REGULATION OF BRANCHING BY PHYTOCHROME AND

PHYTOHORMONES

A Dissertation

by

SRIRAMA R KRISHNAREDDY

May 2011

Major Subject: Agronomy

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Approved by:

Chair of Committee,	Scott Finlayson
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ABSTRACT

Regulation of Branching by Phytochrome and Phytohormones.

(May 2011)

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Chair of Advisory Committee: Dr. Scott A. Finlayson

Light is the fundamental source of energy and information throughout the plant life cycle. Light signals regulate plant architecture and branching, key processes that determine biomass production and grain yield. Low red (R) to far-red (FR) light ratios (R:FR) perceived by phytochromes serve as a warning signal about impending competition for light resources and lead to shade avoidance responses (SARs), including reduced branching. The R:FR regulates branching in both a bud autonomous and non-bud autonomous manner, however a detailed mechanistic understanding of the process remains unclear. We hypothesized that high R:FR promotes bud outgrowth by differentially regulating branching-related genes (transcriptome) within the axillary bud and that increased apical dominance under low R:FR or with phyB deficiency is mediated by auxin or other novel signal/s. We analyzed the branching phenotype of Arabidopsis Columbia-60000 ecotype in response to different R:FR treatments and conducted a microarray study to identify early (within 3 hours) changes in the transcriptome of buds from different rosette positions in response to altered R:FR.

Physiological experiments were also conducted to determine if auxin concentration, transport rate, sensitivity, and establishment of an auxin transport stream were important in determining the branching phenotype of shade avoiding plants.

The results revealed that the duration of low R:FR determines plant architecture and the branching phenotype and that bud outgrowth is regulated by the R:FR in a spatial and temporal manner. Low R:FR promoted the elongation of branches at top rosette nodes while it suppressed the outgrowth of axillary buds at lower nodes. High R:FR could reverse the effects of previous low R:FR by promoting the outgrowth of buds from lower axils within 24 hours of treatment. Transcriptomic analysis revealed that the R:FR differentially regulated the expression of genes related to hormone biosynthesis/transport/signaling, cell-cycle regulation and cell wall modification. Cis-elements responsive to light and hormone signaling pathways were overrepresented in several gene clusters. Apical dominance related studies discovered that loss of phyB function results in a slower auxin transport rate, fewer xylem parenchyma cells, and reduced sensitivity to auxin. These results, in addition to estimates of correlative inhibition, suggested that auxin is at least partially responsible for increased apical dominance under low R:FR or with phyB deficiency, but may be acting in conjunction with other undefined regulators.

DEDICATION

This dissertation is dedicated to Dr. B.A. Stewart for triggering my thought process to work on branching.

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This is one the proudest moments in my life. Several people have contributed towards my success and this dissertation; I take this opportunity to thank them.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Branching is one of the key agronomic traits that have been selected during the process of domestication of wild cultivars by early plant breeders. Since then, improved agronomic practices and advanced breeding techniques have shaped plant architecture to produce better yields (Duvick, 2001, 2005). Over the last century, the increase in total food production was not only due to better cultivars and improved agronomic practices but also came at the expense of ecological imbalance. Nearly 5 million hectares of forest land were converted into arable land to meet the global demand food, fodder, fiber and recently, fuel (Pachauri, 2008). With incessant growth in human population and persistent dependence on agricultural commodities, limitations on the availability of cultivable land pose a great challenge to increase food production. One of the several ways to achieve increased food production is to alter the plant architecture in accordance with environment and resource availability. The potential to alter the branching habit, the key determinant of plant architecture, presents us with a great opportunity to design modern cultivars adaptable to variable climatic conditions and produce better yields.

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This dissertation follows the style and format of Plant Physiology.

The branching phenotype is known to depend on intrinsic factors like phytohormones, and the genetic makeup of the plant in addition to being influenced by external cues such as light, temperature, water, and nutrients (Phillips, 1975; Deregibus et al., 1983; Casal et al., 1986; Cline, 1991; Li and Bangerth, 2003). Among these regulators, the effects of hormones and light quality on branching have been extensively studied for several decades, but a comprehensive understanding of the branching process at the molecular level has not yet been achieved. Earlier studies have shown that light and hormone signals influence branching by acting through independent pathways, though there may be crosstalk between their signaling pathways. This study was designed to investigate in detail the role of phytohormones and phytochromes (R:FR) in regulating branch outgrowth using molecular and transcriptomic approaches to discover the key genes and cis-regulatory regions involved in the process.

1.1 Dissecting the process of shoot branching

Shoot branches arise in the axils of leaves adjacent to the main stem as a result of the activity of axillary meristems (AM). The branching potential of a plant is determined by the formation/initiation of axillary buds and their subsequent outgrowth (McSteen and Leyser, 2005; Schmitz and Theres, 2005). In many cases it appears that the axillary meristem is derived from a small population of meristematic cells that detach from the shoot apical meristem (SAM) at approximately the time of formation of the leaf primordium. The situation in Arabidopsis is less clear since axillary meristems are not

obvious until the floral transition, which can occur long after leaf/leaf axil formation. However, the presence of meristematic marker genes in the axils soon after leaf formation suggests that cryptic axillary meristems, likely derived from the SAM, are produced in concert with the leaf.

In eudicots axillary meristem formation is dependent on meristem identity genes such as, *REVOLUTA (REV)*, *LATERAL SUPPRESSOR (LS)*, *LAS* (ortholog of *LS* in *Arabidopsis*), while *MONOCULM1 (MOC1)* and *UNICULM2 (CUL2)* are known to contribute to this process in monocots (Wang and Li, 2008) and *SHOOTMERISTEMLESS (STM)* is necessary for AM formation in both eudicots and monocots (Ward and Leyser, 2004; McSteen and Leyser, 2005). Loss of function of these genes results in reduced branching/tillering due to the failure to initiate and maintain axillary meristems. When these genes were over expressed, plants produced more branches (Shimizu-Sato and Mori, 2001; Greb et al., 2003). The *LS*/*LAS*/*MOC1* genes are known to encode transcription factors belonging to the GRAS family that function in the initiation and development of axillary meristems (Shimizu-Sato and Mori, 2001; Greb et al., 2003).

Upon initiation, the axillary meristem can differentiate and develop into a branch or remain dormant until favorable conditions for outgrowth occur (Burg and Burg, 1968; McSteen and Leyser, 2005). Bud dormancy is dictated by several factors including; environment (ecodormancy), where changing environmental cues can release bud arrest;

the effects of distal plant organs (paradormancy), as in apical dominance where the SAM regulates axillary bud outgrowth; and factors within the bud (endodormancy), such as hormones and other signaling components regulating bud outgrowth in association with environmental signals (Tucker, 1975). The transition from dormancy to outgrowth is a complex process resulting from interactions between genotype, hormones, and environmental cues. Despite the fact that dormancy dictates key aspects of plant growth and development such as branching, the physiological and molecular mechanisms are poorly understood because of inherent complexity and overlapping pathways. The following review of the literature is an effort to summarize the roles of various signaling compounds in determining the branching phenotype.

1.2 The role of hormones in branching

The role of auxin in branching has been investigated since its discovery in the early 1930's. It was initially believed that apically-derived auxin directly regulated bud outgrowth (Thimann and Skoog, 1933; Phillips, 1975; Cline, 1991). However, later studies showed that apically-derived auxin does not enter the axillary buds to inhibit bud outgrowth (Morris, 1977). While investigating the role of auxin in correlative inhibition, it was proposed that auxin inhibits bud outgrowth through unknown second messenger(s) (Snow, 1937). Since then, the search for the elusive second messenger has suggested several key players including cytokinins (Sachs and Thimann, 1967), ethylene (Burg and Burg, 1968), abscisic acid (Tucker, 1975), gibberellic acid (Phillips, 1975)

(Isbell and Morgan, 1982), and more recently a strigolactone-derived hormone (Brewer et al., 2009; Waldie et al., 2010). Inspite of the research efforts made and the various hypotheses put forth, the possibility of other novel second messengers to auxin in mediating apical dominance, especially under competitive environmental conditions cannot be ruled out. A detailed understanding of the roles of each hormone in mediating plant architecture would provide a starting point to conduct further research into novel regulators of plant branching.

1.2.1 Auxin

Classical plant physiology studies have demonstrated that apically synthesized auxin is transported basipetally and results in suppression of axillary bud outgrowth in a process called *apical dominance* (Skoog and Thimann, 1934; Thimann, 1937). Decapitation studies showed that removing the growing tip resulted in promotion of the outgrowth of axillary shoots (due to depletion of the basipetal auxin stream) and apical dominance was restored by auxin application to the tip (Skoog and Thimann, 1934; Thimann, 1937). The effects of apical auxin appear to be indirect since auxin levels have actually been shown to increase in *Phaseolus* axillary buds within 24 hours following decapitation (Hillman et al., 1977; Gocal et al., 1991). Experiments in pea (*Pisum sativum* L.) also showed that auxin exported from the SAM travels basipetally but is not translocated into axillary buds to inhibit the outgrowth (Morris, 1977; Morris and Johnson, 1990). Later studies in Arabidopsis mutants resistant to auxin (*axr-1*) showed that the reduced auxin

sensitivity resulted in an abnormal phenotype with decreased hypocotyl elongation, plant height, and apical dominance confirming the role of auxin in suppressing branching (Estelle and Somerville, 1987; Lincoln et al., 1990). A reduction in auxin biosynthesis in Arabidopsis (via mutation in the *YUCCA* pathway) (Cheng et al., 2006) and auxin transport in rice (mutation in *OsPIN1* gene) also resulted in increased branching confirming a pivotal role for auxin in determining plant architecture (Xu et al., 2005).

Auxin has long been known to play an important role in plant tropic responses (to light and gravity) by influencing cell division, elongation, differentiation and tissue patterning. The relationship between light and auxin was established long ago, reports suggest that increased stem elongation in response to FR light correlates with increased auxin levels (Fletcher and Zalik, 1964). Later investigations showed that auxin transport (Furuya et al., 1969) and biosynthesis (Iino, 1982a, 1982b) mediate increased stem elongation under FR light (Behringer and Davies, 1992). The observation that the expression of the *ATHB-2* and *ATHB-4* HD-ZIP encoding genes increased under low R:FR (Carabelli et al., 1993) provided a basis to connect auxin and shade via these transcriptional regulators (Steindler et al., 1999). Recent studies in Arabidopsis showed that shade environments induce auxin biosynthesis via a novel tryptophan-dependent pathway (Tao et al., 2008).

1.2.2 Cytokinin

Cytokinins are known to influence plant growth and morphogenesis through cell cycle regulation, especially in the development of the SAM and leaf primordia (Werner et al., 2001). Cytokinin is required for the formation of leaf cells and to induce and maintain shoot meristems (Werner et al., 2001) and their level of abundance is regulated by auxin (Li et al., 1995; Nordstrom et al., 2004). Several recent studies also shed light on the relationship between shade and cytokinins. Low R:FR regulated reduction in leaf numbers has been shown to be mediated by auxin induction of a cytokinin oxidase (*AtCKX6*) gene, resulting in cytokinin breakdown in veins of developing leaf primordia (Ciarbelli et al., 2008). The role of cytokinins in bud outgrowth is both independent of, and associated with, auxin. Cytokinins help to promote bud outgrowth when applied directly to dormant buds and higher concentrations of cytokinins were detected in outgrowing buds (Chatfield et al., 2000; Shimizu-Sato and Mori, 2001). It has been suggested that the CK:IAA (cytokinin to auxin) ratio is crucial in determining the fate of the bud, since buds elongated when the CK:IAA ratio was high and were suppressed when it was lower (Emery et al., 1998). Other work has demonstrated that basipetally-transported auxin supplied from shoot tips inhibited the expression of a cytokinin biosynthetic gene (*IPT*) at the stem nodal region, and resulted in decreased cytokinin concentration (Tanaka et al., 2006). The authors also suggested that decapitation increases the abundance of cytokinins in the stem, which may then be translocated to axillary buds to promote outgrowth.

1.2.3 Ethylene

The potential role of ethylene in apical dominance and branching should not be overlooked, especially under low R:FR conditions, since auxin is known to induce ethylene production. A conflicting evidence concerning the relationship between auxin and ethylene in the control of branching has been noted (Cline, 1991). Although it has been reported that ethylene is not involved in auxin-meditated inhibition of bud outgrowth (Chatfield et al., 2000), ethylene has been shown to play a crucial role in neighbor detection and shade avoidance responses. Higher ethylene production was documented in sorghum (*Sorghum bicolor* L. Moench) *phyB* mutants and was attributed to increased ACC oxidase activity (Finlayson et al., 1998; Finlayson et al., 1999). Higher ethylene production under low R:FR resulted in increased stem and petiole length in tobacco (Pierik et al., 2004) and decreased leaf blade:leaf sheath elongation in sorghum (Finlayson et al., 2007).

1.2.4 Abscisic acid

Abscisic acid (ABA) is involved in the suppression of growth and its role in maintaining bud dormancy has been well documented, with studies showing that dormant buds contain higher ABA concentrations compared to elongating buds (Cline, 1991). Early studies (Tucker, 1975, 1976a, 1976b, 1976c, 1977, 1978) suggested a direct role of ABA in mediating apical dominance and showed that ABA content under low R:FR increased

in the axillary buds of *Xanthium* and tomato (Tucker and Mansfiel, 1972). Other studies

have suggested that there is cross-talk between auxin and ABA. Stem auxin levels

(Eliasson, 1975), and basipetal auxin transport were associated with high levels of ABA

in the axillary buds. ABA concentrations in the buds of *Lupinus angustifolius* L. were

shown to be inversely related to CK:IAA with ABA potentially acting as a secondary

inhibiter of bud outgrowth (Emery et al., 1998). In split plate experiments using excised

nodal sections from *Arabidopsis*, it was demonstrated that inhibition of bud outgrowth

by ABA was related to the auxin supply/source (Chatfield et al., 2000). A basal source

of ABA was shown to increase auxin-mediated bud suppression whereas an apical

source of ABA decreased the effect. These conclusions were further supported by a

study that showed that acropetal movement of ABA is involved in suppression of bud

outgrowth in association with auxin (Cline and Oh, 2006). Though several lines of

evidence suggest a key role for ABA in mediating auxin and low R:FR regulated apical

dominance, the molecular events underling the process remain obscure.

1.2.5 Gibberellic acid

The interaction between GA and light signaling in the processes of germination,

elongation, and flowering has been extensively reviewed (Halliday and Fankhauser,

2003). Although increased apical dominance of plants grown in shaded environment

compels one to suspect that gibberellins (GA) may play a critical role, little supporting

evidence is available. Research addressing the role of GA in correlative inhibition and

apical dominance and reported that no evidence supports the direct involvement of GA

in suppressing bud outgrowth (Phillips, 1975; Cline, 1991). However, the role of GA

has been associated with promoting bud outgrowth following the release of apical

dominance (Cline, 1991). However, similarities in the phenotypes of plants grown under

low R:FR (or with *phyB* deficiency) with plants treated with exogenous GA demands

more evidence at the molecular level to rule out the possible involvement of GA in the

mediation of apical dominance.

1.2.6 Strigolactone

Studies initiated in pea (*Pisum sativum*) have shed light on the function of a novel long

range signaling compound involved in suppressing axillary bud outgrowth (Beveridge,

2000, 2006). The authors concluded that a graft transmissible signal, which is likely

directly involved in bud outgrowth, is produced in both roots and shoots through the

activity of RAMOSUS1 and 2 (RMS1 and RMS2). The graft transmissible long range

signaling compound has been suggested to move acropetally in the shoots to suppress

branch outgrowth, acting as a second messenger to auxin (Foo et al., 2001). The authors

showed that branching in *rms1* can be decreased by grafting WT root stock to *rms1*

scion, demonstrating the transmissible role of *RMS1* in suppressing branching. Later, it

was reported that the novel hormone-like signal is in turn regulated by auxin dependent

and auxin independent pathways (Foo et al., 2005). Studies in Arabidopsis showed that

MORE AXILLARY BRANCHING4 (*MAX4*), an ortholog of pea *RMS1* encoding an

enzyme with homology to carotenoid cleavage dioxygenases, was likely involved in the synthesis of an uncharacterized mobile branch repressing hormone that acts downstream of auxin (Sorefan et al., 2003). Several other studies reported that mutations in *MAX1*, *MAX2*, and *MAX3* also resulted in increased lateral branching (Stirnberg et al., 2002; Booker et al., 2004). *MAX2* is shown to encode a leucine-rich repeat F-box protein, a group that is known to function in ubiquitin mediated degradation of targeted proteins (Stirnberg et al., 2002). Mutations in *MAX1*, *MAX3*, and *MAX4* genes of Arabidopsis resulted in increased branching similar to *RMS1, RMS2* and *RMS5* found in pea, and *DECREASED APICAL DOMINANCE1* (*DAD1*) of petunia (Napoli, 1996; Turnbull et al., 2002; Sorefan et al., 2003; Booker et al., 2004). Recently the hormone was identified as a strigolactone or strigolactone derivative (Gomez-Roldan et al., 2008; Umehara et al., 2008). Strigolactones are terpenoid lactones that are exuded by roots and help in symbiotic relationships with arbuscular mycorrhizal fungi.

Increased branching in the *max* mutants is shown to be associated with increased auxin transport in the stem resulting from an increased level of PIN proteins (Bennett et al., 2006). The authors also suggested that increased expression of *PIN* genes in the stem facilitates auxin movement out of the buds into the stem which acts as a sink. Conversely, some recent studies have shown that strigolactones act downstream of the auxin signaling pathway in regulating bud outgrowth (Brewer et al., 2009). Also recent studies suggest that auxin and the strigolactone derived hormone are components of a dynamic feedback loop that regulates branch outgrowth (Hayward et al., 2009). The

authors demonstrated that the feedback regulation involving auxin is via the AXR1/TIR1 pathway and that increased amounts of strigolactone resulted in suppression of auxin and another unknown feedback signal (Beveridge and Kyozuka, 2010). The interaction of ABA, which is also a carotenoid derived hormone involved in the suppression of branching, with the novel MAX-related hormone, remains uncertain.

1.3 Role of light/shade/R:FR signals

Plants acquire information about their environments from various sources, including signals transmitted by neighboring plants that lower the R:FR. Low R:FR warns plants about impending competition for resources, including light, and helps alter the developmental program accordingly. Plants are equipped with at least three types of photoreceptors; red (R) and far-red (FR) absorbing *phytochromes*, UV-A/blue light absorbing *cryptochromes*, and *phototropins*. Phototropins (PHOT) and cryptochromes (CRY) are flavoproteins involved in the perception of blue light and blue light-mediated signaling pathways (Schafer and Bowler, 2002; Gyula et al., 2003). Phytochromes may be the most extensively studied photoreceptors due to their role in modulating plant growth and development at all stages of the plant life cycle. Phytochromes absorb light in the R and FR regions of the spectrum and are encoded by a small gene family containing five members in Arabidopsis, *PHYA* through *PHYE*, with each member existing in two photointerconvertible forms, Pr and Pfr. Reduced R:FR due to the proximity of neighbors is sensed by the phytochrome system, with *phyB* playing a major

role (Ballare et al., 1990; Ballare and Scopel, 1997). Loss of phyB function results in a constitutive shade avoidance phenotype where the plants have elongated internodes, fewer branches, and flower early (Reed et al., 1993).

The architectural differences between maize and its progenitor teosinte were shown to be due to a limited number of quantitative trait loci (QTL) – including the *TB1* locus (Doebley et al., 1995). TB1 belongs to the TCP domain protein family that is believed to regulate the cell cycle through transcriptional regulation (Cubas et al., 1999). It was demonstrated that the *TB1* gene in maize is responsible for suppressing lateral meristems from developing into branches (Doebley et al., 1997). *tb1* mutants showed an increased number of branches due to a release of dormancy resulting in the elongation of previously initiated buds (Hubbard et al., 2002). The expression of *TB1* was shown in the axil, axillary meristem, and in branches and coincided with suppression of shoot branching. A recent study found that an orthologous gene, *SbTB1*, is expressed at high levels in the axillary buds of sorghum (Kebrom et al., 2006). In eudicots, the homologous genes *BRC1/TBL1* and *BRC2* were identified in unelongated axillary buds of Arabidopsis and were shown to be involved in regulating axillary bud development (Aguilar-Martinez et al., 2007; Finlayson et al., 2007).

In monocots, upon sensing light, phyB is believed to regulate branching in combination with the branching related gene *TEOSINTE BRANCHED1* (*TB1*). The expression of *TB1* has been shown to depend on environmental conditions, especially light quality.

When WT sorghum plants were grown under the influence of supplemental far red light or high density planting to simulate a shaded environment, axillary buds were initiated but remained arrested, as were the buds of *phyB-1* (Kebrom et al., 2006). The suppressed branching was associated with increased expression of *SbTB1* (suppressor of branching) and *SbDRM1* (a marker for dormancy). Recent studies showed that reduced branching in Arabidopsis *phyB-9* is due to a reduction in the bud outgrowth capacity and that BRC1 and BRC2 fall into two different gene networks where BRC2 is part of R:FR signaling pathway (Finlayson et al., 2010). The authors also demonstrated that mutation in *phyB* resulted in an increased amount of correlative inhibition suggesting the possibility of involvement of an auxin mediated pathway. R:FR regulation of the branch outgrowth process has not been studied in detail at the molecular level. Transcriptomic approaches to be used in the present study are expected to identify novel genes and transcription factor binding sites mediating bud outgrowth in response to altered R:FR and bud position. In addition to microarray studies, hormonal regulation of branching in *phyB* will be investigated to understand cross talk between light and hormonal pathways in mediating shade induced apical dominance.

CHAPTER II

RATIONALE, OBJECTIVES, AND HYPOTHESES

2.1 Rationale

The yields of any crop vary under different environmental conditions, resource availability, genotype and agronomic practices. Together, all these factors determine yield, grain quality, biomass production and other aspects of plant growth by altering resource mobilization and plant architecture/branching under given conditions. In order to achieve better yields one needs to have a comprehensive understanding of factors regulating branching (Sawers et al., 2005; Devlin et al., 2007; Kebrom and Brutnell, 2007). Increased demand for food, fodder, and fuel has put enormous pressure on agriculture to increase food production and mobilize agricultural produce to meet global demands. Since the branching potential is crucial in determining crop biomass production and yield, efforts to understand the branching process could have significant ramifications on future food, fodder, and fuel production. Understanding branching responses to light signals at the gene level using transcriptome analysis is expected to help identify candidate genes involved in determining plant architecture. It is also critical to understand the involvement of hormones and related genes in promoting or repressing bud outgrowth. The results of this study can be extended to improve biomass and grain yields by regulating plant architecture through controlling the number of branches/tillers in agronomically important crops.

2.2 Objectives and hypotheses

The overall objectives of my dissertation research are to:

1. Characterize the plant architectural parameters and bud outgrowth kinetics in responses to altered R:FR (CHAPTER III). Quantify and compare the differences in the transcriptome of unelongated axillary buds under low and high R:FR conditions and describe the role of candidate genes and/or cis-elements in promotion and/or suppression of bud outgrowth (CHAPTER IV).

> *Hypothesis*: High R:FR promotes bud outgrowth as a result of differential expression of branching-related genes, whereas low R:FR suppresses bud outgrowth by likewise modulating specific genes.

2. Seek physiological and molecular explanations for 'apical dominance' and 'apical control' related phenotypes in *phyB* mutant Arabidopsis and evaluate the role of different phytohormones and related genes in regulating axillary bud outgrowth (CHAPTER V).

> *Hypothesis:* Auxin dictates the fate of the bud without entering the bud, possibly through a second messenger. The auxin transport capacity through the PAT stream and or transportability/exportability of auxin into the PAT by the axillary bud dictates bud outgrowth.

3. Integrate the results from above studies to address the crosstalk between light and

hormone regulated pathways in determining the branching habit (CHAPTER V and VI).

    Hypothesis: R:FR alters hormone (specifically, auxin) concentration, transport,

and/or sensitivity to influence branching via changes in gene expression.

CHAPTER III

ARABIDOPSIS ARCHITECTURAL RESPONSES TO ALTERED R:FR

3.1 Introduction

The sessile growth habit of plants necessitates that they adapt to environmental conditions, since they cannot escape them. Plants are able to alter their phenotype to improve fitness in response to environmental cues including light. Light has profound effects on plant development from seed germination to flowering. The early developmental changes in response to light sensing are termed photomorphogenesis, a process whereby plants begin to de-etiolate and assemble the photosynthetic apparatus to enable autotrophy. Specialized photoreceptor molecules sense light quality, quantity, and duration to mediate growth responses. Under densely populated agricultural environments, selective absorption of red light (R, 660 nm) and increased reflection of far-red light (FR, 730 nm) from neighboring vegetation results in a reduced R:FR (Ballare et al., 1987; Ballare et al., 1990, 1997). The reduction in the R:FR or increased amount of 'shade signals' elicits a suite of developmental events termed shade avoidance responses (SARs), including increased stem elongation, reduced leaf and branch number, reduced leaf angle, early flowering, etc., (Smith and Whitelam, 1997; Franklin and Whitelam, 2005). Among these SARs, the reduction in branching is a key developmental program since numbers of branches determine plant architecture, resource allocation, biomass production, and grain yield. Constitutive shade signals in

densely planted agricultural environments present a serious challenge to crop production, since shade avoiding plants may undergo increased stem elongation at the expense of the number of harvestable organs/branches, a result of increased apical dominance (Ballare et al., 1997). Though SARs have been studied for several decades, our understanding of the molecular mechanisms resulting in reduced branching under shade environments is still incomplete (Smith, 2000).

Branching (or tillering in monocots), generally follows a three step developmental process; 1) initiation of an axillary meristem, 2) formation of an axillary bud and, 3) outgrowth of the axillary bud to form a branch. Once an axillary bud is formed it may enter a phase of extended dormancy, it may begin to grow immediately, or it may enter a phase of temporary dormancy and begin to grow after an indeterminate delay (Shimizu-Sato and Mori, 2001). Several studies have described genes responsible for the initiation of axillary meristems and formation of axillary buds in the leaf axil and highlighted the importance of the outgrowth process in agricultural environments (McSteen and Leyser, 2005; Wang and Li, 2008). The genetic makeup of the plant is a critical factor in determining the branching habit as demonstrated using the *Teosinte branched1* (*TB1*) gene in maize, rice, sorghum (Doebley et al., 1995; Takeda et al., 2003; Kebrom et al., 2006), and an orthologous gene *BRANCHED1* (*BRC1*) in Arabidopsis (Aguilar-Martinez et al., 2007; Finlayson, 2007). In addition to the genotype, endogenous cues like hormones (auxin, cytokinin, strigolactone, etc.,), and external cues such as water,

nutrients, light quality (R:FR) and day length regulate axillary bud outgrowth (McSteen, 2009; Waldie et al., 2010).

The specialized photoreceptor molecule phytochrome (PHY) exists in two photo-interconvertible forms, a biologically inactive Pr (red light absorbing) form and a biologically active Pfr (far-red light absorbing) form (Smith, 2000). Under continuous FR and/or dark conditions phytochrome exists in the Pr form. Upon sensing R, the Pr form is converted into the Pfr form and is translocated into nucleus, a process that can be reversed by FR treatment or continued exposure to dark (Sakamoto and Nagatani, 1996; Nagatani, 2004). Since phyA is photolabile, its abundance is greatly reduced under high R:FR (Pr A is converted to Pfr A and targeted for degradation), while the active form of phyB (Pfr B) and a small portion of PfrA are translocated into the nucleus to interact with PHYTOCHROME INTERACTING FACTORs (PIFs) to suppress shade avoidance (Franklin, 2008). Under low R:FR conditions, the amount of active Pfr B is reduced (converted to inactive Pr B) resulting in increased transcription of genes promoting shade avoidance (Franklin, 2008). The roles of PHYs and PIFs in mediating SARs have been extensively studied to describe events like seed germination, de-etiolation, stem elongation, flowering etc. PHYs have been shown to serve as a bridge between external cues (light/shade) and endogenous signals (hormones) and hardwired genetic components to modulate axillary bud outgrowth (Finlayson et al., 2010). Hence it is important to target phytochrome signaling pathways to gain a deeper understanding of

phenotypic plasticity and targets for selection and manipulation to produce crop plants with better agronomic performance (Smith, 2000; Sawers et al., 2005).

The significance of high density or low R:FR on tillering has previously been reported in several studies (Deregibus et al., 1985; Casal et al., 1986, 1987; Pigliucci and Schmitt, 1999). Recently, studies from our lab characterized genes within the axillary bud that are regulated by phytochrome or R:FR signals to influence branching (Kebrom et al., 2006; Finlayson et al., 2010). These studies formed the basis for investigations on the effects of R:FR on the bud transcriptome and shaping of plant architecture. As a first step leading to microarray studies, we were interested to describe the effects of R:FR on plant architecture and identify the best approach to study the transcriptomic differences due to altered R:FR. The objectives were to treat Arabidopsis seedlings with different durations of R:FR to test the hypothesis that length of low R:FR treatment determines branching phenotype, where prolonged exposure to low R:FR would result in fewer branches. The architectural analysis and branch outgrowth kinetics showed that moving plants to low R:FR at 1 DAP resulted in promotion of the outgrowth of branches at upper nodes (n), while greatly suppressing the bud outgrowth at lower nodes (n-2 and below).

3.2 Results

3.2.1 Duration of exposure to R:FR determines plant architecture

In Arabidopsis grown under long days, axillary meristems are not obvious until the plants make a transition from vegetative to reproductive growth and bud outgrowth occurs after bolting/anthesis. Buds first begin to grow out from the topmost position, followed by those at sequentially lower nodes. Plants grown under low R:FR have an accelerated developmental program, making the floral transition and achieving bolting/anthesis earlier than those grown under high R:FR. In our quest to understand the key regulators of branch outgrowth under varying light quality, we first analyzed the plant architectural parameters and branch outgrowth kinetics in response to R:FR. Since the R:FR under canopy shade can be below 0.1 (can be as low as 0.05 to 0.07), we choose to study the effects of R:FR on plant architecture at R:FR 0.08 (±0.05) (Smith, 1982).

Visual phenotypes of plants at 10 days post anthesis (DPA), after being exposed to either high R:FR (3.5) or different durations of low R:FR, are presented in Figure 3.1. Plants exposed to low R:FR from very early in development, one day after planting (DAP), had significantly fewer leaves compared to plants moved at later stages (Figure 3.2). Exposure to low R:FR from 1 DAP resulted in fewer branches, while a delay in exposure to low R:FR or continued growth in high R:FR resulted in increased branching (Figure

Figure 3.1 Visual phenotypes of plants grown under variable duration of low R:FR. Representatives of the plants moved to A. low R:FR (0.08) at 1 day after planting (DAP); B. low R:FR at 7 DAP; C. low R:FR on 14 DAP; and D. plants grown only under high R:FR (3.5). The image was captured on 10 days post anthesis.
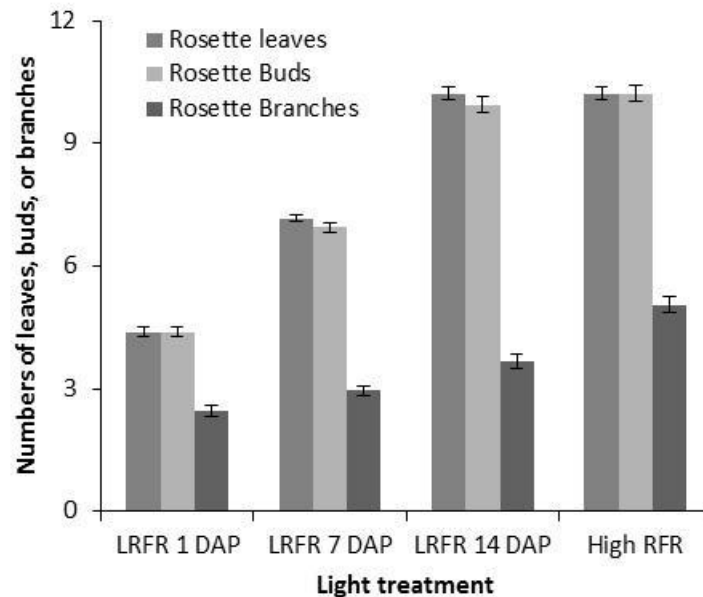
Figure 3.2 Number of rosette leaves, buds, and branches in response to variable duration of exposure to low R:FR. These architectural parameters were measured at 10 DPA across all treatments. Error bars represent standard error, n=18. HRFR – high R:FR (3.5); LRFR – low R:FR (0.08); DAP – days after planting; LRFR 14 DAP – plants moved from high R:FR to low R:FR on 14 days after planting; LRFR 7 DAP – plants moved to low R:FR 7 DAP; LRFR 1DAP – plants moved to low R:FR at 1 DAP.

3.2). The longer the duration of exposure to low R:FR (0.08), the greater the reduction in leaf and branch numbers. Since leaf and branch numbers are directly related, the effect of light conditions were also quantified using a standardization technique as previously described (Finlayson et al., 2010). In a comparison between the plants grown continuously under high R:FR and the plants moved to low R:FR on 14DAP; the leaf numbers were similar in both treatments, while the plants grown continuously under high R:FR had 1.4 additional branches (Figure 3.2).

Numbers of secondary leaves initiated on rosette branches also varied in response to variable duration of R:FR treatment (Figure 3.3). Plants moved to low R:FR on 1 DAP and 7 DAP had fewer secondary leaves and branches compared to the plants allowed to grow under high R:FR for 14 DAP or throughout the experimental duration (Figure 3.3). Similar trends were seen in cauline branch leaf numbers supporting the role of low R:FR in the suppression of leaf production (Figure 3.4). Other parameters such as height, and the lengths of cauline and rosette branches demonstrated that the duration of exposure to shade signals determines the overall plant architecture (Figure 3.5). Since cauline branches show a different developmental progression and appear to be regulated by internal physiological conditions rather than external cues, they are not discussed in greater detail.

3.2.2 Bud outgrowth at different positions is programmed by different signals

Branch outgrowth kinetics in response to varying durations of exposure to low R:FR are presented in Figure 3.6. The topmost rosette bud (n) grew out irrespective of light conditions, although differences were found in the onset of outgrowth and rate of outgrowth. The outgrowth of buds at lower positions (n-2 and below) was found to be strongly influenced by the R:FR signal (Figure 3.6). Buds at position n-2 of plants grown under low R:FR from 1 and 7 DAP were suppressed, while those of plants continuously grown under high R:FR grew into branches (Figure 3.6). Interestingly,
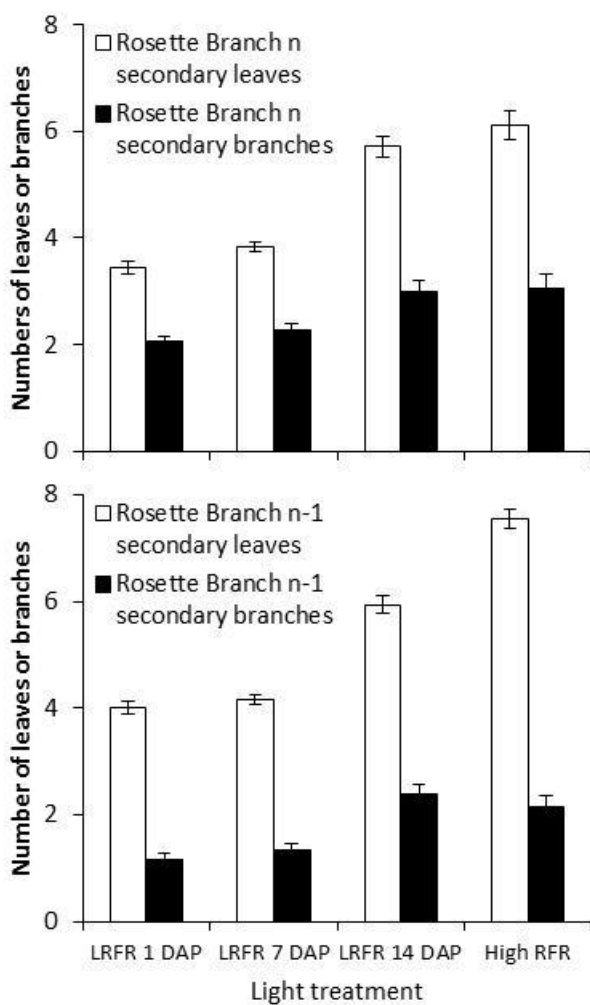
Figure 3.3 Numbers of secondary leaves and secondary branches on rosette branch n and n-1 in response to variable duration of exposure to low R:FR. These architectural parameters were measured at 10 DPA across all treatments. Error bars represent standard error, n=18. HRFR – high R:FR (3.5); LRFR – low R:FR (0.08); DAP – days after planting; LRFR 14 DAP – plants moved from high R:FR to low R:FR on 14 days after planting; LRFR 7 DAP – plants moved to low R:FR 7 DAP; LRFR 1DAP – plants moved to low R:FR at 1 DAP.
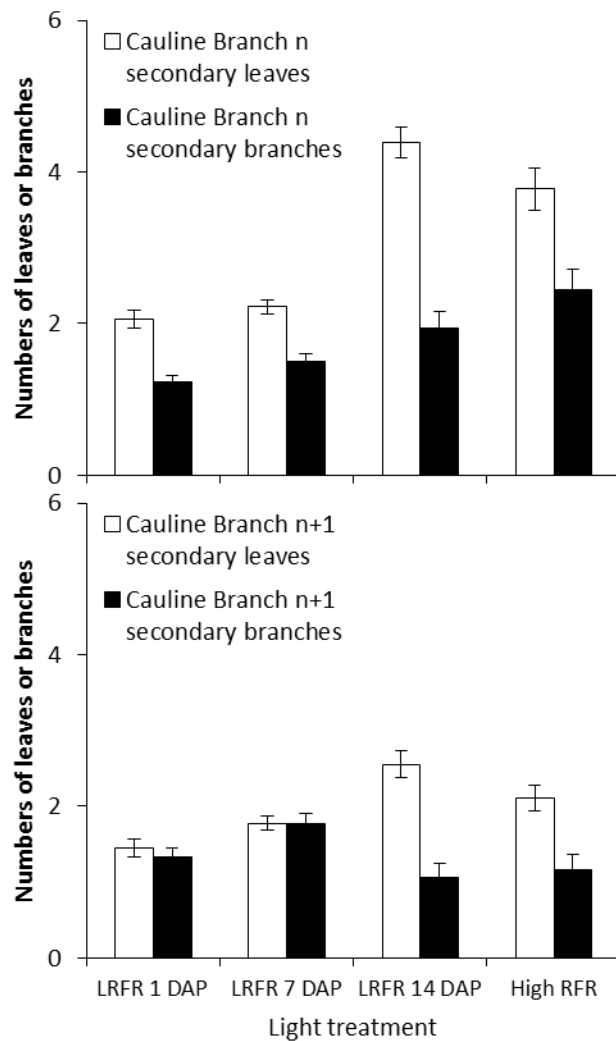
Figure 3.4 Numbers of secondary leaves and secondary branches on cauline branch n and n+1 in response to variable duration of exposure to low R:FR. These architectural parameters were measured at 10 DPA across all treatments. Error bars represent standard error, n=18. HRFR – high R:FR (3.5); LRFR – low R:FR (0.08); DAP – days after planting; LRFR 14 DAP – plants moved from high R:FR to low R:FR on 14 days after planting; LRFR 7 DAP – plants moved to low R:FR 7 DAP; LRFR 1DAP – plants moved to low R:FR at 1 DAP.
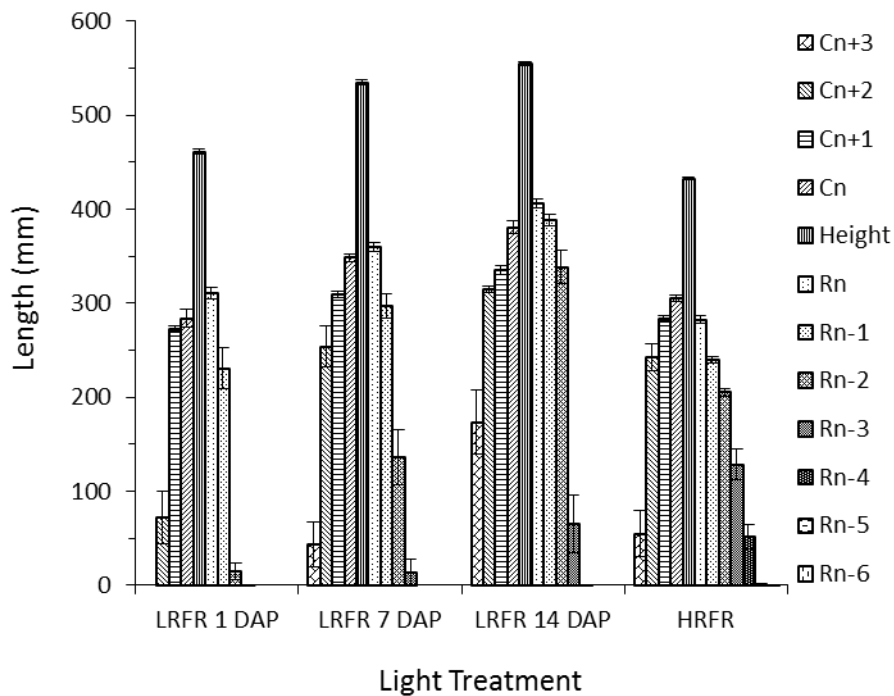
Figure 3.5 Plant height, lengths of rosette and cauline branches in response to variable duration of exposure to low R:FR. These architectural parameters were measured at 10 DPA across all treatments. Error bars represent standard error, n = 18. HRFR – high R:FR; LRFR – low R:FR; DAP – days after planting; LRFR 14 DAP – plants moved from high R:FR to low R:FR on 14 days after planting; LRFR 7 DAP – plants moved to low R:FR 7 DAP; LRFR 1DAP – plants moved to low R:FR at 1 DAP; Height – plant height measuring from rosette to tip of shoot apical meristem; Rn – length of rosette branch n, branch appearing at first rosette leaf; Rn-1 – length of rosette branch below branch n and so on; Cn – Length of cauline branch n, this branch appears from cauline leaf located close to the rosette; cn+1 – length of cauline branch above the Cn and so on.
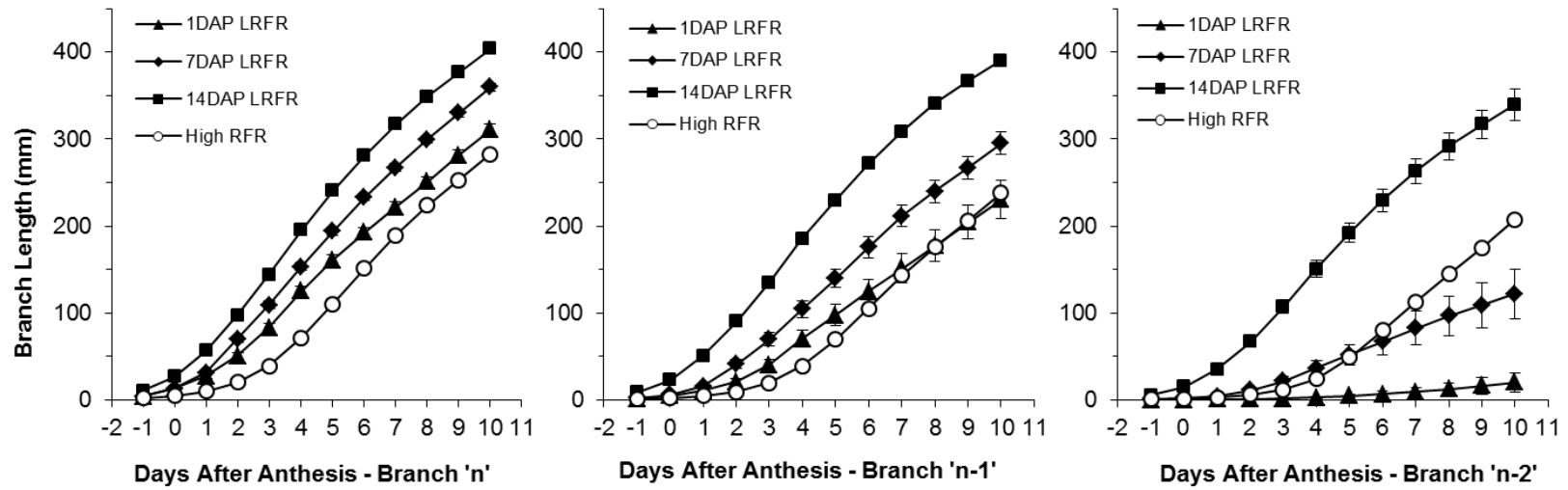
Figure 3.6 Branch outgrowth kinetics of top three rosette buds of plants exposed to low R:FR for different durations. Bud outgrowth was measured from one day before anthesis to 10 days post anthesis. Error bars are standard error, n=18. LRFR – low R:FR (0.08); DAP – days after anthesis; LRFR 14 DAP – plants moved from high R:FR to low R:FR on 14 days after planting and so on; n – top most rosette branch; n-1 – branch below n; and n-2 – branch below n-1.

plants moved to low R:FR on 14 DAP (before anthesis) had greater branch lengths than any other treatment (Figure 3.6). The growth rates of the top three rosette branches in plants moved to low R:FR at different times also supports the hypothesis that low R:FR accelerates the elongation of the buds programmed to grow out while it suppresses the buds that are in a phase of temporary dormancy (Figure 3.7).

3.2.3 Low R:FR promotes correlative inhibition

Plant architectural parameters and outgrowth kinetics suggested the suppressive nature of low R:FR signals on outgrowth of lower buds. In an effort to understand the nature of the inhibition process mediated by R:FR, we derived the correlative inhibition index (CII), a measure of apical dominance and/or apical control. The correlative inhibition index reflects the inhibitory signals mobilized from remote parts of the plant to suppress branch elongation. The CII is the slope derived by fitting a regression line to the top three rosette branch lengths (Finlayson et al., 2010). More negative slope values indicate greater inhibition from distant plant organs like other branches or the shoot apex. The duration of treatment with low R:FR had a profound effect on the correlative inhibition indices (Figure 3.8). Plants moved to low R:FR on 1 DAP and 7 DAP had more negative slopes compared to plants moved on 14 DAP and plants grown only in high R:FR (Figure 3.8). Interestingly, plants moved to low R:FR on 14 DAP had the lowest correlative inhibition index values.
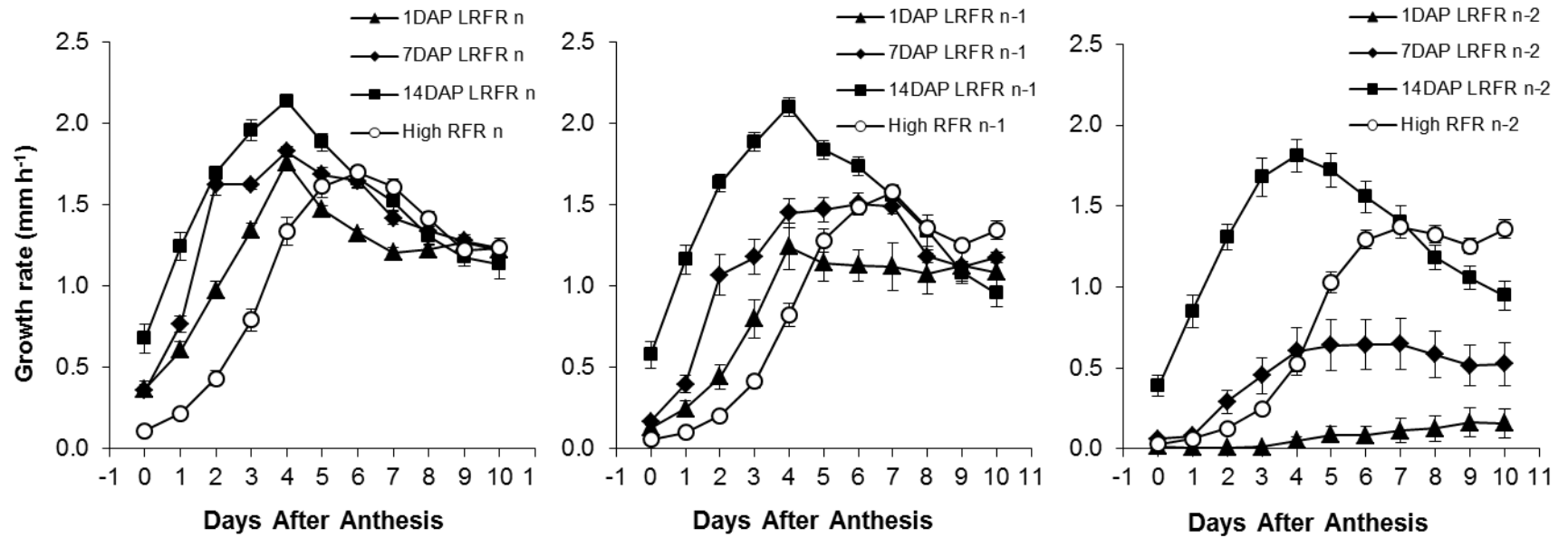
Figure 3.7 Rates of outgrowth for top three rosette buds of plants exposed to low R:FR for different durations. Bud outgrowth was measured from one day before anthesis to 10 days post anthesis. Error bars are standard error, n=18. LRFR – low R:FR (0.08); DAP – days after anthesis; LRFR 14 DAP – plants moved from high R:FR to low R:FR on 14 days after planting and so on; n – top most rosette branch; n-1 – branch below n; and n-2 – branch below n-1.
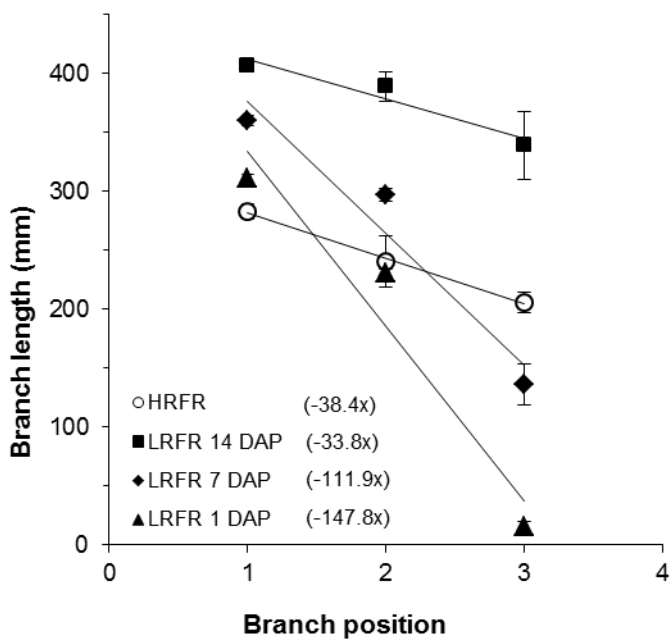
Figure 3.8 Slopes of correlative inhibition index generated by plotting lengths of top three rosette branches against their nominal positions. The lengths of the branches were measured on 10 DPA. HRFR – high R:FR; LRFR – low R:FR; DAP – days after planting; LRFR 14 DAP – plants moved from high R:FR to low R:FR on 14 days after planting; LRFR 7 DAP – plants moved to low R:FR 7 DAP; LRFR 1DAP – plants moved to low R:FR at 1 DAP. Error bars represent standard error, n = 18.

3.3 Discussion and perspectives

3.3.1 Cumulative shade signals determine plant architecture

Light regulates the branching habit in concert with various other signals to produce architecture suitable for the environment. Cumulative light/shade signals are likely to influence plant growth (especially plant architecture) in a manner analogous to the concept of temperature regulation of plant growth by growing degree days. Plants may perceive neighbors or a reduction in the R:FR from very early growth stages, even before they are actually being shaded. From the moment of perception of shade, plants continue to adapt by demonstrating SARs, unless shade signals are removed. Hence, the duration of exposure to R:FR becomes a key factor in determining the branching phenotype and overall plant architecture. In our experiment with varying durations of low R:FR treatment, plants showed considerable differences in phenotype as a result of varying cumulative shade signals. The cumulative shade signals perceived by plants moved to low R:FR from 1 DAP were greater than plants moved at 7 and 14 DAP resulting in greater inhibition of lower branches.

3.3.2 Low R:FR specifically targets the process of bud outgrowth

To relate the phenotypic changes resulting from altered R:FR with molecular events it was very important to understand which of the branching developmental steps are

modulated by R:FR. Previous reports from our lab using sorghum and Arabidopsis suggested that R:FR influenced the process of branch outgrowth (Kebrom et al., 2006; Finlayson et al., 2010). Our results using Arabidopsis showed that low R:FR treatment from 14 DAP produced fewer branches compared to high R:FR treatment despite producing similar numbers of axillary buds (Figure 3.2). This suggests that R:FR has very specific effects on regulating bud outgrowth, in addition to the inhibitory effects it has on leaf numbers. Low R:FR resulted in a greater percentage of unelongated axillary buds compared to high R:FR. These results are in agreement with the reports that low R:FR or *phyB* loss of function results in suppression of axillary bud outgrowth in sorghum (Kebrom et al., 2006).

3.3.3 R:FR likely acts as a non-bud autonomous signal

Several studies suggest that bud outgrowth is determined by signals native to the bud (bud autonomous), such as ABA and cytokinin and also by signals mobilized from distant organs (non-bud autonomous) like auxin and strigolactone (Dun et al., 2009a; Dun et al., 2009b; Waldie et al., 2010). However, apart from Finlayson et al. (2010), there is little information about the role of R:FR in regulating branching by these bud autonomous and non-bud autonomous signals. The correlative inhibition indices (Figure 3.8) suggest that the inhibitory signals may have been generated from distant plant organs to suppress bud outgrowth under low R:FR, implying a role for non-bud autonomous signals.

Our results show that buds at different positions show differential regulation by R:FR, thus revealing the complex nature of hierarchy among various signaling pathways. The results demonstrate that the buds at lower positions of plants moved to low R:FR on 1 DAP were suppressed compared to those of plants moved at later dates. Conversely low R:FR promoted the elongation of branches from upper nodes suggesting that they are regulated by different signaling pathways. These results support the earlier claims that *phyB* loss of function results in increased elongation responses in outgrowing branches while suppressing lower branches (Finlayson et al., 2010).

3.4. Conclusion

The architectural analysis of plants grown for varying durations under low R:FR indicated that low R:FR inhibited the outgrowth of axillary buds at lower positions, while promoting the elongation of outgrowing branches. The correlative inhibition indices suggested that low R:FR may act via non-bud autonomous signaling pathways to alter the outgrowth relationships between sequentially lower branches. The results raised the questions of whether buds at lower positions that are inhibited by low R:FR are capable of outgrowth and also what are the transcriptomic differences in buds that are suppressed and/or promoted by R:FR.

3.5 Methods and material

3.5.1 Plant growth conditions

Wild type (WT) *Arabidopsis thaliana* of the Columbia-60000 ecotype were used as the

experimental material. Plants were grown in trays with six-cell inserts (36 plants tray^{-1})

using Metro-Mix 200 potting mixture. Seeds were stratified at 4°C in tubes containing

distilled water for 3 days prior to planting. The water in the tubes was changed after 24 h

to remove any inhibitory substances released during stratification. Before planting,

seeds were exposed to white fluorescent light for 6 h. Approximately 4-6 seeds were

planted in each tube using water to aid dispersal. After germination, plants were thinned

periodically for one week to retain only the healthiest plant in each tube. Hoagland's

solution (1x) was used to fertilize the plants every 7 days. Plants were grown in a

growth chamber set to 18 h light/24°C daytime and 6 h dark/18°C nighttime conditions.

Light was maintained at 190 (±5) µmol m^{-2} sec^{-1} PPFD and R:FR of either 0.08 (low

R:FR) or 3.5 (high R:FR) using a mixture of T12 VHO fluorescent and compact

fluorescent lamps with an overhead array of LEDS mounted in a clear acrylic sheet to

provide supplemental FR. Light was measured using a Li-1800 Spectroradiometer

(Licor) and the spectra of light used in the study are presented in Figure 3.9. The FR
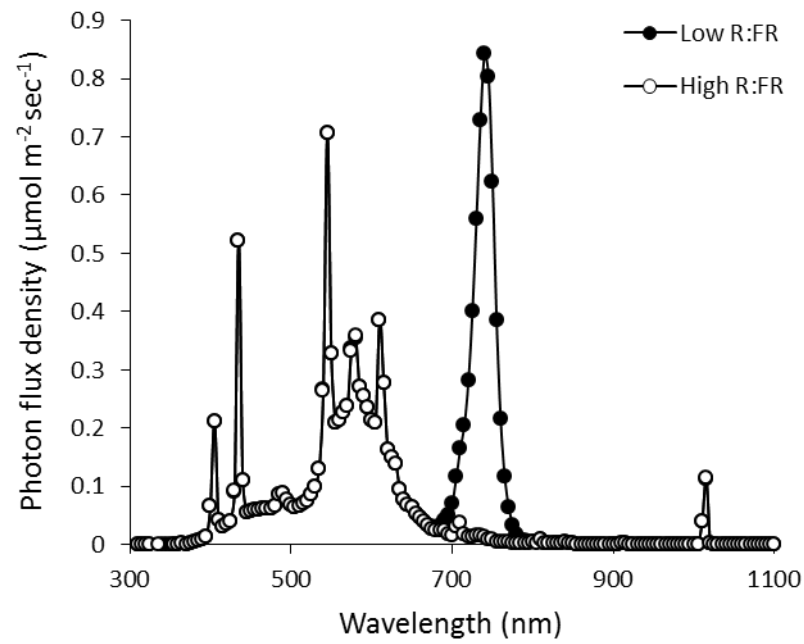
Figure 3.9 The spectra of light used in the study.

treatment was initiated on different days after planting (1 DAP, 7 DAP, 14 DAP) or plants were grown only under high R:FR. Both low and high R:FR conditions were maintained in the same growth chamber using a barrier in the middle to prevent light from one side of the chamber reaching the other.

3.5.2 Analysis of branch outgrowth kinetics and plant architecture

The length of the top three primary rosette buds/branches were measured every day (~24 hour interval) from one day before anthesis (DBA) or 0 DPA to 10 DPA to determine the effect of R:FR on bud outgrowth kinetics. At 10 DPA, various architectural parameters were measured from plants used for measuring branch outgrowth kinetics. Axillary buds/meristems formed at each leaf axil were noted using a dissection microscope. A branch was counted if the length of the axillary bud was greater than or equal to 3 mm. Number of rosette and cauline leaves were counted and lengths of each rosette and cauline branch were measured. Numbers of secondary and accessory branches formed on each branch were also recorded.

CHAPTER IV

TRANSCRIPTOMICS OF AXILLARY BUD OUTGROWTH

IN RESPONSE TO ALTERED R:FR

4. 1 Introduction

The increased apical dominance of shaded plants has been proposed to be mediated by hormones acting as both bud autonomous and non-bud autonomous signals (Finlayson et al., 2010), but there is little evidence to support these claims. In addition to hormone signals, regulators native to the bud such as cell cycle related genes, cell wall modifying genes, and novel light responsive cis-elements and transcription factors (TFs) likely determine bud outgrowth. Though phytochromes have been shown to mediate increased apical dominance triggered by SARs, the understanding of molecular mechanisms is unclear except for the role played by *BRC1*, a bHLH family transcription factor (Finlayson, 2007; Finlayson et al., 2010). There is a pressing need to uncover other novel cis-regulatory regions and TFs that are native to the axillary bud and integrate light signals to influence branch outgrowth. Previous transcriptomic approaches targeting early signaling events in young Arabidopsis seedlings in response to shade signals have identified key genes, promoter motifs and transcription factors mediating the SAR (Devlin et al., 2003; Hudson and Quail, 2003; Sessa et al., 2005; Tepperman et al., 2006). On the other hand, gene expression profiling studies have also addressed meristem development (Van Aken et al., 2007), and axillary bud outgrowth in response

to various other signals like decapitation (Tatematsu et al., 2005) and strigolactones (Mashiguchi et al., 2009). However little is known about the gene regulatory networks leading to axillary bud outgrowth in response to light signals.

Our preliminary studies showed that low R:FR treatment from very early in the life cycle results in differential bud outgrowth at different bud positions (CHAPTER III). Plants moved to low R:FR from 1 DAP had elongated branches at position n while axillary buds at position n-2 remained suppressed. The axillary buds at positions n and n-2 appeared to follow different developmental programs and also responded differently to altered R:FR, possibly due to changes in the bud transcriptome. Previous studies in two grass species showed that increasing the R:FR below the canopy using red light emitting diodes in natural environments resulted in increased tillering and reduced tiller death (Deregibus et al., 1985). However, to date, molecular events addressing axillary bud dynamics in response to supplemental red light or an increased R:FR have not been reported. Hence, for the present experiment, we hypothesized that outgrowth potentials of bud n and n-2 could be reversed by moving low R:FR grown plants to high R:FR and that bud transcriptome differences are associated with differential outgrowth kinetics. The results indicated that R:FR treatments that altered bud outgrowth were associated with changes in the expression of genes related to hormone metabolism, signaling, transport and transcriptional regulation, and other functions and processes as well.

4.2 Results

4.2.1 R:FR has contrasting effects on rosette axillary bud outgrowth depending on bud position

To extend our knowledge about the role of R:FR on parameters of bud outgrowth associated and gene expression responses, plants were grown under low R:FR from 1 DAP and the R:FR was then manipulated near the time of anthesis. Based on the architectural analysis and branch outgrowth kinetics of plants treated with low R:FR for different durations (Chapter III), it was evident that bud positions 'n' and 'n-2' were differentially regulated by R:FR. The onset of outgrowth also varied with bud positions suggesting the possibility of different developmental programs for buds n and n-2. Under the growth conditions used bud n began to grow out coincident with flowering (anthers were visible in the flowers – referred as *anthesis*) in response to developmental signals, while bud n-2 remained dormant. To understand further how effective R:FR signals are in determining the bud outgrowth of buds at different position, plants grown under low R:FR (0.08) from 1 DAP were moved to high R:FR (3.5) on 0 and 3 DPA. The treatments were imposed by moving half the plants to high R:FR at 0 DPA (~ 18±1 DAP) while the other half remained under low R:FR. In a companion experiment examining the response of the third bud down (bud n-2) to R:FR, similar treatments were made at 3 DPA (~ 21±1 DAP). The visual phenotypes of plants either allowed to grow

continually under low R:FR or moved to high R:FR at 3 DPA are presented in Figure

4.1.
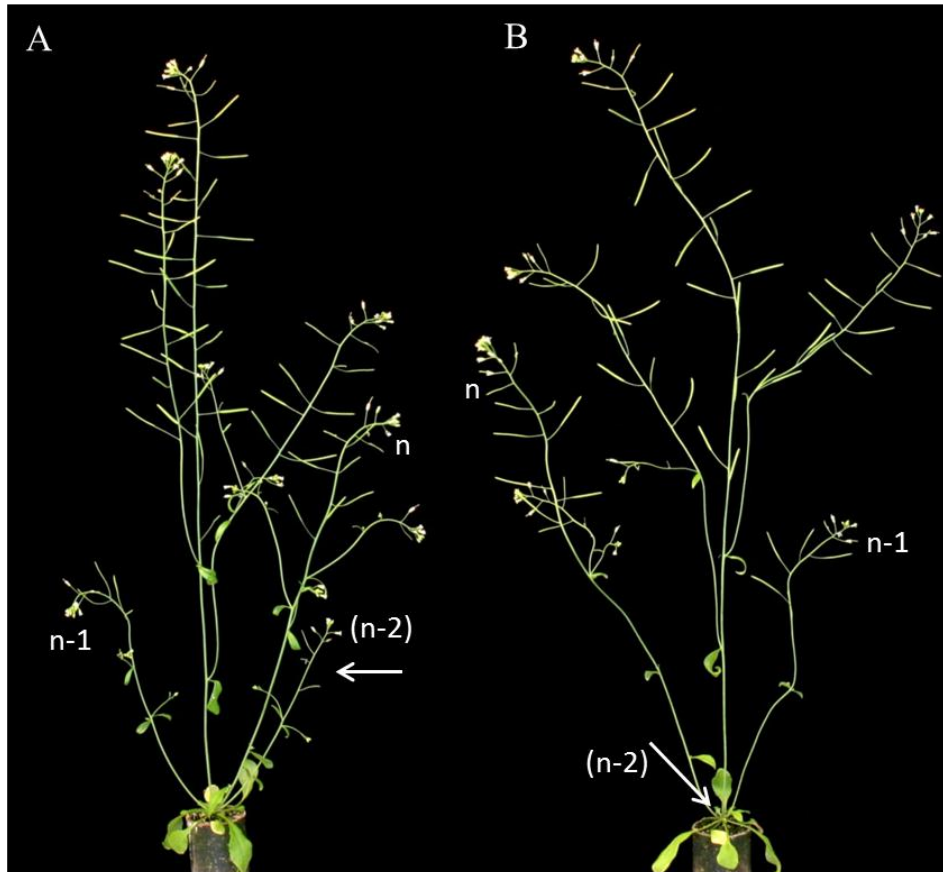
Figure 4.1 Visual phenotypes of plants responding to altered R:FR at 10 days post anthesis (DPA). A) Plant moved to high R:FR (3.5) at 3 DPA; B) Plant grown continuously under low R:FR from 1 DAP. Bud n-2 (horizontal arrow) grew out after moving to high R:FR (A), while the counterpart (diagonal arrow) in low R:FR remained dormant (B). The labels 'n' and 'n-1' represent branches at top-most rosette leaf axil and the leaf axil below it, respectively.
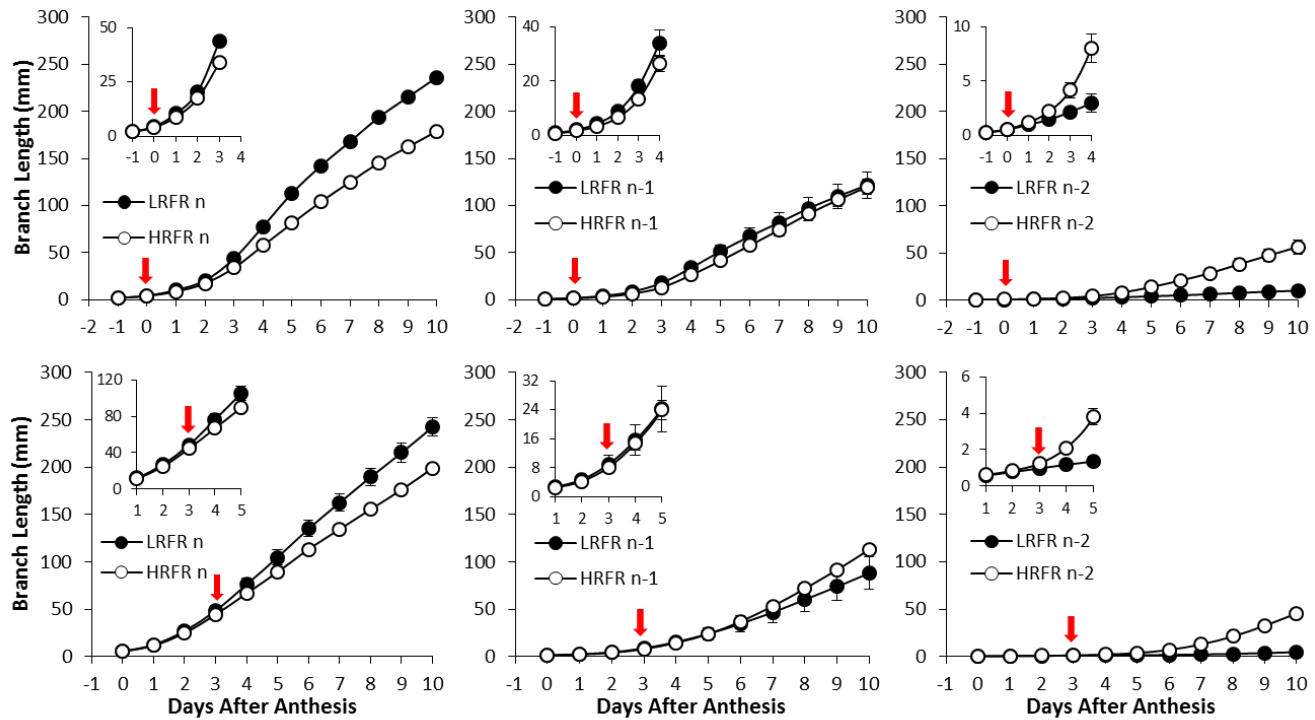
Figure 4.2 Branch outgrowth kinetics of top three rosette buds of plants exposed to different light quality. Plants were either continued to grow in low R:FR (0.08) or moved to high R:FR (3.5) without any changes to PPFD [190 ($\pm$5) μmol m^{-2} sec^{-1}] on 0 DPA (n = 44) or 3 DPA (n = 75). Inset figures show the same graph with a different scale. Red down-arrow represent the day of HRFR treatment. Bud outgrowth was measured from the day of anthesis to 10 days post anthesis. Error bars are standard error. LRFR – low R:FR (0.08); HRFR – high R:FR; n – top most rosette branch; n-1 – branch below n; and n-2 branch below n-1.

The plots of branch outgrowth kinetics after moving to high R:FR showed that bud outgrowth dynamics were contrasting in buds n and n-2 while n-1 was relatively unresponsive (Figure 4.2). Increasing the R:FR at either 0 or 3 DPA promoted the outgrowth of bud n-2, but inhibited the elongation of bud 'n' (Figure 4.2). The inset plots in Figure 4.2 of bud n-2 demonstrate that high R:FR applied 3 DPA promoted outgrowth within 24 hours of treatment. The graphs presenting elongation rates clearly demonstrated that bud n had initiated to grow out by 0 DPA even under low R:FR, while bud n-2 grew out only under the influence of high R:FR (Figure 4.3). The architectural analysis at 10 DPA showed that plant height was greater under low R:FR (Figure 4.4). The correlative inhibition index measured at 10 DPA on plants moved to high R:FR at 0 and 3 DPA indicated that R:FR altered the inter-branch coordination of outgrowth, promoting at upper positions and suppressing at lower positions (Figure 4.5).

4.2.2 Identification of bud autonomous genes that show differential expression in response to R:FR and bud position

Changes in the transcriptome of axillary buds at positions n and n-2 in response to altered R:FR (1 and 3 hours after treatment with high R:FR) were analyzed using Affymetrix ATH1 genome arrays. Following MAS5 normalization and filtering, a two-way factorial analysis of variance (ANOVA, $p < 0.05$) and the Benjamini Hochberg method to discover false positives (FDR, $q < 0.05$) was performed on 15466 genes to
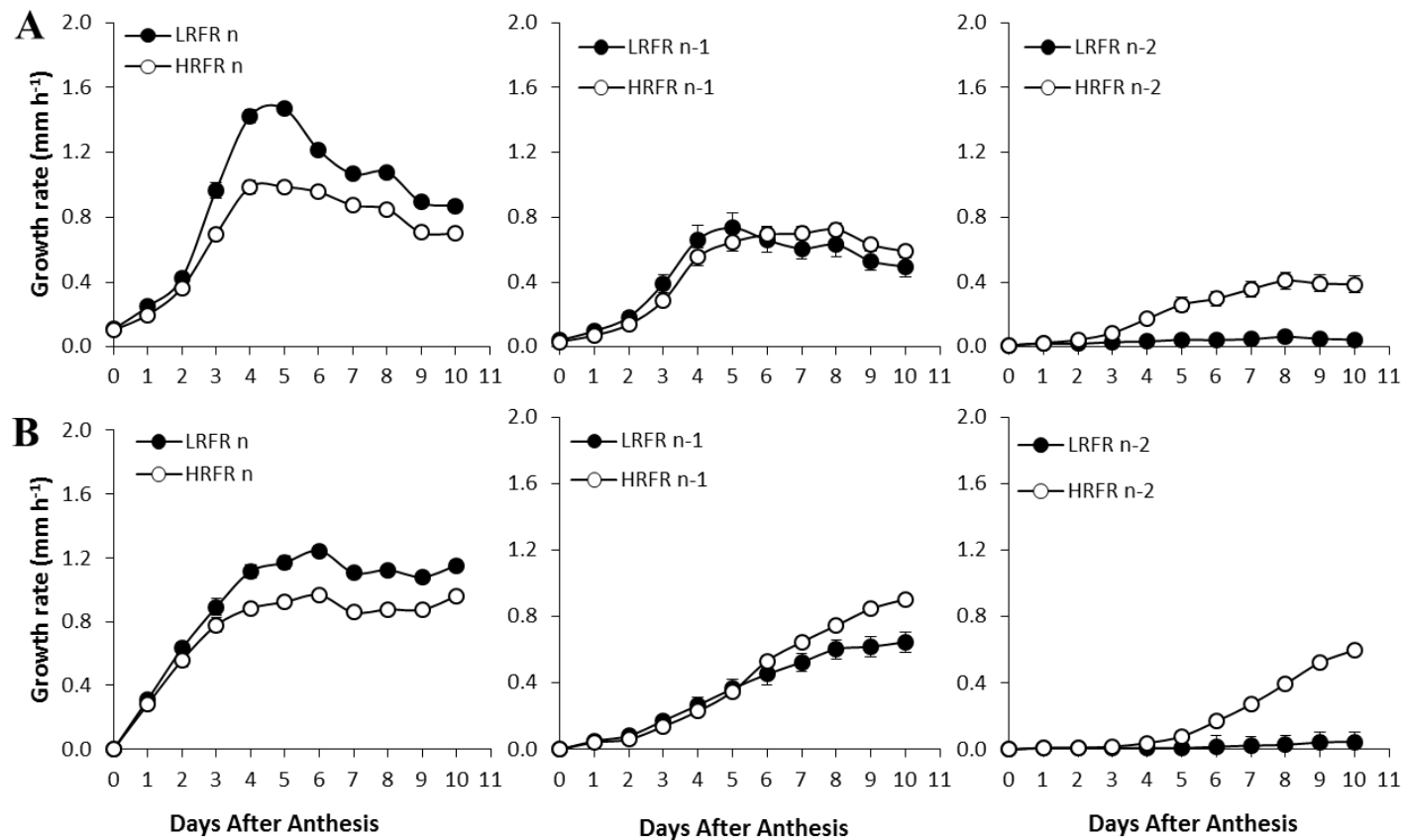
Figure 4.3. Elongation rates of top three rosette branches in response to altered R:FR 0 DPA (A) and 3 DPA (B). Bud outgrowth was measured from the day of anthesis to 10 days post anthesis. Plants were either continued to grow in low R:FR (0.08) or moved to high R:FR (3.5) without any changes to PPFD [190 ($\pm$5) μmol m^{-2} sec^{-1}] on 0 DPA (n = 44) or 3 DPA (n = 75). LRFR – low R:FR (0.08); HRFR – high R:FR; n – top most rosette branch; n-1 – branch below n; and n-2 branch below n-1. Error bars are standard error.
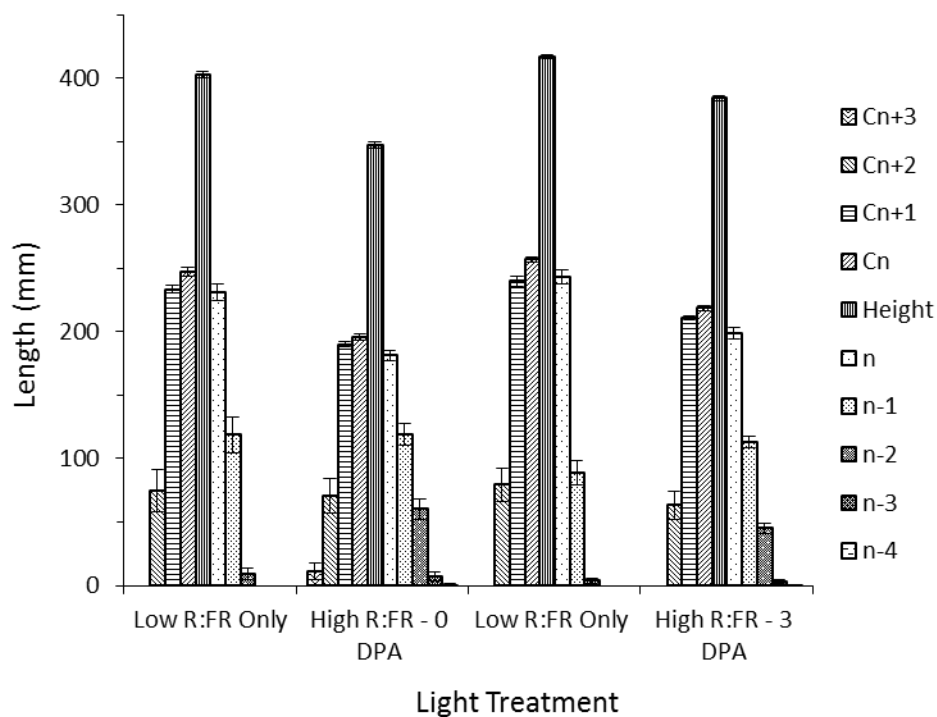
Figure 4.4 Architectural parameters plant height, lengths of rosette and cauline branches in response to variable light quality. The plants were treated with different R:FR on either 0 DPA (n = 44) or 3 DPA (n = 75) and the architectural parameters were measured at 10 DPA. Error bars represent standard error. DPA – days post anthesis; Height – plant height measuring from rosette to tip of shoot apical meristem; Rn – length of rosette branch n, branch appearing at first rosette leaf; Rn-1 – length of rosette branch below branch n and so on; Cn – Length of cauline branch n, this branch appears from cauline leaf located close to the rosette; cn+1 – length of cauline branch above the Cn and so on.
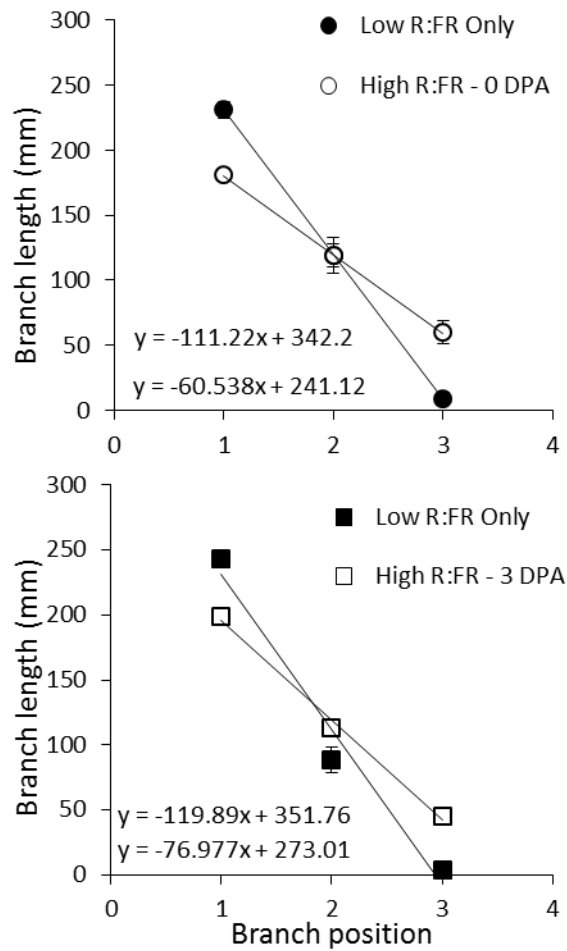
Figure 4.5 Slopes of correlative inhibition index generated by plotting lengths of top three rosette branches against their nominal positions. The lengths of the branches were measured on 10 DPA in response to different light quality, plants were either continued to grow in low R:FR (0.08) or moved to high R:FR (3.5) from either 0 (n = 44) or 3 (n = 75) DPA . Error bars represent standard error.

identify those that were differentially regulated. The statistical tests showed that the expression of 2486 and 9022 genes showed significant main effects of light and bud position respectively and the expression responses of 2049 genes were significant for the interaction between light and position. The statistical grouping showed overlapping gene sets for main effects of light and position which was rectified using Venn diagrams to create four major groups of differentially regulated genes. These groups were labeled 1, 2, 3, and 4 based on gene expression specific to light effects (387), bud position effects (6170), combined effects of light and bud position (1210), and any interaction effects (2049), respectively. The flow chart describing the steps of microarray data analysis is presented in Figure 4.6 and is also discussed in detail in the Methods and Material section. The gene groups were used to discover differentially expressed genes, cis-elements, and GO categories associated with bud outgrowth responses to bud position and R:FR.

4.2.3 Light regulates the expression of hormone related genes in the axillary buds

Light has been proposed to influence bud outgrowth by regulating the expression of genes related to hormone metabolism, transport, and signaling. In support of this our data revealed several hormone related and growth regulating genes differentially expressed in response to altered R:FR both in bud n and n-2. In bud n similar numbers of genes were either up-regulated (96) or down-regulated (92) under low R:FR compared to high R:FR, while in bud n-2 about 20% more genes were up-regulated (104) compared to down-regulated genes (84). Auxin, cytokinin, ethylene, and ABA
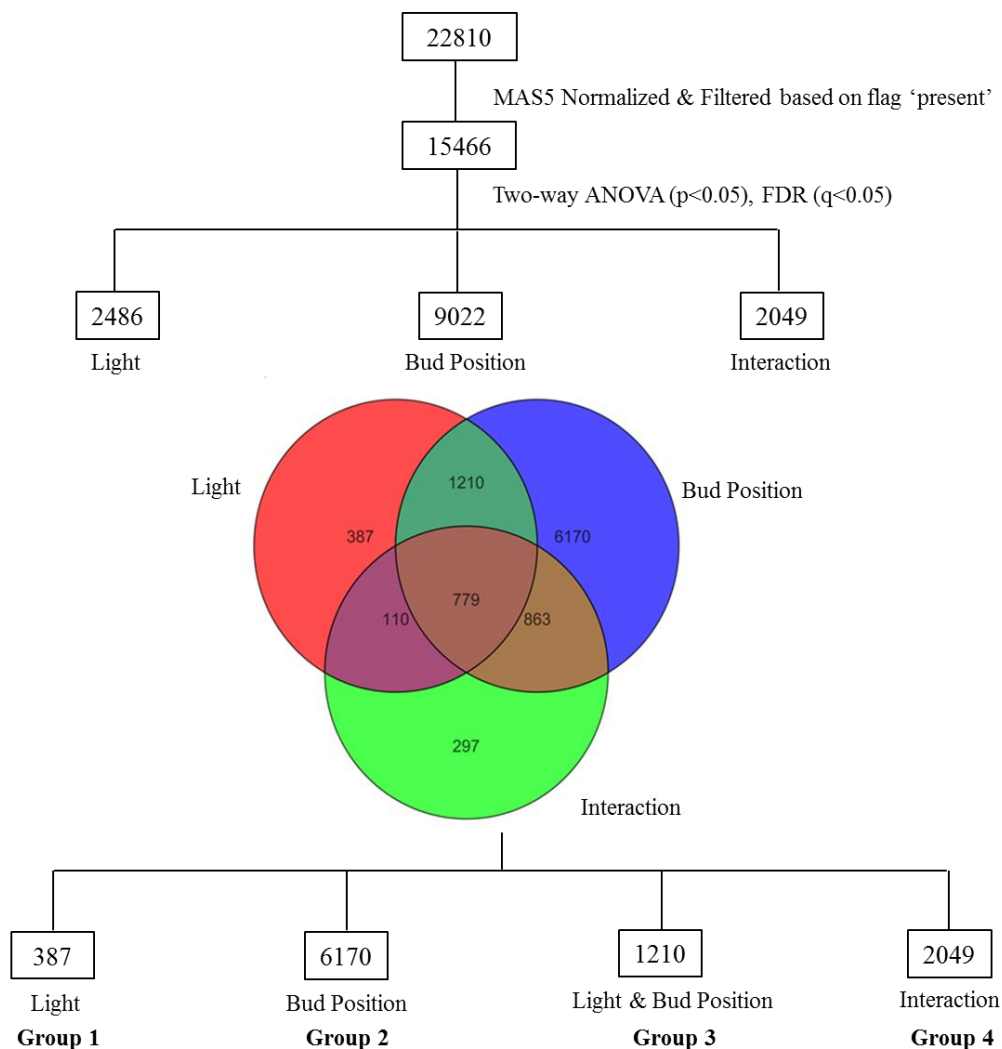
Figure 4.6 A flow chart illustrating steps in microarray data analysis. Following MAS5 normalization on initial set of 22810 genes, 15466 genes were filtered based on flag "present" to retain entities that were detected in all three replications. These genes were subjected to two-way factorial analysis of variance (ANOVA, p<0.05) to identify differentially regulated genes and Benjamini Hochberg method to control false positives and estimate the false discovery rate (FDR, q<0.05). The resulting categories with significantly different genes for main effects (light-2486, bud position-9022, and interaction-2049) were further grouped/filtered to remove overlapping genes using Venn diagram. This ensured that genes specific to light effects (387), position effects (6170), light position effects (1210), and interaction effects (2049) were grouped discretely.
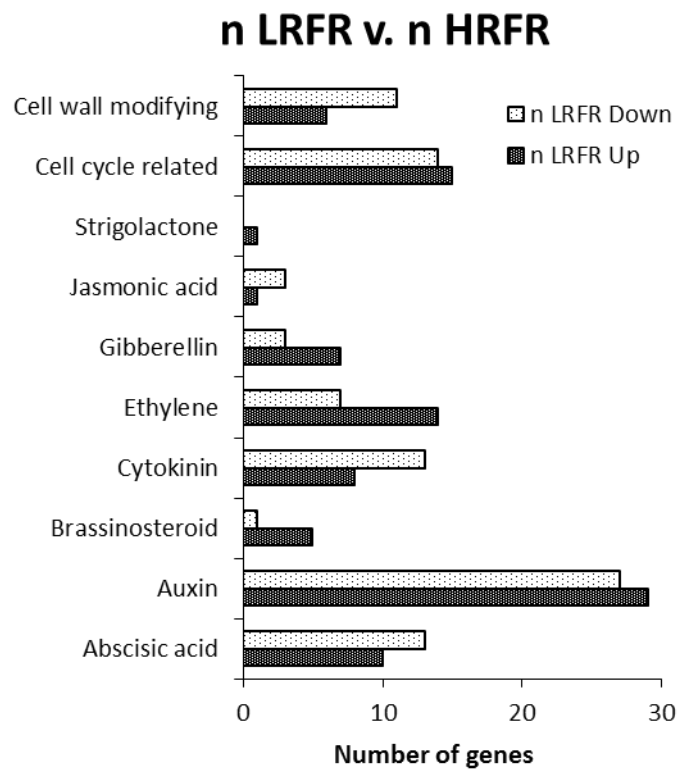
Figure 4.7 Numbers of hormone related and growth regulating genes differentially expressed in response to altered R:FR in bud n. LRFR – low R:FR; HRFR – high R:FR; n – top most rosette bud; n-2 – third rosette bud, two positions below bud n.
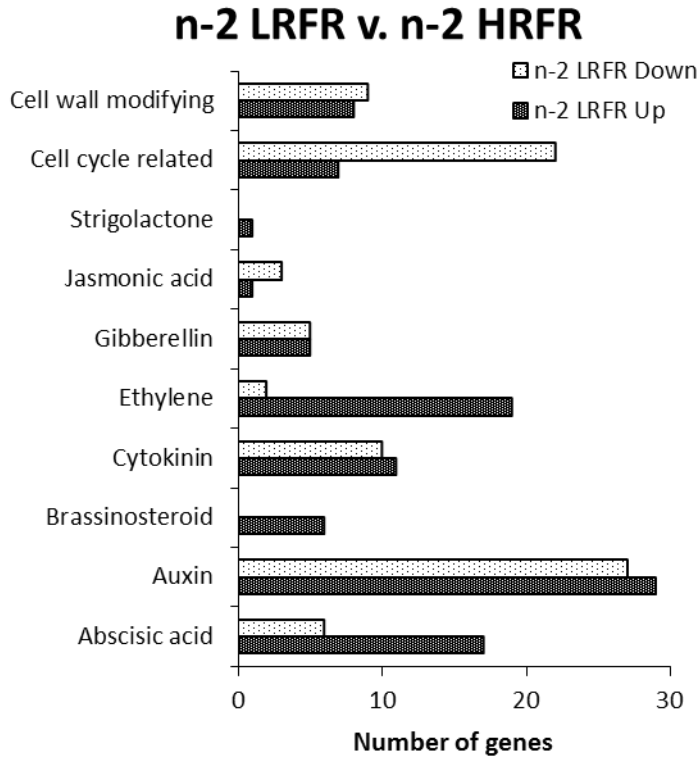
Figure 4.8 Numbers of hormone related and growth regulating genes differentially expressed in response to altered R:FR in bud n-2. LRFR – low R:FR; HRFR – high R:FR.

associated genes were highly responsive to altered R:FR in both bud n (Figure 4.7) and

bud n-2 (Figure 4.8). It is interesting to note that ABA, and ethylene related genes are

up-regulated in greater numbers in bud n-2 and the majority of cell-cycle related genes

are down-regulated. Hormone associated and growth regulating genes were also

differentially regulated in different bud positions in response to altered R:FR (Figure

4.9). Most of the hormone related genes were down-regulated in bud n under both low

and high R:FR, while cell cycle regulating genes were found to be up-regulated. A list

of genes related to hormones and growth that are differentially regulated are presented in

appendix 4.7.

4.2.4 Clustering and motif analysis

The groups of genes showing significant effects specific to light, position, light and

position, and the interaction between light and position were used for clustering and

motif analysis. Appendix 4.1 shows the basis for clustering, the individual clusters and

the number of genes falling into each cluster. The analysis conducted on individual

clusters did not identify significant motifs (p<10e-3) for light effects, but did find

significant motifs in clusters from the other three groups (Table 4.1). A total of 66

motifs were discovered; 16 motifs were found in clusters from group 2 (position), 16

motifs were found in clusters from group 3 (light and position), and 34 motifs were

found in clusters from group 4 (interaction). Of the motifs identified, there were 22

unique motifs and these were scattered among various clusters (Table 4.2). Most of the

motifs represented G-box promoters which are involved in the transcription of light

regulated genes, and ABA-responsive elements. A detailed table illustrating motifs,

consensus sequences, and the corresponding p values of individual clusters is presented

in Appendix 4.2. Individual clusters were then used to construct super-clusters and

supersub-clusters based on many different combinations of light and position effects on
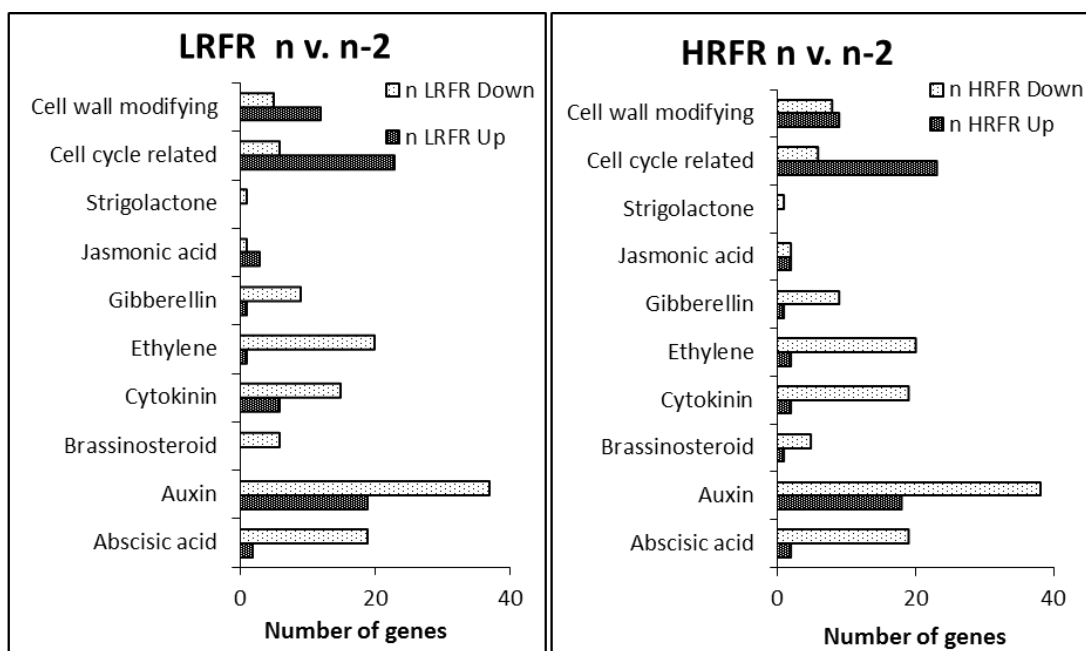
Figure 4.9 Numbers of hormone related and growth regulating genes differentially
expressed in response to altered R:FR in different bud positions. LRFR – low R:FR; HRFR
– high R:FR; n – top most rosette bud; n-2 – third rosette bud, two positions below bud n.

Table 4.1 Summary of motif analysis for individual clusters from all groups. Group 2 is specific for genes showing position effects, 3 for genes showing both light and position effects, and 4 for genes showing interactions between light and position. No motifs were identified for group 1 (specific for light effects). The significance level used in the motif analysis was p<10e-3.

Sl #	Gr #	Cl #	LRFR v HRFR n "a"	LRFR v HRFR n-2 "b"	n v n-2 LRFR "c"	n v n-2 HRFR "d"	No. of genes	No. of motifs
1	2	1	-	-	Up	Up	2535	4
2	2	2	-	-	Down	Down	3627	12
3	3	1	Up	Up	Up	Up	57	1
4	3	2	Down	Down	Down	Down	144	4
5	3	3	Up	Up	Down	Down	622	11
6	4	5	-	-	Up	-	197	1
7	4	6	-	-	Down	-	200	7
8	4	22	-	Up	Down	-	141	8
9	4	23	-	Down	Up	-	212	1
10	4	32	-	-	Down	Down	134	4
11	4	48	Up	Down	-	Down	3	1
12	4	64	-	Up	Down	Down	163	8
13	4	78	Down	Up	Down	Down	7	4

Sl# - serial number; GR# - group number; Cl# - cluster number; LRFR - low R:FR; HRFR - high R:FR; n - top most rosette bud; n-2 - third rosette bud from top.

Table 4.2 Motifs, consensus sequences, and number of times the motif appeared in individual clusters.

Sl#	TF /Motif Name	Consensus Sequence	# times appeared
1	ABFs binding site motif	CACGTGGC	5
2	ABRE binding site motif	YACGTGGC	6
3	ABREATRD22	RYACGTGGYR	3
4	ABRE-like binding site motif	BACGTGKM	9
5	ACGTABREMOTIFA2OSEM	ACGTGKC	7
6	ATHB1 binding site motif	CAATWATTG	1
7	ATHB5ATCORE	CAATNATTG	1
8	CACGTGMOTIF	CACGTG	8
9	CArG promoter motif	CCWWWWWWGG	1
10	DRE core motif	RCCGAC	1
11	DREB1A/CBF3	RCCGACNT	1
12	E2F binding site motif	TTTCCCGC	1
13	EveningElement promoter motif	AAAATATCT	2
14	GADOWNAT	ACGTGTC	5
15	GBOXLERBCS	MCACGTGGC	5
16	Hexamer promoter motif	CCGTCG	1
17	Ibox promoter motif	GATAAG	2
18	TATA-box Motif	TATAAA	1
19	TELO-box promoter motif	AAACCCTAA	3
20	TGA1 binding site motif	TGACGTGG	1
21	UPRMOTIFIAT	CCACGTCA	1
22	UPRMOTIFIIAT	CCNNNNNNNNNNNNNCCACG	1

the four meaningful comparisons (bud n, low R:FR v. high R:FR; bud n-2, low R:FR v. high R:FR; low R:FR, bud n v. bud n-2; high R:FR, bud n v. bud n-2). The layout and basis for constructing super-clusters and supersub-clusters is illustrated in Appendix 4.1. Summaries of motif analysis based on super-clusters and sub-clusters showing any effects of light are presented in Appendix 4.3 and Appendix 4.4, and for position effects are presented in Appendix 4.5 and Appendix 4.6. The details of overrepresented motifs and their corresponding sequences for light and position are presented in Tables 4.3 and 4.4 respectively.

4.3 Discussion

4.3.1 R:FR can be used as a 'switch' to regulate the bud outgrowth

The suppressive effects of low R:FR on branching has been documented both in natural and controlled environment (Deregibus et al., 1985; Casal et al., 1986; Wan and Sosebee, 1998; Kebrom et al., 2006; Finlayson et al., 2010). However, a detailed understanding of the mechanisms regulating bud/branch outgrowth in response to altered R:FR is not available. The branch outgrowth kinetics (Figure 4.2) and architectural analysis (Figure 4.4) demonstrate that the suppressive effects of low R:FR can be reversed by switching to a high R:FR environment. Interestingly, plants moved to high R:FR on 3 DPA showed promotion of outgrowth in bud n-2 within 24 hours after

movement and suppressed elongation of bud n in about 48 hours suggesting that R:FR

can be used a switch to regulate bud outgrowth (Figure 4.2, inset drawings).

Table 4.3 Motifs, consensus sequences, and number times appeared in sub-clusters based

on any light effects.

Sl#	TF /Motif Name	Consensus Sequence	# times appeared
1	ABFs binding site motif	CACGTGGC	3
2	ABRE binding site motif	YACGTGGC	3
3	ABREATRD22	RYACGTGGYR	3
4	ABRE-like binding site motif	BACGTGKM	5
5	ACGTABREMOTIFA2OSEM	ACGTGKC	3
6	ATHB1 binding site motif	CAATWATTG	2
7	ATHB5ATCORE	CAATNATTG	2
8	CACGTGMOTIF	CACGTG	5
9	EveningElement promoter motif	AAAATATCT	2
10	GADOWNAT	ACGTGTC	3
11	GBF1/2/3 BS in ADH1	CCACGTGG	1
12	GBOXLERBCS	MCACGTGGC	3
13	Ibox promoter motif	GATAAG	3
14	TATA-box Motif	TATAAA	1
15	TELO-box promoter motif	AAACCCTAA	1
16	TGA1 binding site motif	TGACGTGG	4
17	UPRMOTIFIAT	CCACGTCA	4
18	UPRMOTIFIIAT	CCNNNNNNNNNNNNCCACG	1

Table 4.4 Motifs, consensus sequences, and number times appeared in sub-clusters based on any position effects.

SI#	TF /Motif Name	Consensus Sequence	# times Appeared
1	ABFs binding site motif	CACGTGGC	4
2	ABRE binding site motif	CACGTGGC	4
3	ABREATRD22	RYACGTGGYR	4
4	ABRE-like binding site motif	BACGTGKM	6
5	ACGTABREMOTIFA2OSEM	ACGTGKC	4
6	ATHB1 binding site motif	CAATWATTG	1
7	ATHB5ATCORE	CAATNATTG	1
8	CACGTGMOTIF	CACGTG	7
9	CArG promoter motif	CCWWWWWWGG	2
10	DRE core motif	RCCGAC	2
11	DREB1A/CBF3	RCCGACNT	2
12	E2F binding site motif	TTTCCCGC	1
13	EveningElement promoter motif	AAAATATCT	3
14	GADOWNAT	ACGTGTC	4
15	GBF1/2/3 BS in ADH1	CCACGTGG	3
16	GBOXLERBCS	MCACGTGGC	4
17	Hexamer promoter motif	CCGTCG	1
18	Ibox promoter motif	GATAAG	2
19	LTRE promoter motif	ACGTGTC	1
20	TATA-box Motif	TATAAA	1
21	TELO-box promoter motif	AAACCCTAA	1
22	TGA1 binding site motif	TGACGTGG	2
23	UPRE2AT	CCACGTCATC	1
24	UPRMOTIFIAT	CCACGTCA	2
25	UPRMOTIFIIAT	CCNNNNNNNNNNNNCCACG	2

4.3.2 R:FR acts through both bud autonomous and non-bud autonomous pathways to regulated the process of outgrowth

Our studies involving different durations of low R:FR treatment helped us to conclude that the cumulative amount of shade signals (from even before axillary buds are initiated) serves as a non-bud autonomous signal to regulate bud outgrowth. In support of these results, the plants moved from low R:FR to high R:FR on either 0 or 3 DPA (Figure 4.5) showed reduced correlative inhibition, bolstering the notion that low R:FR promotes apical dominance. Taken together, these results suggest that R:FR affects bud outgrowth, in part, through bud non-autonomous pathways. Interestingly, in the case of plants moved from low R:FR to high R:FR on 0 or 3 DPA, buds showed R:FR responsiveness in terms of changes in the bud transcriptome. Since the high R:FR treated buds (n-2) grew twice the length of those in low R:FR within 24 h, and many of the genes with altered expression are known to be light responsive, the results suggest that R:FR also directly influences bud outgrowth kinetics by altering the bud transcriptome (bud autonomous pathway).

4.3.3 Branch outgrowth is a process of integration of signals from various gene networks

The results of the axillary bud transcriptomic analysis in response to altered R:FR provided evidence for the role of several hormone related, cell-cycle related, and cell

wall modifying genes in regulating bud outgrowth. These results add to our understanding that bud outgrowth mediated by light signals is a complex process involving several downstream targets, with intricate interactions between them. Previous studies have indicated that light regulates plant growth and development by the action of hormones (Halliday and Fankhauser, 2003; Franklin, 2008; Alabadi and Blazquez, 2009; Jaillais and Chory, 2010). Nevertheless, information pertaining to the role of light in regulating bud autonomous, hormone related genes is unavailable except in recent work (Finlayson et al., 2010; Kebrom et al., 2010).

Auxin is an essential hormone in the process of bud outgrowth since it promotes cell division, and cell elongation. Recent studies indicate that low R:FR induces auxin biosynthesis via a tryptophan dependent pathway by increased expression of *TAA1* (*TRYPOPHAN AMINOTRANSFERASE OF ARABIDOPSIS 1*) (Tao et al., 2008), but its role in the axillary bud appears complicated (Finlayson et al., 2010). An increased level of expression of *ATBH2* and *ATHB4* under shading has been shown to increase auxin levels, and alter auxin transport and/or sensitivity (Carabelli et al., 1993; Sorin et al., 2009). Our gene list contains numerous genes involved in auxin biosynthesis, transport, and signaling that are responsive to R:FR and bud position suggesting a key role for auxin in the outgrowth process.

Brassinosteroids have been shown to mediate SARs by promoting hypocotyl elongation under low R:FR and the responses are suggested to be mediated by *ATHB4* and *HAT3*

(Luccioni et al., 2002; Sorin et al., 2009). However, no reports of brassinosteroid involvement in branching are available. Our data suggests a role for brassinosteroids in axillary bud outgrowth in response to R:FR (Appendix Table 4.7).

There is no direct information concerning the role of cytokinins in bud outgrowth responses to R:FR, however recently it was demonstrated that low R:FR regulated suppression of leaf growth in Arabidopsis is mediated by *AtCKX6*, a cytokinin oxidase gene which is in turn induced by increased auxin levels (Carabelli et al., 2007). The involvement of cytokinin in cell division, an essential part of bud outgrowth, and the large number of cytokinin responsive genes differentially regulated by altered R:FR (Appendix Table 4.7) may indicate a role for cytokinin in promoting bud outgrowth in response to high R:FR.

Ethylene-insensitive tobacco mutants have reduced SARs (Pierik et al., 2003) and simulated shading resulted in increased ethylene production, which promoted stem and petiole elongation (Pierik et al., 2004). However, there is no evidence relating ethylene and SARs to branching. Appendix Table 4.7 presents more than 20 ethylene related genes differentially expressed in the axillary buds, but their involvement is inconclusive.

Gibberellins and SARs seem to go hand in hand since both result in elongation of stem like organs. Low R:FR, end-of-day FR, and *phyB* loss of function have been shown to increase GA metabolism and bioactive GAs in some species but not in others (Martinez-

Garcia et al., 2010). However, there is no direct evidence for GA regulation of branching.

The role of ABA has been established as promoting bud dormancy and apical dominance, especially under low R:FR (Cline, 1991), but information pertaining to genes involved in the process is unavailable. Previous studies showed a correlation between auxin and ABA in bud outgrowth, where decapitation resulted in increased IAA concentrations but decreased ABA levels in the axillary buds (Gocal et al., 1991). Our results show that a great number of ABA related genes are differentially regulated in a bud autonomous manner in response to altered R:FR and bud position (Appendix Table 4.7) supporting ABA's role in regulating bud outgrowth.

The interaction between light and strigolactone pathways in shaping the branching habit was suggested by *MAX2*, an F-box gene involved in suppressing branching (Shen et al., 2007; Stirnberg et al., 2007). Our data suggest that *MAX2* is bud autonomous and that low R:FR promotes the expression in the axillary buds resulting in suppression of outgrowth, in agreement with observations made by (Kebrom et al., 2010).

4.3.4 Motifs associated with genes responsive to R:FR and bud position

Studies on SARs of young Arabidopsis seedling have shown that R:FR regulates several transcription factor families to modulate plant development (Franklin, 2008; Leivar and

Quail, 2011). Phytochrome signal transduction involves nuclear localized Pfr conformer binding with basic helix-loop-helix (bHLH) family transcription factors called PIFs which in turn bind to a G-box motif (CACGTG) to regulate SARs (Toledo-Ortiz et al., 2003; Leivar and Quail, 2011). Our data shows an overrepresentation of the CACGTG motif in individual clusters (Table 4.2), light regulated super and super-sub clusters (Table 4.3) and position dependent super and super-sub clusters (Table 4.4) suggesting a role for PIFs in mediating bud outgrowth. The I-box promoter is also another important region on light regulated genes encoding a GATAAG consensus sequence (Giuliano et al., 1988) found in our data set.

The role of ABA responsive promoter regions in suppressing bud axillary bud outgrowth under low R:FR has not been reported in the past, however our motif analysis results suggest that they may be important. Several ABA-responsive element (ABRE) binding sites, and ABA-responsive element binding factors (ABFs binding site motif) are overrepresented in response to altered R:FR and different bud positions indicating a role for ABA in mediating axillary bud outgrowth (Tables 4.2, 4.3, and 4.4). ABF, ABRE, and ABRE-like binding site motifs have previously been shown to play role in drought, salt tolerance, and glucose signaling suggesting roles in multiple abiotic stresses (Narusaka et al., 2003; Kim et al., 2004; Fujita et al., 2005).

Low R:FR has been reported to increase the abundance of members of the homeodomain leucine zipper (HDZip) transcription factor family like ATHB2 and ATHB4 (Carabelli

et al., 1993). The HDZip family contains 47 genes that are grouped into four classes, HDZip I through IV, and each class is further subdivided into different subclasses/clades (Carabelli et al., 1993; Henriksson et al., 2005). Genes belonging to the HDZip family have been shown to be involved in mediating light and hormone pathways to regulate shade avoidance, apical dominance, leaf development, vascular tissue differentiation, etc. (Carabelli et al., 1993; Henriksson et al., 2005; Franklin, 2008). Our results (Tables 4.1, 4.2, and 4.3) show that some bud autonomous genes contain overrepresented CAATNATTG and CAATWATTG motifs that are bound by ATHB5 and ATHB1 transcription factors respectively, implying the role of HDZip family proteins in mediating bud outgrowth.

4.4 Conclusions

Low R:FR mediated axillary bud outgrowth seem to be regulated both by bud autonomous and non-bud autonomous pathways. It is likely that light regulated branching involves hormones as modulators at both levels. Altered R:FR induced differential expression of hormone and cell-cycle related genes. Also, identification of novel cis-regulatory elements associated with bud outgrowth in response to light signals and bud position could help in designing strategies to alter plant architecture in agronomically important crops.

4.5 Methods and material

4.5.1 Plant growth conditions, branch outgrowth kinetics, and architectural analysis

The plant growth conditions, methods, and light environment used for the study are described in detail in CHAPTER III. To generate the numbers of plants required to collect enough tissue for transcriptomic analysis (including three biological replicates) in a small area, the plants were grown in 50 ml plastic conical tubes (cut to the 25 ml mark). The FR treatment was initiated one day after planting (DAP) and was continued until ten days after anthesis (low R:FR) or was discontinued on the day of anthesis or three days post anthesis (DPA) by turning off far-red diodes (high R:FR) to increase the R:FR to 3.5 without altering the PPFD. Bud outgrowth kinetics and architectural analysis revealed that bud n and n-2 responded differently to altered R:FR, and were therefore studied separately using two different experiments. Treatments with high R:FR on 0 DPA and 3 DPA were applied in two different experiments to capture changes in the bud transcriptome separately in bud n (0 DPA) and n-2 (3 DPA). Both low and high R:FR conditions were maintained in the same growth chamber using a barrier in the middle to prevent light from one side of the chamber reaching the other. Plant outgrowth kinetic and architectural analyses were performed on subsets of plants equivalent to those used for microarray analyses as described in CHAPTER III.

4.5.2 Axillary bud harvest and sample preparation

Unelongated axillary buds < 2.5 mm at the topmost (n) and third from top (n-2) rosette

leaf positions from plants grown under both low and high R:FR treatments were

harvested separately at 1 h and 3 h after altering the R:FR. Buds at different positions

were harvested on different DPA (on the day of anthesis for bud 'n' and three DPA for

'n-2') to ensure they were at comparable growth stages. The buds included one or two

of the youngest bud leaves and were harvested directly into Lysis binding solution

(Ambion) maintained on ice. Total RNA was extracted using TRIzol (Invitrogen) and

quantified using a NanoDrop ND-1000 Spectrophotometer. The quality of the RNA was

assessed by observing the ethidium bromide stained ribosomal bands following

separation on a denaturing glyoxal agarose gel. The quality of RNA for microarray

experiments was assessed using the RNA integrity number (RIN) determined using an

RNA 6000 Pico LabChip in Bioanalyzer 2100. The samples used for microarray

analysis had RIN values greater than 7.0.

4.5.3 Microarray experiments

Transcriptome analysis was performed using Affymetrix ATH1 genome arrays. Probe

synthesis, hybridization, and chip scanning were performed at the Nottingham

Arabidopsis Stock Center (http://arabidopsis.info/).

4.5.4 Microarray data analysis

Microarray data analysis was performed using GeneSpring GX software version 11.0 and other tools. First, the .CEL files were grouped to pool the replicates based on bud position (n and n-2) and light treatment (low R:FR and high R:FR). This experimental grouping resulted in four experimental conditions (samples); bud n low R:FR, bud n high R:FR, bud n-2 low R:FR, and bud n-2 high R:FR. The MAS5 summarization algorithm was used to normalize the data based on the median values and to assign flags to each feature indicating "present", "absent" or "marginal". The data was filtered to retain only features that were flagged "present" in all three replicates of at least one sample and this reduced the number of genes from an initial 22810 (for which some signal was detected) to 15466. This set was subjected to a two-way factorial analysis of variance (ANOVA) to identify differentially expressed features (p<0.05), and false positives were subsequently controlled using "Q" to apply the Benjamini Hochberg method to estimate the false discovery rate (FDR) with q<0.05. The statistics (ANOVA and FDR) produced three major categories of features showing significant expression responses; main effect of light (2486), main effect of bud position (9022), and interaction effect of light and position (2049). The statistical grouping resulted in features occurring in overlapping categories. Venn diagrams were used to sort the features into groups that were specific for light effects (387), bud position effects (6170), combined effects of light and bud position (1210), and interaction effects (2049) that created a basis for the cluster analysis.

For cluster analysis, the four biologically meaningful comparisons used were: bud n, low R:FR v. high R:FR; bud n-2, low R:FR v. high R:FR; low R:FR, bud n v. bud n-2; high R:FR, bud n v. bud n-2. Supervised clustering was used to generate clusters showing main effects of light (2 clusters; up in low R:FR; down in low R:FR), bud position (2 clusters; up in bud n; down in bud n), and the combined effects of light and bud position (4 clusters; up in low R:FR, up in bud n; up in low R:FR, down in bud n; down in low R:FR, up in bud n; down in low R:FR, down in bud n). Clustering of the interaction group was challenging since there were numerous possible combinations of interactions that were difficult to interpret. A pairwise t-test was first conducted based on the four biologically meaningful comparisons and features with expression differences with $p < 0.05$ in any comparison were retained. The features were then grouped into clusters based on significance and expression; up-regulated, down-regulated, not significantly different giving rise to 81 theoretical clusters.

4.5.6 Motif and GO analysis

Motif analysis was performed using the visualization tool on the Athena website (http://www.bioinformatics2.wsu.edu/cgi-bin/Athena/cgi/visualize_select.pl) to search for overrepresented cis-regulatory elements and putative transcription factor binding sites within 1000bp upstream region of the selected genes. GO analysis was performed using the GO enrichment tool on the AtCOECiS website (http://bioinformatics.psb.ugent.be/ATCOECIS/).

CHAPTER V

IS PHYTOCHROME REGULATED BRANCHING

MEDIATED BY AUXIN?

5.1 Introduction

Shoot branching (tillering in monocots) determines overall plant architecture, efficient

resource utilization, and final crop yield (Gerik and Neely, 1987; Bandaru et al., 2006).

In addition to intrinsic genetic programs, phytohormones and abiotic factors like light,

temperature, water, and nutrients play a critical role in determining the branching habit

(Jones and Kirby, 1977; Deregibus et al., 1983; Casal et al., 1986). The effects of light

quality on plant growth and development have been studied for several decades. In

crowded environments, reduced transmittance of red light (R, ~660 nm) to the lower

strata and/or increased reflectance of far-red light (FR, ~730 nm) from neighboring

plants results in lower R:FR (Ballare et al., 1990). Perception of low R:FR by

phytochromes triggers a series of developmental responses termed the *'shade avoidance*

responses' (SARs) (Smith and Whitelam, 1997). Plants with a typical shade avoidance

phenotype have elongated internodes, fewer branches and leaves, reduced leaf area,

smaller inflorescences with fewer and smaller grains, and an early flowering pattern

(Smith and Whitelam, 1997; Aphalo et al., 1999). R:FR acts as both a bud non-

autonomous signal influencing the branching process from remote organs like leaves,

roots, and/or the shoot apical meristem and as a bud autonomous signal. However, a

detailed mechanistic understanding about how plants adapt to diverse environmental conditions is lacking, especially with respect to shade avoidance responses and branching. Plants with a lesion in *PHYB*, which encodes a R:FR sensor, show a constitutive shade avoidance phenotype (similar to plants grown under high density) with increased apical dominance.

Auxin was the first plant hormone to be discovered and is still regarded as the master controller of plant growth and development. Auxin has been shown to promote cell division, cell elongation and inhibit shoot axillary branching. However, its role in shaping these basic developmental processes in shade environments is unclear. Auxin has been proposed to play a critical role in modulating developmental plasticity in response to environmental cues such as light, shade, and temperature. In typical agricultural environments, dense planting causes a decreased red to far-red light ratio (R:FR) which results in increased elongation of stem-like organs (including stem, petiole, and internodes) and a reduction in leaf and branch numbers. The role of auxin in modulating shade avoidance responses, especially stem elongation, has been demonstrated by (Tao et al., 2008). In a different study it was reported that auxin is involved in the reduction of leaf numbers and suggested that it may play a major role in regulating branching (Carabelli et al., 2007). *Apical dominance*, is a type of *correlative inhibition*, where auxin produced in the shoot apex and transported basipetally through the polar auxin transport (PAT) stream determines branching. A similar process where upper dominating branches regulate the outgrowth of lower branches is termed *apical*

control, and is also a type of correlative inhibition. Auxin regulation of apical

dominance has been studied for many years but its role in regulating the apical

dominance phenotype of plants deficient in phyB has not been described.

Branches form at the leaf axils as a result of the activity of axillary meristems that give

rise to axillary buds. Upon initiation, axillary buds may enter a state-of-transition, where

they either grow out to produce branches or remain dormant until they perceive suitable

signals to grow. The phytohormone auxin and light/shade signals are key regulators of

the process of axillary bud outgrowth. Auxin is known to influence the bud outgrowth

in organ-autonomous and non-organ-autonomous means by acting either directly or in

association with other signaling compounds. In the organ-autonomous mode of

regulation, auxin levels in the axillary bud are critical to regulate the outgrowth process.

Auxin concentration has been shown increase in the axillary buds during the bud

outgrowth process and during formation of new organs, since auxin is required to

promote cell division and cell elongation (Gocal et al., 1991).

Apart from the organ-autonomous mode where, auxin concentrations in the bud

determining bud outgrowth, two other schools of thought to explain the role of auxin in

bud outgrowth: one school supports the vascular connection theory, another non-organ-

autonomous theory. The vascular connection theory contends that high stem auxin

levels prevent the bud from exporting auxin and establishing vascular connections with

the main shoot, resulting in inhibition of bud outgrowth (Lazar and Goodman, 2006;

Ongaro and Leyser, 2008). According to the non-organ-autonomous theory, auxin travelling through the PAT stream inhibits bud outgrowth without entering the buds, but by signaling through second messenger(s) like cytokinin (Tanaka et al., 2006), and strigolactone (Brewer et al., 2009).

Previous studies from our lab addressing branching in response to R:FR signals and/or *phyB* loss of function noted that increased apical dominance is determined at both the organ level (axillary bud) and at the whole plant level (Kebrom et al., 2006; Finlayson et al., 2010). These studies, along with other observations that *phyB* null sorghum (58M) tillers profusely after harvesting heads (much greater than WT, 100M), raised a series of questions. Is auxin mediating the increased apical dominance phenotype of *phyB* null mutants? Can disruption of the PAT stream defeat the apical dominance phenotype? Does auxin abundance or its transport rate in the stem dictate the branching phenotype of shade avoiding plants? Will disruption of auxin transport from outgrowing branches prevent outgrowth and release apical control? Since sorghum is not the best model system to study apical dominance and related processes, we choose to use Arabidopsis to test these questions. The results suggested that phytochrome regulation of branch outgrowth is very complex and may involve other novel components in addition to auxin.

5.2 Results

5.2.1 Disruption of the PAT stream by decapitation or TIBA treatment reduces apical dominance of *phyB* and WT plants grown in low R:FR

Increased apical dominance is one of the major characteristics of loss of phyB function and implies a role for auxin in the process. We investigated the involvement of auxin by decapitating the entire main stem of WT plants grown under low R:FR and high R:FR and compared branching parameters. Similar experiments were also conducted using WT and *phyB* null mutant plants grown under high R:FR. The results showed that removing the auxin source from organs above the rosette results in the release of apical dominance in plants grown under low R:FR but not in plants grown under high R:FR. The release of apical control was measured by the correlative inhibition index (CII), which is an estimate of inhibitory signals from the shoot apex or superior branches (Thimann et al., 1934; Finlayson et al., 2010). Decapitation had little effect on the CII of plants grown under high R:FR (Figure 5.1). However, the CII was reduced in decapitated WT plants compared to undecapitated plants grown under low R:FR, and similar observations in *phyB* mutant plants under high R:FR suggested auxin could be involved. The role of auxin was investigated further by treating the main stem (just above the rosette) of WT and *phyB* plants with a ring of TIBA, a polar auxin transport inhibitor. TIBA reduced the CII of *phyB* to levels similar to WT. The results indicate that either decapitation or TIBA treatment could restore the CII of *phyB* to levels similar

to WT, supporting a role for auxin in suppressing branching under low R:FR or with

phyB deficiency (Figure 5.2).
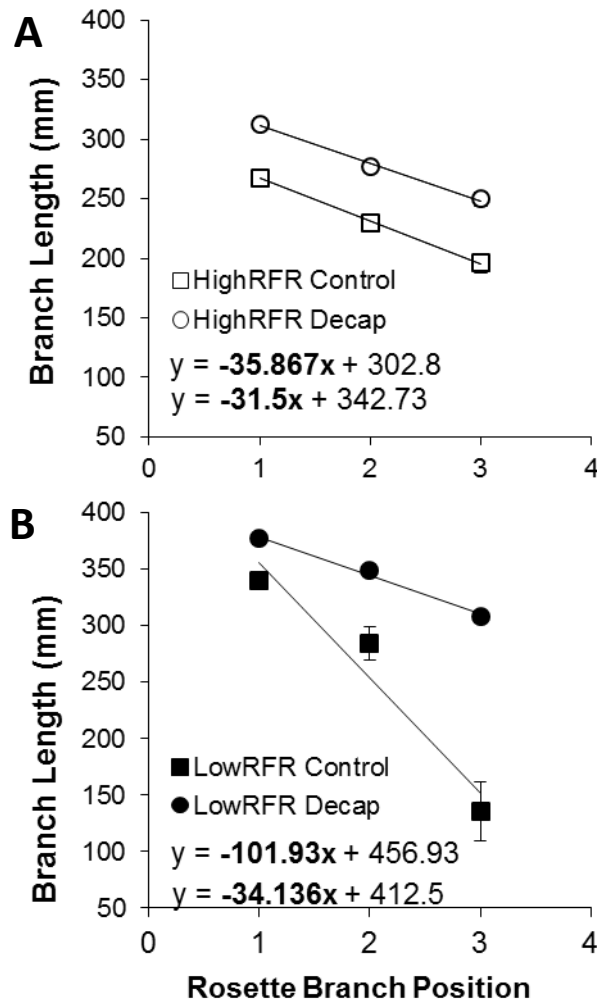
Figure 5.1 Slopes of correlative inhibition index of plants grown under high
and low R:FR. The error bars represent standard error, n=24 (±2). Decap –
decapitation or removal of entire inflorescence stem.
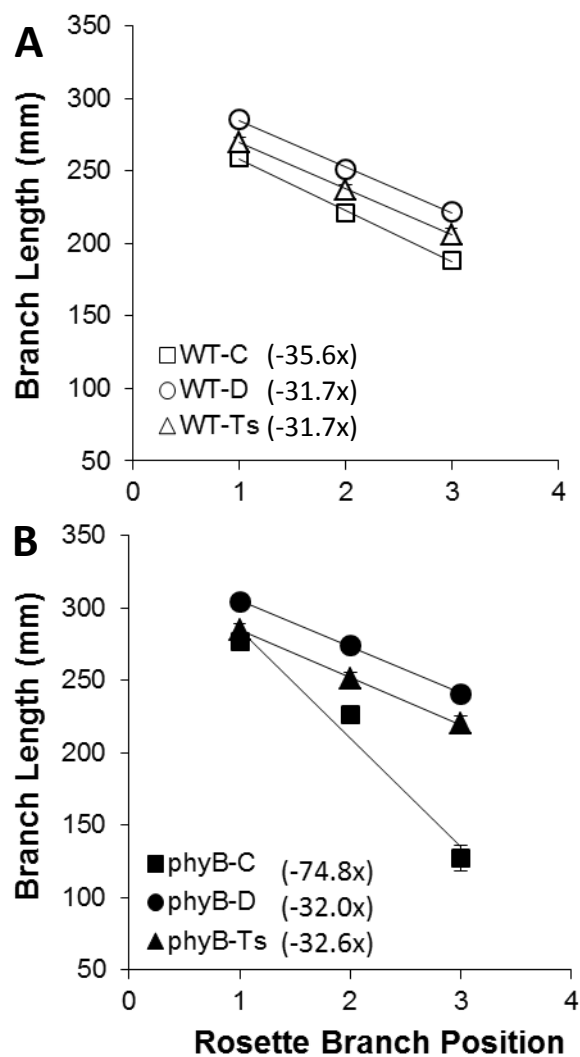
Figure 5.2 Slopes of correlative inhibition index of WT and *phyB* mutants in response to decapitation and TIBA treatment to the main stem just above the rosette. The slopes of the corresponding treatments are presented in the parenthesis next to the label. The error bars represent standard error, n = 105 (±10), 70 (±2), 54 (±1) for control, decapitation, and TIBA treatment (20mg/g of lanolin) respectively. WT – wild type; phyB – phyB mutant; C – control; D – decapitation or removal of entire inflorescence stem; Ts – TIBA treatment to main stem.

5.2.2 Decapitation of *phyB* results in increased auxin signaling in the axillary buds

Decapitation resulted in a reduced CII and increased branching in *phyB* mutants.

Previous studies have shown that auxin levels were higher in outgrowing buds following

decapitation (Gocal et al., 1991). To investigate if a similar phenomenon might occur in

the case of *phyB* buds promoted to grow out by decapitation, we crossed transgenic

plants containing a synthetic auxin responsive promoter (*DR5*) driving expression of the

GUS reporter gene with *phyB* and recovered plants homozygous for both traits.

Decapitation of *phyB* resulted in increased expression of the *DR5:GUS* reporter in

axillary buds in less than 3 hours which demonstrates that auxin signaling is elevated

(Figure 5.3). WT buds showed less change in DR5:GUS expression with the same

treatment.

5.2.3 Bud outgrowth can be inhibited by preventing auxin export from the growing

buds

The role of auxin moving through the PAT stream in suppressing branch outgrowth is

well documented, but very little is known about the influence of auxin transport in the

axillary buds/branches on the process. Several reviews suggested that auxin export from

the growing bud is critical to its outgrowth (Ongaro and Leyser, 2008; Leyser, 2009).

To test if establishment of a polar auxin transport stream in the outgrowing branches is

necessary for outgrowth, TIBA was applied as a ring to each of the top three rosette buds

in WT, *phyB*, and *max-4* mutants on 2 DPA. Preventing auxin transport from the top

three rosette branches resulted in suppression of outgrowth compared to the untreated

control. Bud outgrowth kinetics (Figure 5.4) and growth rate estimates (Figure 5.5)

revealed that TIBA treatment resulted in arrest of bud outgrowth in less than 24 hours.

Branching analysis at 10 DPA showed that suppression of outgrowth was similar among
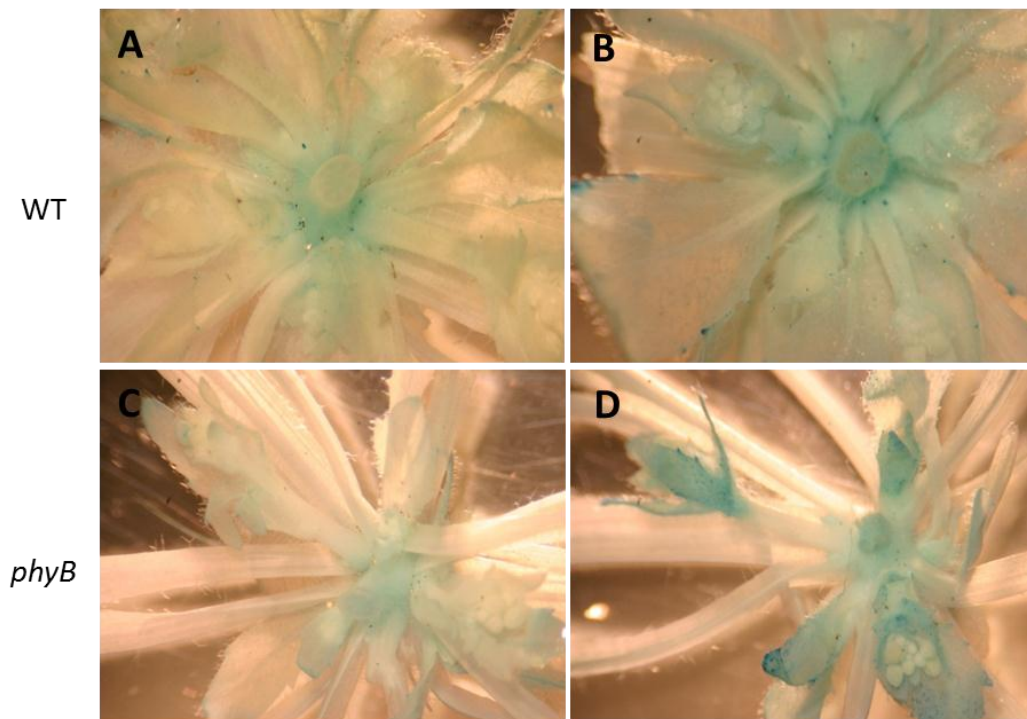
Figure 5.3 Auxin responsiveness in axillary buds 3 hours after decapitation using
DR5:GUS reporter system in WT and *phyB*. Pictures A and C represent rosette
region of control plants in WT and *phyB* respectively while, B and D after 3 hours
following decapitation of main stem in WT and *phyB* respectively.
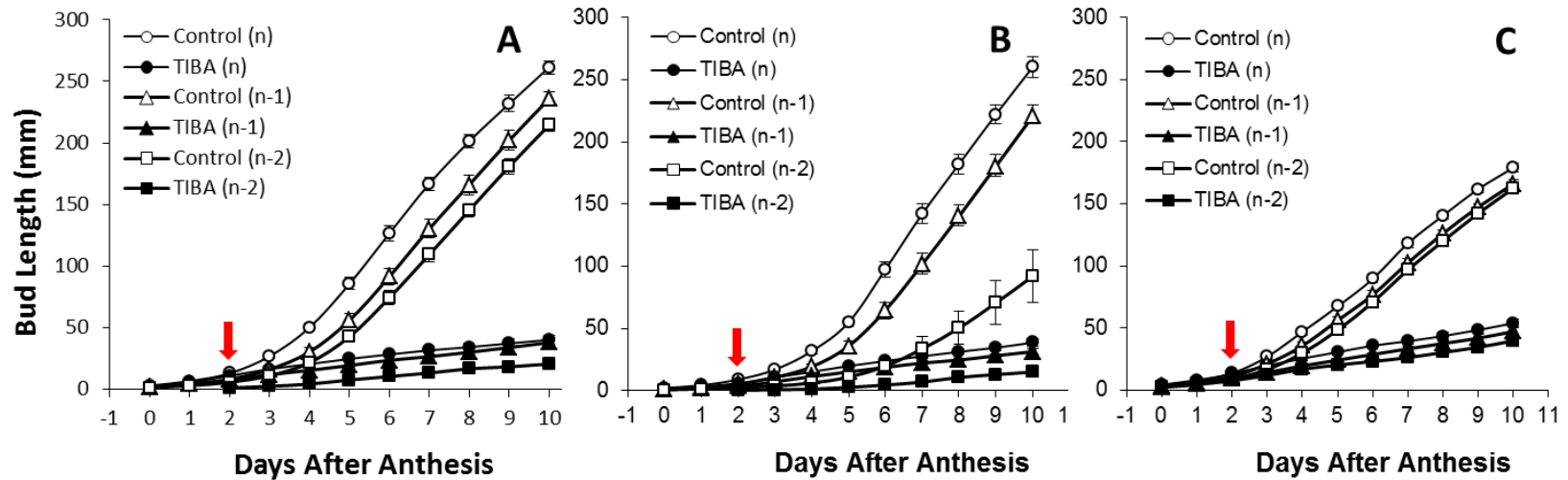
Figure 5.4 Branch outgrowth kinetics of top three rosette braches in WT (A), *phyB* (B), and *max4* (C) mutants treated with either TIBA (20mg/g of lanolin) or control (Lanolin). The red arrow indicate the day of TIBA treatment. The error bars represent standard error; n = 17 (±1); the experiment was repeated one more time with similar number of plants to confirm the results. WT – wild type; TIBA – TIBA treatment to the axillary bud; n – top most rosette branch; n-1 – branch below n; and n-2 branch below n-1.
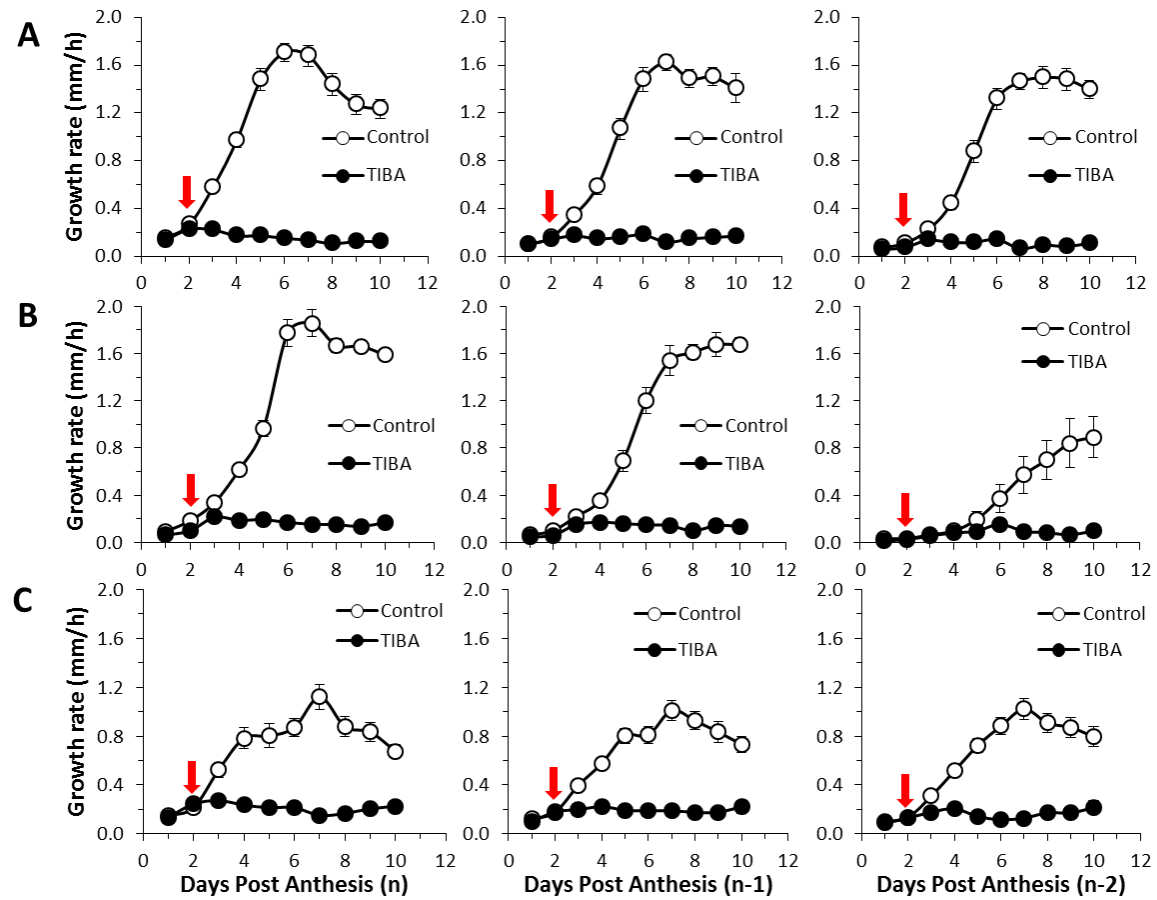
Figure 5.5 Branch outgrowth rates of top three rosette braches in WT (A), *phyB* (B), and *max4* (C) mutants treated with either TIBA (20mg/g of lanolin) or control (Lanolin). The red arrow indicate the day of TIBA treatment. The error bars represent standard error; n = 17 (±1); WT – wild type; TIBA – TIBA treatment to the axillary bud; n – top most rosette branch; n-1 – branch below n; and n-2 branch below n-1.

all TIBA treated buds across all three genotypes (WT, *phyB*, and *max-4*). This indicates

that export of auxin from growing buds is necessary to allow outgrowth.

5.2.4 Superior branches restrict the outgrowth of axillary buds at lower positions

The role of auxin in apical control was examined by preventing bud outgrowth by

application of TIBA to the top three rosette buds. The numbers of rosette leaves and

axillary buds initiated in control and TIBA treated plants were similar but TIBA

treatment of superior branches promoted the outgrowth of lower branches compared to

untreated controls (Figure 5.6). The total number of rosette branches increased to 10.2

and 6.8 in WT and *phyB* after TIBA treatment compared to 5.0 and 3.3 in untreated

controls. In *max4*, the number of rosette branches after TIBA treatment remained

unaltered because *max4* had already branched to its potential (one branch from each

axil). The increase in branch numbers following TIBA treatment in WT and *phyB* was

due to increased outgrowth of branches at lower positions.

5.2.5 Correlative inhibition is quantitative

To determine if the process of correlative inhibition is quantitative, various sources of

inhibitory signals (such as auxin) including the shoot apex, cauline branches, and the

entire main stem were removed. The results showed that the CII of *phyB* was reduced

by all treatments and that the effect was greatest with complete decapitation compared to
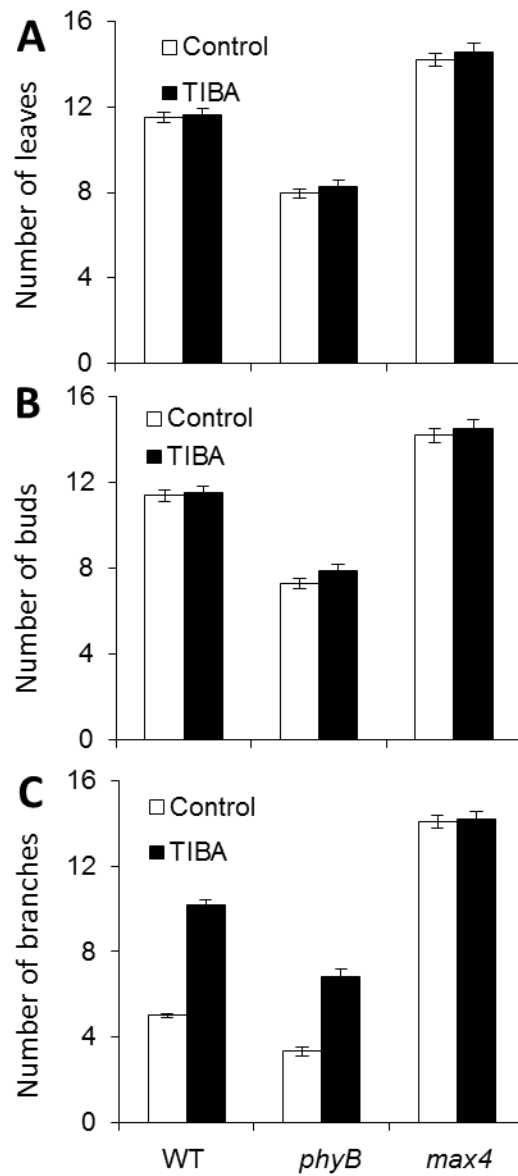
Figure 5.6 Number of rosette leaves (A), axillary buds (B), and rosette branches (C) in response to TIBA (20mg/g of lanolin) treatment in WT, *phyB*, and *max4* mutants. The TIBA treatment to axillary buds was made on 2 days post anthesis (DPA) and branching data was recorded on 10 10 DPA. The error bars represent standard error; n = 17 (±1).

the removal of just the shoot apex or cauline branches (Figure 5.7). The CII of WT was largely unresponsive to any of the treatments. This suggests that there may be greater auxin transport through the PAT stream (or some other inhibitory signal) in *phyB*, resulting in a greater CII. The axillary branches of *phyB* grew longer when two or more sources of auxin were removed compared to just one, suggesting a quantitative nature of branch outgrowth regulation.

5.2.6 Lesion in *PHYB* results in reduced auxin transport capacity and slower PAT stream

To test if increased apical dominance in *phyB* was due to higher auxin concentrations in the stem we estimated the amount of auxin in WT and *phyB* stem segments using GC-MS. The results showed that IAA concentrations of WT and *phyB* stem segments were relatively similar (data not presented). Since auxin levels were not different, it was possible that an increased auxin transport rate promotes apical dominance in *phyB*. To examine this possibility 1µl of ^{3}H labeled IAA was applied to the shoot apical meristem at three different concentrations (2.5 µM, 25 µM, and 250 µM) and the radiolabelled auxin was allowed to be transported for 150 minutes in both WT and *phyB* inflorescence stems. It was found that WT transports more auxin with greater velocity than *phyB* at all three concentrations (Figure 5.8). These results conflict with the conclusions drawn from the decapitation and TIBA treatment experiments but may suggest that auxin could

be promoting apical dominance in *phyB* in conjunction with an additional unknown

component.
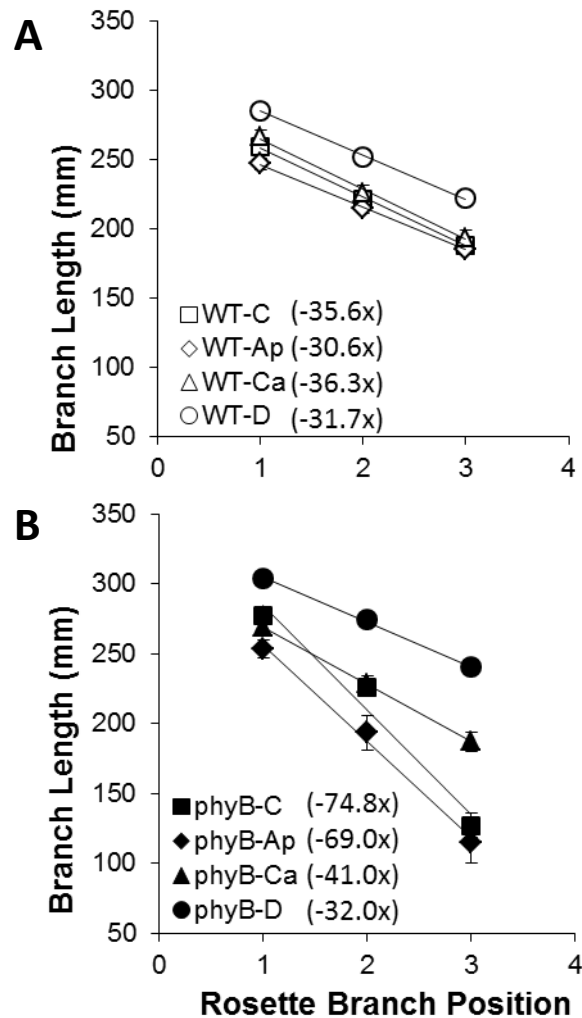
Figure 5.7 Slopes of correlative inhibition index of WT (A) and *phyB* (B) mutant in response to removal of various sources of inhibitory signals. The slopes of the corresponding treatments are presented in the parenthesis next to the label. Branching was measured 10 days post anthesis. The error bars represent standard error; n = 105 (±10), 33 (±2), 35 (±3) 70 (±2), for control (C), only shoot apex removed (Ap), cauline branches removed (Ca), and decapitation (D) respectively; WT – wild type; phyB – *phyB* mutant.
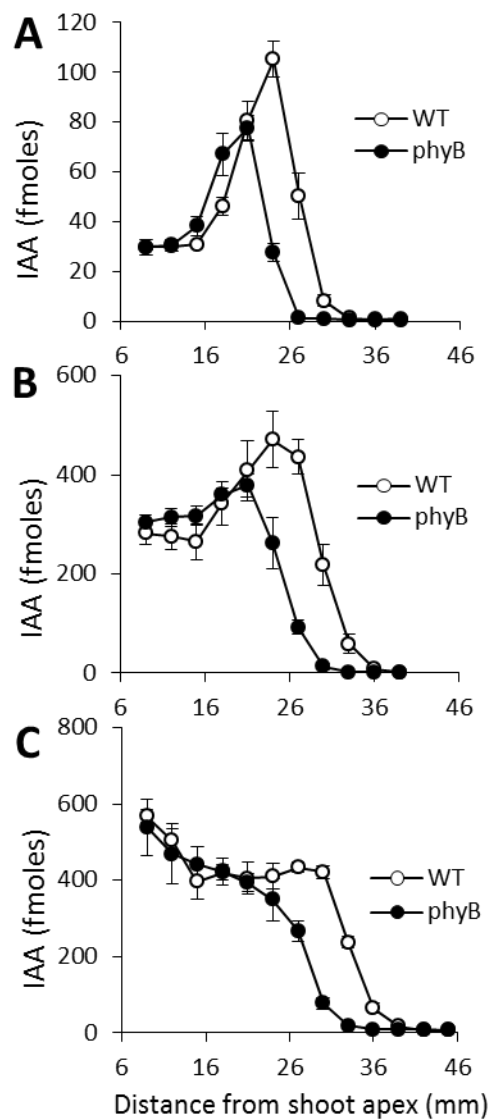
Figure 5.8 Estimates of polar auxin transport rate of ^{3}H labeled IAA applied at shoot apex in WT and *phyB* mutant. The radiolabelled IAA was applied at three different concentrations 2.5 μM IAA (A), 25 μM IAA (B), and 250 μM IAA (C) and 3mm long stem sections were cut after 150 min after treatment to determine the transport rate. The error bars represent standard error; n = 5.

5.2.7 Reduction of auxin transport in *phyB* is associated with fewer xylem parenchyma cells

Auxin transport has been shown to be facilitated by xylem parenchyma cells in the vascular bundles. We hypothesized that the reduction in auxin transport in *phyB* stems could be due to either differences in vascular bundle numbers or a differential arrangement of the vascular tissue. Histological sections of WT and *phyB* stem segments revealed that both genotypes had similar numbers of vascular bundles, but the arrangement of the associated cells differed (Figure 5.9). WT stem sections showed increased numbers of xylem parenchyma cells compared to *phyB*, which likely explains the greater auxin transport rate in WT.

5.2.8 *phyB* is less sensitive to exogenous auxin compared to WT

Confounded by results from auxin transport studies and auxin quantification, we investigated the sensitivity of *phyB* to auxin. Arabidopsis WT and *phyB* seedlings with the *DR5:GUS* reporter gene were grown in agar media and treated with several IAA concentrations (control, 1 μM, 10 μM and 100 μM) for various duration (3, 6, 12, and 24 hours) on 5-6 DAP. At 10 μM and 100 μM concentration, both WT and *phyB* demonstrated similar (excessive) staining while, at 1 μM differential staining was apparent. WT plants at 1 μM showed increased expression of early DR5 as indicated by blue staining in cotyledon leaves, shoot apex, root tips, and elongating regions of root

and stem segments compared to *phyB* (Figure 5.10). Reduced staining was also noticed

in shoot tissues compared to roots.

Figure 5.9 Cross section of WT and *phyB* stem segments portraying vascular arrangement. P – phloem; XP – xylem parenchyma; PX – proto xylem; MX – meta xylem.

5.2.9 Treatment with IAA following decapitation to the main stem does not reverse the release of apical dominance

IAA suspended in lanolin was applied to decapitated stems of both WT and *phyB* at 500µM and 5mM concentration to test if supplemental auxin would re-impose apical dominance by preventing bud outgrowth. IAA was ineffective in inhibiting bud outgrowth at both rates, indicating that apical dominance lost after decapitation cannot be restored (Figure 5.11).
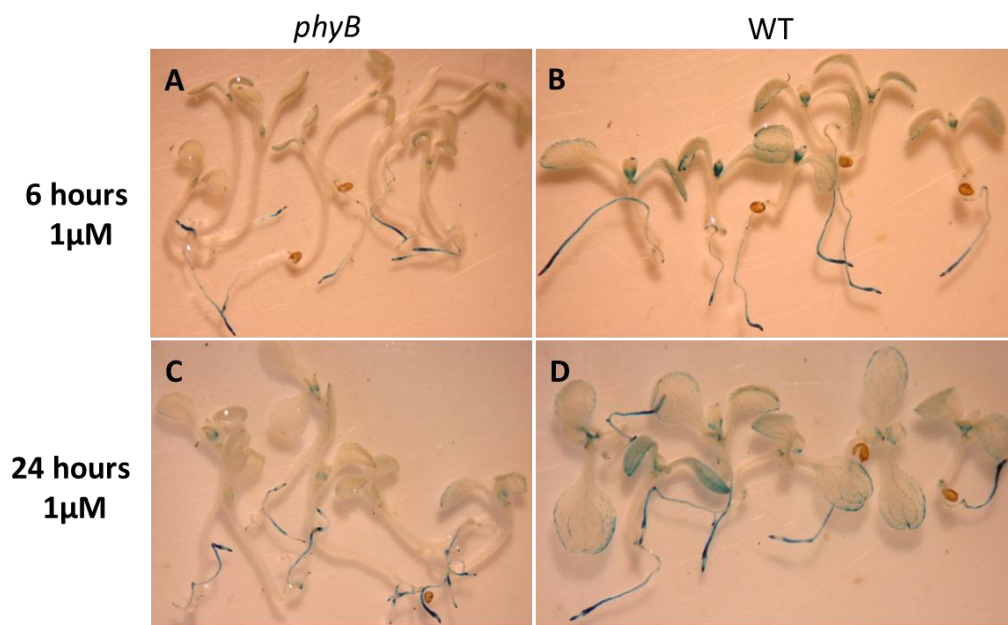
Figure 5.10 Auxin sensitivity of WT and *phyB* seedling in response to 1 µM exogenous IAA using DR5:GUS reporter system. Pictures A and C represent *phyB* seedlings 6 and 12 hours after treatment with exogenous IAA respectively; B and D display similar treatments in WT.
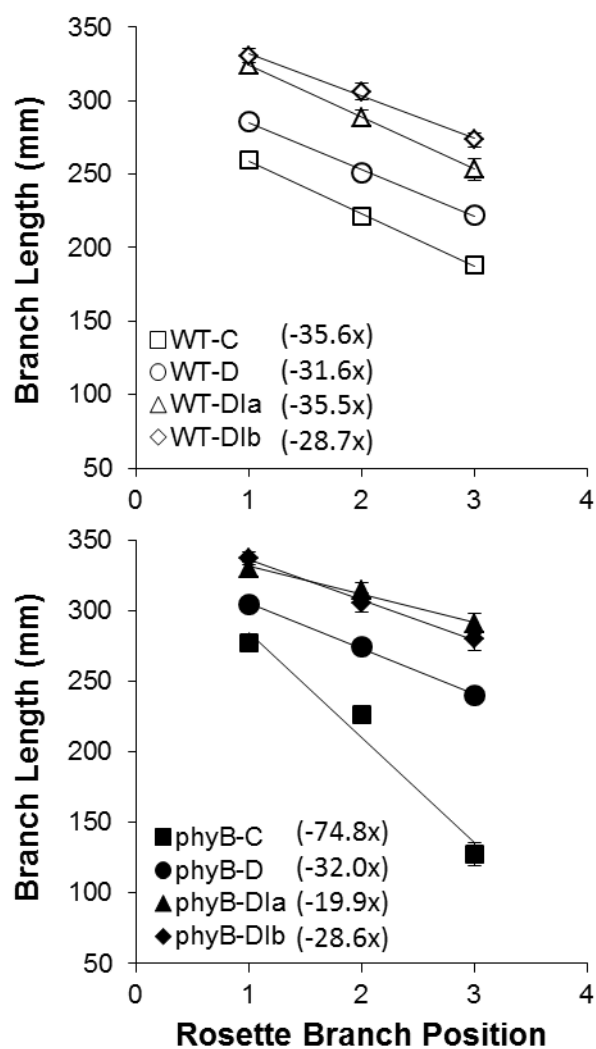
Figure 5.11 Slopes of correlative inhibition index of WT (A) and *phyB* (B) mutant in response to decapitation followed by IAA treatment. The slopes of corresponding treatment are presented in the parenthesis next to the label. The branching was measured 10 days post anthesis. The error bars represent standard error; n = 105 (±10), 72 (±2), 18, and 18 for control (C), decapitation (D), decapitation followed by IAA (500µM) in lanolin (DIa) and decapitation followed by IAA (5 mM) in lanolin (DIb) respectively; WT – wild type; phyB – *phyB* mutant.
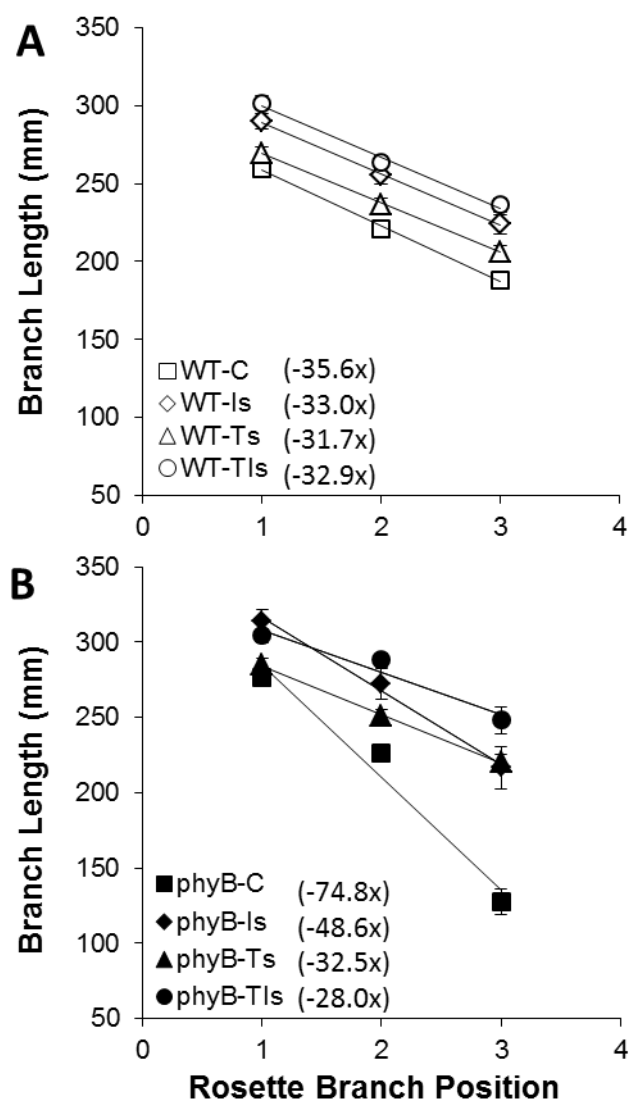
Figure 5.12 Slopes of correlative inhibition index of WT (A) and *phyB* (B) mutant in response to treatment with auxin and TIBA. The slopes of corresponding treatment are presented in the parenthesis next to the label. The branching was measured 10 days post anthesis. The error bars represent standard error; n = 105 (±10), 21, 22 (±2), and 54 (±1), for control (C), a ring of IAA (500μM) in lanolin to main stem (Is), TIBA (20mg/g of lanolin) to main stem (Ts), and ring of TIBA followed by ring of IAA (TIs) respectively; WT – wild type; phyB – *phyB* mutant.

5.3 Discussion

5.3.1 Removal of apical auxin source releases apical dominance in *phyB* mutants

Auxin is one of the most important hormones in plant biology given the fact that it

regulates a vast number of developmental events throughout the plant life cycle. Auxin

mediated apical dominance has been studied for about 80 years, yet how auxin works,

especially in mediating SARs, is not well established. The lack of a definitive

mechanism could possibly be due to multiple pathways and complex interactions among

signals (Jaillais and Chory, 2010; Leyser, 2010; Martinez-Garcia et al., 2010).

Light/shade regulated morphological changes in young seedlings have been attributed to

differential auxin biosynthesis and distribution (Morelli and Ruberti, 2000; Tao et al.,

2008), while regulation of the branching habit in mature plants involves auxin transport,

fluxes, and long range root-shoot signaling (Morelli and Ruberti, 2000; Leyser, 2009).

Our results show that removal of the auxin source by decapitation or specifically

blocking stem polar auxin transport using an auxin transport inhibitor results in release

of apical dominance (measured using CII) in *phyB* but not in WT (Figure 4.2). Another

experiment conducted using WT plants grown in high and low R:FR also showed similar

results, where the CII of decapitated plants under low R:FR was reduced to match that of

the high R:FR treatment (Figure 5.1). In fact, our results also showed that auxin

mediated apical dominance in Arabidopsis is quantitative, since removal of multiple

auxin sources resulted in greater release of apical dominance compared to removal of just one (Figure 5.7).

5.3.2 Auxin transport from the growing bud is essential for bud outgrowth

In a recent paper, it has been reported that bud outgrowth following decapitation is associated with the establishment of an auxin transport stream between the axillary bud and the main stem via *PIN1* mediated canalization (Balla et al., 2011). The authors noted that before decapitation buds failed to transport ^{14}C labeled IAA, while after decapitation, outgrowing buds were able to export IAA into the stem, suggesting that dormant buds fail to establish a basipetal auxin transport stream. It is likely that the *phyB* mutation suppresses establishment of an auxin transport stream from lower axillary buds. After decapitation, auxin levels in the axillary buds increase resulting in relocalization of auxin transport proteins resulting in increased outgrowth (Gocal et al., 1991). Figure 5.3 supports this hypothesis since increased expression of *DR5:GUS* is apparent in 3 hours after decapitation in *phyB* but not WT. The auxin transport hypothesis proposed by several labs (Li and Bangerth, 1999; Leyser, 2005; Dun et al., 2006) suggests that enhanced auxin transport capacity in the stem and from the axillary buds determines outgrowth. Failure to establish an auxin transport stream from the growing buds would prevent bud outgrowth. This hypothesis is supported by our data showing that treatment with TIBA to the top three rosette buds resulted in suppression of bud outgrowth (Figure 5.4). The inhibition of bud outgrowth was evident within 24

hours after treatment with TIBA, especially in the rosette buds at higher nodes (Figure 5.5).

5.3.3 Auxin partially mediates apical dominance in *phyB*

Previous studies showed that increased branching in the *max4* mutant is due to an increased capacity to transport auxin (Bennett et al., 2006). The authors showed that isolated *max4* inflorescence stem sections transported more radiolabeled IAA compared to WT as a result of increased expression of PIN1 protein, a polar auxin efflux carrier. However, the role of auxin transport capacity in regulating branching is questionable, since application of strigolactone to the buds of decapitated plants immediately stops the outgrowth, while TIBA treatment resulted in slowing down the outgrowth rate (Beveridge et al., 2009; Brewer et al., 2009; Ferguson and Beveridge, 2009). Decapitation induced axillary bud outgrowth may not be due to a direct involvement of auxin in releasing the repression (auxin is still believed to regulate the elongation process following release of repression), but may be due to the action of second messenger/s like strigolactone or other novel signal/s (Brewer et al., 2009).

Overall our results strongly suggest that auxin mediates apical dominance in *phyB*, but auxin transport assays using radiolabelled IAA and measurements of auxin concentrations in the stem sections suggest otherwise, indicating the potential for a novel additional component. The apical dominance theory suggests that increased polar

transport from the shoot apex would result in suppression, but we found that the auxin concentration in WT and *phyB* stem sections was similar (data not presented). Also, our results in intact plants showed that *phyB* transported less ^{3}H IAA compared to WT (Figure 5.8). Further examination of vascular tissue revealed that the reduced auxin transport capacity of *phyB* mutants is associated with a reduction in xylem parenchyma cells involved in basipetal auxin transport (Figure 5.9). Auxin transport and concentrations in *phyB* stem regions contradicted the decapitation and TIBA experiments suggesting that auxin might mediate apical dominance in association with an additional component. In addition to auxin fluxes in the stem, the increased auxin sensitivity of *phyB* roots compared to shoots may indicate that loss of *phyB* function could promote the activity of an upward moving inhibitor. It seems likely that the increased apical dominance phenotype of *phyB* mutants or WT with low R:FR treatment results from the PAT stream acting with other unidentified components to restrict the ability of the axillary buds to export auxin.

5.4 Conclusions

Taken together, our results demonstrate that auxin is likely a key hormone in mediating the increased apical dominance phenotype of *phyB*. Apically synthesized auxin may play an important role in correlating the outgrowth of branches at various nodes. Auxin dynamics in the plant, including transport (in the stem and from the growing bud), sensitivity, and cross-talk with other hormones mediate plant architecture. The

involvement of additional unidentified components acting with auxin to regulate branching in plants exhibiting SARs cannot be ruled out.

5.5 Methods and material

5.5.1 Plant materials and growth conditions

The Arabidopsis (*Arabidopsis thaliana*) Columbia ecotype was used throughout. Sources of Wild-type col-60000, *axr1-12*, *phyB* (*phyB-9*), and *max4* (*max4-538*) seeds were previously described (Finlayson et al., 2010). Seeds were stratified in 2ml micro-centrifuge tubes at 4°C for 3 days and planted on to 36-cell trays containing Metromix 200 soilless medium top dressed with Ready-Earth soilless medium. Plants were fertilized with 7ml Hoagland solution, 1x concentration, applied every week. Plants were grown in two growth chambers, both maintained at 16/8 h light/dark photoperiod and 24/18°C day/night temperature. In both growth chambers, light was maintained at 190 (±5) μMol m^{-2} sec^{-1} photosynthetic photon flux density (PPFD), using a mixture of either T12 very high output lamps with compact fluorescence or using T8 cool-white high output lamps with incandescence lamps. For high R:FR treatments the ratio was maintained at >3.5 while, for low R:FR treatments the ratio was brought down to 0.08.

5.5.2 Treatments

Several different sets of experiments were conducted as following: 1) The top 3 rosette buds of WT, *phyB*, and *max4* plants were treated with either lanolin with 20% ethanol (control) or lanolin with 20% ethanol and 2% TIBA (TIBA treated) at 2 days post anthesis (DPA); 2) One day before anthesis (DBA) or 0 DPA, the PAT stream was disturbed in WT or *phyB* by either decapitation or 2% TIBA treatment to the main stem just above the rosette; 3) Decapitation of the main stem of WT grown under low and high R:FR at 1 DBA; 4) Decapitation, removal of cauline branches, or removal of bud n at 1 DBA in WT grown under low and high R:FR; 5) Removal of shoot apex, removal of cauline branches, decapitation, or removal of bud n on 0 DPA in WT and *phyB* grown under high R:FR. 6) The spatial and temporal distribution of auxin responsiveness was determined in the top three rosette buds of WT and *phyB* following decapitation using DR5::GUS reporter activity; 7) Application of auxin to decapitated stems in *phyB* and WT. 8) Determination of polar auxin transport rate using ^{3}H labeled IAA in phyB and WT. 9) Quantification of auxin content in the stem segments of *phyB* and WT using GC-MS. 10) Determination of arrangement of vascular tissue in *phyB* and WT stem sections below and above the cauline branches.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The regulation of plant architecture, and especially the branching habit, in higher plants is one of the most complex processes given the fact that there are multiple signals with hierarchy, crosstalk, and feedback regulation among the key players. Even though the process has been studied for several decades and a lot of information about these signaling components has been generated, many crucial details remain unknown. Researchers are beginning to lay more emphasis on elucidating interactions between environmental signals and so called modulators/messengers within the plant and their downstream targets to understand the process of branching (Doust, 2007; Kebrom and Brutnell, 2007; Leyser, 2009).

Among several environmental signals, light is one of the key regulators of phenotypic plasticity and the associated branching habit. In agricultural environments, high density planting produces increased canopy shade and greater reflectance of far-red light from surrounding vegetation, resulting in a reduced R:FR (Ballare et al., 1997). The reduction in R:FR is perceived by phytochrome photoreceptor molecules, and serves as a warning signal about impending competition for light resources. This warning signal elicits SARs, characterized by reduced leaf growth, early flowering, and increased apical dominance (Martinez-Garcia et al., 2010). The molecular events leading to SARs have been studied in great detail in young seedlings by dissecting the process of

photomorphogenesis and to some extent flowering (Halliday and Fankhauser, 2003;

Franklin and Whitelam, 2005; Devlin et al., 2007; Jiao et al., 2007; Leivar and Quail,

2011). Very few studies have attempted to describe molecular events addressing R:FR

regulated branch outgrowth (Kebrom et al., 2006; Finlayson et al., 2010), a key

determinant of grain yield and biomass production under agricultural environments.

Low R:FR mediated axillary bud outgrowth has been shown to be regulated at the bud

autonomous (Kebrom et al., 2006; Finlayson et al., 2010) and non-bud autonomous

levels (Finlayson et al., 2010), possibly in association with phytohormones.

Nevertheless, a detailed mechanistic understanding of R:FR regulation of axillary bud

outgrowth at the hormonal and transcriptomic levels addressing the interactions remains

unclear. Our data suggested that low R:FR treatment from very early in the plant life

cycle promotes increased apical dominance and greater correlative inhibition. The

inhibitory signals under prolonged low R:FR accumulate over the period of time.

Treatment with low R:FR beginning just prior to anthesis (when the buds from upper

nodes are preparing to grow out) resulted in increased elongation of buds that were

triggered to grow out (from top three rosette positions) compared to high R:FR

treatment. Interestingly, the CII of the plants moved to low R:FR on 14 DAP was lower

than those grown continuously under high R:FR, demonstrating the promotive potential

of low R:FR on buds programmed to grow out.

The branching responses of plants grown with varying durations of low R:FR treatment suggested that low R:FR promotes a non-bud autonomous signal to suppress the outgrowth of axillary buds at lower positions. Architectural analysis prior to transcriptomic experiments revealed that low R:FR grown plants moved to high R:FR released the repression at lower nodes allowing bud outgrowth, but suppressed the elongation of outgrowing branches at top nodes. These results suggested that low R:FR acts as both a promotive and suppressive signal and the nature of this activity is imposed in a spatial and temporal manner.

In support of the phenotypic data, microarray experiments (3 hours after high R:FR treatment) indicated that many genes are differentially regulated in response to altered R:FR and different bud positions. Stringent statistical analysis using two-way factorial ANOVA ($p<0.05$) and Benjamini Hochberg FDR ($q<0.05$) to eliminate false positives yielded 9804 genes that showed significant differences in expression. This expression of this set of genes, representing more than 1/3 of the genome, was altered to produce the phenotype in response to altered light conditions and bud position. Careful observations suggested that only 387 genes (4%) were specifically regulated by light while 6170 (63%) genes were influenced by position effects. It is not surprising to see a huge number of genes being differentially regulated in response to bud position given the robust phenotypic differences between bud n and n-2. Additional analysis indicated that the expression of vast numbers of genes related to hormone biosynthesis, transport and signaling were altered in a bud autonomous manner to regulate the outgrowth process.

Motif analysis on individual clusters, super clusters, and super-sub clusters revealed interesting cis-regulatory elements being overrepresented in response to both light and bud position. Several of these motifs interact with hormone and light regulated transcription factor family proteins signifying the potential interaction between two key signaling pathways in mediating plant architecture.

Experiments investigating the increased apical dominance phenotype of the *phyB* mutant revealed that auxin is a key player mediating the process and that the effect of the apical auxin source is quantitative. Disturbance of the auxin source by decapitation or treatment with an auxin transport inhibitor results in release of apical dominance in *phyB* (lesser CII) compared to WT. The role of auxin in mediating apical dominance could be supported by an additional unknown component as indicated by reduced auxin transport rates/capacity in *phyB* compared to WT and similar auxin concentrations in stem sections of both genotypes.

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APPENDIX

Appendix Table 4.1 Systematic classification of genes into groups, clusters, super-clusters, and supersub-clusters based on four meaning comparisons labeled "a" through "d".

Sl #	Gr #	Cl #	Ldir "SUP"	Lpos "SUB"	Pdir "SUP"	Plite "SUB"	LRFR v HRFR n "a"	LRFR v HRFR n-2 "b"	n v n-2 LRFR "c"	n v n-2 HRFR "d"	No. of genes
1	1	1	1	A	4	Z	Up	Up	-	-	203
2	1	2	2	A	4	Z	Down	Down	-	-	183
3	2	1	4	Z	1	A	-	-	Up	Up	2535
4	2	2	4	Z	2	A	-	-	Down	Down	3627
5	3	1	1	A	1	A	Up	Up	Up	Up	57
6	3	2	2	A	2	A	Down	Down	Down	Down	144
7	3	3	1	A	2	A	Up	Up	Down	Down	622
8	3	4	2	A	1	A	Down	Down	Up	Up	385
9	4	1	1	B	4	Z	Up	-	-	-	16
10	4	2	2	B	4	Z	Down	-	-	-	17
11	4	3	1	C	4	Z	-	Up	-	-	53
12	4	4	2	C	4	Z	-	down	-	-	83
13	4	5	4	Z	1	B	-	-	Up	-	197
14	4	6	4	Z	2	B	-	-	Down	-	200
15	4	7	4	Z	1	C	-	-	-	Up	33
16	4	8	4	Z	2	C	-	-	-	Down	31
17	4	9	1	A	4	Z	Up	Up	-	-	0
18	4	10	3	D	4	Z	Up	Down	-	-	3
19	4	11	3	E	4	Z	Down	Up	-	-	4
20	4	12	2	A	4	Z	Down	Down	-	-	1
21	4	13	1	B	1	B	Up	-	Up	-	38
22	4	14	1	B	2	B	Up	-	Down	-	1
23	4	15	2	B	1	B	Down	-	Up	-	1
24	4	16	2	B	2	B	Down	-	Down	-	40
25	4	17	1	B	1	C	Up	-	-	Up	0
26	4	18	1	B	2	C	Up	-	-	Down	11

Appendix Table 4.1 continued…

27	4	19	2	B	1	C	Down	-	-	Up	10
28	4	20	2	B	2	C	Down	-	-	Down	1
29	4	21	1	C	1	B	-	Up	Up	-	0
30	4	22	1	C	2	B	-	Up	Down	-	141
31	4	23	2	C	1	B	-	Down	Up	-	212
32	4	24	2	C	2	B	-	Down	Down	-	0
33	4	25	1	C	1	C	-	Up	-	Up	17
34	4	26	1	C	2	C	-	Up	-	Down	0
35	4	27	2	C	1	C	-	Down	-	Up	2
36	4	28	2	C	2	C	-	Down	-	Down	26
37	4	29	4	Z	1	A	-	-	Up	Up	118
38	4	30	4	Z	3	D	-	-	Up	Down	3
39	4	31	4	Z	3	E	-	-	Down	Up	1
40	4	32	4	Z	2	A	-	-	Down	Down	134
41	4	33	1	A	1	B	Up	Up	UP	-	0
42	4	34	2	A	2	B	Down	Down	Down	-	0
43	4	35	1	A	2	B	Up	Up	Down	-	4
44	4	36	2	A	1	B	Down	Down	Up	-	3
45	4	37	3	D	1	B	Up	Down	Up	-	13
46	4	38	3	E	2	B	Down	Up	Down	-	4
47	4	39	3	E	1	B	Down	Up	Up	-	0
48	4	40	3	D	2	B	Up	Down	Down	-	0
49	4	41	1	A	1	C	Up	Up	-	UP	0
50	4	42	2	A	2	C	Down	Down	-	Down	1
51	4	43	1	A	2	C	Up	Up	-	Down	0
52	4	44	2	A	1	C	Down	Down	-	Up	0
53	4	45	3	D	1	C	Up	Down	-	Up	0
54	4	46	3	E	2	C	Down	Up	-	Down	0
55	4	47	3	E	1	C	Down	Up	-	Up	0
56	4	48	3	D	2	C	Up	Down	-	Down	3
57	4	49	1	B	1	A	Up	-	Up	UP	15
58	4	50	2	B	2	A	Down	-	Down	Down	13
59	4	51	1	B	3	D	Up	-	Up	Down	2
60	4	52	2	B	3	E	Down	-	Down	Up	0

Appendix Table 4.1 continued…

61	4	53	1	B	3	E	Up	-	Down	Up	0
62	4	54	2	B	3	D	Down	-	Up	Down	0
63	4	55	2	B	1	A	Down	-	Up	Up	2
64	4	56	1	B	2	A	Up	-	Down	Down	3
65	4	57	1	C	1	A	-	Up	Up	UP	3
66	4	58	2	C	2	A	-	Down	Down	Down	2
67	4	59	1	C	3	D	-	Up	Up	Down	0
68	4	60	2	C	3	E	-	Down	Down	Up	0
69	4	61	1	C	3	E	-	Up	Down	Up	2
70	4	62	2	C	3	D	-	Down	Up	Down	1
71	4	63	2	C	1	A	-	Down	Up	Up	105
72	4	64	1	C	2	A	-	Up	Down	Down	163
73	4	65	1	A	1	A	Up	Up	Up	Up	0
74	4	66	2	A	2	A	Down	Down	Down	Down	2
75	4	67	1	A	3	D	Up	Up	Up	Down	0
76	4	68	1	A	3	E	Up	Up	Down	Up	0
77	4	69	3	D	1	A	Up	Down	Up	Up	5
78	4	70	3	E	1	A	Down	Up	Up	Up	0
79	4	71	1	A	2	A	Up	Up	Down	Down	12
80	4	72	3	D	3	E	Up	Down	Down	Up	0
81	4	73	2	A	1	A	Down	Down	Up	Up	4
82	4	74	3	D	3	D	Up	Down	Up	Down	1
83	4	75	3	E	3	E	Down	Up	Down	Up	0
84	4	76	3	E	3	D	Down	Up	Up	Down	0
85	4	77	3	D	2	A	Up	Down	Down	Down	0
86	4	78	3	E	2	A	Down	Up	Down	Down	7
87	4	79	2	A	3	D	Down	Down	Up	Down	1
88	4	80	2	A	3	E	Down	Down	Down	Up	0
89	4	81					-	-	-	-	283

Sl# - serial number; GR# - group number; Cl# - cluster number; LRFR - low R:FR; HRFR - high R:FR; n - top most rosette bud; n-2 - third rosette bud from top; SUP – super-cluster; SUB – supersub-cluster.

Ldir: Direction of light effect

1   Promoted by low R:FR

2   Inhibited by low R:FR

3   Promoted by low R:FR at one position and inhibited at another

4   Unaffected by light

Lpos: position of light effect

A   Up or down in both buds

B   Up or down in bud n

C   Up or down in bud n-2

D   Up in bud n and down in bud n-2

E   Down in bud n and up in bud n-2

Z   Not changed in either bud

Pdir: direction of position effect

1   Promoted in bud n

2   Inhibited in bud n

3   Promoted in bud n in one light condition and inhibited in another

4   Unaffected by position

Plite: light effect on position

A   Up or down in both light conditions

B   Up or down in LRFR

C   Up or down in HRFR

D   Up in LRFR and down in HRFR

E   Down in LRFR and up in HRFR

Z   Not changed by either light condition

Appendix Table 4.2 Motif analysis details for individual clusters.

Sl#	G#	Cl#	MoE#	TF /Motif Name	Consensus Sequence	p-value
1	2	1	1	ABRE-like binding site motif	BACGTGKM	< 10e-4
2	2	1	2	CACGTGMOTIF	CACGTG	< 10e-4
3	2	1	3	CArG promoter motif	CCWWWWWWGG	< 10e-3
4	2	1	4	E2F binding site motif	TTTCCCGC	< 10e-3
5	2	2	1	EveningElement promoter motif	AAAATATCT	< 10e-9
6	2	2	2	ABFs binding site motif	CACGTGGC	< 10e-8
7	2	2	3	GBOXLERBCS	MCACGTGGC	< 10e-7
8	2	2	4	ABREATRD22	RYACGTGGYR	< 10e-7
9	2	2	5	DRE core motif	RCCGAC	< 10e-4
10	2	2	6	Hexamer promoter motif	CCGTCG	< 10e-4
11	2	2	7	UPRMOTIFIIAT	CCNNNNNNNNNNNNNCCACG	< 10e-4
12	2	2	8	ABRE-like binding site motif	BACGTGKM	< 10e-10
13	2	2	9	ACGTABREMOTIFA2OSEM	ACGTGKC	< 10e-10
14	2	2	10	CACGTGMOTIF	CACGTG	< 10e-10
15	2	2	11	GADOWNAT	ACGTGTC	< 10e-10
16	2	2	12	ABRE binding site motif	YACGTGGC	< 10e-10
17	3	1	1	CACGTGMOTIF	CACGTG	<0.001
18	3	2	1	TATA-box Motif	TATAAA	< 10e-3
19	3	2	2	ABRE-like binding site motif	BACGTGKM	< 10e-3
20	3	2	3	CACGTGMOTIF	CACGTG	< 10e-3
21	3	2	4	EveningElement promoter motif	AAAATATCT	< 10e-10
22	3	3	1	ABRE-like binding site motif	BACGTGKM	< 10e-7
23	3	3	2	ACGTABREMOTIFA2OSEM	ACGTGKC	< 10e-7
24	3	3	3	GADOWNAT	ACGTGTC	< 10e-6
25	3	3	4	Ibox promoter motif	GATAAG	< 10e-5
26	3	3	5	ABRE binding site motif	YACGTGGC	< 10e-4
27	3	3	6	ABFs binding site motif	CACGTGGC	< 10e-4
28	3	3	7	GBOXLERBCS	MCACGTGGC	< 10e-4
29	3	3	8	ATHB5ATCORE	CAATNATTG	< 10e-3
30	3	3	9	ABREATRD22	RYACGTGGYR	< 10e-3
31	3	3	10	ATHB1 binding site motif	CAATWATTG	< 10e-3
32	3	3	11	CACGTGMOTIF	CACGTG	< 10e-10
33	4	5	1	TELO-box promoter motif	AAACCCTAA	< 10e-4
34	4	6	1	ABRE-like binding site motif	BACGTGKM	< 10e-8
35	4	6	2	ACGTABREMOTIFA2OSEM	ACGTGKC	< 10e-7
36	4	6	3	GADOWNAT	ACGTGTC	< 10e-5
37	4	6	4	CACGTGMOTIF	CACGTG	< 10e-4
38	4	6	5	GBOXLERBCS	MCACGTGGC	< 10e-4
39	4	6	6	ABRE binding site motif	YACGTGGC	< 10e-3
40	4	6	7	ABFs binding site motif	CACGTGGC	< 10e-3

Appendix Table 4.2 continued…

41	4	22	1	ABRE-like binding site motif	BACGTGKM	< 10e-8
42	4	22	2	ACGTABREMOTIFA2OSEM	ACGTGKC	< 10e-7
43	4	22	3	CACGTGMOTIF	CACGTG	< 10e-4
44	4	22	4	ABRE binding site motif	YACGTGGC	< 10e-4
45	4	22	5	ABFs binding site motif	CACGTGGC	< 10e-4
46	4	22	6	GBOXLERBCS	MCACGTGGC	< 10e-4
47	4	22	7	GADOWNAT	ACGTGTC	< 10e-3
48	4	22	8	ABREATRD22	RYACGTGGYR	< 10e-3
49	4	23	1	TELO-box promoter motif	AAACCCTAA	< 10e-9
50	4	32	1	ABRE-like binding site motif	BACGTGKM	< 10e-4
51	4	32	2	DREB1A/CBF3	RCCGACNT	< 10e-4
52	4	32	3	ACGTABREMOTIFA2OSEM	ACGTGKC	< 10e-3
53	4	32	4	ABRE binding site motif	YACGTGGC	< 10e-3
54	4	48	1	TELO-box promoter motif	AAACCCTAA	< 10e-3
55	4	64	1	ABRE binding site motif	YACGTGGC	< 10e-6
56	4	64	2	Ibox promoter motif	GATAAG	< 10e-5
57	4	64	3	CACGTGMOTIF	CACGTG	< 10e-5
58	4	64	4	ABFs binding site motif	CACGTGGC	< 10e-3
59	4	64	5	GBOXLERBCS	MCACGTGGC	< 10e-3
60	4	64	6	ABRE-like binding site motif	BACGTGKM	< 10e-10
61	4	64	7	ACGTABREMOTIFA2OSEM	ACGTGKC	< 10e-10
62	4	64	8	GADOWNAT	ACGTGTC	< 10e-10
63	4	78	1	ACGTABREMOTIFA2OSEM	ACGTGKC	< 10e-4
64	4	78	2	ABRE-like binding site motif	BACGTGKM	< 10e-3
65	4	78	3	UPRMOTIFIAT	CCACGTCA	< 10e-3
66	4	78	4	TGA1 binding site motif	TGACGTGG	< 10e-3

Sl# - serial number; GR# - group number; Cl# - cluster number; Mo E# - motif entity number; TF – transcription factor.

Appendix Table 4.3 Super-clusters and supersub-clusters based on any effects of light.

Sl#	Ldir "SUP"	Lpos "SUB"	Pdir "SUP"	Plite "SUB"	SUP;SUB	# of Clusts	# of Genes	# of Motifs
	1	ABC/n	n/1234	n	**Ldir-1**	**18**	**1363**	**15**
1	1	A	n	n	**Ldir-1; LposA**	5	898	13
2	1	B	n	n	**Ldir-1; LposB**	7	86	0
3	1	C	n	n	**Ldir-1; LposC**	6	379	12
4	1	n	1	n	**Ldir-1; Pdir1**	5	130	1
5	1	n	2	n	**Ldir-1; Pdir2**	8	957	14
6	1	n	3	n	**Ldir-1; Pdir3**	2	4	0
7	1	n	4	n	**Ldir-1; Pdir4**	3	272	2
	2	ABC/n	n/1234	n	**Ldir-2**	**23**	**1239**	**4**
8	2	A	n	n	**Ldir-2; LposA**	9	724	1
9	2	B	n	n	**Ldir-2; LposB**	7	84	0
10	2	C	n	n	**Ldir-2; LposC**	7	431	1
11	2	n	1	n	**Ldir-2; Pdir1**	9	724	0
12	2	n	2	n	**Ldir-2; Pdir2**	8	229	2
13	2	n	3	n	**Ldir-2; Pdir3**	2	2	0
14	2	n	4	n	**Ldir-2; Pdir4**	4	284	0
	3	DE/n	n/1234	n	**Ldir-3**	**8**	**40**	**1**
15	3	D	n	n	**Ldir-3; LposD**	5	25	0
16	3	E	n	n	**Ldir-2; LposE**	3	15	3
17	3	n	1	n	**Ldir-3; Pdir1**	2	18	0
18	3	n	2	n	**Ldir-3; Pdir2**	3	14	0
19	3	n	3	n	**Ldir-3; Pdir3**	1	1	0
20	3	n	4	n	**Ldir-3; Pdir4**	2	7	0

Sl#- serial number; SUP – super-cluster; SUB – supersub-cluster; n – any effect.

Ldir: Direction of light effect

1 Promoted by low R:FR

2 Inhibited by low R:FR

3 Promoted by low R:FR at one position and inhibited at another

4 Unaffected by light

Lpos: position of light effect

A Up or down in both buds

B   Up or down in bud n

C   Up or down in bud n-2

D   Up in bud n and down in bud n-2

E   Down in bud n and up in bud n-2

Z   Not changed in either bud

Pdir: direction of position effect

1   Promoted in bud n

2   Inhibited in bud n

3   Promoted in bud n in one light condition and inhibited in another

4   Unaffected by position

Plite: light effect on position

A   Up or down in both light conditions

B   Up or down in LRFR

C   Up or down in HRFR

D   Up in LRFR and down in HRFR

E   Down in LRFR and up in HRFR

Z   Not changed by either light condition

Appendix Table 4.4 Motif analysis details for light effect based on super-clusters and supersub-clusters.

Sl#	Ldir "SUP"	Lpos "SUB"	Pdir "SUP"	Plite "SUB"	SUP;SUB	Mo E#	TF /Motif Name	p-value
1	1	ABC/n	n/1234	n	**Ldir-1**	1	Ibox promoter motif	< 10e-10
2	1	ABC/n	n/1234	n	**Ldir-1**	2	ABRE-like binding site motif	< 10e-10
3	1	ABC/n	n/1234	n	**Ldir-1**	3	CACGTGMOTIF	< 10e-10
4	1	ABC/n	n/1234	n	**Ldir-1**	4	ACGTABREMOTIFA2OSEM	< 10e-10
5	1	ABC/n	n/1234	n	**Ldir-1**	5	GADOWNAT	< 10e-10
6	1	ABC/n	n/1234	n	**Ldir-1**	6	ABRE binding site motif	< 10e-10
7	1	ABC/n	n/1234	n	**Ldir-1**	7	UPRMOTIFIIAT	< 10e-3
8	1	ABC/n	n/1234	n	**Ldir-1**	8	ABFs binding site motif	< 10e-10
9	1	ABC/n	n/1234	n	**Ldir-1**	9	ATHB5ATCORE	< 10e-4
10	1	ABC/n	n/1234	n	**Ldir-1**	10	UPRMOTIFIAT	< 10e-5
11	1	ABC/n	n/1234	n	**Ldir-1**	11	TGA1 binding site motif	< 10e-5
12	1	ABC/n	n/1234	n	**Ldir-1**	12	GBOXLERBCS	< 10e-10
13	1	ABC/n	n/1234	n	**Ldir-1**	13	ABREATRD22	< 10e-7
14	1	ABC/n	n/1234	n	**Ldir-1**	14	ATHB1 binding site motif	< 10e-4
15	1	ABC/n	n/1234	n	**Ldir-1**	15	GBF1/2/3 BS in ADH1	< 10e-3
16	1	A	n	n	**Ldir-1; LposA**	1	Ibox promoter motif	< 10e-6
17	1	A	n	n	**Ldir-1; LposA**	2	ABRE-like binding site motif	< 10e-10
18	1	A	n	n	**Ldir-1; LposA**	3	CACGTGMOTIF	< 10e-10
19	1	A	n	n	**Ldir-1; LposA**	4	ACGTABREMOTIFA2OSEM	< 10e-10
20	1	A	n	n	**Ldir-1; LposA**	5	GADOWNAT	< 10e-8
21	1	A	n	n	**Ldir-1; LposA**	6	ABRE binding site motif	< 10e-5
22	1	A	n	n	**Ldir-1; LposA**	7	ABFs binding site motif	< 10e-5
23	1	A	n	n	**Ldir-1; LposA**	8	ATHB5ATCORE	< 10e-4
24	1	A	n	n	**Ldir-1; LposA**	9	TGA1 binding site motif	< 10e-3
25	1	A	n	n	**Ldir-1; LposA**	10	UPRMOTIFIAT	< 10e-3
26	1	A	n	n	**Ldir-1; LposA**	11	GBOXLERBCS	< 10e-5
27	1	A	n	n	**Ldir-1; LposA**	12	ABREATRD22	< 10e-3
28	1	A	n	n	**Ldir-1; LposA**	13	ATHB1 binding site motif	< 10e-4
29	1	C	n	n	**Ldir-1; LposC**	1	Ibox promoter motif	< 10e-6
30	1	C	n	n	**Ldir-1; LposC**	2	ABRE-like binding site motif	< 10e-10
31	1	C	n	n	**Ldir-1; LposC**	3	CACGTGMOTIF	< 10e-10
32	1	C	n	n	**Ldir-1; LposC**	4	ACGTABREMOTIFA2OSEM	< 10e-10
33	1	C	n	n	**Ldir-1; LposC**	5	GADOWNAT	< 10e-10
34	1	C	n	n	**Ldir-1; LposC**	6	ABRE binding site motif	< 10e-10
35	1	C	n	n	**Ldir-1; LposC**	7	UPRMOTIFIIAT	< 10e-3

Appendix Table 4.4 continued…

36	1	C	n	n	**Ldir-1; LposC**	8	ABFs binding site motif	< 10e-7
37	1	C	n	n	**Ldir-1; LposC**	9	TGA1 binding site motif	< 10e-4
38	1	C	n	n	**Ldir-1; LposC**	10	UPRMOTIFIAT	< 10e-4
39	1	C	n	n	**Ldir-1; LposC**	11	GBOXLERBCS	< 10e-8
40	1	C	n	n	**Ldir-1; LposC**	12	ABREATRD22	< 10e-5
41	1	n	1	n	**Ldir-1; Pdir1**	1	CACGTGMOTIF	< 10e-3
42	1	n	2	n	**Ldir-1; Pdir2**	1	Ibox promoter motif	< 10e-10
43	1	n	2	n	**Ldir-1; Pdir2**	2	ABRE-like binding site motif	< 10e-10
44	1	n	2	n	**Ldir-1; Pdir2**	3	CACGTGMOTIF	< 10e-10
45	1	n	2	n	**Ldir-1; Pdir2**	4	ACGTABREMOTIFA2OSEM	< 10e-10
46	1	n	2	n	**Ldir-1; Pdir2**	5	GADOWNAT	< 10e-10
47	1	n	2	n	**Ldir-1; Pdir2**	6	ABRE binding site motif	< 10e-10
48	1	n	2	n	**Ldir-1; Pdir2**	7	ABFs binding site motif	< 10e-10
49	1	n	2	n	**Ldir-1; Pdir2**	8	ATHB5ATCORE	< 10e-4
50	1	n	2	n	**Ldir-1; Pdir2**	9	UPRMOTIFIAT	< 10e-4
51	1	n	2	n	**Ldir-1; Pdir2**	10	TGA1 binding site motif	< 10e-4
52	1	n	2	n	**Ldir-1; Pdir2**	11	GBOXLERBCS	< 10e-10
53	1	n	2	n	**Ldir-1; Pdir2**	12	ABREATRD22	< 10e-7
54	1	n	2	n	**Ldir-1; Pdir2**	13	ATHB1 binding site motif	< 10e-4
55	1	n	2	n	**Ldir-1; Pdir2**	14	GBF1/2/3 BS in ADH1	< 10e-4
56	1	n	4	n	**Ldir-1; Pdir4**	1	ABRE-like binding site motif	< 10e-3
57	1	n	4	n	**Ldir-1; Pdir4**	2	CACGTGMOTIF	< 10e-3
58	2	ABC/n	n/1234	n	**Ldir-2**	1	DRE core motif	< 10e-3
59	2	ABC/n	n/1234	n	**Ldir-2**	2	CACGTGMOTIF	< 10e-3
60	2	ABC/n	n/1234	n	**Ldir-2**	3	EveningElement promoter motif	< 10e-6
61	2	ABC/n	n/1234	n	**Ldir-2**	4	LTRE promoter motif	< 10e-4
62	2	A	n	n	**Ldir-2; LposA**	1	EveningElement promoter motif	< 10e-9
63	2	C	n	n	**Ldir-2; LposC**	1	TELO-box promoter motif	< 10e-5
64	2	n	2	n	**Ldir-2; Pdir2**	1	TATA-box Motif	< 10e-4
65	2	n	2	n	**Ldir-2; Pdir2**	2	EveningElement promoter motif	< 10e-10
66	3	DE/n	n/1234	n	**Ldir-3**	1	ABRE-like binding site motif	< 10e-3
67	3	E	n	n	**Ldir-2; LposE**	1	ABRE-like binding site motif	< 10e-3
68	3	E	n	n	**Ldir-2; LposE**	2	UPRMOTIFIAT	< 10e-3
69	3	E	n	n	**Ldir-2; LposE**	3	TGA1 binding site motif	< 10e-3

Sl# - serial number; GR# - group number; Cl# - cluster number; Mo E# - motif entity number; TF – transcription factor; SUP – super-cluster; SUB – sub-cluster.

Ldir: Direction of light effect

1   Promoted by low R:FR

2   Inhibited by low R:FR

3   Promoted by low R:FR at one position and inhibited at another

4   Unaffected by light

Lpos: position of light effect

A   Up or down in both buds

B   Up or down in bud n

C   Up or down in bud n-2

D   Up in bud n and down in bud n-2

E   Down in bud n and up in bud n-2

Z   Not changed in either bud

Pdir: direction of position effect

1   Promoted in bud n

2   Inhibited in bud n

3   Promoted in bud n in one light condition and inhibited in another

4   Unaffected by position

Plite: light effect on position

A   Up or down in both light conditions

B   Up or down in LRFR

C   Up or down in HRFR

D   Up in LRFR and down in HRFR

E   Down in LRFR and up in HRFR

Z   Not changed by either light condition

Appendix Table 4.5 Super-clusters and supersub-clusters based on any effects of position.

Sl#	Ldir "SUP"	Lpos "SUB"	Pdir "SUP"	Plite "SUB"	SUP;SUB	# of Clusts	# of Genes	# of Motifs
	n/1234	n	1	ABC/n	**Pdir-1**	**20**	**3755**	**2**
1	n	n	1	A	**Pdir-1; PliteA**	10	3299	4
2	n	n	1	B	**Pdir-1; PliteB**	6	464	1
3	n	n	1	C	**Pdir-1; PliteC**	4	62	0
4	1	n	1	n	**Pdir-1; Ldir1**	5	130	1
5	2	n	1	n	**Pdir-1; Ldir2**	9	724	0
6	3	n	1	n	**Pdir-1; Ldir3**	2	18	0
7	4	n	1	n	**Pdir-1; Ldir4**	4	2896	4
	n/1234	n	2	ABC/n	**Pdir-2**	**23**	**5192**	**19**
8	n	n	2	A	**Pdir-2; PliteA**	11	4729	14
9	n	n	2	B	**Pdir-2; PliteB**	6	390	8
10	n	n	2	C	**Pdir-2; PliteC**	6	73	0
11	1	n	2	n	**Pdir-2; Ldir1**	8	957	14
12	2	n	2	n	**Pdir-2; Ldir2**	8	229	2
13	3	n	2	n	**Pdir-2; Ldir3**	3	14	0
14	4	n	2	n	**Pdir-2; Ldir4**	4	3992	14
	n/1234	n	3	DE/n	**Pdir-3**	**7**	**11**	**0**
15	n	n	3	D	**Pdir-3; LposD**	5	8	0
16	n	n	3	E	**Pdir-3; LposE**	2	3	0
17	1	n	3	n	**Pdir-3; Ldir1**	2	4	0
18	2	n	3	n	**Pdir-3; Ldir2**	2	2	0
19	3	n	3	n	**Pdir-3; Ldir3**	1	1	0
20	4	n	3	n	**Pdir-3; Ldir4**	2	4	0

Sl#- serial number; SUP – super-cluster; SUB – sub-cluster; n – any effect.

Ldir: Direction of light effect

1　Promoted by low R:FR

2　Inhibited by low R:FR

3　Promoted by low R:FR at one position and inhibited at another

4　Unaffected by light

Lpos: position of light effect

A   Up or down in both buds

B   Up or down in bud n

C   Up or down in bud n-2

D   Up in bud n and down in bud n-2

E   Down in bud n and up in bud n-2

Z   Not changed in either bud

Pdir: direction of position effect

1   Promoted in bud n

2   Inhibited in bud n

3   Promoted in bud n in one light condition and inhibited in another

4   Unaffected by position

Plite: light effect on position

A   Up or down in both light conditions

B   Up or down in LRFR

C   Up or down in HRFR

D   Up in LRFR and down in HRFR

E   Down in LRFR and up in HRFR

Z   Not changed by either light condition

Appendix Table 4.6 Motif analysis details for position effects based on super-clusters and supersub-clusters.

Sl#	Ldir "SUP"	Lpos "SUB"	Pdir "SUP"	Plite "SUB"	SUP;SUB	MoE#	TF /Motif Name	p-value
1	n/1234	n	1	ABC/n	**Pdir-1**	1	CACGTGMOTIF	< 10e-8
2	n/1234	n	1	ABC/n	**Pdir-1**	2	LTRE promoter motif	< 10e-4
3	n	n	1	A	**Pdir-1; PliteA**	1	CACGTGMOTIF	< 10e-7
4	n	n	1	A	**Pdir-1; PliteA**	2	ABRE-like binding site motif	< 10e-4
5	n	n	1	A	**Pdir-1; PliteA**	3	LTRE promoter motif	< 10e-4
6	n	n	1	A	**Pdir-1; PliteA**	4	CArG promoter motif	< 10e-3
7	n	n	1	B	**Pdir-1; PliteB**	1	TELO-box promoter motif	< 10e-10
8	1	n	1	n	**Pdir-1; Ldir1**	1	CACGTGMOTIF	< 10e-3
9	4	n	1	n	**Pdir-1; Ldir4**	1	ABRE-like binding site motif	< 10e-4
10	4	n	1	n	**Pdir-1; Ldir4**	2	CACGTGMOTIF	< 10e-4
11	4	n	1	n	**Pdir-1; Ldir4**	3	CArG promoter motif	< 10e-3
12	4	n	1	n	**Pdir-1; Ldir4**	4	E2F binding site motif	< 10e-4
13	n/1234	n	2	ABC/n	**Pdir-2**	1	CARGCW8GAT	< 10e-6
14	n/1234	n	2	ABC/n	**Pdir-2**	2	Ibox promoter motif	< 10e-8
15	n/1234	n	2	ABC/n	**Pdir-2**	3	DRE core motif	< 10e-7
16	n/1234	n	2	ABC/n	**Pdir-2**	4	ABRE-like binding site motif	< 10e-9
17	n/1234	n	2	ABC/n	**Pdir-2**	5	CACGTGMOTIF	< 10e-10
18	n/1234	n	2	ABC/n	**Pdir-2**	6	ACGTABREMOTIFA2OSEM	< 10e-10
19	n/1234	n	2	ABC/n	**Pdir-2**	7	Hexamer promoter motif	< 10e-3
20	n/1234	n	2	ABC/n	**Pdir-2**	8	GADOWNAT	< 10e-10
21	n/1234	n	2	ABC/n	**Pdir-2**	9	DREB1A/CBF3	< 10e-7
22	n/1234	n	2	ABC/n	**Pdir-2**	10	EveningElement promoter motif	< 10e-10
23	n/1234	n	2	ABC/n	**Pdir-2**	11	ABRE binding site motif	< 10e-10
24	n/1234	n	2	ABC/n	**Pdir-2**	12	UPRMOTIFIIAT	< 10e-8
25	n/1234	n	2	ABC/n	**Pdir-2**	13	ABFs binding site motif	< 10e-10
26	n/1234	n	2	ABC/n	**Pdir-2**	14	TGA1 binding site motif	< 10e-7
27	n/1234	n	2	ABC/n	**Pdir-2**	15	UPRMOTIFIAT	< 10e-7
28	n/1234	n	2	ABC/n	**Pdir-2**	16	GBOXLERBCS	< 10e-10
29	n/1234	n	2	ABC/n	**Pdir-2**	17	ABREATRD22	< 10e-10
30	n/1234	n	2	ABC/n	**Pdir-2**	18	GBF1/2/3 BS in ADH1	< 10e-7
31	n/1234	n	2	ABC/n	**Pdir-2**	19	UPRE2AT	< 10e-4
32	n	n	2	A	**Pdir-2; PliteA**	1	Ibox promoter motif	< 10e-9
33	n	n	2	A	**Pdir-2; PliteA**	2	DRE core motif	< 10e-7
34	n	n	2	A	**Pdir-2; PliteA**	3	ABRE-like binding site motif	< 10e-10
35	n	n	2	A	**Pdir-2; PliteA**	4	CACGTGMOTIF	< 10e-10
36	n	n	2	A	**Pdir-2; PliteA**	5	ACGTABREMOTIFA2OSEM	< 10e-10
37	n	n	2	A	**Pdir-2; PliteA**	6	GADOWNAT	< 10e-10
38	n	n	2	A	**Pdir-2; PliteA**	7	DREB1A/CBF3	< 10e-6
39	n	n	2	A	**Pdir-2; PliteA**	8	EveningElement promoter motif	< 10e-10
40	n	n	2	A	**Pdir-2; PliteA**	9	ABRE binding site motif	< 10e-10

Appendix Table 4.6 continued…

41	n	n	2	A	**Pdir-2; PliteA**	10	UPRMOTIFIIAT	< 10e-8
42	n	n	2	A	**Pdir-2; PliteA**	11	ABFs binding site motif	< 10e-10
43	n	n	2	A	**Pdir-2; PliteA**	12	TGA1 binding site motif	< 10e-5
44	n	n	2	A	**Pdir-2; PliteA**	13	UPRMOTIFIAT	< 10e-5
45	n	n	2	A	**Pdir-2; PliteA**	14	GBOXLERBCS	< 10e-10
46	n	n	2	A	**Pdir-2; PliteA**	15	ABREATRD22	< 10e-10
47	n	n	2	A	**Pdir-2; PliteA**	16	GBF1/2/3 BS in ADH1	< 10e-5
48	n	n	2	A	**Pdir-2; PliteA**	17	UPRE2AT	< 10e-4
49	n	n	2	B	**Pdir-2; PliteB**	1	ABRE-like binding site motif	< 10e-10
50	n	n	2	B	**Pdir-2; PliteB**	2	CACGTGMOTIF	< 10e-7
51	n	n	2	B	**Pdir-2; PliteB**	3	ACGTABREMOTIFA2OSEM	< 10e-10
52	n	n	2	B	**Pdir-2; PliteB**	4	GADOWNAT	< 10e-7
53	n	n	2	B	**Pdir-2; PliteB**	5	ABRE binding site motif	< 10e-7
54	n	n	2	B	**Pdir-2; PliteB**	6	ABFs binding site motif	< 10e-6
55	n	n	2	B	**Pdir-2; PliteB**	7	GBOXLERBCS	< 10e-7
56	n	n	2	B	**Pdir-2; PliteB**	8	ABREATRD22	< 10e-4
57	1	n	2	n	**Pdir-2; Ldir1**	1	Ibox promoter motif	< 10e-10
58	1	n	2	n	**Pdir-2; Ldir1**	2	ABRE-like binding site motif	< 10e-10
59	1	n	2	n	**Pdir-2; Ldir1**	3	CACGTGMOTIF	< 10e-10
60	1	n	2	n	**Pdir-2; Ldir1**	4	ACGTABREMOTIFA2OSEM	< 10e-10
61	1	n	2	n	**Pdir-2; Ldir1**	5	GADOWNAT	< 10e-10
62	1	n	2	n	**Pdir-2; Ldir1**	6	ABRE binding site motif	< 10e-10
63	1	n	2	n	**Pdir-2; Ldir1**	7	ABFs binding site motif	< 10e-10
64	1	n	2	n	**Pdir-2; Ldir1**	8	ATHB5ATCORE	< 10e-4
65	1	n	2	n	**Pdir-2; Ldir1**	9	TGA1 binding site motif	< 10e-4
66	1	n	2	n	**Pdir-2; Ldir1**	10	UPRMOTIFIAT	< 10e-4
67	1	n	2	n	**Pdir-2; Ldir1**	11	GBOXLERBCS	< 10e-10
68	1	n	2	n	**Pdir-2; Ldir1**	12	ABREATRD22	< 10e-7
69	1	n	2	n	**Pdir-2; Ldir1**	13	ATHB1 binding site motif	< 10e-4
70	1	n	2	n	**Pdir-2; Ldir1**	14	GBF1/2/3 BS in ADH1	< 10e-4
71	2	n	2	n	**Pdir-2; Ldir2**	1	TATA-box Motif	< 10e-4
72	2	n	2	n	**Pdir-2; Ldir2**	2	EveningElement promoter motif	< 10e-10
73	4	n	2	n	**Pdir-2; Ldir4**	1	DRE core motif	< 10e-4
74	4	n	2	n	**Pdir-2; Ldir4**	2	ABRE-like binding site motif	< 10e-9
75	4	n	2	n	**Pdir-2; Ldir4**	3	CACGTGMOTIF	< 10e-10
76	4	n	2	n	**Pdir-2; Ldir4**	4	ACGTABREMOTIFA2OSEM	< 10e-10
77	4	n	2	n	**Pdir-2; Ldir4**	5	Hexamer promoter motif	< 10e-4
78	4	n	2	n	**Pdir-2; Ldir4**	6	GADOWNAT	< 10e-9
79	4	n	2	n	**Pdir-2; Ldir4**	7	DREB1A/CBF3	< 10e-4
80	4	n	2	n	**Pdir-2; Ldir4**	8	EveningElement promoter motif	< 10e-9
81	4	n	2	n	**Pdir-2; Ldir4**	9	ABRE binding site motif	< 10e-10
82	4	n	2	n	**Pdir-2; Ldir4**	10	UPRMOTIFIIAT	< 10e-5
83	4	n	2	n	**Pdir-2; Ldir4**	11	ABFs binding site motif	< 10e-10
84	4	n	2	n	**Pdir-2; Ldir4**	12	GBOXLERBCS	< 10e-10
85	4	n	2	n	**Pdir-2; Ldir4**	13	ABREATRD22	< 10e-9
86	4	n	2	n	**Pdir-2; Ldir4**	14	GBF1/2/3 BS in ADH1	< 10e-3

Sl# - serial number; GR# - group number; Cl# - cluster number; Mo E# - motif entity number; TF – transcription factor; SUP – super-cluster; SUB – supersub-cluster.

Ldir: Direction of light effect

    1   Promoted by low R:FR

    2   Inhibited by low R:FR

    3   Promoted by low R:FR at one position and inhibited at another

    4   Unaffected by light

Lpos: position of light effect

    A   Up or down in both buds

    B   Up or down in bud n

    C   Up or down in bud n-2

    D   Up in bud n and down in bud n-2

    E   Down in bud n and up in bud n-2

    Z   Not changed in either bud

Pdir: direction of position effect

    1   Promoted in bud n

    2   Inhibited in bud n

    3   Promoted in bud n in one light condition and inhibited in another

    4   Unaffected by position

Plite: light effect on position

    A   Up or down in both light conditions

    B   Up or down in LRFR

    C   Up or down in HRFR

    D   Up in LRFR and down in HRFR

    E   Down in LRFR and up in HRFR

    Z   Not changed by either light condition

Appendix Table 4.7 Genes related to; hormone biosynthesis, transport, and signaling, cell-cycle regulation; and cell wall modification that are differentially expressed in response to altered R:FR. The values represent the relative expression levels.

Sl #	Hormone	Gr	Cl#	AGI Code	Gene Symbol	n, L v H "a"	n-2, L v H "b"	L, n v n-2 "c"	H, n v n-2 "d"
1	ABA	2	2	AT2G36270	ABI5	-0.152	0.395	-1.697	-1.150
2	ABA	4	64	AT5G57050	ABI2	-0.005	0.919	-2.464	-1.540
3	ABA	4	64	AT4G26080	ABI1	0.199	0.789	-1.336	-0.746
4	ABA	2	2	AT2G46225	ABI1L1	-0.025	-0.023	-0.167	-0.165
5	ABA	3	3	AT5G42030	ABIL4	0.133	0.237	-0.262	-0.157
6	ABA	2	2	AT1G16540	ABA3	0.027	-0.299	-0.534	-0.859
7	ABA	2	2	AT5G67030	ABA1	-0.026	0.104	-0.713	-0.583
8	ABA	2	2	AT3G19270	CYP707A4	-0.440	-0.321	-0.907	-0.788
9	ABA	2	2	AT2G29090	CYP707A2	-0.156	0.108	-1.827	-1.563
10	ABA	2	2	AT5G45340	CYP707A3	0.526	0.113	-0.696	-1.110
11	ABA	2	2	AT4G19230	CYP707A1	-0.156	0.049	-0.842	-0.637
12	ABA	4	32	AT5G66880	SNRK2-3	-0.055	0.372	-1.107	-0.680
13	ABA	3	3	AT5G63650	SNRK2-5	0.208	0.223	-0.247	-0.232
14	ABA	2	1	AT1G48270	GCR1	0.066	-0.025	0.365	0.274
15	ABA	2	2	AT1G52920	GCR2	-0.348	0.026	-0.616	-0.242
16	ABA	2	2	AT1G17550	HAB2	0.219	0.140	-0.332	-0.411
17	ABA	4	64	AT1G72770	HAB1	-0.030	0.584	-1.113	-0.499
18	ABA	2	2	AT2G27150	AAO3	-0.247	0.345	-1.216	-0.624
19	ABA	2	2	AT1G49720	ABF1	-0.068	-0.087	-0.875	-0.894
20	ABA	4	64	AT4G34000	ABF3	0.092	0.775	-2.204	-1.520
21	ABA	4	64	AT3G19290	ABF4	-0.043	0.533	-1.170	-0.594
22	ABA	2	1	AT2G28740	HIS4	0.025	-0.236	0.435	0.174
23	ABA	2	2	AT2G18050	HIS1-3	0.553	0.607	-7.083	-7.029
1	Auxin	2	2	AT3G49870	ATARLA1C	0.025	-0.673	-0.242	-0.940
2	Auxin	2	1	AT2G47750	GH3.9	-0.008	-0.182	0.462	0.289
3	Auxin	2	1	AT2G23170	GH3.3	0.160	-0.337	1.613	1.116
4	Auxin	4	22	AT1G28130	GH3.17	-0.291	0.867	-1.624	-0.466
5	Auxin	4	64	AT4G27260	GH3.5	0.392	1.032	-2.256	-1.616
6	Auxin	2	1	AT1G23080	PIN7	0.116	0.058	0.345	0.288
7	Auxin	2	1	AT2G18040	PIN1AT	0.074	-0.168	0.343	0.101
8	Auxin	3	2	AT2G01420	PIN4	-0.379	-0.315	-0.388	-0.323
9	Auxin	3	4	AT1G73590	PIN1	-0.157	-0.376	0.737	0.518
10	Auxin	3	4	AT1G77110	PIN6	-0.200	-0.118	0.358	0.440
11	Auxin	2	1	AT3G15540	IAA19	-0.061	-0.332	0.993	0.723
12	Auxin	2	2	AT3G23030	IAA2	0.095	0.487	-1.934	-1.542
13	Auxin	2	2	AT5G25890	IAA28	0.896	0.098	-0.967	-1.765
14	Auxin	2	2	AT4G14550	IAA14	-0.017	0.386	-0.741	-0.339
15	Auxin	2	2	AT1G04100	IAA10	-0.299	-0.078	-0.534	-0.313

Appendix Table 4.7 continued…

16	Auxin	2	2	AT1G51950	IAA18	0.197	-0.028	-0.529	-0.754
17	Auxin	2	2	AT2G33310	IAA13	-0.063	0.002	-0.527	-0.461
18	Auxin	3	3	AT3G04730	IAA16	0.018	0.221	-0.841	-0.639
19	Auxin	3	3	AT3G23050	IAA7	0.315	0.564	-0.873	-0.624
20	Auxin	3	3	AT4G28640	IAA11	0.276	0.138	-0.351	-0.489
21	Auxin	3	3	AT3G62100	IAA30	0.530	0.363	-0.318	-0.484
22	Auxin	4	30	AT1G04550	IAA12	0.057	-0.326	0.191	-0.191
23	Auxin	1	2	AT1G34310	ARF12	-0.358	-1.013	-0.148	-0.804
24	Auxin	2	1	AT1G10630	ATARFA1F	0.015	-0.064	0.291	0.211
25	Auxin	2	1	AT2G47170	ARF1A1c	-0.077	-0.120	0.215	0.172
26	Auxin	2	2	AT3G62290	ATARFA1E	0.054	0.056	-0.552	-0.550
27	Auxin	2	2	AT1G77850	ARF17	0.038	-0.086	-0.418	-0.542
28	Auxin	2	2	AT2G46530	ARF11	-0.016	0.105	-0.403	-0.282
29	Auxin	2	2	AT5G60450	ARF4	-0.147	-0.152	-0.271	-0.276
30	Auxin	2	2	AT4G30080	ARF16	-0.054	0.009	-0.243	-0.179
31	Auxin	2	2	AT1G59750	ARF1	0.050	0.077	-0.240	-0.213
32	Auxin	3	2	AT1G30330	ARF6	-0.080	-0.191	-0.080	-0.191
33	Auxin	3	3	AT3G03120	ATARFB1C	0.367	0.182	-0.538	-0.723
34	Auxin	3	4	AT5G37020	ARF8	-0.116	-0.182	0.208	0.142
35	Auxin	4	1	AT1G70490	ATARFA1D	0.132	-0.145	0.295	0.018
36	Auxin	4	6	AT5G62000	ARF2	-0.295	0.344	-0.802	-0.163
37	Auxin	4	64	AT5G62000	ARF2	-0.072	0.226	-0.707	-0.409
38	Auxin	2	1	AT1G10230	ASK18	-0.083	0.020	0.410	0.513
39	Auxin	2	2	AT4G34210	ASK11	-1.790	0.057	-4.283	-2.437
40	Auxin	2	2	AT3G21860	ASK10	0.237	0.471	-3.703	-3.469
41	Auxin	2	2	AT3G60020	ASK5	-0.671	0.847	-2.355	-0.836
42	Auxin	2	2	AT3G60010	ASK13	-0.974	0.280	-2.090	-0.835
43	Auxin	2	2	AT2G03170	ASK14	0.392	-0.143	-1.628	-2.163
44	Auxin	2	2	AT1G10940	ASK1	0.040	0.046	-0.270	-0.264
45	Auxin	4	5	AT1G20140	ASK4	0.088	-0.291	0.396	0.017
46	Auxin	3	1	AT3G44300	NIT2	0.602	0.743	0.163	0.305
47	Auxin	4	4	AT1G31340	RUB1	0.151	-0.593	0.759	0.015
48	Auxin	2	2	AT5G20960	AAO1	1.404	-0.006	-0.794	-2.203
49	Auxin	4	29	AT2G20610	SUR1	-0.023	-0.406	1.144	0.761
50	Auxin	4	50	AT5G54510	DFL1	-0.335	0.342	-2.006	-1.329
51	Auxin	2	2	AT5G43700	ATAUX2-11	0.135	-0.484	-0.474	-1.092
52	Auxin	2	2	AT2G38120	AUX1	0.073	0.053	-0.219	-0.239
53	Auxin	4	4	AT1G04240	SHY2	0.117	-0.949	0.151	-0.914
54	Auxin	2	2	AT3G62980	TIR1	-0.033	0.313	-0.410	-0.064
55	Auxin	2	1	AT1G15690	AVP1	-0.112	-0.012	0.294	0.394
56	Auxin	4	31	AT1G78920	AVP2	-0.096	0.147	-0.043	0.200
1	BR	4	71	AT2G44080	ARL	1.010	1.952	-2.184	-1.242
2	BR	2	2	AT4G18710	BIN2	0.027	0.333	-0.653	-0.347

Appendix Table 4.7 continued…

3	BR	2	2	AT1G69010	BIM2	0.121	0.144	-0.245	-0.222
4	BR	2	2	AT3G50660	DWF4	0.049	0.086	-0.911	-0.874
5	BR	2	2	AT5G05690	CPD	-0.005	0.011	-0.527	-0.511
6	BR	1	1	AT4G33430	BAK1	0.127	0.258	-0.074	0.057
1	CK	1	2	AT5G56970	CKX3	-0.733	-0.735	-0.321	-0.323
2	CK	1	2	AT1G75450	CKX5	-0.121	-0.459	0.221	-0.117
3	CK	2	1	AT2G40670	ARR16	0.052	-0.285	0.469	0.132
4	CK	2	2	AT3G16857	ARR1	-0.466	0.798	-1.626	-0.363
5	CK	2	2	AT3G57040	ARR9	0.014	0.345	-1.516	-1.185
6	CK	2	2	AT3G16857	ARR1	0.146	0.333	-0.633	-0.447
7	CK	2	2	AT2G01760	ARR14	0.002	0.169	-0.364	-0.198
8	CK	3	2	AT3G48100	ARR5	-0.636	-1.833	-0.885	-2.081
9	CK	3	2	AT5G62920	ARR6	-1.481	-1.849	-1.617	-1.985
10	CK	4	4	AT1G19050	ARR7	-0.513	-1.574	0.065	-0.997
11	CK	4	12	AT1G74890	ARR15	-0.684	-2.593	0.714	-1.195
12	CK	4	28	AT1G10470	ARR4	-0.303	-0.824	-0.118	-0.639
13	CK	4	32	AT4G16110	ARR2	-0.069	0.450	-1.078	-0.559
14	CK	2	2	AT1G03430	AHP5	-0.085	0.175	-0.675	-0.416
15	CK	2	2	AT5G39340	AHP3	-0.038	0.183	-0.372	-0.151
16	CK	2	2	AT3G29350	AHP2	0.045	0.051	-0.167	-0.161
17	CK	4	6	AT3G21510	AHP1	-0.265	0.515	-1.333	-0.553
18	CK	1	2	AT5G53290	CRF3	-0.184	-0.996	0.062	-0.750
19	CK	2	1	AT4G11140	CRF1	0.102	-0.457	1.477	0.918
20	CK	2	2	AT3G61630	CRF6	0.243	0.561	-1.875	-1.557
21	CK	3	3	AT1G27320	AHK3	0.263	0.432	-0.693	-0.523
1	ETH	2	2	AT2G25490	EBF1	-0.121	0.145	-0.429	-0.162
2	ETH	4	22	AT5G25350	EBF2	0.057	1.096	-1.172	-0.133
3	ETH	3	3	AT2G40940	ERS1	0.445	0.791	-0.627	-0.281
4	ETH	4	22	AT1G04310	ERS2	0.062	0.910	-1.032	-0.183
5	ETH	2	1	AT3G04580	EIN4	-0.053	-0.004	0.203	0.252
6	ETH	3	3	AT3G20770	EIN3	0.086	0.287	-0.632	-0.431
7	ETH	4	3	AT5G03280	EIN2	-0.020	0.277	-0.338	-0.041
8	ETH	2	2	AT1G66340	ETR1	0.195	0.057	-0.229	-0.367
9	ETH	4	3	AT3G23150	ETR2	0.246	1.626	-0.229	1.151
10	ETH	3	3	AT1G05010	EFE	0.456	0.488	-0.393	-0.361
11	ETH	2	2	AT4G17500	ATERF-1	-0.363	-0.073	-0.908	-0.617
12	ETH	2	2	AT1G50640	ATERF3	0.023	0.291	-0.581	-0.314
13	ETH	2	2	AT3G20310	ERF7	-0.083	0.136	-0.327	-0.108
14	ETH	3	3	AT5G47220	ATERF-2	0.377	1.040	-1.823	-1.159
15	ETH	3	3	AT1G28360	ATERF12	0.147	0.476	-0.732	-0.402
16	ETH	4	6	AT1G53170	ATERF-8	-0.011	0.698	-1.034	-0.325
17	ETH	2	2	AT5G13330	RAP2.6L	0.107	0.427	-1.486	-1.166
18	ETH	2	2	AT1G78080	RAP2.4	-0.128	0.313	-0.760	-0.319

Appendix Table 4.7 continued…

19	ETH	2	2	AT1G53910	RAP2.12	0.167	0.085	-0.417	-0.500
20	ETH	3	3	AT1G43160	RAP2.6	1.114	2.103	-6.084	-5.095
21	ETH	3	3	AT3G14230	RAP2.2	0.017	0.179	-0.613	-0.451
1	GA	2	2	AT3G05120	ATGID1A	0.290	-0.074	-0.572	-0.936
2	GA	3	3	AT3G63010	ATGID1B	0.070	0.462	-1.394	-1.002
3	GA	4	22	AT5G27320	ATGID1C	0.039	0.600	-1.297	-0.736
4	GA	1	2	AT3G03450	RGL2	-0.109	-0.234	-0.046	-0.170
5	GA	2	2	AT5G14420	RGLG2	0.067	0.147	-0.458	-0.379
6	GA	4	13	AT1G66350	RGL1	0.198	-0.448	1.027	0.381
7	GA	2	2	AT1G22070	TGA3	0.002	-0.023	-0.659	-0.685
8	GA	2	2	AT5G25900	GA3	-0.015	0.123	-0.322	-0.184
9	GA	2	2	AT1G78440	ATGA2OX1	-0.611	-0.107	-1.299	-0.795
10	GA	3	3	AT1G30040	ATGA2OX2	0.586	0.794	-2.357	-2.149
1	JA	3	4	AT1G17380	JAZ5	-0.120	-1.204	1.450	0.366
2	JA	4	5	AT1G70700	JAZ9	-0.023	-0.651	0.895	0.267
3	JA	4	5	AT1G72450	JAZ6	0.083	-0.428	0.420	-0.091
4	JA	2	2	AT2G39940	COI1 F-box	-0.010	0.126	-0.394	-0.258
1	Strigolactone	3	3	AT2G42620	MAX2	0.195	0.629	-0.503	-0.070
1	Cell wall	1	1	AT3G60570	ATEXPB5	1.236	0.713	0.197	-0.327
2	Cell wall	2	1	AT2G37640	ATEXPA3	-0.160	-0.205	0.792	0.747
3	Cell wall	2	1	AT2G28950	ATEXPA6	0.101	-0.227	0.397	0.069
4	Cell wall	2	1	AT4G28250	ATEXPB3	-0.085	0.021	0.290	0.396
5	Cell wall	2	2	AT2G40610	ATEXPA8	-0.165	0.091	-0.873	-0.618
6	Cell wall	3	2	AT1G69530	ATEXPA1	-0.255	-0.729	-0.583	-1.057
7	Cell wall	4	5	AT3G03220	ATEXPA13	0.006	-0.269	0.362	0.087
8	Cell wall	4	23	AT2G03090	ATEXPA15	-0.243	-0.797	0.415	-0.138
9	Cell wall	4	23	AT2G39700	ATEXPA4	0.004	-0.293	0.202	-0.094
10	Cell wall	4	36	AT1G26770	ATEXPA10	-0.214	-0.728	0.910	0.396
11	Cell wall	4	63	AT3G29030	ATEXPA5	-0.083	-0.991	1.575	0.667
12	Cell wall	2	1	AT4G38400	ATEXLA2	-0.072	0.134	0.351	0.557
13	Cell wall	2	2	AT3G45970	ATEXLA1	-0.316	0.356	-1.314	-0.641
14	Cell wall	2	1	AT1G14720	XTR2	-0.095	-0.010	0.390	0.475
15	Cell wall	3	1	AT4G25810	XTR6	0.199	0.705	2.284	2.790
16	Cell wall	3	3	AT5G57550	XTR3	1.103	0.994	-1.846	-1.954
17	Cell wall	4	71	AT4G14130	XTR7	1.145	1.903	-1.393	-0.635
1	cell-cycle	1	2	AT2G45080	CYCP3;1	-1.158	-1.066	-0.145	-0.053
2	cell-cycle	2	1	AT3G11520	CYCB1;3	-0.035	-0.034	1.576	1.577
3	cell-cycle	2	1	AT4G35620	CYCB2;2	0.127	-0.142	1.486	1.217
4	cell-cycle	2	1	AT1G76310	CYCB2;4	0.203	-0.270	1.448	0.976
5	cell-cycle	2	1	AT1G44110	CYCA1;1	-0.049	-0.213	1.355	1.191
6	cell-cycle	2	1	AT2G17620	CYCB2;1	-0.046	-0.099	1.282	1.228
7	cell-cycle	2	1	AT4G37490	CYC1	-0.071	-0.086	1.247	1.231

Appendix Table 4.7 continued…

8	cell-cycle	2	1	AT1G16330	CYCB3;1	0.077	-0.030	1.126	1.019
9	cell-cycle	2	1	AT2G26760	CYCB1;4	-0.177	0.008	1.066	1.252
10	cell-cycle	2	1	AT1G34460	CYCB1;4	0.055	-0.078	0.926	0.793
11	cell-cycle	2	1	AT1G15570	CYCA2;3	0.046	-0.104	0.713	0.563
12	cell-cycle	2	1	AT1G20590	CYCB2;3	0.027	0.109	0.702	0.783
13	cell-cycle	2	1	AT1G80370	CYCA2;4	0.060	0.203	0.607	0.750
14	cell-cycle	2	1	AT5G11300	CYC3B	-0.088	0.020	0.435	0.542
15	cell-cycle	2	1	AT4G34160	CYCD3;1	0.113	-0.046	0.413	0.254
16	cell-cycle	2	1	AT1G70210	CYCD1;1	0.146	0.045	0.352	0.251
17	cell-cycle	2	1	AT3G50070	CYCD3;3	-0.019	-0.070	0.230	0.180
18	cell-cycle	2	2	AT4G19600	CYCT1;4	0.044	-0.081	-0.104	-0.229
19	cell-cycle	3	2	AT1G27630	CYCT1;3	-0.100	-0.185	-0.359	-0.444
20	cell-cycle	3	2	AT5G25380	CYCA2;1	-0.695	-0.325	-0.755	-0.385
21	cell-cycle	3	3	AT3G21870	CYCP2;1	0.193	0.282	-1.058	-0.969
22	cell-cycle	3	4	AT5G43080	CYCA3;1	-0.061	-0.344	0.648	0.364
23	cell-cycle	1	2	AT1G66750	AT;CDKD;2	-0.188	-0.320	-0.042	-0.174
24	cell-cycle	2	1	AT1G20930	CDKB2;2	-0.067	-0.080	0.995	0.982
25	cell-cycle	2	1	AT2G38620	CDKB1;2	0.041	-0.201	0.989	0.747
26	cell-cycle	2	1	AT1G76540	CDKB2;1	-0.008	0.078	0.944	1.030
27	cell-cycle	2	1	AT5G64960	CDKC;2	0.074	-0.051	0.270	0.145
28	cell-cycle	4	13	AT2G29570	PCNA2	0.154	-0.079	0.461	0.228
29	cell-cycle	4	63	AT1G07370	PCNA1	0.140	-0.563	1.097	0.394

Sl# - serial number; GR# - group number; Cl# - cluster number; n – top most rosette bud; n-2 – third rosette bud from top; L – low R:FR; H – high R:FR; ABA – abscisic acid; BR – brassinosteroid; CK – cytokinin; ETH – ethylene; GA – gibberellin; JA – jasmonic acid, Strigo – strigolactone.

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