	7	8.5
PI 639826	Japan	15.8	7	32.3	7	7.0

PI 598836	Morocco	21.3	9	64.0	4	6.5
PI 225572	South Africa	28.7	10	51.8	5	7.5
W6 16085	Tunisia	19.9	8	51.4	5	6.5
PI 632582	Italy	16.1	7	57.5	5	6.0
PI 598932	Italy	15.9	7	78.7	3	5.0
PI 234720	France	19.0	8	56.3	5	6.5
GA-186	United States	22.5	9	33.9	7	8.0
PI 610920	Tunisia	13.2	6	70.9	3	4.5
PI 598863	Morocco	9.5	4	67.0	4	4.0
PI 598942	Italy	2.2	1	71.7	3	2.0
PI 639825	Japan	22.7	10	43.0	6	8.0
PI 598929	Italy	4.9	2	79.5	3	2.5
PI 184041	Serbia	15.8	7	66.2	4	5.5
PI 260245	Germany	17.7	8	60.7	4	6.0
PI 265354	Netherlands	15.4	7	57.4	5	6.0
PI 598925	Italy	14.1	6	68.1	4	5.0
PI 251823	Italy	17.8	8	47.1	6	7.0
PI 598903	Morocco	16.5	7	79.2	3	5.0
PI 634229	Tunisia	14.4	6	59.6	5	5.5
PI 659607	Tunisia	9.7	4	73.2	3	3.5
PI 595048	N/A	18.1	8	54.1	5	6.5
PI 234906	Switzerland	20.0	8	46.5	6	7.0
PI 234890	Switzerland	16.9	7	55.1	5	6.0
PI 231563	Portugal	10.2	5	70.1	3	4.0
PI 233237	Israel	9.2	4	69.2	4	4.0
PI 610956	Tunisia	3.5	2	65.0	4	3.0
PI 232876	Algeria	15.0	6	42.2	6	6.0
PI 265352	Netherlands	11.5	5	41.8	6	5.5
PI 632494	Tunisia	8.8	4	57.0	5	4.5
PI 598882	Morocco	12.6	6	58.6	5	5.5
PI 659884	N/A	6.9	3	85.6	2	2.5
PI 208680	Algeria	17.2	7	70.6	3	5.0
PI 207769	Israel	9.1	4	68.7	4	4.0
PI 598841	Morocco	22.5	9	35.6	7	8.0
PI 633833	Tunisia	15.9	7	48.6	6	6.5
PI 234883	Switzerland	23.1	10	35.9	7	8.5
PI 253438	Former Serbia and Montenegro	16.9	7	57.2	5	6.0
PI 598849	Morocco	18.3	8	51.2	5	6.5

PI 221927	Afghanistan	5.1	3	67.0	4	3.5
PI 598846	Morocco	23.8	10	31.9	7	8.5
PI 598927	Italy	4.9	2	72.0	3	2.5
PI 289651	N/A	2.1	1	85.5	2	1.5
PI 659897	N/A	15.7	7	74.6	3	5.0
PI 211032	Afghanistan	21.9	9	52.5	5	7.0
GA 29	United States	19.7	8	54.8	5	6.5
PI 232880	Algeria	1.6	1	85.4	2	1.5
PI 251583	Former Serbia and Montenegro	6.9	3	77.8	3	3.0
PI 234717	France	10.8	5	78.9	3	4.0
PI 227505	Iran	9.0	4	67.0	4	4.0
PI 610937	Morocco	9.7	4	79.3	3	3.5
PI 174210	Turkey	4.0	2	84.3	2	2.0
PI 598842	Morocco	10.8	5	63.8	4	4.5
PI 235470	Switzerland	16.1	7	50.8	5	6.0
PI 264766	Netherlands	3.4	2	71.8	3	2.5
PI 598834	Morocco	22.9	10	42.9	6	8.0
PI 231552	Algeria	0.0	1	75.2	3	2.0
PI 224975	South Africa	15.3	7	64.2	4	5.5
GAFall01	United States	18.8	8	32.8	7	7.5
PI 639824	Japan	7.8	4	49.0	6	5.0
PI 229451	Iran	2.3	1	79.0	3	2.0
PI 204446	Turkey	11.0	5	81.7	2	3.5
PI 232879	Algeria	12.8	6	69.7	4	5.0
PI 250963	Macedonia	6.7	3	81.1	2	2.5
PI 208679	Algeria	4.6	2	78.5	3	2.5
AGRFA-126	New Zealand	10.7	5	83.3	2	3.5
T0706-1	United States	9.4	4	70.9	3	3.5

† Regrowth height and senescence = mean regrowth height and mean senescence percentage in the field over two years and three replications.
‡ Regrowth height score and senescence score = Average regrowth height or senescence score on the 1-10 scale across two years and three replications.
§ SDRS: Summer dormancy rating score = Phenotypic score based on the average of the regrowth height and senescence scores for each accession.

Table 2.2. Mean squares and variance components of regrowth height and senescence of tall fescue accessions evaluated for summer dormancy in the field in Watkinsville, GA for 2014 and 2015 years, and 3 replications.

		Regrowth height (cm)		Senescence (%)	
Source	DF	Mean Squares	Variance components	Mean Squares	Variance components
Accession	208	117.08 **	17.68	657.51**	166.5
Rep	2	7.16	0.035	163.50	0.4
Year	1	1214.29 **	6.23	275.91	48.8
Accession*Year	208	42.89 **	19.07	126.11	28.56
Error	832	14.31	14.31	83.34	83.34

** denotes statistical significance at the 0.01 probability level.

Table 2.3. Mean differences in germination rates (%) between non-dormant and dormant tall fescue check cultivars. The seed was germinated in growth chambers at various combinations of temperatures and photoperiods with three replications.

Photoperiod	Reps	Temperature					
		18℃	20℃	22℃	30℃	32℃	34℃
24 h light	3	20.0 *	10.6 ns	32.8 **	71.7**	70.6 **	62.8 **
16 h light	3	28.9 **	24.4 **	12.2 *	28.9 **	56.7 **	42.8 **
14 h light	3	28.9 **	17.2 *	20.6*	32.2 **	39.4 **	53.9 **
12 h light	3	9.4 ns	10.0 ns	12.8 *	33.9 **	48.9 **	60.6 **
0 h light	3	28.9 **	18.9 **	22.8 **	77.2 **	67.2 **	37.8 **

* Indicates significance at $P < 0.05$.
** Indicates significance at $P < 0.01$.
ns Indicates non sigificnat t test.

Table 2.4. Least square means of regrowth height, senescence, germination ratio (30/20ºC) under light (GRL), and their scores on a 1-10 scale for 56 tall fescue entries selected based on their dormancy status in the field. SDRS represents summer dormancy rating score based on the average of regrowth height and senescence.

Accession name	Regrowth height (cm) †	Regrowth height score ‡	Senescence (%) †	Senescence score ‡	GRL §	GRL score ¶	SDRS#
PI 249738	9.2	5	75.6	3	0.4	4	4.0
PI 234747	20.6	9	50.9	5	0.1	2	7.0
PI 289654	12.7	6	68.3	4	0.6	7	5.0
PI 598889	20.0	8	34.4	7	1.0	10	7.5
PI 610949	18.9	8	40.9	6	0.9	9	7.0
PI 234748	7.5	3	70.4	3	1.1	10	3.0
PI 251383	11.7	5	65.0	4	0.7	7	4.5
PI 610957	16.3	7	67.7	4	0.3	4	5.5
PI 231553	11.9	5	78.8	3	0.5	5	4.0
PI 610948	7.6	4	67.0	4	0.6	6	4.0
PI 639781	11.1	5	66.2	4	0.8	9	4.5
PI 610954	25.2	10	39.1	7	1.2	10	8.5
GA 186-T	26.6	10	34.4	7	1.3	10	8.5
PI 632587	24.3	10	34.6	7	1.0	10	8.5
PI 227446	10.0	5	72.1	3	0.3	3	4.0
GAFAll03	22.4	9	35.7	7	0.9	10	8.0
PI 223369	9.0	4	74.7	3	0.6	6	3.5
PI 610953	13.1	6	60.3	4	0.6	6	5.0
PI 598848	23.7	10	45.7	6	0.9	10	8.0
PI 234719	24.5	10	46.6	6	0.5	6	8.0
KY 31	21.8	9	35.8	7	1.0	10	8.0
PI 234047	25.7	10	36.5	7	0.6	6	8.5
PI 610951	23.7	10	38.7	7	0.8	9	8.5
PI 234892	17.1	7	62.5	4	1.0	10	5.5
PI 174209	4.8	2	70.5	3	0.6	7	2.5
Max Q	21.5	9	35.0	7	1.0	10	8.0
W6 16094	14.6	6	65.7	4	0.9	9	5.0
PI 598829	8.8	4	78.9	3	0.8	9	3.5
PI 598858	18.1	8	37.5	7	1.1	10	7.5
PI 598844	20.4	9	45.1	6	1.2	10	7.5
PI 198088	2.5	1	81.3	2	0.4	5	1.5
PI 208681	25.8	10	30.3	7	0.9	10	8.5
PI 598932	15.9	7	78.7	3	0.5	6	5.0
GA-186	22.5	9	33.9	7	0.9	10	8.0

PI 598942	2.2	1	71.7	3	0.2	3	2.0
PI 639825	22.7	10	43.0	6	0.6	6	8.0
PI 598929	4.9	2	79.5	3	0.4	5	2.5
PI 598925	14.1	6	68.1	4	0.4	5	5.0
PI 251823	17.8	8	47.1	6	0.3	4	7.0
PI 598903	16.5	7	79.2	3	0.6	7	5.0
PI 659607	9.7	4	73.2	3	0.9	10	3.5
PI 632494	8.8	4	57.0	5	0.2	2	4.5
PI 598841	22.5	9	35.6	7	0.8	9	8.0
PI 234883	23.1	10	35.9	7	0.3	4	8.5
PI 253438	16.9	7	57.2	5	0.7	7	6.0
PI 598846	23.8	10	31.9	7	1.1	10	8.5
PI 598927	4.9	2	72.0	3	0.1	2	2.5
PI 251583	6.9	3	77.8	3	0.1	1	3.0
PI 227505	9.0	4	67.0	4	0.4	4	4.0
PI 174210	4.0	2	84.3	2	0.5	5	2.0
PI 235470	16.1	7	50.8	5	0.7	8	6.0
PI 598834	22.9	10	42.9	6	0.9	9	8.0
GAFall01	18.8	8	32.8	7	0.9	10	7.5
PI 232879	12.8	6	69.7	4	0.5	5	5.0
AGRFA-126	10.7	5	83.3	2	0.4	5	3.5
T0706-1	9.4	4	70.9	3	0.7	7	3.5

† Regrowth height/senescence: Average regrowth height or senescence of each of 56 putative accessions across two years and three replications.
‡ Regrowth height/senescence score: Average regrowth height or senescence score based on the score scale 1-10 across two years and three replications.
§ GR Light = Germination ratio of percent germination a 30℃ over that at 20℃ under 24 hours of light.
¶ GRL score = The score of germination ratio of germination rate under 30℃ over that under 20℃ under 24 hours of light conditions of each putative accession based on the same score scale as senescence percentage described in material and methods (scale 1-10).
# SDRS = Field phenotypic score calculated as the average of regrowth height and senescence scores for each accession.

Table 2.5. Mean squares and variance components of germination ratios (30/20°C) under 24 hours of light for 56 tall fescue accessions germinated grown in growth chambers

Source	DF	Mean Square of GR Light	Variance components
Accession	55	0.28 **	0.08
Rep	2	0.011	0.06
Error	110	0.05	0.05

** denotes statistical significance at the 0.01 probability level.

Table 2.6. Spearman Correlation coefficient between regrowth height scores, senescence scores, germination ratio under light scores, and summer dormancy rating scores (SDRS) in 56 accessions of tall fescue evaluated for summer dormancy.

	Senescence	GR Light	Phenotypic score (SDRS)
Height	-0.7 **	0.5 **	
Senescence		-0.5 **	
GR Light score			0.7**

** denotes statistical significance at the 0.01 probability level.

Table 2.7. Broad sense heritability of regrowth height and senescence percentage and germination ratio (30/20°C) under 24 hours of light of tall fescue accessions evaluated in the field and growth chamber for summer dormancy.

	Regrowth height (cm)	**Senescence (%)**	**GR Light**
H^2†	0.59	0.85	0.87

† H^2 stands for broad sense heritability, the percentage of summer dormancy phenotypic variance which can be explained by genetic variance

Figure 2.1. Photos of dormant (left) and non-dormant (right) with green percentage highlighted in red area with a Digital image analysis method on the area of interest with a GC value of 11.2% (left) and 72.0% (right), which lead to a senescence percentage of 88.8% (left) and 28.0% (right).

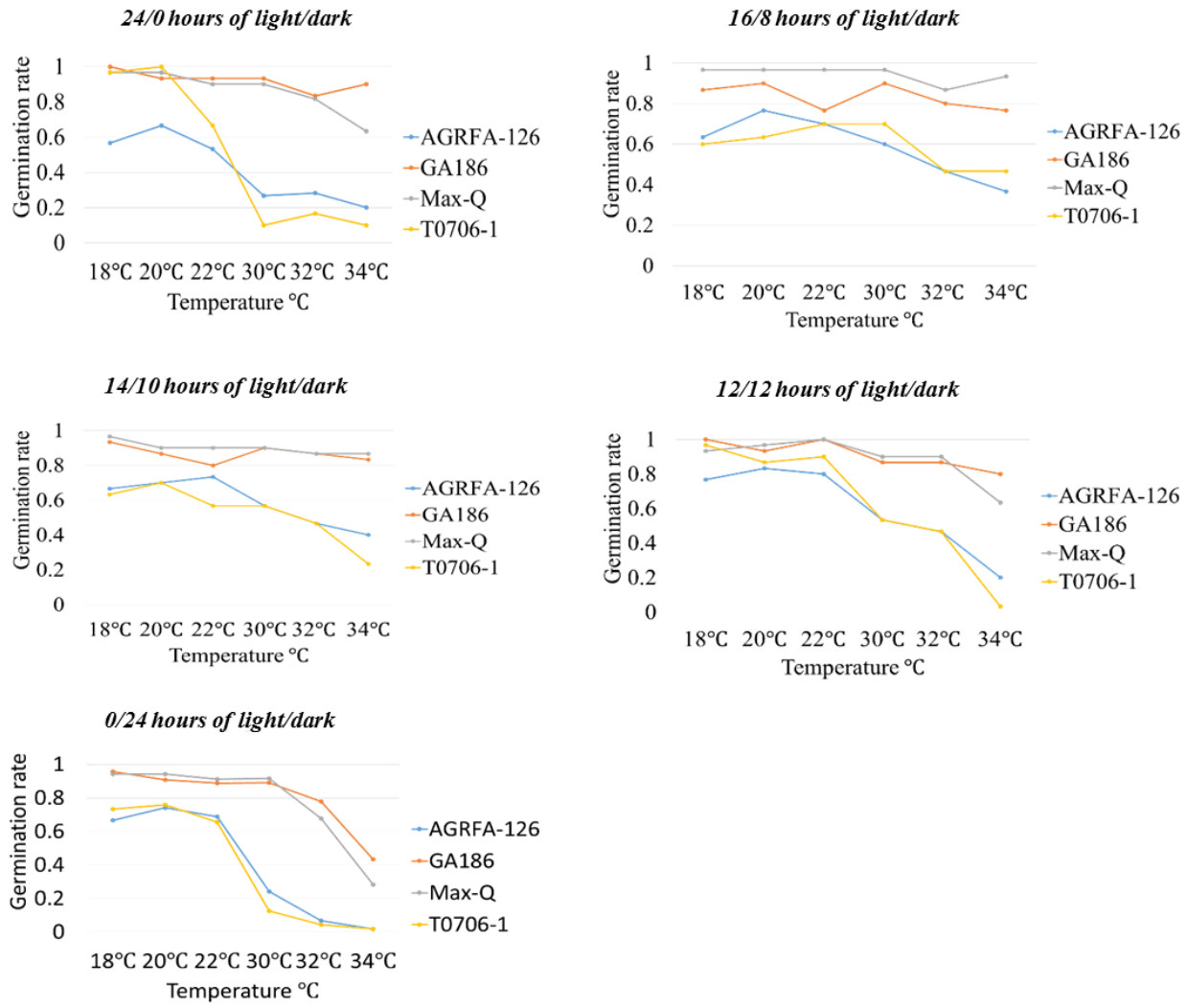

Figure 2.2. Germination rate of summer dormant checks (AGRFA-126 and T0706-1) and two non-dormant checks (Max-Q and GA186) under different temperature conditions under five different photoperiods (24 hours of light (top left); 16 hours of light (top right); 14 hours of light (middle left); 12 hours of light (middle right) and 0 hour of light (bottom left).

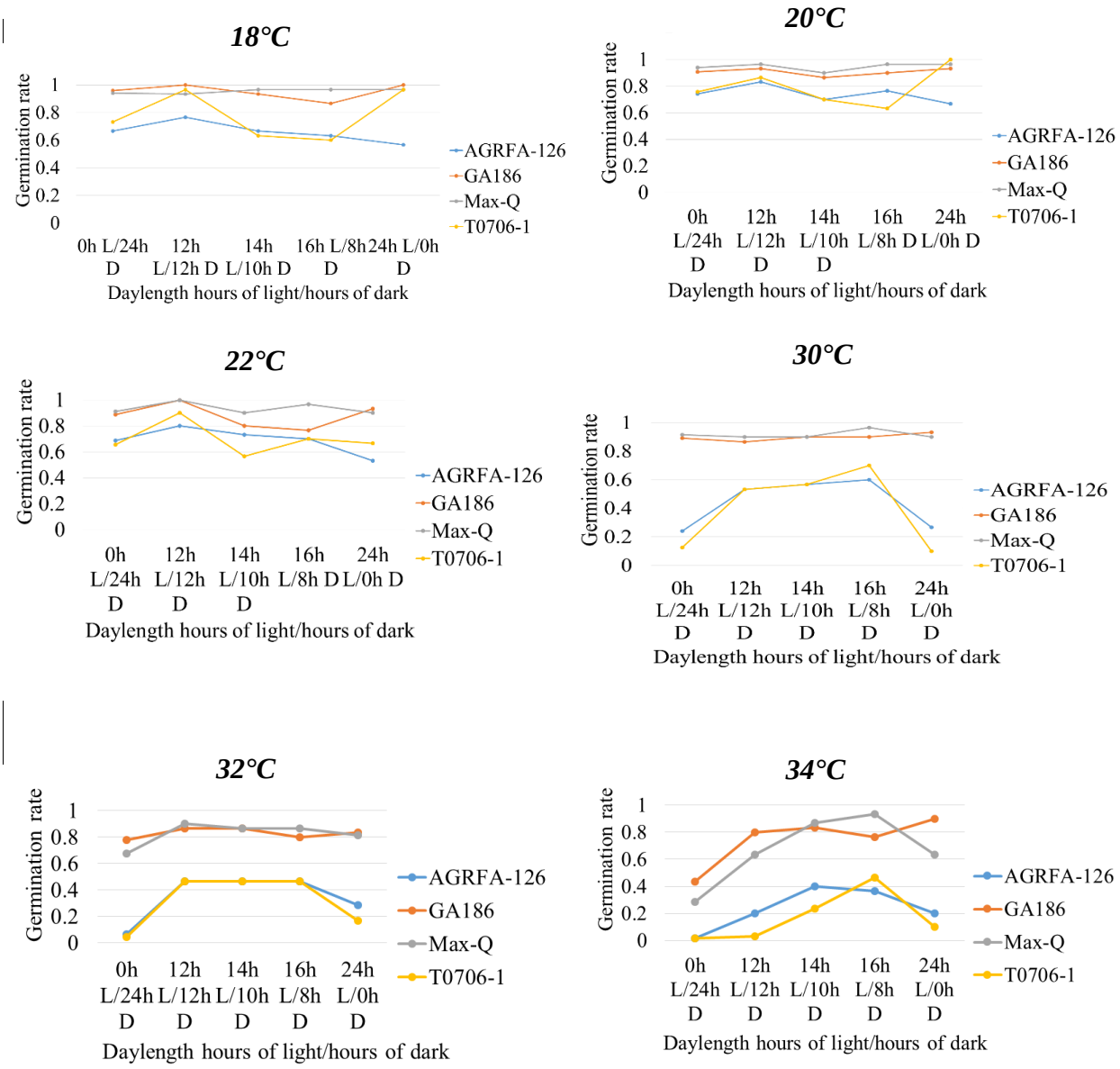

Figure 2.3. Germination rate of summer dormant checks (AGRFA-126 and T0706-1) and two non-dormant checks (Max-Q and GA186) under different photoperiod conditions within 6 temperatures (18°C , 20°C, 22°C, 30°C, 32°C, and 34°C).

CHAPTER 3

CANDIDATE GENE ASSOCIATION WITH SUMMER DORMANCY IN TALL FESCUE AND POSSIBLE ROLE OF DETERMINCAY

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Abstract

There are two main types of summer dormancy: eco-dormancy and endo-dormancy. The regulation of endo-dormancy is usually associated with a slowing of metabolic activity in meristematic tissues and most likely controlled by circadian clock genes. Endo-dormancy, therefore, is genetically inherited and allelic variation among and between summer-dormant and non-dormant varieties is expected. Understanding the mechanisms and genetic factors underlying summer dormancy will enable efficiency in selection and cultivar development. The main objective of this study was to test if various candidate genes are associated with summer dormancy. According to literature reviews, twenty-three candidate genes were amplified and sequenced on two dormant and two non-dormant checks. Nucleotide variants unique to each group were converted to KASP (kompetitive allele specific PCR) markers. Twenty-six markers were tested on 56 dormant and non-dormant accessions including four checks. Five markers, from the genes CONSTANS and TERMINAL FLOWER showed significant associations ($p<0.05$) with field phenotypic scores. These two genes are known to modulate meristem determinacy and growth. Another five markers showed significant associations at $p<0.05$ with the surrogate germination phenotype ($R^2 = 0.13$ to 0.20). 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. Relationship between summer dormancy and determinate growth was tested on two dormant (T0706-1, AGRFA-126) and two non-dormant (Kentucky 31, GA186) checks, where half the entries in each check were continuously clipped and prevented from flowering and the second half were not clipped and allowed to flower and set seed. The number of new tiller buds was not different between the clipped and non-clipped entries in the non-dormant checks, but

was significantly different ($p<0.05$) between clipped and non-clipped treatments within the dormant checks. These results suggest that meristem determinacy is probably one of the mechanisms involved in summer dormancy in tall fescue. A more extensive study needs to be conducted to draw a firm conclusion.

Keywords: Summer dormancy, KASP assay, Molecular marker, Marker-assisted selection, determinacy

Introduction

Tall fescue is an outcrossing allohexaploid (2n=6x=42) cool season species with a high level of self-incompatibility (Hopkins et al., 2009) resulting in highly heterozygous and heterogeneous populations. The genomic constitution is considered to be PPG1G1G2G2 (Chandras. and Thomas, 1971a; Chandras. and Thomas, 1971b). The P genome is from the diploid (2n=2x=14) meadow fescue (*F. Pratensis* Huds.), which is closely related to tall fescue morphologically. The G1 and G2 genomes are from the tetraploid (2n=4x =28) *F. arundinacea* var. *glaucescens* Boiss (Xu et al., 1991). The tall fescue genome contains approximately 5.27 to 5.83×10^6 kilobases (Seal, 1983)].

Tall fescue germplasm has been classified by taxonomists into five botanical groups, from which, plant breeders defined three major pools, Continental, Mediterranean, and Rhizomatous (Hand, et al., 2010). The Continental pool predominates in Northern Europe, is characterized by wide leaf blades, relative winter hardiness, and summer activity, like 'Kentucky 31'(Hopkins, et al., 2009). The Mediterranean germplasm, originated mostly from North Africa and is characterized by its 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). Dormancy is a temporary suspension of growth of plant structures containing meristems (Lang et al., 1987). It is an evolutionary response enabling plants to survive during seasons when environmental conditions are unfavorable for growth (Vegis, 1964). Dormancy has been studied extensively in woody species adapted to temperate environments. The two major environmental cues that plants use to sense environmental changes are day length and temperature (Olsen, 2010; Tanino et al., 2010).

Summer dormancy is controlled by a series of endogenous processes leading to the cessation of leaf growth and senescence under non-limiting moisture conditions (Norton et al., 2008) while keeping the plant meristems alive (Koller, 1969). The transition from active growth to the resting stage necessitates major developmental changes, such as increased energy storage and inhibition of meristematic activity, followed by senescence of above ground parts (Rees, 1992). Summer dormancy is beneficial in enabling heat and drought survival and allowing faster fall recovery and increased persistence (Malinowski et al., 2005; Norton et al., 2006a; Norton et al., 2006b; Shaimi et al., 2009a; Shaimi et al., 2009b). It is was described in several grasses such as pine bluegrass [*Poa scabrella* (Laude, 1953)], bulbous bluegrass [*P. bulbosa* (Ofir and Kigel, 1999; Volaire et al., 2001)], tall fescue [*Festuca arundinacea* Schreb. (Norton et al., 2006a; Norton et al., 2006b)], and orchardgrass [*Dactylis glomerata* L. (Norton et al., 2006a; Norton et al., 2006b; Shaimi et al., 2009a)].

Two common types of summer dormancy were reported, eco-dormancy and endo-dormancy. Eco-dormancy is a response to environmental stress and involves the interaction of various stress response mechanisms that are not well understood (Volaire and Norton, 2006). Endo-dormancy is a physiological response to environmental signals, such as temperature and photoperiod and is regulated by circadian clock genes (Salome et al., 2008). Endo-dormancy is usually associated with flowering and decreased metabolic activity in meristematic tissues (Volaire and Norton, 2006).

Summer dormancy has been described as complete or incomplete. Under complete dormancy, plants undergo full senescence and suspension of growth for at least four weeks during the summer (Norton et al., 2006a). Under incomplete dormancy, plants undergo moderate levels of senescence and noticeable reduction in growth as result of partial inhibition of leaf

development without dehydration of leaf bases (Norton et al., 2006b). Different morphotypes of tall fescue express different levels of summer dormancy (Hand et al., 2010).

During dormancy and growth cessation, meristems and crowns sense the eliciting signals and become inactive even when growth conditions are favorable (Rohde and Bhalerao, 2007). During physiological dormancy, increased expression levels of growth hormones, such as abscisic acid [ABA (Avendaño López et al., 2011)], and reduced levels of gibberellins and cytokinins (Powell, 1987) were observed. Genetic studies in *Arabidopsis thaliana* and Solanaceae species indicated that ABA is involved in induction and maintenance of dormancy along with other hormones like brassinosteroids, auxin, and cytokinins (Kucera et al., 2005).

Various genes are implicated in the regulation of dormancy. PHY-interacting transcription factors (PIFs) play a significant role in sensing environmental signals linked to the timing of the onset and release of dormancy and are influenced by other genes and proteins, such as DELLA proteins (de Lucas et al., 2008). The basic leucine zipper (bZIP) transcription factor, encoded by ABA-insensitive 5 (ABI5), interacts with ABI3 and impacts the expression of ABA responding genes (Carles et al., 2002; Nakamura et al., 2001). Homeodomain leucine-zipper (HD-Zip) protein mutants in *Arabidopsis* seed (ATHB20) increase ABA sensitivity and seed dormancy (Barrero et al., 2010). Auxin response factors (ARFs) were reported to activate the transcription of auxin-responsive genes involved in growth responses, which are also related to seasonal dormancy (Shim et al., 2014). In peaches, growth cessation and dormancy require a family of *dormancy-associated MADS-box* genes [*DAM*] (Bielenberg et al., 2008)] which are a group of transcription factors related to short vegetative phase (*SVP*) in *Arabidopsis* that have been shown to regulate bud dormancy processes in leafy spurge (Horvath et al., 2010) and Japanese apricot (Sasaki et al., 2011). Expression analysis of three *DAM* genes in leaf buds of a dormant and a

less dormant pear (*Pyrus*) cultivar showed down-regulation in the expression of *MADS13-1* levels in the less dormant cultivar prior to dormancy release. The transcripts differed by an insertion of 3.2 kb in the first intron of the dormant cultivar (Saito et al., 2013). Transcripts coding for dehydrins, GAST1, LTI65, no apical meristem (NAC), HTA8, HTA12 and RAP2.12-like proteins were considered putative factors involved in the dormancy process in apple trees (da Silveira Falavigna et al., 2014).

The onset of dormancy is also associated with remobilization of photosynthates and nutrients from the shoots to reserve structures such as buds and crowns to support re-growth of new shoots in spring (Millard and Neilsen, 1989; Schwartz and Amasino, 2013). Expression analysis of genes involved in carbohydrate metabolism and bud dormancy of leafy spurge (*Euphorbia esula*), showed a steep increase in up-regulation of the expression of a specific *Euphorbia esula* beta-amylase gene (*Ee-BAM1*) after growth induction (Chao and Serpe, 2010).

Heat is another player in the initiation of summer dormancy. Increasing heat tolerance in plants has been suggested to be affected by transformation with the *BADH* gene (Yang et al., 2005). Several genes associated with the synthesis of HSPs have been identified and isolated in various plant species, including tomato and maize (Liu et al., 2006; Momcilovic and Ristic, 2007; Sun et al., 2006). A recent study showed that a positive feedback loop formed by two heat-inducible genes, *HSP101* and heat stress-associated 32-KD protein (HSA32), at the post-transcriptional level extends the effect of heat acclimation in rice seedlings. The interaction between *HSP101* and HSA32 also affects basal thermo-tolerance of rice seeds (Lin et al., 2014).

Various genomic studies identified genes involved in the control of dormancy induction and growth cessation including circadian clock regulators, such as *GIGANTEA (GI)* and circadian clock associated 1 (Cao et al., 2005; Chiang et al., 2009; Ibanez et al., 2010; Oliverio et

al., 2007; Penfield and Hall, 2009), *FLOWERING LOCUS T* (*FT*) and the *CO*/*FT* complex (Pin and Nilsson, 2012), *CEN1*/ *TERMINAL FLOWER1* [*TFL1* (Mohamed et al., 2010)], as well as *dormancy-associated MADS-box* factors (Horvath et al., 2010). Growth cessation in aspen exposed to short photoperiod was prevented by overexpression of *FT1* and *CO* homologues of poplar [*Populus trichocarpa* (Böhlenius et al., 2006)]. Molecular models for photoperiod and temperature associated dormancy have been suggested (Campoy et al., 2011; Horvath, 2009). In these models, short day and short-term exposure to low temperatures induced dormancy and is regulated by the *CO/FT* module and *DAM* gene expression (Böhlenius et al., 2006; Horvath, 2009). Effects of the *FRIGIDA* gene at flowering time was also well documented in *Arabidopsis* (Fribourg et al., 1984), including the positive influence of *FT* expression in *Arabidopsis* (Caicedo et al., 2004; Korves et al., 2007; Lee and Amasino, 1995; Lee et al., 1994; Michaels and Amasino, 2001; Shindo et al., 2005; Stinchcombe et al., 2004; Werner et al., 2005).

It is possible that this information could be extrapolated to understand the genetic basis and mechanisms underlying summer dormancy in cool season grasses. The variations in gene expression occurring in crowns and buds during the onset of dormancy may be the result of allelic variation among and between summer dormant and non-dormant genotypes (Ibanez et al., 2010; Mohamed et al., 2010; Pin and Nilsson, 2012; Saito et al., 2013; Schwartz and Amasino, 2013; Tian et al., 2010). This variation may allow for the discovery of quantitative trait loci (QTL) underlying dormancy phenotypes and development of molecular markers that can be used for selection during early generations in breeding programs. The primary aim of this research is to explore the association between candidate genes involved in seasonal dormancy, flowering time, heat and photoperiod sensing with summer dormancy phenotypes in tall fescue. Understanding the genetic basis and environmental determinants of dormancy is essential for

interpreting the evolution history of seasonal dormancy and could help provide tools to increase the precision of selection in populations segregating for summer dormancy and accelerate the development of cultivars.

Materials and methods

Candidate genes, Orthology assessment and primer design

Candidate genes known for their putative involvement in seasonal dormancy, heat sensing, and flowering time were selected for analysis of their potential association with summer dormancy in tall fescue (Table 3.1).

The National Center for Biotechnology Information (NCBI) databases were first searched for published candidate gene sequences derived from *Festuca arundinacea* and *Festuca pratensis*. As the database for fescue is relatively limited, sequences from related species such as perennial ryegrass (*Lolium perenne*) and *Brachypodium distachyon* were considered (Table 3.1). For some candidate genes including *MADS-box*, drought inducible 22kD protein, Phytochrome A, B, C etc., there were no sequences available in tall fescue or related grasses, and since these genes are most likely conserved across plant taxa, sequences found in other species including *Pyrus pyrifolia, Euphorbia esula, Oryza sativa* cv. *Japonica*, *Zea mays, Arabidopsis thaliana*, *Glycine max*, and *Triticum* spp. were used. Blast or tblastn (NCBI BLAST, Bethesda, MD, USA) queries of the nucleotide sequences were conducted in *Festuca, Lolium perenne* expressed sequence tags (EST), and *Brachypodium distachyon* genome sequence release. Sequences meeting the threshold level around $E < 10^{-10}$ or with the highest level of similarity in blast results were selected as reference sequences for primer design. Primers were designed to target PCR products of 400-500 bp. One hundred and thirty two primer pairs were designed from the

candidate sequences using Primer3 Version 0.4.0 (Koressaar T, 2007; Untergasser, 2012) with the following parameter settings: primer length from 18 to 24 nucleotides, with 21 as the optimum, PCR product size from 300 to 500 bp, optimum annealing temperature 60°C, and GC contents from 40% to 70% with 50% as optimum. The primers were obtained from Integrated DNA Technologies, Inc. Coralville, IA, USA (Table 3.2) and used to amplify orthologues from dormant (T0706-1, AGRFA-126) and non-dormant (GA186, and Kentucky 31) tall fescue checks.

Plant materials

The dormant check cultivars (AGRFA-126 and T0706-1) 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. To enrich the dormant and non-dormant alleles, only the extreme 52 dormant and non-dormant putative accessions selected based on field evaluation of 209 tall fescue PI accessions were used in the analysis of association with candidate gene allele variants (Ding and Missaoui, 2016). The accessions were planted for evaluation of field summer dormancy at the J. Phil Campbell (JPC) farm at Watkinsville, GA in November 2013. The soil was a Cecil sandy loam, Fine, kaolinitic, thermic Typic Kanhapludults. The average annual precipitation is 1219 mm. The average high temperature is 32^{o}C in July and the lowest is 11^{o}C in January. Seed of each accession was planted in single rows with three replications. The rows were evaluated for dormancy during summers 2014 and 2015 by measuring the percentage of senescence and regrowth height after clipping. An average phenotypic score of summer dormancy was assigned to each accession, using a combined average of digital image analysis

senescence percentage scores (SPS) and regrowth height scores (RHS) of 3 replications and 2 years for each accession as in the following equation:

Summer Dormancy Rating Score (SDRS) = (SPS + RHS)/2

The senescence score was determined based on the percentage of dead plant material in each row. Digital image analysis was used to estimate senescence percentage in each row. Digital images were captured in JPEG (joint photographic experts group, or ".jpg") format with an image resolution of 5152 x 3864 pixels using a Canon PowerShot ELPH 150IS digital camera (Canon USA, Melville, NY). The camera was set up on a light box equipped with four incandescent light bulbs (800 lumens, Longstar) inside and on the four corners of the box to provide a consistent lighting background. Digital images of each plant row were captured on July 28th 2014 and August 7th 2015. The image files were analyzed using the software Assess version 2.0 from APS Press (American Phytopathological Society, St. Paul, MN), developed originally for disease analysis on turf grass (Horvath and Vargas, 2005). The images were analyzed using the options "Agronomist panel" and "GC-Auto". The percentage of green area is obtained from "GC-Auto" for the selected area of interest [AOI (Figure 1.1)]. Senescence percentage is calculated as 1-"GC-Auto". Rating scores were assigned to each accession based on senescence percentage, where: 90-100% =1, 80-90% =2, 70-80% = 3, 60-70% = 4, 50-60% = 5, 40-50% = 6, 30-40% = 7, 20-30% = 8, 10-20% = 9, and 0-10% = 10. The lower scores indicate higher summer dormancy (dormant), and the high scores indicate higher summer activity (non-dormant). Plants were clipped to a residual height of 15cm on June 28th, 2014 and on July 8th, 2015. Regrowth heights for each accession were measured after four weeks, on July 29th, 2014 and August 7th, 2015, and scores were assigned according to the following rating system where measurements are rounded off to the nearest half: 2.5cm or below = 1, 3 -5cm = 2, 5.5 -7.5cm

=3, 8 -10cm = 4, 10.5 -12.5cm = 5, 13 -15cm = 6, 15.5 -17.5cm = 7, 18 -20cm = 8, 20.5 -22.5 cm = 9, and 23 cm or above = 10. Higher scores indicate a lower level of summer dormancy (non-dormant) and the lower scores indicate higher summer dormancy (dormant). To avoid confounding effects of water deficiency, supplemental irrigation was provided weekly as needed. Weed control was conducted by spraying herbicides for broadleaf weeds, and manually for summer grasses within and between rows. Based on field summer dormancy rating scores and visual inspection adjustment of the 209 accessions, 24 accessions with high scores (>7.5) were selected as potential non-dormant and 28 accessions with low scores (<3.5) were selected as dormant to test the association between summer dormancy scores in the field, surrogate phenotyping score based on germination and marker genotypes.

DNA extraction

Young leaf tissue of the 52 accessions was collected from the field for DNA extraction and marker analysis. Approximately 10g of leaf tissue from each accession was collected in a 15 ml plastic centrifuge tube and freeze-dried for 48 hours at -45 ºC in a Virtis Freezemobile 12 ES (SP Industries, Gardiner, NY, USA). The freeze-dried samples were ground into fine powder using a 2010 Geno/Grinder® (SPEX Sample Prep., Metuchen, NJ). DNA was extracted using DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). The DNA concentrations were quantified using an Infinite® M1000 PRO plate reader with NanoQuant Plate (Tecan Group Ltd., Männedorf, Switzerland). The DNA products were then diluted to approximately 10 ng μL^{-1} and used for PCR amplification.

PCR amplification, sequence alignment and polymorphism identification

For initial screening of candidate genes, the 132 primer pairs (Table 3.2), were tested using a Taq PCR Kit (New England Biolabs, Ipswich, MA, USA) under the same PCR conditions with DNA templates of dormant checks: T0706-1, AGRFA-126, and non-dormant checks: GA186, Kentucky 31. The PCR reactions were performed in 96-well plates in 20 µL reactions. Each well contained 1µL of 10ng μL^{-1} DNA as template, 2µL of 10x PCR reaction buffer, 1.2 µL of 25 mM $MgCl_2$, 0.4 µL of 10 mM dNTPs, 0.08 µL of Taq polymerase 5000 units ml^{-1}, 0.5 µL of 10 mM forward and reverse primers and 14.32 µL of sterilized distilled water. The PCR conditions were 15 min at 95°C; 10 cycles of 30 sec at 95°C, 30 sec at 65°C, with 1°C decreased each cycle, 2 min at 72°C; 25 cycles of 30 sec at 95°C. 30 sec at 55°C, 2 min at 72°C and a final extension step of 7 min at 72°C. Each of the 132 primer pairs was tested on a three-replicates of each check cultivar to confirm the repeatability of the amplification. The PCR products were electrophoresed on 1% agarose gel by combining 4 µL PCR product with 3 µL 6x loading dye under 180 mA, 250 V for 30 min. Fluorescent bands were detected with Gel Doc™ EZ Imager software (Bio-Rad Laboratories, Inc, Hercules, CA, USA) and analyzed by Image Lab™ Version 5.1 software (Bio-Rad Laboratories, Inc, Hercules, CA, USA).

Forty of the primer pairs that resulted in PCR products with single bands were selected for sequencing. Sequencing reactions were conducted using separate reactions for forward and reverse primers. Sequencing was done at University of Georgia Genomics Facility (GGF) using Capillary Electrophoresis. The sequence data was assembled and analyzed in Geneious software version 7.1.2 (Drummond et al. 2014) to identify potential polymorphisms unique to dormant and non-dormant checks.

Multiple sequence alignment of dormant and non-dormant check sequences was conducted in Geneious using Clustal option, and nucleotide variants including SNPs and Indels between dormant and non-dormant checks were identified. One hundred and fifteen polymorphisms were identified in 8 of the 23 candidate genes (*CEN*, *CO*, Phytochrome A, heat shock protein, *MADS-box*, *TF*, ARF and DELLA protein).

KASP markers development and validation

Thirty-eight of the 115 polymorphisms, mostly SNPs, were successfully converted to KASP™ (competitive allele-specific PCR) markers to enable genotyping of the alleles at each single nucleotide polymorphism (SNP) and insertion and deletion (Indel) locus.

SNP specific primers were designed with Primer 3 Plus Version 2.3.6 (Untergasser et al., 2012). Two allele-specific forward primers were designed for each locus, in addition to one common reverse primer. One allele-specific primer harbors a unique tail sequence labelled with FAM™. The other allele specific primer harbors a tail sequence labeled with VIC® (Table 3.3).

The genotyping assay consisted of SNP-specific KASP Assay mix, universal KASP Master mix (KBS-1016-016 KASP V4.0 2X, LGC Genomics, Beverly, MA, USA), and DNA from each dormant and non-dormant checks. The PCR reactions were performed in 384-well plates with approximately 4 µL per reaction containing 2 µL of 10 ng μL^{-1} DNA template, 2µL of 2X Mastermix, 0.3 µL of 10% Polyvinylpyrrolidone (PVP) solution and 0.11 µL of primer assay which contained 12 µM of each forward primer for both SNPs and 30 µM of common reverse primer. The PCR conditions were 15 min at 94°C; 10 cycles of 20 sec at 94°C, 1 min at 65°C, with 0.8°C decreased each cycle; 30 cycles of 20 sec at 94°C and 1 min at 57°C. The PCR plates were read using a LightCycler® 480 Instrument II (Roche Life Science, Indianapolis, IN,

USA). The genotype call results were analyzed with LightCycler® 480 Software release 1.5.1.62. Plates were also read with a Tecan Infinite® M1000 PRO plate reader (Tecan Trading AG, Switzerland) and analyzed by Klustercaller Version 2.24.0.11 software (KBiosciences, Hoddesdon, UK) for confirmation. Ambiguous results were run with an additional 10 to 20 cycles of PCR at 20 min at 94°C and 1 min at 57°C to improve separation. Each of the 38 markers were screened and tested twice on dormant and non-dormant check varieties to detect and confirm the repeatability of KASP marker performance.

Twenty-six KASP markers that showed clear resolution between the 2 alleles were tested on 52 tall fescue accessions that were identified as dormant and non-dormant based on 2 year-field evaluation in addition to the surrogate phenotype based on the ratio of seed germination at $30^oC/20^oC$ at 24 hrs light, together with the 4 checks (Figure 3.1; Table 3.3).

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 $\rho = 0$ (dormancy scores are not associated with marker genotypes). The probability of a false positive was maintained at $P < 0.05$. PROC CORR with the option <Spearman> in SAS 9.4 was used to determine the association between dormancy scores and genotypes at each marker under the null hypothesis $\rho = 0$ (dormancy scores do not co-vary with marker genotypes). The significance level of the correlations was maintained at $P < 0.05$. Spearman rank correlation was chosen because it is non-parametric statistic that does not assume normality or homoscedasticity of the

measurements as well as it doesn't assume a linear association between variables, like regression models and linear models.

Testing the possible relationship between determinacy and summer doramncy

To explore the possible relationship between summer dormancy and determinacy, a greenhouse experiment was initiated in September 2014 in the greenhouse at the University of Georgia, Athens. Seed of two non-dormant checks (GA186 and Kentucky 31) and 2 dormant checks [T0706-1 (a half-sib from the experimental population GA95101T) and AGRFA-126 (Ag Research New Zealand)] according to personal communication were planted in trays in the greenhouse and then transplanted into 6 inch pots after eight weeks. Twenty pots containing one plant per pot were used for each check. Greenhouse conditions were maintained at a temperature of 27 ºC and natural light conditions. The plants were watered once a day and fertilized once a week. One group of 10 plants for each check was allowed to flower and set seed (non-clipped group). The other 10 plants were kept in the vegetative stage by frequently clipping them starting at the first signs of stem elongation, and were prevented from flowering and setting seed (clipped group). Two of the 10 dormant check AGRFA-126 plants died, therefore only 8 plants were maintained in each treatment. In August 2014, all the plants were removed from the pots. The roots were washed, and the number of tiller buds sprouting from the crowns was counted on each plant. Separation of differences in tiller count means between the clipped and no-clipped treatments within each accession was analyzed using the PROC TTEST procedure in SAS 9.4 at alpha = 0.05.

Results

Screening of candidate genes and polymorphism identification

Twenty-three candidate genes were selected based on their known involvement in photoperiod and temperature regulation functions (Table 3.1). The genes included phytochromes, growth hormones related genes, dormancy related or regulating genes, seed dormancy related or regulating genes, heat shock proteins, drought inducible genes, and flowering time and circadian clock genes that were reported in various species, including *Festuca arundinacea, Pyrus pyrifolia, Brachypodium distachyon, Euphorbia esula*, *Oryza sativa* cv.Japonica, *Zea mays*, *Arabidopsis thaliana*, *Glycine max*, *Triticum* spp. and *Lolium perenne* (Table 3.1).

Among the 132 primer pairs designed targeting average PCR products of less than 500 bp length to insure accurate sequencing, 33 primer pairs amplified fragments in all 4 check accessions. Forty-two of these primer pairs amplified fragments in only 1, 2, or 3 check accessions. Fifty-seven of these primer pairs did not amplify any of the 4 check accessions. The amplification products resulted in sequence lengths ranging from 153 bp to 587 bp (Table 3.2). Sequence alignment of the 33 PCR products in all 4 check accessions resulted in 115 polymorphisms including SNPs and insertion/deletions that have one allele specific to the dormant group and one allele specific to the non-dormant group of checks (Table 3.2). The number of polymorphisms identified within each amplified sequence ranged from 0 to 30 (Table 3.2). Thirty-eight of the 115 polymorphisms that appeared to be unique to dormant or non-dormant checks were selected and converted to KASP markers.

The polymorphisms were selected based on the location of the polymorphism and distance from nearby variants (Table 3.3). Among the 38 KASP markers, 26 showed clear fluorescence

distinction between the dormant and non-dormant alleles in the genotyping scatterplots (Figure 3.1; Table 3.3).

Association between marker genotypes and summer dormancy rating scores

The R^2 values between marker alleles and field summer dormancy rating scores ranged from 0 to 0.13 in the 52 dormant and non-dormant accessions (Table 3.4). The R^2 values between marker alleles and the surrogate phenotypic scores based on seed germination ratio 32ºC/20ºC) under 24 hours of light ranged from 0 to 0.20 (Table 3.4). Genotypes of the markers CO1-TF-1598l, CO1-TF-1576, CO4-TF-1591l, and CO1-TF-15180 showed significant association ($p<0.05$) with the field phenotypic scores and explained 12 %, 11%, 11% and 13% of the phenotypic variation, respectively. Genotypes of the markers ARF6-TF-15278l, VER-MADS-TF-1586o, HEAT1-TF-15130, ARF1-TF-15383 and ARF6-TF-1556s showed significant association ($p<0.05$) with the germination ration scores and explained 13%, 20%, 15%, 14% and 16 % of the of the variation, respectively).

Correlation coefficients between marker alleles and field summer dormancy rating scores ranged from -0.02 to 0.37 in the 52 dormant and non-dormant accessions (Table 3.4). Correlation coefficients between marker alleles and the surrogate phenotypic scores based on seed germination ratio 32ºC/20ºC) under 24 hours of light ranged from -0.27 to 0.31. Genotypes of the KASP markers TFNEW-TF-15219-3, CO4-TF-1591l, and CO1-TF-15180 showed significant correlations ($p<0.05$) with the field phenotypic scores (0.33, 0.33, and 0.37 respectively). The same markers showed correlations of 0.26, 0.26, and 0.31 with the germination surrogate phenotypic scores. Only CO1-TF-15180 marker was significant ($p<0.05$).

Testing the relationship between summer dormancy and determinacy

In this experiment, a high number of tiller buds in the crown area indicates more regrowth, and suggests less dormancy. A low number of buds indicates less regrowth and suggests higher level of summer dormancy. The average number of tiller buds in the dormant checks was much higher in the clipped treatment, where the plants were prevented from flowering and setting seed. In the non-dormant checks, both clipped and non-clipped had the same number of tiller buds (Figure 3.2).

The average difference in number of tiller buds between clipped and non-clipped treatment groups ranged from -0.1 to 0.1 for the non-dormant checks. For the dormant checks, the average differences between clipped and non-clipped treatments ranged from 0.8 to 1.2 (Figure 3.2). Paired T test analysis showed a significant difference ($p<0.01$) between the average number of buds under clipped and non-clipped treatments of the dormant checks T0706-1 and AGRFA (Table 3.5). There was no significant difference ($p>0.05$) between clipped treatments and non-clipped treatments of the non-dormant check accessions (GA186 and KY31).

Discussion

Screening of candidate genes and polymorphism identification

Several changes occur during summer dormancy including growth cessation, change in expression levels of growth related hormones, environmental signal perception, senescence, and remobilization of nutrient reserves (Avendaño López et al., 2011; Millard and Neilsen, 1989; Powell, 1987; Rohde and Bhalerao, 2007; Schwartz and Amasino, 2013). Genetic studies in different species showed that several genes and proteins may have a direct or indirect connection with dormancy including *CEN, TFL1, CO, FT, DAM, BAM,* heat shock proteins, drought inducible 22 kD protein, ABA stimulation MAP kinase, GAST1 protein, NAC, viviparous-1

(VP1), *SVP*, HD-Zip, Per1, Phytochrome A, Phytochrome B, Phytochrome C, *FRIGIDA*, Gigantea, PHY-interacting transcription factor, auxin response factor, and *DELLA* protein (Barrero et al., 2010; Bielenberg et al., 2008; Böhlenius et al., 2006; Caicedo et al., 2004; Cao et al., 2005; Chao and Serpe, 2010; da Silveira Falavigna et al., 2014; Danilevskaya et al., 2010; de Lucas et al., 2008; Fribourg et al., 1984; Furuya, 1968; Giraudat et al., 1992; Horvath, 2009; Horvath et al., 2008; Horvath et al., 2010; Lin et al., 2014; McCarty et al., 1991; Oliverio et al., 2007; Shaimi et al., 2009b; Shim et al., 2014; Stacy et al., 1996).

The 23 candidate genes included in this study were all reported to have a connection to mechanisms related to seasonal dormancy. The fact that only 33 out of 132 primers developed from the candidate gene sequences resulted in amplified fragments on all 4 check accessions might be due to sequence divergence between tall fescue and relatives such as *Lolium perenne* and *Brachypodium distachyon*. The dormant checks belong to the continental tall fescue germplasm, while the non-dormant checks belong to the Mediterranean pool. Therefore, each may have different genetic constitutions, which could explain why some of the primers only amplified in 1 to 3 of the 4 check accessions. Mediterranean (dormant) and continental tall fescue may trace back to different progenitors. The genomic constitution of continental tall fescue is believed to be PPG1G1G2G2 (Chandras and Thomas, 1971a; Chandras. and Thomas, 1971b). The P genome originated from the diploid (2n=2x=14) meadow fescue (F. Pratensis Huds.), which is closely related to tall fescue morphologically and the G1 and G2 genomes are from the tetraploid (2n=4x =28) *F. arundinacea* var. glaucescens Boiss. Mediterranean tall fescue may have different genome sets derived from different progenitors than continental tall fescue (Hand et al., 2010). Some of the sequences, such as Phytochrome showed no

polymorphism between and within dormant and non-dormant accessions, indicating the possibility that these genes are highly conserved even among different genera.

The five markers that showed significant association at $p<0.05$ with field dormancy rating scores ($R^2 = 0.10$ to 0.13) were all derived from sequences of the genes *Terminal flower*, and *Constans (CO).* The same markers showed significant correlations at $p<0.05$ with field dormancy rating scores ($r = 0.31$ to 0.37). Another five markers showed significant associations at $p<0.05$ with the surrogate germination phenotype ($R^2 = 0.13$ to 0.20). One marker originated *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.

Auxin response factors (ARFs) were reported to activate the transcription of auxin-responsive genes involved in growth responses, which are also related to seasonal dormancy (Shim et al., 2014). In peaches, growth cessation and dormancy require a family of *dormancy-associated MADS-box genes (DAM)* (Bielenberg et al., 2008)] which are a group of transcription factors related to *short vegetative phase (SVP)* in Arabidopsis that have been shown to regulate bud dormancy processes in leafy spurge (Horvath et al., 2010) and Japanese apricot (Sasaki et al., 2011). Expression analysis of three DAM genes in leaf buds of a dormant and a less dormant pear (*Pyrus*) cultivar showed down-regulation in the expression of *MADS13-1* levels in the less dormant cultivar prior to dormancy release. Heat is another player in the initiation of summer dormancy. Several genes associated with the synthesis of *HSPs* have been identified and isolated in various plant species, including tomato and maize (Liu et al., 2006; Momcilovic and Ristic, 2007; Sun et al., 2006). A recent study showed that a positive feedback loop formed by two heat-inducible genes, HSP101 and heat stress-associated 32-KD protein (HSA32), at the post-

transcriptional level extends the effect of heat acclimation in rice seedlings. The interaction between HSP101 and HSA32 also affects basal thermotolerance of rice seeds (Lin et al., 2014).

Plant growth relies on cell division in apical meristems. In determinate meristems, cell division is stopped (Sablowski, 2007). Initiation of flowering is often triggered by change in photoperiod sensed by the endogenous timer called circadian clock (Thomas and Vince-Prue, 1996). Genes, including *FT*, *TFL*, *CEN* and *CO*, have been implicated in the initiation of flowering process (Kim et al., 2008). *TFL1*-like genes are highly conserved in plants and are believed to maintain meristem indeterminacy (Danilevskaya et al., 2010). Several regulator genes were implicated in the control of the transition from vegetative to reproductive stage and determinacy, such as *APETALA1 (AP1)/CAULIFLOWER (CAL)* and *LEAFY* [*LFY* (Blazquez et al., 2006; Komeda, 2004)]. Both *AP1/CAL* and *LFY* are believed to be activated by *FT* (Huang et al., 2005; Ruiz-García et al., 1997) and to be prevented by *TFL*, which is a homologue of *FT* but serves an opposite function (Bradley et al., 1997; Kardailsky et al., 1999; Kobayashi et al., 1999). There are other genes in *TFL1* family, such as *CEN* which is the closest homolog of *TFL*1 in *Arabidopsis* (Danilevskaya et al., 2010). In *Antirrhinum majus*, the *CEN* gene is expressed to maintain the inflorescence meristem after the floral transition (Bradley et al., 1996; Cremer et al., 2001). *ZEA CENTRORADIALIS (ZCN)* in maize was suggested to play a role in floral transition and maintenance of meristem indeterminacy (Danilevskaya et al., 2010). Evaluation of the gene *CEN* for potential phylogenetic classification of tall fescue morphotypes suggested that there might be selective pressure on this gene in different germplasm (Hand et al. 2010).

CONSTANS (CO) are proteins involved in day length sensing and promoting flowering in response to long photoperiods (Böhlenius et al., 2006) and the level of expression of *CO* is regulated by the circadian clock (Kim et al., 2008; Valverde et al., 2004; Yanovsky and Kay,

2002). The circadian expression of *CO* mRNA is related to *GI* due to the decrease of *CO* transcript levels in *GI* mutants (Suárez-López et al., 2001). *CO* is a B-box-containing protein promoting the expression of *FT* (Kobayashi et al., 1999; Samach et al., 2000; Valverde et al., 2004). The expression of *FT* is restricted to a similar time of day as the expression of *CO* (Suárez-López et al., 2001).

Summer dormancy is most likely a quantitative trait controlled by several genes with small effects, which would explain the low to moderate associations detected in the candidate gene analysis. The R^2 values identified in this study are within the range of reported prediction accuracies from genomic selection in *Lolium perenne* (L.) breeding populations that varied from 0 to 0.59 (Grinberg et al., 2016). Summer dormancy QTL identified in an orchardgrass mapping population explained 12-13 % of the phenotypic variation (Kallida et al., 2016). These markers will need to be validated as a selection index to increase the allele frequency in recurrent selection of dormant or non-dormant progenies of tall fescue breeding populations.

Testing the relationship between summer dormancy and determinacy

One of the processes preceding dormancy is the slowing down of plant growth and the arrest of cell division in meristems. Meristems in determinate cultivars cease vegetative activity at, or soon after, photoperiod-induced floral initiation, and undergo a transition to reproductive phase (Bernard, 1972). The ectopic expression of *TFL1*-like genes in maize modifies flowering time and inflorescence architecture through maintenance of the indeterminacy of the vegetative and inflorescence meristems (Danilevskaya et al., 2010). The fact that the *TERMINAL FLOWER1 (TFL1*) marker showed significant correlation with dormancy in this study, suggests a potential relationship between dormancy and determinacy. It is well established that several

circadian clock regulators are also associated with determinacy, an agronomically important trait associated with domestication in many crop species (Tian et al., 2010).

In this study, tiller buds data from 10 replications of two summer dormant and two non-dormant tall fescue check accessions showed significant differences between the clipped and non-clipped treatments only within the dormant group. There was also variation within each treatment in both groups, which can be explained by the fact that tall fescue is an outcrossing species and therefore heterozygosity and heterogeneity are expected within populations.

Future work

A collection of 780 tall fescue accessions were planted in the field for the purpose of fine tuning the summer dormancy rating score system and selection of a set of accessions that can be used as checks for determining summer dormancy. The three markers that showed significant correlation and the nine markers (two of the nine also showed significant correlation with summer dormancy rating scores) showed significant association with summer dormancy rating scores need to be validated in a larger number of accessions. A larger scale experiment involving a higher number of dormant and non-dormant accessions should be undertaken under rigorously controlled growing conditions of light and temperature to confirm the connection between determinacy and summer dormancy observed in this study.

Segregating tall fescue populations are under development to generate mapping populations to understand the genetic basis of summer dormancy and map the trait. A non-dormant continental genotype GA186 is currently being crossed with the summer dormant genotypes T0706-1 and AGRFA-126, to generate bi-parental mappings populations segregating for summer dormancy. To maximize recombination between chromosomal segments associated

with the trait in the progeny, an F_2 population involving sib-mating between F_1 will be developed in the greenhouse. The F_2 mapping population will be used to identify QTL associated with dormancy and related factors in future work.

Table 3.1. Summary of 23 candidate genes used to search sequences available in tall fescue and related grasses and develop primers.

Candidate genes †	**Gene name**	**GenBank reference No.** ‡	**Organism** §	**Function** ¶
CEN	*Centroradialis*	HM453168.1	*Festuca arundinacea*	Terminal flower, circadian clock regulators
		HM453158.1	*Lolium perenne*	
TFL1	*Terminal Flower 1*	AF316419.1	*Lolium perenne*	Terminal flower, circadian clock regulators
CO	*CONSTANS*	GU214996.1	*Festuca arundinacea*	Terminal flower, circadian clock regulators
		AY600919.1	*Lolium perenne*	
FT	*FLOWERING LOCUS T*	HM453168.1	*Festuca arundinacea*	Terminal flower, circadian clock regulators
DAM	*Dormancy-associated MADS-box*	HQ588325.1	*Brachypodium distachyon*	Growth cessation and dormancy
		DQ110009.1	*Lolium perenne*	
		AY198334.1	*Lolium perenne*	
		DQ110010.1	*Lolium perenne*	
		GU574698.1	*Festuca arundinacea*	
BAM	*Beta-amylase*	JX536620.1	*Lolium perenne*	Carbohydrate metabolism and bud dormancy
HSPs	Heat shock proteins	GR513742.1	*Lolium perenne*	Heat stress tolerance
		GR512355.1	*Lolium perenne*	
		GT759932.1	*Brachypodium distachyon*	
		GT817782.1	*Brachypodium distachyon*	
		CK801781.1	*Festuca arundinacea*	
		DT708898.1	*Festuca arundinacea*	
		DT693671.1	*Festuca arundinacea*	
		CK801816.1	*Festuca arundinacea*	
		GR514569.1	*Lolium perenne*	
Dip22	Drought inducible 22kD protein	DT684265.1	*Festuca arundinacea*	Inducible by drought condition
		DT682806.1	*Festuca arundinacea*	
ABA stimulation MAP kinase	ABA stimulation MAP kinase	GT034880.1	*Festuca arundinacea*	Biotic and abiotic stress responses
		DT688857.1	*Festuca arundinacea*	
GAST1	Gibberellin (GA)-stimulated transcript 1	GO894856.1	*Festuca pratensis*	Involeved in dormancy process
		GT840451.1	*Brachypodium distachyon*	
		GT833628.1	*Brachypodium distachyon*	
NAC	No Apical Meristem	DT691784.1	*Festuca arundinacea*	Involved in dormancy process
		DT698157.1	*Festuca arundinacea*	
		DT711125.1	*Festuca arundinacea*	

VP1	*Viviparous-1*	DV472471.1	*Brachypodium distachyon*	Seed development and dormancy
SVP	*Short Vegetative Phase*	HM439237.1	*Festuca arundinacea*	Regulate bud dormancy processes
HDZip	Homeodomain leucine-zipper proteins	AU250641.1	*Lolium multiflorum*	Increases ABA sensitivity and seed dormancy
Per1	*Period circadian clock 1*	DV476103.1	*Brachypodium distachyon*	Involved in dormancy process
PHY A	Phytochrome A	GO846881.1	*Festuca pratensis*	Regulate seed dormancy and germination
		DT681161.1	*Festuca arundinacea*	
		GT034246.1	*Festuca arundinacea*	
		GO872741.1	*Festuca pratensis*	
PHY B	Phytochrome B	DT699539.1	*Festuca arundinacea*	Regulate seed dormancy and germination
PHY C	Phytochrome C	GT034246.1	*Festuca arundinacea*	Regulate seed dormancy and germination
FRI	*FRIGIDA*	NC_016134.1	*Brachypodium distachyon*	Flowering time
		DT688729.1	*Festuca arundinacea*	
		GO799556.1	*Festuca pratensis*	
GI	*Gigantea*	DT703770.1	*Festuca arundinacea*	Circadian clock regulators
		GT040256.1	*Festuca arundinacea*	
PIFs	PHY-interacting transcription factors	DT696010.1	*Festuca arundinacea*	Sense environmental signals and dormancy
ARFs	Auxin response factors	GR516228.1	*Lolium perenne*	Growth responses and growth-dormancy
		GR513352.1	*Lolium perenne*	
		DT687146.1	*Festuca arundinacea*	
		DT683024.1	*Festuca arundinacea*	
		GT046076.1	*Festuca arundinacea*	
DELLAs	DELLA proteins	DT703665.1	*Festuca arundinacea*	Influence transcriptional activity PHY-interacting transcription factor
		HX842708.1	*Brachypodium distachyon*	

† Candidate Genes= Name of selected candidate genes according to previous studies and references

‡ GenBank/NCBI Reference No = The reference number of sequences selected as candidate genes from GenBank or NCBI.

§ Organism= The species from which reference sequences were used to develop primers.

¶ Function= Function of the candidate genes.

Table 3.2. Sequences of 132 primer pairs, amplification status, sequence length (bp), and number polymorphisms identified in the amplification of candidate genes in tall fescue dormant and non-dormant checks.

Primers name†	Primer sequences (forward/reverse) ‡	Amplification (Y/P/N) §	Sequence length (bp) ¶	No. of polymorphisms #
CENf1/CENr1	TAAGCAGCCCAAGCCCTTCAAAG/CGAGGAAGTAATGTAGAAGAGGAGC	P	N/A	N/A
CENf2/CENr2	CGAGGAAGTAATGTAGAAGAGGAGC/CACTGCGAAAGAAGAAAACAAG	P	N/A	N/A
CENf3/CENr3	GGGTGGCTACTAAGCACCTAAA / AAGGACCTACCAAATGAAGCAT	Y	196	2
CENf4/CENr4	GGGTGGCTACTAAGCACCTAAA/AAGGACCTACCAAATGAAGCAT	Y	197	2
CENf5/CENr5	AGGTTCATTTTTGTGCTCTTCA/TTATTTGGCACAACTGGGATAG	P	N/A	N/A
TFf1/TFr1	ACTTGCCTCTCCAACCATGT/GAAGATGCTCCCGCAGATAC	N	N/A	N/A
TFf2/TFr2	AGGTTCATTTTTGTGCTCTTCA/TTATTTGGCACAACTGGGATAG	P	N/A	N/A
TFf3/TFr3	TGGACCATTGCTTCTTTACTACA/GGTTGTCTACCTGACCTTCCTT	P	N/A	N/A
TFf1/TFr1-new	CCCAAGCCACTTCAAAGC/GAAGATGCTCCCGCAGATAC	P	N/A	N/A
TFf2/TFr2-new	CTGGGACAACAGATGCTTCA/GAAGGTGCATAACTCCAAAAGC	Y	386	2
TFf3/TFr3-new	TGGACCATTGCTTCTTTACTACA/TTTTTGCCAGTTTGGGTTGT	P	N/A	N/A
Consf1/Consr1	ACACATAAACAGATGCGTAGGG/GAGTCAGCGTGGCAGTACAC	Y	208	4
Consf2/Consr2	CCTATGAAGCACAGGTGCACTA/CCCGACAAGATCAAAGTACTCA	P	N/A	N/A
Consf3/Consr3	TATGTGATGCAAGAACAGCAAC/CCTTCCATTGATGAGAACGATA	P	N/A	N/A
Consf4/Consr4	GGTCCTCAGGTACAAGGAGAAG/TCATTAAAACCATGGAACGGTA	Y	285	1
Consf5/Consr5	GAACAATAAGGTCCAGTGCAAG/CATTCACAATCCATCCTTCAAT	P	N/A	N/A
MADSf1/MADSr1	GAAGCGGATCGAGAACAAGA/ACTCGCGCTTTCAGTTTGAG	N	N/A	N/A
MADSf2/MADSr2	GATCTCCAAAGGAAGGAGCA/CGCATGAGCTACTCATCTGC	N	N/A	N/A
MADSf3/MADSr3	CTCTCCCCTCCTCTGCTTTC/CTTCCCTGTGGAGGAGAAGA	N	N/A	N/A
MADSf4/MADSr4	AGGGTGCTTCAGACGAAAGA/CACTGC	N	N/A	N/A

	AGGGTAATCCCAAT			
MADSf5/MADSr5	TGGAGAAGCTCTTGTGTAATCG/ACCATGAACAAATAGTGCCAAC	P	N/A	N/A
MADSf6/MADSr6	GAGAGGGAAAGGTGGGAGAG/TTGCTTCCTGTGAGTTGTGG	N	N/A	N/A
MADSf7/MADSr7	CCCTCAAGCATATCAGGTCAA/TGTTCAGCTGGTCCGTGTAG	N	N/A	N/A
MADSf8/MADSr8	CGACCTGCATGTAGCCACTA/AACGCGAAATTTCTGAGTGAA	N	N/A	N/A
MADSf9/MADSr9	GAAGAAAGGAGAGGGGATGG/GTCCTCACGCAAGTCCAACT	N	N/A	N/A
MADSf10/MADSr10	TGCTGAAAACCAAGAGCAAA/AAGTGGTAATCCGAGCCTGA	N	N/A	N/A
MADSf11/MADSr11	CGCCGCATCGAATAAACC/TTGAACATCCTAACAGACCCTTT	P	N/A	N/A
VER-MADSf1/VER-MADSr1	GCAGCTGAATGAAACAACACAT/AGAGATTACAGTAGTAAGAACCCTTGA	Y	302	1
VER-MADSf2/VER-MADSr2	CTTCGACAAGCCATCGAGT/CTGTTTGCTTGTGCTGGAGAT	P	N/A	N/A
BAMf1/BAMr1	CAAGAGCGACCTAGACATTTTC/CATCCAGGAACTGTTTCATGTT	Y	356	0
BAMf2/BAMr2	GTCATCGTGGACATTGAGGT/AGTAGCGATAAGCCAAAATTGC	P	N/A	N/A
BAMf3/BAMr3	GCAATTTTGGCTTATCGCTACT/TATGTTCCGTTGTCCTTGAAGA	P	N/A	N/A
BAMf4/BAMr4	GACCCAATTCTTCAAGGACAAC/TCCATTGGATCATCTCTCTCAT	N	N/A	N/A
BAMf1/BAMr1-new	GGCAAGAGCGACCTAGACAT/TTCATGTTCTCCCTGAAGCTC	P	N/A	N/A
BAMf2/BAMr2-new	TCATCGTGGACATTGAGGTG/GCGATAAGCCAAAATTGCAG	P	N/A	N/A
BAMf3/BAMr3-new	TGGAAGCAGACTTCAAAGCA/GGAAAAACTTCCCCTTCTCG	N	N/A	N/A
BAMf4/BAMr4-new	ACTGATCGAGCACGGTGAC/GCATGGTTTGGAACCCTGTA	P	N/A	N/A
HEATf1/HEATr1	AACCAGCCGCTCTTAGACAA/TAGGCTGTGAGCAGAGAGCA	Y	575	28
HEATf2/HEATr2	ATATCCGCGCAAAGGTACAC/AACCATGGGTCTTCTTACGAG	P	N/A	N/A
HEATf3/HEATr3	GAGGTGGAACTGGGTGAGTT/GGGAAAATAAACCACACAACC	P	N/A	N/A
HEATf4/HEATr4	GGCTGAAGAAGGAGGAGGTC/CCGGAGATCTGGATGAACTG	N	N/A	N/A
HEATf5/HEATr5	GGAGATCTGGATGGACTTAC/GCATCGACTGGAAGGAGAC	Y	329	8

HEATf6/HEATr6	CATCGTGGAGGACGATAAGG/CTTGCGTTCAGTCTCCGTCT	Y	308	30
HEATf7/HEATr7	GTTCGACGGTTTTCCCTTC/CTTCTTGGCCTCCTCCTTG	P	N/A	N/A
HEATf9/HEATr9	TCTATCTCCGCATCCGAAAC/TCAGGCAAGCTTCATCACAC	N	N/A	N/A
HEATf10/HEATr10	CGCGTACCTCCTCTGACAC/CGCGTACCTCCTCTGACAC	N	N/A	N/A
HEATf11/HEATr11	TTCGACCCCTTCTCTCTTGA/CTTCTTGGCCTCCTCCTTG	N	N/A	N/A
HEATf12/HEATr12	ACGTGTTCGACCCCTTCTC/CCTTCACGTCCTCCACCTT	Y	348	7
DRYf1/DRYr1	GGCACGAGGGATTATGTTGA/CAATCAGTGCTGAAGAAGCAA	N	N/A	N/A
DRYf2/DRYr2	TGTTATTGCTGTCCATTCTTTTG/TGGATGCATTTACATCCGTATC	P	N/A	N/A
DRYf3/DRYr3-new	GGCACGAGGGATTATGTTGA/TTTTCAAAAGAATGGACAGCAA	N	N/A	N/A
DRYf4/DRYr4-new	AGGAATGCAGTCTTCAGTGTCA/TGTCGCAACTTTGTCTAGATATG	P	N/A	N/A
DRYf1/DRYr1-new	TACGCGTACACCAGCGAGAC/CTCCTCCGCGATCTTGTG	P	N/A	N/A
DRYf2/DRYr2-new	TACGCGTACACCAGCGAGA/CTCCTCCGCGATCTTGTG	P	N/A	N/A
ABAf1/ABAr1	TCAAGCTTTATCGGAGGAACA/GAAAGCGATTGACTAGCATGG	N	N/A	N/A
ABAf1/ABAr1-new	AACGCCTTCGACAACAAGAT/GCAAGCCCAAAATCACAAAT	N	N/A	N/A
ABAf2/ABAr2-new	AACGCCTTCGACAACAAGAT/GTACGAGCAAGCCCAAAATC	N	N/A	N/A
ABAf3/ABAr3-new	TGGTCCGTGGGTTGTATTTT/ACAATGCATGCTGCTCAAAG	N	N/A	N/A
ABAf4/ABAr4-new	TGGTCCGTGGGTTGTATTTT/CAATGCATGCTGCTCAAAGT	N	N/A	N/A
ABAf5/ABAr5-new	CGGAGGAACATTGCCAGTAT/CCCGATGAGCTCCATTAGAA	N	N/A	N/A
ABAf6/ABAr6-new	ATCAAATTCTCCGTGGCTTG/CCCGATGAGCTCCATTAGAA	Y	331	0
GASTf1/GASTr1	GTGCCTCACCTACTGCAACA/CACCGTATAGGCCGAAACAC	P	N/A	N/A
GASTf1/GASTr1-new	GTCCAAGTGCTCATCGAGGT/AATCCTCGCTCTCCCTTCTC	N	N/A	N/A
GASTf2/GASTr2-new	GTCCAAGTGCTCATCGAGGT/GAATCCTCGCTCTCCCTTCT	N	N/A	N/A
GASTf2/GASTr2	ATTCCCCGTGGAGGTGAT/GAATCCTCGCTCTCCCTTCT	N	N/A	N/A

GASTf3/GASTr3-new	GTGCCTCACCTACTGCAACA/CACCGTATAGGCCGAAACAC	P	N/A	N/A
GASTf4/GASTr4-new	TGCCTCACCTACTGCAACAA/CACCGTATAGGCCGAAACAC	Y	582	0
GASTf3/GASTr3	ATGGCCAAGATCTCCTTCCT/AATCCTCGCTCTCCCTTCTC	N	N/A	N/A
NACf1/NACr1	CTCTCAAGTCGCCATGAACA/GGTCGAGGCTGTAGAGGTTG	P	N/A	N/A
NACf1/NACr1-new	CTCTCAAGTCGCCATGAACA/GGTCGAGGCTGTAGAGGTTG	P	N/A	N/A
NACf2/NACr2	AACGTTCCAAGCAAATGCAC/ATACTGCAGCACCAGCTCCT	N	N/A	N/A
NACf2/NACr2-new	ACTGCAAGTCGCCATGAAC/GGTCGAGGCTGTAGAGGTTG	N	N/A	N/A
NACf3/NACr3	CTCTCAAGTCGCCATGAACA/GGTCGAGGCTGTAGAGGTTG	P	N/A	N/A
NACf3/NACr3-new	GCACGAGGCTAGACTCTCTCA/GGTCGAGGCTGTAGAGGTTG	N	N/A	N/A
VPf1/VPr1	AATAATAAGCCGGCAGCAGA/CAAAATCCCCTGCAAGTTTC	P	N/A	N/A
VPf2/VPr2	CGTGATTTACTCTGATGTCAAGG/AGATCCTCACCGCCATCATA	N	N/A	N/A
VPf1/VPr1-new	AGAAGGTGCTGAAGCAGAGC/CAAAATCCCCTGCAAGTTTC	N	N/A	N/A
VPf2/VPr2-new	CATCTGCAAGCTAGCCACAC/AGATCCTCACCGCCATCATA	N	N/A	N/A
SVPf1/SVPr1	CTCGTCGTCTTCTCCTCCAC/TCTCTTCTGCCAGCTGTGTG	N	N/A	N/A
SVPf1/SVPr1-new	CTCGTCGTCTTCTCCTCCAC/CAAGCGCATGTTCTCTTCTG	N	N/A	N/A
SVPf2/SVPr2-new	GGAAGCTCCAGTATGGACGA/CAAGCGCATGTTCTCTTCTG	N	N/A	N/A
HDf1/HDr1	GGAGGAGTACTACGACGAGCA/CCAGCGTCTTTGTCTTCCAC	P	N/A	N/A
HDf1/HDr1-new	CAGGTGCATCTGCTTGAGAG/CCAGCGTCTTTGTCTTCCAC	Y	153	0
HDf2/HDr2-new	AGGTGCATCTGCTTGAGAGG/CCAGCGTCTTTGTCTTCCAC	P	N/A	N/A
Perf1/Perr1	CGAACCTGGAGCTGGACTC/GTGGCCACTTTGCCCTTG	N	N/A	N/A
Perf1/Perr1-new	CCGACTCCTACGTCATCCTC/GACGGGTACAGGAAGCTGAG	N	N/A	N/A
Perf2/Perr2-new	ACTACGTCGCCGACTCCTAC/GACGGGTACAGGAAGCTGAG	N	N/A	N/A
PHYAf1/PHYAr1	TGAACAACCAGGGCAACAGC/TTGCCCCCATACAGGAGAGC	Y	587	0

PHYAf2/PHYAr2	GCGTCTGCGTAATGCTCCAA/AGGGTGCATCCTTCTGCTGTC	Y	279	0
PHYAf3/PHYAr3	AATGATGCCAGCAAGCCAAA/GCATCCTCTGGACACCTGGTA	Y	278	2
PHYf1/PHYr1-new	CTGGAGCTTCTGCTCTTGGT/TGCATCCTTCTGCTGTCATC	Y	162	0
PHYf2/PHYr2-new	TTGCATTATCCTGCCACTGA/CCATGTTTGCCATGTACTGC	Y	198	0
PHYf3/PHYr3-new	TTGTGCTTGGTTGGCTCTACT/CACAGCAGCGTCTGTGTTCT	Y	333	0
PHYAf4/PHYAr4	TCACAACCCGAACCCACTCA/GACGTCTGCTCCGAGGCTTG	Y	413	0
PHYAf5/PHYAr5	CAGGGATTCCACTGGCCTGA/CCAAGACAAGCTCTTCATCTTGACA	Y	344	0
PHYAf6/PHYAr6	GAATGATGCCAGCAAGCCAAC/AGCATCCTCTGGACAACTGG	P	N/A	N/A
PHYAf7/PHYAr7	CGTGGCTGCAGGAGTACCAT/TGGATTGCATCCATTTCAACA	P	N/A	N/A
PHYAf8/PHYAr8	CGTGGCTCCCTGCAAGATG/TCAAGATCTGTTTAACCACTTCCACA	Y	303	0
PHYBf1/PHYBr1	GCCTCCATGATGCTGGCTAT/GCATCCATCTCATAGTCAGTCCAAGA	Y	323	0
PHYf4/PHYr4-new	GGATTCCACTGGCCTGAGT/TTGCAATGAATGAATAGCATCC	Y	303	0
PHYCf1/PHYCr1	CGAGCCGTACCTTGGTTTGC/AGGCCTGCATGAGGAACTCG	P	N/A	N/A
PHYf5/PHYr5-new	AAAACTTGATGGGCTTGCTG/GCATCCTCTGGACAACTGGT	Y	291	0
FRI-Bf1/FRI-Br1	TCGCACCACCTAGTACTCCTC/TGCCAGATAGTTGACGGTTG	N	N/A	N/A
FRI-Bf2/FRI-Br2	ACGGGATTGGGCGTAACTAT/TCCTCGAGTTGCTTCCACTT	N	N/A	N/A
FRI-Bf3/FRI-Br3	TGAGCAGTCGCTGAAGAAAA/AGCACTGCTGTCTTGCATGT	N	N/A	N/A
FRI-Bf4/FRI-Br4	TCAGACAACCGAAAGAACTTGA/TGGATTTCCACTCAAAAGCA	N	N/A	N/A
FRI-Bf5/FRI-Br5	TTGGATAGCCTTGACGTTGA/GAAAACTAAGAGTTGTTCCAGTGAA	N	N/A	N/A
FRI-Bf6/FRI-Br6	TGATGGTTAGTAGGTGTGCTGAA/ACCAAACCTAGAACTCAACAAAAA	N	N/A	N/A
FRI-Bf7/FRI-Br7	TGCACATTCCTTACTTTTGTCTG/AAACAAAATCCTCAAACATGTGG	N	N/A	N/A
FRI-Bf8/FRI-Br8	GTGGATAGCGCAAAACCAAT/CCATGAAAGGAGAAGGCACT	N	N/A	N/A
FRI-Bf9/FRI-Br9	CATATGTTGCTGGCCTCAAT/CCACAACCTACCTCAAACTGC	N	N/A	N/A

FRI-Bf10/FRI-Br10	GTGCATGTATGCAAGGTATGC/ACCGAAGCTGGTAACACGAG	N	N/A	N/A
FRI-Bf11/FRI-Br11	TAAACGCAGCTCCCTACACC/TCATCATGGGCAGAAGATACA	N	N/A	N/A
FRIf1/FRIr1	CTACCGGAGGATCGTTCTCA/CGGTCCTCGATGCATTTTAT	P	N/A	N/A
FRIf2/FRIr2	GCCTCATGCTGATGGAGTCT/ATCTTTTGCGACAACCCAAG	Y	379	0
FRIf3/FRIr3	GTGTCACATGCCAAGAATGG/TCGGCACCATAGACAAACTG	N	N/A	N/A
GIf1/GIr1	GGGCATCCGAGTTCGTTAG/AATCTGCTGAAGTGCCGTCT	Y	367	0
GIf2/GIr2	TCTCGTTGCTGTGGCATAAG/ACGGAGCAGAAGGTCTGAAG	P	N/A	N/A
GIf3/GIr3	GAGCCATCCATCTCATCGTT/GTTGTGGGCTTCCCACTCTA	Y	386	0
PIFf1/PIFr1	ATAACCAGGCCGAGAGGAG/TATCGATGCCTTGTCGCTCT	N	N/A	N/A
PIFf2/PIFr2	GCCGACGTCCATAACCAG/CTTGTCGCTCTTGTTGCAGT	N	N/A	N/A
ARFf1/ARFr1	CGTTGGGATGAGCCTTCTAC/GCACCAGAAATTGCATCCTT	Y	527	4
ARFf2/ARFr2	CAACGTGCCTTCTTCAGTCA/TTTGACCCGAGAAAGAGGAA	P	N/A	N/A
ARFf3/ARFr3	TCCCTGGCAAATAGAACCTG/TTGACTGTGTGCCAGACGTT	P	N/A	N/A
ARFf4/ARFr4	ACTGGATATGAGCCGTCAGC/TGCCATGCTGTAGCAAGAAC	P	N/A	N/A
ARFf5/ARFr5	AGTCCAGCTGAGTTTGTGGT/CACCGCACCTTAAGGGAAC	N	N/A	N/A
ARFf6/ARFr6	ATACTCAGCGGGTTGGTTTG/CAAGTGGCCTCGGAATCTTA	Y	309	2
ARFf7/ARFr7	AAGAAGGTCCACAAGCAAGG/CAGCTGAGCCTCTTTCCATC	P	N/A	N/A
DELf1/DELr1	CAACGAAGAACCCGAGGTAA/GAGGTACACCTCGGACATGA	Y	315	11
DELf2/DELr2	CAACGAAGAACCCGAGGTAAT/GAGGTACACCTCGGACATGA	Y	315	11
DELf3/DELr3	CAGATCTGCAACGTCGTG/AGTGTGCCACCCAAGTGTC	N	N/A	N/A
DELf4/DELr4	CAGATCTGCAACGTCGTG/GAGTGTGCCACCCAAGTGT	N	N/A	N/A

† Primer names: Name of the primer pairs based on the gene name and reference sequences listed in Table 3.1.
‡ Primer Sequences (forward/reverse): The sequences of the primer forward sequence /reverse sequence.
§ Amplification (Y/P/N): PCR amplification status for each primer pair. Y= amplified all 4 check accessions; P= partial amplification in only 1, 2, or 3 of the 4 checks; N= no amplification for any of the 4 checks.
¶ Sequence Length: The length in bp of the amplified fragments for each primer pair.
# No. of Polymorphisms: The number of polymorphisms, including SNPs and indels, identified from the alignment of 4 check sequences for each primer pair.

Table 3.3. Summary of 38 KASP markers, clear separation between dormant and non-dormant checks, and putative accessions for selected identified polymorphisms (SNPs).

Marker name †	Marker sequences (D/ND/R) ‡	Separation on checks (Y/N) §	Separation on 52 accessions (Y/N) ¶
CEN3-TF-15s	ACATCTTCTTCTTGCACATAT/ ACATCTTCTTCTTGCACATATAT/GAAGCATCT GTTGTCCCGGGT	N	N
CEN3-TF-15116s	GAAGGTCGGAGTCAACGGATTTGAGAGTCAT TTACCAATACCTTTTGC/GAAGGTGACCAAGT TCATGCTTGAGAGTCATTTACCAATACCTTTT GA/TGAAGCATCTGTTGTCCCGG	N	N
CEN4-TF-1574	GAAGGTGACCAAGTTCATGCTTCAGTACATC TTCTTCTTGCACATAT/GAAGGTCGGAGTCAA CGGATTTCAGTACATCTTCTTCTTGCACATAT AT/ACTCTCATTGGAAGTGCTGGT	Y	Y
CEN4-TF-15136	GAAGGTCGGAGTCAACGGATTGAGAGTCATT TACCAATACCTTTTGC/GAAGGTGACCAAGTT CATGCTGAGAGTCATTTACCAATACCTTTTGA /CCCGGGTATATTACTGACAATCCTGT	N	N
TFNEW-TF-1590s	GAAGGTGACCAAGTTCATGCTGTTCTGCATTT TGAATGAATAACCATTC/GAAGGTCGGAGTCA ACGGATTGTTCTGCATTTTGAATGAATAACCA TTG/AACTGGCGGGTGTTGAAATG	N	N
TFNEW-TF-15219-3	GAAGGTGACCAAGTTCATGCTACTGTATCTGT GCCTTCCTTCAGA/GAAGGTCGGAGTCAACGG ATTACTGTATCTGTGCCTTCCTTCAGG/TGGCA CAACTGGGATAACCA	Y	Y
CO1-TF-1576	GAAGGTCGGAGTCAACGGATTCCAGCTCCCC TCTCGTCCAT/GAAGGTGACCAAGTTCATGCT CCAGCTCCCCTCTCGTCCAA/CCACAAGGCCT CCTCGTTCA	Y	Y
CO1-TF-1598l	GAAGGTCGGAGTCAACGGATTCGCCTCCTTG AAAATGGTGCC/GAAGGTGACCAAGTTCATGC TCGCCTCCTTGAAAATGGTGCA/TAGCCACAA GGCCTCCTCGTTC	Y	Y
CO1-TF-15180	GAAGGTGACCAAGTTCATGCTTGTAATGGGT GTTAGGGAATGTAGTAC/GAAGGTCGGAGTC AACGGATTTGTAATGGGTGTTAGGGAATGTA GTAG/ACAGATGCGTAGGGAAACAGT	Y	Y
CO4-TF-1591l	GAAGGTGACCAAGTTCATGCTCACTTCGATTT CCGCTTCTGATATTTTC/GAAGGTCGGAGTCA ACGGATTCACTTCGATTTCCGCTTCTGATATT TTT/TCGAGAAGACCACACGTTACGC	Y	Y
VER-MADS-TF-1586o	GAAGGTGACCAAGTTCATGCTGGCGGTCCTA GATTCCGTGG/GAAGGTCGGAGTCAACGGATT GGCGGTCCTAGATTCCGTGT/TGGAGCATAAA GGAGAACAGTCAGT	Y	Y
HEAT1-TF-15130	GAAGGTCGGAGTCAACGGATTACGCCTTGAT CTGCTCCGTC/GAAGGTGACCAAGTTCATGCT ACGCCTTGATCTGCTCCGTA/CAGCAGCGGCA AGTTTCTG	Y	Y
HEAT1-TF-1572/140	GAAGGTGACCAAGTTCATGCTCGGGCTTCTT GGCCTCCTC/GAAGGTCGGAGTCAACGGATTC GGGCTTCTTGGCCTCCTG/GAAGTCCGACACC TGGCACC	Y	N
HEAT1-TF-15262	GAAGGTCGGAGTCAACGGATTAGCACGTTGC CGTCGTCG/GAAGGTGACCAAGTTCATGCTAG CACGTTGCCGTCGTCC/ACGCCAGAGGCTCAC GTGTT	Y	N
HEAT1-TF-15409	GAAGGTGACCAAGTTCATGCTATGCTGCCGC TGCCGGAA/GAAGGTCGGAGTCAACGGATTA TGCTGCCGCTGCCGGAG/CAGTTCATCGCCAA CCCGCC	Y	Y

HEAT1-TF-15292s	GAAGGTGACCAAGTTCATGCTACCTTCACCTC CTCCTTCTTCAGC/GAAGGTCGGAGTCAACGG ATTACCTTCACCTCCTCCTTCTTCAGT/GCCAG AGGCTCACGTGTTCA	Y	Y
HEAT1-TF-15472	GAAGGTCGGAGTCAACGGATTGGGTCGAACA CGTCGGCT/GAAGGTGACCAAGTTCATGCTGG GTCGAACACGTCGGCG/TAAACATTCAACAGT TCATCGCCAACC	Y	Y
HEAT1-TF-15494	GAAGGTCGGAGTCAACGGATTGCGAATCAGC GACATCGTCT/GAAGGTGACCAAGTTCATGCT GCGAATCAGCGACATCGTCG/TAAACATTCAA CAGTTCATCGCCAACC	N	N
HEAT5-TF-1545s	GAAGGTCGGAGTCAACGGATTGCGCACGTGT TCAAGGCG/GAAGGTGACCAAGTTCATGCTGC GCACGTGTTCAAGGCA/CGTTGCCGTCCTCCA CCTC	Y	N
HEAT5-TF-1576	GAAGGTCGGAGTCAACGGATTGGGCTGAAGA AGGAGGAGGTG/GAAGGTGACCAAGTTCATG CTGGGCTGAAGAAGGAGGAGGTC/GGTGCCA GGTGTCCGTCTTC	N	N
HEAT5-TF-15105l	GAAGGTGACCAAGTTCATGCTGGAGGACGGC AACGTGCTC/GAAGGTCGGAGTCAACGGATTG GAGGACGGCAACGTGCTG/CGTTCTCCATGGA CGCCTTC	Y	N
HEAT12-TF-1536	GAAGGTCGGAGTCAACGGATTAACAGCGAGA CGGCCGCT/GAAGGTGACCAAGTTCATGCTAA CAGCGAGACGGCCGCG/CCGTCCTCCACCTCC ACCTT	Y	N
HEAT12-TF-1581	GAAGGTGACCAAGTTCATGCTGAGACCCCCG AGGCGCAC/GAAGGTCGGAGTCAACGGATTG AGACCCCCGAGGCGCAT/CTGACGAACTTGCC GCTGCT	Y	N
HEAT12-TF-15174	GAAGGTGACCAAGTTCATGCTGTCAGCGGCG AGCGCACC/GAAGGTCGGAGTCAACGGATTG TCAGCGGCGAGCGCACG/CGTTCTCCGGCAGG CGGAAG	Y	N
PHYA3-TF-15101s	GAAGGTCGGAGTCAACGGATTCCAGTCAACT CCGCTGCTTTG/GAAGGTGACCAAGTTCATGC TCCAGTCAACTCCGCTGCTTTC/TGCTGAATTG CAGGCGGTGA	N	N
PHYA3-TF-1580s	GAAGGTGACCAAGTTCATGCTTCTTCCTATTG CATCATCAACTCTTAGC/GAAGGTCGGAGTCA ACGGATTTCTTCCTATTGCATCATCAACTCTT AGG/GGACTGGTCAATGGGTGGAA	N	N
ARF1-TF-15167m	GAAGGTCGGAGTCAACGGATTGGTGGCATCA GACTCATTACTGTCAG/GAAGGTGACCAAGTT CATGCTGGTGGCATCAGACTCATTACTGTCAA /TGCAGGTGCAACTAAGGCTGA	Y	Y
ARF1-TF-15351s	GAAGGTGACCAAGTTCATGCTGCTTCCACTA CGACTAACGACCTTC/GAAGGTCGGAGTCAAC GGATTGCTTCCACTACGACTAACGACCTTG/A ACCCGCTTCCTCTTTCTCG	Y	N
ARF1-TF-15383	GAAGGTCGGAGTCAACGGATTAGTACCTTCT TTAGTAAGAACAGATGAT/GAAGGTGACCAA GTTCATGCTAGTACCTTCTTTAGTAAGAACAG ATGAC/TCTACTATTCCACGCCCGGA	Y	Y
ARF1-TF-1530s	GAAGGTCGGAGTCAACGGATTAAATTGCATC CTTGAAGTCAGG/GAAGGTGACCAAGTTCATG CTAAATTGCATCCTTGAAGTCAGC/TGGATGC AGTTGGGAAGGCG	N	N
ARF6-TF-1556s	GAAGGTCGGAGTCAACGGATTCCAAGTTCTC ACATGGATGACACG/GAAGGTGACCAAGTTC ATGCTCCAAGTTCTCACATGGATGACACA/CG CTGGGCAGTCAAGATGAA	Y	Y
ARF6-TF-15278l	GAAGGTGACCAAGTTCATGCTCGCAACTGAA CTAGAAAAAGTTTCTGA/GAAGGTCGGAGTCA ACGGATTCGCAACTGAACTAGAAAAAGTTTC TGG/GGTGTCAAGTGGCCTCGGAA	Y	Y

DEL1-TF-15131s	GAAGGTGACCAAGTTCATGCTCCGAGGATCG TGACCGTGGTA/GAAGGTCGGAGTCAACGGA TTCCGAGGATCGTGACCGTGGTG/GGACTCGG TGAATCGGTCCAG	Y	N
DEL1-TF-15153s	GAAGGTCGGAGTCAACGGATTGCAGGAGGCC AACCACAACG/GAAGGTGACCAAGTTCATGCT GCAGGAGGCCAACCACAACA/TCGAACATGG TGGAGTAGTAGT	Y	Y
DEL1-TF-15158	GAAGGTGACCAAGTTCATGCTGCCAACCACA ACGCCGGC/GAAGGTCGGAGTCAACGGATTG CCAACCACAACGCCGGT/ACATGACCTGGTCC GTGCCA	N	N
DEL1-TF-15212l	GAAGGTCGGAGTCAACGGATTGCACTACTAC TCCACCATGTTCGAT/GAAGGTGACCAAGTTC ATGCTGCACTACTACTCCACCATGTTCGAC/T CGGACATGACCTGGTCCGT	Y	Y
DEL1-TF-15245	GAAGGTGACCAAGTTCATGCTGGCTCCGGCC CGTCCGAA/GAAGGTCGGAGTCAACGGATTG GCTCCGGCCCGTCCGAG/CCTCGGACATGACC TGGTCCGT	N	N
DEL1-TF-15269	GAAGGTCGGAGTCAACGGATTTCGGGGCCTG CTGCTGCT/GAAGGTGACCAAGTTCATGCTTC GGGGCCTGCTGCTGCC/GGACATGACCTGGTC CGTGCC	N	N

† Marker name: Name of the KASP Markers. The first letters correspond to the gene name, followed by TF (tall fescue), and then sequence and position of the polymorphism.

‡ Marker Sequences (D/ND/R): The sequences of designed KASP markers, listed as allele specific primer for dormant genotypes / allele specific primer for non-dormant genotypes/ reverse primer sequence.

§ Separation on Checks (Y/N): Resolution of separation between dormant and non-dormant alleles in the checks based on genotyping scatterplots. Y= clear separation; N= poor separation.

¶ Separation on 52 Accessions (Y/N) = Resolution of separation between dormant and non-dormant alleles between 52 dormant and non-dormant accessions based on genotyping scatterplots. Y= clear separation; N= poor separation.

Table 3.4. 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.

KASP SNP markers	SNP alleles (D/ND)†	No. of heterozygous‡	Correlation with SDRS§	Correlation with GRL score¶	R2 with SDRS§	R^2 with GRL score¶
CO1-TF-1598l	C/A	2	0.27	0.23	0.12*	0.01
ARF1-TF-15167m	G/A	1	0.11	-0.13	0.02	0.07
CO1-TF-1576	T/A	4	0.27	0.23	0.11*	0.00
ARF6-TF-15278l	A/G	5	0.09	-0.20	0.00	0.13*
TFNEW-TF-15219-3	A/G	1	0.33*	0.26	0.10*	0.01
CO4-TF-1591l	C/T	0	0.33*	0.26	0.11*	0.00
CO1-TF-15180	C/G	1	0.37*	0.31*	0.13*	0.06
VER-MADS-TF-1586o	G/T	8	0.16	-0.29	0.00	0.20*
HEAT1-TF-15130	C/A	0	0.16	-0.26	0.01	0.15*
ARF1-TF-15383	T/C	4	0.04	-0.24	0.00	0.14*
HEAT1-TF-15292s	C/T	4	0.09	-0.26	0.03	0.07
HEAT1-TF-15472	T/G	8	0.14	0.11	0.06	0.00
HEAT1-TF-15409	A/G	5	-0.02	-0.24	0.01	0.03
CEN4-TF-1574	Del. AT in dormant	2	0.23	0.16	0.08	0.04
ARF6-TF-1556s	G/A	16	0.16	-0.23	0.02	0.16*
DEL1-TF-15212l	T/C	22	0.13	-0.12	0.04	0.03
DEL1-TF-15153s	G/A	17	0.05	0.09	0.01	0.02

* Significant at the 0.05 probability level.
† SNP Alleles (D/ND): Polymorphism alleles specific to dormant (D) genotypes and non-dormant (ND) genotypes.
‡ No. of heterozygous: The number of accessions heterozygous for dormant and non-dormant alleles.
§ Correlation/R^2 with SDRS = Correlation coefficients/association R^2 between KASP Marker scores and SDRS. SDRS indicates summer dormancy rating score based on the average of field regrowth height and senescence scores for 2 years.
¶ Correlation/R^2 with GRL score= Correlation coefficients/association R^2 between KASP Markers score and the score of ratio of germination rate under 30℃over that under 20℃under 24 hours of light/dark conditions of each accession based on a scale 1-10.

Table 3.5. Average difference in the number of tiller buds between Clipped and Non-clipped treatment group of two dormant tall fescue checks and two non-dormant checks. In the clipped treatments, the plants were prevented from flowering by continuous clipping. In the non-clipped treatment, the plants were allowed to flower and set seeds.

Accession	Replications	Ave. difference
T0706-1 (dormant)	10	0.8*
AGRFA (dormant)	8	1.2*
GA186 (non-dormant)	10	-0.1
K31 (non-dormant)	10	0.1

* Significant at the 0.05 probability level.

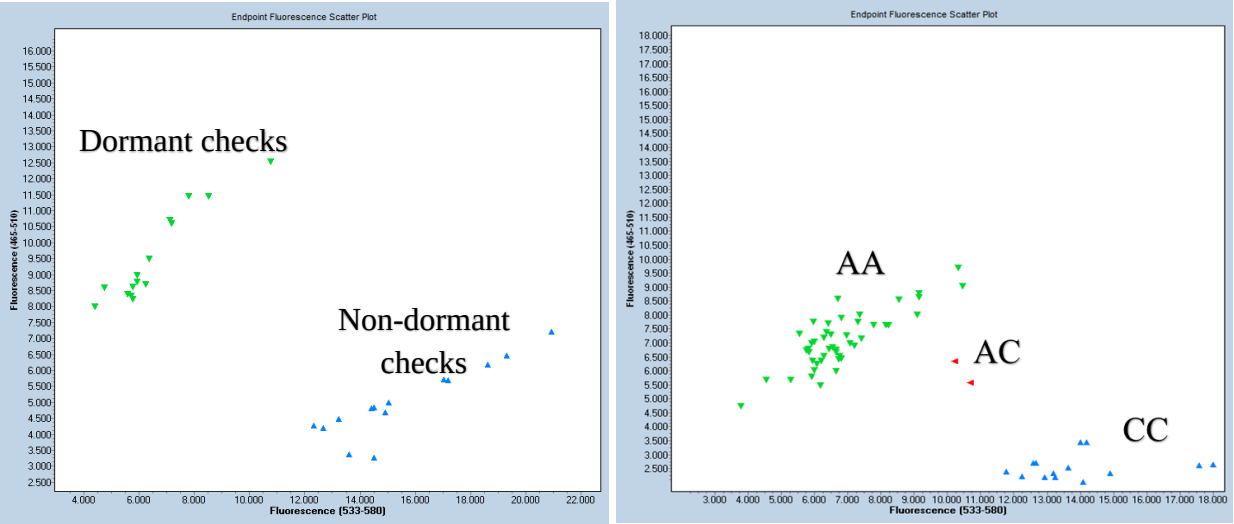

Figure 3.1. Genotyping graph of the KASP marker CO1-TF-1598 on 4 checks with 8 replications for each (left), and genotyping graph of 52 putative accessions and 4 checks (right). Dormant checks include T0706-1 and AGRFA-126. Non-dormant checks include Kentucky 31 and GA186. AA= homozygous alleles from sequencing data of non-dormant check; AC= heterozygous alleles from sequencing data of both dormant and non-dormant check accessions; CC= homozygous alleles from sequencing data of dormant check.

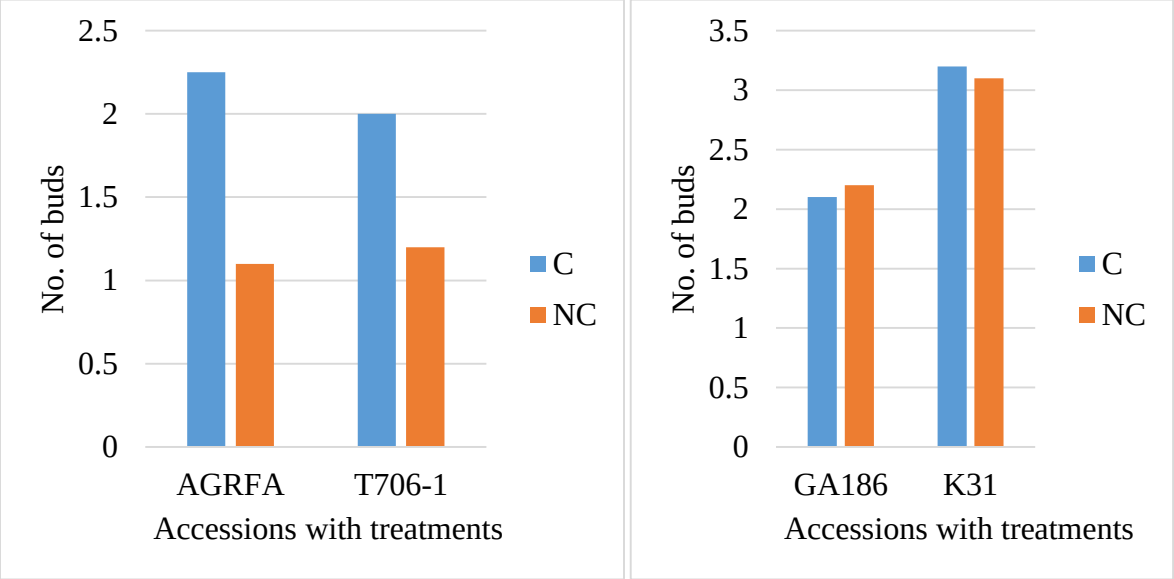

Figure 3.2. Average number of buds between clipped and non-clipped treatments of dormant checks AGRFA-126 and GA186 (left) and non-dormant checks Kentucky 31 and T0706-1 (right). C= clipped treatment; NC= non-clipped treatment.

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CHAPTER 4

SUMMARY

Tall fescue is a widely used cool-season forage grass. Tall fescue germplasm can be classified into two major groups, continental and Mediterranean. Most Mediterranean germplasm exhibit summer dormancy even when conditions are favorable. Understanding the mechanisms and genetic factors underlying summer dormancy in tall fescue can aid in a faster transfer of favorable traits across germplasm pools and speed up the process of cultivar development. Phenotyping summer dormancy in the field is difficult and costly. The objectives of this study were to: i) develop a fast and low-cost surrogate phenotyping approach and a rating system of summer dormancy in tall fescue, ii) analyze candidate genes for association with summer dormancy and possible identification of markers that can discriminate between dormant and non-dormant genotypes iii) test the relationship between summer dormancy and determinacy.

Environmental cues that trigger dormancy in mother plants should act similarly on the embryo during seed dormancy/germination, and thus seed germination may be used as a surrogate phenotype. Germination tests were conducted under various combinations of photoperiod and temperature using 2 non-dormant and 2 dormant check varieties in growth chamber conditions. Combinations of six temperatures (18°C, 20°C, 22°C, 28°C, 30°C, 32°C, and 34°C) and 5 photoperiods (0/24, 12/12 14/8, 16/8 and 24/0 hours of light/dark) were tested for their effect on seed germination. The data showed that temperature had a greater effect on germination than photoperiod, with higher temperatures reducing the germination of dormant but

not non-dormant checks. 168 accessions of tall fescue were evaluated for summer dormancy in field conditions using a combined score of regrowth height and senescence based on Digital Image Analysis. Twenty six putative non-dormant and 30 dormant accessions were selected for seed germination testing to establish a correlation between seed germination and field dormancy rating scores. The data showed that the ratio of seed germination at 30°C over 20°C under 24 hrs photoperiod correlates well ($r = 0.7$) with field dormancy phenotypes and has potential to be used as proxy for phenotyping summer dormancy in tall fescue. The high correlation between summer dormancy rating scores in the field and the surrogate phenotype scores based on seed germination, in addition to the fairly high heritability of the two parameters, suggests that the surrogate phenotyping method may have potential application in the breeding process to select for summer dormancy or summer activity. Research to determine the genetic gain from selection based on the surrogate phenotype which would require making crosses and developing structured populations should be carried out in the future.

The regulation of endo-dormancy has been associated with flowering, slowing of metabolic activity in meristematic tissues, and translocation of reserves to storage structures like crowns. Endo-dormancy is most likely genetically controlled and inherited. Allelic variation among and between summer dormant and non-dormant varieties were suggested and therefore identifying the genetic factors underlying summer dormancy may enable the development of molecular markers that will be useful for breeding and selection. Since there are no genomic resources available for tall fescue, we relied on candidate genes that have been documented for their involvement in seasonal dormancy in trees and other crop species. Twenty-three candidate genes were amplified and sequenced in two dormant and two non-dormant tall fescue checks. Sequence data was used to develop kompetitive allele specific PCR (KASP) markers. Twenty-

six markers were tested in 56 dormant and non-dormant accessions including 4 check accessions. Five markers showed significant associations ($p<0.05$) with the field phenotypic scores. Even though these markers have small effects, typical of polygenic quantitative traits, they can be combined to increase the allele frequency in tall fescue breeding populations through marker assisted recurrent selection. Validation work in a larger set of diverse genotypes in underway. These markers are from the genes CONSTANS and TERMINAL FLOWER 1 known to be involved in determinacy. The meristems in determinate plants cease vegetative activity at or soon after photoperiod-induced floral induction, similar to what takes place during dormancy. The relationship between summer dormancy and determinate growth was tested on two dormant (T0706-1 and AGRFA-126) and two non-dormant (Kentucky 31 and GA186) checks, where half the entries in each check were continuously clipped and prevented from flowering and the second half were not clipped and allowed to flower and set seed. In the non-dormant checks, no difference in the number of new tiller buds was observed between the clipped and non-clipped treatments. But, there was a significant ($p<0.05$) difference between clipped and non-clipped treatments within the dormant checks. These results suggest that meristem determinacy is probably one of the mechanisms involved in summer dormancy of tall fescue. A more extensive study needs to be conducted on a large set of germplasm to draw a firm conclusion.

Tall fescue populations segregating for summer dormancy are being developed for the genetic mapping of trait. A non-dormant genotype GA186 is currently being crossed with the summer dormant genotypes T0706-1 and AGRFA-126, to generate bi-parental mappings populations segregating for summer dormancy. To maximize recombination between chromosomal segments associated with the trait in the progeny, an F_2 population involving sib-mating between F_1 will be developed in the greenhouse. The F_2 mapping populations will be

used to identify QTL associated with dormancy and related factors such as determinacy in future work. The mapping populations will also be used to validate potential application of the KASP markers that showed significant association with summer dormancy rating scores in potential application in increasing allele frequency in breeding populations through marker assisted recurrent selection.