# THE MOLECULAR AND PHYSIOLOGICAL BASIS FOR TEMPERATURE MEDIATED REGULATION OF DWARFNESS IN TIFDWARF BERMUDAGRASS

A Dissertation

by

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# ABSTRACT

Tifdwarf (*C. dactylon* (L.) Pers. x *C. transvaalensis* (Burtt-Davies) has been used on putting greens in the southern US for over 50 years. Dwarfism in Tifdwarf (TD) bermudagrass is a conditional trait. Tifdwarf internodes and leaves elongate when exposed to suboptimal temperatures. This study further quantified physiological aspects of this response and investigated the role of gibberellins in the temperature mediated release of TD dwarfism.

In controlled environment studies, TD internode and leaf lengths were two times longer in suboptimal (27°C/19°C day/night) compared to optimal temperatures (35/27°C). In NuMex Sahara (NM), a non-dwarf bermudagrass, internode and leaf length decreased or showed no response to suboptimal temperatures.

When grown under suboptimal temperatures, TD accumulated the same or less biomass than optimal treatments. NM accumulated less biomass. Suboptimal temperature reduced respiration in TD but had no affect on photosynthesis.

To investigate the role of gibberellins in conditional dwarfism, expression patterns for *GA20ox1*, *GA20ox2*, *GA3ox*, *GA2oxa*, *GA2oxb* and *GAMyb* were analyzed. Under optimal temperatures, *GA20ox2* and *GA3ox* expression were higher and *GA2oxa* expression was lower in TD than NM. Similar expression patterns are common in many GA associated dwarf mutants.

Despite limited phenotypic differences in NM given different temperature treatments, *GA20ox2* and *GA3ox* were elevated and *GA2oxa* and *GAMyb* were depressed

in suboptimal treatments. Unlike NM, and despite robust phenotypic changes, TD displayed minimal molecular responses to suboptimal temperatures. Only *GA2oxa* and *GA2oxb* displayed differential expression patterns between treatments. Both were higher in the suboptimal temperature regime.

The GA biosynthetic inhibitors CCC and flurprimidol decreased TD internode length while $GA_3$ increased length under both temperature treatments, however internodes from suboptimal treatments remained longer than optimal treatments. Trinexapac-ethyl also decreased internode length in both temperature treatments, but at the high application rate, no difference was measured between temperature treatments. Therefore, functional late-stage GA metabolic and/or catabolic enzymes are required for temperature mediated adjustments in TD morphology.

No difference due to temperature was observed in bermudagrass internode length when an inhibitor combination plus $GA_3$ was applied. This suggests that the temperature mediated adjustments in morphology are not the result of altered GA sensitivity.

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# CHAPTER I

# INTRODUCTION

Bermudagrass *Cynodon dactylon* (L.) Pers., a $C_4$ monocot, is the best-adapted turfgrass species for use on golf course greens in the hot, humid regions of the southern United States. Tifdwarf Bermudagrass (*C. dactylon* (L.) Pers. x *C. transvaalensis* Burtt-Davies) has been cultured on golf greens for more than 50 years (Beard, 1973). However, there is little published information available describing temperature's influence on Tifdwarf bermudagrass growth and development. Stanford *et al*., 2005 documented a previously undescribed temperature mediated response in Tifdwarf bermudagrass, where internode length was two-fold longer at 27°C day / 19°C night compared to 35°C day / 27°C night, producing plants that exhibited a non-dwarf phenotype. A similar response has also been witnessed in several newer ultradwarf bermudagrass cultivars (Unpublished Data).

The molecular and physiologic basis for temperature mediated regulation of dwarfness is currently unknown. Also, the general dwarfing mechanism in bermudagrass is not known. The conditional nature of Tifdwarf's dwarf phenotype could provide a model system for the study of the physiological and genetic mechanisms of dwarfism in bermudagrass. It is likely that this response causes significant management problems. However, since temperature's influence on plant morphology is not well understood, problems are likely misdiagnosed.

This project continues an ongoing effort by Texas A&M University to provide support for the turfgrass industry. A key component of that support is the development of best management practices for new and existing dwarf bermudagrass cultivars as well as providing information for cultivar improvement. To accomplish these goals, research must be conducted to understand how key environmental conditions impact physiological and molecular processes within the plant. The temperature response in Tifdwarf bermudagrass characterized by Stanford et al., 2005 has highlighted a gap in knowledge that could significantly alter prescribed management programs as well as provide a real opportunity for germplasm improvement through a deeper understanding of the molecular basis of dwarfism. The following work investigated the physiological and molecular basis for dwarfism by studying temperature induced changes in the dwarf phenotype in turf-type bermudagrasses.

Two central hypotheses guided this research. First, the conditional release of dwarfism as reported by Stanford et al., 2005, is due to a leaky dwarfing gene whose product quantity or function is altered by temperature. Second, the dwarfing gene in Tifdwarf bermudagrass codes for a gibberellin (GA) biosynthetic enzyme or a protein involved in GA signal transduction. Therefore, at optimal temperatures for C4 monocots (35°C day / 27°C night), GA synthesis and/or sensitivity is limited resulting in a dwarf phenotype. Under suboptimal temperatures (27°C day / 19°C night), bioactive GA synthesis or sensitivity increases resulting in a non-dwarf phenotype.

The first objective of this research was to further characterize temperature's influence on dwarf bermudagrass morphology and physiology. The second objective

was to better understand the physiological and genetic ramifications of dwarfism in bermudagrass. The third objective was to determine if the conditional nature of dwarfism in bermudagrass is associated with altered synthesis and/or sensitivity to GA.

# CHAPTER II

# REVIEW OF THE LITERATURE

To understand how temperature regulates Tifdwarf's dwarf phenotype, the mechanisms responsible for dwarfism and plant responses to temperature must be understood. A logical connection might exist between dwarfism and plant responses to temperature that explains the conditional nature of Tifdwarf's dwarf phenotype. This review of the literature will highlight important dwarfing mechanisms in other species, plant responses to temperature and documented interactions between dwarfism and plant temperature responses. It will also address other topics key to this research.

## Dwarfism in Plants

Shoot elongation is controlled largely by the gibberellin group (GA) of phytohormones . Brassinosteriods (BR) and auxin are two additional phytohormones that influence shoot elongation. For more detailed information on BR and auxin, refer to the review articles authored by Fridman and Savaldi-Goldstein, 2013 and Andrea Gallavotti, 2013.

In many instances, a dwarf growth habit is the results of either a lesion in the GA biosynthetic pathway, which results in reduced levels of GA (Hedden and Kamiya, 1997), or a lesion in the GA sensing/signal transduction pathway, which results in a plant with reduced responsiveness to GA (Richards et al., 2001). It is logical to investigate the possibility that Tifdwarf's plastic dwarf characteristics are regulated by a

GA associated “leaky” mutant gene. Therefore, this review will focus on GA synthesis, signaling and their associated dwarf mutants.

## GA Biosynthesis

Gibberellin (GA), a plant phytohormone, plays a critical regulatory role in key plant processes such as seed development, flower development, and stem and leaf expansion. Synthesis and deactivation of bioactive GA includes multi-step pathways that are both complex and tightly regulated. For a detailed explanation of the GA metabolic and catabolic pathways, reference the review publication authored by Shinjiro Yamaguchi, 2008.

GA synthesis and catabolism can been divided into three stages. Each stage and its associated enzymes and products are presented in Appendix A (Yamaguchi, 2008).

GA metabolism begins with geranylgeranyl diphosphate (GGDP) and concludes with the production of bioactive gibberellic acid ($GA_1$ or $GA_4$). Deactivation by GA2ox occurs at multiple steps along the pathway and serves to tightly regulate endogenous GA levels.

## Key GA Synthesis Genes and Their Associated Dwarf Mutants

The genes associated with both GA biosynthesis and GA signal transduction are often comprised of gene families whose expression are tightly regulated in a tissue and/or developmentally specific manner. This research focuses on temperature induced adjustments to vegetative growth and development. Therefore, only genes whose

expression profiles and/or mutant phenotypes are associated with vegetative growth will be highlighted in this review.

Also, since Tifdwarf is responsive to exogenous GA, only GA associated candidate genes whose mutant phenotype displays GA responsive semi-dwarf or dwarf phenotypes will be described (Dudeck and Peacock, 1985). *Arabidopsis* and rice are the two well characterized plant species for GA regulation of vegetative growth and will therefore be the primary focus. Other species will be discussed where information is available.

*Early GA Biosynthesis Genes*

*ent*-copalyl diphosphate synthase (*CPS)*, *ent*-kaurene synthase (*KS)*, *ent*-kaurene oxidase (KO), and *ent*-kaurenoic acid oxidase (KOA) are GA biosynthesis genes whose products convert geranylgeranyl diphosphate (GGDP) into $GA_{12}$. They are primarily single copy genes that are expressed in most actively growing tissues (Silverstone et al., 1997; Sakamoto et al., 2004; Yamaguchi et al., 1998).

In general, a loss of function mutation of CPS produces dwarf plants that contain limited bioactive GA and are responsive to exogenous bioactive GA (Koorneef and van der Veen, 1980; Reid and Ross 1993; Sun and Kamiya, 1994; Benson et al., 1995; Phillips et al., 1995; AitAli et al., 1997; Silverstone et al., 1997; Cowling et al., 1998; Silverstone et al., 1998; Elliott et al., 2001; Sakamoto et al., 2004). Also, expression levels of those *GA20ox, GA3ox* GA biosynthesis gene and *GID1* GA receptor gene family members that are feedback regulated are elevated compared to wild type (WT).

The *Arabidopsis* CPS loss-of-function mutant, *ga1* is GA responsive and displays a dark green dwarf phenotype (Koorneef and van der Veen, 1980; Sun at al., 1992; Sun and Kamiya, 1994). Compared to WT, *ga1* contains limited bioactive GA, elevated *AtGA20ox1*, *AtGA20ox2*, *AtGA20ox3*, *AtGA3ox1*, *GID1a*, *GID1b*, and *GID1c* expression and reduced RGA expression (Koorneef and van der Veen, 1980; Sun and Kamiya, 1994; Phillips et al., 1995; Silverstone et al., 1997; Cowling et al., 1998; Silverstone et al., 1998; Thomas et al., 1999; Griffiths et al., 2006). Application of bioactive GA is capable of restoring the WT phenotype.

The pea CPS loss-of-function mutant *ls-1* is also a GA responsive dwarf with limited bioactive GA, elevated *GA20ox1* and *GA3ox1* and reduced *GA2ox1* and *GA2ox2* GA catabolic gene expression (Reid and Ross 1993; AitAli et al., 1997; Elliott et al., 2001). The maize *an1* and tomato *gib-1* are also CPS loss-of-function mutants with GA responsive dwarf phenotypes that contain limited bioactive GA. (Zeevaart, 1986; Bensen and Zeevaart, 1990; Benson et al., 1995).

Like CPS loss-of-function mutants, KS, KO, and KOA loss-of-function mutants are GA responsive dwarfs that contain reduced bioactive GA (Hedden and Phinney, 1979; Koorneef and van der Veen, 1980; Ingram et al., 1984; Zeevaart, 1986; Fujioka et al., 1988a; Bensen and Zeevaart; 1990, Proebstring et al., 1992; Reid and Ross 1993; Martin et al., 1996; Helliwell et al., 1998; Yamaguchi et al., 1998; Sakamoto et al., 2004;). Examples of loss-of-function KS mutants include *Arabidopsis ga2*, maize *dwarf-5* (*d5*) and tomato *gib-3* (Hedden and Phinney, 1979; Koorneef and van der Veen, 1980; Fujioka et al., 1988a; Zeevaart, 1986; Bensen and Zeevaart, 1990; Yamaguchi et

al., 1998). Examples of loss-of-function KO mutants include *Arabidopsis ga3*, maize *dwarf-3* (*d3*) and pea *lh* (Fujioka et al., 1988a; Reid and Ross 1993; Swain et al., 1995; Helliwell et al., 1998). Examples of loss of function KOA mutants include maize *dwarf-2* (*d2*), pea *na*, and tomato *gib-2* (Ingram et al., 1984; Zeevaart, 1986; Fujioka et al., 1988a; Reid and Ross 1993).

GA20ox *and* GA3o*x*

GA20ox is responsible for the conversion of $GA_{12}$ to $GA_9$ or $GA_{53}$ to $GA_{20}$ and is encoded by a multi-member gene family. Expression of *GA20ox* family members is tissue and/or developmentally specific (Phillips et al., 1995; Ashikari et al., 2002; Sakamoto et al., 2004)

*Arabidopsis* has five *GA20ox* members, *AtGA20ox1* through *AtGA20ox5* but not all play a significant role in regulating GA associated vegetative growth (Phillips et al., 1995 and Rieu, et al, 2008a). *AtGA20ox1* is expressed in most actively growing tissue but seems to be the primary gene encoding GA20ox responsible for regulating GA associated vegetative growth (Phillips et al., 1995; Xu et al., 1997; Rieu et al., 2008a). *AtGA20ox2* and *AtGA20ox3* are also expressed in most tissues tested including leaves and reproductive tissue but are not highly expressed in stem tissue. They likely play a more minor role than *AtGA20ox1* in regulating vegetative growth and a more prominent role in regulating reproductive growth and development. *AtGA20ox4* and *AtGA20ox5* are either minimally expressed throughout the plant or are expressed primarily in

reproductive organs and thus play only a minor role in regulating vegetative growth (Phillips et al., 1995 and Rieu et al., 2008a).

Rice has four *GA20ox* family members, *OsGA20ox1* through *OsGA20ox4*. *OsGA20ox2* and *GA20ox4* are expressed in most tissue tested but are highest in stems, sheaths, and leaf blades respectively and are likely the primary *OsGA20ox* genes responsible for GA regulated vegetative growth (Ashikari et al., 2002; Kaneko et al., 2003; Sakamoto et al., 2004). *OsGA20ox1* is expressed in most tissues tested but likely contributes primarily to GA associated reproductive growth and development. *OsGA20ox3* is expressed in the panicles and likely only contributes to reproductive growth and development.

GA3ox converts $GA_{20}$ to bioactive $GA_1$ or $GA_9$ to bioactive $GA_4$ and is also encoded by a gene family. Like *GA20ox*, expression of *GA3ox* gene members is tissue and/or developmental stage specific. *Arabidopsis* has four *GA3ox* gene members but only *AtGA3ox1* and *AtGA3ox2* are predicted to regulate GA associated vegetative growth (Yamaguchi et al., 1998; Mitchum et al., 2006; Matsushita et al., 2007). *AtGA3ox3* and *AtGA3ox4* expression is primarily limited to reproductive organs (Mitchum et al., 2006 and Matsushita et al., 2007).

Rice GA3ox is encoded by two family members. *OsGA3ox1* is expressed only in reproductive organs and likely plays little to no role in regulating GA associated vegetative growth (Itoh et al., 2001; Kaneko et al., 2003; Sakamoto et al., 2004). *OsGA3ox2* is expressed in all tissue tested and is considered the primary gene encoding

GA3ox during vegetative growth in rice (Itoh et al., 2001; Kaneko et al., 2003; Sakamoto et al., 2004).

Since GA20ox and GA3ox are normally encoded by gene families, loss-of-function mutations of a single *GA20ox* or *GA3ox* gene normally result in GA responsive plants that exhibit either a WT or semi-dwarf phenotype depending on the specific gene affected (Talon et al., 1990a; Chiang et al., 1995; Xu et al., 1995**)**. Semi-dwarf plants contain reduced bioactive GA and gene expression patterns consistent with active feedback regulation.

The *Arabidopsis ga5* mutant is caused by a loss-of-function mutation of *AtGA20ox1* (Xu et al., 1995). It is a GA responsive, semi-dwarf that contains reduced bioactive GA compared to WT (Talon et al., 1990a; Xu et al., 1995; Coles et al., 1999). Transformations using an *AtGA20ox1* antisense transcript produced semi-dwarf plants that contained elevated *AtGA20ox2, GA3ox1* and *AtGID1b* expression and reduced *AtGA2ox1* expression (Coles et al., 1999 and Rieu et al., 2008a). Transformations using an *AtGA20ox2* or *AtGA20ox3* antisense transcript produced plants with little phenotypic variation from WT (Coles et al., 1999). Transformations using both *AtGA20ox1* and *AtGA20ox2* antisense transcripts produced dwarf plants.

The rice *sd1* mutant, which is considered the "Miracle Rice" and contributed to the Green Revolution, is caused by a loss-of-function deletion within the *OsGA20ox2* gene (Ashikari et al., 2002; Monna et al., 2002; Spielmeyer et al., 2002). It is a GA-responsive, semi-dwarf with decreased bioactive GA content compared to WT (Ashikari et al., 2002; Sasaki et al., 2002; Spielmeyer et al., 2002; Sakamoto et al., 2004).

*Arabidopsis ga4* has a loss-of-function mutation in the *AtGA3ox1* gene and is a GA responsive semi-dwarf that contains reduced bioactive GA (Talon et al., 1990a and Chiang et al., 1995). Loss-of-function of *AtGA3ox2* results in a WT phenotype (Mitchum et al., 2006). Loss-of-function of both *AtGA3ox1* and *AtGA3ox2* produced plants that share a similar phenotype with *ga1* mutants.

Mendel's famous *le* pea results from a *PsGA3ox1* loss-of-function mutation that produces GA responsive dwarf plants that contain reduced bioactive GA and elevated *PsGA20ox* expression compared to WT (Ross et al., 1992 and Martin et al., 1996). The maize *dwarf-1* (*d1*), a *ZmGA3ox1* loss-of-function mutation, is also a GA responsive dwarf with limited bioactive GA (Fujioka et al., 1988a).

GA2ox

GA2ox is a catabolic enzyme that hydroxylates gibberellins at different points along the GA biosynthesis pathway and is a key component of GA homeostasis. Like GA20ox and GA3ox, GA2ox is encoded by a gene family.

*Arabidopsis* contains 8 *GA2ox* genes and their expression is tightly regulated (Thomas et al., 1999 and Schomburg et al., 2003). *AtGA2ox1* and *AtGA2ox6* are highly expressed in most tissues tested and are likely the dominant *GA2ox* genes responsible for regulation of GA associated vegetative growth in *Arabidopsis* (Thomas et al., 1999; Wang et al., 2004; Rieu et al., 2008b). *AtGA2ox2* is also expressed in most tissue types but likely plays a more minor role in regulating vegetative growth than *AtGA2ox1* or *AtGA2ox6*. *AtGA2ox3*, *AtGA2ox4*, *AtGA2ox5* are either expressed at very low levels or

their expression in limited to reproductive organs (Thomas et al., 1999 and Rieu et al., 2008b). AtGA2ox6 and AtGA2ox7 only hydroxylate C20-GA compounds and are not as well characterized as the other AtGA2ox proteins (Schomburg et al., 2003).

Rice contains four GA2ox family members. *OsGA2ox3* and *OsGA2ox4* are likely the two rice *GA2ox* genes primarily responsible for regulating vegetative growth (Sakamoto et al., 2001 and Sakamoto et al., 2004). *OsGA2ox1* is expressed mainly in roots and panicles while *OsGA2ox2* expression was not detected in any tissue tested.

Since GA2ox is a catabolic enzyme, loss-of-function of one or more GA2ox genes results in either a WT or a "GA overdose" phenotype (Rieu et al., 2008b). In order to achieve a dwarf phenotype, *GA2ox* must be over-expressed (Sakamoto et al., 2003). There are a number of engineered plants that constitutively express or over-express a specific *GA2ox* gene but no reports of naturally occurring gain-of-function *GA2ox* dwarf mutants could be found in the literature (Sakai et al., 2003; Sakamoto et al., 2003; Schomburg et al., 2003; Wang et al., 2004; Lee and Zeevaart, 2005). Plants that over-express *GA2ox* are typically GA responsive dwarfs that contain reduced bioactive GA.

## GA Signal Transduction and Associated Dwarf Mutants

GA signaling dwarf mutants exhibit reduced to no sensitivity to bioactive GA (Ross et al., 1997). Tifdwarf is responsive to bioactive GA however absolute sensitivity compared to non-dwarf bermudagrass has not been determined (Dudeck and Peacock,

1985). Therefore, GA perception and GA signaling related dwarf mutants will also be described in this review.

In recent years, significant insight has been gained into GA perception and signal transduction. The GA receptor, GID1 has been identified, DELLA protein function is now better understood and the role the SCF ubiquitin-ligase complex plays has been defined. However, the pathway down-stream of DELLA is less understood. Here, only a brief summary of GA perception and signal transduction will be described. See the review authored by Achard and Genschik, 2009 for a more in-depth overview. Appendix B displays an overview of the GA signal transduction pathway.

*DELLA Proteins*

In the absence of bioactive GA, DELLA proteins negatively regulate or inhibit GA responses (Harberd, 1998; Dill and Sun, 2001; King et al., 2001; Silverstone et al., 2001). Bioactive GA binds the soluble GA receptor GID1 (Ueguchi-Tanaka et al., 2005). This interaction causes a conformational change in the receptor protein which promotes binding to the DELLA domain of DELLA proteins (Ueguchi-Tanaka et al., 2005 and Ueguchi-Tanaka et al., 2007). Binding of GA-GA Receptor to a DELLA protein increases the affinity of the DELLA protein to the SCF E3 ubiquitin-ligase complex. This interaction promotes the unbiquitinylation of the DELLA protein which targets it for degradation via the 26S proteosome. Thus, bioactive GA causes DELLA degradation via the GA receptor and SCF E3 ubiquitin-ligase complex which releases "the brakes" or inhibition of GA responses (Appendix B).

In *Arabidopsis*, the DELLA proteins are encoded by a five member gene family: *AtGAI*, *AtRGA*, *AtRGL1*, *AtRGL2* and *AtRGL3*. AtGAI and AtRGA are the primary DELLAs responsible for vegetative growth (Silverstone et al., 1998 and Lee et al., 2002). The rice DELLA protein is encoded by a single gene, *OsSLR1* which is expressed in all rapidly elongating or dividing tissue tested (Ogawa et al., 2000 and Kaneko et al., 2003).

A loss-of-function mutation of the DELLA protein results in a tall "GA overdose" phenotype (Croker at al., 1990 and Silverstone et al., 1998). However, a mutation that affects only the DELLA domain inhibits the ability of the DELLA protein to bind the GA-GID1 complex and thus prevents DELLA from being targeted for proteasome degradation regardless of GA status (Gubler et al., 2002). Since this mutation constitutively inhibits GA responses, it is considered a gain-of-function mutation. The resulting phenotypes are dwarf plants with elevated bioactive GA, decreased sensitivity to bioactive GA and impaired feedback regulation mechanisms (Talon et al., 1990b; Xu et al., 1995; Peng et al., 1997; Dill and Sun, 2001).

The *Arabidopsis* DELLA domain mutant *gai* is a GA insensitive dark green dwarf that contains reduced levels of C20-dicarboxylic acids ($GA_{53}$, $GA_{44}$, $GA_{19}$, $GA_{12}$, $GA_{15}$, $GA_{24}$), elevated levels of C19-dicarboxylic acids ($GA_{20}$, $GA_1$, $GA_8$, $GA_9$, $GA_{51}$, $GA_4$, $GA_{34}$) and elevated expression of *GA20ox* and *GA3ox1* (Koorneef et al., 1985; Talon et al., 1990b; Xu et al., 1995; Peng et al., 1997; Dill and Sun, 2001). Application of bioactive GA to *gai* plants results in minimal to no changes in phenotype or gene expression (Xu et al., 1995; Cowling et al., 1998; Silverstone et al., 1998). Also, typical

GA associated feedback regulation responses are impaired. GA associated feedback regulation is explained later in this review.

Maize *dwarf-8* (*d8*) has a DELLA gain of function mutation that results in a GA-nonresponsive dwarf with elevated $GA_{20}$, $GA_1$, and $GA_8$ and elevated *GA20ox* and *GA3ox* expression (Fujioka et al., 1988b and Winkler and Freeling, 1994). Application of the GA biosynthesis inhibitor, paclobutrazol further increases the dwarf phenotype (Winkler and Freeling, 1994). The wheat DELLA mutant *Rht*, which was made famous by Dr. Borlaug, produces plants that are semi-dwarf to dwarf and contain elevated $GA_{20}$ and $GA_1$ compared to WT (Webb et al., 1998).

*GA Receptor*

The GA receptor, GID1 is responsible for GA perception (Ueguchi-Tanaka et al., 2005). In rice, *GID1* is a single-copy gene that is expressed in all tissue tested (Ueguchi-Tanaka et al., 2005). AtGID1 in *Arabidopsis* is encoded by a three member gene family (Nakajima et al., 2006). *GID1a, GID1b* and *GID1c* are expressed in all tissue tested with the exception of *GID1b* where expression was not detected in dry seeds (Nakajima et al., 2006). Expression levels of the three *GID1* members are different (Griffiths et al., 2006). In most tissues, *GID1a*'s expression is highest followed by *GID1b* and then *GID1c*.

Loss-of-function mutation of *GID1* results in plants that are GA insensitive and exhibit a severe dwarf phenotype (Ueguchi-Tanaka et al., 2005). Loss-of-function of a single *AtGID1* gene member does not produce a dwarf phenotype (Griffiths et al., 2006).

Loss of two *GID1* members produces variable phenotypes depending on the genes affected. Loss of all three produces a GA insensitive severe dwarf that contains elevated DELLA protein levels (Griffiths et al., 2006 and Willige et al., 2007)

*F-Box Protein*

The F-Box protein is part of the SCF E3 ubiquitin-ligase complex which ubiquitinylates DELLA protein in the presence of bioactive GA. Based on current findings, the F-box protein is encoded by a single gene that is likely broadly expressed. The F-box protein in rice is called OsGID2 and in *Arabidopsis* is called AtSLY1 (Sasaki et al., 2003; Dill et al., 2004; Fu et al., 2004).

Loss-of-function of the F-box protein prevents proteasome degradation of DELLA proteins resulting in dwarf plants that have reduced sensitivity to bioactive GA. The *Arabidopsis* F-box loss-of-function mutant *sly1* is a GA insensitive dark green dwarf with elevated DELLA protein levels and reduced fertility and apical dominance (Dill et al., 2004 and Ariizumi et al., 2008). *Osgid2* has a loss-of-function mutation of the rice F-box protein. It is a GA-insensitive dwarf with wide, dark green leaves that contains elevated bioactive GA, *GA20ox2*, and *GID1* expression and DELLA protein levels (Sasaki et al., 2003 and Ueguchi-Tanaka et al., 2008). Its dwarf phenotype is less severe than *gid1* or loss-of-function *cps* mutants even though *Osgid1* accumulates higher levels of DELLA proteins (Ueguchi-Tanaka et al., 2008). It is believed that DELLA proteins' repressive ability decreases when they are complexed with GA-GID1. Addition of $GA_3$ increased SLR1 DELLA protein levels but reduced dwarfism.

## Other GA Related Dwarf Mutants

Other loss-of-function GA associated mutants have been classified that show similar characteristics to Tifdwarf bermudagrass. *OsGAE1* is a rice GA up-regulated gene that is expressed primarily in growing leaf sheath and likely acts downstream of DELLA (Jan et al., 2006). Loss-of-function of GAE1 produced dwarf plants that are 55-70% shorter than WT.

The tobacco RSG (REPRESSION OF SHOOT GROWTH) is a transcription factor that participates in GA feedback regulation (Fukazawa et al., 2010). In the absence of bioactive GA, RSG binds to the promoter of *GA20ox1* and activates its expression. In the presence of bioactive GA, RSG is quickly translocated out of the nucleus to the cytoplasm reducing expression of *GA20ox1*. Loss of function of RSG produces GA responsive dwarf plants with reduced internode elongation and reduced bioactive GA.

The rice *DWARF1* (*D1*) gene encodes the α-subunit of heterotrimeric G-proteins (Ashikari et al., 1999). It is expressed in rapidly elongating or dividing tissue, especially internodes (Fujisawa et al., 1999 and Kaneko et al., 2003). Loss-of-function of *DWARF1* results in semi-dwarf plants that have broad, dark green leaves and contain elevated bioactive GA content (Ueguchi-Tanaka et al., 2000). Sensitivity to bioactive GA in internodes is significantly reduced.

Other GA associated dwarf mutants have been generated; however they are the result of significant over-expression of the gene of interest. Results for ectopic over-

expression studies have limited application for this study and therefore they will not be highlighted in this review.

## GA Homeostasis/ Feedback & Feed Forward Regulation

For normal growth and development to occur, plants must tightly regulate bioactive GA. GA homeostasis is accomplished primarily via feedback and feed forward regulation of GA biosynthetic and catabolic genes (Hedden and Phillips, 2000; Olszewski et al., 2002; Yamaguchi, 2008**)**. Feedback regulation requires functional GA receptors, SCF ubiquitin ligase complexes and DELLA proteins (Cowling et al., 1998 and Dill and Sun 2001). A mutation in any of these genes inhibits normal feedback regulation.

As discussed previously, GA-responsive dwarf mutants contain limited bioactive GA. Gene expression patterns within these mutants reflect appropriate feedback/feed forward responses. Expression of select *GA20ox*, *GA3ox*, and *GID1* genes are elevated while expression of select *GA2ox* and DELLA genes are depressed (Cowling et al., 1998; Elliott et al., 2001; Ashikari et al., 2002; Sakai et al., 2003; Griffiths et al., 2006; Rieu et al., 2008a; Rieu et al., 2008b). Application of bioactive GA causes a rapid shift in these expression patterns (Silverstone et at., 1998; Sakai et al., 2003; Zentella et al., 2007; Rieu et al., 2008a; Rieu et al., 2008b).

In GA-insensitive dwarf mutants, appropriate feedback/feed forward regulation is impaired. Despite a dwarf phenotype, both bioactive GA and DELLA protein levels are elevated. Expression of *GA20ox* and *GA3ox* is also elevated while expression of *GA2ox*

in depressed (Cowling et al., 1998; Dill and Sun, 2001; Dill et al., 2004). Elevated GA should decrease DELLA protein levels which in turn should initiate GA homeostatic responses to decrease bioactive GA levels. This does not occur in the mutants due to defects in GA perception and/or early signal transduction.

If Tifdwarf's dwarf phenotype is caused by a GA associated mutation, then characteristic GA feedback/feed forward expression patterns would be expected. Therefore it is important to know which genes are likely under homeostatic control.

*Early GA Biosynthesis Genes*

The early GA biosynthesis genes *CPS*, *KS*, *KO* and *KOA* do not appear to be feedback regulated (Helliwell et al., 1998).

GA20ox

In *Arabidopsis*, only *AtGA20ox1*, *AtGA20ox2* and *AtGA20ox3* are feedback regulated (Xu et al., 1999 and Rieu et al., 2008a). In a GA deficient background, their expression was elevated compared to WT. When bioactive GA was exogenously applied, expression levels decreased significantly. GA status did not impact expression of *AtGA20ox4* or *AtGA20ox5*, therefore they are not feedback regulated (Rieu et al., 2008a). In rice, pea, and tobacco, *OsGA20ox2* (Ashikari et al., 2002 and Sakamoto et al., 2004), *PsGA20ox1*, and *NtGA20ox1* are feedback regulated (Martin et al., 1996; Tanaka-Ueguichi et al., 1998; Elliott et al., 2001; Reid et al., 2002; Gallego-Giraldo et

al., 2008; Weston et al., 2008). It has not been determined whether the other *GA20ox* family members are under feedback control for these species.

GA3ox

In *Arabidopsis*, only *AtGA3ox1* is subject to feedback regulation (Mitchum et al., 2006 and Matsushita et al., 2007). In a GA deficient background, *AtGA3ox1* expression is elevated compare to WT however, when bioactive GA is applied, expression drops to undetectable levels (Chiang et al., 1995; Cowling et al., 1998; Thomas et al., 1999; Matsushita et al., 2007; Zentella et al., 2007). Since maintenance of elevated *AtGA3ox2* expression is believed to be critical to overcome homeostatic responses during seed germination, it is important that it is not subject to feedback regulation (Matsushita et al., 2007). In rice, only *OsGA3ox2* is subject to feedback regulation (Itoh et al., 2001 and Sakamoto et al., 2003).

GA2ox

*AtGA2ox1*, *AtGA2ox2*, *AtGA2ox4*, and *AtGA2ox6* are feedback regulated by bioactive GA (Thomas et al., 1999 and Rieu et al, 2008b). In a GA-deficient background, expression of these genes is not detectable. When bioactive GA is applied, expression levels increase significantly. *AtGA2ox3* and *AtGA2ox5* are not feed back up-regulated (Thomas et al., 1999 and Rieu et al., 2008b). In rice, *OsGA2ox3* is feed back up-regulated while *OsGA2ox1* is not (Sakai et al., 2003 and Sakamoto et al., 2004). It has not yet been determined whether *OsGA2ox2*, *OsGA2ox4*, *OsGA2ox5* or *OsGA2ox6*

are feedback regulated. In tobacco, *NtGA2ox1*, *NtGA2ox2* and *NtGA2ox3* are feedback regulated however *NtGA2ox2* and *NtGA2ox3* react to small increases in bioactive GA while *NtGA2ox1* only reacts to large increases in bioactive GA (Gallego-Giraldo et al., 2008).

GID1

In rice, *OsGID1* is feed forward regulated by bioactive GA (Ueguchi-Tanaka et al., 2005). In a GA deficient background, all three *Arabidopsis GID1* gene members are feed forward regulated (Griffiths et al., 2006). In a GA deficient background, *AtGID1a*, *AtGID1b*, and *AtGID1c* expression is elevated compared to WT. Application of bioactive GA causes a rapid decrease in *GID1* expression.

F-BOX *and* DELLA

The *Arabidopsis* F-box protein AtSLY1 is feed forward regulated (Dill et al., 2004 and Fu et al., 2004). Expression of select DELLA genes in some species appears to be under GA feed forward regulation. *AtRGA* and *OsSLR1* are feed forward regulated while barley *HvSLN1* is not (Gubler et al., 1995; Siverstone et at., 1998; Ueguchi-Tanaka et al., 2008; Zhang et al., 2008).

## The Impact of GA Associated Dwarfism on Photosynthesis and Biomass Allocation

A tomato (*Solanum lypopersicum* L.) GA deficient dwarf partitioned more biomass to roots and less to stems than WT plants (Nagel et al., 2001 and Nagel and

Lambers, 2002). Biomass partitioned to leaves was unchanged. Photosynthesis (Pn) was also similar but specific leaf area (SLA) was lower in dwarf plants and the root mass ratio (g root/g plant) was higher in dwarf than WT plants. Leaf thickness was also higher in dwarf plants than WT. Application of the GA biosynthesis inhibitor paclobutrazol to two different *Aegilops* species reduced phyllochron, and leaf elongation rate (LER), and shifted biomass allocation from the leaves to the roots (Bultynck and Lambers, 2004).

Dwarfism did not impact photosynthesis in GA-insensitive *rht* dwarf wheat plants compared to tall WT, however, root weight was higher in dwarf plants (Bush and Evans, 1988).

## Plant Responses to Suboptimal Temperature

Since plants are sessile, they must possess the ability to respond to environmental stimuli. A great deal of research has been done on the impact temperature has on plant morphology and function. Temperatures low enough to induce chilling injury or cold acclimation responses can produce profound plant metabolic changes. Photosynthesis in bermudagrass plants acclimated to a 35°C day/25°C night temperature regime decreased over 50% following 18 hours of exposure to 7°C (Karnok and Beard, 1983). When bermudagrass plants were exposed to chilling temperatures, photosynthesis decreased resulting in a significant reduction in growth rate.

However, this research deals with suboptimal and not cold temperatures. In this study, suboptimal temperatures are those that fall below optimal but are sufficient to

maintain plant growth and development. The optimum temperature range for bermudagrass growth and development is 30°C to 35°C (Beard, 1973). The minimum degree-hours that supports growth in bermudagrass was 1280 which was supplied by a 15.6°C day/4.4°C night temperature regime (Youngner, V.B., 1959). Cold acclimation responses in bermudagrass are not triggered until temperatures fall below 10°C (Zhang et al., 2008). Basal temperature for bermudagrass, or the lowest temperature that supports growth, ranges from 3.1 to 4.9°C when under a 14-hour photoperiod (Unruh et al., 1996). Therefore, for this study, temperatures between 29°C and 15.6°C comfortably fall within the suboptimal range for bermudagrass.

Suboptimal temperatures can impact plant growth characteristics. Typically, internode length, leaf length, and growth rate decrease when warm-season grass plants are exposed to suboptimal temperatures (Mitchell, 1955 and Youngner, 1961). Zoysiagrass plants grown under a 30°C day (estimated) /27°C night temperature regime produced 2.5 times more top material, 1.3 times more roots and 1.2 times more rhizomes than a 24°C day/21°C night temperature regime (Youngner, 1961). Blade length and internode length were 1.36 and 2.2 times longer respectively in plants grown under a 30°C day/27°C night temperature regime compared to a 24°C day/21°C night temperature regime. P*aspalum dilitatum* plants grown under a mean temperature of 15°C produced 10 times less tissue per day and had three times shorter leaf length than plants grown under a mean temperature of 28.3°C (Mitchell, 1955). Leaf area and leaf dry weight were also three times lower at 15°C compared to 28.3°C.

As temperature decreases below optimum, photosynthesis also decreases. Maximum apparent photosynthesis in bermudagrass, or photosynthesis minus respiration, occurs at 35°C and then decreases as temperature decreases (Miller, 1960). At 27°C, apparent photosynthesis in bermudagrass was predicted to be between 85 and 95% of the maximum.

Tifdwarf bermudagrass exhibits a response to cool temperatures that is opposite that of other grasses. When Tifdwarf acclimated at an optimum temperature regime of 35°/27°C day/night was exposed to a 27°/19°C day/night temperature regime, internode and leaf length increased (Stanford et al., 2005). Following 35 days of exposure, shoot weight in Tifdwarf plants grown under the cool temperature treatments was three times greater than plants grown under the optimal temperature treatment. This temperature mediated adjustment is inconsistent with any other characterized temperature response found in the literature.

## The Influence of Temperature on a GA Dwarf's Morphology

Internodes in the wheat *Rht3* GA-insensitive dwarf do not elongate under cool temperatures like Tifdwarf, however it does display atypical responses to cool/cold temperatures. Exposure of *Rht3* seeds to low temperatures (5°C) for 20 hours prior to $GA_3$ treatment significantly increased α-amylase production (Singh and Paleg, 1984). *Rht3* is resistant to bioactive $GA_3$. Singh and Paleg (1984) speculated that low temperature either eliminated or bypassed the *rht3* lesion resulting in plants that are sensitive to GA and function as wild-type.

Leaf length of wild-type wheat plants is longer when grown at 25°C than when grown at 11°C (Pinthus et al., 1989). Leaf length in the *Rht3* dwarf mutant slightly increased as temperature decreased to 11°C but the difference was not significant. Sensitivity of wild-type leaves to $GA_3$ application was unaffected by decreasing temperatures. While *Rht3* leaves showed no sensitivity to $GA_3$ application at 25°C, it did respond to $GA_3$ application at11°C.

Appleford and Lenton, 1991 found that WT wheat leaf length was 35% longer when grown at 20°C than at 10°C. Leaf length in the *Rht3* dwarf however, was 24% shorter at 20°C than at 10°C. This numeric difference was not significant. Temperature treatments did not alter $GA_1$ content in wild-type plants but it did in *Rht3* plants. *Rht3* lines contained 24 times more $GA_1$ than WT lines at 20°C but decreased to only 5 times more $GA_1$ at 10°C.

Tonkinson et al. (1997) found that leaf length and maximum absolute growth rate of WT and *Rht3* were similar at 10°C. At 20°C, WT leaf length was 33% longer than at 10C while *Rht3* leaf length was the same as at 10°C. At 20°C, maximum absolute growth rate of wild-type plants was 39% higher than *Rht3* dwarf plants. $GA_{20}$ content was higher in both wild-type and *Rht3* plants at 20°C than 10°C and overall content was similar in WT and *Rht3* at both temperatures. $GA_1$ content in wild type plants did not change due to temperature treatment. *Rht3* plants contained 2.5 times more $GA_1$ at 20°C than at 10°C. At both temperatures, *Rht3* contained more $GA_1$ than wild-type plants.

In wild-type wheat, the rate of leaf extension increased as temperature increased from 5°C to 30°C (Stoddart and Lloyd, 1986) The rate of leaf extension in *Rht3* dwarf

was similar to wild-type from 5°C to 15°C. Above 15°C, extension was less in *Rht3* than wild-type. Pinthus and Abraham (1996) found that in wild-type plants, the rate of leaf expansion was lower at 11°C than at 25°C but duration of leaf expansion was 50% longer at 11°C than at 25°C.

## GAMYB: An Indicator of GA Responses

GAMYB is a GA regulated transcription factor that acts downstream of DELLA to regulate expression of many GA-inducible genes and therefore its expression level can provide a quantitative estimation of GA response (Gubler et al., 1995 and Gocal et al., 1999**)**. In seed aleurone tissue, GAMYB, in response to bioactive GA, binds to the GA response element (GARE) in the promoter region of α-amylase and initiates transcription (Gubler et al., 1995).

GAMYB also acts as a transcriptional activator for GA-inducible genes associated with floral initiation, anther development and stem elongation (Gocal et al., 1999; Chen et al., 2001; Lee and Kende, 2002; Murray et al., 2003; Achard et al., 2004; Millar and Gubler, 2005). A good correlation between bioactive GA levels, GAMYB protein levels, and α-amylase protein levels does not always exist however (Gubler at al., 2002). Also, it has been theorized that GAMYB is likely regulated at the posttranslational level (Diaz et al., 2002; Achard et al., 2004; Miller and Gubler, 2005; Cao et al., 2006).

## GA Associated Plant Growth Regulators

The GA associated plant growth regulators $GA_3$, CCC, flurprimidol and trinexapac-ethyl were used throughout this study. $GA_3$ is a readily available bioactive GA that can substitute for endogenous bioactive GAs. It is important to note that $GA_3$ is not deactivated by GA2ox due to an extra double-bond within its chemical structure (Grindal et al., 1998) Chlorocholine Chloride (CCC) is GA biosynthetic inhibitor that primarily disrupts the function of copalyl-diphosphate synthase (CPS) but also displays activity of ent-kaurene synthase (KS) (Rademacher, 2000 review). Flurprimidol is a GA biosynthetic inhibitor that disrupts the function of ent-kaurene oxidase (KO). Trinexapac-ethyl (TE) is a GA biosynthetic inhibitor that primarily disrupts the function of GA3ox. However, TE also inhibits GA20ox and GA2ox function. Therefore trinexapac-ethyl impacts both the formation and deactivation of bioactive GA and GA intermediates.

Appendix C displays the GA metabolic pathway with each endogenous PGR used in this study displayed next to the enzyme it affects or the GA it substitutes for.

# CHAPTER III

# MATERIALS AND METHODS

## The Influence of Temperature on Dwarf Bermudagrass Morphology, Photosynthesis and Respiration

### *Plant Establishment and Growth Conditions*

Tifdwarf bermudagrass [*Cyanodon dactylon* (L.) Pers. x *C. transvaalensis* Burtt-Davy] sprigs containing three nodes each were harvested from a single stock plant grown in a greenhouse. Six sprigs were planted in each 25.4 cm diameter pot containing a mix of sand:fritted clay:peat (2:1:1 by volume). Sprigs were established in a greenhouse for 10 days. After establishment, they were clipped to a canopy height of 5 cm, arranged in two 3.35 $m^2$ growth chambers (Environmental Growth Chambers, Chagrin Falls, OH) in a completely random design and acclimated for seven days. During acclimation, growth chambers maintained a 14 hour photoperiod, a 35/27°C day/night temperature regime and photosynthetic photon flux density (PPFD) of 450 µmol $m^{-2}s^{-1}$. PPFD was provided by 30 243.8 cm VHO fluorescent lamps and nine 60 watt incandescent bulbs. PPFD was measured weekly using a Li-1800 spectroradiometer (Li-Cor). Pots were watered as needed to prevent water stress and nutrients were applied weekly at 12 kg N $ha^{-1}$ as 20-8-16 dissolved in 60 mL $H_2O$.

*Temperature Treatments*

Upon treatment initiation, the day/night temperature regime in one chamber was maintained at 35/27°C while the temperature regime in the other chamber was lowered to 27/19°C for the remainder of the experiment. All other conditions within both chambers remained unchanged from acclimation conditions. Upon completion, the experiment was repeated using the same growth chambers and procedures with new plant material. Temperature treatments were randomly assigned to growth chambers for the initial experiment and then switched to the opposite chamber for its repetition.

*Internode and Leaf Length*

Internode (IN) and lamina lengths (LL) were measured 28 days after treatment initiation (DAI). Internode length was the distance between the second and third most recently formed nodes (Stanford et al, 2005). Lamina length was the length of the youngest leaf from the second most recently formed phytomer (Stanford et al, 2005). Three pots per temperature treatment were measured and three IN and LL measurements were made per pot.

*Shoot and Leaf Measurements*

Twenty-eight DAI, all above ground plant material was removed from three pots per temperature treatment. Two blind grab sub-samples were taken per sample. Sub-sample shoots were counted; leaves were removed from stem tissue, counted, scanned on a digital scanner, and then packaged in a coin envelope. Sub-sample stem tissue was

packaged in a second coin envelop and all remaining sample plant material was packaged in a third coin envelope. All coin envelopes were dried in an oven at 60°C for 14 days and then weighed. Sub-sample leaf area was calculated from sub-sample leaf scans using SigmaScan image analysis software. Sub-sample shoot number, leaf number, leaf area, leaf weight, and shoot weight were used to predict sample shoot number, leaf number, average area per leaf, total leaf area, and specific leaf area (SLA). Specific leaf area is leaf area per unit leaf mass.

*Photosynthesis and Respiration Measurements*

Photosynthesis and respiration were measured 28 DAI between 10 am and 2 pm on stolons comprised of the two most recently formed nodes. Measurements were made using a Licor-6400 Portable Photosynthesis System (Licor Biosciences) equipped with a 6400-02B LED Light Source. Leaf chamber $CO_2$ concentration was maintained at 400 µmol $mol^{-1}$. Temperature was set to match the temperature of the respective growth chamber. Plants remained in the growth chamber throughout the process. First, respiration was calculated by determining $CO_2$ flux in the dark. Then the light levels were set to 450 µmol $m^{-2}s^{-1}$ and photosynthesis was determined. For each measurement, data were logged every six seconds for one minute and then averaged to compute a single respiration or photosynthesis rate. Leaf area within the chamber was less than that of the total chamber area. Therefore, all leaf material that was within the chamber during the measurements was scanned and leaf area was calculated using SigmaScan image analysis software. Finally, all leaves were removed and stem tissue

photosynthesis was measured. Stem tissue photosynthesis was determined to be negligible (data not shown). Therefore, photosynthesis and respiration rates were adjusted based solely on leaf area calculations. Three replicate pots per temperature treatment were measured. One randomly selected stolon was measured per pot.

*Statistical Analysis*

All data were subject to analysis of variance and showed no significant difference between experiments or any experiment by treatment interaction effects. Therefore, all data for the repeated experiments were pooled and subjected to analysis of variance. When a significant difference occurred for a treatment effect, Tukey's multiple range test was used for mean comparison.

## The Response of Dwarf Bermudagrass to GA Associated Plant Growth Regulators (PGRs) When Grown Under Optimal or Suboptimal Temperature Regimes

*Plant Establishment and Growth Conditions*

Sixty-six 58.06 $cm^2$ plugs were pulled from an existing Tifdwarf bermudagrass green, potted in 18 well trays, established in the greenhouse for seven days and then arranged in 3.35 $m^2$ growth chambers (Environmental Growth Chambers, Chagrin Falls, OH) in a completely random design where they were allowed to acclimate for seven days. During acclimation, growth chambers maintained a 14 hour photoperiod, a

35/27°C day/night temperature regime and photosynthetic photon flux density (PPFD) of 450 µmol $m^{-2}s^{-1}$. PPFD was provided by 30 243.8 cm VHO fluorescent lamps and nine 60 watt incandescent bulbs. PPFD was measured weekly using a Li-1800 spectroradiometer (Li-Cor). Pots were watered as needed to prevent water stress and nutrients were applied weekly at 12 kg N $ha^{-1}$ as 20-8-16 dissolved in 60 mL $H_2O$.

*Bioactive $GA_3$ and GA Biosynthetic Inhibitor Treatments*

Following acclimation, pots were treated with one of the following: control, 0.01g $m^{-2}$ CCC ($CCC_{(Low)}$), 0.1g $m^{-2}$ of CCC ($CCC_{(High)}$), 0.005 g $m^{-2}$ of Flurprimidol ($Flur_{(Low)}$), 0.02 g $m^{-2}$ of Flurprimidol ($Flur_{(High)}$), 0.005 ml $m^{-2}$ of Trinexapac-ethyl ($TE_{(Low)}$), 0.02 ml/$m^2$ of Trinexapac-ethyl ($TE_{(High)}$), 0.001 g $m^{-2}$ of $GA_3$ ($GA_{3(Low)}$), 0.01 g $m^{-2}$ of $GA_3$ ($GA_{3(High)}$), $CCC_{(Low)}$ + $Flur_{(Low)}$ + $TE_{(Low)}$ (Inh), $GA_{3(Low)}$ + Inh, or $GA_{3(High)}$ + Inh. All treatments contained 0.05% by volume nonionic surfactant. Chemical amounts were applied in a total water volume of 0.5 mL using a spray bottle that applied .125ml per spray. Plugs were irrigated 10 hours after application. Three replicate plugs were sprayed per chemical/temperature treatment. Immediately following application, the temperature regime in one chamber was reduced to 27/19°C. Three more applications were made at 10 day intervals. Temperature treatments were randomly assigned to growth chambers for the initial experiment and then switched to the opposite chamber for its repetition.

*Internode and Leaf Length*

Eight days after the 4th application, three internode length and lamina length measurements were made per plug. See previous description for measurement methodology. Three IN and LL measurements were made per plug.

*Statistical Analysis*

All data were subject to analysis of variance and showed no significant difference between experiment nor any experiment by treatment interaction effects. Therefore, all data for the experiment and its repetition were pooled and subjected to analysis of variance. When a significant difference occurred for a treatment effect, Tukey's multiple range test was used for mean comparison.

## Comparing the Influence of temperature on Dwarf and Non-dwarf Bermudagrass Morphology and Physiology

*Plant Establishment and Growth Conditions*

Tifdwarf bermudagrass [*C. dactylon* (L.) Pers. x *C. transvaalensis* Burtt-Davy] and NuMex Sahara (*C. dactylon* (L.) Pers.) sprigs containing three nodes each were harvested from a single stock plant grown in a greenhouse. Two sprigs were planted in each 10.16 cm X 10.16 cm pot containing a root-zone mix of sand:fritted clay:peat (2:1:1 by volume). Forty-eight pots sprigged with Tifdwarf and forty-eight pots sprigged with NuMex Sahara were established in a greenhouse for 10 days. Then they

were then clipped to a canopy height of 5 cm, arranged in two growth chambers (Model Q 2936, Environmental Growth Chambers, Chagrin Falls, OH) in a completely random design and acclimated for seven days. During acclimation, both growth chambers maintained a 14 hour photoperiod, a 35/27°C day/night temperature regime, photosynthetic photon flux density (PPFD) of 525 µmol $m^{-2}s^{-1}$ and a red to far-red ratio (R:FR) of 2. PPFD was provided by 30 243.8 cm fluorescent lamps, six 121.9 cm fluorescent lamps, and 15 incandescent bulbs. Temperature was measured and logged every 30 seconds using an EL-USB-2 Temperature, Humidity, and Dew Point Data Logger. PPFD and R:FR were measured weekly using a Li-1800 spectroradiometer (Li-Cor). The R:FR was calculated as the quantum flux density from 655 to 665 nm divided by the quantum flux density from 725 to 735 nm. Plants were watered as needed to prevent water stress and nutrients were applied weekly at 12 kg N $ha^{-1}$ as 20-8-16 dissolved in 60 mL $H_2O$.

*Temperature Treatments*

Upon treatment initiation, the day/night temperature regime in one chamber was maintained at 35/27°C while the temperature regime in the other chamber was lowered to 27/19°C for the remainder of the experiment. All other conditions within both chambers remained unchanged from acclimation conditions. Upon completion of the experiment, it was repeated using the same growth chambers and procedures with new plant material. Temperature treatments were randomly assigned to growth chambers for the initial experiment and then switched to the opposite chamber for its repetition.

*Internode and Leaf Length*

Internode (IN) and lamina lengths (LL) were measured 0, 2, 4, 6, and 8 days after treatment initiation (DAI). See previous description for measurement methodology. Six pots per species/temperature treatment were measured at each sampling date. Three IN and LL measurements were made per pot per sampling date.

*Biomass Allocation*

Ten DAI, all plant material from six pots per species/temperature treatment were removed from their pots and washed to remove all sand. Roots and leaves were then removed from stem tissue, packaged individually in coin envelopes, dried in an oven at 60°C for 14 day and weighed. Total weight is the combined dry weight of all stem, leaf, and root tissue. Shoot weight combined dry weight of all stem and leaf tissue. Biomass allocation was presented as % Biomass for stem, leaf and root tissue and was calculated as % Biomass ($structure_n$) = Dry Weight ($structure_n$)/ Total Dry Weight *100.

*Statistical Analysis*

All data were subject to analysis of variance and showed no significant differences between experiments or for any experiment by treatment interaction effects. Therefore, all data for the experiment and its repetition were pooled and subjected to analysis of variance. When a significant difference occurred for a treatment effect, Tukey's multiple range test was used for mean comparison.

## The Influence of Suboptimal Temperatures on GA Associated Gene Expression in Dwarf and Non-dwarf Bermudagrass

*Plant Establishment and Growth Conditions*

Tifdwarf bermudagrass [*C. dactylon* (L.) Pers. x *C. transvaalensis* Burtt-Davy] and NuMex Sahara (*C. dactylon* (L.) Pers.) sprigs containing three nodes each were harvested from a single stock plant material grown in a greenhouse. Two sprigs were planted in each 12.7 cm diameter pot containing a root-zone mix of sand:fritted clay:peat (2:1:1 by volume). Eighteen pots sprigged with Tifdwarf and 18 pots sprigged with NuMex Sahara were established in a greenhouse for 10 days. Then they were clipped to a canopy height of 5 cm, arranged in two growth chambers in completely random design and acclimated for seven days. During acclimation, growth chambers maintained a 14 hour photoperiod, a 35/27°C day/night temperature regime and photosynthetic photon flux density (PPFD) of 400 µmol $m^{-2}s^{-1}$. PPFD was provided by 30 243.8 cm VHO fluorescent lamps and 10 60 watt incandescent bulbs. PPFD was measured weekly using a Li-1800 spectroradiometer (Li-Cor). Pots were watered as needed to prevent water stress and nutrients were applied weekly at 12 kg N ha-1 as 20-8-16 dissolved in 60 mL $H_2O$.

*Temperature Treatments*

Upon treatment initiation, the day/night temperature regime in one chamber was maintained at 35/27°C while the temperature regime in the other chamber was lowered

to 27/19°C for the remainder of the experiment. All other conditions within both chambers remained unchanged from acclimation conditions. Upon completion of the first experiment, it was repeated using the same growth chambers and procedures with new plant material. Temperature treatments were randomly assigned to growth chambers for the initial experiment and then switched to the opposite chamber for its repetition.

*Internode and Leaf Length*

Internode (IN) and lamina lengths (LL) were measured 0, 7, and 14 days after treatment initiation (DAI). See previous description for measurement methodology. Three pots per species/temperature treatment were measured at each sampling date. Three IN and LL measurements were made per pot per sampling date.

*Tissue Collection for Analysis of Gene Expression*

Stolons comprised of the three most recently formed nodes were harvested from three replicates for each species/temperature treatment. Samples were collected between 11:00am and 2:00 pm, immediately frozen in liquid nitrogen, and then stored at -80°C until gene expression could be analyzed. Tissue samples were collected on 0, 7, and 14 DAI.

*Primer Development*

Primer sets used to sequence the target genes in Tifdwarf and NuMex Sahara were developed from highly conserved regions across numerous monocot species using BLAST search results and ClustalW sequence alignment software.

*Analysis of Gene Expression*

Procedures for total RNA extraction, cDNA synthesis, and quantitative PCR used in this experiment are consistent with those described in Finlayson et al, 2010 with two exceptions. First, during RNA extraction, 5 μg of total RNA from each sample was digested with 4 units of DNase I for 60 min and then re-extracted with TRIzol (Invitrogen). Second, the standard curve for all gene targets were generated from cloned genes in plasmid vectors. Three replicates were measured for each species/temperature treatment. RT-PCR primer combinations are provided in Appendix D.

*Statistical Analysis*

All data were subject to analysis of variance and showed no significant difference between experiment or any experiment by treatment interaction effects. Therefore, all data for the experiment and its repetition were pooled and subjected to analysis of variance. When a significant difference occurred for a treatment effect, Tukey's multiple range test was used for mean comparison.

*Plant Establishment and Growth Conditions*

Tifdwarf bermudagrass [*C. dactylon* (L.) Pers. x *C. transvaalensis* Burtt-Davy] and NuMex Sahara (*C. dactylon* (L.) Pers.) sprigs containing three nodes each were harvested from a single stock plant material grown in a greenhouse. Two sprigs were planted in each 10.16 cm X 10.16 pot containing a root-zone mix of sand:fritted clay:peat (2:1:1 by volume). Forty-eight pots sprigged with Tifdwarf and forty-eight pots sprigged with NuMex Sahara were established in a greenhouse for 10 days. They were then clipped to a canopy height of 5 cm, arranged in two growth chambers in completely random design and acclimated for seven days. During acclimation, both growth chambers (Model Q 2936, Environmental Growth Chambers, Chagrin Falls, OH) maintained a 14 hour photoperiod, a 35/27°C day/night temperature regime, photosynthetic photon flux density (PPFD) of 525 µmol $m^{-2}s^{-1}$ and a red to far-red ratio (R:FR) of 2. PPFD was provided by 30 243.8 cm fluorescent lamps, six 121.9 cm fluorescent lamps, and 15 incandescent bulbs. Temperature was measured and logged every 30 seconds using an EL-USB-2 Temperature, Humidity, and Dew Point Data Logger. PPFD and R:FR were measured weekly using a Li-1800 spectroradiometer (Li-Cor). R:FR was calculated as the quantum flux density from 655 to 665 nm divided by the quantum flux density from 725 to 735 nm. Plants were watered as needed to prevent water stress and nutrients were applied weekly at 12 kg N $ha^{-1}$ as 20-8-16 dissolved in 60 mL $H_2O$.

*Temperature Treatments*

Upon treatment initiation, the day/night temperature regime in one chamber was maintained at 35/27°C while the temperature regime in the other chamber was lowered to 27/19°C for the remainder of the experiment. All other conditions within both chambers remained unchanged from acclimation conditions. Upon completion of the experiment, it was repeated using the same growth chambers and procedures with new plant material. Assignment of treatments to growth chambers was random for both the initial experiment and its repetition.

*Tissue Collection for Analysis of Gene Expression*

Stolons comprised of the three most recently formed nodes were harvested from three replicates for each Tifdwarf Temperature treatment 0, 1, and 2 DAI. Samples were collected between 11:00 am and 2:00 pm, immediately frozen in liquid nitrogen, and then stored at -80°C until gene expression could be analyzed.

*Primer Development*

Primer sets used to sequence the target genes in Tifdwarf and NuMex Sahara were developed from highly conserved regions across numerous monocot species using BLAST search results and ClustalW sequence alignment software.

*Analysis of Gene Expression*

Procedures for total RNA extraction, cDNA synthesis, and quantitative PCR used in this experiment are consistent with those described in Finlayson et al, 2010 with three exceptions. First, during RNA extraction, 5 μg of total RNA from each sample was digested with 4 units of DNaseI for 60 min and then re-extracted with TRIzol (Invitrogen). Second, cDNA was synthesized with the SuperScript III kit using a combination of oligo dT(Invitrogen) and 18s specific primers as described by (Zhu and Altmann, 2005) and then the cDNA was diluted 1:15. Third, the standard curve for all gene targets were generated from cloned genes in plasmid vectors. Three replicates were measured for each species/temperature treatment. RT-PCR primer combinations are given in Appendix D.

*Statistical Analysis*

All data were subject to analysis of variance and showed no significant difference between experiment or any experiment by treatment interaction effects. Therefore, all data for the experiment and its repetition were pooled and subjected to analysis of variance. When a significant difference occurred for a treatment effect, Tukey's multiple range test was used for mean comparison.

## Comparing the Effects of Temperature on GA Sensitivity in Dwarf and Non-dwarf Bermudagrass

*Plant Establishment and Growth Conditions*

Tifdwarf bermudagrass [*C. dactylon* (L.) Pers. x *C. transvaalensis* Burtt-Davy] and NuMex Sahara (*C. dactylon* (L.) Pers.) sprigs containing three nodes each were harvested from a single stock plant material grown in a greenhouse. Two sprigs were planted in each 12.7 cm diameter pot containing a root-zone mix of sand: fritted clay: peat (2:1:1 by volume). Tifdwarf and NuMex Sahara pots were established in a greenhouse for 10 days. Then they were clipped to a canopy height of 5 cm, arranged in two growth chambers (Model Q 2936, Environmental Growth Chambers, Chagrin Falls, OH) in a completely random design and acclimated for seven days. During acclimation, both growth chambers maintained a 14 hour photoperiod, a 35/27°C day/night temperature regime, photosynthetic photon flux density (PPFD) of 525 µmol $m^{-2}s^{-1}$ and a red to far-red ratio (R:FR) of 2. PPFD was provided by 30 243.8 cm fluorescent lamps, six 121.9 cm fluorescent lamps, and 15 incandescent bulbs. Temperature was measured and logged every 30 seconds using an EL-USB-2 Temperature, Humidity, and Dew Point Data Logger. PPFD and R:FR were measured weekly using a Li-1800 spectroradiometer (Li-Cor). R:FR was calculated as the quantum flux density from 655 to 665 nm divided by the quantum flux density from 725 to 735 nm. Plants were watered as needed to prevent water stress and nutrients were applied weekly at 12 kg N $ha^{-1}$ as 20-8-16 dissolved in 60 mL $H_2O$.

*Temperature Treatments*

Upon treatment initiation, the day/night temperature regime in one chamber was maintained at 35/27°C while the temperature regime in the other chamber was lowered to 27/19°C for the remainder of the experiment. All other conditions within both chambers remained unchanged from acclimation conditions. Upon completion of the experiment, it was repeated using the same growth chambers and procedures with new plant material. Assignment of treatments to growth chambers was random for both the initial experiment and its repetition.

*Bioactive $GA_3$ and GA Biosynthetic Inhibitor Treatments*

At 3:45 am two DAI, pots were treated with one of the following: control, 0.001 g $m^{-2}$ of $GA_3$ ($GA_{3(Low)}$), or $GA_{3(Low)}$ + ($CCC_{(Low)}$ + $Flur_{(Low)}$ + $TE_{(Low)}$) (Inh). All treatments contained 0.05% by volume nonionic surfactant. Chemical amounts were applied in a total water volume of 0.5 mL using a spray bottle that applied 0.125 mL per spray. Three replicate pots were sprayed per chemical/temperature treatment.

*Tissue Collection for Analysis of Gene Expression*

Nine hours after application, stolons comprised of the three most recently formed nodes were harvested from three replicates for each species/application/temperature treatment. Samples were immediately frozen in liquid nitrogen, and then stored at -80°C until gene expression could be analyzed.

*Internode and Leaf Length*

Internode (IN) and lamina lengths (LL) were measured 11 days after chemical treatment. See previous description for measurement methodology. Three pots per species/chemical/temperature treatment were measured at each sampling date. Three IN and LL measurements were made per pot.

*Primer Development*

Primer sets used to sequence the target genes in Tifdwarf and NuMex Sahara were developed from highly conserved regions across numerous monocot species using BLAST search results and ClustalW sequence alignment software.

*Analysis of Gene Expression*

Procedures for total RNA extraction, cDNA synthesis, and quantitative PCR used in this experiment are consistent with those described in Finlayson et al, 2010 with three exceptions. First, during RNA extraction, 5 μg of total RNA from each sample was digested with 4 units of DNaseI for 60 min and then re-extracted with TRIzol (Invitrogen). Second, cDNA was synthesized with the SuperScript III kit using a combination of oligo dT(Invitrogen) and 18s specific primers as described by (Zhu and Altmann, 2005) and then the cDNA was diluted 1:15. Third, the standard curve for all gene targets were generated from cloned genes in plasmid vectors. Three replicates were measured for each species/temperature treatment. RT-PCR primer combinations are given in Appendix D.

*Statistical Analysis*

All data were subject to analysis of variance and showed no significant difference between experiment or any experiment by treatment interaction effects. Therefore, all data for the experiment and its repetition were pooled and subjected to analysis of variance. When a significant difference occurred for a treatment effect, Tukey's multiple range test was used for mean comparison.

# CHAPTER IV

# RESULTS AND DISCUSSION

## The Influence of Temperature on Dwarf Bermudagrass Morphology, Photosynthesis, and Respiration

To better classify temperature's influence on dwarf bermudagrass, Tifdwarf was grown under optimal (35/27°C) or suboptimal (27/19°C) temperature regimes. Twenty-eight days after initiation of temperature treatments (DAI), plants were harvested and internode length, leaf length, shoot number, leaf number, average leaf area, total leaf area, and total biomass were determined (Table 1). Both internode and leaf length were longer in plants grown under suboptimal temperatures compared to optimal temperatures. These findings were consistent with Stanford et al., 2005, who reported Tifdwarf internodes and leaves were three and two times longer respectively in plants grown under a 27/19°C regime compared to plants grown under a 35/27°C temperature regime. Internode and leaf elongation was not consistent with typical plant responses to suboptimal temperatures. For example, Zoysiagrass leaf and internode length were 1.36 and 2.2 times longer, respectively, in plants grown under a 30°C day/27°C night temperature regime compared to a 24°C day/21°C night temperature regime (Youngner, 1961). *Paspalum dilitatum* plants grown under a mean temperature of 15°C had leaves one third the length of plants grown under a mean temperature of 28.3°C (Mitchell, 1955).

Shoot and leaf numbers were higher in optimal compared to suboptimal temperature treatments. Average leaf size was larger in suboptimal treatments but total leaf area was higher in optimal treatments due to a higher total leaf number.

Stanford et al., 2005 found that above ground herbage production was higher in suboptimal than optimal temperature treatments. However, in this experiment, temperature did not affect total biomass accumulation (Table 2). Also, temperature did not affect photosynthesis. In bermudagrass, apparent photosynthesis is predicted to be approximately 90% of the maximum at 27°C (Miller, 1960). Therefore, minor or no significant difference in net photosynthesis would be expected between the two temperature treatments used in this study. Therefore, the mechanism in Tifdwarf that causes internode and leaf elongation under suboptimal temperatures does not appear to differentially affect photosynthesis. Dwarfism itself does not impact photosynthesis in a number of species. For example, photosynthesis was unchanged in two tomato GA deficient dwarfs and the wheat GA-insensitive *rht* dwarf, compared to their respective wild types (Bush and Evans, 1988 and Nagel and Lambers, 2002)

Respiration was higher in optimal temperature treatments compared to suboptimal treatments (Table 2). This result would be expected as respiration typically increases with increasing temperature (Beinhart, G. 1962).

Table 1. Internode length, leaf length, total shoot number, total leaf number, average leaf area, total leaf area and total biomass from dwarf bermudagrass following 28 days exposure to optimal or suboptimal temperatures regimes

| Temperature | Internode Length | Leaf Length | Shoots | Leaves | Ave Leaf Area | Total Leaf Area | Biomass |
|---|---|---|---|---|---|---|---|
| Day/Night (°C) | (cm) | (cm) | (Shoots $cm^{-2}$) | (Leaves $cm^{-2}$) | ($cm^2$) | ($cm^2$ $cm^{-2}$) | (g $cm^{-2}$) |
| 27/19 | 1.39 | 0.94 | 0.58 | 1.70 | 0.079 | 0.135 | 0.0050 |
| 35/27 | 0.71 | 0.76 | 0.87 | 2.91 | 0.074 | 0.215 | 0.0055 |
| *LSD†* | *0.12* | *0.09* | *0.12* | *0.31* | *0.003* | *0.036* | *ns* |

† Represents the least significant difference at the 0.05 probability level for mean comparisons.
ns, Not significant.

Table 2. Net photosynthesis and respiration from dwarf bermudagrass following 28 days exposure to optimal or suboptimal temperature regimes

| Temperature Day/Night (°C) | Net Photosynthesis (μmol $CO_2$ $m^{-2}$ $sec^{-1}$) | Respiration (μmol $CO_2$ $m^{-2}$ $sec^{-1}$) |
|---|---|---|
| 27/19 | 16.1 | -2.3 |
| 35/27 | 14.9 | -6.2 |
| *LSD†* | *ns* | *1.0* |

†Represents the least significant difference at the 0.05 probability level for mean comparisons.
ns, Not significant.

## The Response of Dwarf Bermudagrass to GA Associated PGRs When Grown Under Optimal or Suboptimal Temperature Regimes

Dwarfism in many other plant species is often the result of altered synthesis or sensitivity to bioactive gibberellins (GA). To explore the possibility of temperature mediated differential GA synthesis/sensitivity, exogenous $GA_3$ and GA biosynthetic inhibitors were applied to Tifdwarf plants grown under optimal or suboptimal temperatures. Applications were made every 10 days over a 30 day period. On day 38, internode and leaf lengths were measured (Figure 1). Trends between internode and leaf lengths were similar. Therefore, only internode length data are reported here.

### *Control Treatments*

Internodes of untreated (control) plants grown under suboptimal temperatures were 30.2% longer than those of untreated (control) plants grown under optimal temperatures (Figure 1). This result is consistent with previous studies.

### *GA Synthesis Inhibitor - CCC*

Chlorocholine Chloride (CCC) is an early GA biosynthetic inhibitor that primarily disrupts the function of copalyl-diphosphate synthase (CPS) but also displays activity on *ent*-kaurene synthase (KS) (Rademacher, 2000 Review). CCC applied at the low and high rate reduced internode length under both temperature regimes (Figure 1).

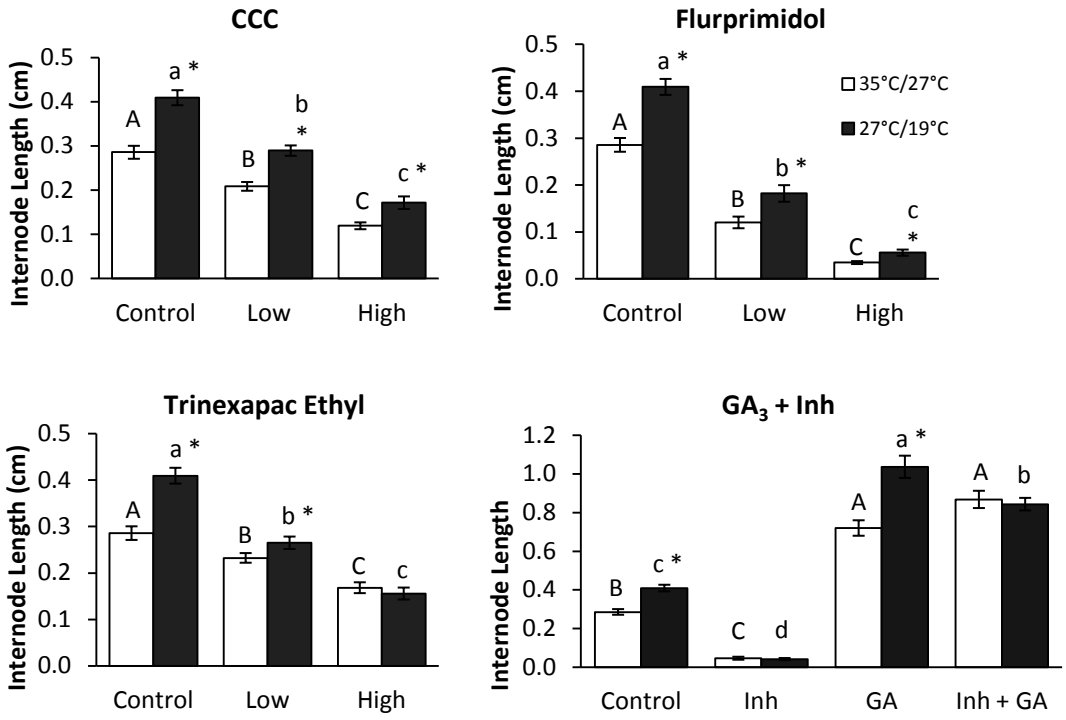


Figure 1. Internode length from Tifdwarf bermudagrass grown under optimal or suboptimal temperature regimes and treated with different PGR chemical combinations. Error bars denote standard error. *, Denotes significant difference at the 0.05 probability level between temperature treatments within the same chemical treatment. Optimal temperature treatments with the same upper case letter do not differ at the 0.05 probability level. Sub-optimal temperature treatments with the same lower case letter do not differ at the 0.05 probability level.

At the high and low rate, internodes from suboptimal temperature treatments remained 28.0% and 30.5% longer respectively than internodes from optimal treatments.

*GA Synthesis Inhibitor - Flurprimidol*

Flurprimidol is a GA biosynthetic inhibitor that disrupts the function of *ent*-kaurene oxidase (KO) (Rademacher, 2000 Review). Like CCC, both rates of flurprimidol reduced internode length in both temperature treatments (Figure 1). Also, at the high and low rate, internodes from suboptimal temperature treatments remained 33.9% and 60.2% longer respectively than internodes from optimal treatments. Therefore, a differential growth response due to temperature was measured in all CCC and flurprimidol treatments.

*GA Synthesis Inhibitor - Trinexapac-ethyl*

Trinexapac-ethyl is a late GA biosynthetic inhibitor that disrupts GA3ox, GA20ox and GA2ox enzyme function (Rademacher, 2000 Review). GA3ox activity is inhibited most strongly but GA20ox and GA2ox activity is also inhibited. Therefore trinexapac-ethyl impacts both the formation and deactivation of bioactive GA and GA intermediates. Trinexapac-ethyl reduced internode length regardless of application rate or temperature treatment (Figure 1). At the low rate, internodes from suboptimal temperature treatments were 12.3% longer than internodes from optimal treatments. However, at the high rate, there was no difference between temperature treatments.

Therefore, sufficient disruption of GA20ox, GA3ox and GA2ox enzyme function lead to an elimination of temperature dependent differential morphology.

*Bioactive $GA_3$*

Application of exogenous bioactive $GA_3$ increased internode length under both temperature regimes (Figure 1). In $GA_3$ treatments, internodes from suboptimal treatments remained 30.6% longer than optimal temperature treatments.

*GA Synthesis Inhibitors Plus Bioactive $GA_3$*

To compare base sensitivity to bioactive GA, CCC, Flurprimidol, and Trinexapac-ethyl were applied in combination with $GA_3$ to plants grown under optimal and suboptimal temperature treatments (Figure 1). The synthesis inhibitor combination effectively eliminates endogenous bioactive GA production allowing quantification of plant response to exogenous $GA_3$.

Application of the inhibitor combination alone reduced internode length to less than 0.1 cm in both temperature regimes (Figure 1). Application of the inhibitor combination with $GA_3$ increased internode length in both temperature treatments compared to control plants. However, there was no difference between temperature regimes. This would indicate that internode elongation in suboptimal temperatures is not the result of increased sensitivity to bioactive GA in Tifdwarf. However, since trinexapac-ethyl alone eliminated any differential response due to temperature at internode lengths that were numerically longer than the high rates of the other PGR's,

definitive conclusions concerning GA sensitivity cannot be made. Future work should investigate temperature mediated sensitivity using GA inhibitor combinations that exclude trinexapac-ethyl.

Application of $GA_3$ plus inhibitors to suboptimal temperature treatments produced internodes that were shorter than $GA_3$ treatments. However, in optimal treatments, internode length was statistically the same in $GA_3$ plus inhibitors and $GA_3$ treatments. Therefore, in optimal treatments, endogenous GA did not contribute to internode length when $GA_3$ was applied. This could be the result of limited endogenous bioactive GA content compared to exogenous levels or higher GA2ox catabolic activity in optimal temperature treatments. Definitive interpretation of these data is difficult however because trinexapac-ethyl inhibits GA2ox function and $GA_3$ is structurally protected from deactivation by GA2ox while endogenous bioactive GA is not (Grindal et al., 1998; Rademacher, 2000 Review).

Regardless of temperature, CCC, flurprimidol and trinexapac-ethyl reduced internode length while $GA_3$ increased internode length. Also, regardless of application rate, when CCC, flurprimidol or $GA_3$ were applied, a differential response due to temperature was measured. Since GA biosynthesis inhibitors and bioactive $GA_3$ alter internode length, the GA metabolic and signal transduction pathways are at least partially functional in Tifdwarf. No differential response to temperature was measured when trinexapac-ethyl was applied at the high rate. Since the inhibitory effects of trinexapac-ethyl target GA20ox, GA3ox and GA2ox, definitive interpretation of these results is difficult. Since no differential response to temperature was measured in high

trinexapac-ethyl treatments, it is reasonable to conclude that GA20ox, GA3ox, and/or GA2ox or factors upstream of these enzymes play a direct or indirect role in regulating this temperature mediated response

## Comparing the Influence of Temperature on Dwarf and Non-dwarf Bermudagrass Morphology and Physiology.

To compare Tifdwarf's response to suboptimal temperatures to that of a non-dwarf bermudagrass genotype, internode and leaf length were measured in Tifdwarf and NuMex Sahara bermudagrass grown under optimal or suboptimal temperature regimes. Measurements were taken 0, 2, 4, 6, and 8 days after initiation of temperature treatments (Figure 2). Under both temperature regimes, internode and leaf length of NuMex Sahara decreased from day 0 to day 8. This is possibly a result of increasing intra-specific competition due to increasing plant density or a natural plant maturation response. Both are purely speculative as it cannot be supported or refuted following a comprehensive review of the literature. On day 0, leaf length was shorter in plants grown under suboptimal temperatures compared to optimal temperatures. This relative difference remained constant through the remaining samplings days. The small statistical difference on day 0 was likely due to minor differences in chamber conditions.

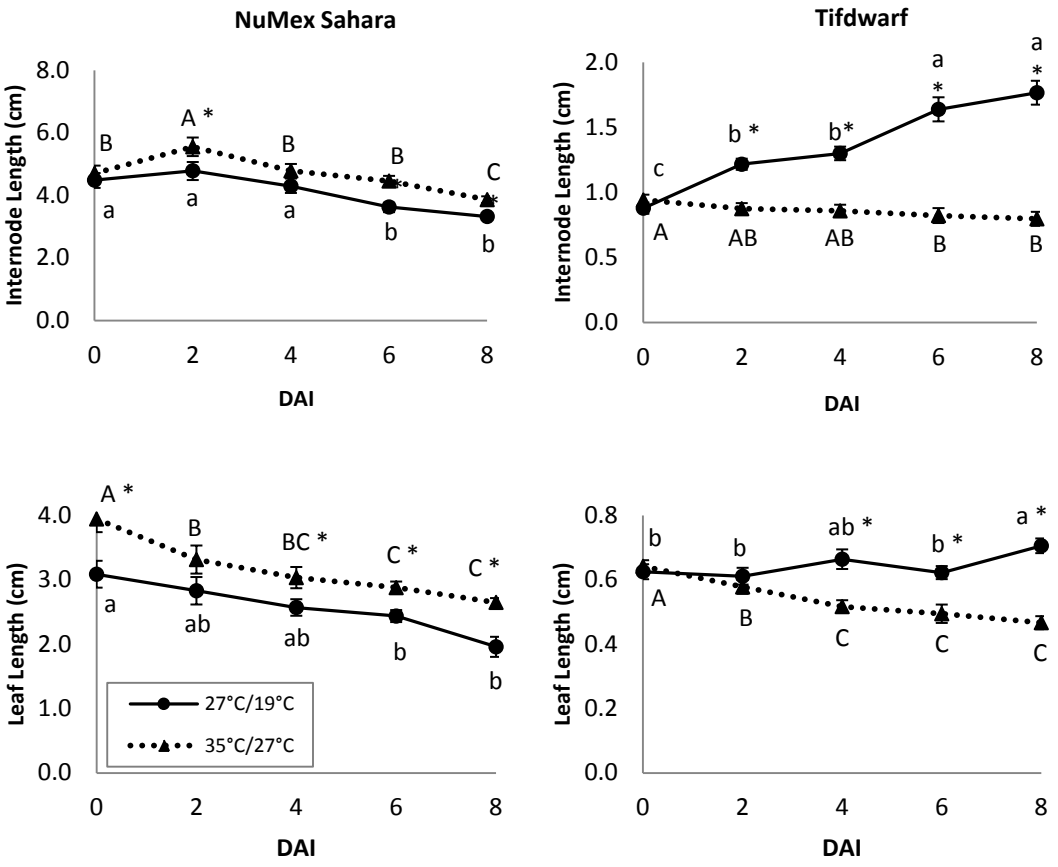


Figure 2. Internode and leaf length from dwarf and non-dwarf bermudagrass grown under optimal and suboptimal temperture regimes. DAI represents days after initiation of suboptimal temperature treatments. Error bars denote standard error. Optimal temperature treatments with the same upper case letter do not differ at the 0.05 probability level. Sub-optimal temperature treatments with the same lower case letter do not differ at the 0.05 probability level. An asterisk denotes significant difference at the 0.05 probability level between temperature treatments within the same sampling day.

Like NuMex Sahara, internode and leaf length in Tifdwarf grown under optimal temperatures decreased from day 0 to day 8 (Figure 2). Again, this might be a response to increased competition or the result of natural plant maturation. Once suboptimal temperature treatments were initiated, internode length increased rapidly. Two days after initiation of temperature treatments, internode length was longer in Tifdwarf grown under suboptimal than optimal temperatures. From day 0 to day 8, internode length doubles in Tifdwarf plants grown under suboptimal temperatures. On day 8, internode length and leaf length were 2.25 and 1.5 times longer respectively in plants grown under suboptimal than optimal temperatures.

The reduction in leaf and internode length of non-dwarf bermudagrass in response to suboptimal temperatures is similar to other documented responses. For example, zoysiagrass lamina and internode length were 1.36 and 2.2 times longer in plants grown under a 30°C day/27°C night temperature regime compared to a 24°C day/21°C night temperature regime (Youngner, 1961). *Paspalum dilitatum* plants grown under a mean temperature of 15°C had 3 times shorter leaves than plants grown under a mean temperature of 28.3°C (Mitchell, 1955). Since 27°/19°C Day/Night is close to optimal, a small reduction in internode and leaf length relative to the control treatments would be expected in this experiment.

Like Tifdwarf, the wheat GA mutant *rht3* displays atypical growth when exposed to suboptimal temperatures. Leaves of wild-type plants were longer when grown at 25°C than when grown at 11°C (Pinthus et al., 1989). However, leaves in *rht3* were numerically longer in 11° than 25°C treatments but the difference was not significant.

Appleford and Lenton (1991) found that wild-type leaves were significantly longer when grown at 20°C than at 10°C, but leaves in *rht3* were not longer at 20°C than at 10°C. Like Tifdwarf, *rht3* does not respond to suboptimal temperature in a manner consistent with WT. However, significant internode elongation in response to suboptimal temperatures seems to be unique to Tifdwarf.

Internode and leaf length in dwarf and non-dwarf bermudagrass grown under optimal temperature regimes decreased over time. The response of dwarf bermudagrass to suboptimal temperatures either reverses, bypasses or overcomes the reduction in growth habit measured in the other species/temperature treatment combinations.

To compare the effects of temperature on biomass acquisition and allocation between dwarf and non-dwarf bermudagrass, Tifdwarf and NuMex Sahara where grown under optimal or suboptimal temperatures for 14 days. Plants were harvested and total biomass, % leaf tissue, % stems tissue, % root tissue, and shoot to root ratios were determined (Table 3 and Table 4).

In non-dwarf bermudagrass, total biomass accumulation was 31% lower in suboptimal temperature treatments compared to optimal treatments (Table 3). This trend is consistent with other non-dwarf grass species. For Example, Zoysiagrass grown under a 30°C day (estimated) /27°C night temperature regime produced 2.5 times more top material, 1.3 times more roots and 1.2 times more rhizomes than when grown under a 24°C day/21°C night temperature regime (Youngner, 1961). *Paspalum dilitatum* grown under a mean temperature of 15°C produced 10 times less tissue per day than when grown under a mean temperature of 28.3°C (Mitchell, 1955).

Table 3. Total biomass, biomass allocation patterns and shoot-to-root ratio from non-dwarf bermudagrass grown under optimal or suboptimal temperatures for 14 days.

| | | Biomass Allocation | | | |
|---|---|---|---|---|---|
| Temperature | Biomass | % Leaf | % Stem | % Root | Shoot:Root |
| Day/Night | (g $cm^{-2}$) | % | % | % | |
| 35/27°C | 0.102 | 22.5 | 60.1 | 17.4 | 4.8 |
| 27/19°C | 0.070 | 20.0 | 54.0 | 26.0 | 2.9 |
| LSD† | 0.012 | 2.0 | 1.5 | 1.9 | 0.5 |

† Represents the least significant difference at the 0.05 probability level for mean comparisons.

Table 4. Total biomass, biomass allocation patterns and shoot to root ratio from dwarf bermudagrass grown for 14 days under optimal or suboptimal temperatures.

| | | Biomass Allocation | | | |
|---|---|---|---|---|---|
| Temperature | Biomass | % Leaf | % Stem | % Root | Shoot:Root |
| Day/Night | (g $cm^{-2}$) | % | % | % | |
| 35/27°C | 0.046 | 32.6 | 45.6 | 21.9 | 3.67 |
| 27/19°C | 0.037 | 23.3 | 45.5 | 31.2 | 2.21 |
| LSD† | 0.009 | 1.6 | 2.9 | 3.0 | 0.51 |

† Represents the least significant difference at the 0.05 probability level for mean comparisons.

In the current experiment, total biomass accumulation was 21% lower in Tifdwarf suboptimal than optimal treatments (Table 4). Again, these findings are opposite of Stanford et al. (2005) who found that above ground herbage production was higher in suboptimal temperature treatments.

Non-dwarf plants grown under optimal conditions allocated 60.1% of its biomass to stems, 22.5% to leaves and 17.4% to roots (Table 3). Dwarf plants grown under optimal temperatures allocated 45.6% of its biomass to stems, 32.6% to leaves and 21.9% to roots (Table 4). Under optimal temperatures, dwarf bermudagrass allocates more biomass to leaves and less to stems than non-dwarf bermudagrass (Figure3). Less allocation to stem tissue has been reported in other dwarf plants. A tomato (*Solanum lycopersicum* L.) GA deficient dwarf partitioned less to stems and more to roots compared to WT plants (Nagel et al., 2001 and Nagel and Lambers, 2002). However, biomass partitioned to leaves was unchanged.

Under suboptimal temperatures, NuMex Sahara partitioned more biomass from leaves and stems toward roots than optimal treatments (Table 3). A similar result was seen in Tifdwarf but reallocation was only from leaves to roots. Allocation to stems remained unchanged.

Therefore, in this experiment, Tifdwarf's biomass acquisition patterns seem to be consistent with other species. When grown under suboptimal temperatures, total biomass accumulation in Tifdwarf decreases and allocation shifts toward the root system.

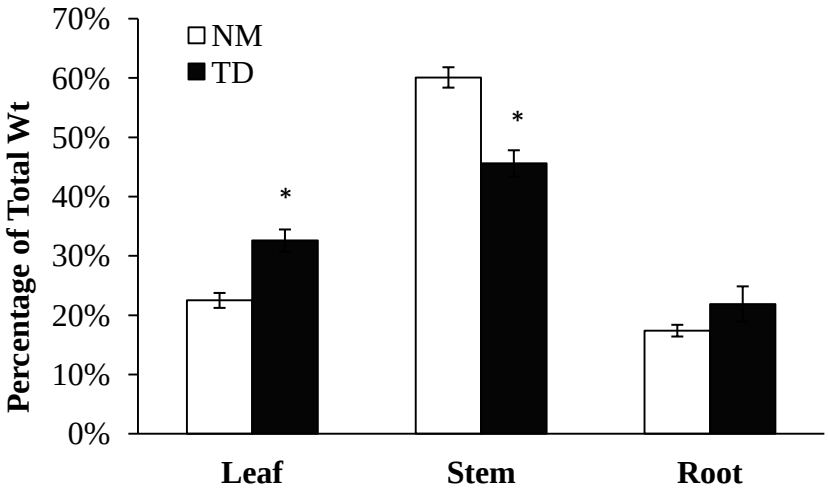


Figure 3. Biomass partitioning patterns in NuMex Sahara (NM) and Tifdwarf (TD) bermudagrass grown under optimal temperatures for 14 days. *, Denotes significant difference at the 0.05 probability level between species within the same plant feature. Error bars denote standard error.

## The Influence of Suboptimal Temperatures on GA Associated Gene Expression in Dwarf and Non-dwarf Bermudagrass

To further investigate GA's role in temperature mediated regulation of bermudagrass morphology, a study was conducted to analyze GA associated gene expression in dwarf and non-dwarf bermudagrass grown under optimal or suboptimal temperature regimes. Tifdwarf and NuMex Sahara were established in a greenhouse and then acclimated in growth chambers at optimal temperatures prior to initiation of temperature treatments. Zero, seven and 14 DAI, internode length was measured and tissue was harvested, frozen in liquid nitrogen, and stored at -80°C until gene expression analysis was conducted. Expression analysis was conducted on the GA synthesis genes *GA20ox1*, *GA20ox2* and *GA3ox1*, the GA catabolic genes *GA2oxa* and *GA2oxb*, and the GA signaling gene *GAMyb*.

### *Base Differences in GA Associated Gene Expression Between Dwarf and Non-Dwarf Bermudagrass Grown*

To identify base differences in GA associated gene expression between dwarf and non-dwarf bermudagrass, day 0, 7 and 14 data from each respective genotype's optimal temperature treatments were pooled and analyzed (Figure 4).

There were no differences between Tifdwarf and NuMex *GA20ox1*, *GA2oxb* and *GAMyb* expression. *GA20ox2* and *GA3ox* were 121.6% and 194.1% higher respectively and GA2oxa was 68% lower in Tifdwarf than NuMex Sahara.

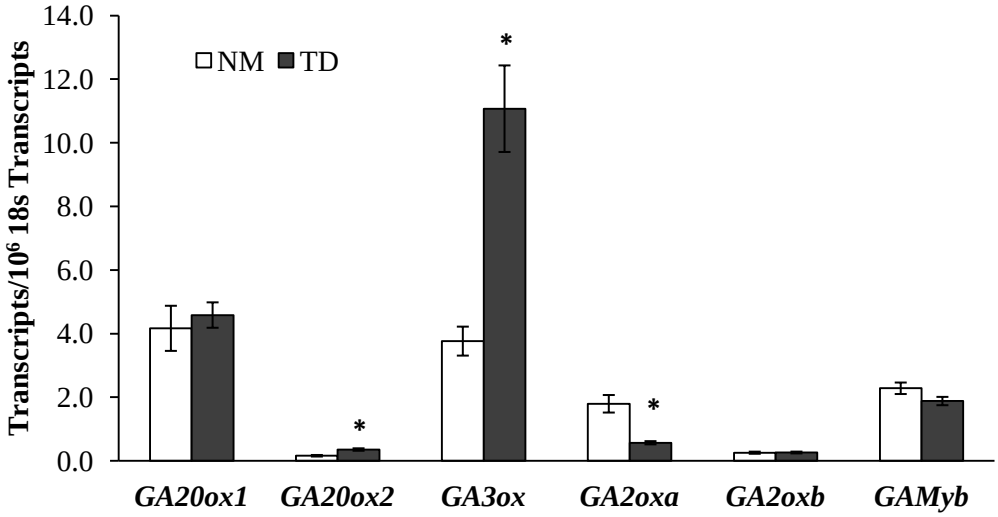


Figure 4. Gene expression of GA synthesis and signaling genes from dwarf and non-dwarf bermudagrass grown under an optimal temperature regime. Results represent pooled data from 0, 7, and 14 DAI. NM represents NuMex Sahara. TD represents Tifdwarf. *, Denotes significant difference at the 0.05 probability level between species within the same gene. Error bars denote standard error.

Elevated expression of GA biosynthesis genes and reduced expression of GA catabolic genes in Tifdwarf compared to NuMex is consistent with patterns often found in GA-deficient or GA-insensitive dwarf mutants. GA-deficient dwarf mutants typically contain limited bioactive GA and gene expression patterns within these mutants reflect appropriate feedback/feed forward regulation responses where expression of select *GA20ox* and *GA3ox* genes are elevated and select *GA2ox* catabolic genes are depressed (Fujioka et al., 1988a; Talon et al., 1990a; Ross et al., 1992; Chiang et al., 1995; Xu et al., 1995; Martin et al., 1996; Coles et al., 1999; Ashikari et al., 2002; Sasaki et al., 2002; Spielmeyer et al., 2002; Sakamoto et al., 2004; Rieu et al., 2008a).

In GA-insensitive dwarf mutants, appropriate feedback/feed forward regulation is also impaired. Despite a dwarf phenotype, bioactive GA is elevated (Fujioka et al., 1988b; Winkler and Freeling, 1994; Webb et al., 1998; Sasaki et al., 2003). Expression of *GA20ox* and *GA3ox* are also elevated while expression of *GA2ox* is depressed (Peng et al., 1997; Cowling et al., 1998; Dill and Sun, 2001; Sasaki et al., 2003). Elevated GA should initiate GA homeostatic processes to decrease bioactive GA levels. This does not occur due to defects in GA perception and/or early signal transduction. Quantification of endogenous bioactive GAs in both dwarf and non-dwarf bermudagrass would provide further evidence whether this expression pattern is associated with active feedback regulation of the GA biosynthetic pathway.

It is possible that *GA20ox2*, *GA3ox* and *GA2oxa* are feedback regulated while *GA20ox1* and *GA2oxb* are not. In many plant species, only select GA biosynthetic genes and select members within multi-gene families are feedback regulated. For example,

three of the five *Arabidopsis* GA20ox family members and one of four GA3ox family members have been shown to be under feedback control (Xu et al., 1999; Mitchum et al., 2006; Matsushita et al., 2007; Rieu et al., 2008a). In rice, one of two GA3ox members is under feedback control (Itoh et al., 2001 and Sakamoto et al., 2003). Four of the eight Arabidopsis GA2ox family members and one of the four rice GA2ox members have been shown to be subject to feed forward control (Rieu et al, 2008b; Thomas et al., 1999; Sakai et al., 2003; Sakamoto et al., 2004).

Due to phenotypic differences, *GAMyb* expression would be expected to be higher in non-dwarf than dwarf bermudagrass. However, that was not the case in this study. Gubler et al., 2002 demonstrated that a good correlation between bioactive GA, GAMyb protein levels, and subsequent GA responses does not always exist. Also, it has been reported that GAMyb is under post-translational control (Diaz et al., 2002; Achard et al., 2004; Millar and Gubler, 2005; Cao et al., 2006). Therefore, *GAMyb* mRNA quantification may not always be an accurate predictor of GA responses.

*The Influence of Temperatures on Gene Expression in Dwarf and Non-Dwarf Bermudagrass*

Internode length in NuMex Sahara grown under optimal temperatures did not change from day 0 to day 14 (Figure 5). NuMex Sahara internode length decreased 12.9% from day 0 to day 14 in plants grown under suboptimal temperature. Again, reduction in internode length in response to suboptimal temperatures is consistent with other documented responses to suboptimal temperatures (Mitchell, 1955; Youngner, 1961; Stanford et al., 2005).

Consistent with previous experiments, Tifdwarf plants grown under suboptimal temperatures possessed longer internodes than plants grown under optimal temperatures (Stanford et al., 2005). From day 0 to day 7, internode length increased 35.2% in plants grown under suboptimal temperatures and then remained constant from day 7 to day 14 (Figure 5). Tifdwarf internodes decreased in plants grown under optimal temperatures 24.2% from day 0 to day 7 and 10.0% from day 7 to 14. On day 14, Tifdwarf internodes from suboptimal temperature treatments were 99.2% longer than internodes from optimal treatments.

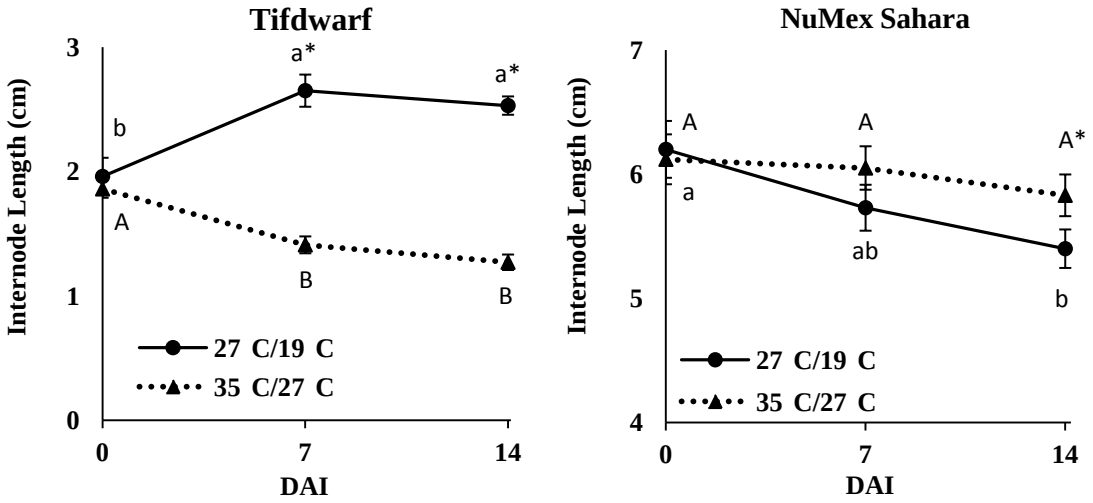


Figure 5. Internode length from Tifdwarf and NuMex Sahara bermudagrass grown at optimal or suboptimal temperatures over a 14 day period. DAI represents days after initiation of suboptimal temperature treatments. Error bars denote standard error. *, Denotes significant difference at the 0.05 probability level between temperature treatments within the same day. Optimal temperature treatments with the same upper case letter do not differ at $p = 0.05$. Sub-optimal temperature treatments with the same lower case letter do not differ at $p = 0.05$. DAI represents days after initiation of suboptimal temperature treatments.

GA20ox1

GA20ox is responsible for the conversion of $GA_{12}$ to $GA_9$ or $GA_{53}$ to $GA_{20}$. In both NuMex Sahara and Tifdwarf, there was no difference in *GA20ox1* expression between temperature treatments at any sampling day (Figure 6). Expression in both genotypes decreased approximately 40% from day 0 to day 14 in both temperature treatments. Since expression patterns of *GA20ox1* are consistent between genotypes and temperature treatments, it does not appear to play a significant role in dwarfism or temperature mediated adjustments in morphology.

GA20ox2

From Day 0 to Day 7, *GA20ox2* expression decreased 51% in NuMex optimal treatments and increased 106% in suboptimal treatments (Figure 6). On day 7, expression was 4.5 times higher in suboptimal than optimal treatments. From day 7 to day 14, expression in optimal treatments increased slightly but suboptimal still remained 2.5 times higher than optimal treatments.

In Tifdwarf, expression of *GA20ox2* decreased approximately 60% from day 0 to day 14 in both temperature treatments and no differences were measured between temperature treatments at any sampling day (Figure 6). Unlike NuMex, *GA20ox2* expression did not increase under suboptimal treatments. It is possible that this demonstrates a failure in an acclimation mechanism once exposed to suboptimal temperatures.

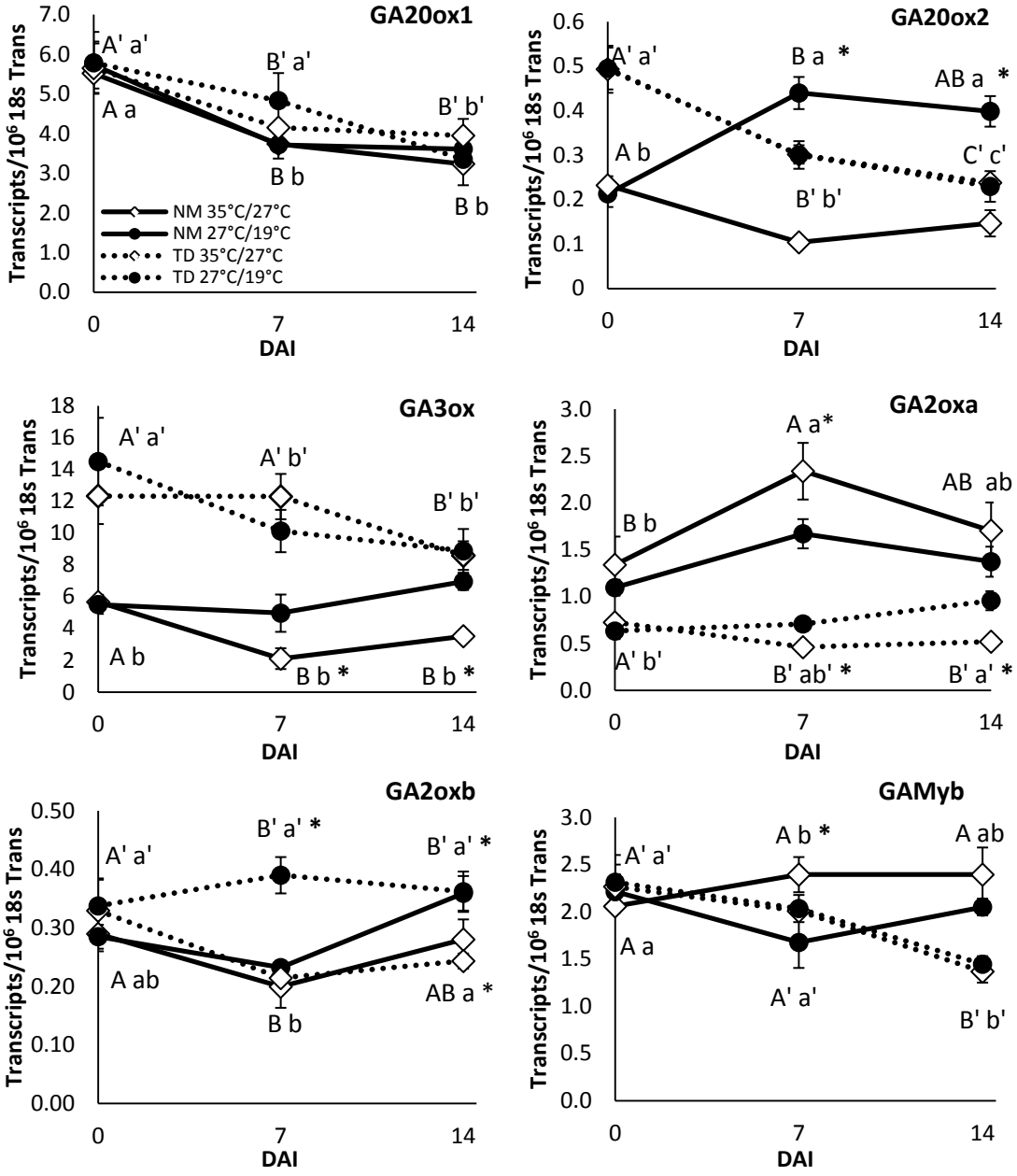


Figure 6. Gene expression from dwarf and non-dwarf bermudagrass grown at optimal or suboptimal temperatures. DAI represents days after initiation of suboptimal temperature treatments. NM represents NuMex Sahara. TD represents Tifdwarf. Error bars denote standard error. *, Denotes significant difference at the 0.05 probability level between temperature treatments within the same genotype. Optimal NM temperature treatments with the same upper case letter do not differ at the 0.05 probability level. Sub-optimal NM temperature treatments with the same lower case letter do not differ at the 0.05 probability level. Optimal TD temperature treatments with the same upper case letter followed by a prime symbol do not differ at the 0.05 probability level. Sub-optimal TD temperature treatments with the same lower case letter followed by a prime symbol do not differ at the 0.05 probability level.

GA3ox

GA3ox converts $GA_{20}$ to bioactive $GA_1$ or $GA_9$ to bioactive $GA_4$. In optimal treatments, NuMex *GA3ox* expression decreased 62.8% from day 0 to day 7 and then remained constant through Day 14 (Figure 6). In suboptimal treatments, expression did not change from day 0 to 14. On day 7 and day 14, *GA3ox* expression was approximately 2.4 and 2 times higher respectively in NuMex suboptimal treatments than optimal treatments. Like *GA20ox2*, *GA3ox* expression was elevated in suboptimal treatments despite shorter internode lengths.

It is possible that suboptimal temperatures cause a reduction in GA synthesis/sensitivity in NuMex which in turn, leads to feedback up-regulation on *GA20ox2* expression. There is precedence for this. Exposing Arabidopsis to cold temperatures caused a reduction in bioactive GA content (Achard et al., 2008). In response, *GA20ox* and *GA3ox* expression were feedback upregulated. As previously demonstrated, *GA20ox2* appears to be under GA feedback regulation in bermudagrass.

In Tifdwarf, expression of *GA3ox* decreased approximately 36% from day 0 to day 14 in both temperature treatments and no differences in expression were measured between temperature treatments at any sampling day (Figure 6). Like *GA20ox2*, suboptimal temperatures affected *GA3ox* expression differently in Tifdwarf than NuMex. It appears the conditional nature of Tifdwarf's dwarf phenotype negates genetic responses similar to those documented in non-dwarf suboptimal treatments.

GA2oxa

GA2ox is a catabolic enzyme that hydoxylates gibberellins at different points along the GA biosynthesis pathway. NuMex *GA2oxa* expression in optimal and suboptimal treatments increased 75 % and 52% respectively from day 0 to day 7 (Figure 6). On day 7, expression was 40% higher in optimal than suboptimal treatments. Day 14 expression in both temperature treatments was the same as day 0 or day 7.

*GA2oxa* expression in Tifdwarf suboptimal treatments increased 35% from day 0 to day 14 (Figure 6). Expression in optimal treatments decreased 37% from day 0 to day 7 and remained unchanged on day 14. On days 7 and 14, expression was 54% and 84% higher respectively in suboptimal than optimal treatments.

*GA2oxa* is the first gene to display temperature mediated differential expression patterns in dwarf bermudagrass. In dwarf and non-dwarf bermudagrass, *GA2oxa* expression levels relative to phenotype was consistent with GA feedback regulation.

GA2oxb

*GA2oxb* expression in NuMex optimal treatments decreased 31% from day 0 to day 7.(Figure 6). There was no significant difference between day 14 and day 0 or day 7. *GA2oxb* expression in NuMex suboptimal treatments did not change from day 0 to day 7 but then increased 55% from day 7 to day 14. On day 14, NuMex *GA2oxb* expression was 28% higher in suboptimal than optimal treatments.

Similar to NuMex optimal treatments, *GA2oxb* expression in Tifdwarf optimal treatments decreased 35% from day 0 to day 7 (Figure 6). There were no differences

between day 7 and day 14. Unlike the other genotype/temperature combinations, *GA2oxb* expression in Tifdwarf suboptimal treatments did not change throughout the study. On day 7 and day 14, expression was 82% and 81% higher respectively in suboptimal than optimal treatments. Like *GA2oxa*, temperature mediated differential expression was measured in Tifdwarf treatments.

GAMyb

GAMYB is a GA regulated transcription factor that regulates expression of a number of GA-inducible genes and therefore its expression levels can provide a quantitative estimation of GA response (Gubler et al., 1995 and Gocal et al., 1999**).** Expression of *GAMyb* in NuMex optimal treatments remained unchanged from day 0 to day 14 (Figure 6). Expression in suboptimal treatments decreased 24% from day 0 to day 7. There was no difference in expression between day 14 and day 0 or day 7. On day 7, expression was 42% lower in suboptimal than optimal treatments.

*GAMyb* expression in both optimal and suboptimal Tifdwarf treatments remained unchanged from day 0 to day 7 and then decreased approximately 30% from day 7 to day 14 (Figure 6). Despite differences in internode length, no differences in *GAMyb* expression were measured between temperature treatments at any sampling day. Again, it has been reported that *GAMyb* mRNA quantification may not always accurately predict GA responses (Diaz et al., 2002; Achard et al., 2004; Millar and Gubler, 2005; Cao et al., 2006).

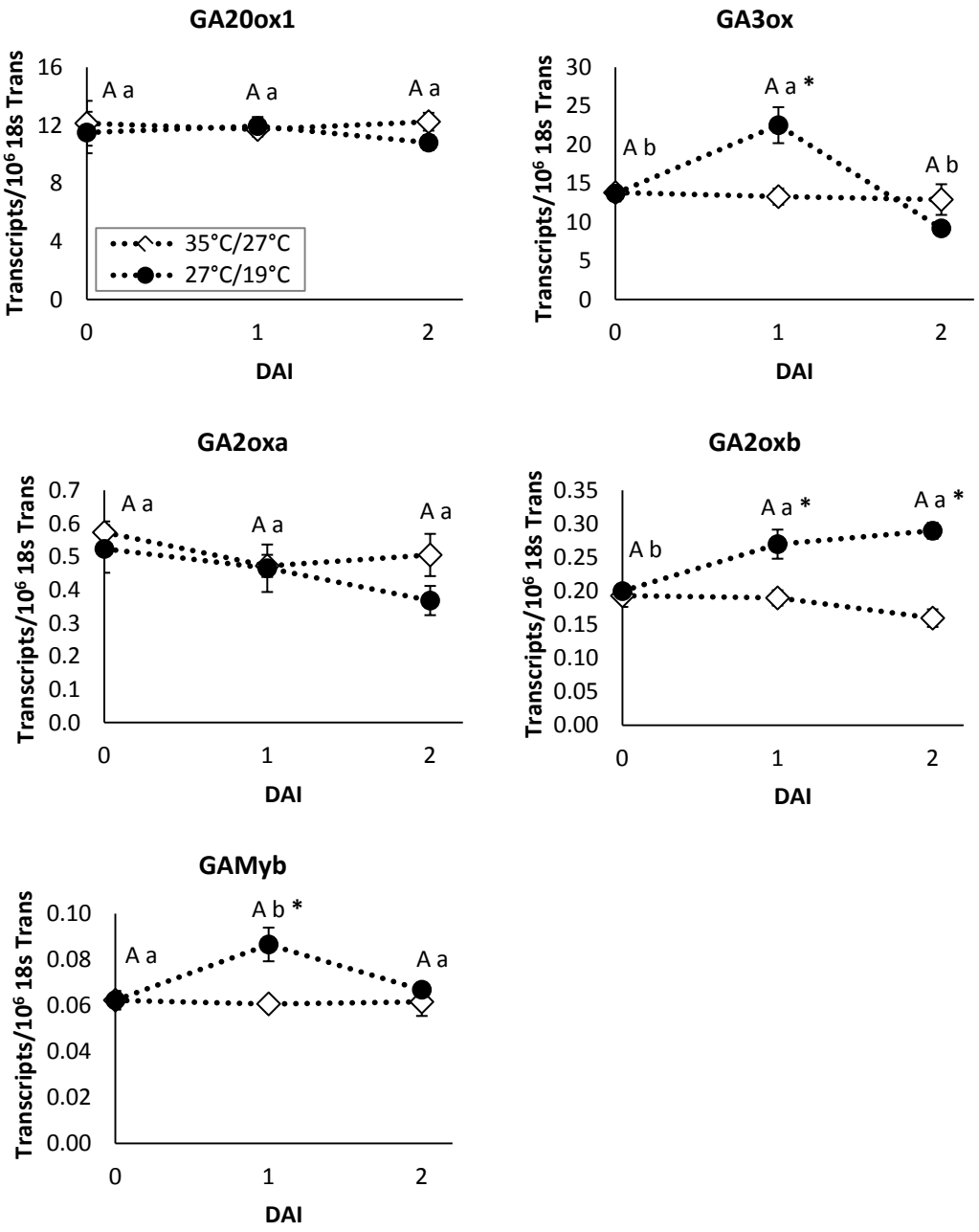


Figure 7. Gene expression from dwarf bermudagrass grown at optimal or suboptimal temperatures. DAI represents days after initiation of suboptimal temperature treatments. Error bars denote standard error. *, Denotes significant difference at the 0.05 probability level between temperature treatments within the same day. Optimal temperature treatments with the same upper case letter do not differ at the 0.05 probability level. Sub-optimal temperature treatments with the same lower case letter do not differ at the 0.05 probability level.

Early Responses to Suboptimal Temperatures in Dwarf Bermudagrass

Changes in GA associated gene expression can occur very quickly following the appropriate stimuli. To better characterize early responses to suboptimal temperature in dwarf bermudagrass, gene expression was analyzed 0, 1, and 2 DAI in Tifdwarf grown under optimal and suboptimal temperature regimes.

In all Tifdwarf optimal treatments, expression levels did not change from day 0 to day 2 for any genes tested (Figure 7). Consistent with the previous study, there were no differences in expression between temperature treatments for *GA20ox1* and *GA2oxa*. There were also no differences in *GA20ox1* and *GA2oxa* expression between sampling days within suboptimal treatments. However, in suboptimal treatments, *GA3ox* expression increased 65% from day 0 to day 1 but returned to day 0 values by day 2. *GA2oxb* expression in suboptimal treatments increased 35% from day 0 to day 1 and then remained unchanged on day 2. Expression of *GA2oxb* was 42% and 81% higher in suboptimal treatments than optimal treatments on days 1 and 2 respectively. Expression of *GAMyb* in suboptimal treatments increased 42% from day 0 to day 1 but returned to day 0 levels by day 2.

Therefore, initiation of suboptimal temperatures caused a brief increase in *GA3ox* and *GAMyb* expression. However, expression of both genes quickly returned to control levels. Suboptimal temperatures also caused an increase in expression of *GA2oxb*. These data combined with morphology data could indicate that expression of key GA biosynthesis/signaling genes increases briefly but are quickly targeted for feedback regulation of GA biosynthesis and catabolic genes. It is difficult to determine if these

gene expression data reflect initial responses to suboptimal treatment initiation, subsequent homeostatic responses or a combination of the two. Future work should investigate the first 24 hours following initiation of the temperature treatment.

## Comparing the Effects of Temperature on GA Sensitivity in Dwarf and Non-dwarf Bermudagrass

To evaluate the effect of temperature on GA sensitivity, a known quantity of exogenous $GA_3$ plus a GA biosynthetic inhibitor combination (GA + Inh) was applied to Tifdwarf and NuMex Sahara plants grown under optimal or suboptimal temperature regimes. Since the inhibitor combination effectively eliminates endogenous bioactive GA production, temperature induced differential sensitivity due to temperature can be quantified. Both internode length and gene expression were measured.

### *Internode Length*

Tifdwarf morphological responses in the second experiment were similar to the first (Figure 8). Eleven days after PGR treatment, control plants grown under suboptimal temperatures were 42% longer than optimal treatments. $GA_3$ application increased internode length more than 60% compared to controls for both temperature treatments. Suboptimal temperature treatments treated with $GA_3$ remained 33% longer than optimal temperature treatments treated with $GA_3$. $GA_3$ + inhibitor application increased internode length in both temperature treatments compared to the control but

there was no difference in internode length between optimal and suboptimal temperature treatments.

In NuMex Sahara, internodes from control treatments grown under suboptimal temperatures were numerically 8% shorter than optimal treatments but this difference was not significant (Figure 8). Internode length increased slightly when $GA_3$ was applied to plants grown under both temperature regimes. There was no difference between temperature treatments. Compared to control treatments, $GA_3$ + Inhibitor had no effect on internode length in plants grown under either temperature treatment. There was no difference due to temperature within $GA_3$ + Inhibitor treatments.

NuMex appears to be less sensitive to exogenous $GA_3$ than Tifdwarf. For example, Tifdwarf grown under optimal temperatures and treated with $GA_3$ possessed internodes that were 93% longer than plants grown under the same temperatures but with no exogenous GA treatment. Comparing the same temperature treatments, NuMex Sahara internodes treated with $GA_3$ were only 28% longer than control treatments. This might indicate that NuMex Sahara internodes grown under these temperature and growth conditions are close to saturated with endogenous bioactive GA and addition of exogenous $GA_3$ elicits little response. A GA response curve and more comprehensive GA sensitivity study are required to better address this possibility in NuMex Sahara.

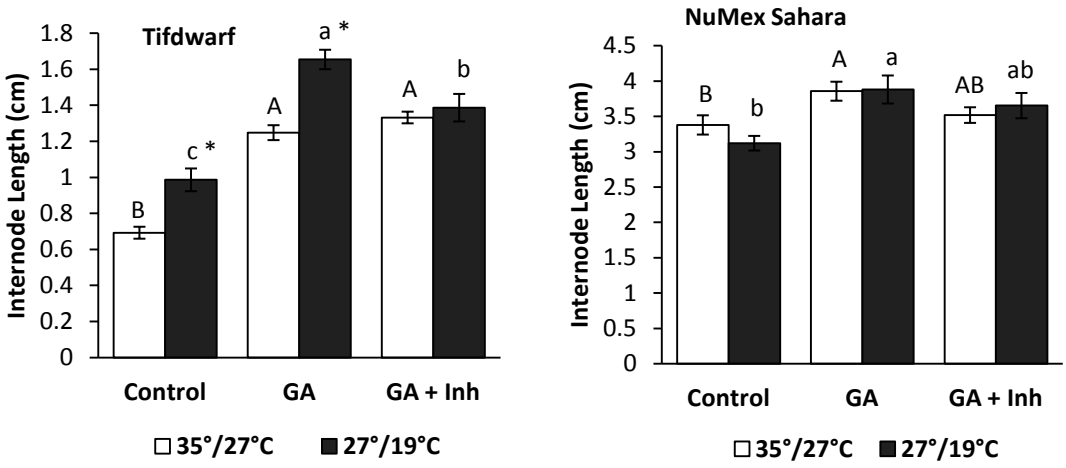


Figure 8. Internodes length in dwarf and non-dwarf bermudagrass grown under optimal or suboptimal temperature regime and treated with different PGRs. Control treatments were sprayed with solution void on PGRs. GA treatments were sprayed with $GA_3$. GA + Inh treatments were sprayed with $GA_3$ plus a 3-way inhibitor combination. Error bars denote standard error. *, Denotes significant difference at the 0.05 probability level between temperature treatments within the same chemical treatment. Optimal temperature treatments with the same upper case letter do not differ at the 0.05 probability level. Sub-optimal temperature treatments with the same lower case letter do not differ at the 0.05 probability level.

*Gene Expression*

To highlight the differential effects of temperature on gene expression in plants treated with $GA_3$ plus an inhibitor combination, data is presented as the percent difference in suboptimal temperature treatments compared to optimal temperature treatments within the same chemical treatment (Figure 9).

In NuMex Sahara, $GA_3$ plus Inhibitor treatments, there was no difference in internode length between optimal and suboptimal treatments. Also, there were no differences in *GA20ox1* and *GAMyb* expression responses to GA3 + inhibitor between optimal and suboptimal treatments. However, *GA3ox*, *GA2oxa* and *GA2oxb* expression responses to GA3 + inhibitor were 69%, 68% and 24% higher respectively in suboptimal treatments than optimal. Therefore, when similar quantities of bioactive GA plus inhibitors were applied, temperature had no effect on NuMex internode length or *GA20ox1* and *GAMyb* expression butdid increase expression of *GA3ox*, *GA2oxa*, and *GA2oxb* compared to optimal treatments.

In Tifdwarf $GA_3$ + Inhibitor treatments, internode length and expression of *GA20ox1*, *GA3ox* and *GA2oxa* were similar between temperature treatments. However, *GA2oxb* and *GAMyb* expression were 95%, and 44% higher respectively in suboptimal than optimal treatments. Therefore, similar bioactive $GA_3$ levels resulted in no differential growth response due to temperature but there were differences in *GA2oxb* and *GAMyb* expression.

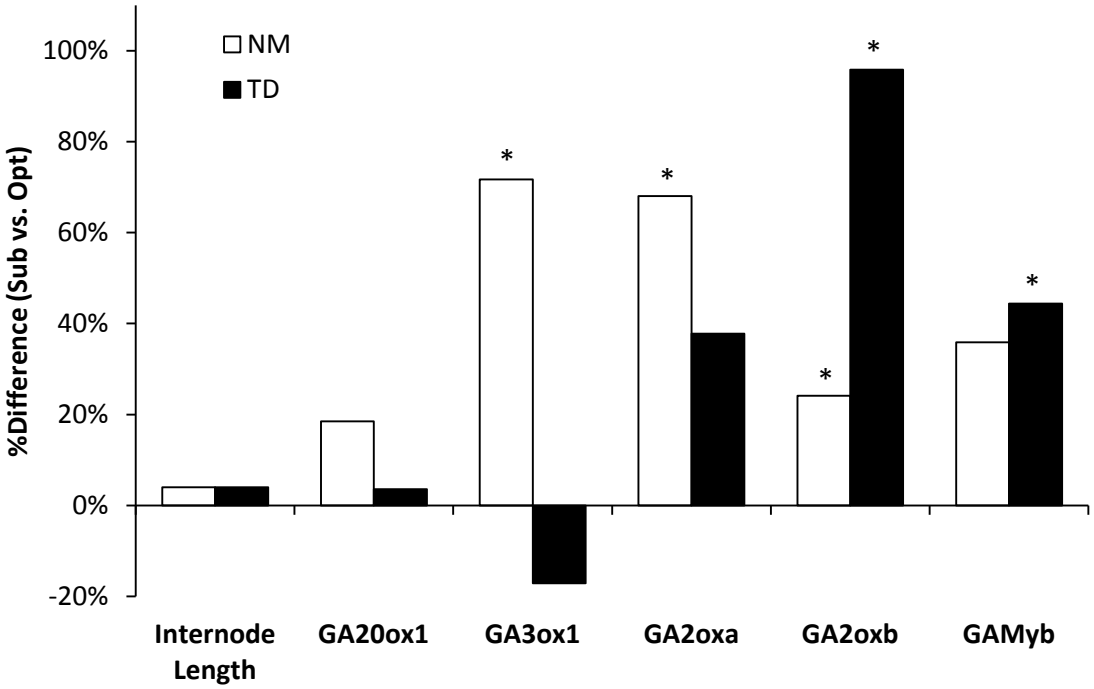


Figure 9. Percent difference in gene expression between bermudagrass treated with $GA_3$ plus a GA inhibitor combination and grown at suboptimal temperatures (27/19°C) or optimal temperatures (35/27°C). NM represents NuMex Sahara. TD represents Tifdwarf. *, Denotes significant difference at the 0.05 probability level between temperature treatments within the same genotype/chemical treatment.

Based on the results of this study, it would appear that differences in NuMex and Tifdwarf internode length are not due to temperature mediated differences in sensitivity to bioactive GA. However, despite identical bioactive GA application, expression of select GA deactivation and signal transduction genes were higher in suboptimal than optimal temperatures in both Tifdwarf and NuMex. Therefore, the possibility of temperature induced differences in sensitivity to bioactive GA cannot be eliminated. Also, since $GA_3$ is not deactivated by GA2ox and trinexapac-ethyl adversely affects GA2ox enzyme activity, growth differences due to GA2ox activity could be masked (Grindal et al., 1998 and Rademacher, 2000 Review). This experiment should be conducted again using different bioactive GAs and biosynthetic inhibitors.

# CHAPTER V

# SUMMARY

## The Effect of Temperature on Bermudagrass Morphology and Physiology

Consistent with Stanford et al., 2005, internode and leaf length were longer in dwarf bermudagrass plants grown under suboptimal compared to optimal temperature regimes. Non-dwarf internode and leaf length either decreased slightly or showed no response to the suboptimal temperature regimes used in this study.

Tifdwarf shoot number and leaf number were lower and average area per leaf was larger in suboptimal than optimal treatments. Total leaf area was higher in optimal treatments due to higher leaf numbers. The suboptimal temperature regime had no effect on photosynthesis in dwarf plants but respiration was lower compared to optimal treatments. Biomass accumulation was unaffected or slightly reduced in dwarf plants given suboptimal treatments but non-dwarf plants provided with suboptimal treatments accumulated less biomass than optimal treatments. Under optimal temperatures, Tifdwarf allocated more biomass to leaves and less to stems than non-dwarf bermudagrass. Suboptimal temperatures caused non-dwarf bermudagrass to shift biomass from stems and leaves toward roots and dwarf bermudagrass to shift biomass from leaves toward roots.

Reduction in internode length, leaf length, and biomass accumulation and allocation of biomass toward roots in non-dwarf plants in response to suboptimal temperatures is consistent with other plant species. Increased internode and leaf length

in response to suboptimal temperatures seems to be unique to dwarf bermudagrass and could be the result of conditional release of its dwarfing mechanism.

## Base Differences in GA Associated Gene Expression between Dwarf and Non-dwarf Bermudagrass

Significant differences in GA associated gene expression exist between dwarf and non-dwarf bermudagrass. *GA20ox2* and *GA3ox* expression were higher and *GA2oxa* expression was lower in Tifdwarf than NuMex Sahara under optimal temperatures. These expression patterns are similar to those documented in a number of GA associated dwarf mutants resulting from feedback/forward homeostasis responses to GA status.

Expression of *GA20ox1*, *GA2oxb* and *GAMyb* were similar between dwarf and non-dwarf bermudagrass grown under optimal temperatures. Therefore, if the proteins that these genes encode function properly under both temperature regimes, they likely do not contribute to Tifdwarf's dwarf phenotype. Temperature dependent protein function would need to be studied to further address this possibility.

## The Influence of Temperatures on GA Associated Gene Expression in Bermudagrass

Depending on the experiment, internode elongation either remained constant or decreased when NuMex Sahara was exposed to suboptimal treatments. Despite limited phenotypic responses, suboptimal temperature did lead to changes in gene expression. Suboptimal temperatures affected expression of NuMex Sahara *GA20ox2*, *GA3ox*,

*GA2oxa* and *GAMyb* in a manner that was consistent with homeostatic feedback/ feed forward regulation of GA synthesis.

Tifdwarf was quite different. Suboptimal temperatures produced robust phenotypic changes. However, with the exception of *GA2oxa* and *GA2oxb*, Tifdwarf displayed minimal molecular responses to suboptimal temperatures at 7 DAI. Suboptimal temperatures did lead to a brief increase in expression of *GA3ox* and *GAMyb* one DAI however both quickly returned to day 0 values by two DAI. Since GA responses can happen within hours following an appropriate stimuli, and GA biosynthesis and signal transduction pathways are actively regulated, it is impossible to know whether day one expression patterns are a response to suboptimal temperatures or corresponding homeostatic processes. Future research efforts should investigate the first 24 hours following temperature treatment initiation.

Tifdwarf's lack of a molecular response relative to non-dwarf bermudagrass may provide insight into the conditional nature of dwarfism in Tifdwarf. If suboptimal temperatures cause the release of the dwarfing mechanism in Tifdwarf which in turn causes an increase in GA production, this could over-ride the temperature response documented in the non-dwarf bermudagrass. Significant research is required before definitive conclusions can be drawn, however there is enough evidence gathered in this study to warrant such future research efforts.

## Plant Growth Regulators (PGRs) and Temperature Mediated Differences in GA Sensitivity

To further investigate GAs role in temperature mediated regulation of dwarfism in bermudagrass, PGR's were applied to Tifdwarf grown under optimal and suboptimal temperatures. CCC and flurprimidol decreased internode length in both temperature treatments but internodes from suboptimal temperature regimes were longer than optimal regimes. Trinexapac-ethyl also decreased internode length in both temperatures treatments but no difference in internode length was measured at the high application rate. Therefore, sufficient disruption of *GA20ox, GA3ox* and *GA2ox* enzyme function effectively eliminated temperature mediated differences in Tifdwarf internode length.

$GA_3$ increased internode length in both temperature treatments but internodes from suboptimal temperature regimes were longer than optimal temperature regimes. Non-dwarf bermudagrass internode length also increased when $GA_3$ was applied but unlike Tifdwarf, there was no difference due to temperature.

Like trinexapac-ethyl, when the inhibitor combination plus $GA_3$ was applied, no difference due to temperature was observed in dwarf internode length. The same was true for non-dwarf bermudagrass. Therefore, it would appear that temperature mediated adjustment in bermudagrass morphology is not the result of altered sensitivity to bioactive GA. Also, since trinexapac-ethyl effectively eliminated any temperature mediated difference in dwarf morphology, it would appear that this temperature response requires functional late stage GA metabolic and/or catabolic enzymes.

However, despite identical bioactive GA content in $GA_3$ plus inhibitor treatments, gene expression data from both bermudagrass genotypes indicate differential responses. *GA2oxb* and *GAMyb* expression in Tifdwarf and *GA3ox*, *GA2oxa* and *GA2oxb* expression in NuMex were higher in plants grown under suboptimal temperatures treated with the inhibitor combination plus $GA_3$ than in plants grown under optimal temperatures treated with the inhibitor combination plus $GA_3$. Therefore, despite no phenotypic differences, expression of some GA-associated genes differed between temperature treatments.

Since trinexapac-ethyl disrupts both synthesis and deactivation of GA, and $GA_3$ is resistant to GA2ox activity, interpretation of these data is difficult. Future work should utilize alternative PGRs that may provide data that is more easily interpreted.

No definitive conclusions can be drawn from this study concerning GA's role in dwarfism in bermudagrass or the conditional dwarf trait in Tifdwarf. Nor has a likely candidate dwarfing gene been identified. The original hypotheses which state the conditional release of dwarfism as reported by Stanford et al., 2005, is due to a leaky dwarfing gene whose product quantity or function is altered by temperature and the dwarfing gene in Tifdwarf bermudagrass codes for a gibberellin (GA) biosynthetic enzyme or a protein involved in GA signal transduction still seem the most plausible explanations. Sufficient evidence was gathered in this study to justify ongoing efforts to further address these hypotheses. Future work should focus on GA quantification and early genetic responses in dwarf and non-dwarf bermudagrass genotypes grown under suboptimal temperatures.

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# APPENDIX A

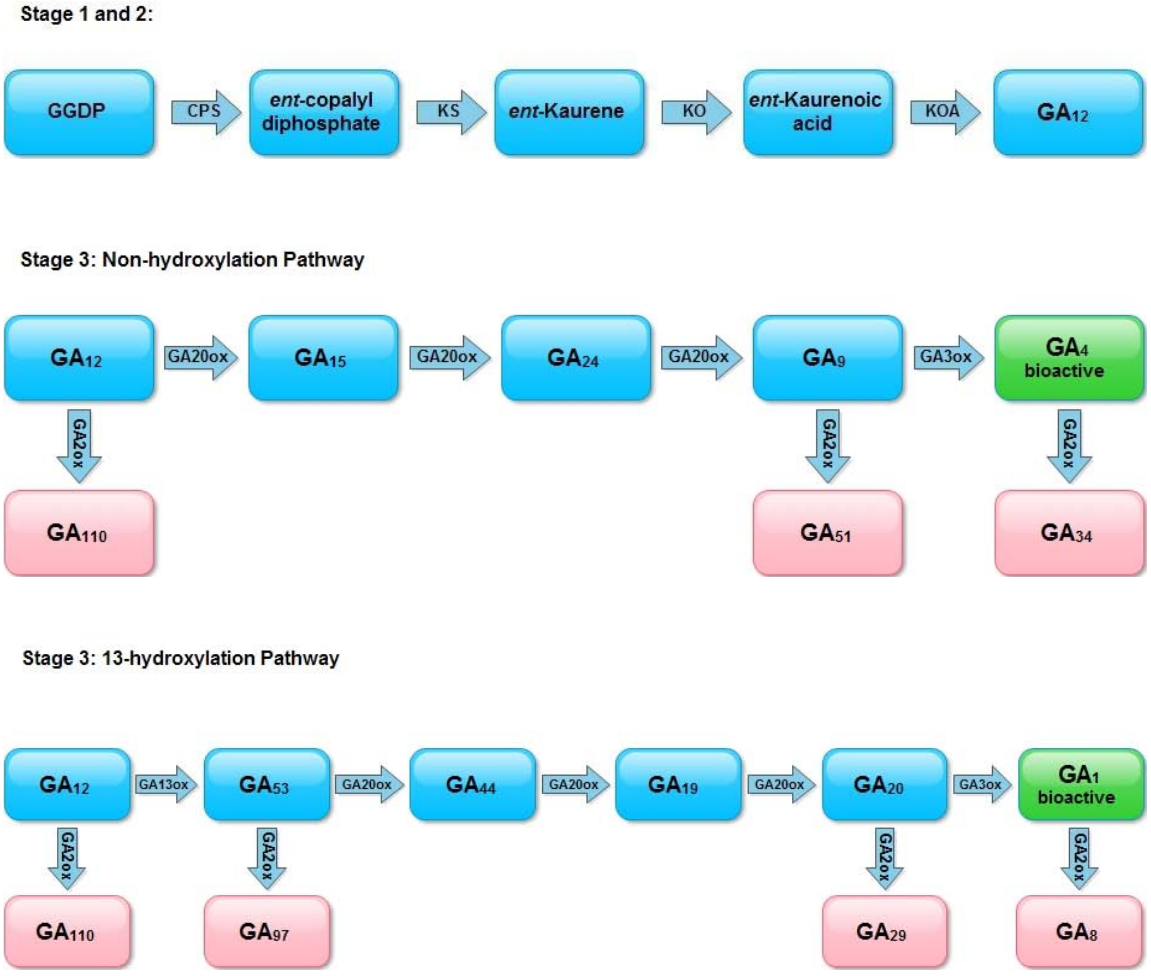


Diagram of the GA Biosynthetic Pathway (Yamaguchi, S., 2008). GGDP, geranylgeranyl diphosphate; CPS, *ent*-copalyl diphosphate synthase; KS, *ent*-kaurene synthase; KO, *ent*-kaurene oxidase; KAO, *ent*-kaurenoic acid oxidase.

APPENDIX B

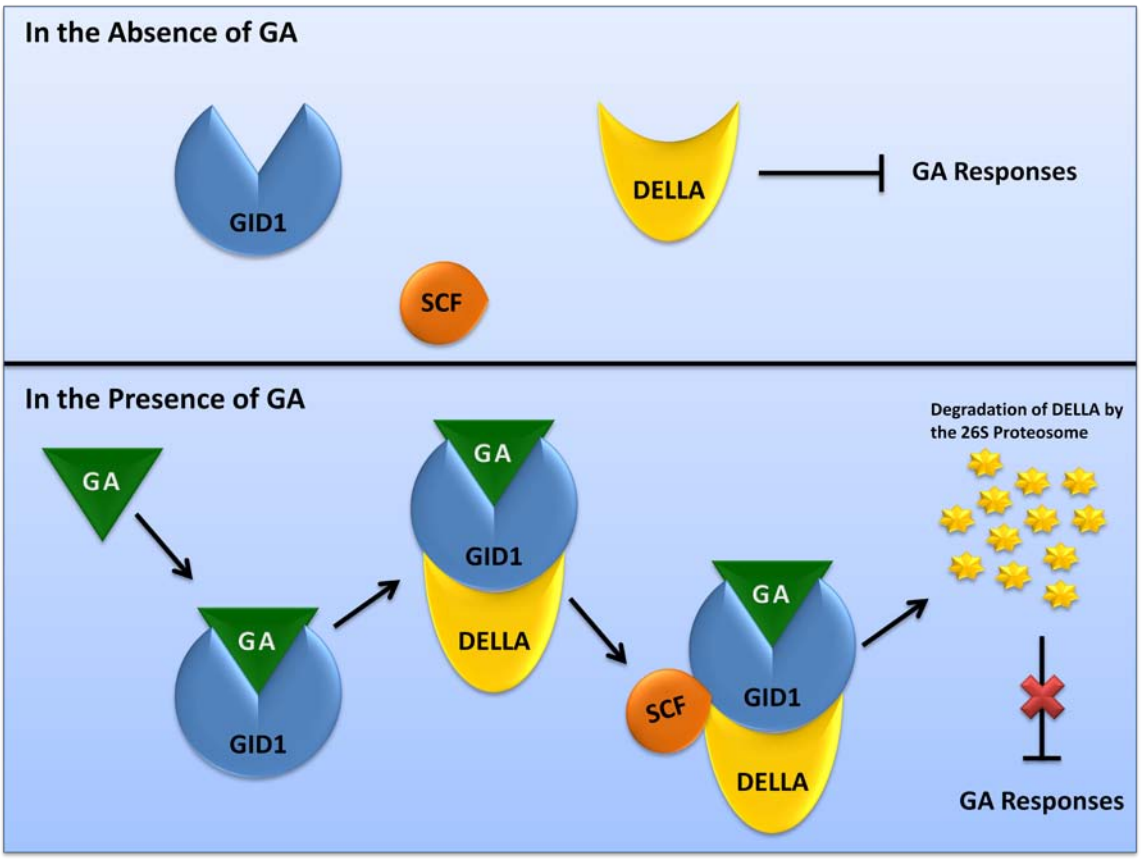


Diagram of the GA Signal Transduction Pathway (Achard and Genschik, 2009). GA, bioactive GA; GID1, GA receptor; SCF, SCF ubiquitin-ligase complex; DELLA, DELLA protein.

APPENDIX C

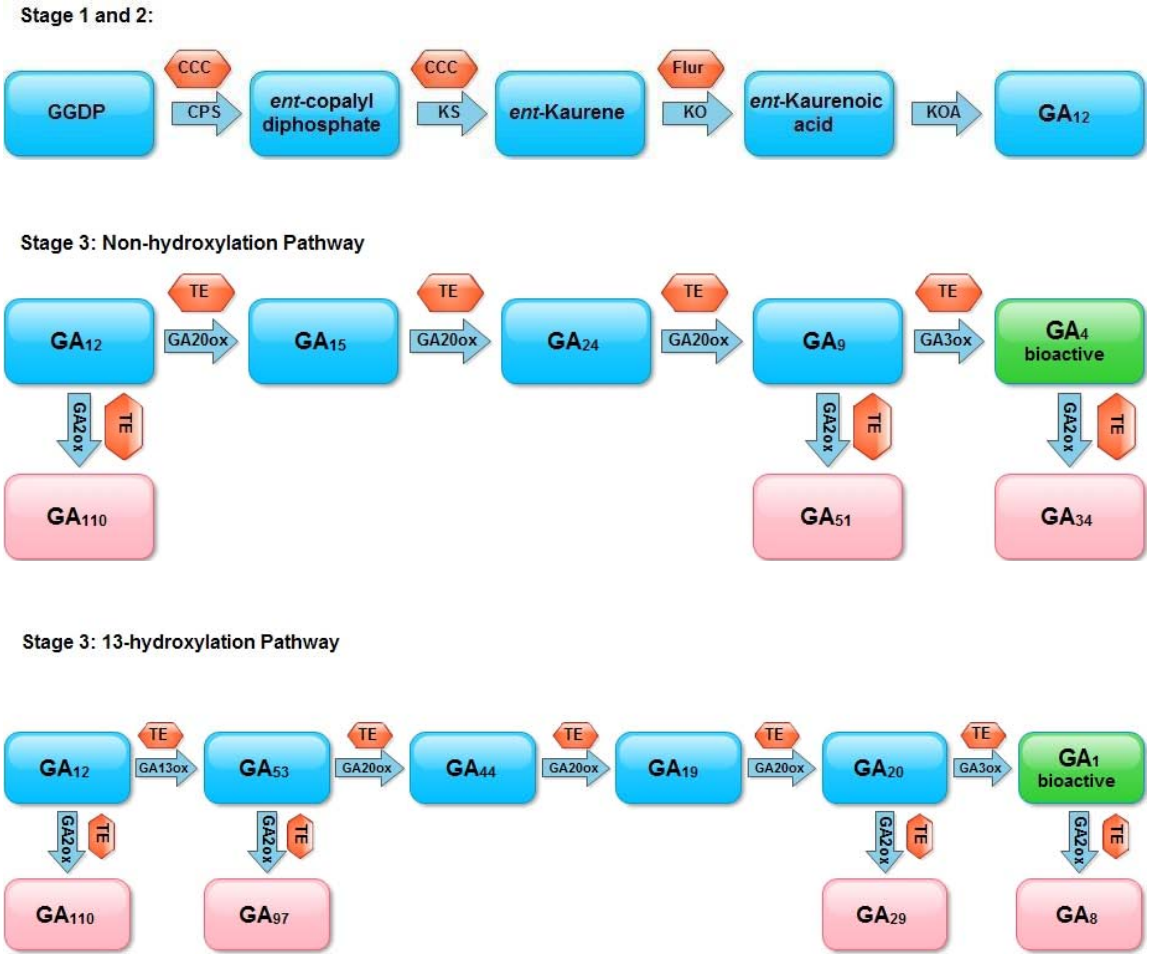


Diagram of GA Biosynthetic Inhibitors impact on the GA Biosynthesis Pathway (Yamaguchi, S., 2008 and Rademacher, 2000 review). GGDP, geranylgeranyl diphosphate; CPS, *ent*-copalyl diphosphate synthase; CCC, chlorocholine chloride; KS, *ent*-kaurene synthase; Flur, flurprimidol; KO, *ent*-kaurene oxidase; KAO, *ent*-kaurenoic acid oxidase; TE, Trinexapac-ethyl.

# APPENDIX D

Sequences of primers used for QPCR

| mRNA target | Forward primer | Reverse primer |
|---|---|---|
| *18s mRNA* | GCGCCCGGTATTGTTATTTA | AAACGGCTACCACATCCAAG |
| *GA20ox1* | ACATCGGCGACACATTCAT | ACACCACCTTGTCCATCTCC |
| *GA20ox2* | GTGTAGGCAGCTCTTGTACCG | CTCTTGCTTCAGGACGACACC |
| *GA3ox* | GGAGTTCCACAAGGAGATGC | TACCAGTTGAGGTGCATGGT |
| *GA2oxa* | AACAACTCTCTTCATCTTGCATTG | AGCAAGCTTATCACAGACACTGAC |
| *GA2oxb* | GAGCTTCTGAACGAGTACATTGC | GTAGTGGTTCACCCTCAGCATC |
| *GAMyb* | GGAGGACCATCCCAATTCTT | TGCACAGGAGACATTTTGGA |

# APPENDIX E

## Gene Sequences

***GA3ox***
ATGTGGGCCGAGGGCTACACCTTCTCGCCGGCCTCCCTCCGCGCCGACCTGCGCAAGCTCTGG
CCCAAGGCCGGCGACGACTACACCGGCTTCTGTGACGTGATGGAGGAGTTCCACAAGGAGAT
GCGCGCCCTCGCCAACAAGCTGCTGGAGCTGTTCCTCAAGGCGCTCGGGCTCACCGACGACC
AGGTCAACTCCGTCGAGGCGGAGCGGAGGCTCGCCGAGACCATGACCGCCACCATGCACCTC
AACTGGTATCCGAGGTGCCCGGACCCGCAGCGCGCTCTGGGCCTGATCGCGCACACCGACTC
GGGCTTCTTCACCTTGGTGCTACAGAGCCTCGTCCCCGGGCTGCAGCTGTTCCGGCGGGACCC
CGACCGCTGGGTGGCGGTGCCGGCCGTGCCGGGCGCCTTTGTCGTCAACGTCGGGGACCTCT
TCCAAATCCTCACGAACGGCCGCTTCC

***GA20ox1***
CCATCATGCGCCTCAACTACTACCCTCCGTGCCAGCGTCCCCTGGAGACGCTCGGCACGGGCC
CGCATTGCGACCCCACCTCCCTCACCATCCTCCACCAGGACCACGTCGGCGGCCTTCAGGTCT
TCGCCGGCGGCCGCTGGCTCTCCATCCGCCCGCACACCGGCGCCTTCGTCGTCAACATCGGCG
ACACATTCATGGCGCTCTCCAACGGACGCTACAGGAGCTGCCTGCATCGGGCCGTCGTCAAC
AGCCGCGTCCCACGCAGGTCGCTGGCCTTCTTCCTCTGCCCGGAGATGGACAAGGTGGTGTG
CCCACCGGAGGAGCTGGTCGGCGCCGGCGAGACCAGGGCGTACCCGGACTTCACGT

***GA20ox2***
TGCAACAACTACCCGCCGTGCCCGGAGCCGGAGCGGACGCTGGGCACGGGGCCCCACTGCG
ATCCGGCGGCCCTCACCCTCTTGCTTCAGGACGACACCGTGGACGGGCTCGAGGTGCTCGTC
GGCGGCGAGTGGAGGCCCGTGAGGCCCAAGCCCGGCGCTCTCGTCGTCAACATCGGGGACAC
ATTCACGGCGCTGTCGAACGGGCGGTACAAGAGCTGCCTACACCGCGCGGTGGTGCACCGGG
ATCGGGCGCGCCGGTCGCTGGCCTTCTTCCTCTGCCCGCGCGACGACCGCGTCGTGCGCCCGC
CGCCGCTTCTCGCGCGGCCGCGCCGCCGGTACCCGGACTTCACGTGGGCCGACCTGGCAAGC
TTCACGCAGCGCCACTA

***GA2oxa***
TGTGAGATCCTGGACCTCTTAGGAGAGGGGCTAGAGCTCAAAGATCCCAGATCATTCAGCAA
GCTTATCACAGACACTGACAGTGACTCCCTCCTGAGGATCAACCACTACCCTTCAGCTTGCAC
CATCCACAAGCTTGACCATGACGACCAATGCAAGATGAAGAGAGTTGTTCGCACCAAGGCTA
GCAATGGCGTGAGCCCAGCTGCAGGTGCACGGATCGGGTTCGGTGAGCACTCTGATCCGCGG
ATACTTAGCTTGCTCCGATCAAACG

***GA2oxb***
CATGGGGTGGGTCGAGTACCTCCTCCTCGGCGTCACCTCTGCCGGCACGCCATTGCCTGAGTC
CTCGGACGCGTCGTCCTCTTCGTTCCGTGAGCTTCTGAACGAGTACATTGCGGCGGTGAGAAG
GTTGACATGCACGGTCCTGGAGCTGATGGCGGAAGGGTTGGGCCTGGACGAGGACGTGTTCA
CCAGGTTGGTGCTGGACAAGGAAAGCGACTCGATGCTGAGGGTGAACCACTACCCGCCGCGC
CCCGAGCTGAAACAGCTCGGAGGGCACGGCAGGCTCACCGGATTCGGCGAGCACACTGACC
CGCAGATCATCTCCGTGCTCCGGTCCAACGACACCTCCGGGCTGGAGATCTCGCTCCGGGAC
GGCAGCTGGGTGTCGGTGCCGGCCGACCGGAACTCGTTCTTCGTCAACGTCGGCGACTC

***GAMyb***
TGGACTACGTCAAGAAGCACGGCGAGGGGAACTGGAACGCGGTGCAGAAGAACACGGGGTT
GTCCCGGTGCGGCAAGAGCTGCCGCCTCCGGTGGGCGAACCACCTCAGGCCCAACCTCAAGA

AGGGGGCCTTCACCCCGGAGGAGGAACGCCTCATCATCCAGCTCCACGCCAAGATGGGGAAC
AAGTGGGCGAGGATGGCTGCTCATTTGCCAGGGCGTACTGACAATGAAATAAAGAACTACTG
GAACACTCGAATAAAGAGATGTCAGCGAGCTGGCCTTCCTATATATCCTGCTAGTGTATGCA
ATCAATCGCCTGACGAAGATGAACAAGTCTCTGATGATTTTAACTGTGGCGAGAATCTGGCC
AGTGATTTTCTGAATGGAAACGGACTCATTTTACCAGATTTTACCAGTGAAAATTTCATCCCA
GATGCTTTATCTTACGCACCACAACTTTCAGCTGTTTCCATAAGCAACTTGCTAGGCCAGAGT
CTTGCATCAAGAAGTTGTAGCTTCATGGATCAGGTAGACCAAACAGGGGTTTTCAAACAATC
TGGTAGCGTGCTTCCTTCATTGAGTGATACAGTCGATGGTGTGCTTTCATCAGTGGATCAATT
TTCGAATGATTCTGATAAGCTCAAGCAGGCTCTAGGTTTTGATTATCTCAGTGAAGCCAATGC
TTGCAGCAAGGCTGTTGCACCTTTTGGTGTTGCACTTTCTGGCAGCCATGCCTTTTTAAATGGC
ACCTTCTCTGCTTCTAGGCCCATGAATGGTCCTCTGAAGATGGAGCTCCCTTCACTCCAAGAT
ACTGAATCTGATCCAAATAGCTGGCTCAAGTATACTGTGGCTCCTGCAATGCAGCCTACTGAG
TTAGTTGATCCTTACCTGCAGTCTCCAGCAGCGTCACCGTCAGTGAAATCTGAGTGTGCATCG
CCGAGGAACAGTGGTCTTTTGGAAGAGCTGCTTCATGAAGCTCAAGTACTAAGATCCGGGAA
GAACCAACACCTGTCTGTTCGAAGTTCAAGTTCCTCTGCTGGTACACCATGTGAGACTACTAC
GGCGGTTAGCCCAGAATTTGATATCTGTCAAGAATACTGGGAGGACCATCCCAATTCTTTCGT
CAATGGATACACTCCTTTTAGTGGAAATTCATTTACTGAATCCACTCCTCCTGTTAGTGCTGC
ATCACCTGATATCTTTCAGCTCTCCAAAATGTCTCCTGTGCAAAGCCCTTCAATGGGTTCTTGT
GATCATGTGACAGAACGTAAATACGAGTCTGGAGGTTCACCTCATCCTGAAAACTTGAGGC

***18s mRNA***

CTATTGGAGCTGGAATTACCGCGGCTGCTGGCACCAGACTTGCCCTCCAATGGATCCTCGTTA
AGGGATTTAGATTGTACTCATTCCAATTACCAGACACTAACGCGCCCGGTATTGTTATTTATT
GTCACTACCTCCCCGTGTCAGGATTGGGTAATTTGCGCGCCTGCTGCCTTCCTTGGATGTGGT
AGCCGTTTCTCAGGCTCCCTCTCCGGAATCGAACCCTAATTCTCCGTCACCCGTCACCACCAT
GGTAGGCCCCTATCCTACCATCGAAAGTTGATAGGGCAGAAA