GENE EXPRESSION DIFFERENCES BETWEEN SHORTLEAF PINE AND LOBLOLLY PINE AFTER TOP-KILLING

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CHAPTER I

INTRODUCTION

Shortleaf pine and loblolly pine

Shortleaf pine (*Pinus echinata* Mill.) and loblolly pine (*Pinus taeda* L.) are two important commercial conifers native to the southeastern United States. Both species have the ability to produce wood in large volumes on lands with soils unsuited to intensive agriculture (Nakane, 1994). These pines have the widest natural ranges among all southern pines, with shortleaf pine having the largest. Much of the geographic range of shortleaf pine and loblolly pine is shared. Shortleaf pine occurs in areas further north where it's too cold for loblolly pine to survive.

However, loblolly pine is more financially attractive than shortleaf pine, due to its higher wood production rate resulting largely from its faster juvenile growth and greater full-grown size. Loblolly pines now occupy 65 percent of the commercial forest land in the southern United States and directly or indirectly contribute $30 billion to the economy of the region (Schultz, 1999; Conner and Hartsell, 2002). Hence, loblolly pine is entitled "the pine for the twenty-first century" (Schultz, 1999).

Fire and pine species

Fire is one of the most important ecological factors associated with pine (Agee, 1998). Fire is responsible in large part for the wide distribution of pines. Much of pine species' current large ranges are due to its ability to quickly spread to open places created by various disturbances such as fire. Disturbances help remove the previous vegetation, recycle nutrition and create space for the development of pine seedlings. Without disturbance like fire, pines on better sites might be completely eliminated and replaced by hardwoods, because without disturbance the hardwood species develop more rapidly on these sites (Denevan, 1961; Kowal, 1966).

Some pine species have developed the capacity to resprout from the stem or root collar to help survive after disturbance (McCune, 1988). Shortleaf pine, pond pine (*Pinus serotina* Michx.) and pitch pine (*Pinus rigida* Mill.) are the three southern pines known to have strong basal sprouting ability (Fowells, 1965; Stone and stone, 1954). Vigorous resprouting from axillary buds can be produced at the base of the stem of seedlings of these species (Agee, 1998). The preformed buds, originally located in axils of primary needles, are well protected by bark and can sprout following fire in these pine species up to age 10 or older (Ledig and Little, 1979). These buds are connected to the pith by a bud trace. They remain almost dormant under the bark and grow only a little each year so that the tip can keep pace with cambial growth (Kozlowski et al., 1991). Under severe conditions when the leaves, or more seriously, the stem are lost by fire, the bud dormancy

is released (Kozlowski et al., 1991). Basel sprouting is, however, largely restricted to seedlings and small saplings (Stone and stone, 1954; McCune, 1988).

Species with sprouting ability may be more competitive than those that reproduce only by seeds because the sprouts grow faster than seedlings (Biswell, 1974; Kozlowski et al., 1991). Liming (1945) reported that shortleaf pines developed from sprouts caught up with other shortleaf pines planted at the same time as the originally top-killed shortleaf pines, and later even surpassed undamaged planted shortleaf pine in height.

Natural regeneration and artificial regeneration of pine

Natural regeneration of pine species includes regeneration through natural seedlings or from sprouts (Butler, 2003). However, most pines have limited ability to regenerate from sprouts, with several exceptions including shortleaf pine. Thus, natural regeneration of pines generally depends on seeds provided by older trees left there to regenerate the site (Duryea, 1992). Natural regeneration has its advantages, such as lower cost, less labor and ensured adaptation of the native stock to the site (Edward, 1987). However, due to limited seed production, and competition for light, nutrition and space, pine species' natural regeneration rates can be quite low in any given year (Liming, 1945). The competition with natural regenerating of pine stands can come from other trees and shrubs, grasses, and woody vines.

Artificial regeneration methods include planting seedlings or direct seeding (Butler, 2003). In recent years, pine management has changed from dependence on natural regeneration to artificial regeneration by planting seedlings or sowing seeds (http://www.forestencyclopedia.net/p/p599). Despite its higher initial cost and labor, artificial regeneration has its own advantage: it reduces the time required for establishment, provides better control of spacing, and allows establishment of genetically improved trees (Edward, 1987). Due to the financial attractiveness of growing loblolly pine compared to shortleaf pine, many more areas of loblolly pine are being regenerated by artificial regeneration, even on those lands which were originally occupied by shortleaf pines (Moser et al., 2008).

Shortleaf pine is declining

The acreage and volume of shortleaf pine has been decreasing during the last several decades (Moser et al., 2008). The decline is due to the following two reasons. One is landowners' preference for loblolly pine. In much of the original shortleaf pine range, shortleaf pine is being replaced by loblolly pine through plantation establishment. Secondly, shortleaf pine's establishment is more dependent on disturbance like fire than most other tree species. However, disturbance is extremely restricted in today's increasingly urbanized world (Johnson et al., 2002; Moser, 2003; Moser et al., 2008). Due to lack of disturbances like fire in shortleaf pine stands, when shortleaf pines become overmature and die, midstory hardwoods tend to dominate the stand and replace the original shortleaf pine.

Prescribed fire

Fire is an important factor in forest ecosystems. It is a major disturbance, and if uncontrolled, it can result in stand replacement. Unlike wildfire, prescribed fire is now utilized in forest management because it is helpful in improving regeneration. First, prescribed fire can effectively reduce built-up fuel levels, and chances for the outbreak of a stand-replacing wildfire are greatly reduced after application of prescribed fire. Second, after prescribed fire, site conditions are more favorable for the establishment of pine seedlings, as non-fire-adapted resource-competing species are eliminated. Third, more nutrition is available for pine seedling development, as otherwise immobilized nutrients from other understory vegetation are released to the soil after fire (Schultz, 1997). Therefore prescribed burning is used for regenerating southern pines either by artificial regeneration, or natural regeneration (Pritchett, 1979). Prescribed fire is considered the most economical site preparation tool (Luke et al., 2000).

Shortleaf pine sprouting and natural regeneration

Shortleaf pine's strong sprouting ability and prescribed fire might be useful for natural regeneration in silvicultural applications. Prescribed fire not only helps to promote shortleaf pine seedling establishment on the site by providing a seed bed and nutrition recycled from other above-ground competing species, but also eliminates species with less fire resistance that would otherwise compete for the resources (Moser, 2003; Moser et al., 2008). When prescribed fire is applied, some shortleaf pine seedlings or saplings

might be killed, but sprouts developed from top-killed stems and new shortleaf pines developed from seeds may provide adequate regeneration. Spouting is much less common in loblolly pine than in shortleaf pine (Schultz, 1997).

Utilizing microarrays to profile gene expression related to sprouting due to dormancy release

Microarrays have been utilized to answer many diverse biological questions since the middle of the 1990s (Chee et al., 1996; van Hal et al., 2000). The microarray method was developed on the principle that complementary nucleic acids hybridize with each other. Unlike other techniques for the analysis of gene expression at the mRNA level, such as Northern blot hybridization (Kevil, 1996), differential display (Liang and Pardee, 1992), or serial analysis of gene expression (SAGE; Adams 1996), microarray technology can be utilized to examine the expression pattern of large subsets of genes simultaneously for any particular organism at any developmental stages and under varied environmental stimuli (Duggan et al., 1999; Richmond and Somerville, 2000).

The microarray method has been used for many different purposes, such as comparing global expression profiles under different environmental conditions (Reymond et al., 2000) and identifying genes of specific functions (Park et al., 2006; Ducreux et al., 2008). Recently several microarray experiments have been carried out to profile genes responsible for dormancy release of buds of perennial trees and perennial grasses (Huang et al., 2008; Mazzitelli et al., 2007; Pacey-Miller et al., 2003). Normally, for a cDNA

array, the cDNA clones printed on the slides and the labeled mRNA hybridized to it are from the same species, but researchers have utilized so called “cross hybridization”, and found that when fluorescent tagged leafy spurge (*Euphorbia esula* L.) mRNA is hybridized to *Arabidopsis* cDNA based array slides, 60% of the cDNAs show successful hybridization (Chao, 2002).

Experimental Objectives

Although there have been many investigations of dormancy, the molecular mechanisms that control the transitions into and out of dormancy are still unclear; and to this author’s knowledge, there is no report on the molecular mechanism involved in dormancy release that leads to sprouting in pine species. This study aimed to identify genes that lead to shortleaf pine’s quick bud break and prolific sprouting after top-kill; and to explain why shortleaf pine and loblolly pine have huge differences in response to dormancy release after top-killing besides the fact that dormant buds are better protected from disturbances by the J-shaped crook of shortleaf pine. The results generated from this experiment might be helpful in shortleaf pine regeneration planning as well as simply understanding the process.

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CHAPTER II

LITERATURE REVIEW

Loblolly pine (*Pinus taeda* L.) and shortleaf pine (*Pinus echinata* Mill.) are commercially important tree species in the southeastern United States, and stands may be successfully regenerated by either natural or artificial methods. In regenerated stands, pine seedlings are frequently top damaged by a wide variety of animals, insects, forestry operations and fire (Bond and Midgley, 2001). Small seedlings in natural strands are especially at risk, as they are not protected like seedlings in a nursery. Animals such as cows and deer may eat them. Harvest activities may damage existing natural regeneration. Wildfire is common in forest areas and prescribed fire is applied to reduce hazardous fuel buildup and help create an exposed soil bed to encourage desired regeneration. Both wildfire and prescribed fire can cause severe damage to pine seedlings. Thus, sprouting ability following top-kill may be an adaptation to insure survival following some of these kinds of damage.

Sprouting is a well-organized mode of vegetative recovery, which helps repair the damaged tree after top-killing (Blake, 1983; Yamada et al., 2001). A species' sprouting ability is responsible for how well plants can recover following top damage. Shortleaf pine's sprouting ability after top-kill by fire is well recognized among southern

pines (Boucher, 1990; Bellingham, 1994; Everham and Brokaw, 1996). Loblolly pine is also found to be able to sprout after top-kill, but its sprouting ability along the base of the stem after fire damage is significantly reduced compared to shortleaf pine (Shelton, et al., 2002). Shortleaf pine's strong ability to sprout following fire is partially due to its characteristic J-crook, by which the stem of young shortleaf pine runs parallel to the ground for a few centimeters before growing vertically. This basal crook helps bring dormant buds in contact with the soil surface and allows shortleaf pine to sprout near ground line by avoiding fire kill of that portion of the stem. For loblolly pine, dormant buds on the stem above the cotyledons are exposed to and usually killed by fire (Shelton, et al., 2002).

Sprouting

Sprouting in plants is a form of vegetative recovery, which helps damaged plants to survive (Yamada et al., 2001; Bond and Midgley, 2001). For some tree species such as the rainforest tree *Nothofagus cunninghamii*, which can regenerate both from seeds and by sprouting, sprouting is more common than regeneration by seed following fire or drought on drier and less fertile sites (Read and Brown, 1996; Bellingham, 2000). After fire, plants that can not sprout (eg. non-fire adapted species) are probably most threatened by extinction from the site, while resprouters survive. Survival by sprouting is therefore a good adaptation for regeneration (Johnston and Lacey, 1983; Ohkubo, 1992; Midgley, 1996). Sprouting following top-kill can occur higher in the canopy or at the base of the plant (Bond and Midgley, 2003). For the latter, there are four basic sprouting types: "collar sprouts from the base of the trunk, sprouts from specialized underground stems (lignotubers and rhizomes), sprouts from roots (root suckering), and opportunistic sprouts

from layered branches" (Del Tredici, 2001; Bond and Midgley, 2003).

Sprouting at the base occurs in certain species when the above-ground portions of the plants are cut down or are killed by fire or other kinds of damage, such as animals browsing, logging, hurricanes, etc. (Putz et al., 1989; Yamada et al., 2001). In temperate and tropical forests, sprouting is a common means of regeneration after forest harvesting (Webb et al., 1972; Knight 1975; Zahner et al., 1985). Plants of different age and size have different sprouting abilities (Bond and Midgley, 2001). Many angiosperm tree species can sprout after top-killing when they are seedlings and saplings, while most conifers can only sprout when they are seedlings (Del Tredici, 2001; Bond and Midgley, 2003). Sprouting abilities also vary according to type and severity of injury (Bellingham and Sparrow, 2000; Bond and Midgley, 2001). Almost all plants are able to resprout when exposed to minor herbivory (Ito and Gyokusen, 1996; Chamberlin and Aarssen, 1996).

It is generally accepted that resources are needed from the remaining stems and roots for sprouting, when trees are top-damaged. But disagreement exists with regard to where these resources come from. Kramer and Kozlowski (1979) suggest that for the sprouting of woody plants, resources come from the stumps and roots; while the investigation of Sakai and Sakai (1998) shows that for the sprouting of a Mediterranean shrub named *Euptelea polyandra*, the resources are from above ground.

Carbohydrates (mainly starch and soluble sugars) are the major resources used for sprouting. Bowen (1993) and Canadell (1998) reported that carbohydrate is most depleted among all nutrients (eg. Carbohydrate, nitrogen, phosphorus and so on) after sprouting of the shrub *Stirlingia latifolia*. It takes two years for the shrub to recover to pre-fire carbohydrate levels (Bowen and Pate, 1993). Will and Tauer (unpublished paper, 2006) proposed that a difference in carbohydrate availability is the reason why shortleaf pine sprouts more vigorously following a winter burn than a summer burn. In addition to carbohydrates, other nutrients such as nitrogen, phosphorus, potassium and magnesium, are required for sprouting (Miyanishi and Kellman, 1986; Canadell and Lopez-Soria, 1998). Pate (1990) reports starch storage is higher in roots for sprouters than for none-sprouters (also called seeders). The signal process involved in the use of these nutritional resources for sprouting is not known.

Sprouting potential of shortleaf pine and loblolly pine

Shortleaf pine and loblolly pine are two southern pines that at a young age can sprout after top-killing. When top damaged, the potential for recovery of both shortleaf pine and loblolly pine is good, with shortleaf pine having a stronger sprouting ability. Top-damaged shortleaf and loblolly pine sprout from dormant buds in the axils of the primary needles and the base of secondary needles. Lateral buds or shoots can also develop into dominant ones (Shelton and Cain, 2002). Spouting in pines is restricted to the portion of the stem above the cotyledons, where primary needles exist. No foliage is produced on the hypocotyl, which is the stem between the root collar and the cotyledons. Therefore, stem death below the cotyledons will lead to the death of the seedlings (Shelton and Cain,

2002). Adventitious buds on roots, which are used by hardwoods for vegetative propagation (Kramer and Kozlowski, 1979), are rarely seen in mature shortleaf pine and loblolly pine. Adventitious rooting ability of shortleaf pine and loblolly pine is lost with maturation (Diaz-Sala et al., 1997).

Top-killed shortleaf pine saplings can sprout from dormant buds, which are previously developed in the axils of the primary needles (Stone and Stone, 1954). Shortleaf pine has a characteristic J-shape-crook in its stem at ground-line, which is usually a few centimeters long. These crooks cause the stems of young shortleaf pine to run parallel to the ground before they grow vertically. These crooks help to keep the dormant buds near the soil surface, which is proposed to be responsible for facilitated sprouting (Shelton and Cain, 2000). Buds on the soil surface or in the litter-layer suffer less damage from animals and fire than erect stems. Loblolly pine has erect stems.

Shelton and Cain (2002) reported that more than 95% of one-year-old shortleaf pine seedlings survived by sprouting after a winter burn, which top-killed almost all the seedlings (>99%). Sprouting ability decreases when shortleaf pine becomes older and larger. But Harlow et al. (1979) reported that shortleaf pine can still sprout even when they are 10 years old, when their main stems are top-killed by fire or cutting. Moore (1936) reported that shortleaf pines of four or more inches in diameter did develop sprouts but with less vigor than younger and smaller trees. Even trees over six-eight inches in diameter may still sprout (Fowells, 1965).

Unlike shortleaf pine, no J-shape crook is developed on loblolly pine seedlings to protect the dormant buds from fire. But if loblolly pine seedlings are planted with the cotyledons below ground level, survival by sprouting is improved flowing top-damage. Shelton and Cain (2002) reported that for one-year-old loblolly pine seedlings, the average length of hypocotyls is 0.6-1.6 inches. For three-year-old seedlings, if cut above the cotyledon, the survival rate from sprouting was 97% for winter cut trees and 96% for summer cut trees (Shelton and Cain, 2002).

Genes related to sprouting

Sugars not only play a central role in metabolism to provide nutrition and energy, but also function in gene expression regulation (Koch, 1996; Smeekens, 1998). Regulation of gene expression by sugar is involved in a number of physiological and developmental processes, such as seed germination (Garciarrubio et al., 1997; Finkelstein and Lynch, 2000), flowering (Corbesier et al., 1998; Bernier et al., 1993), photosynthesis (Krapp et al., 1993; Araya et al., 2006) and tuber formation in potatoes (Muller-Rober et al., 1992; Gibson, 2000). Change of sugar concentration may be associated with the sprouting process, and it is possible that this kind of change may be responsible for regulation of gene expression involved in sprouting. However, to this author's knowledge there is no report on sugar signal transduction associated with tree sprouting. What genes, how many genes, and how they are involved in sprouting is still obscure. With more and more research reported related to dormancy release in buds and seeds, a detailed review of genes related to dormancy release which leads to sprouting is of interest.

What is dormancy?

By definition, dormancy is "the temporary suspension of visible growth of any plant structure containing a meristem" (Lang et al., 1987). Woody perennial plants rely on dormancy for survival; bud break of woody perennials plants is regulated by a dormancy release mechanism seasonally (Olsen, 2002; Viemont and Crabbe, 2000; Rohde and Bhalerao, 2007). In addition, dormancy in some plant organs (such as seeds) or meristems also plays an important role in controlling plant morphogenetic characters (Horvath et al., 2003). Dormancy makes it possible for axillary buds to replace a damaged primary shoot (Shimizu-Sato and Mori, 2001; Olsen, 2002).

Dormancy can be classified into different types according to the different dormancy developmental stages: induction, maintenance, and breakage (Olsen, 2002). More commonly, dormancy is divided into three types: eco-, para- and endo-dormancy (Lang et al., 1987). Ecodormancy is suspension of growth provoked by limitations in environmental factors. Paradormancy is arrest of growth imposed by physiological factors coming from another part of the plant outside of the dormant tissue (Olsen, 2002). Endodormancy is a type of dormancy controlled by internal factors within the dormant tissue and it is released only when a chilling requirement is met (Lang et al., 1987). Dormancy of axillary buds fall into the paradormant category, as such dormancy is caused by apical dominance, an inhibitory effect caused by growing apical buds, which is a factor in the plant but outside of the dormant tissue (Olsen, 2002). Shortleaf pine and loblolly pine have axillary buds located in the axils of the primary needles. The buds remain in a paradormant state after formation. The buds are released from dormancy if

apical dominance is removed by loss of the main stem.

Bud dormancy vs. seed dormancy

There is some commonality between bud and seed dormancy. For example, seed germination and bud break may be induced or inhibited by similar growth regulators, and these processes are regulated similarly as well (Powell, 1987; Dennis, 1996; Olsen, 2002). In seeds of specific plant species, such as sweetgum (*Liquidambar styraciflua*), the chilling requirement for breaking dormancy is similar to that for the buds. However, seeds have potential internal controls, while buds are part of the plant and may be affected by other parts of the plant (Crabbé and Barnola, 1996; Olsen, 2002). It is reasonable to assume that differences exist between bud dormancy and seed dormancy of the same species, and also for bud dormancy or seed dormancy between different species. But until now, there is little detailed information on these differences.

Types of bud dormancy

Dormancy of axillary buds

Axillary meristems, so called plant stem cells, are formed in the axils of leaves on the primary shoot axis (Geier et al., 2008). After initiation, axillary meristems form axillary buds (Schmitz and Theres, 2005; Beveridge, 2006). After formation, the buds may continue growth to form axillary shoots. Or, most often, the bud remains dormant indefinitely unless its growth is triggered by one or more cues from its developmental program or from the outside environment (McSteen and Leyser, 2005; Shimizu-Sato and

Mori, 2001).

Indefinite dormancy is caused by “apical dominance”, which is “the inhibitory control of the shoot apex over the outgrowth of lateral buds” (Cline, 1991; Napoli et al., 1999). Apical dominance was demonstrated by a well-known decapitation study, which examined the inhibiting function of the shoot tip on the outgrowth of axillary buds (Thimann and Skoog, 1933; Bangerth, 1994). Environmental cues or developmental programs or both can function to release apical dominance (Shimizu-Sato and Mori, 2001). Most interestingly, apical dominance plays an important role in plant survival mechanisms. If the primary shoot is damaged or removed after disturbance like grazing, pruning or fire, axillary meristems in indefinite dormancy might help the plant to survive by replacing the damaged primary shoot, as is seen in some tree and annual plant species (Klimesova and Klimes, 2003 and 2007; Anderson et al., 2001; Shimizu-Sato and Mori, 2001). For tree species, it seems that apical dominance is primarily limited to the juvenile stages (Cline, 2000), which may be why some tree species, like shortleaf pine, can resprout at an early age following top-kill or serious damage, but looses this ability with maturity.

It is known from the decapitation study that it is auxin, which is produced mainly in the growing shoot apex, that inhibits the immediate continued development of axillary meristems and results in so called “apical dominance”. It was further reported that by adding auxin to tops of decapitated *Vicia faba* plants, growth inhibition of their axillary buds is resumed (Thimann and Skoog, 1933). Transgenic studies also confirmed the role

of auxin in apical dominance, showing reduced branching ability with an increased level of auxin, and vice versa (Klee and Lanahan, 1995). Despite intensive research on the function of auxin, how it acts in plants is still much a mystery. To date, it is known that auxin works indirectly on the dormant bud, and direct application of auxin to buds does not inhibit their outgrowth (Klee and Lanahan, 1995; Ferguson and Beveridge 2009). It has been noted that levels of auxin in buds rise as they resume growth (Lincoln et al., 1990).

Although auxin is the major player in apical dominance, its function is regulated by several other secondary messengers, including cytokinin, abscisic acid (ABA), and a newly discovered hormone which inhibits bud growth in pea (*Pisum sativum* L.) and branching in *Arabidopsis*, named *rms* and *max*, respectively (Beveridge, 2000; Morris et al., 2001). Cytokinin functions to promote bud outgrowth, and by applying cytokinin to tops of decapitated *Arabidopsis* plants, buds begin to grown in spite of the existence of apical auxin (Chatfield et al., 2000). ABA is found to be associated with the maintenance of dormancy in both apical and axillary buds of woody plants (Frewen et al., 2000). The level of dominance can be determined by assessing the ABA concentration, which is found in both the decapitation study with regard to the effect of indefinite dormancy on European white birch (*Betula pendula Roth*) and in the study of isolated buds of *Rosa hybrida* cultured in vitro (Galoch et al., 1998; LeBris et al., 1999). In *Arabidopsis*, ABA application to tops of decapitated plants functions to enhance the inhibitory effect of apical dominance due to apical auxin (Chatfield et al., 2000).

Dormancy of buds of perennial plants

In perennial wood plants, dormancy is a key factor in their survival. Many temperate trees grow in a fashion of alternative bud flush and growth arrest (Crabbé and Barnola, 1996). By adopting a dormant state in meristems, during which the buds are insensitive to growth-promoting signals, woody plants remain freeze-tolerant to protect themselves against severe weather conditions in the winter (Kozlowski, 1943; Weiser, 1970). When weather conditions become favorable, woody plants resume growth with the release of dormancy in the bud meristems. There is little known about the mechanism behind the seasonal cycling between growth and dormancy of perennial trees and it is as yet unknown whether these dormancy mechanisms are similar to those of the axillary buds of herbaceous and woody plants (Rohde and Bhalerao, 2007).

Photoperiod is known to control the establishment of dormancy by triggering growth cession of many trees (Nitsch, 1957; Rohde and Bhalerao, 2007). The photoperiod signal is sent to the plant apex by leaves (Hemberg, 1949; Wareing, 1956), in which photochrome and two newly found genes *FLOWERING LOCUS T* (*FT*) and *CONSTANS* (*CO*) play an important role in sensing short-day signals for growth cessation (Bohlenius et al., 2006). Interestingly, the *FT* and *CO* genes had been previously found to be involved in floral meristem transition in photoperiodic controlled flowering in long- and short-day plants (Hayama and Coupland, 2004).

It is known that for most plant species, dormancy can only be released when a chilling requirement is met (Falusi and Calamassi 1990; Myking and Heide 1995). The most

interesting discovery with regard to woody plant dormancy release is the finding of a *FLC* (*FLOWERING LOCUS C*)-like gene, which shows differential expression during the completion of the chilling requirement in *Populus* (Chen and Coleman, 2006; Rohde and Bhalerao, 2007). *FLC* is found to be involved in vernalization of *Arabidopsis* (Sung and Amasino, 2005). The similarity between vernalization and dormancy release is that a chilling requirement must be met before growth resumes (Rohde and Bhalerao, 2007).

Hormone controlled dormancy release in seeds and buds

Most of what is known about hormone controlled dormancy release in seeds and buds is obtained from research reports on seed dormancy release before germination. Multiple factors work cooperatively to achieve seed dormancy release, including environmental cues, endogenous hormones, and other small molecules in the plants or seeds (Finkelstein et al., 2008). The relative abundance and sensitivity of endogenous ABA and GA are the key regulators of breaking dormancy, with ABA functioning to maintain dormancy, while GA progresses toward release and germination (Thomas et al., 2005).

Gibberellins (GA) are plant hormones belonging to a subfamily of tetracyclic diterpenes (Thomas et al., 2005). Increased GA levels and sensitivity are both reported to be associated with dormancy release in seeds of most species, but in no species is germination found to be stimulated by GA treatment alone (Finkelstein et al., 2008; Ali-Rachedi et al., 2004; Bewley, 1997; Derkx et al., 1994). GA functions to promote germination by inducing multiple enzymes, which work not only to induce mobilization of seed storage reserves, but also to promote embryo expansion (Bewley and Black, 1994). Resultant mobilized reserves and softened seed tissues ready the seed for growth

(Finkelstein et al., 2008).

GA synthesis is required for dormancy release. Investigations have shown that in dormancy released *Arabidopsis* seeds, the expression level of one GA biosynthetic gene GA3ox2 (GIBBERELLIN 3 OXIDASE) is about 40 times of that of seeds still in dormancy (Finch-Savage et al., 2007). Experiments on *Arabidopsis* and tomato (*Solanum lycopersicum* L.) show that those plants with mutant genes for GA synthesis fail to germinate (Mitchum et al., 2006; Steber et al., 2007). Seeds of some species have to go through stratification before germination. During stratification, function of genes involved in GA biosynthesis is increased, while function of those genes involved in GA catabolism is decreased (Yamauchi et al., 2004). One of the known negative regulators of GA is DELLA (named after a conserved amino acid motif; Sun and Gubler, 2004). GA stimulates germination by degradation of the DELLA proteins (Ariizumi and Steber, 2007; Tyler et al., 2004).

ABA can inhibit germination, and ABA's concentration is positively correlated with the level of dormancy (Morris et al., 1991). Genes involved in ABA catabolism have been found to function in dormancy release (Cadman et al., 2006; Millar et al., 2006). Accumulated H_2O_2 can promote dormancy release through pathways that lead to ABA breakdown (Bailly, 2004).

Ethylene is another kind of phytohormone found to promote dormancy release. It stimulates seed germination through antagonistic interaction with ABA signaling.

Increased dormancy, increased sensitivity to ABA during seed germination, and increased ABA synthesis are found in seeds of ethylene resistant receptor mutants (Beaudoin et al., 2000; Chibani et al., 2006; Ghassemian et al., 2000).

Sugar signaling and dormancy release

Sugars have been shown to function as signaling molecules in plant development through regulation of gene expression (Jang et al., 1997; Sheen et al., 1999; Ho et al., 2001). Sugar signaling and its role in dormancy release are best documented for leafy spurge (*Euphorbia esula* L.), an invasive perennial weed in North America (Horvath and Anderson, 2002). Chao et al. (2006) reported that bud break and new shoot growth results in decreased level of endogenous starch and sucrose, but an increased level of fructose, which is the exact opposite of what is found in buds that are in dormancy (Anderson et al., 2005). The increased level of fructose might not only supply an energy source for sprouting, but also function as a signal molecule to cross talk with other signalling molecules such as plant hormones, calcium, phosphatase and kinase. Interestingly, GA is proposed to promote the synthesis and activity of α-amylases and invertase, two key enzymes in carbon metabolism (Jones et al., 1998; Nakayama et al., 2002; Koch, 2004). Sugar is proposed to interact with GA (Gesch et al., 2007). A direct application of sucrose and glucose to leafy spurge roots inhibited root bud growth after decapitation, while the application of GA cancelled the effect, thus it was suggested that sucrose or its metabolites inhibited the GA response pathway or abundance of active GA's is decreased due to the presence of excessive extrinsic sucrose (Gibson, 2004; Chao et al., 2006). Sugar-signaling is proposed to play an important role in regulating dormancy of leafy spurge's underground adventitious buds (Anderson et al., 2005;

Horvath et al., 2003). Unlike in axillary buds, the paradormancy in the adventitious buds is not only controlled by auxin from the shoot apices, but also by sugar signals transmitted from the leaves (Horvath, 1998; Horvath, 1999; Horvath et al., 2002).

Oxidative stress and dormancy release

In general, active oxygen species (AOS) are highly reactive and can cause damage to a majority of biomolecules, such as nucleic acids, enzymatic proteins, and cell membranes, and therefore AOS are considered to be toxic to the cell (Foyer et al., 1997; Beckman and Ames, 1997; Bailly, 2004). AOS are produced during electron transport processes, where oxygen is originally involved as an electron acceptor (Bailly, 2004). Dormancy break in seeds and buds is associated with a sudden increase in respiratory activity and an enhanced production of AOS, as mobilization of lipid requires beta-oxidation, which produces H_2O_2 (Huang et al., 1983). Antioxidant molecules and enzymes, such as catalase (CAT) and reduced glutathione, have been widely considered as being of particular importance for the AOS accumulation process (Kranner and Grill, 1993; De Gara et al., 2003; Tommasi et al., 2001). In addition, a rapid accumulation of H_2O_2 is seen in wounded plants (Angelini et al., 1990). H_2O_2 has been found to function as a second messenger for the induction of defense genes in the plant wounding response (Orozco-Cardenas et al., 2001).

Besides wounding, AOS are proposed to function as signaling molecules in plant responses to many other various stimuli through interactions with other proteins and molecules, such as MAP kinase (mitogen-activated protein kinase; Samuel et al., 2000),

calcium (Bowler and Fluhr, 2000; Rentel and Knight, 2004), phytohormones and jasmonic acid (Vranova et al., 2002). AOS are also found to be involved in the regulation of gene expression (Desikan et al., 1998; Desikan et al., 2001).

Of interest, AOS and antioxidants also play an important role in seed dormancy regulation (Bailly, 2004). Increased levels of H_2O_2 tend to promote dormancy release in seeds of barley (*Hordeum vulgare* L.; Fontaine et al., 1994; Stacy et al., 1996) and apple (*Malus* Mill.; Bogatek et al., 2003). It was shown that by using chemicals that inhibit catalase activity, germination of sunflower (*Helianthus annuus* L.) seeds (Oracz et al., 2007) and sprouting of potato (*Solanum tuberosum* L.) are promoted due to dormancy release (Hendricks and Taylorson 1975; Bajji et al., 2007). With regard to bud dormancy break, high H_2O_2 levels were seen during dormancy release in the buds of grapevine (*Vitis vinifera* L.; Pacey-Miller et al., 2003; Perez and Lira, 2005). It is proposed that H_2O_2 might decrease ABA content and activity through H_2O_2 signaling, and decreased ABA activity in turn leads to dormancy release. There is abundant evidence supporting this hypothesis. For example, Bogatek et al. (2003) have shown that dormancy release of apple embryos by cyanide includes an increase in H_2O_2 concentration associated with a decrease in ABA concentration. In addition to ABA, increased ethylene production promoted by H_2O_2 signaling is demonstrated to be involved in breaking seed dormancy (Corbineau and Côme, 1995 and 2007).

Although there have been many investigations of dormancy, the molecular mechanisms that control the transitions into and out of dormancy are still unclear; to this author's

knowledge, there is no report on the molecular mechanism involved in dormancy release that leads to sprouting in pine species. This study aimed to identify what genes lead to shortleaf pine's quick bud break and prolific sprouting after top-kill; and to explain why shortleaf pine and loblolly pine have huge differences in response via dormancy release after top-killing. Hopefully, the results generated from this study might be helpful in shortleaf pine regeneration planning.

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CHAPTER III

METHODOLOGY

Materials

Four hundred one-year-old shortleaf pine and 400 one-year-old loblolly pine bare-root seedlings, from the Missouri Department of Natural Resources (MoDNR) nursery and the Oklahoma Department of Agriculture Food and Forestry (ODAFF) nursery, respectively, were planted in potting compost soil in plastic pots in the NREM greenhouse (Figure 1). The loblolly pines were planted on April 16, 2007, and the shortleaf pines were planted on April 18, 2007. For convenient tissue collection from both the cutting treatment and the corresponding control treatment, the seedlings of both shortleaf pine and loblolly pine were divided into two groups, with 200 seedlings in each group. One group was used for a cutting treatment, and the other was for a control treatment (Figure 2). On May 6, 200 shortleaf pines and 200 loblolly pines in the cutting treatment groups were top cut with one inch of stem remaining above the soil. In the following days, tissues were collected from shortleaf pine and loblolly pine every day. For the treatment groups, the remaining one-inch stems on 10 individuals were collected daily. For the control groups, 10 seedlings were cut every day to collect the one-inch stem segment above ground level. If sprouts were seen in the treatment group, stems with sprouts and stems without sprouts

sprouts were collected separately. The collected materials were kept on ice for about one half hour until they were transferred to a -80°C freezer. For shortleaf pine, sprouts were seen on the stumps two days after top-cutting (Figure 3A and 3C). Stems without sprouts collected on the first and second day were used for subtractive cDNA libraries. For loblolly pine, sprouts were seen seven days after treatment, and stems collected on day six and seven after treatment were used (Figure 3B and 3D). After tissue collection, the experimental approach used is outlined in Figure 4.

Construction of subtractive cDNA libraries

Total RNA was extracted from shortleaf pine and loblolly pine tissue samples collected as described above. As we aimed to identify genes responsible for sprouting in shortleaf pine and loblolly pine after top-killing, only tissues collected just before visual sprouting occurred were used for cDNA library construction. For shortleaf pine, samples collected 24 hours and 48 hours after the cutting treatment were used; and for loblolly pine, samples collected six and seven days after treatment were used. Stem tissue was ground into a fine powder in liquid nitrogen and total RNA was extracted following the pine tree RNA isolation method described by Chang, et al. (1993). cDNAs were obtained by using the Super SMART cDNA synthesis kit (Clonetech, Palo Alto, CA), and cDNA subtraction was carried out using the PCR-Select cDNA subtraction kit (Clonetech, Palo Alto, CA) according to the manufacturer's recommendations. In brief, two different cDNA subtractions (forward subtraction and reverse subtraction) were carried out to construct forward and reverse cDNA libraries, respectively, for both shortleaf pine and loblolly pine. For the forward libraries, cDNA from cutting-treated shortleaf pine and

loblolly pine was used to produce the “tester” (Tester is the cDNA that contains differentially expressed transcript to be identified), and cDNA from uncut shortleaf pine and loblolly pine collected at the same time was used to synthesize the “driver” (Driver is the cDNA that is used as the reference). For the reverse libraries, cDNA from uncut shortleaf pine and loblolly pine was used to produce the “tester”, while cDNA from cutting-treated shortleaf pine and loblolly pine was used to produce the “diver”. Two rounds of subtractive hybridization and PCR amplifications were performed according to CLONTECH instructions. The resultant PCR products were cloned into the pCR8-TOPO T/A cloning vector (Invitrogen, Carlesbad, CA), and transformed into *E. coli* TOP10 cells (Invitrogen, Carlesbad, CA). Spectinomycin-resistant colonies were picked and grown overnight in liquid LB medium containing spectinomycin. The LB medium was incubated at 37°C on a shaker (250 rpm). Transformed cells were stored in liquid LB medium containing 15% glycerol.

Amplification of cDNA inserts and preparation of the cDNA microarray

The cDNA inserts ligated to the pCR8-TOPO T/A cloning vector were amplified by PCR using the primer pair corresponding to the flanking adaptor sequences (Nested 1, 5'-TCGAGCGGCCGCCCGGGCAGGT-3'; Nested 2R, 5'-AGCGTGGTCGCGGCCGAGGT-3'; Clontech).

Transformed bacterial cell lysates rather than the purified plasmid DNA were used for PCR reactions. To lyse cells, five microliters of bacterial culture was added into 95 µl of double-distilled water, and then the mixture was incubated at 98 °C for seven minutes. After incubation, 0.6 µl of burst cell templates were added to 10 µl of PCR mixture

containing 0.20 mM of each nucleotide, 0.25 µM of each primer, 1X buffer, 1.5mM $MgCl_2$ and 0.25 units of Taq DNA polymerase (Promega). The PCR protocol included an initial step of 5 min at 95 °C, followed by 35 cycles of the following incubation pattern: 95 °C for 45 sec, 68 °C for 45 sec, and 72 °C for 1 min. A final step at 72 °C for 7 min concluded the reaction. PCR products were subjected to agarose gel electrophoresis and the gels were inspected to find positive transformants. Then for the positive transformants, two microliters of burst cell templates were added to 50 µl of PCR mixture. The PCR product was cleaned up by ethanol precipitation. For precipitation, the PCR product was mixed with 125µl ethanol and 5 µl of 5 M NH_4OAc (pH 7.4). The mixture was inverted several times and then stored at -80°C for one hour. To recover the precipitated DNA, the mixture was centrifuged at 4,600 rpm (3,650 G) for 40 min at 4°C. The DNA pellet was rinsed with 70% ethanol and centrifuged again. After the second centrifugation, the DNA pellet was dried and resuspended in 15 µl 3X SSC, which was diluted from 20X SSC (3 M NaCl, 0.3 M sodium citrate). Normalization control DNAs (spike_1, spike_3, spike_5, spike_7, and spike_9) from the microarray control set provided by the *Arabidopsis* functional genomics consortium (AFGC) were included in the printing as well. These control DNAs were amplified by PCR reactions and purified with ethanol precipitation for use. As intensities of the two fluorescent dyes Cy3 and Cy5 are affected by many systematic sources of variation, normalization is applied to remove such systematic sources of variation in order to make measured intensities within and between slides comparable. Spiked controls from a different organism spotted on the array slides and included in the two different samples at certain amounts are one frequently used normalization method. Each cDNA clone was

printed three times on Arrayit Superamine2 slides (Telechem International, Sunnyvale, CA) using the GeneMachines OmniGrid 100 system (Genomic solution, Ann Arbor, MI) for technical replication. After printing, the slides were left in the machine for one hour to be rehydrated with hot vapor. Then, the slides were baked at 80°C overnight to immobilize the printed cDNAs.

Preparation of probes and microarray hybridization

Microarray probes were produced from total RNA from stem segments of seedlings from cut and uncut treatments of both shortleaf pine and loblolly pine seedlings, which were collected as described earlier. Total RNA was quantified and the A260:280 ratio was checked using a NanoDrop 1000A spectrophotometer. RNA amplification was performed with 200 nanograms of total RNA using the Amino Allyl MessageAmp II aRNA Amplification kit (Ambion Inc., Austin, TX, USA) following the manufacturers' instructions. In addition, 100 pg of each spike control (spike_1, spike_3, spike_5, spike_7, and spike_9) DNA was mixed to the total RNA of each sample for normalization. In brief, RNA was reverse transcribed into first strand cDNA, which was primed from an oligo(dT) primer containing a phage T7 RNA polymerase promoter sequence. The second-strand was synthesized by DNA polymerase, which was primed by fragments of the original RNA partially digested by RNase H. Then doubled-stranded cDNA was purified and *in vitro* transcription was performed to generate antisense RNA (aRNA), during which amino allyl-labeled dUTPs were included. The amino allyl UTP residues on the aRNA were coupled to Cy (Cy3 for control samples; Cy5 for cutting treated samples) dyes (Amersham Biosciences) following aRNA purification.

Quantification was carried out on products of each step with the NanoDrop spectrophotometer. OD (optical density) and gel electrophoresis were used to check nucleic acid integrity. Thus RNA from each sample was comparable and the quality was assured for each step. The resultant cDNA probes were mixed with formamide-based hybridization buffer and nuclease free water. Then the cDNA probe mixture (from both cutting treatment samples and control samples) was transferred to the slide without creating any bubbles. A 24x60 mm LifterSlip (Erie Scientific Company, Portsmouth, NH) cover slip was placed on top of the array slide and the slide was kept in the hybridization chamber at 42°C overnight for hybridization. After hybridization, stringent washes were carried out according to the manufacturer's instructions. Probes for the replicate hybridizations were independently prepared from cutting treated and control shortleaf pine and loblolly pine tissues to minimize technical errors.

Microarray scanning and data analysis

Fluorescent intensities of Cy3 and Cy5 dyes at each spot on the array slides were determined by using ScanArray Express scanner (Perkin-Elmer, Wellesley, MA, USA). Separate images for Cy3 and Cy5 dyes were obtained through array scanning at the wavelength of 633nm and 543nm, respectively. The images were then combined, and ScanArray Express microarray analysis software was used to identify combined spots. Laser power and PMT (Photo Multiplier Tube) settings were adjusted during the scanning process to balance overall intensities in two channels (i.e. Cy3 and Cy5) while avoiding a high number of saturated spots. Signal ratios at spike control spots (Spike_1,

spike_3, spike_5, spike_7, and spike_9) were watched during adjustment, and setting adjustment was complete when most of spike control spots had Cy5 to Cy3 ratios equal to one and appeared yellow on balanced images. Local background was subtracted from intensity of each spot. The ratio of the resultant adjusted intensities of Cy5 to Cy3 was computed for each spot. The normalization process was conducted according to GenePix Pro program (version 6.0), during which spots with bad quality and low signal intensity (less than 200 Relative Fluorescence Units) were removed. Differences in expression were considered significant for a ≥2-fold change between the treated tree sample and the control tree sample, therefore log2 ratios of less than minus one or greater than one were deemed significant.

DNA sequencing and database search

The differentially expressed cDNAs after top-killing identified by array experiments were sequenced as follows. Cell lysates used for the synthesis of microarray cDNA were used as PCR templates. Inserts of the cDNA clones were amplified by PCR using M13 forward primer (5'-GTAAAACGACGGCCAG-3') and M13 reverse primer (5'-CAGGAAACAGCTATGAC-3'). Shrimp alkaline phosphatase (SAP) and exonuclease I (EXO I) were used to purify PCR products for sequencing. Five microliters of PCR product was mixed with 0.4 µl of enzyme mix (0.5 U/ µl of SAP and 0.5 U/ µl EXO I), and then the mixture was incubated at 37 °C for 30 min and 85 °C for 15 min. Two microliters of purified PCR product was added to the following reaction mixture: 1µl 5X sequencing buffer (400 mM Tris, 10 mM $MgCl_2$, pH 9), 1 µl M13 forward primer (100 ng/ µl), 2µl BigDye Terminator (Applied Biosystems, Forster City, CA), and 4 µl of

deionized water. The PCR protocol for sequencing reactions included an initial step of 30 sec at 95 °C, followed by 36 cycles of the following incubation pattern: 96 °C for 10 sec, 50 °C for 5 sec, and 60 °C for 4min. The resultant PCR products were purified by ethanol precipitation and were sequenced using the ABI Model 3700 DNA Analyzer (Applied BioSystem). BLAST search was performed to determine functions of differentially expressed genes. The sequences were searched against GenBank databases, protein (nr) and EST (dbEST), by BLASTX and BLASTN, respectively. BLASTN was used for cDNAs which had no significant hits (with E value cutoff at 0.001) when BLASTX was performed.

Real time "quantitative" PCR (q RT-PCR) analysis

Real time "quantitative" PCR analyses were performed to validate the microarray results and also to further examine when genes important for sprouting were expressed. Total RNA was isolated from tissues collected at several different time points following the cut treatment, as well as from non-treated control shortleaf pine and loblolly pine seedlings (Figure 5). Reverse transcription was carried out on 5μg of DNase-treated mRNA with the SuperScript III (Invitrogen) reagent set. Primers were designed for the various specific genes which were proposed to be significant for triggering sprouting. Five nanograms of cDNA along with 250 nM of each primer pair were subjected to real time PCR using an ABI Prism 7500 sequence detection system (Applied Biosystems) and SYBR Green master mix, according to manufacturer's recommendations (Applied Biosystems). In brief, qRT-PCR amplification mixtures (15 μl) containing 15 ng template cDNA, 2x SYBR Green I Master Mix buffer (7.5 μl), and 300 nM forward and

reverse primer were prepared. Target mRNA values were normalized using actin mRNA as an internal control. Primer pairs used for actin amplification were as follows: 5'-TCCATCGTCCACAGAAAATG-3' (forward primer), and 5'-CAAGATGCGTCATCCCACTA-3' (reverse primer). PCR was performed as follows: a) 50°C for 2 min; b) 95°C for 10 min; c) 95°C for 40 sec; d)55°C for 40 sec; e)72°C for 40 sec; f) repeat step c to step e for 45 cycles. A comparative threshold cycle (C_T) was used to determine gene expression relative to the control. For each sample, the C_T values were calculated using the formula $\Delta C_T = C_{Treference} - C_{Ttarget}$. To determine relative expression levels, the following formula was used $\Delta\Delta C_T = \Delta C_{Tcontrol} - \Delta C_{Ttreatment}$ and the value used to indicate relative gene expression was calculated using the formula $2^{-\Delta\Delta C_T}$.

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CHAPTER IV

RESULTS

Shortleaf pine and loblolly pine were studied to profile sprouting responsive genes after top-killing to provide a better understanding of (1) shortleaf pine's prolific sprouting after top-killing and (2) gene expression differences that may relate to sprouting between shortleaf pine and loblolly pine after top-killing. As seen during tissue collection, shortleaf pine and loblolly pine showed huge differences in sprouting speed and number of sprouts after cutting treatment (Figure 3A-3D). Numerous sprouts became apparent between 24h-48h after cutting treatment on shortleaf pine, while on loblolly pine, only a few sprouts were observed seven days after cutting treatment. As we wanted to identify genes responsible for sprouting after top-killing, only tissues collected just before sprouting were used for cDNA library construction and the array experiment. Therefore, for shortleaf pine, tissues collected on the first and second day were used, and for loblolly pine, tissues collected on the sixth and seventh day were used.

cDNA library construction and array slides preparation

Two SSH (suppression subtractive hybridization) cDNA libraries enriched in genes responsive to top-killing were constructed from the shortleaf pine and loblolly pine tissue samples. One thousand and eighteen shortleaf pine cDNA inserts and 1,319 loblolly pine

cDNA inserts were included in the cDNA libraries. In total, a collection of 2,337 cDNA clones were obtained from the SSH cDNA libraries and printed on specially designed Arrayit Superamine2 slides (Telechem International, Sunnyvale, CA) for the microarray analysis.

The cDNA clones were arranged on the slides as shown in Figure 6. The left part of the slides contained cDNA from shortleaf pine and the right part of the slides contained cDNA from loblolly pine. As each slide contained both shortleaf pine cDNA inserts and loblolly pine cDNA inserts, the hybridization conducted on the slide actually included self hybridization and cross hybridization. When labeled shortleaf pine RNA was applied to the slides, on the left part of the slide, the result was shortleaf pine self hybridization, on the right part of the slide, the result was cross hybridization between labeled shortleaf pine RNA and loblolly cDNA. The converse was true when using labeled loblolly pine RNA.

Based on the collected cDNAs, two microarray analyses were performed. Each microarray analysis was designed to investigate expression patterns of transcriptomes from shortleaf pine and loblolly pine, respectively. In the microarray analyses, expression profiles of shortleaf pine and loblolly pine genes showed induction or suppression in response to cutting treatment after top-killing. Three technical replicates were used to minimize variability of the results.

Differentially expressed genes in shortleaf pine and loblolly pine detected by array experiments

In this study, genes were considered differentially regulated if intensity ratios of cDNA clones from the microarray analyses showed more than a two-fold change of expression up or down. Genes with more than two fold up or down regulation were sequenced. Ultimately, 139 unique genes showing differential expression were identified. The partial sequence of each of these genes is included in Appendix I (page 190). These genes have been deposited in the GenBank EST database and they can be accessed with the corresponding accession numbers given in the appendix. One hundred and six of these genes were of shortleaf pine origin, with the gene names starting with SLP, and 38 were of loblolly pine origin, with the gene names starting with LLP. As shown in Figure 7, five out of the 139 unique genes were identified from both shortleaf pine and loblolly pine, with the names starting with SLP_LLP.

Although 106 differentially expressed genes were of shortleaf pine origin, not every gene was identified during shortleaf pine self-hybridization; rather, four genes were sent for sequencing because of their detected differential expression during cross hybridization. As shown in Figure 1, 61 and 42 genes of shortleaf pine origin showed up and down regulation during shortleaf pine self-hybridization, respectively. Four genes of shortleaf pine origin did not show differential expression during shortleaf pine self-hybridization, but showed differential expression during shortleaf pine-loblolly pine cross hybridization when labeled loblolly pine cDNAs were applied to the array slides and hybridized to the shortleaf pine cDNA on the array slides. On the other

hand, 25 out of 34 genes of loblolly pine origin were sent for sequencing due to differential expression during cross hybridization. The cross hybridization provided us with information of genes which were differentially expressed but were not included in the cDNA libraries of either shortleaf pine or loblolly pine. For loblolly pine, six and seven genes showed up- and down- regulation during loblolly pine self hybridization, respectively; while another 15 and four genes showed up- and down- regulation during the cross hybridization when labeled loblolly pine cDNAs were applied to the array slides and hybridized to the shortleaf pine cDNAs on the array slides.

Function of diffentially expressed genes determined by BLAST search

cDNA sequences were blasted against the NCBI database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). BLAST hits for each query cDNA sequence showed up in order according to the similarity between specific sequences in the database and the query cDNA sequence, with the most similar sequence detected in the database showing up in the first place. The E value provided information regarding the possibility of the query cDNA sequence matching any random sequences in the database. A smaller E value indicated a higher possibility for the query sequence to have the exact same function as one specific "fished" gene in the database. Initially, each cDNA was blasted against GenBank's non-redundant protein (NR) database, during which the cDNA was first translated into proteins according to different open reading frames (ORFs) and then the translated protein sequences were blasted against the protein database. If no hit was found (with E value cutoff at 0.001), the query

cDNA was then blasted against GenBank EST databases (dbEST, None-human, none mouse ESTs, EST others) with BLASN.

Functional classification of genes

A total of 139 genes differentially regulated in response to cutting treatment were listed and categorized according to the putative function of each gene from BLAST search (Tables 2-16). The signal intensity ratios of these genes from microarray analyses are also provided in the tables. The putative functions of these genes were inferred from metabolic processes known to be related to each gene. Although some genes act in multiple metabolic processes, they were classified according to their main functions in plant metabolism. The genes found responsive to cutting treatments were classified into 15 functional categories (Tables 2-16), including transcription factors, cell growth and maintenance, carbohydrate metabolism, signal transduction, ubiquitin related, pathogenesis related, hormone related, fatty acid metabolism, transport, protein and amino acid metabolism, stress responsive, translation, photosynthesis, transcribed loci with unknown function, and genes with no hit in the databases (Figure 8). The largest two categories of genes were genes for transcribed loci with unknown function and genes with no hit in the databases searched; genes that were stress responsive and genes that were involved in cell growth and maintenance were ranked as the third and fourth largest groups, respectively; followed by protein and amino acid metabolism related genes (Figure 8).

Sequence annotation and analysis for differentially expressed genes

Based on the preliminary results generated by NCBI BLAST search, cDNAs encoding interesting and important proteins from each functional category were further analysized and annotated. Determination of a gene as "interesting and important" was based on literature search results. If the results suggested the possibility of any specific gene to function in bud dormancy release and/or sprouting processes in some way, this gene was included in the detailed analysis. Housekeeping genes and genes with no documentation for bud dormancy release and/or sprouting function were not included. First, DNA translation analysis was performed on cDNAs of interest by using ExPASy Translate tool (http://ca.expasy.org/tools/dna.html), during which each cDNA was translated into proteins according to six different open reading frames (ORFs). Deduced proteins with reasonable length (at least 10 amino acids long) were selected, and amino acid sequences of these deduced proteins were recorded and put in order according to their length. Each deduced protein was then blasted against GenBank's non-redundant protein (NR) database to identify if it had conserved domains for one specific protein family or if it had homologs from other species. If no conserved domains or homologs were detected for any deduced proteins from the query cDNA sequence, this query cDNA was then blasted against NCBI EST databases, and if found, a longer EST with high similarity (close to 100%) to this cDNA was used for DNA translation analysis. (Deduced) protein sequences from several other species sharing conserved domains or homology with the deduced protein sequence from the query cDNA were identified during blast search. Multiple sequence alignment (MSA)

was performed using ClustalW2 (http://www.ebi.ac.uk/Tools/clustalw2/index.html) on these (deduced) protein sequences including the one from the query cDNA.

Transcription factor related genes

Three transcription factors were identified, SLP1, SLP2 and SLP3, as shown in Table 2. These three genes are all of shortleaf pine origin, as indicated by the names. In Table 2, values for signal ratio for genes with at least a two-fold up-or down-regulation were shaded in blue and yellow, respectively. As seen in Table 2, SLP1 showed downregulation in shortleaf pine, while SLP2 and SLP3 showed upregulation. No transcription factor of loblolly pine origin was identified. Neither did cross hybridization of loblolly pine cDNA with shortleaf pine cDNA printed on the array slides produce strong signals.

SLP1 encodes a NAM transcription factor, which functions in plant shoot meristem formation (Aida and Tasaka, 2006), as well as in stress response (Olsen et al., 2005; Chen et al., 2008; Seo et al., 2008). SLP1 was 380 base pairs in length and the deduced protein from SLP1 was 95 amino acids long (Figure 9). Figure 10 provides the MSA of SLP1 with NAM proteins from several other tree species, including white spruce (*Picea glauca* (Moench) Voss), Norway spruce (*Picea abies* L.), and black spruce (*Picea mariana* Mill.). As seen from this alignment, sequences for NAM transcription factor from different tree species shared a high level of similarity.

SLP2 is homologous to the homeobox transcription factor KN3 in eastern white pine (*Pinus strobes* L.). In *Arabidopsis*, this gene has another name: STM (SHOOT

MERISTEMLESS). SLP2 was 466 base pairs in length. By translation, the deduced protein was 114 amino acids long (Figure 11). Protein blast showed that this protein had a conserved homeodomain, which is known to function in transcriptional regulation during plant development. Figure 12 shows the MSA of SLP2 with STM proteins from several other species. As seen from this alignment, sequences for the STM transcription factor from diverse species shared a high level of similarity.

SLP3 encodes a GT-1-like transcription factor. SLP3 was 481 base pairs in length. No significant hits were observed during BLASTX search. SLP3 was then blasted against GenBank EST databases using BLASTN. Although BLASTN identified SLP3 as a homolog to *Arabidopsis* DNA binding protein GT-1, DNA translation analysis of SLP3 did not detect any deduced protein sequences (of at least 10 amino acids) containing any conserved domains; neither did those deduced sequences have GT-1-like homologs from other species. Therefore, query SLP3 cDNA sequence might only include the five or three prime untranslated region (5' UTR or 3'UTR) of the full GT-1 cDNA sequence, or part (less than 10 amino acids long) of the GT-1 coding region, and it was difficult to detect any conserved domain or homologs based on such short deduced protein sequences. SLP3 was then blasted against the NCBI EST database again, and a longer EST sequence (903bp, accession number: 148815838) from sitka spruce (*Picea sitchensis* (Bong.) Carrière) aligning very well with SLP3 was identified. This EST was used for DNA translation analysis, and one deduced protein sequence (49 amino acids long) had GT-l homologs from several other species. Figure 13 shows the MSA

for all these GT-1-like proteins. As seen from this alignment, sequences for the GT-1-like transcription factor from diverse species shared a high level of similarity.

Genes in cell growth and maintenance

Thirteen cell growth and maintenance related genes were differentially expressed as shown in Table 3. All these genes are of shortleaf pine origin, with the exception of LLP1. Eleven out of the 13 genes showed differential expression in shortleaf pine (except SLP10 and LLP1). LLP1 is of loblolly pine origin and it showed downregulation in loblolly pine. Besides LLP1, five genes of shortleaf pine origin showed differential expression during shortleaf pine-loblolly pine cross hybridization when labeled loblolly pine cDNAs were applied to the array slides for hybridization. For genes both differentially expressed in shortleaf pine and loblolly pine (either through self hybridization or cross hybridization), SLP4 showed differential expression in opposite directions in the two species, with downregulation in shortleaf pine while upregulated in loblolly pine. The other three genes (SLP6, SLP7 and SLP12) showed differential expression in the same direction, either upregulation or downregulation, in both species.

SLP4 encodes an expansin-like protein. SLP4 was 273 base pairs long. A much longer loblolly pine EST (873bp, accession number: 67488878) with high similarity (close to 100%) to SLP4 was used for DNA translation analysis. The deduced protein from this EST was 99 amino acids long. Protein blast showed that this protein had a conserved domain among expansin-like proteins. Figure 14 shows the MSA for the expansin-like proteins from several species. As seen from this alignment, the deduced protein

sequence from SLP4 shared a high level of similarity with ATEXPA4-like proteins from diverse species, including China fir (*Cunninghamia lanceolata* (Lamb.) Hook.), castorbean (*Ricinus communis* L.), tomato (*Solanum lycopersicum* L.) and soybean (*Glycine max* L. Merr.).

SLP5 encodes a pectin-methylesterase-like protein. SLP5 was 502 base pairs long. The deduced protein was 55 amino acids long. Protein BLAST showed that this protein has a pectin-methylesterase-specific domain. Figure 15 shows the MSA for pectin-methylesterase-like proteins from several species. As seen from this alignment, the deduced protein sequence from SLP5 shares a high level of similarity with pectin-methylesterase-like proteins from diverse species, including black cottonwood (*Populus trichocarpa* L.), castorbean (*Ricinus communis* L.), banana (*Musa acuminata*), and coffin tree (*Taiwania cryptomerioides*).

SLP7 and SLP8 both encode glycine-rich cell wall proteins. SLP7 was 83 base pairs in length, and SLP8 was 307 base pairs long. No significant hits were observed during BLASTX search for both sequences. Individual BLAST search with SLP7 and SLP8 against NCBI EST databases showed that both sequences had a high similarity to one loblolly pine EST sequence with the accession number of 68089089. SLP7 and SLP8 shared 15 base pairs at the end of the sequences, where sequencing error rate was normally high. Due to the high sequencing error, SLP7 and SLP8 were regarded as different genes initially and deposited into GenBank. EST68089089 was 814 base pairs long, and the deduced protein was 154 amino acids long. Figure 16 shows the MSA for

(deduced) amino acid sequences for glycine-rich cell wall like proteins from different species, including the loblolly pine EST with the accession number of 68089089, which was used as substitute for SLP7 and SLP8 during analysis due to high sequence similarity shared among EST68089089, SLP7 and SLP8.

SLP11 encodes STT3B (staurosporine and temperature sensitive 3-like B), which acts as an oligosaccharyl transferase. SLP11 was 330 base pairs long, and the deduced protein was 109 amino acids long. Figure 17 shows the MSA of SLP11 with STT3B-like proteins from several other species, including *Arabidopsis*, black cottonwood (*Populus trichocarpa* L.), and grape (*Vitis vinifera* L.). As seen from this alignment, the deduced protein sequence from SLP11 shared a high level of similarity with STT3B-like proteins from diverse species.

LLP1 encodes an O-methyltransferase-like protein. It was 315 base pairs in length. The deduced protein was 104 amino acids long, and it had a conserved domain shared by methyltransf_2 superfamily proteins. Figure 18 shows the MSA of LLP1 with O-methyltransferase-like proteins from several different species, including loblolly pine, black cottonwood (*Populus trichocarpa* L.), barrel clover (*Medicago truncatula* Gaertn.), and *Arabidopsis*.

SLP13 encodes a TET8 (TETRASPANIN8)-like protein. SLP13 was 363 base pairs long, and the deduced protein was 68 amino acids long. This protein had a conserved domain shared by proteins belonging to the tetraspanin_LEL (large extracellular loop)

superfamily. Figure 19 shows the MSA for TET8-like proteins from several species. As seen from this alignment, the deduced protein sequence from SLP13 shared a high level of similarity with TET8-like proteins from diverse species, including black cottonwood (*Populus trichocarpa* L.), wild cabbage (*Brassica oleracea* L.), and *Arabidopsis*.

SLP15 encodes a pentatricopeptide repeat-containing protein. SLP15 was 317 base pairs long. The deduced protein was 105 amino acids long. Protein blast showed that this protein contained a conserved pentatricopeptide repeat domain (PPR motif). This conserved domain consists of 33 amino acids. Figure 20 shows the MSA for PPR motifs in several species. As seen from this alignment, the deduced PPR motif-containing protein sequence from SLP15 shared a high level of similarity with PPR motifs from diverse species, including black cottonwood (*Populus trichocarpa* L.), rice (*Oryza sativa* L.), and *Arabidopsis*.

Carbohydrate metabolism related genes

Seven carbohydrate metabolisms related genes showed differential expression as shown in Table 4. All these seven genes are of shortleaf pine origin, as indicated by their names. All these seven genes showed upregulation in shortleaf pine, except SLP23, which showed downregulation. None of these seven genes showed differential expression in loblolly pine.

SLP17 encodes malate synthase, which is involved in glycolysis. SLP17 was 437 base pairs long, and the deduced protein was 92 amino acids long. This protein contained a

conserved domain which belongs to the malate synthase superfamily. Figure 21 shows the MSA for malate synthase from diverse species. As seen from this alignment, the deduced protein sequence from SLP17 shared a high level of similarity with (deduced) protein sequences for malate synthase from diverse species, including black cottonwood (*Populus trichocarpa* L.), rice (*Oryza sativa* L.), and castorbean (*Ricinus communis* L.).

SLP18 encodes pyruvate kinase, which is also involved in glycolysis. SLP18 was 499 base pairs long, and the deduced protein was 95 amino acids long. When blasted against the NCBI protein database, this protein was identified as having a conserved domain shared by the PK (pyruvate kinase)_C superfamily. Figure 22 shows the MSA for pyruvate kinase from diverse species. As seen from this alignment, the deduced protein sequence from SLP18 shared a high level of similarity with (deduced) protein sequences for pyruvate kinase from diverse species, including corn (*Zea mays* L.), barrel clover (*Medicago truncatula* Gaertn.), and castorbean (*Ricinus communis* L.).

SLP19 encodes fructose-bisphosphate aldolase. SLP19 was 400 base pairs long, and the deduced protein was 70 amino acids long. This protein had a conserved domain belonging to the TIM_phosphate_binding superfamily. Figure 23 shows the MSA for fructose-bisphosphate aldolase from several different species. As seen from this alignment, the deduced protein sequence from SLP19 shared a high level of similarity with (deduced) protein sequences for fructose-bisphosphate aldolase from diverse

species, including corn (*Zea mays* L.), tobacco (*Nicotiana tabacum* L.), and castorbean (*Ricinus communis* L.).

SLP20 encodes glucose-6-phosphate 1-dehydrogenase (G6PD), which functions in the pentose pathway. SLP20 was 339 base pairs long. When SLP20 sequence was blasted against the EST database, a longer loblolly pine EST (619bp, accession number 34350332) with close to 100% similarity to SLP20 was identified. This longer EST was used for DNA translation analysis. The deduced protein from this longer EST was 85 amino acids long. Protein blast showed that this protein had a conserved domain belonging to the G6PD_C (Glucose-6-phosphate dehydrogenase, C-terminal domain) superfamily. Figure 24 shows the MSA for G6PD from several species. As seen from this alignment, the deduced protein sequence from SLP20 shared a high level of similarity with (deduced) protein sequences for G6PD from diverse species, including common wheat (*Triticum aestivum* L.), poplar (*Populus suaveolens*), tobacco (*Nicotiana tabacum* L.), and *Arabidopsis*.

SLP21 encodes invertase, which is involved in carbohydrate metabolism by breaking down sucrose to fructose and glucose (Bocock et al., 2008). SLP12 was 416 base pairs in length, and the deduced protein was 96 amino acids long (Figure 25). When blasted against the protein database, this protein showed a conserved domain belonging to the plant neutral invertase superfamily. Figure 26 shows the MSA for invertase. As seen from this alignment, the deduced protein sequence from SLP21 shared a high level of similarity with (deduced) protein sequences for invertase from diverse species,

including legume (*Lotus japonicus*), tomato (*Solanum lycopersicum* L.), rice (*Oryza sativa* L.), and corn (*Zea mays* L.).

SLP22 encodes amylase, which is involved in carbohydrate metabolism by breaking down starch to sucrose (Lao et al., 1999; Yamasaki, 2003). SLP22 was 344 base pairs in length. The deduced protein was 68 amino acids long (Figure 27). This protein had a conserved domain belonging to the glyco_hydro (glycosyl hydrolase) family 14. Figure 28 shows the MSA for amylase. As seen from this alignment, the deduced protein sequence from SLP22 shared a high level of similarity with (deduced) protein sequences for amylase from diverse species, including barley (*Hordeum vulgare* L.), soybean (*Glycine max* L. Merr.), corn (*Zea mays* L.), and *Arabidopsis*.

Genes in signal transduction

Four signal transduction related genes were differentially expressed in this study as shown in Table 5. SLP_LLP1 was identified in both species, and in this case, only the signal ratio shown during self-hybridization is included in the table. SLP_LLP1 showed upregulation only in shortleaf pine, while no strong signal was detected in loblolly pine whether during self-hybridization or cross hybridization. The other three genes (SLP24, SLP25 and SLP27) are all of shortleaf pine origin. SLP24 and SLP25 were both upregulated in shortleaf pine, while SLP27 was downregulated. Of these four differentially expressed genes, only SLP25 showed a more than two-fold upregulation in loblolly pine during cross hybridization.

SLP24 encodes a phosphatase 2C (PP2C)-like protein, which acts in plant signal transduction by adding phosphate groups to target proteins. SLP24 was 287 base pairs in length, and the deduced protein was 45 amino acids long (Figure 29). This protein had a conserved domain belonging to the PP2Cc (serine/threonine phosphatases, family 2C, catalytic subunit) protein family. Figure 30 shows the MSA for PP2C. As seen from this alignment, the deduced protein sequence from SLP24 shared a high level of similarity with (deduced) protein sequences for PP2C from diverse species, including barrel clover (*Medicago truncatula* Gaertn.), rice (*Oryza sativa* L.), castorbean (*Ricinus communis* L.), and *Arabidopsis*.

SLP25 encodes the catalytic subunit of protein phosphatase 2A (PP2A). SLP25 was 497 base pairs in length, and the deduced protein was 65 amino acids long (Figure 31). When blasted against NCBI protein database, this protein showed a conserved domain for protein family PP2Ac (protein phosphatase 2A homologues, catalytic domain). Figure 32 shows the MSA for the catalytic subunit of PP2A. As seen from this alignment, the deduced protein sequence from SLP25 shared a high level of similarity with (deduced) protein sequences for the catalytic subunit of PP2A from diverse species, including corn (*Zea mays* L.), rice (*Oryza sativa* L.), tomato (*Solanum lycopersicum* L.) and *Arabidopsis*.

SLP_LLP1 was 328 base pairs long. When the SLP_LLP1 sequence was blasted against the GenBank EST databases, SLP_LLP1 was identified as a weak homolog of an *Arabidopsis* receptor-like protein kinase. The deduced protein of SLP_LLP1 was 99

amino acids long, and when it was blasted against the protein database, hypothetical protein kinases from several species were identified. Figure 33 shows the MSA for these protein kinases. As seen from this alignment, the deduced protein sequence from SLP_LLP1 shared similarity with (deduced) protein sequences for hypothetical receptor kinases from diverse species, including corn (*Zea mays* L.), grape (*Vitis vinifera* L.), and *Arabidopsis*.

Hormone related genes

Six hormone related genes were differentially expressed in this study as shown in Table 8. All these genes are of shortleaf pine origin with the exception of LLP3. All six genes were differentially expressed in shortleaf pine, with SLP34, SLP35 and SLP37 showing upregulation, and the other three showing downregulation (LLP3's downregulation was detected during cross hybridization). Five out of the six genes did not show differential expression in loblolly pine either through self-hybridization or cross hybridization, and the only exception was SLP34, which showed upregulation during cross hybridization.

SLP34 encodes AP2/ERF (APETALA2/ethylene-responsive factor), which is an ethylene responsive transcription factor. SLP34 was 397 base pairs in length. The deduced protein was 132 amino acids long (Figure 34). Blast search showed that this protein contained a 59 amino acids-long DNA-binding domain unique to plant transcription factors such as APETALA2 and EREBP. Figure 35 shows the MSA for the DNA binding domain of AP2/ERF. As seen from this alignment, the deduced protein sequence from SLP34, which contains the 59 amino acid-long DNA binding

domain, shared a high level of similarity with (deduced) protein sequences for the DNA binding domain of AP2/ERF from diverse species, including chickpea (*Cicer arietinum* L.), soybean (*Glycine max* L. Merr.), corn (*Zea mays* L.), and black cottonwood (*Populus trichocarpa* L.).

SLP35 encodes an auxin-repressed protein-like protein. SLP35 was 606 base pairs long, and the deduced protein was 136 amino acids in length (Figure 36). Protein blast search showed that this protein had a conserved domain belonging to the auxin-repressed protein superfamily. Figure 37 shows the MSA for auxin-repressed protein-like proteins from several species. As seen from this alignment, the deduced protein sequence from SLP35 shared a high level of similarity with (deduced) protein sequences for auxin-repressed protein-like proteins from diverse species, including tree peony (*Paeonia suffruticosa* Andrews), citrus (*Shiranuhi*), bonnet bellflower (*Codonopsis lanceolata*), corn (*Zea mays* L.), and *Solanum virginianum*.

SLP37 was 370 base pairs long, and the deduced protein was 123 amino acids long. This protein had a conserved domain belonging to the superfamily of isopenicillin N synthase and related dioxygenases. This protein showed a high sequence similarity to gibberellin 7-oxidase found in squash (*Cucurbita maxima* Duchesne). Figure 38 shows the sequence alignment between deduced protein sequences for SLP37 and squash gibberellin 7-oxidase.

SLP38 encodes anthocyanidin reductase. SLP38 was 438 base pairs long, and the deduced protein was 146 amino acids long. Protein blast showed that this protein had a conserved domain belonging to the DADM (nucleoside-diphosphate-sugar epimerases) Rossmann superfamily. Figure 39 shows the MSA for anthocyanidin reductase from several species. As seen from this alignment, the deduced protein sequence from SLP38 shared a high level of similarity with (deduced) protein sequences for anthocyanidin reductase from diverse species, including upland cotton (*Gossypium hirsutum* L.), black cottonwood (*Populus trichocarpa* L.), and maidenhair tree (*Ginkgo biloba* L.).

LLP3 encodes a flavanone 3-hydroxylase-like protein. LLP3 was 667 base pairs in length, and the deduced protein was 69 amino acids long. Figure 40 shows the MSA for flavanone 3-hydroxylase like proteins from several species. As seen from this alignment, the deduced protein sequence from LLP3 shared similarity with (deduced) protein sequences for flavanone 3-hydroxylase like proteins from two other species wheat (*Triticum aestivum* L.) and castorbean (*Ricinus communis* L.).

SLP39 encodes a tetratricopeptide repeat (TPR)-containing protein. SLP39 was 897 base pairs long, and the deduced protein was 298 amino acids long. Protein blast showed that this protein contained a conserved TPR (tetratricopeptide repeat) domain. Figure 41 shows the MSA for TPR domains from several species. As seen from this alignment, the deduced protein sequence from SLP39, which contained a TPR domain,

shared a high level of similarity with (deduced) protein sequences for TPR domains from two other species *Arabidopsis* and rice (*Oryza sativa* L.).

Fatty acids metabolism related genes

Three fatty acids metabolism related genes were differentially expressed in this study as shown in Table 9. All three genes are of shortleaf pine origin, and they were differentially expressed in shortleaf pine, with SLP40 and SLP41 showing upregulation, while SLP42 showing downregulation. In loblolly pine, during cross hybridization, SLP40 was upregulated, no signal was detected for SLP41, and no strong signal (greater than a two-fold change in expression) was detected for SLP42.

SLP40 encodes caleosin. SLP40 was 560 base pairs long, and the deduced protein was 127 amino acids long. Protein blast showed that this protein had a conserved domain belonging to the caleosin superfamily. Figure 42 shows the MSA for caleosin from several species. As seen from this alignment, the deduced protein sequence from SLP40 shared a high level of similarity with (deduced) protein sequences for caleosin-like proteins from diverse species, including sago cycad (*Cycas revolute* Thunb.), rape (*Brassica napus* L.), Easter lily (*Lilium longiflorun* Thunb.), and corn (*Zea mays* L.).

SLP41 encodes a triacylglycerol lipase (Class III)-like protein. SLP41 was 475 base pairs long, and the deduced protein was 86 amino acids long. This protein contained a 45 amino acids-long protein sequence, which shared a high sequence similarity to triacylglycerol lipase proteins in many other species, including sitka spruce (*Picea*

sitchensis (Bong.) Carrière), castorbean (*Ricinus communis* L.), and rice (*Oryza sativa* L.), as shown by the MSA in Figure 43.

SLP42 encodes a GNS1/SUR4 membrane protein. SLP42 was 373 base pairs long. When blasted against the EST databases, a longer loblolly pine EST sequence (accession number: 48949032) was identified as having close to 100% similarity to SLP42, with only a few base pairs differences at the beginning of SLP42, which might be due to sequencing errors. This loblolly pine EST was used for DNA translation analysis. The deduced protein from this EST was 165 amino acids long. Protein blast showed that this protein had a conserved domain belonging to the ELO (elongation) and GNS1/SUR4 protein family. Figure 44 shows the MSA for GNS1/GNS1 proteins from several species. As seen from this alignment, the deduced protein sequence from SLP42 shared a high level of similarity with (deduced) protein sequences for GNS1/SUR4 membrane proteins from diverse species, including barrel clover (*Medicago truncatula* Gaertn.), corn (*Zea mays* L.), and *Arabidopsis*.

Transporter related genes

Seven transporter related genes were differentially expressed in this study as show in Table 10. Three of them are of shortleaf pine origin, and the other four are of loblolly pine origin. All seven genes showed differential expression in shortleaf pine either during self-hybridization or cross hybridization, with SLP43, SLP44 and LLP6 showing upregulation, and the remaining (LLP4, LLP5 LLP7, and SLP45) showing downregulation. Only SLP44 showed upregulation during cross hybridization in loblolly pine.

SLP43 encodes an ABC transporter protein. SLP43 was 681 base pairs long, and the deduced protein was 87 amino acids long. This protein had a conserved domain belonging to the ABC transporter superfamily. Figure 45 shows the MSA for ABC transporter proteins from several species. As seen from this alignment, the deduced protein sequence from SLP43 shared a high level of similarity with (deduced) protein sequences for ABC transporter proteins from diverse species, including black cottonwood (*Populus trichocarpa* L.), pepper (*Capsicum chinense* Jacq.), and *Arabidopsis*.

LLP6 encodes a nitrate transporter-like protein. It was 95 base pairs in length. It had close to 100% similarity with a longer loblolly EST sequence (783bp) in GenBank EST databases, with the accession number of 67487349. This longer EST sequence was then used in DNA translation analysis. EST 67487349 encoded a protein of 163 amino acids long, which had a conserved PTR2 (peptide transporter 2) domain. Figure 46 shows the MSA for nitrate transporter-like proteins from several species. As seen from this alignment, the deduced protein sequence from LLP6 shared a high level of similarity with (deduced) protein sequences for nitrate transporter-like proteins from diverse species, including black cottonwood (*Populus trichocarpa* L.), tobacco (*Nicotiana tabacum* L.), and *Arabidopsis*.

Protein and amino acid metabolism genes

Thirteen protein and amino acid metabolism related genes were differentially expressed in this study as shown in Table 11. Ten out of these 13 genes are of shortleaf pine

origin, and the other three are of loblolly pine origin. All 13 genes showed differential expression in shortleaf pine during either self-hybridization or cross hybridization, with ten showing upregulation and three downregulation. None of these 13 genes showed differential expression in loblolly pine either in self-hybridization or cross hybridization.

SLP47 encodes a serine-type peptidase/ signal peptidase. SLP47 was 518 base pairs long. The deduced protein was 50 amino acids long. This protein had a conserved domain belonging to the peptidase superfamily. Figure 47 shows the MSA for signal peptidase from several different species. As seen from this alignment, the deduced protein sequence from SLP47 shared a high level of similarity with (deduced) protein sequences for signal peptidase proteins from diverse species, including black cottonwood (*Populus trichocarpa* L.), rice (*Oryza sativa* L.), corn (*Zea mays* L.), and *Arabidopsis*.

SLP48 encodes an aleurain-like protease. SLP48 was 185 base pairs long. The deduced protein was 61 amino acids long. This protein had a conserved domain which belongs to the peptidase C1A superfamily. Figure 48 shows the MSA for the aleurain-like protease from several species. As seen from this alignment, the deduced protein sequence from SLP48 shared a high level of similarity with (deduced) protein sequences for aleurain-like protease from diverse species, including corn (*Zea mays* L.), rape (*Brassica napus* L.), sunflower (*Helianthus annuus* L.), plan (*Plantago major* L.), and tobacco (*Nicotiana tabacum* L.).

SLP49 encodes a subtilisin-like protein, subtilase. Subtilase acts in protein reserve mobilization (Liu et al., 2001; Fontanini and Jones, 2002). SLP49 was 390 base pairs long. The deduced protein was 126 amino acids long. Homologs of sublilase from several other species were identified when the deduced protein was blasted against the NCBI protein database. Figure 49 shows the MSA for subtilisin-like proteins from several species. As seen from this alignment, the deduced protein sequence from SLP49 shared a high level of similarity with (deduced) protein sequences for subtilase from diverse species, including Norway spruce (*Picea abies* L.), grape (*Vitis vinifera* L.), and *Arabidopsis*.

SLP51 encodes a thioredoxin *h*-like protein. SLP51 was 777 base pairs long. The deduced protein was 120 amino acids long. It contained a conserved domain belonging to the thioredoxin like superfamily. The conserved domain was 88 amino acids long. Figure 50 shows the MSA for the conserved domain from several species. As seen from this alignment, the deduced protein sequence from SLP51 shared a high level of similarity with (deduced) protein sequences for thioredoxin *h* from diverse species, including rubber tree (*Hevea brasiliensis* Muell. Arg.), grape (*Vitis vinifera* L.), black cottonwood (*Populus trichocarpa* L.), and *Arabidopsis*.

Stress responsive genes

Fifteen stress responsive genes were differentially expressed in this study as shown in Table 12. Two out of the 15 genes were identified from both species (SLP_LLP3 and SLP_LLP4). For the remaining 13 genes, 10 are of shortleaf pine origin, and the other

three are of loblolly pine origin. In shortleaf pine, five out of the 15 genes showed downregulation, including LLP12, SLP60, SLP62, SLP63 and SLP64, either through self-hybridization or cross hybridization. Among these five genes, four were oxidative stress related, including LLP12, SLP60, SLP62, and SLP63. The other eight genes showed upregulation in shortleaf pine during self-hybridization, and the remaining two genes of loblolly pine origin did not show differential expression during shortleaf pine-loblolly pine cross hybridization. Five out of the 15 genes showed differential expression in loblolly pine, either through self-hybridization (SLP_LLP3, LLP11, LLP13, and LLP14) or cross hybridization (SLP59). For the corresponding differentially expressed oxidative stress related genes, which were downregulated in shortleaf pine, none of them showed downregulation in loblolly pine, with one of them (LLP11) showing upregulation.

SLP57 encodes a cystatin-like protein. SLP57 was 226 base pairs long. The deduced protein was 75 amino acids long. This protein had a conserved domain belonging to the CY (cystatin-like domain) superfamily. Figure 51 shows the MSA for cystatin-like proteins from several species. As seen from this alignment, the deduced protein sequence from SLP57 shared a high level of similarity with (deduced) protein sequences for cystatin-like proteins from diverse species, including tomato (*Solanum lycopersicum* L.), soybean (*Glycine max* L. Merr.), black cottonwood (*Populus trichocarpa* L.), and common wheat (*Triticum aestivum* L.).

LLP11 and LLP12 both encode peroxidase-like proteins. LLP11 was 236 base pairs long and LLP12 was 330 base pairs long. LLP11 and LLP12 had no sequence overlap and were initially thought to be two different genes. Each was deposited into the GenBank databases. However, detailed BLAST search showed that both cDNAs were part of a loblolly pine EST with the accession number of 67962276. This longer loblolly pine EST was used for DNA translation analysis. This EST was 826 base pairs long, and the deduced protein was 188 amino acids long. This protein had a KatG (catalase/hydroperoxidase) domain unique to the plant peroxidase superfamily. This protein belong to the class I superfamily of peroxidases, which is responsible for hydrogen peroxide removal in chloroplasts and cytosol of higher plants. Figure 52 shows the MSA for peroxidase from several different species.

SLP60 encodes a peroxidase-like protein. SLP60 was 403 base pairs long. When SLP60 was blasted against the EST database, a longer loblolly pine EST sequence (accession number 66976703) was identified with close to 100% similarity to SLP60. This loblolly pine EST was 707 base pairs long and was used for DNA translation analysis. The deduced protein from this EST was 156 amino acids in length. Protein blast showed that this deduced protein had a conserved domain belonging to the plant peroxidase superfamily. More specifically, this protein was a member of the class III subfamily of peroxidases, which included secretory peroxidases functioning in hydrogen peroxide detoxification and stress response. Figure 53 shows the MSA for peroxidase-like proteins from several species. As seen from this alignment, the deduced protein sequence from SLP60 shared a high level of similarity with (deduced)

protein sequences for secretory peroxidase-like protein from diverse species, including upland cotton (*Gossypium hirsutum* L.), soybean (*Glycine max* L. Merr.), and *Arabidopsis*.

SLP61 encodes cytochrome P450. SLP61 was 478 base pairs long, and the deduced protein was 159 amino acids in length. Protein blast showed that this protein had a conserved domain belonging to the CypX superfamily. Figure 54 shows the MSA for cytochrome P450 from several species. As seen from this alignment, the deduced protein sequence from SLP61 shared a high level of similarity with (deduced) protein sequences for cytochrome P450 from diverse species, including sweetleaf (*Stevia rebaudiana*), potato (*Solanum tuberosum* L.), and black cottonwood (*Populus trichocarpa* L.).

SLP62 encodes an aldo/keto reductase-like protein. SLP62 was 432 base pairs long. The deduced protein was 143 amino acids in length. Protein blast showed that this protein had a conserved domain belonging to the AKRs (aldo-keto reductase) superfamily. Figure 55 shows the MSA for aldo/keto reducatase from several species. As seen from this alignment, the deduced protein sequence from SLP62 shared a high level of similarity with (deduced) protein sequences for aldo/keto reductase from diverse species, including castorbean (*Ricinus communis* L.), grape (*Vitis vinifera* L.), *Arabidopsis*, and black cottonwood (*Populus trichocarpa* L.).

SLP63 encodes a glutathione S-transferase (GST). SLP63 was 88 base pairs long. When blasted against EST databases, a much longer loblolly pine EST with close to 100% sequence similarity with SLP63 was identified. This EST was 721 base pairs long, and its accession number was 34490708. This longer EST was used for DNA translation analysis. The deduced protein from this EST was 222 amino acids long. Protein blast showed that this protein had a 76 amino acids-long conserved domain, which was unique to the GST protein family. Figure 56 shows the MSA for GST from several different species. As seen from this alignment, the deduced protein sequence from SLP63 (substituted by a loblolly pine EST with the accession number 34490708) shared a high level of similarity with (deduced) protein sequences for GST from diverse species, including rice (*Oryza sativa* L.), green alga *Ostreococcus tauri* (Prasinophyceae), *Arabidopsis*, and black cottonwood (*Populus trichocarpa* L.).

SLP_LLP4 was differentially upregulated in both species. SLP_LLP4 was 282 base pairs long and the deduced protein was 56 amino acids long. Protein blast showed that this protein had a conserved domain belonging to the LEA (late embryogenesis abundant)_3 superfamily. Figure 57 shows the MSA for LEA proteins from several different species. As seen from this alignment, the deduced protein sequence from SLP_LLP4 shared a high level of similarity with (deduced) protein sequences for LEA from diverse species, including tobacco (*Nicotiana tabacum* L.), aleppo pine (*Pinus halepensis* Mill.), and white spruce (*Picea glauca* (Moench) Voss).

LLP13 encodes galactinol synthase (GolS). LLP13 was 682 base pairs long, and the deduced protein was 107 amino acids long. When this protein was blasted against NCBI protein databases, homologs from several species were identified. Figure 58 shows the MSA for these GolS proteins. As seen from this alignment, the deduced protein sequence from LLP13 shared a high level of similarity with (deduced) protein sequences for GolS from diverse species, including ajuga (*Ajuga reptans* L.), showy mullein (*Verbascum phoeniceum* L.), rape (*Brassica napus* L.), and *Arabidopsis*.

SLP36 encodes a water deficit inducible LP3-like protein. SLP36 was 517 base pairs long. The deduced protein was 128 amino acids long. Protein blast showed that this protein had a conserved domain belonging to the ABA_WDS (water deficit stress) superfamily. Figure 59 shows the MSA for the LP3-like proteins from diverse species. As seen from this alignment, the deduced protein sequence from SLP36 shared a high level of similarity with (deduced) protein sequences for LP3-like proteins from different species, including loblolly pine, maidenhair tree (*Ginkgo biloba* L.), and douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco).

Translation related genes

Twelve translation related genes were differentially expressed in this study as shown in Table 13. Ten out of these 12 genes are of shortleaf pine origin. 11 genes were differentially expressed in shortleaf pine, either through self-hybridization (10 genes) or cross hybridization (only one gene, LLP16), with most of the ribosomal proteins showing upregulation. Most of these genes did not show differential expression in loblolly pine, with the exception of these three genes, LLP15, SLP66, and LLP16.

LLP15 and LLP16 were identified through self-hybridization, while SLP66 was identified through cross hybridization.

SLP70 encodes a DCP1-like decapping protein. SLP70 was 381 base pairs long, and the deduced protein was 93 amino acids long. When this protein was blasted against the NCBI protein databases, homologs from several species were identified. Figure 60 shows the MSA for DCP1-like proteins from several species. As seen from this alignment, the deduced protein sequence from SLP70 shared a high level of similarity with (deduced) protein sequences for DCP1-like proteins from diverse species, including rice (*Oryza sativa* L.), corn (*Zea mays* L.), and *Arabidopsis*.

Real-time PCR to confirm the microarray results

Six genes were selected to further confirm the microarray results and to study gene expression patterns using quantitative real-time PCR (qRT-PCR). The quantitative PCR experiment was performed using actin as the endogenous control. Genes used for verification were KN3 (SLP2, GO479091), invertase (SLP21, GO479110), amylase (SLP22, GO479111), AP2/ERF (SLP34, GO479123), a water deficit inducible LP3-like protein (SLP36, GO479125), and a putative receptor kinase (listed as kinase in Table 17, SLP_LLP1, GO479192). These selections represent genes from five different functional categories: KN3 is a developmental related transcription factor; invertase and amylase function in carbohydrate metabolism; AP2/ERF is a hormone related gene; the LP3-like protein is involved in plant stress response; and the receptor kinase functions in signal transduction.

The software Primer 3 (http://frodo.wi.mit.edu/) was used to design primers based on sequence information obtained in this study. The sequence information is available from GenBank with the corresponding accession numbers. Normal PCR was applied to test the specificity of each pair of primers. Only primers with high specificity showing one band in an agarose gel were used in qRT-PCR. Table 16 lists all the primers used for qRT-PCR in this study.

In general, the qRT-PCR and array results were in agreement (Figure 61-66). There were some differences between them. For some cases, results from qRT-PCR showed a higher gene expression level in mRNA abundance than did the array. The higher expression level in qRT-PCR might result from qRT-PCR's greater sensitivity to detect RNA abundance. Another possible reason for the difference was that the time points for the array experiment and qRT-PCR were not exactly the same: for shortleaf pine, tissues used in the array experiment were a combination of tissues collected at 24 hour and 48 hour after top-killing, and tissues for qRT-PCR had clear-cut time points, from two hour after cutting treatment to 48h after cutting treatment; for loblolly pine, tissues used in the array experiment were a combination of tissues collected on the sixth and seventh day after top-killing, and again tissues for qRT-PCR were tissues collected from one day after cutting treatment to one week after cutting treatment. Therefore, qRT-PCR results provided us with information on the detailed expression pattern of each gene at different time points after cutting treatment, while array result only

provided us with general information on transcriptome profile for an extended period of time.

From the qRT-PCR results, shortleaf pine's quick response to top removal was more obvious, as five out of six genes showed expression peaks between four to eight hours after top-killing. For example, expression of invertase and amylase in shortleaf pine after top-killing reached expression peaks after four hour and eight hour after topkilling, respectively. For loblolly pine, the expression peaks for these two carbohydrate metabolism genes were seen three days after cutting treatments.

As shown in Figure 61, the abundance of invertase transcripts increased within two hours after cutting treatment in shortleaf pine, and continued to increase to reach an expression peak four hours after cutting treatment, showing a ten-fold upregulation. Shortleaf pine invertase RNA abundance had a second but smaller peak at 24 hours after cutting treatment, showing a two-fold upregulation. Correspondingly, array experiments detected a two-fold upregulation on mixed shortleaf pine samples collected at 24hours and 48hours after cutting treatment. For loblolly pine, expression peak of invertase was at three days after cutting treatment, showing a two-fold upregulation. The abundance of invertase transcripts began to decrease thereafter, and at five days and seven days after cutting treatment, there was almost no difference in loblolly pine invertase RNA abundance between control samples and cutting treated samples, which was in agreement with array results.

The abundance of amylase transcripts also increased within two hours after cutting treatment in shortleaf pine (Figure 62), and continued to increase to reach an expression peak eight hours after the cutting treatment, showing a 3.5-fold upregulation. For loblolly pine, the expression peak was at three days after cutting treatment, showing a 2.5-fold upregulation.

The abundance of AP2/ERF transcripts increased within two hours after cutting treatment in shortleaf pine (Figure 63), and reached the expression peak eight hours after cutting treatment, showing a 12-fold upregulation. AP2/ERF RNA abundance had a small peak at 24 hours after cutting treatment, showing a 2.8-fold upregulation in shortleaf pine. Correspondingly, the array experiment detected a 2.2-fold upregulation in mixed shortleaf pine samples collected at 24hours and 48hours after cutting treatment. For loblolly pine, the expression peak was at three days after cutting treatment, showing an 11-fold upregulation. The abundance of AP2/ERF transcripts began to decrease thereafter, but began to increase again at five days after cutting treatment. And at seven days after cutting treatment, there was a four-fold change in AP2/ERF RNA abundance between cutting treated samples and control samples, which was in agreement with array results.

The abundance of transcripts encoding KN3 increased within two hours after cutting treatment in shortleaf pine (Figure 64), and continued to increase to reach the expression peak after four hours, showing a five-fold upregulation. For loblolly pine, KN3 showed no significant (more than two-fold) upregulation at any of the four time

points; rather, at five days after cutting treatment, KN3 transcript was significantly less in treated samples than in control samples.

The abundance of LP3-like gene transcripts increased within two hours after cutting treatment in shortleaf pine (Figure 65), and continued to increase to reach an expression peak eight hours after cutting treatment, showing a six-fold upregulation. LP3-like gene RNA abundance decrease thereafter, but at 24 hours and 48 hours after cutting treatment, it reaches a plateau, maintaining a two-fold upregulation. Correspondingly, the array experiment detected a two-fold upregulation in mixed shortleaf pine samples collected at 24 hours and 48 hours after cutting treatment. For loblolly pine, the expression peak was at three days after cutting treatment, showing a 6.5-fold upregulation. The abundance of the LP3-like transcripts began to decrease thereafter, and at seven days after cutting treatment, there was almost no difference in LP3-like RNA transcript abundance between control samples and cutting treated samples, which was in agreement with array results.

The abundance of kinase transcripts increased within four hours after cutting treatment in shortleaf pine (Figure 66), and transcripts reached peak abundance at 48 hours after cutting treatment, showing a 2.6-fold upregulation. For loblolly pine, the expression peak was at seven days after cutting treatment, showing a seven-fold upregulation. With the exception of this time point, the abundance of loblolly pine kinase transcripts were significantly less in cutting-treated sample than in control samples.

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CHAPTER V

DISCUSSION

Shortleaf pine and loblolly pine are two species possessing large differences in sprouting capability after top cutting treatment. These pines were used for cDNA subtraction and microarray experiments to identify genes responsible for prolific shortleaf pine sprouting and the gene expression differences that lead to the large differences between shortleaf pine and loblolly pine's sprouting ability following top-killing. In these comparative analyses with a 2400 cDNA microarray, a total of 139 transcripts were identified to be responsive for sprouting after top-killing.

There have been several recent studies carried out using microarray to study dormancy in buds of other plants, including leafy spurge, raspberry (*Rubus idaeus* L.) and poplar (*Populus tremula* L.; Horvath et al., 2005; Mazzitelli et al., 2007; Schrader et al., 2004). These studies were focused on dormancy release of either primary or axillary buds of perennial woody species, adventitious buds on perennial weeds, or buds on a perennial vine. This study might be the first study designed to examine pine species' sprouting ability due to bud dormancy release after top-killing. In this study of shortleaf pine and loblolly pine, dormancy release was a main response to top-death. Outside environmental cues like temperature and light might not play a pivotal role in this

system as it does in the other systems reported in study of dormancy release. However, it might still be of interest to find common genes and mechanism involved in the different dormancy release systems, and these concerns will be included in the discussion of genes in different functional categories of genes identified.

Optimum tissue collection for gene profiling

As our goal was to identify genes responsible for sprouting after top-killing of both shortleaf pine and loblolly pine, it was important to identify the appropriate time points for gene profiling. By a careful watch of the two pine species' response after top killing, we found that shortleaf pine sprouted 24 hours-48 hours after top-killing, while loblolly pine sprouted seven days after top-killing. Therefore, the tissues collected on the first and second day for shortleaf pine were used for the array experiment, while for loblolly pine tissues collected on the sixth and seventh day were used.

Through the array experiment 130 differentially expressed genes responsible for dormancy release were identified for shortleaf pine; while for loblolly pine, only 32 genes were found. It seems that the time points for shortleaf pine tissue collection are optimal, but the time points for loblolly pine tissue collection may not be optimized. Perhaps loblolly pine's sprouting stimulus at the molecular level is a result of a combination of genes with low level changes in expression which in turn could explain the limited and slow sprouting response observed. The results demonstrated that six-seven days after treatment, limited gene activity was noted in the remaining loblolly pine stumps. Few differentially expressed genes related to loblolly pine bud dormancy

release and sprouting were found. In a study reporting the influence of defoliation on the dormancy release of underground adventitious buds of leafy spurge, it was found that genes, such as histone H3 and tubulin, were differentially expressed between 24h to 48h after the defoliation treatment (Horvath et al., 2002). A similar study reported that 24-h time points displayed the greatest number of differentially expressed genes in leafy spurge (Horvath et al., 2005). And of note, growth of underground buds was detected four to five days after defoliation treatments (Horvath et al., 2005).

Functional categorization of differentially expressed genes

Carbohydrate metabolism

Seven genes were found involved in carbohydrate metabolism. Six of them were upregulated in shortleaf pine. All of these seven genes were of shortleaf pine origin. Not a single gene came from loblolly pine. Even the cross hybridization of these genes with loblolly pine RNA did not produce strong signals. These genes encode well-known enzymes involved in glycolysis (malate synthase by SLP17; pyruvate kinase by SLP18; fructose-bisphosphate aldolase by SLP19) and the pentose phosphate pathway (glucose-6-phosphate dehydrogenase by SLP20). Upregulation of genes involved in glycolysis and the pentose pathway suggested that active carbohydrate metabolism was involved in dormant bud release in shortleaf pine after top-killing to provide energy for bud growth. And of note, increased expression levels of genes involved in glycolysis and the pentose pathway were also found associated with poplar dormant bud release (Canam et al., 2008).

Amylase and invertase were also found upregulated after top-killing in shortleaf pine. As key enzymes in carbohydrate catabolism, amylase and invertase play significant roles in the regulation of sugar concentration, which in turn influences gene expression through sugar signalling pathways (Roitsch et al., 2003). And of note, in trees, two invertase genes were found involved in processes related to poplar dormant bud release (Canam et al., 2008). High levels of expression of amylase and invertase genes in this study might promote dormant bud release in shortleaf pine by not only providing energy through mobilization of carbohydrate reserves, but also functioning in sugar signaling pathways.

The one gene found downregulated was glycogenin glucosyltransferase. This enzyme catalyzes an essential step in glycogen synthesis (Lomako et al., 1988; Qi et al., 2005). Downregulation of a glycogenin glucosyltransferase gene in shortleaf pine suggests that the series of chemical reactions to store glucose as glycogen might be suppressed during dormant bud release after top-killing. The lowered rate of these chemical reactions might be due to the fact that sprouting is an energy consuming process and no excessive glucose is available to be stored as glycogen.

Hormone related genes

As shown in Table 7, six hormone related genes were found differentially expressed after top-killing in shortleaf pine: two genes related to gibberellin, three to auxin, and one to ethylene. Gibberellin 7-oxidase functions to finely modulate GA biosynthesis (Israelsson et al., 2004; Lange et al., 1994, 1997). A gibberellin 7-oxidase-like gene (SLP37) was found upregulated in shortleaf pine after top-killing. The high expression

levels of gibberellin 7-oxidase suggested that GA was actively involved in dormant bud release in shortleaf pine after top-killing. Another GA related gene encoded a putative tetratricopeptide repeat (TPR) protein similar to SPINDLY(SPY), which is a putative O-linked N-acetyl-glucosamine transferase, and is deemed as a negative regulator of the GA signal transduction pathway (Tseng et al., 2001; Swain et al., 2002; Maymon et al., 2009). In this study, the SPY-like TPR-containing gene (SLP39) was downregulated in shortleaf leaf pine after top-killing, and it might function in one of the pathways that lead to abundant GA accumulation during dormant bud release.

An auxin-repressed protein (ARP; SLP35) was upregulated in shortleaf pine after top-killing. ARP showed enhanced expression in the root nodules of Japanese silverberry (*Elaeagnus umbellata*; Kim et al., 2007) and seemed to have a positive role in plant development. High expression levels of ARP were also reported to be associated with dormant bud release in tree peonies (*Paeonia suffruticosa* Andrews; Huang et al., 2008). Like in tree peonies, the upregulation of ARP might promote sprouting in shortleaf pine after top-killing.

The other two auxin-related differentially expressed genes were a gene encoding anthocyanidin reductase (SLP38) and a gene encoding flavanone 3-hydroxylase (LLP3). Both genes are involved in flavonoid biosynthesis. Flavonoid biosynthesis genes were found to be rapidly down-regulated during loss of paradormancy in root buds of leafy spurge (*Euphorbia esula*), and they were documented to be negative regulators of auxin transporters (Horvath et al., 2005). Therefore the downregulation of the two genes

involved in flavonoid biosynthesis in shortleaf pine might lead to a higher expression level of auxin transporter genes which might be responsible for auxin transport from other minor sources (such as primary root tip) after top-killing, as auxin is required for further bud growth after dormancy release (Lincoln et al., 1990). In this study, the stimulation of auxin transporter genes by downregulation of flavonoid biosynthesis genes might be helpful for dormant bud growth. The apical dominance of shortleaf pine was released by top-killing, but auxin might be required for sprout growth and perhaps to inhibit growth of remaining lateral buds on stems of shortleaf pine.

The differentially expressed gene related to ethylene was the AP2/ERF domain-containing transcription factor. AP2/REF, like its counterpart ERF1, is a component of the ethylene signaling pathway, and might play the same role during shortleaf pine bud dormancy release as ERF1 does in promoting sunflower (*Helianthus annuus* L.) seed dormancy release (Oracz et al., 2009). Investigations have also shown that ethylene plays a role during tiller release from apical dominance (Harrison and Kaufman, 1982). Ethylene might have the same role during dormant bud release in shortleaf pine after top-killing.

Cell growth and maintenance

Fourteen differentially expressed genes were found involved in cell growth and maintenance in shortleaf pine and loblolly pine after top-killing. SLP11 is a homolog to the STT3B, which functions in protein N-glycosylation (Koiwa et al., 2003). Specific protein glycosylation might be important for cell cycle progression under stress

conditions. In this study, the upregulation of the STT3B-like gene SLP11 might help cell growth of shortleaf pine under wounding stress.

Glycine-rich gene (SLP8) was upregulated in shortleaf pine following top-kill. The function of glycine-rich proteins (GRPs) is obscure, some were found to be components of plant cell walls (Ringli et al., 2001) and some were proposed to be players in plant defense mechanisms (Mousavi and Hotta, 2005). In this study, the increased expression level of GRPs might help the remaining stems of shortleaf pine to survive wounding stress after top-killing. This glycine-rich gene is of shortleaf pine origin, and cross hybridization of loblolly pine cDNAs to the array slides showed upregulation.

Two cell-wall loosening genes were downregulated in shortleaf pine: an expansin like gene (SLP4) and a pectin methylesterase-like gene (SLP5). Both genes play important roles in cell wall modification during plant growth and development (Lee et al., 2003; Phan et al., 2007). The downregulation of expansin and pectin methylesterase genes seen in this study may be due to their more active role in seedling development rather than dormancy release and sprouting.

A methyltransferase (MTase)-like gene (SLP9) was downregulated in shortleaf pine after top-killing. MTases are essential enzymes functioning in DNA and protein methylation (Wang et al., 2005). There are reports showing decreased MTase expression during dormancy release in potato tubers (Campbell et al., 2008).

Downregulation of MTase in shortleaf pine might play a similar role in bud dormancy release after top-killing.

Tetraspanins were found involved in regulation of cell differentiation (Olmos et al., 2003). One homolog of tetraspanin (SLP13) was upregulated in both shortleaf pine and loblolly pine. One pentatricopeptide repeat-containing, EMBRYO-DEFECTIVE similar gene (SLP15) was found to be upregulated in shortleaf pine after top-killing. This is a newly discovered gene, which was found to be important for *Arabidopsis* seed development (Devic, 2008). Upregulation of the homologs of tetraspanin and EMBRYO-DEFECTIVE genes in shortleaf pine after top-killing might be helpful for dormant bud release after top-killing because of their positive function in plant development.

Signal transduction related genes

There were four differentially expressed genes in this category, including one receptor-kinase like gene, two phosphatase-like genes and one PB1domain-containing protein. The two phosphateases related genes, protein phosphatase 2A (PP2A) catalytic subunit-like gene (SLP25) and protein phosphatase 2C (PP2C)-like gene (SLP24), were both upregulated in shortleaf pine after top-killing. Investigations have shown that they both can negatively regulate abscisic acid (ABA) response (Pernas et al., 2007; Schweighofer et al., 2004; Yoshida et al., 2006), with PP2A's possible involvement in the signal transduction pathway mediated by GAs (Chang et al., 1999) and PP2C's direct function as an ABA-insensitive locus to attenuate ABA signal (Leung et al., 1994, 1997; Meyer et al., 1994; Rodriguez et al., 1998).

One receptor-kinase like protein (SLP_LLP1) was significantly upregulated in shortleaf pine after top-killing. One investigation showed that a protein kinase mRNA level was low in dormant pea (*Pisum sativum* L.) axillary buds on intact plants and the level increased when buds were stimulated to grow during loss of apical dominance by decapitating the terminal bud (Devitt and Stafstrom, 1995). The upregulation of one receptor-kinase like protein in shortleaf pine and loblolly pine following removal of the top may represent a similar response.

Transcription factor related genes

One NAM transcription factor-like gene (SLP1) was downregulated in shortleaf pine after top-killing. NAM not only functions in the initial development of plant lateral organs from shoot meristems (Aida and Tasaka, 2006), but also is responsive to various stresses (Olsen et al., 2005; Chen et al., 2008; Seo et al., 2008). Under stress conditions, NAM was found upregulated where ABA might be induced due to water loss (Ganesan et al., 2008). In this study, the NAM-like gene might be downregulated due to the lower ABA levels associated with dormant bud break in the shortleaf pine sprouting process.

A SHOOT MERISTEMLESS (STM) homolog KN3-like gene (SLP2) was found highly expressed in shortleaf pine after top-killing. The STM gene belongs to the *Arabidopsis* Knotted1-like homeobox (KNOX) gene subfamily and encodes homeodomain transcriptional regulators that regulate shoot growth in the shoot apical meristem (SAM) (Long et al., 1996; Scofield et al., 2007). In this study, the high expression

level of a KN3-like gene in shortleaf pine suggests increased meristem activity involved in sprout formation following dormant bud break in shortleaf pine.

The DNA binding protein GT-1 is a light-modulated transcription factor (Marechal et al., 1999). In this study, a GT-1-like gene was found upregulated in shortleaf pine after top-killing. GT-1 might interact with light responsive genes to promote dormant bud release in shortleaf pine.

Protein and amino acid metabolism

Thirteen genes related to protein and amino acid metabolism were differentially expressed during sprouting following cutting treatment, including genes involved in protein reserve mobilization, amino acid metabolism and genes involved in posttranslational modulation or degradation of enzymes (such as plant hormones). Both the serine-type peptidase/signal peptidase and prolyl endopeptidase are proteins involved in the maturation and degradation of peptide hormones. Homoglogs of these two genes (SLP47 and LLP8) were both found upregulated in shortleaf pine. The upregulation is probably an indicator of active involvement of plant hormones in dormant bud release in shortleaf pine after top-killing.

Aleurain (cysteine protease), subtilase (endopeptidase) and thioredoxin *h* (small redox regulating proteins) were all documented to be associated with mobilization of storage protein in seed germination (Rogers et al., 1997; Fontanini and Jones, 2002; Gelhaye et al., 2004). Homologs of these three genes (SLP48, SLP49 and SLP51) were upregulated in shortleaf pine after top-killing. The upregulation of these genes functioning in protein

degradation suggested active reserve mobilization during dormant bud release in shortleaf pine after top-killing.

Several genes functioning in amino acid metabolism were also differentially expressed; they tend to be upregulated in shortleaf pine. These genes might function to provide more energy for sprouting by further breakdown of proteins and amino acids. Druart et al. (2007) reported similar results from research on dormant bud release in poplar.

Fatty acid metabolism genes

Three differentially expressed genes were found to be associated with fatty acid metabolism after top-killing: one caleosin-like gene (SLP40), one triacylglycerol (TAG) lipase-like gene (SLP41), and one gene encoding proteins belonging to GNS1/SUR4 membrane family (SLP42). In shortleaf pine, the first two genes were upregulated, while the third one was downregulated.

Caleosins (a group of calcium binding proteins) and TAG lipase are both involved in lipid degradation during seed germination to provide an energy source to support the early development of seedlings (Quettier and Eastmond, 2008; Padham et al., 2007). The upregulation of the two fatty acid catabolism genes (triacylglycerol lipase-like, SLP41; caleosin-like, SLP40) suggested that fatty acids were actively mobilized during dormant bud release in shortleaf pine after top-killing. Fatty acid metabolism related genes were also found upregulated during poplar dormant bud release (Druart et al., 2007).

A gene encoding proteins belonging to GNS1/SUR4 membrane family was downregulated in shortleaf pine after top-killing. The GNS1/SUR4 membrane family genes were found associated with fatty acid elongation (Baudry et al., 2001). The downregulation of GNS1/SUR4-like gene (SLP42) further indicates that in top-killed shortleaf pine, fatty acids were broken down to provide energy for dormant bud release and bud growth.

Transport genes

Seven differentially expressed genes related to transport were found in this study, including two ABC (ATP-binding cassette) transporter-like genes, a sulfate transporter-like gene, an nitrate transporter-like gene, and two genes encoding transporters for unknown proteins and a transporter for basic amino acids (carnitine:acyl carnitine antiporter).

Nitrate transporters help mediate NO_3^- uptake from external sources (Chopin et al., 2007). Nitrate can promote dormancy release and seed germination by positively regulating the activities of enzymes involved in ABA catabolism and GA biosynthesis (Finch-Savage et al., 2007; Bethke et al., 2007). Hence the upregulation of a nitrate transporter-like gene (LLP6) in shortleaf pine after top-killing might function not only to provide nutrition for sprouting, but also to play a role in dormancy release.

The ATP binding cassette (ABC) functions to transport various molecules across cell membranes by use of energy from ATP (Theodoulou, 2000; Jasinski et al., 2003). Two ABC-transporter related proteins were found either up regulated (SLP43) or

downregulated (LLP4) after top-killing in shortleaf pine. These two ABC transporters might be involved in transporting different molecules, either toxic or nutritional in reverse directions, during shortleaf pine dormant bud release after top-killing. The reason why the two ABA transporter genes did not have the same direction of differential expression (either upregulation or downregulation) might be due to the different functions they have in the bud dormant release process.

Stress responsive genes

Fifteen stress responsive genes were found either upregulated or downregulated after top-killng. A cystatin (cystein protease inhibitor)-like gene (SLP57) was found upregulated in shortleaf pine. In plants, high-level expression of cystatin was associated with enhanced resistance to various abiotic stresses (Zhang et al., 2008). In this study, the upregulation of a cystatin-like gene (SLP57) might better protect shortleaf pine from wound stress.

LEA (late embryo abundant) proteins are extremely hydrophilic proteins, and they are proposed to play an important role in protecting cells from dehydration stress (Gilles et al., 2007; Baker et al., 1988). After the top-cutting treatments, both shortleaf pine and loblolly pine were in the danger of losing too much water from the wounds, which would affect further development. Upregulation of LEA-like genes (SLP_LLP4) probably function to protect both shortleaf pine and loblolly pine from extreme water loss.

In this study, four genes involved in oxidative stress release were all downregulated in shortleaf pine, including two peroxidase-like genes (LLP11_LLP12, SLP60), one glutathione S-transferase-like gene (SLP63) and one aldo-keto reductase-like gene (SLP62). Peroxidase, glutathione S-transferase and aldo-keto reductase are known to be components of the complex network of active oxygen species (AOS) enzymes in plant cells.

Glutathione S-transferase (GST) functions to add reduced glutathione (GSH) to a variety of substrates (Yu et al., 2003), and can protect cells from oxidative damage (McGonigle et al., 2000). Decreased expression of glutathione S-transferase (GST) was found associated with dormancy breakage in *Trollius ledebouri* seeds (Bailey et al., 1996) and grape buds (Halaly et al., 2008). On the other hand, accumulation of GST was found associated with dormancy introduction in *Castanea crenata* trees (Japanese chestnut) (Nomura et al., 2007). The downregulation of the GST-like gene (SLP63) after top-killing in this study might have an important role in keeping high levels of oxidative stress in dormant buds and the increased levels of oxidative stress in turn helps to promote dormant bud release in shortleaf pine.

One homolog of the *Arabidopsis* peroxidase gene At5g64120 was found downregulated after top-killing in shortleaf pine. As catalase, At5g64120 is capable of decreasing oxidative stress by decreasing H_2O_2 levels (Riganti et al., 2004; Rouet et al., 2006). In this study, an At5g64120-like gene (LLP11_LLP12) showed decreased expression levels in shortleaf pine after cutting treatment. Downregulation of the At5g64120-like gene

suggested that oxidative stress might be resultant from the decreased level of antioxidant genes, and oxidative stress might function to promote dormant bud release in shortleaf pine.

Aldo-keto reductases function primarily to reduce aldehydes and ketones to primary and secondary alcohols (Jin and Penning, 2007; Oberschall et al., 2000). In wild oat (*Avena fatua* L.), aldose reductase was found associated with seed dormancy, with high expression levels in dormant seeds but low expression levels in afterripened ones (Li and Foley, 1995). In this study, an aldo-keto reductase-like gene (SLP62) was downregulated in shortleaf pine after top-killing. This suggested that decreased expression of aldo-keto reductase might be one pathway leading to the accumulation of AOS other than H_2O_2, such as primary and secondary alcohols. These primary and secondary alcohols could function cooperatively with H_2O_2 to promote dormant bud release in shortleaf pine. Therefore decreased expression of all these genes involved in antioxidant systems might produce oxidative stress and promote dormant bud break in shortleaf pine.

Cytochrome P450 genes catalyse multiple important reactions in plant secondary metabolism and are responsive to plant stress (Bolwell et al., 1994). It was found that plant cytochrome P450 enzymes were involved in wound healing and pest resistance of *Arabidopsis* plants (Noordermeer et al., 2001). In this study, a cytochrome P450-like gene (SLP61) was upregulated after top-killing in shortleaf pine. Increased P450 expression might help shortleaf pine survive wounding stress after cutting treatment.

Galactinol synthase (GolS) is an essential enzyme functioning in the synthesis of raffinose family oligosaccharides that act as osmoprotectants in plant cells (Wakiuchi et al., 2003). It was proposed that galactinol and raffinose might function to scavenge hydroxyl radicals to protect plant cells from oxidative damage caused by stress like high salinity and chilling (Nishizawa et al., 2008; Kim et al., 2008). Decreased expression of GolS-like gene (LLP13) in loblolly pine might be a sign of decreased tolerance to environmental stress, and hence reduced sprouting ability after top-killing.

Translation genes

Ribosomal proteins are involved in the cellular process of translation (Sohal et al., 2008). Five ribosomal-like genes were upregulated in shortleaf pine after top-killing. Upregulation of ribosomal genes suggested that active translation might occur after top-killing, and this translation activity most probably resulted in abundant hormones and transporter proteins that might be especially important for dormancy release and sprouting.

A DCP1 (an mRNA-decapping enzyme)-like gene (SLP70), was found upregulated in shortleaf pine after top-killing. DCP1 was proposed to be important for shoot apical meristem formation (Xu et al., 2006). In this study, the upregulation of a DCP1-like gene (SLP70) in shortleaf pine after top-killing suggested that DCP1 might be important for shortleaf pine dormant bud break and or shoot apical meristem development.

Transcribed loci with unknown function and genes with no hit in the databases

The genes with unknown function or no hit in the databases ranked as the two largest groups of all the categories. A total of 17 cDNAs failed to match any sequence in the GenBank databases by the BLAST search, and 28 had matched sequences but their functions had not yet been characterized. These two categories represented 32 percent of the sequenced differentially expressed genes. Some of them showed strong up or down regulation after top-killing, suggesting that these genes might be intimately involved in regulation of dormant bud release and development after top-killing. However due to limited sequence information available in the GenBank database, we do not know their function. Hopefully, as more information is reported in the near future, more can be inferred from the results in this study with regard to what genes are involved in shortleaf pine's prolific sprouting after top-killing, and the differences in gene expression between shortleaf pine and loblolly pine.

Oxidative stress and dormancy release

In this study four genes (two peroxidase-like genes, one glutathione S-transferase-like gene and one aldo-keto reductase-like gene) involved in antioxidant systems were cooperatively downregulated in shortleaf pine after top-killing, suggesting oxidative stress' possible role in dormant bud release. Simultaneous downregulation of four genes acting in antioxidant systems might result efficient promotion of dormancy release in shortleaf pine buds after top-killing through oxidative stress.

Dormant bud release in shortleaf pine was primarily a response to top-death, which was a different stimulus from that of other studies of dormant bud release, e.g. in grape and

perennial trees. However, one common point exists for all species, that is, abiotic stress was associated with the dormancy release processes. For this study, shortleaf pine underwent wound stress, and for the other studies, plants endured low temperature stress to fulfill chilling requirements needed for dormancy release. Both wound stress and low temperature stress can lead to active oxygen species (AOS) build-up and oxidative stress (Swindell, 2006). Decreased catalase activity and oxidative stress were proposed to be associated with dormant bud release in perennial trees (Shulman et al., 1983). In this study, AOS resulting from top-killing might contribute to dormant bud release in shortleaf pine.

Sprouting was an energy-consuming process, during which fatty acid reserve might be broken down to provide energy for sprouting. In this study, genes (one triacylglycerol lipase-like gene, one caleosin-like and one gene encoding proteins belonging to GNS1/SUR4 membrane family) involved in fatty acid metabolism were cooperatively regulated, which suggests that fatty acid was actively mobilized during dormancy release after top-killing to provide energy for sprouting. Beta-oxidation, an essential step in fatty acid breakdown, might be activated (although no genes for beta-oxidation were identified in this study) and lead to the accumulation of H_2O_2, because beta-oxidation is an active H_2O_2 producing reaction (Huang et al., 1983). As its role in promoting seed dormancy release (Finkelstein et al., 2008), resultant H_2O_2 from beta-oxidation might play a positive role in bud dormancy release in shortleaf pine.

Taken together, three major factors might play essential roles in AOS build-up and

oxidative stress during dormant bud release in shortleaf pine after top-killing: 1) wound stress due to cutting treatments; 2) a reduced antioxidant system; 3) beta-oxidation in fatty acid reserve mobilization. Elevated oxidative stress was effectively achieved due to the combinational effect of all three factors, and an increased level of oxidative stress probably promoted dormant bud release in shortleaf pine, which leads to prolific sprouting.

Interestingly, increased intrinsic levels of H_2O_2 were proposed to stimulate the pentose pathway, which is important for dormancy release (Hendricks and Taylorson, 1975). In this study, a Glucose-6-phosphate dehydrogenase (G6PD6)-like gene (a gene involved in the pentose pathway), was upregulated in shortleaf pine after top-killing. Upregulation of G6PD6 suggests an enhanced pentose pathway after cutting treatments. The stimulated pentose pathway might be due to excessive endogenous H_2O_2 resulting from accumulated oxidative stress in shortleaf pine after top-killing. Therefore, increased pentose pathway activity in this study might be an indicator of the oxidative stress associated with dormant bud break in shortleaf pine after top-killing.

Hormone regulated dormancy release

Hormone plays a central role in plant development. GA and ethylene have been shown to be involved in dormancy release in seeds and buds. Auxin was demonstrated to act in axillary bud dormancy release after removal of apical dormancy through decapitation. In this study, genes related to auxin, GA and ethylene were differentially expressed and

they might play an essential role in regulating various developmental pathways leading to dormant bud release in shortleaf pine.

An AP2/ERF transcription factor-like gene was upregulated during dormancy release of shortleaf pine and loblolly pine buds. Upregulation of AP2/ERF might promote dormant bud release in shortleaf pine and loblolly pine because of AP2/ERF's positive role in ethylene production. Interestingly, ethylene's activity and abundance might be affected by AOS, which has been reported to be able to enhance ethylene accumulation through its interaction with ethylene response factors (Oracz et al., 2009). An increased level of AOS in shortleaf pine and loblolly pine after the cutting treatments might further elevate ethylene expression levels, which would function to promote dormant bud release.

GA's interaction with various genes in dormant bud release in shortleaf pine

Hormones, as central players in plant development, are demonstrated to cross-talk with components of various signal transduction pathways. In this study, together with increased levels of GA, various other potentially functionally related genes were upregulated. With GA's documented roles in gene regulation of expression of various genes, it is possible that in this study GA's active interaction with genes of diverse function might help explain dormant bud release in shortleaf pine after top-killing. A number of such possible interactions are discussed below.

1) GA's involvement in reserve mobilization

GA was proposed to promote dormancy release due to its possible role in mobilization of storage reserves by inducing enzymes functional in the mobilization processes (Bewley

and Black, 1994). For example, GA was proposed to promote the synthesis and activity of α-amylases and invertase, two key enzymes in carbon metabolism (Jones et al., 1998; Nakayama et al., 2002; Koch, 2004). GA also induces expression of aleurain, a type of cysteine protease associated with the mobilization of storage proteins during seed germination (Koehler and Ho, 1990; Phillips and Wallace, 1989). In this study, amylases-like, invertase-like and aleurain-like genes were all upregulated in shortleaf pine after top-killing. It is possible that GA played a positive role in carbohydrate and protein reserve mobilization by regulating activities of genes involved in the reserve mobilization processes, including invertase and aleurain.

2) GA's possible role in sugar signal transduction

In addition to their role in carbohydrate degradation, invertase and amylase were proposed to be important players of sugar signal transduction (Koch, 2004). Therefore, GA might be directly or indirectly involved in sugar signal transduction because of its interaction with pathways associated with amylase and invertase activity and abundance. Cross-talk might exist between sugar signal pathways and GA-mediated plant developmental pathways, and the cross-talk might play an important role in dormant bud release in shortleaf pine after top-killing.

3) GA's possible role in signal transduction mediated by PP2A

GA might play a positive role in dormancy release by interacting with other components in signal transduction pathways. For example, protein phosphatase 2A (PP2A) is an important phosphatase involved in reversible protein phosphorylation to regulate many

cellular processes (Hunter, 1995; Millward et al., 1999). GA was proposed to be involved in the PP2A signal transduction pathway (Chang et al., 1999). Therefore, GA's abundance and activity might affect various pathways which had reversible protein phosphorylation mediated by PP2A, because of GA's involvement in PP2A signal transduction. Interestingly, it was found that PP2C acts to negatively regulate ABA activities by functioning as an ABA insensitive locus to attenuate ABA signal (Meyer et al., 1994; Rodriguez et al., 1998). In this study, one PP2A-like was upregulated in shortleaf pine after cutting treatments. It is possible that cross-talking between GA and components from other signal transduction pathways (including ABA mediated pathways) might be involved in dormant bud release in shortleaf pine.

4) GA's regulation of light sensitive genes

GA synthesis was proposed to be influenced by light because some GA synthesis genes were known to be light sensitive. For example, light promotes GA synthesis in imbibing lettuce seeds (Toyomasu et al., 1998). In this study, a GT-1-like gene (a light-modulated transcription factor), was upregulated in shortleaf pine after cutting treatments. The increased expression levels of the GT-1-like gene might have an important role in regulating light responsive genes, such as those genes involved in GA synthesis, which would function to promote dormancy release.

5) GA might be regulated by nitrate

Nitrate was proposed to be able to positively regulate the activities of enzymes involved in GA biosynthesis (Finch-Savage et al., 2007). In this study, a nitrate transporter gene

was upregulated in shortleaf pine after cutting treatments. Increased levels of nitrate resultant from elevated levels of nitrate transporter genes might function to promote GA biosynthesis during dormant bud release in shortleaf pine.

Application of array results to pine regeneration planning

Reserve mobilization appears to play an important role in shortleaf pine bud dormancy release and sprouting. To insure good sprouting, it may be important to optimize the timing of prescribed fire. A winter burn might be better than summer burn, because following a winter burn, adequate reserves can be mobilized for sprouting. Conversely, following a summer burn, as most of the reserves have already been used for the growth in the spring and early summer, sprouting could be limited due to limited reserves.

Oxidative stress might play an important role in bud dormancy release and sprouting. Chemicals could be applied to loblolly pine stems to decrease activities of enzymes (such as catalase) involved in the antioxidant systems to attempt to induce oxidative stress. The resultant oxidative stress might function to promote loblolly pine sprouting after top-killing.

In conclusion, by gene profiling with about 2400 cDNA clones obtained from suppression subtractive hybridization, 139 differentially expressed genes were found to be associated with sprouting, including genes functioning in reserve (carbohydrates, protein and fatty acid) mobilization, transcriptional regulation, stress response, plant development, signal transduction and hormone regulation. 130 differentially expressed

genes were found to be responsible for the dormancy release of axillary buds of shortleaf pine after top-killing. Shortleaf pine responds actively to top-killing at the molecular level. In contrast, only 32 differentially expressed genes were detected for loblolly pine. It seems that loblolly pine's sprouting stimulus at the molecular level was a result without much change in expression level, and may explain loblolly pine's slow and limited sprouting compared to shortleaf pine.

As reported for dormancy release of buds of other perennial plants, oxidative stress might be the major factor in dormancy release of axillary buds of shortleaf pine. It is apparent that cross talking between plant hormones (especially gibberellins and auxins), carbohydrates, and other players of signal transduction work cooperatively to promote sprouting of shortleaf pine after top-killing.

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Table 1. Number of upregulated and downregulated genes identified in microarray experiments

	Upregulated[a]	Downregulated[a]	Upregulated in cross hybridization	Downregulated in cross hybridization
Shortleaf pine	60[b]	42	3	24
Loblolly pine	6	7	15	4

[a] Only differentially expressed genes identified during self-hybridization were included.
[b] Values of signal intensity ratios showing a more than two-fold up- or down- regulation were included.

Table 2. Transcription factor genes and their expression levels in shortleaf pine (SLP) and loblolly pine (LLP) after top-killing

Name	Accession number[a]	Annotation[b]	E value[c]	Source	SLP	LLP
SLP1	GO479090	NAM; transcription factor	1e-17	*Picea glauca*	-2.75[d]	1.67
SLP2	GO479091	homeobox transcription factor KN3	2e-49	*Pinus strobus*	2.47	1.22
SLP3	GO479092	DNA binding protein GT-1, transcription factor	2e-10	*Zea mays*	2.18	*

[a] GenBank accession number. All cDNA sequences were submitted to GenBank.

[b] BLASTX was used to identify homologous genes and putative functions of genes. BLASTN was used in case no hits were found by BLASTX.

[c] The Expect value (E) is a parameter that describes the number of hits one can "expect" to see by chance when searching a database of a particular size. The lower the E-value, or the closer it is to zero, the more "significant" the match is.

[d] Values of signal intensity ratios showing a more than two-fold up- or down- regulation are shaded with blue or yellow, respectively. The median intensity value of the replicates was used to calculate the signal intensity ratio.

* Data not available due to the low significance.

Table 3. Genes related to cell growth and maintenance and their expression levels in shortleaf pine (SLP) and loblolly pine (LLP) after top-killing

Name	Accession Number[a]	Annotation[b]	E value[c]	Source	SLP	LLP
SLP4	GO479093	expansin 2	2e-10	*Cunninghamia lanceolata*	-2.64[d]	2.11
SLP5	GO479094	pectin-methylesterase	4e-26	*Musa acuminata*	-3.42	1.56
SLP6	GO479095	actin 1	7e-29	*Picea abies*	2.79	2.41
SLP7	GO479096	glycine-rich protein	7e-26	*Arabidopsis thaliana*	3.98	2.09
SLP8	GO479097	glycine-rich protein	4e-132	*Arabidopsis thaliana*	2.86	1.77
SLP9	GO479098	methyltransferase	6e-14	*Arabidopsis thaliana*	-3.01	1.37
SLP10	GO479099	histone H4	5e-13	*Zea mays*	1.54	-2.60
SLP11	GO479100	STT3B	1e-19	*Arabidopsis thaliana*	2.74	-1.88
SLP12	GO479101	endomembrane protein 70	7e-22	*Oryza sativa*	2.23	2.55
SLP13	GO479102	TETRASPANIN8	3e-21	*Arabidopsis thaliana*	2.13	1.75
SLP14	GO479103	pyridoxine biosynthesis protein	7e-24	*Lotus corniculatus*	2.77	1.56
SLP15	GO479104	pentatricopeptide repeat-containing protein; similar to EMBRYO DEFECTIVE 2745	4e-10	*Ricinus communis*	2.59	-1.16
SLP16	GO479105	thioesterase family protein	1e-46	*Arabidopsis thaliana*	2.49	1.44
LLP1	GO479197	O-methyltransferase	2e-17	*Arabidopsis thaliana*	1.42	-3.13

[a] GenBank accession number. All cDNA sequences were submitted to GenBank.

[b] BLASTX was used to identify homologous genes and putative functions of genes. BLASTN was used in case no hits were found by BLASTX.

[c] The Expect value (E) is a parameter that describes the number of hits one can "expect" to see by chance when searching a database of a particular size. The lower the E-value, or the closer it is to zero, the more "significant" the match is.

[d] Values of signal intensity ratios showing a more than two-fold up- or down- regulation are shaded with blue or yellow, respectively. The median intensity value of the replicates was used to calculate the signal intensity ratio.

Table 4. Carbohydrate metabolism genes and their expression levels in shortleaf pine (SLP) and loblolly pine (LLP) after top-killing

Name	Accession Number[a]	Annotation[b]	E value[c]	Source	SLP	LLP
SLP17	GO479106	malate synthase	2e-10	*Glycine max*	3.48[d]	1.31
SLP18	GO479107	pyruvate kinase-like	4e-31	*Deschampsia antarctica*	2.04	1.01
SLP19	GO479108	fructose-bisphosphate aldolase	4e-17	*Ricinus communis*	2.91	1.56
SLP20	GO479109	glucose-6-phosphate dehydrogenase(G6PD6)	2e-59	*Populus suaveolens*	2.90	-1.24
SLP21	GO479110	invertase	8e-23	*Lotus japonicus*	2.18	1.17
SLP22	GO479111	beta-amylase	1e-14	*Solanum tuberosum*	3.16	1.36
SLP23	GO479112	glycogenin-related; transferring glycosyl groups	2e-11	*Ricinus communis*	-2.72	1.52

[a] GenBank accession number. All cDNA sequences were submitted to GenBank.

[b] BLASTX was used to identify homologous genes and putative functions of genes. BLASTN was used in case no hits were found by BLASTX.

[c] The Expect value (E) is a parameter that describes the number of hits one can "expect" to see by chance when searching a database of a particular size. The lower the E-value, or the closer it is to zero, the more "significant" the match is.

[d] Values of signal intensity ratios showing a more than two-fold up- or down- regulation are shaded with blue or yellow, respectively. The median intensity value of the replicates was used to calculate the signal intensity ratio.

Table 5. Signal transduction genes and their expression levels in shortleaf pine (SLP) and loblolly pine (LLP) after top-killing

Name	Accession Number[a]	Annotation[b]	E value[c]	Source	SLP	LLP
SLP24	GO479113	protein phosphatase 2C	2e-25	*Zea mays*	3.52[d]	1.82
SLP25	GO479114	protein phosphatase 2A catalytic subunit	6e-37	*Zea mays*	3.50	2.09
SLP27	GO479116	octicosapeptide/Phox/Bem1p (PB1) domain-containing protein	3e-25	*Medicago truncatula*	-2.88	1.34
SLP_LLP1	GO479192	serine-threonine protein kinase	5e-04	*Ricinus communis*	7.66	1.22

[a] GenBank accession number. All cDNA sequences were submitted to GenBank.

[b] BLASTX was used to identify homologous genes and putative functions of genes. BLASTN was used in case no hits were found by BLASTX.

[c] The Expect value (E) is a parameter that describes the number of hits one can "expect" to see by chance when searching a database of a particular size. The lower the E-value, or the closer it is to zero, the more "significant" the match is.

[d] Values of signal intensity ratios showing a more than two-fold up- or down- regulation are shaded with blue or yellow, respectively. The median intensity value of the replicates was used to calculate the signal intensity ratio.

Table 6. Ubiquitin related genes and their expression levels in shortleaf pine (SLP) and loblolly pine (LLP) after top-killing

Name	Accession Number[a]	Annotation[b]	E value[c]	Source	SLP	LLP
SLP28	GO479117	ubiquitin extension protein-like protein	2e-26	*Elaeis guineensis*	2.43[d]	*
SLP29	GO479118	ubiquitin system component Cue domain containing protein	1e-22	*Zea mays*	3.22	1.50
SLP30	GO479119	20S proteasome subunit alpha-1	1e-36	*Carica papaya*	-2.14	1.48
SLP31	GO479120	26S protease regulatory subunit 8	3e-59	*Pinus taeda*	2.90	1.64

[a] GenBank accession number. All cDNA sequences were submitted to GenBank.

[b] BLASTX was used to identify homologous genes and putative functions of genes. BLASTN was used in case no hits were found by BLASTX.

[c] The Expect value (E) is a parameter that describes the number of hits one can "expect" to see by chance when searching a database of a particular size. The lower the E-value, or the closer it is to zero, the more "significant" the match is.

[d] Values of signal intensity ratios showing a more than two-fold up- or down- regulation are shaded with blue or yellow, respectively. The median intensity value of the replicates was used to calculate the signal intensity ratio.

* Data not available due to the low significance.

Table 7. Pathogenesis related genes and their expression levels in shortleaf pine (SLP) and loblolly pine (LLP) after top-killing

Name	Accession Number[a]	Annotation[b]	E value[c]	Source	SLP	LLP
SLP_LLP2	GO479193	PR1a preprotein	1e-37	*Capsicum annuum*	-3.87[d]	1.79
LLP2	GO479198	TIR/NBS/LRR disease resistance protein	5e-05	*Pinus taeda*	-3.76	1.52
SLP32	GO479121	PR4 (Pathogenesis-Related 4)	0.0	*Arabidopsis thaliana*	2.64	1.49
SLP33	GO479122	NBS/LRR	0.001	*Pinus taeda*	-2.33	1.90

[a] GenBank accession number. All cDNA sequences were submitted to GenBank.

[b] BLASTX was used to identify homologous genes and putative functions of genes. BLASTN was used in case no hits were found by BLASTX.

[c] The Expect value (E) is a parameter that describes the number of hits one can "expect" to see by chance when searching a database of a particular size. The lower the E-value, or the closer it is to zero, the more "significant" the match is.

[d] Values of signal intensity ratios showing a more than two-fold up- or down- regulation are shaded with blue or yellow, respectively. The median intensity value of the replicates was used to calculate the signal intensity ratio.

Table 8. Hormone related genes and their expression levels in shortleaf pine (SLP) and loblolly pine (LLP) after top-killing

Name	Accession Number[a]	Annotation[b]	E value[c]	Source	SLP	LLP
SLP34	GO479123	AP2/ERF domain-containing transcription factor	6e-05	*Populus trichocarpa*	2.23[d]	2.81
SLP35	GO479124	auxin-repressed protein-like protein ARP1	6e-23	*Manihot esculenta*	2.31	-1.07
SLP37	GO479126	gibberellin 7-oxidase	4e-09	*Cucurbita maxima*	3.20	-1.50
SLP38	GO479127	anthocyanidin reductase	9e-35	*Ginkgo biloba*	-2.79	1.19
SLP39	GO479128	tetratricopeptide repeat protein, tpr; similar to SPY (SPINDLY)	2e-65	*Ricinus communis*	-2.89	1.54
LLP3	GO479199	flavanone 3-hydroxylase	6e-04	*Triticum aestivum*	-4.42	1.29

[a] GenBank accession number. All cDNA sequences were submitted to GenBank.

[b] BLASTX was used to identify homologous genes and putative functions of genes. BLASTN was used in case no hits were found by BLASTX.

[c] The Expect value (E) is a parameter that describes the number of hits one can "expect" to see by chance when searching a database of a particular size. The lower the E-value, or the closer it is to zero, the more "significant" the match is.

[d] Values of signal intensity ratios showing a more than two-fold up- or down- regulation are shaded with blue or yellow, respectively. The median intensity value of the replicates was used to calculate the signal intensity ratio.

Table 9. Fatty acid metabolism genes and their expression levels in shortleaf pine (SLP) and loblolly pine (LLP) after top-killing

Name	Accession Number[a]	Annotation[b]	E value[c]	Source	SLP	LLP
SLP40	GO479129	caleosin	2e-45	*Cycas revoluta*	2.69[d]	1.99
SLP41	GO479130	triacylglycerol lipase	1e-06	*Ricinus communis*	1.95	*
SLP42	GO479131	GNS1/SUR4 membrane family protein	6e-45	*Medicago truncatula*	-3.03	1.58

[a] GenBank accession number. All cDNA sequences were submitted to GenBank.

[b] BLASTX was used to identify homologous genes and putative functions of genes. BLASTN was used in case no hits were found by BLASTX.

[c] The Expect value (E) is a parameter that describes the number of hits one can "expect" to see by chance when searching a database of a particular size. The lower the E-value, or the closer it is to zero, the more "significant" the match is.

[d] Values of signal intensity ratios showing a more than two-fold up- or down- regulation are shaded with blue or yellow, respectively. The median intensity value of the replicates was used to calculate the signal intensity ratio.

* Data not available due to the low significance.

Table 10. Transport genes and their expression levels in shortleaf pine (SLP) and loblolly pine (LLP) after top-killing

Name	Accession Number[a]	Annotation[b]	E value[c]	Source	SLP	LLP
SLP43	GO479132	ABC transporter	2e-88	*Populus nigra*	4.27[d]	1.38
SLP44	GO479133	protein transport protein SEC61 gamma subunit	5e-19	*Zea mays*	2.58	2.84
SLP45	GO479134	ATMBAC2/BAC2	2e-12	*Arabidopsis thaliana*	-2.27	1.62
LLP4	GO479200	ATMRP15	2e-62	*Arabidopsis thaliana*	-3.64	1.73
LLP5	GO479201	sulfate transporter (SULTR3)	5e-23	*Arabidopsis thaliana*	-4.53	1.42
LLP6	GO479202	nitrate transporter (NTP2)	7e-21	*Arabidopsis thaliana*	5.23	-1.85
LLP7	GO479203	protein transport ATGDI1	6e-69	*Neurospora crassa*	-20.44	-1.56

[a] GenBank accession number. All cDNA sequences were submitted to GenBank.

[b] BLASTX was used to identify homologous genes and putative functions of genes. BLASTN was used in case no hits were found by BLASTX.

[c] The Expect value (E) is a parameter that describes the number of hits one can "expect" to see by chance when searching a database of a particular size. The lower the E-value, or the closer it is to zero, the more "significant" the match is.

[d] Values of signal intensity ratios showing a more than two-fold up- or down- regulation are shaded with blue or yellow, respectively. The median intensity value of the replicates was used to calculate the signal intensity ratio.

Table 11. Protein and amino acid metabolism genes and their expression levels in shortleaf pine (SLP) and loblolly pine (LLP) after top-killing

Name	Accession Number[a]	Annotation[b]	E value[c]	Source	SLP	LLP
LLP8	GO479204	prolyl endopeptidase	2e-07	*Ricinus communis*	3.14[d]	1.33
LLP9	GO479205	serine carboxypeptidase	2e-77	*Oryza sativa*	-2.42	1.77
LLP10	GO479206	homoserine O-acetyltransferase	1e-21	*Pyrenophora tritici-repentis*	-2.89	1.69
SLP47	GO479136	serine-type peptidase/ signal peptidase	7e-12	*Arabidopsis thaliana*	2.15	1.72
SLP48	GO479137	aleurain-like protease	6e-71	*Arabidopsis thaliana*	2.08	-1.32
SLP49	GO479138	subtilase	7e-43	*Picea abies*	2.57	1.44
SLP50	GO479139	ATP-dependent Clp protease proteolytic subunit	5e-08	*Ricinus communis*	2.13	1.41
SLP51	GO479140	thioredoxin *h*	7e-22	*Hevea brasiliensis*	2.17	1.22
SLP52	GO479141	O-acetylserine(thiol)-lyase	9e-35	*Sesamum indicum*	2.93	-1.17
SLP53	GO479142	tryptophan synthase	0.0	*Physcomitrella patens*	2.55	1.57
SLP54	GO479143	ketol-acid reductoisomerase	5e-76	*Spinacia oleracea*	2.51	1.49
SLP55	GO479144	fumarylacetoacetate hydrolase	5e-79	*Ricinus communis*	2.10	-1.03
SLP56	GO479145	peptidase M3 family protein	3e-48	*Arabidopsis thaliana*	-2.05	1.72

[a] GenBank accession number. All cDNA sequences were submitted to GenBank.

[b] BLASTX was used to identify homologous genes and putative functions of genes. BLASTN was used in case no hits were found by BLASTX.

[c] The Expect value (E) is a parameter that describes the number of hits one can "expect" to see by chance when searching a database of a particular size. The lower the E-value, or the closer it is to zero, the more "significant" the match is.

[d] Values of signal intensity ratios showing a more than two-fold up- or down- regulation are shaded with blue or yellow, respectively. The median intensity value of the replicates was used to calculate the signal intensity ratio.

Table 12. Stress responsive genes and their expression levels in shortleaf pine (SLP) and loblolly pine (LLP) after top-killing

Name	Accession Number[a]	Annotation[b]	E value[c]	Source	SLP	LLP
SLP36	GO479125	deficit inducible LP3-like protein	2e-34	*Pseudotsuga menziesii*	2.25[d]	1.21
SLP46	GO479135	metallothionein-like protein class II	6e-08	*Picea abies*	4.41	1.58
SLP57	GO479146	cystatin, cysteine protease inhibitor	5e-22	*Glycine max*	3.01	-1.72
SLP59	GO479148	peroxiredoxin (PRX)-like 2 family	8e-04	*Oryza sativa*	2.17	2.00
SLP60	GO479149	secretory peroxidase	8e-04	*Catharanthus roseus*	-2.28	1.16
SLP61	GO479150	cytochrome P450	8e-63	*Picea sitchensis*	2.33	*
SLP62	GO479151	aldo-keto reductases	5e-44	*Ricinus communis*	-2.00	1.46
SLP63	GO479152	glutathione S-transferase	4e-33	*Ostreococcus tauri*	-2.94	1.35
SLP64	GO479153	strictosidine synthase family protein	8e-17	*Marinobacter aquaeolei*	-2.95	1.45
SLP65	GO479154	type 3 metallothionein	2e-09	*Prosopis juliflora*	3.25	-1.52
SLP_LLP3	GO479194	class I chitinase	2e-22	*Pinus elliottii*	2.24	-5.44
SLP_LLP4	GO479195	LEA	3e-25	*Pinus halepensis*	5.17	1.79
LLP11	GO479207	peroxidase_ At5g64120	4e-11	*Solanum lycopersicum*	-1.68	2.47
LLP12	GO479208	peroxidase_ At5g64120	4e-11	*Solanum lycopersicum*	-3.74	1.77
LLP13	GO479209	galactinol synthase	8e-15	*Ajuga reptans*	1.52	-3.50
LLP14	GO479210	low molecular weight HSP	9e-08	*Pseudotsuga menziesii*	1.62	4.20

[a] GenBank accession number. All cDNA sequences were submitted to GenBank.

[b] BLASTX was used to identify homologous genes and putative functions of genes. BLASTN was used in case no hits were found by BLASTX.

[c] The Expect value (E) is a parameter that describes the number of hits one can "expect" to see by chance when searching a database of a particular size. The lower the E-value, or the closer it is to zero, the more "significant" the match is.

[d] Values of signal intensity ratios showing a more than two-fold up- or down- regulation are shaded with blue or yellow, respectively. The median intensity value of the replicates was used to calculate the signal intensity ratio.

* Data not available, due to the low significance.

Table 13. Translation related genes and their expression levels in shortleaf pine (SLP) and loblolly pine (LLP) after top-killing

Name	Accession Number[a]	Annotation[b]	E value[c]	Source	SLP	LLP
LLP15	GO479211	40S ribosomal protein S17	4e-5	*Solanum tuberosum*	1.35[d]	-3.60
LLP16	GO479212	60S ribosomal protein L21	4e-86	*Arabidopsis thaliana*	3.91	12.81
SLP66	GO479155	60S ribosomal protein L30	7e-35	*Pisum sativum*	1.98	1.98
SLP67	GO479156	60S ribosomal protein L27	1e-50	*Elaeis guineensis*	2.86	1.27
SLP68	GO479156	40S ribosomal protein S14	2e-57	*Elaeis guineensis*	1.97	1.02
SLP69	GO479158	eukaryotic translation initiation factor 5 (eIF-5)	4e-35	*Ricinus communis*	2.84	1.31
SLP70	GO479159	DCP1 (DECAPPING 1)	2e-6	*Oryza sativa*	2.16	*
SLP71	GO479160	XS domain-containing protein	2e-35	*Ricinus communis*	-2.74	1.46
SLP72	GO479161	CDK-activating kinase assembly factor MAT1	2e-09	*Ricinus communis*	-4.41	1.43
SLP73	GO479162	MIF4G domain containing RNA binding protein	1e-101	*Physcomitrella patens*	4.88	1.58
SLP74	GO479163	RNA-binding protein, similar to GR-RBP5 (glycine-rich RNA-binding protein 5)	5e-26	*Ricinus communis*	-2.08	1.69

[a] GenBank accession number. All cDNA sequences were submitted to GenBank.

[b] BLASTX was used to identify homologous genes and putative functions of genes. BLASTN was used in case no hits were found by BLASTX.

[c] The Expect value (E) is a parameter that describes the number of hits one can "expect" to see by chance when searching a database of a particular size. The lower the E-value, or the closer it is to zero, the more "significant" the match is.

[d] Values of signal intensity ratios showing a more than two-fold up- or down- regulation are shaded with blue or yellow, respectively. The median intensity value of the replicates was used to calculate the signal intensity ratio.

* Data not available, due to the low significance.

Table 14. Photosynthesis genes and their expression levels in shortleaf pine (SLP) and loblolly pine (LLP) after top-killing

Name	Accession Number[a]	Annotation[b]	E value[c]	Source	SLP	LLP
SLP75	GO479164	photosystem I reaction center subunit XI	5e-15	*Zea mays*	-2.82[d]	1.38
SLP76	GO479165	PSBQ-2; calcium ion binding	1e-08	*Arabidopsis thaliana*	-3.21	1.66

[a] GenBank accession number. All cDNA sequences were submitted to GenBank.

[b] BLASTX was used to identify homologous genes and putative functions of genes. BLASTN was used in case no hits were found by BLASTX.

[c] The Expect value (E) is a parameter that describes the number of hits one can "expect" to see by chance when searching a database of a particular size. The lower the E-value, or the closer it is to zero, the more "significant" the match is.

[d] Values of signal intensity ratios showing a more than two-fold up- or down- regulation are shaded with blue or yellow, respectively. The median intensity value of the replicates was used to calculate the signal intensity ratio.

Table 15. Transcribed loci with unknown function and their expression levels in shortleaf pine (SLP) and loblolly pine (LLP) after top-killing

Name	Accession Number[a]	Annotation[b]	E value[c]	Source	SLP	LLP
SLP77	GO479166	DR060572.1	1e-172	*pinus taeda*	2.49[d]	2.98
SLP78	GO479167	DR055374.1	0.0	*Pinus taeda*	-1.94	2.11
SLP79	GO479168	DR072693.1	0.0	*Pinus taeda*	1.53	-4.78
SLP80	GO479169	DR017586.1	6e-80	*Pinus taeda*	-3.07	1.23
SLP81	GO479170	AT1G16210	1e-52	*Arabidopsis thaliana*	-2.38	1.64
SLP82	GO479171	AT2G45990	7e-47	*Oryza sativa Indica Group*	-2.76	1.83
SLP83	GO479172	DR117814.1	0.0	*Pinus taeda*	-4.22	1.49
SLP84	GO479173	AT4G24330	0.0	*Arabidopsis thaliana*	3.27	*
SLP85	GO479174	DT624870.1	4e-22	*Pinus taeda*	2.30	1.38
SLP86	GO479175	AT5G46090	5e-120	*Arabidopsis thaliana*	3.05	2.83
SLP87	GO479176	DV986162.2	6e-79	*Picea glauca*	-2.52	1.28
SLP88	GO479177	BX784157.1	5e-121	*Pinus pinaster*	-2.53	1.41
SLP89	GO479178	CO158582.1	1e-52	*Pinus teada*	3.23	*
SLP90	GO479179	AT4G30790	0.0	*Arabidopsis thaliana*	2.04	1.92
SLP91	GO479180	AT4G02880	0.0	*Arabidopsis thaliana*	2.76	1.07
SLP92	GO479181	CT576025.1	0.0	*Pinus pinaster*	-2.55	1.49
SLP93	GO479182	BX680450.1	2e-130	*Pinus pinaster*	-3.86	1.45
SLP94	GO479183	BQ655588.1	6e-28	*Pinus teada*	-2.72	1.95
SLP95	GO479184	EG967606.1	5e-92	*Tamarix hispida*	-2.80	1.63
SLP26	GO479115	DR017133.1	0.0	*Pinus taeda*	1.05	-4.45
LLP17	GO479213	CN852425.1	4e-116	*Pinus taeda*	-3.22	-9.49
LLP18	GO479214	CX648522.1	2e-45	*Pinus taeda*	-2.57	1.73
LLP19	GO479215	ES248885.1	2e-27	*Pinus taeda*	-3.49	2.13
LLP20	GO479216	DR072326.1	2e-123	*Pinus taeda*	-6.02	1.62
LLP21	GO479217	DR021735.1	8e-64	*Pinus taeda*	-2.84	1.53
LLP22	GO479218	BM493742.1	1e-43	*Pinus taeda*	-2.67	2.33
LLP23	GO479219	DR017133.1	0.0	*Pinus taeda*	-1.71	-3.96
LLP24	GO479220	CAN72731	4e-04	*Vitis vinifera*	-2.60	1.75
SLP_LLP5	GO479196	CO362028.1	3e-73	*Pinus taeda*	-3.43	-11.26

[a] GenBank accession number. All cDNA sequences were submitted to GenBank.

[b] BLASTX was used to identify homologous genes and putative functions of genes. BLASTN was used in case no hits were found by BLASTX.

[c] The Expect value (E) is a parameter that describes the number of hits one can "expect" to see by chance when searching a database of a particular size. The lower the E-value, or the closer it is to zero, the more "significant" the match is.

[d] Values of signal intensity ratios showing a more than two-fold up- or down- regulation are shaded with blue or yellow, respectively. The median intensity value of the replicates was used to calculate the signal intensity ratio.

* Data not available due to the low significance.

Table 16. Genes with no hit in the databases and their expression levels in shortleaf pine (SLP) and loblolly pine (LLP) after top-killing

Name	Accession number[a]	Annotation[b]	E value	Source	SLP	LLP
SLP58	GO479147	No hit			-3.53[c]	1.49
SLP96	GO479185	No hit			-4.17	1.49
SLP97	GO479186	No hit			0.77	-2.99
SLP98	GO479187	No hit			-2.87	1.42
SLP99	GO479188	No hit			-2.94	1.44
SLP100	GO479189	No hit			-2.62	1.49
SLP101	GO479190	No hit			-3.95	1.44
SLP102	GO479191	No hit			-2.22	1.60
LLP25	GO479221	No hit			-4.11	1.51
LLP26	GO479222	No hit			1.11	2.08
LLP27	GO479223	No hit			-3.07	1.27
LLP28	GO479224	No hit			-3.08	1.52
LLP29	GO479225	No hit			-3.53	1.45
LLP30	GO479226	No hit			-2.85	1.38
LLP31	GO479227	No hit			-2.46	1.64
LLP32	GO479228	No hit			-3.06	1.45
LLP33	GO479229	No hit			-2.67	1.53
LLP34	GO479230	No hit			-3.17	1.22

[a] GenBank accession number. All cDNA sequences were submitted to GenBank.

[b] BLASTX was used to identify homologous genes and putative functions of genes. BLASTN was used in case no hits were found by BLASTX.

[c] Values of signal intensity ratios showing a more than two-fold up- or down- regulation are shaded with blue or yellow, respectively. The median intensity value of the replicates was used to calculate the signal intensity ratio.

Table 17. Primers used in qRT-PCR.

Gene name	Accession number[a]	Forward primer (5→3) [b]	Reverse primer (5→3)
SLP2	GO479091	AAGCGACATTGGAAACCATC	TCCATTGAAAAGGCAGTTCC
SLP21	GO479110	CGAGCAATTGAACTTGCAGA	TGGCGGCTTTATCTTCTTGT
SLP22	GO479111	CAGTCCGGAGGGTCTCATTA	CTGAACAGTGCCTCCCTCAT
SLP34	GO479123	CATTAGGGTTTGGCTTGGAA	AATCAGGGTTTTTGGCACAG
SLP36	GO479125	GCCTATGGATCGTCCGATTA	ACGCTTGTGGTGTTTCTCCT
SLP_LLP1	GO479192	GGCTTTGTCGGATCCTTGTA	AATCCACCACATTCGGAAAA

[a] GenBank accession number.

[b] Primers were designed using Primer 3.

Figure 1. Shortleaf pine and loblolly pine in the greenhouse seven days after planting. One-year-old shortleaf pine and loblolly pine were planted in plastic pots in the NREM greenhouse.

Figure 2. Top cut pines (foreground) and untreated controls (background). Shortleaf pine and loblolly pine in the treatment groups were topcut with one-inch of stem left seven days after planting.

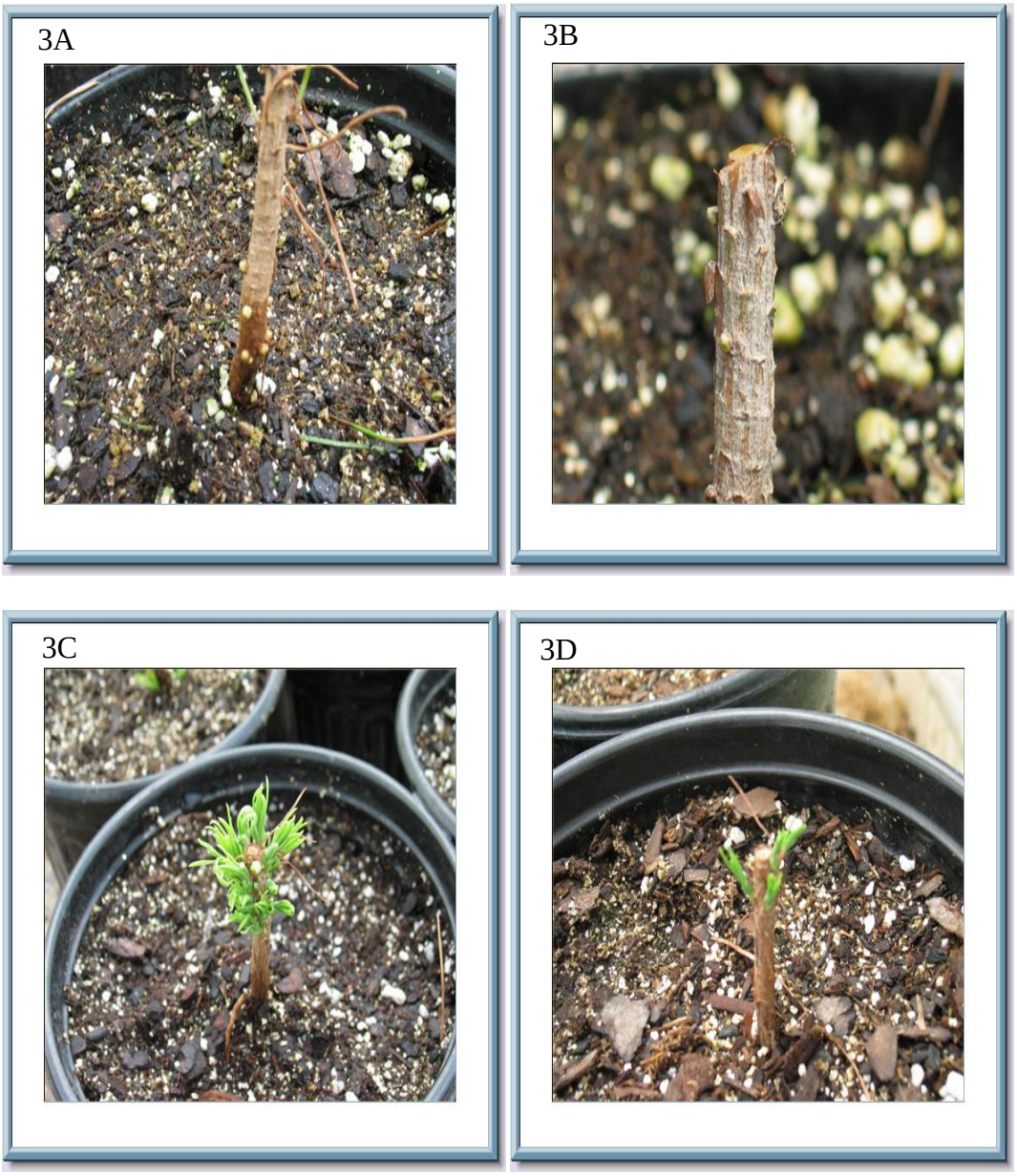

Figure 3. Pictures taken during tissue collection. 3A was taken two days after cutting treatments on shortleaf pine; 3B was taken seven days after cutting treatments on loblolly pine; 3C was taken one week after sprouting (nine days after cutting treatments) on shortleaf pine; 3D was taken one week after sprouting (14 days after cutting treatments) on loblolly pine.

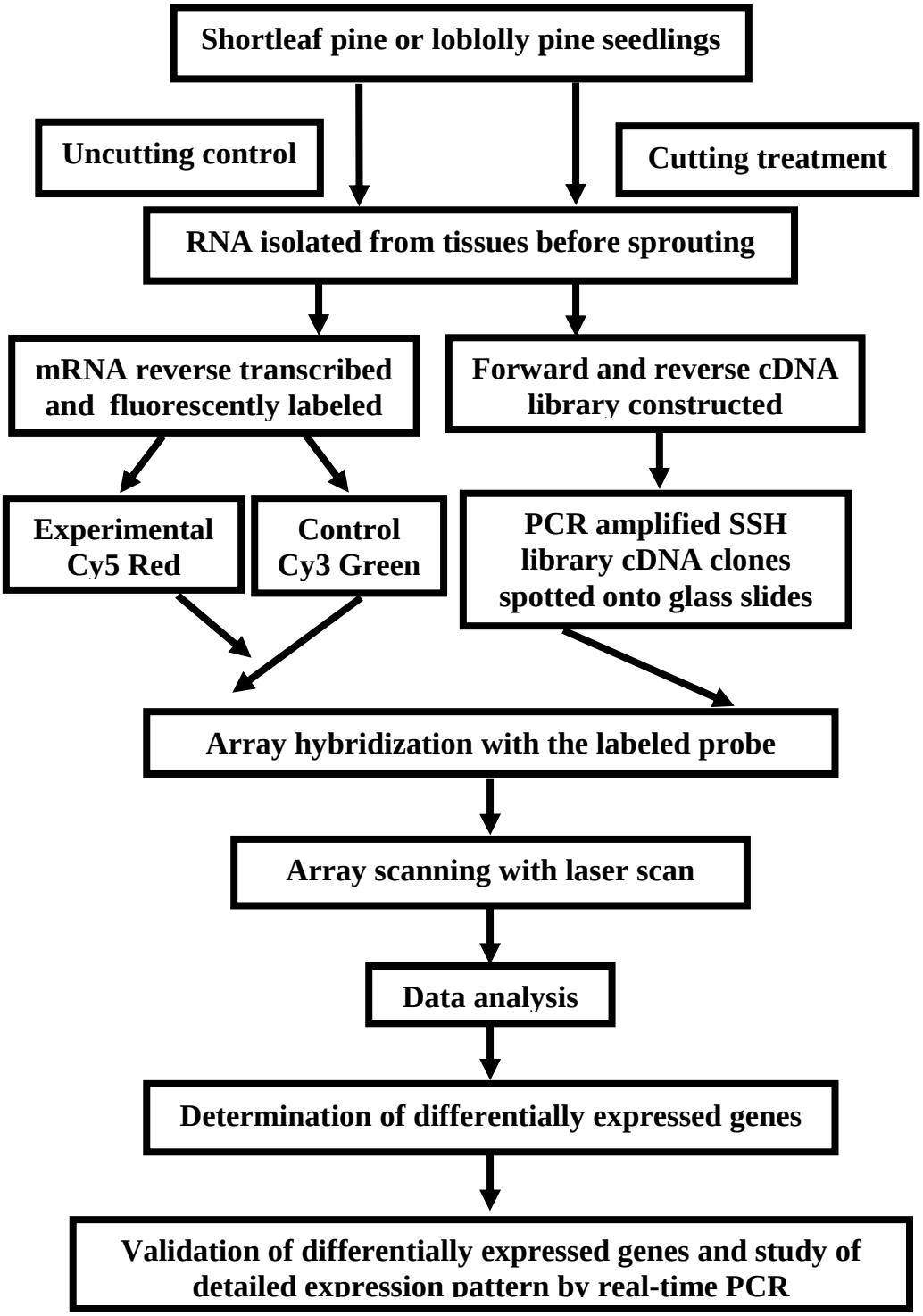

Figure 4. Detailed experimental outline. Tissues were collected at different time points, and tissues collected at the optimum time points (shortleaf pine, the first and second day; loblolly pine, the sixth and the seventh day) were used for preparation of array cDNA, cDNA labeling and the array experiment.

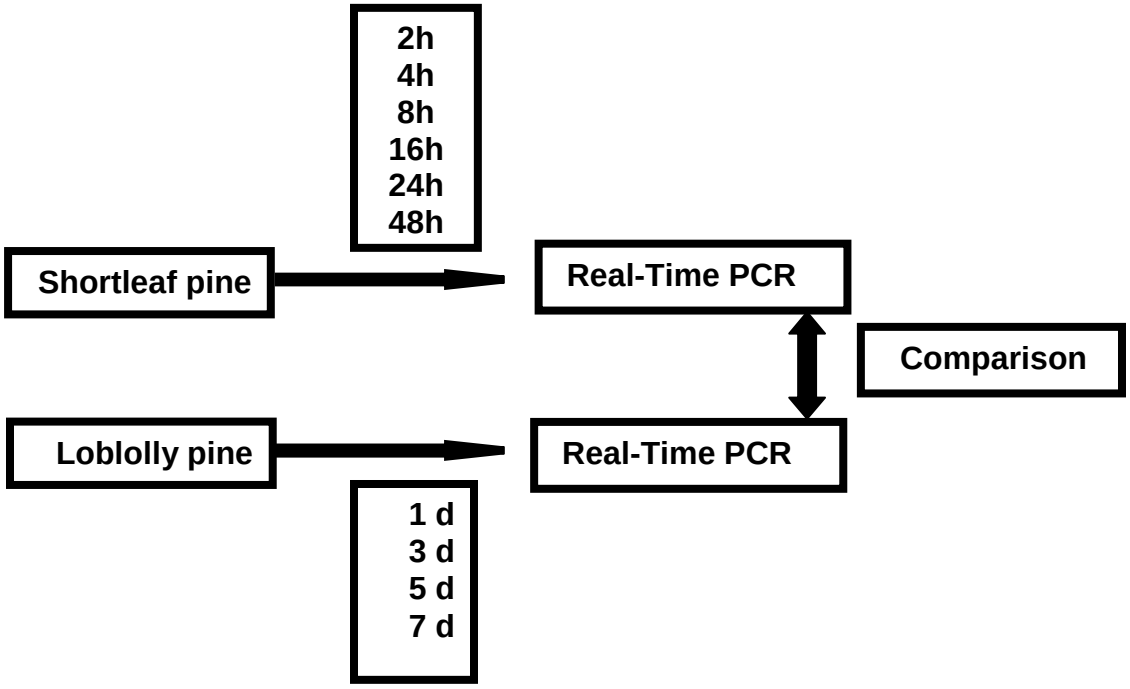

Figure 5. Tissue collection for Real-time PCR. Tissues were collected at different time points after top-killing for quantitative realtime PCR experiments. For shortleaf pine, tissues were collected at two, four, eight, 16, 24 and 48 hours after top-killing. For loblolly pine, tissues were collected at one, three, five and seven days after top-killing.

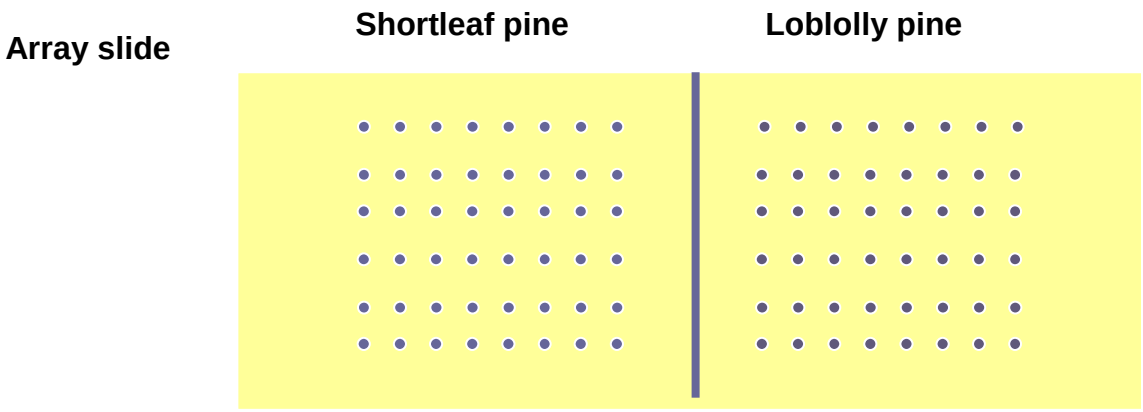

Figure 6. Array slides layout. The left side of the array slides printed 1,018 cDNAs originated from the shortleaf pine cDNA library, and the right side printed 1,319 cDNA from the loblolly pine cDNA library. In total, 2,337 cDNA clones were obtained from the cDNA libraries and printed on the array slides, and each cDNA spot had three technical replications.

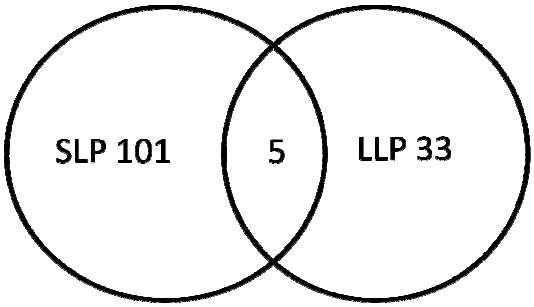

Figure 7. Venn diagrams of the origin of differentially expressed genes, which showed at least a two-fold change in expression either during self-hybridization or cross hybridization. SLP indicates differentially expressed genes coming from shortleaf pine cDNA library, and LLP indicates differentially expressed genes coming from loblolly pine cDNA library.

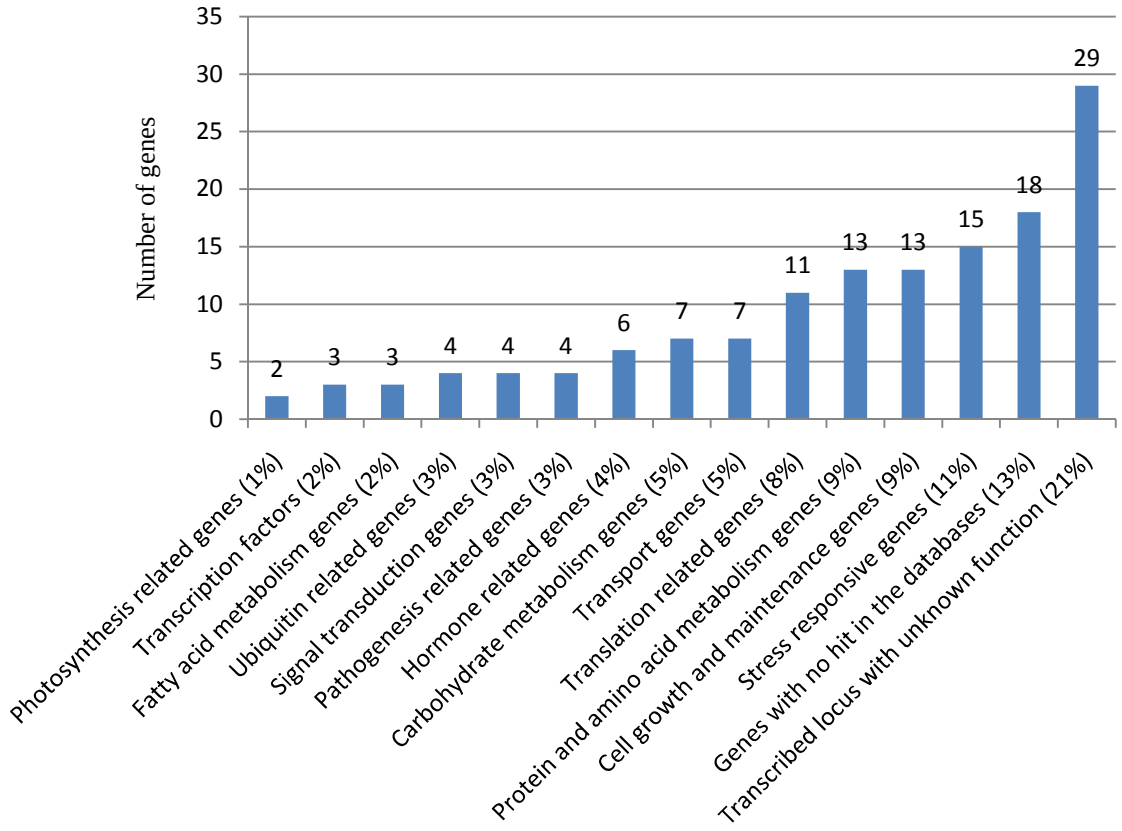

Figure 8. Functional categories of differentially expressed genes after top-killing treatment. In this chart, functional groups and the proportion of genes in each group are indicated under each column. Numbers of genes in individual functional groups are shown above each column.

```
cttagatcgtgcagatacagtattcacccaatctccccctcacagacaaattacaattca  60
 L  R  S  C  R  Y  S  I  H  P  I  S  P  S  Q  T  N  Y  N  S
acagagttaatctcaggattgcatgatgatttcagtcgttccaaggcatcatcatcttca  120
 T  E  L  I  S  G  L  H  D  D  F  S  R  S  K  A  S  S  S  S
gaacccatctgggagaaagaagctgagagcagccccagaacggaaaatccctcgcagaag  180
 E  P  I  W  E  K  E  A  E  S  S  P  R  T  E  N  P  S  Q  K
cagcaacaatcattatttaatatggatctggaaggtctacaaagttccttccctcatcta  240
 Q  Q  Q  S  L  F  N  M  D  L  E  G  L  Q  S  S  F  P  H  L
gaccaaatatcttttagcgatgcttatcaagactggcttttactc                285
 D  Q  I  S  F  S  D  A  Y  Q  D  W  L  L  L
```

Figure 9. Nucleotide and deduced amino acid sequences of SLP1. The deduced amino acid sequences are shown below the second nucleotide of each corresponding codon.

```
gi|50910848|norway_spruce      APVQNTAFNPISSSINHQWTNCNSTDLMSGLHNDSSCSKPSSFSEPISEK 50
gi|2982275|black_spruce        APVQNTTFNPISSSINHQWTNCNSTDLMSGLHNDSSCSKPSSFSEPISEK 50
gi|50910844|white_spruce       APVQNTAFNPISSSINHQWTNCNSTDLMSGLHNDSSCSKPSSFSEPISEK 50
SLP1                           LRSCRYSIHPISPSQ----TNYNSTELISGLHDDFSRSKASSSSEPIWEK 46
                                   . :::***.*     ** ***:*:****:* * **.** **** **

gi|50910848|norway_spruce      EEVQSSFRLENFSQEQQQSLFNFGLEGLQNTFTHLDQITFPGAYQDWFYP 100
gi|2982275|black_spruce        EEVQSSFRLENFSQEQQQSLFNFGLEGLQNTFTHLDQITLPGAYQDWFYP 100
gi|50910844|white_spruce       EEVESSFRLENFSQEQQQSLFNFGLEGLQNTFTHLDQITFPGAYQDWFYP 100
SLP1                           -EAESSPRTENPSQKQQQSLFNMDLEGLQSSFPHLDQISFSDAYQDWLLL 95
                                *.:** * ** **:*******:.*****.:*.*****::..*****:
```

Figure 10. Alignment of amino acid sequences of SLP1 with NAM proteins from several tree species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SPL1 used in the alignments were deduced from nucleotide sequences.

```
agcgtgtcgcggccgaggtacttgagcagtctcaagcaagaatttcttaagaagaaaagg  60
 S  V  S  R  P  R  Y  L  S  S  L  K  Q  E  F  L  K  K  K  R
Aaaggcaaactccccaaggaagcaaggcaaaagttgttggattggtggaccagaaactat  120
 K  G  K  L  P  K  E  A  R  Q  K  L  L  D  W  W  T  R  N  Y
Aagtggccatatccttcggaaagtcaaaagatagcattggcagaatctaccgggctggat  180
 K  W  P  Y  P  S  E  S  Q  K  I  A  L  A  E  S  T  G  L  D
Cagaagcaaataaataactggtttataaatcagcgcaagcgacattggaaaccatctgaa  240
 Q  K  Q  I  N  N  W  F  I  N  Q  R  K  R  H  W  K  P  S  E
Gagatgcagttcgtggttatggatagtcctaatcctcacaacgctgctttttcctggag  300
 E  M  Q  F  V  V  M  D  S  P  N  P  H  N  A  A  F  F  L  E
Ggacatctcaggacagatggaactgcctttcaatggattgt                    342
 G  H  L  R  T  D  G  T  A  F  S  M  D  C
```

Figure 11. Nucleotide and deduced amino acid sequences of SLP2. The deduced amino acid sequences are shown below the second nucleotide of each corresponding codon.

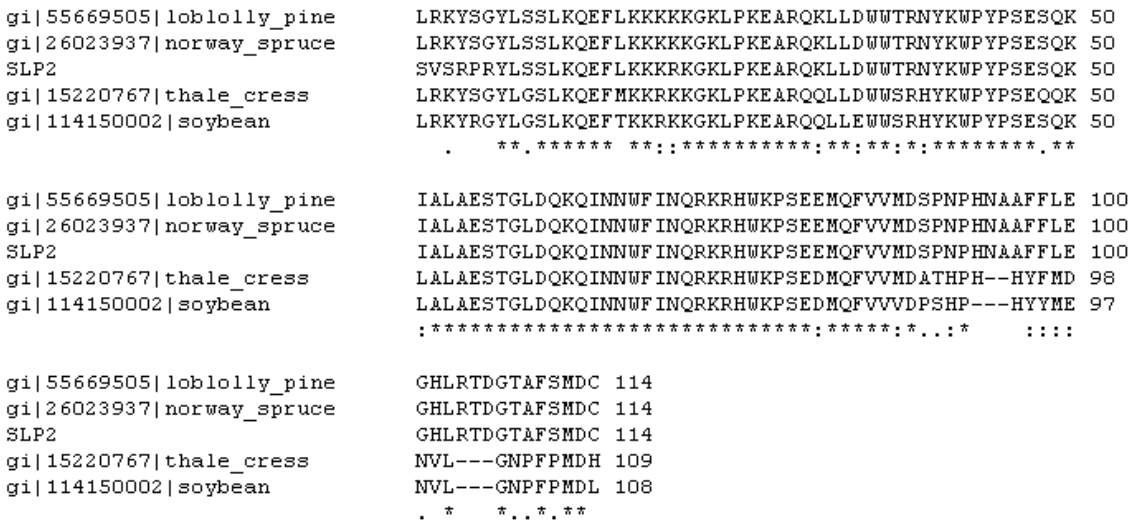

Figure 12. Alignment of amino acid sequences of SLP2 with STM proteins from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SPL2 used in the alignments were deduced from nucleotide sequences.

```
gi|195624118|corn           TFYTEEDFRDFLSRRGWTFLREYGGYRNVDSLDDLRPGVMYQGLRSLGD 49
gi|170271|tobacco           TFYTANDFRDFLSHRGWTCLREYNGYRHVDMLDELCPGAVYRGVN---- 45
SLP3                        TLYTEEDFRDFLTRRGWSGLQEVGGFRAIDSLDDLRPLCVYQRAGLLGE 49
gi|161789859|soybean        IFYTEDDFRDFLTRRGWICLREFDSYRNIDNMDDLRPGAIYRGVS---- 45
gi|30683296|thale_cress     IFYTEEDYREFLARQGWSSL-QVDGFRNIENMDDLQPGAVYRGVR---- 44
                             :** :*:*:**:::**  * : ..:* :: :*:* *  :*:
```

Figure 13. Alignment of amino acid sequences of SLP3 with GT-l-like proteins from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SPL3 used in the alignments were deduced from a loblolly pine EST sequence (accession number: 148815838).

```
gi|223527109|castorseed      LYSQGYGVNTAALSTALFNNGLSCGACFEIKCANDPKWCHSGSPSILITA 50
gi|27464179|soybean          LYSQGYGVNTAALSTALFNNGLSCGACFEIKCDQDPRWCNPGNPSILITA 50
gi|4886515|tomato            LYSQGYGVNNGALSTALFNNGLSCGACFEIKCDNYPQWCHPGSPSIFITA 50
gi|121484275|China_fir       LYSQGYGVQTAALSTALFNDGLSCGACFEIKCVNDPEWCHPGSPSIFITA 50
SLP4                         PVQPGIWSSSAALSTALFNSGLSCGACFEIKCVNDPEWCHPGNPSILVTA 50
                               . *   ...********.************ : *.**:.*.***::**

gi|223527109|castorseed      TNFCPPNFALPNDNGGWCNPPRPHFDLAMPMFLKIAEYRAGIVPVAYRR 99
gi|27464179|soybean          TNFCPPNFALPNDNGGWCNPPRPHFDLAMPMFLKIAQYRAGIVPVAYRR 99
gi|4886515|tomato            TNFCPPNFALPNDNGGWCNPPRPHFDLAMPMFLHIAEYRAGIVPVVYRR 99
gi|121484275|China_fir       TNFCPPNYALPNDNGGWCNPPRPHFDLSMPIFLKFAEYRAGIVPVLHRR 99
SLP4                         TNFCPPNYALPNDNGGWCNPPRPHFDLSMPIFLKMAEYRAGIVPVLFRR 99
                             *******:********************:**:**::*:******** .**
```

Figure 14. Alignment of amino acid sequences of SLP4 with expansin-like proteins from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SPL4 used in the alignments were deduced from a loblolly pine EST sequence (accession number: 67488878).

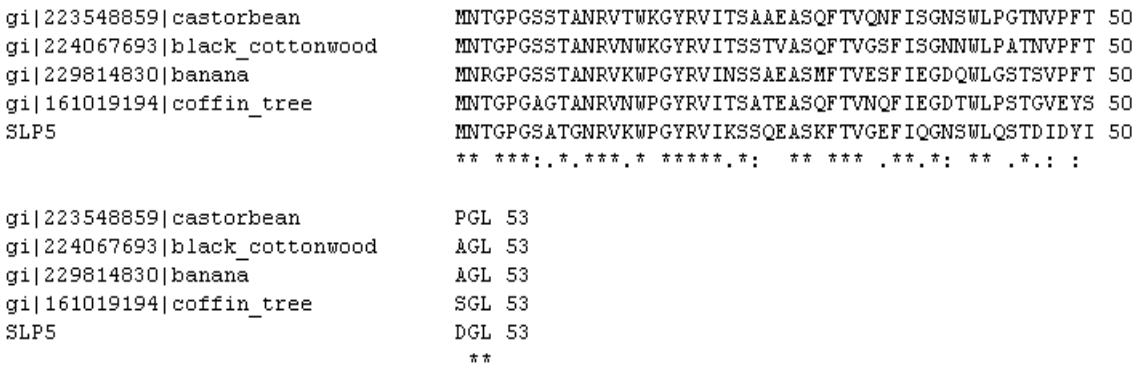

Figure 15. Alignment of amino acid sequences of SLP5 with pectin-methylesterase-like proteins from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SPL5 used in the alignments were deduced from nucleotide sequence.

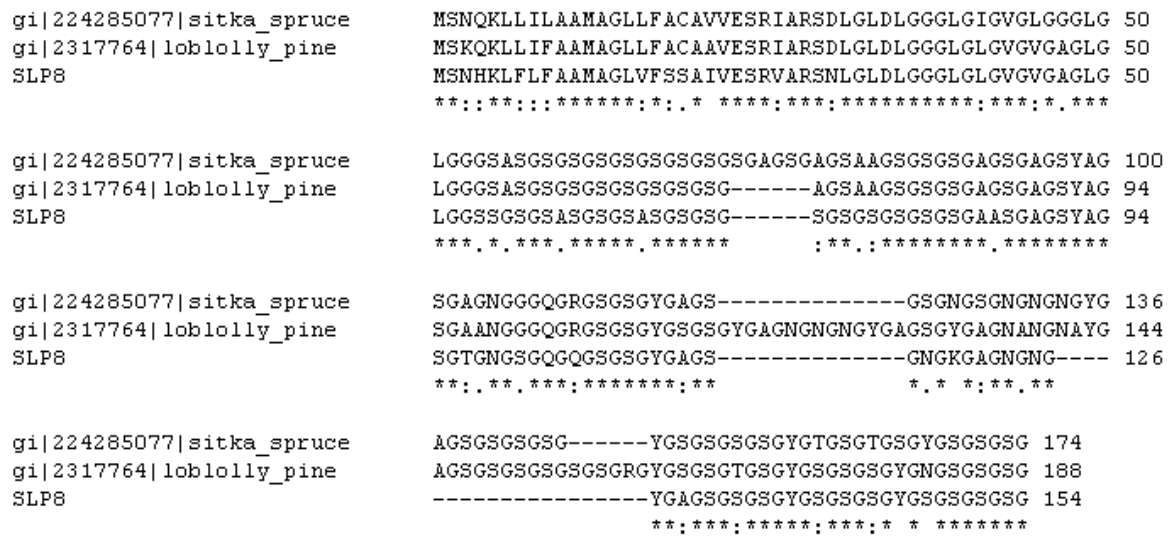

Figure 16. Alignment of amino acid sequences of SLP8 with cell wall like-proteins from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SLP8 used in the alignments were deduced from a loblolly pine EST sequence with the accession number of 68089089.

```
gi|30693010|thale_cress           LRQNTATDAKIMSWWDYGYQITAMGNRTVIVDNNTWNNTHIATVGRAMSS 50
gi|225457500|grape                LRQNTPPDAKVMSWWDYGYQITAMGNRTVIVDNNTWNNTHIATVGRAMSS 50
gi|224120506|black_cottonwood     LRQNTPPDAKVMSWWDYGYQITAMGNRTVIVDNNTWNNTHIATVGRAMSS 50
SLP11                             SAAAIPLLSQVMSWWDYGYQITAMGNRTVIVDNNTWNNTHIATVGRAMSS 50
                                       .  :::**************************************

gi|30693010|thale_cress           YEDDAYDIMRSLDVNYVLVVFGGVTGYSSDDINKFLWMVRIGGGVFPVIK 100
gi|225457500|grape                YEDEAYEIMRSLDVDYVLVVFGGVTGYSSDDINKFLWMVRIGGGVFPVIK 100
gi|224120506|black_cottonwood     YEDEAYEIMKSLDVDYVLVVFGGVTGYSSDDINKFLWMVRIGGGVFPVIK 100
SLP11                             YEHEAYEIMQSLDVDHVLVVFGGVTGYSSDDINKFLWMVRIGGGVFPVIK 100
                                  **.:**:**:****::**********************************

gi|30693010|thale_cress           EPDYLVNGE 109
gi|225457500|grape                EPDYLVNGE 109
gi|224120506|black_cottonwood     EPDYLVNGE 109
SLP11                             EADYLVNGE 109
                                  *.*******
```

Figure 17. Alignment of amino acid sequences of SLP11 with STT3B-like proteins from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SPL11 used in the alignments were deduced from nucleotide sequences.

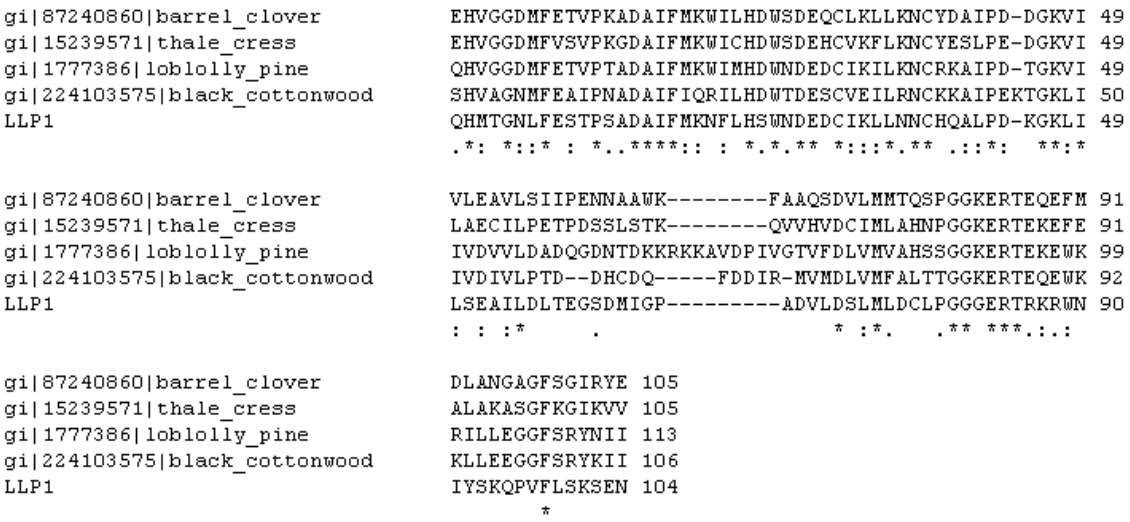

Figure 18. Alignment of amino acid sequences of LLP1 with O-methyltransferase-like proteins from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of LLP1 used in the alignments were deduced from nucleotide sequences.

```
SLP13                          TTWSNNSTQLCYDCNSCKAGVLANLKHDWRKVAVVNIVMLIFLIIVYSVG 50
gi|79592093|thale_cress        QTWDNAKEKLCFDCQSCKAGLLDNVKSAWKKVAIVNIVFLVFLIIVYSVG 50
gi|171921097|wild_cabbage      GAWSNVQTELCFNCNACKAGVLANIREKWRNLLIFNVCLIVLLITVYSCG 50
gi|224130182|black_cottonwood  AAWSNRQDTLCFNCESCKAAYVVTSRKQWGQLAIANACFIAFTVIFYSIG 50
                                :*.* .  **::*::***. : . :  * :: : *  :: : : .** *

SLP13                          CCAFRNNRSDNSYGKGYL 68
gi|79592093|thale_cress        CCAFRNNKRDDSYSRTYG 68
gi|171921097|wild_cabbage      CCAHRNNRMARKSGFKTM 68
gi|224130182|black_cottonwood  CCARSNNQQDSHHRYRGY 68
                               ***  **:
```

Figure 19. Alignment of amino acid sequences of SLP13 with TET8-like proteins from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SPL13 used in the alignments were deduced from nucleotide sequences.

```
SLP15                              FTIVINCHCQSGRMNNALKVFEEMEAQGHVPNI 33
gi|83744088|rice                   FNSIIDSHCKEGRVIESEKLFELMVRIGVKPNV 33
gi|15218284|thale_cress            YNMLIDAYCKLGKIDDGFALKEEMEREGIVPDV 33
gi|224123734|black_cottonwood      YTTLIDAYCKDGRMEDAFALYNMMIDRGIFPEV 33
                                   :. :*:.:*: *:: :.  : : *   *  *::
```

Figure 20. Alignment of amino acid sequences of SLP15 with PPR motif from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SPL15 used in the alignments were deduced from nucleotide sequences.

```
gi|223550340|castorbean          QNWQWLKYGVELDGDGLGVKVTFDLLGRVVEDEMARIEREVGKEKFKKGM 50
gi|224136065|black_cottonwood    QNWQWLKYGVELDGDGLGVKVNNDLFGKVVEEEMARIEREVGKEKFKRGM 50
gi|90265259|rice                 QNWQWLRHGAVLDAGGVEVRATPELLARVVEEEMARVEAEVGAERFRRGR 50
SLP17                            QNWQWIHYEVVLDGEVVPVKVTRELVGRILAEEMARIEREVGTKKFKGGR 50
                                 *****::: . **.  : *:.. :*..::: :****:* *** ::*: *

gi|223550340|castorbean          YKEACKMFVRQCAAPTLDDFLTLDAYNNIVIHYP-KGSS-RL 90
gi|224136065|black_cottonwood    YKEACKIFARQCTAPTLDDFLTLNAYDNIVIHHP-MGSSSRL 91
gi|90265259|rice                 YAEAGRIFSRQCTAPELDDFLTLDAYNLIVVHHPGASSPCKL 92
SLP17                            YEEAAKMFGRQCTAPSLDDFLTLDVYTSILQFHPTPVASSRI 92
                                 * ** ::* ***:** *******:.*  *: .:*   :. ::
```

Figure 21. Alignment of amino acid sequences of SLP17 with malate synthase from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SPL17 used in the alignments were deduced from nucleotide sequences.

```
gi|226502865|corn              MPVLSVVIPRLKTNQLKWSFTGAFEARQSLIVRGLFPMLADPRHPAES-- 48
gi|92870921|barrel_clover      MPVLSVVIPRLKTNQLKWSFSGAFEARQSLIVRGLFPMLADPRHPAESET 50
gi|223526090|castorbean        MPVISVVIPRLKTNQLRWTFTGAFEARQSLIVRGLFPMLADPRHPAES-- 48
SLP18                          MPVLSVVIPRLTTNQLRWSFTGAFQARQTLVVRGLFPMLADPRHPAES-- 48
                               ***:*******.****:*:*:***:***:*:******************

gi|226502865|corn              TSATNESVLKVALDHGKASGVIKSHDRVVVCQKVGDSSVVKIIELDD 95
gi|92870921|barrel_clover      TTASNESILKVALDHGKALGVIKSHDRVVVCQKLGDASVVKIIELED 97
gi|223526090|castorbean        TNATNESVLKVALDHGKAIGVIKPHDRVVVFQKVGDSSVVKILELED 95
SLP18                          INATNESVLKIALDHGKTVGLIKPHDRIVVCQKIGDSAVVKILELED 95
                                .*:***:**:******: *:**.***:** **:**::****:**:*
```

Figure 22. Alignment of amino acid sequences of SLP18 with pyruvate kinase from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SPL18 used in the alignments were deduced from nucleotide sequences.

```
gi|223534389|castorseed      NPWHVSFSYARALQNSVLKTWQGHPENVEAAQKALLVRAKANSLAQLGKY 50
gi|226502756|corn            NPWHVSFSYVRALQNSVLKTWQGRPENVEAAQKALLVRAKANSLAQLGRY 50
gi|4827251|tobacco           NPWHVSFSYARALQNTCLKTWGGRPENVQAAQEALLIRANANSLAQLGKY 50
SLP19                        QGGACFLLYARALQNTSLKTWKGLPENVEAAQRALLIRAKANSLAQLGRY 50
                             :     : *.*****: **** * ****:***.***:**:********:*

gi|223534389|castorseed      SAEGENEEAKKGMFVKGYTY 70
gi|226502756|corn            TGEGESDDAKKGMFQKGYTY 70
gi|4827251|tobacco           TGEGESEEAKKGMFVKGYVY 70
SLP19                        SAEGESEESKKGMFVKGYTY 70
                             :.***.:::***** ***.*
```

Figure 23. Alignment of amino acid sequences of SLP19 with fructose-bisphosphate aldolase from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SPL19 used in the alignments were deduced from nucleotide sequences.

```
gi|8918504|common_wheat          IPEAYERLILDTIRGDQQHFVRRDELKAAWQIFTPLLHDIDAGKLKAVSY 50
gi|5732195|thale_cress           IPEAYERLILDTIRGDQQHFVRRDELKAAWEIFTPLLHRIDKGEVKSVPY 50
gi|3021510|tobacco               IPEAYERLILDTIRGDQQHFVRRDELKAAWEIFTPLLHRIDDGEVKPIPY 50
gi|89214190|Mongolian_poplar     IPEAYERLILDTIRGDQQHFVRRDELKAAWEIFTPLLHRIDNGELKPKEY 50
SLP20                            IPEAYERLILDTIRGDQQHFVRRDELKVAWEIFTPLLNRIDNGEIKPYTY 50
                                 ****************************.**:******: ** *::*.  *

gi|8918504|common_wheat          KPGSRGPKEADELSEKVGYMQTHGYIWIPPTLA-- 83
gi|5732195|thale_cress           KQGSRGPAEADQLLKKAGYMQTHGYIWIPPTL--- 82
gi|3021510|tobacco               KPGSRGPAEADELLQNVGYVQTHGYICIPPTL--- 82
gi|89214190|Mongolian_poplar     QPGSRGPVEADELLAKAGYVQTHGYIWIPPTL--- 82
SLP20                            TPGSRGPNEADELAARVGYKQTHGYIWIPPSLQTD 85
                                   ***** ***:*  ..** ****** ***:*
```

Figure 24. Alignment of amino acid sequences of SLP20 with glucose-6-phosphate 1-dehydrogenase from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SPL20 used in the alignments were deduced from a loblolly pine EST (accession number: 34350332).

```
atattctgtggttctctgctgcctgcatcaaactggaaggcccatattgcaaaacgagca 60
 I  F  C  G  S  L  L  P  A  S  N  W  K  A  H  I  A  K  R  A
attgaacttgcagaacggagattatctaaagatggatggcctgaatactatgatggtaaa 120
 I  E  L  A  E  R  R  L  S  K  D  G  W  P  E  Y  Y  D  G  K
cttggaagatacattggaaagcaagctcggaaatttcagacatggtctgttgctggctat 180
 L  G  R  Y  I  G  K  Q  A  R  K  F  Q  T  W  S  V  A  G  Y
ctggtagctaagatgatgcttgaagatccatcccacttaggtatgatatcacttgaggaa 240
 L  V  A  K  M  M  L  E  D  P  S  H  L  G  M  I  S  L  E  E
gacaagaagataaagccgccactcaccagatcacattcctggacatgt            288
 D  K  K  I  K  P  P  L  T  R  S  H  S  W  T  C
```

Figure 25. Nucleotide and deduced amino acid sequences of SLP21. The deduced amino acid sequences are shown below the second nucleotide of each corresponding codon.

```
gi|51587334|wild_legume      LWLVTAACIKTGRPQIARRAIELAESRLLKDGWPEYYDGKLGRYVGKQAR 50
gi|146395463|tomato          LWLLTAAAIKTGRPQIARRAIELAESRLLKDSWPEYYDGKLGRFIGKQAR 50
gi|54112232|rice             LWLLTAACIKTGRPQIARRAIDLAERRLLKDGWPEYYDGKLGRYVGKQAR 50
gi|226504262|corn            LWLLTAACIKTGRLKIARRAIDLAEARLARDGWPEYYDGKLGRYIGKQAR 50
SLP21                        IFCGSLLPASNWKAHIAKRAIELAERRLSKDGWPEYYDGKLGRYIGKQAR 50
                             ::  :    .. : :**:***:*** ** :*.***********::*****

gi|51587334|wild_legume      KYQTWSIAGYLVAKMMLEDPSHLGMISLEEDKQMKPVIKRSSSWTC 96
gi|146395463|tomato          KFQTWSIAGYLVARMMLEDPSHLGMISLEEDKQMKPTMKRSASWTC 96
gi|54112232|rice             KFQTWSIAGYLVAKMMLEDPSHLGMISLEEDKAMKPVLKRSASWTN 96
gi|226504262|corn            KLQTWSIAGYLVAKMMVEDPSHLGMISLEEEKPTKPVLRRSASWTG 96
SLP21                        KFQTWSVAGYLVAKMMLEDPSHLGMISLEEDKKIKPPLTRSHSWTC 96
                             * ****:******:**:**************:*  ** : ** ***
```

Figure 26. Alignment of amino acid sequences of SLP 21 with invertase from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SPL21 used in the alignments were deduced from nucleotide sequences.

```
atgaaagaagttgcaaggcgaggaaatatacctttaacaggtgaaaatgcaattgaacgc  60
 M  K  E  V  A  R  R  G  N  I  P  L  T  G  E  N  A  I  E  R
tttgataaggaggctttctctcaaattgtgagaaatgcttacaatcgtcctcaagatgtg  120
 F  D  K  E  A  F  S  Q  I  V  R  N  A  Y  N  R  P  Q  D  V
agagcctttacgtatttccgaatgagggaggcactgttcaggactgataattggaaatca  180
 R  A  F  T  Y  F  R  M  R  E  A  L  F  R  T  D  N  W  K  S
ttcgtgaactttgttaagcagaag                                      204
 F  V  N  F  V  K  Q  K
```

Figure 27. Nucleotide and deduced amino acid sequences of SLP22. The deduced amino acid sequences are shown below the second nucleotide of each corresponding codon.

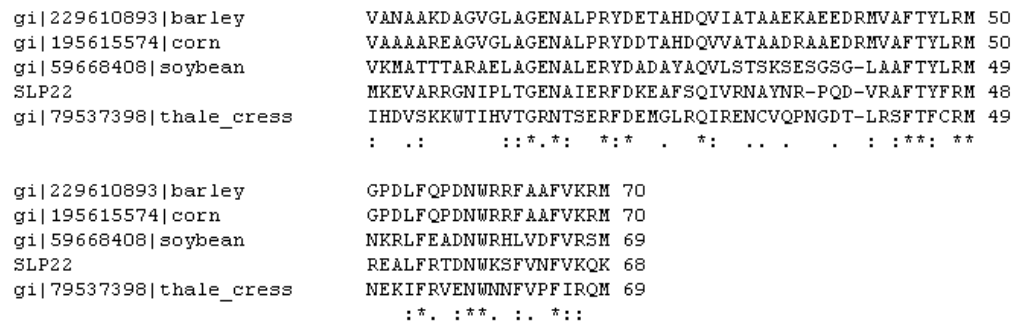

Figure 28. Alignment of amino acid sequences of SLP 22 with invertase from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SPL22 used in the alignments were deduced from nucleotide sequences.

```
atgaaaaatcaagaggctgttgatttggttagaaaaatcaaggaccctcaggtggcagcc  60
 M  K  N  Q  E  A  V  D  L  V  R  K  I  K  D  P  Q  V  A  A
aagtgtctgactgaaaatgcagttgcaagaaagagcaaagatgatatttcatgcattgtt  120
 K  C  L  T  E  N  A  V  A  R  K  S  K  D  D  I  S  C  I  V
gtgcgtttccagcat                                               135
 V  R  F  Q  H
```

Figure 29. Nucleotide and deduced amino acid sequences of SLP24. The deduced amino acid sequences are shown below the second nucleotide of each corresponding codon.

```
SLP24                          MKNQEAVDLVRKIKDPQVAAKCLTENAVARKSKDDISCIVVRFQH 45
gi|51969968|thale_cress        MSNQEAVDLIKSIKDPQAAAKELIEEAVSKQSTDDISCIVVRFQ- 44
gi|115460446|rice              MKNQEAVDLVKSIKDPQAAAKRLTTEALARKSKDDISCIVIRFRC 45
gi|223546392|castorbean        MQNQEAVDLVKPIKDPQAAAKRLTTEALARKSKDDISCIVIRFG- 44
gi|124361192|barrel_clover     MANQEAVDIARKVKDPLKAAKQLTAEALKRESKDDISCVVVRFR- 44
                               * ******: : :***  *** *  :*: ::*.*****:*:**
```

Figure 30. Alignment of amino acid sequences of SLP 24 with protein phosphatase 2C (PP2C) from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SPL24 used in the alignments were deduced from nucleotide sequences.

```
atggagggtttcaattggtgtcagggcaacaatgttgttacagttttagcgcaccgaat  60
 M  E  G  F  N  W  C  Q  G  N  N  V  V  T  V  F  S  A  P  N
tattgctataggtgtggtaatatggcagctataatggagattagtgagactatggagcaa  120
 Y  C  Y  R  C  G  N  M  A  A  I  M  E  I  S  E  T  M  E  Q
aacttcattcaatttgagccagcacccaggcaaattgaacctgatatgacacgcaagaca  180
 N  F  I  Q  F  E  P  A  P  R  Q  I  E  P  D  M  T  R  K  T
cctgattatttttg                                                195
 P  D  Y  F  L
```

Figure 31. Nucleotide and deduced amino acid sequences of SLP25. The deduced amino acid sequences are shown below the second nucleotide of each corresponding codon.

```
gi|195639542|corn           MEGFNWCQDKNVVTVFSAPNYCYRCGNMAAILEIGENMDQNFLQFDPAPR 50
gi|1218054|rice             MEGFNWCQDKNVVTVFSAPNYCYRCGNMAAILEIGENMDQNFLQFDPAPR 50
gi|15218524|thale_cress     MEGFNWCQDKNVVTVFSAPNYCYRCGNMAAILEIGENMEQNFLQFDPAPR 50
gi|34398261|tomato          MEGFNWCQDKNVVTVFSAPNYCYRCGNMAAILEISENMEQNFLQFDPAPR 50
SLP25                       MEGFNWCQGNNVVTVFSAPNYCYRCGNMAAIMEISETMEQNFIQFEPAPR 50
                            ********.:**********************:**.*.*:***:**:****

gi|195639542|corn           QIEPDMTRKTPDYFL 65
gi|1218054|rice             QIDPDTTRKTPDYFL 65
gi|15218524|thale_cress     QVEPDTTRKTPDYFL 65
gi|34398261|tomato          QIEPDTTRKTPDYFL 65
SLP25                       QIEPDMTRKTPDYFL 65
                            *::** *********
```

Figure 32. Alignment of amino acid sequences of SLP 25 with the catalytic subunit of protein phosphatase 2A (PP2A) from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SPL25 used in the alignments were deduced from nucleotide sequences.

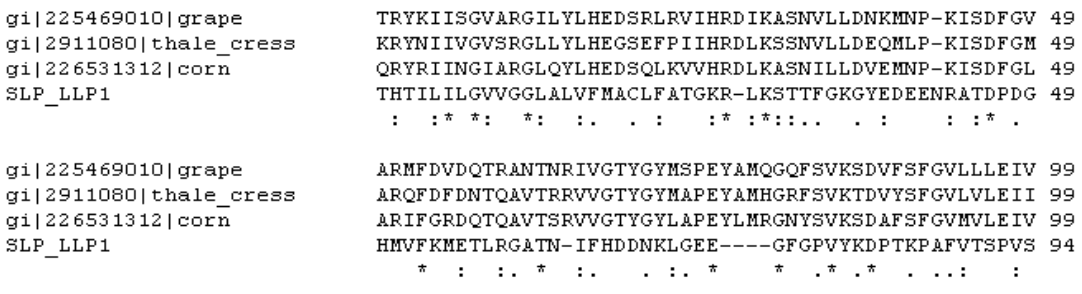

Figure 33. Alignment of amino acid sequences of SLP_LLP1 with receptor kinase-like proteins from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SLP_LLP1 used in the alignments were deduced from nucleotide sequences.

```
ctggggaggagggcacgggattcgagatcccagaagggcattagggtttggcttggaaca 60
 L  G  R  R  A  R  D  S  R  S  Q  K  G  I  R  V  W  L  G  T
tttaacactgcggaagaggccgccaaggcgtatgatgcagaggctaaaaagatcagaggc 120
 F  N  T  A  E  E  A  A  K  A  Y  D  A  E  A  K  K  I  R  G
aagaaagccaagcttaactttgctgatggctcctgctctgtaaaagaggacagtcgcaac 180
 K  K  A  K  L  N  F  A  D  G  S  C  S  V  K  E  D  S  R  N
aaaatgtcaaggaagaaagtaaagtcctgtgccaaaaaccctgatttattattggctttg 240
 K  M  S  R  K  K  V  K  S  C  A  K  N  P  D  L  L  L  A  L
aatataaagagtaaggtaaaatcttcatattcaccaaagcctgatttattagaggattgc 300
 N  I  K  S  K  V  K  S  S  Y  S  P  K  P  D  L  L  E  D  C
tatcttcaaatggaacgctctttgaaggatgtccgcagatccgatctttcaatctatggc 360
 Y  L  Q  M  E  R  S  L  K  D  V  R  R  S  D  L  S  I  Y  G
tacgatgatatggagtacctcggccgcgacaccgct                       396
 Y  D  D  M  E  Y  L  G  R  D  T  A
```

Figure 34. Nucleotide and deduced amino acid sequences of SLP34. The deduced amino acid sequences are shown below the second nucleotide of each corresponding codon.

```
gi|24817250|chickpea              QYRGIRQRPWGKWAAEIRDPRKGVRVWLGTFNTAEEAARAYDAEARRIRG 50
gi|188039906|soybean              QYRGIRQRPWGKWAAEIRDPRKGVRVWLGTFNTAEEAARAYDAEARRIRG 50
gi|224119670|black_cottonwood     QYRGIRQRPWGKWAAEIRDPRKGVRVWLGTFNTAEEAARAYDAEARRIRG 50
gi|226499014|corn                 QYRGIRQRPWGKWAAEIRDPQKGVRVWLGTFNSPEEAARAYDAEARRIRG 50
SLP34                             LYRGIRQRPWGKWAAEIRDPRKGIRVWLGTFNTAEEAAKAYDAEAKKIRG 50
                                   ********************:**:********:.****:******::***

gi|24817250|chickpea              KKAKVNFPE 59
gi|188039906|soybean              KKAKVNFPE 59
gi|224119670|black_cottonwood     KKAKVNFPD 59
gi|226499014|corn                 KKAKVNFPD 59
SLP34                             KKAKLNFAD 59
                                  ****:**.:
```

Figure 35. Alignment of amino acid sequences of SLP34 with the DNA binding domain of AP2/ERF from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SLP34 used in the alignments were deduced from nucleotide sequences.

```
atgttggataagatatgggatgacactcttggcggcccgcagccagacaagggcctcagg  60
 M  L  D  K  I  W  D  D  T  L  G  G  P  Q  P  D  K  G  L  R
aggcttcgcaataattcaggcaaattgcaggtgagcccagtagatattgacgagatgaat  120
 R  L  R  N  N  S  G  K  L  Q  V  S  P  V  D  I  D  E  M  N
ctaaaagaagggagtgggggaattcgtgtgattggaaagccgcagaaattcgcttttcaa  180
 L  K  E  G  S  G  G  I  R  V  I  G  K  P  Q  K  F  A  F  Q
cgctcgttatccctggaaaatagccccccatcttcaccaactgcagcctcttcctcatcc  240
 R  S  L  S  L  E  N  S  P  P  S  S  P  T  A  A  S  S  S  S
gcttcctctactccacgagatcgggagaatgtatggagaagtgtgttcaatccggggagt  300
 A  S  S  T  P  R  D  R  E  N  V  W  R  S  V  F  N  P  G  S
aatatcaattccaagacaattgggtctgcaaaattcgacaaaccagaaccacagagccct  360
 N  I  N  S  K  T  I  G  S  A  K  F  D  K  P  E  P  Q  S  P
acggtgtatgactggctctacagtggagagactaaatccaaatggcgt              408
 T  V  Y  D  W  L  Y  S  G  E  T  K  S  K  W  R
```

Figure 36. Nucleotide and deduced amino acid sequences of SLP35. The deduced amino acid sequences are shown below the second nucleotide of each corresponding codon.

Figure 37. Alignment of amino acid sequences of SLP35 with auxin-repressed protein from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SLP35 used in the alignments were deduced from nucleotide sequences.

```
SLP37                   RCDQVGIHGLQCVSPCRWDSYENLYYVTDPAACNAFPEDLPQLRLVIEKLLVFLARTVEF 60
gi|2224892|squash       KPGAPLLAGFNKQKTNCVDKNEYVLVFPPGSKFNIYPQEPPQFKETLEEMFLKLSDVSLV 60
                        : .   : *::  ..   *. * :  ..  :  * :*:: **:: .:*:::: *: .  .

SLP37                   IESLISQSLGLPANFLKEFNGDGIEAFKVLCYPKARSQEEEVGARAHQDSSCITIVGQDG 120
gi|2224892|squash       IESILNVCLGLPPGFLKQFNNDRSWDFMTNLYYYPAADVGENGLIHHEDANCITLVIQDD 120
                        ***::. .****..***:**.*    * .  *  . ::  * *   *:*:.***:* **.

SLP37                   SGG 123
gi|2224892|squash       AGG 123
                        :**
```

Figure 38. Alignment of amino acid sequences of SLP37 with gibberellin 7-oxidase from squash. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SLP37 used in the alignments were deduced from nucleotide sequences.

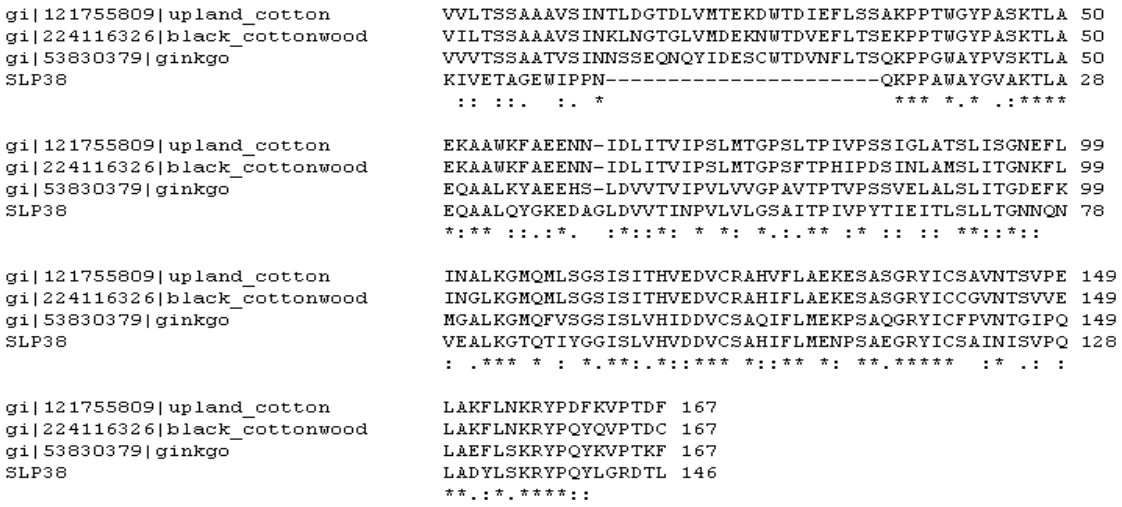

Figure 39. Alignment of amino acid sequences of SLP38 with anthocyanidin reductase from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SLP38 used in the alignments were deduced from nucleotide sequences.

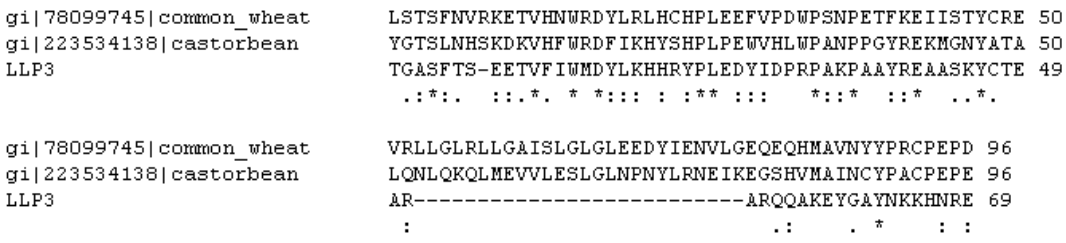

Figure 40. Alignment of amino acid sequences of LLP3 with flavanone 3-hydroxylase like proteins from two other species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of LLP3 used in the alignments were deduced from nucleotide sequences.

```
gi|22329737|thale_cress      TELFELGAVMLRRKFYPAANKFLQQAIQKWDGDDQDLAQVYNALGVSYVR 50
gi|78708787|rice             TEYFELGAVMLRRKFYPAAIKYLQQAIQKWDRDEQDLAQVYNALGVSYKR 50
SLP39                        VEYFELGAVMLRKKFYPLAAKYLEQAIAKWEGDVQDLAQVHNALGFSYAS 50
                             .* *********:**** * *:*:*** **: * ******:****.**

gi|22329737|thale_cress      EDKLDKGIAQFEMAVKLQPGYVTAWNNLGDAYEKKKELPLALNAFEEVLL 100
gi|78708787|rice             DNKLDKSIQQFEKAVELQPGYVTAWNNLGDAYEQKKDLKSALKAFEEVLL 100
SLP39                        DGKLDKGITHHEKAVELQPGYVTAWNNMGDAFEKKKDLKAALKAYNQALI 100
                             :.****.* :.* **:************:***:*:**:*  **:*:::.*:

gi|22329737|thale_cress      FDP 103
gi|78708787|rice             FDP 103
SLP39                        FDP 103
                             ***
```

Figure 41. Alignment of amino acid sequences of SLP39 with anthocyanidin reductase from two other species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SLP39 used in the alignments were deduced from nucleotide sequences.

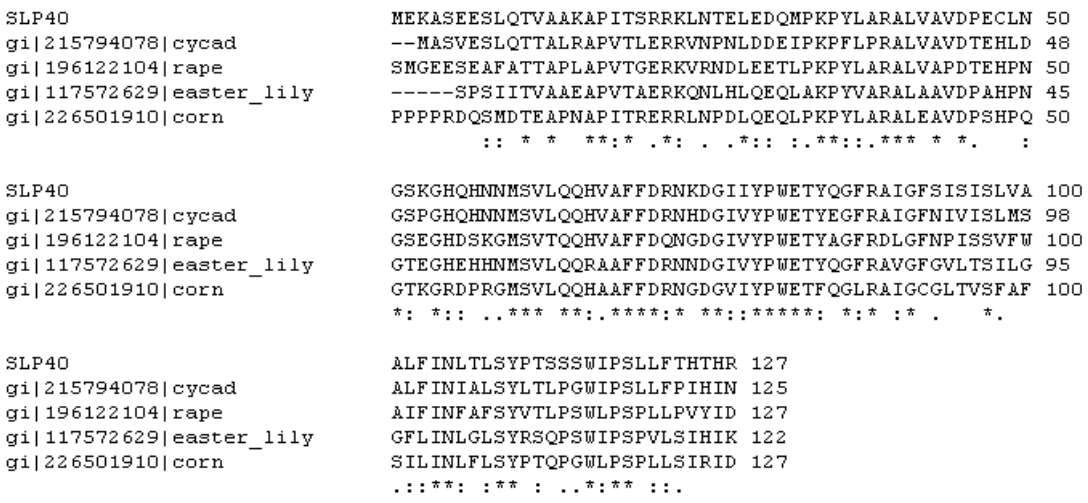

Figure 42. Alignment of amino acid sequences of SLP40 with caleosin from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SLP40 used in the alignments were deduced from nucleotide sequences.

```
gi|148905868|sitka_spruce      LFLQYTKGREFKETKLSIFFRILGLMIPGVSAHSPVNYINAVRLG 45
gi|38259660|castorbean         FILNHVYGAEYKETWESRMFRILGLFLPGVAAHSPVNYVNSVRLG 45
SLP41                          MVCTAKRGERFREGWFALCFRFMGILLPGMSAHSPVNYVNAIRLG 45
gi|115462157|rice              MFLWAKEGKDYREGPVSIVYRAAGLLFPGLASHSPRDYVNAIRLG 45
                               :.     *  ::*   :  :*  *:::**:::*** :*:*::***
```

Figure 43. Alignment of amino acid sequences of SLP41 with triacylglycerol lipase-like proteins from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SLP41 used in the alignments were deduced from nucleotide sequences.

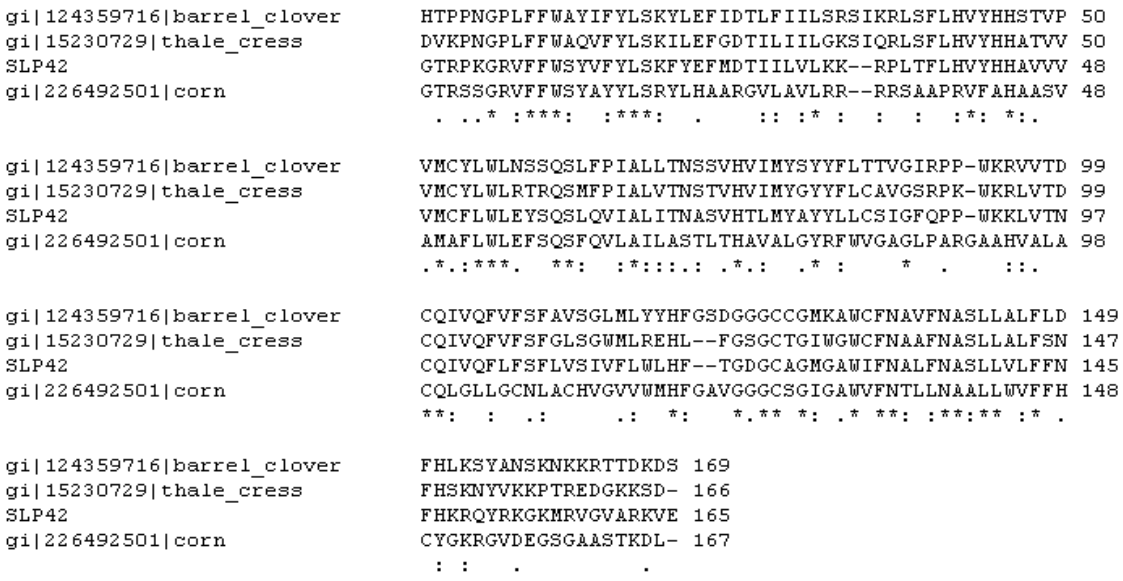

Figure 44. Alignment of amino acid sequences of SLP42 with GNS1/SUR4 from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SLP42 used in the alignments were deduced from a loblolly pine EST sequence (accession number: 48949032).

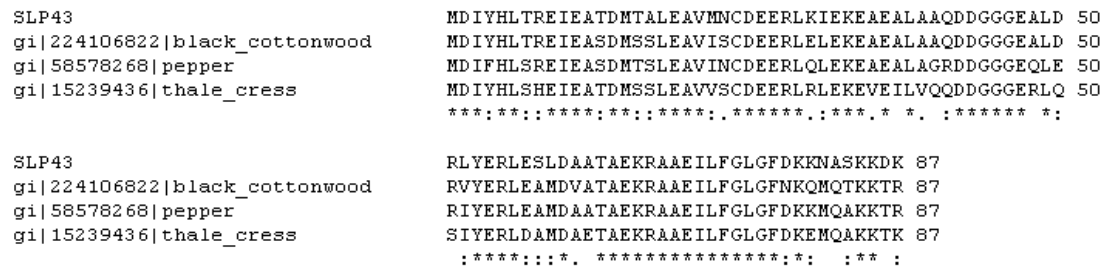

Figure 45. Alignment of amino acid sequences of SLP43 with ABC transporters from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SLP43 used in the alignments were deduced from nucleotide sequences.

```
gi|28273096|tobacco              LHGLTNDANAKVPLSVFWLVPQFLLVGAGEAFTYIGQLDFFLRECPKGMK 50
gi|224071551|black_cottonwood    LHGLANDPTAEIPLSVFWLVPQFFFVGSGEAFTYIGQLDFFLRECPKGMK 50
LLP6                             QHQLTNKENAIVPLSVYWLIPQFLLVGAGEAFAYVGQLEFFIKQAPVSMK 50
gi|21536775|thale_cress          ----NDKK-----ISAFWLVPQYFLVGAGEAFAYVGQLEFFIREAPERMK 41
                                      :.      :*.:**:**:::**:****:*:***:**:::.*  **

gi|28273096|tobacco              TMSTGLFLSTLSLGFFFSSILVTIVHKVTGKNP---WLADNLNQGRLYDF 97
gi|224071551|black_cottonwood    TMSTGLFLSTLSLGFFVSSSLVTIVHKVTINKP---WLADNLNQGRLHDF 97
LLP6                             SMSTGLFLSTLSLGFFWSTLLVTLVNGLTGHGGSPGWLPDNLNRGRLDYF 100
gi|21536775|thale_cress          SMSTGLFLSTISMGFFVSSLLVSLVDRVTDKS----WLRSNLNKARLNYF 87
                                 :**********:*:*** *: **::*. :* :     ** .***:.**  *

gi|28273096|tobacco              YWLLATLSVLNLMIFLFISRRYVYKEKRLAECGIEME 134
gi|224071551|black_cottonwood    YWLLAILSALNFVIYLICARWYVYKDKRLADEGIELD 134
LLP6                             YWLLTVLSFLNLLIFFVFAHFYKYTKESTGGQKESKS 137
gi|21536775|thale_cress          YWLLVVLGALNFLIFIVFAMKHQYKADVITVVVTDDD 124
                                 ****. *. **::*::. :  : *. .       . .
```

Figure 46. Alignment of amino acid sequences of LLP6 with nitrogen transporter-like proteins from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of LLP6 used in the alignments were deduced from a loblolly pine EST sequence (accession number: 67487349).

```
SLP47                           MSDILGRVIYCLRSSVDHGPIKNSETAMQRDSPVLAVELDIDELARSPKT 50
gi|15221421|thale_cress         MADIVGRAIYCLRTAVDHGPVSNSEFAMDEDSPILAVELDVDELAKGHK- 49
gi|38344766|rice                MTDILGRVIYSLRTAVDHGPVENSRMAMNQDSPVLAVELDVEEMAKNNK- 49
gi|212721620|corn               MTDILGRVIYSLRTAVDHGLVENSGMATKLDGPVLAVELDVEELAKNNK- 49
gi|224066245|black_cottonwood   MSNIVGRVIYCLQTAVDHGPVQNSHFSMRKDSPVLEVELDVEEMAKNHK- 49
                                *::*:**.**.*:::**** :.**  :   *.*:* ****::*:*:. *
```

Figure 47. Alignment of amino acid sequences of SLP47 with serine-type peptidase/ signal peptidase from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SLP47 used in the alignments were deduced from nucleotide sequences.

```
gi|162460343|corn          DHCGTTPMDVNHAVLAVGYGVED-GVPYWLIKNSWGADWGDEGYFKMEMG 49
gi|77379397|rape           SHCGQTPMDVNHAVLAVGYGIED-GVPYWLIKNSWGADWGDKGYFKMEMG 49
gi|211953197|sunflower     GDCGSGPMDVNHAVVAVGYGVED-GVPYWLIKDSWGADWGLNGYFKMEMG 49
gi|53748483|plan           TTCGNSPMDVNHAVLAVGYGVEN-GIPYWLVKNSWGADWGDNGYFKMEMG 49
gi|8347420|tobacco         TECGNTPMDVNHAVLAVGYGVEN-GVPYWLIKNSWGADWGDNGYFKMEMG 49
SLP48                      TTCGQGPMDVNHAVLAVGYGVSDEGTPHWIIKNSWGKSWGVDGYFKMELG 50
                             **  ********:*****:.: * *:*::*:*** .** .******:*

gi|162460343|corn          KNMCGVATCAS 60
gi|77379397|rape           KNMCGIATCAS 60
gi|211953197|sunflower     KNMCGVATCAS 60
gi|53748483|plan           KNMCGVATCAS 60
gi|8347420|tobacco         KNMCGIATCAS 60
SLP48                      KNMCGVATCAS 61
                           *****:*****
```

Figure 48. Alignment of amino acid sequences of SLP48 with aleurain-like protease from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SLP48 used in the alignments were deduced from nucleotide sequences.

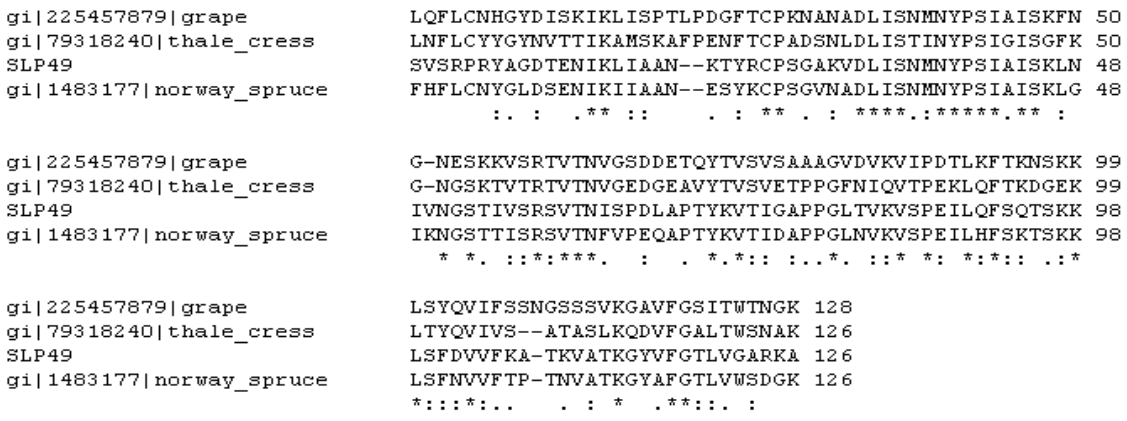

Figure 49. Alignment of amino acid sequences of SLP49 with subtilisin-like protein from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SLP49 used in the alignments were deduced from nucleotide sequences.

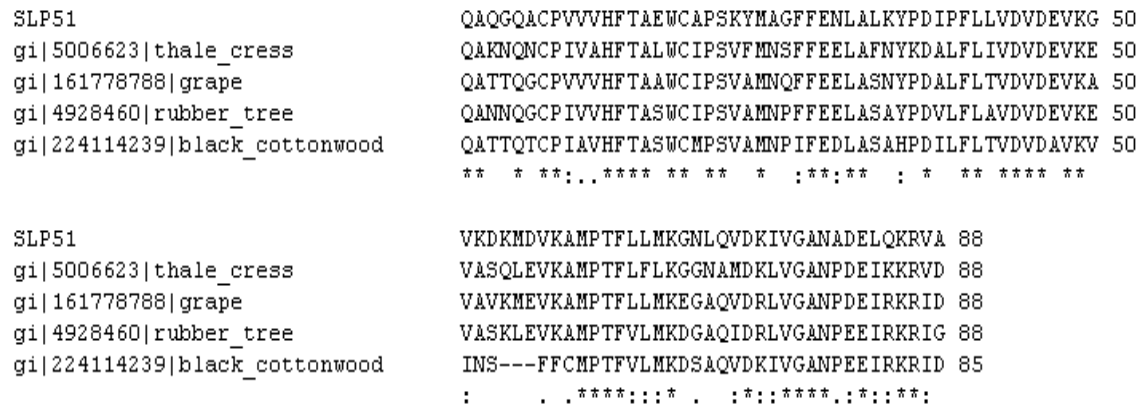

Figure 50. Alignment of amino acid sequences of SLP51 with thioredoxin *h* from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SLP51 used in the alignments were deduced from nucleotide sequences.

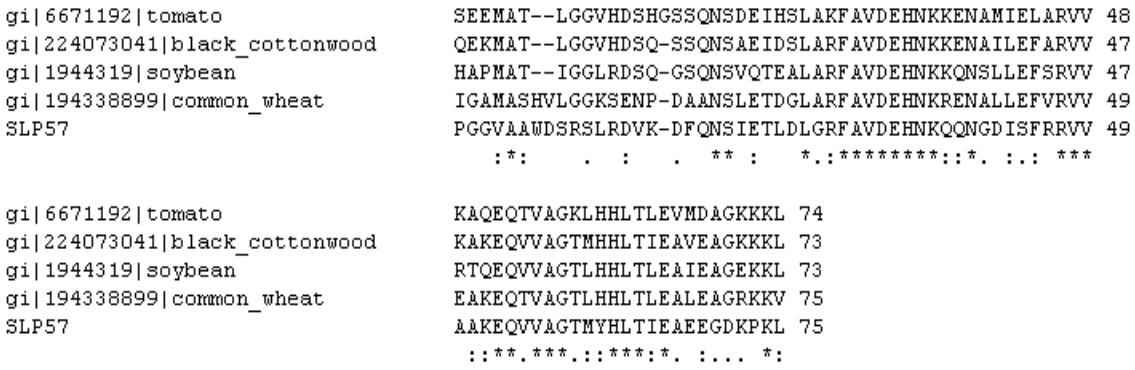

Figure 51. Alignment of amino acid sequences of SLP57 with cystatin-like proteins from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SLP57 used in the alignments were deduced from nucleotide sequences.

Figure 52. Alignment of amino acid sequences of LLP11 and LLP12 with peroxidase-like proteins from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of LLP11 and LLP12 used in the alignments were deduced from one loblolly pine EST (accession number: 67962276), due to LLP11 and LLP12's close to 100% similarity with this EST.

```
gi|167367|upland_cotton      FAAMGIDTPGVVALLGAHSVGRTHCVKLVHRLYPEVDPALSPDHVPHMLH 50
gi|5002234|soybean           FGAMGIDTPGVVALLGAHSVGRTHCVKLVHRLYPEIDPALNPDHVPHILK 50
gi|1403136|thale_cress       FKSIGIDTPGLVALLGSHSVGRTHCVKLVHRLYPEVDPSLNPDHVPHMLH 50
SLP60                        FKAMGIDTRGVVALLGAHSVGRTHCVKLVHRLYPEVDPTLDPGHVEHMKH 50
                             * ::**** *:*****:******************:**:*.*.** *: :

gi|167367|upland_cotton      KCPDQIPDPKAVQYVRNDRGTPMVLDNNYYRNILDNKGLLIVDHQLAYDK 100
gi|5002234|soybean           KCPDAIPDPKAVQYVRNDRGTPMILDNNYYRNILDSKGLLIVDHQLANDK 100
gi|1403136|thale_cress       KCPDSIPDPKAVQYVRNDRGTPMVLDNNYYRNILDNKGLLLVDHQLAHDK 100
SLP60                        KCPDAIPNPKAVQYVRNDRGTPMKLDNNYYVNLMNNKGLLIVDQQLYADS 100
                             **** **:*************** ****** *:::.****:**:**  *.

gi|167367|upland_cotton      RTRPYVKKMAKSQDYFFKEFSRAITLLSENNPLTGSKGEIRKQCNLANKL 150
gi|5002234|soybean           RTKPYVKKMAKSQDYFFKEFSRAITLLSENNPLTGTKGEVRKQCNVANKH 150
gi|1403136|thale_cress       RTRPIVKKMAKDQAYFFKEFTRAIQILSENNPLTGSKGEIRKQCNLANKN 150
SLP60                        RTRPYVKKMAKSQEYFFKYFSRALTILSENNPLTGARGEIRRQCSLKNKL 150
                             **:* ******.* **** *:**: :**********::**:*:**.: **

gi|167367|upland_cotton      H----- 151
gi|5002234|soybean           HDQDP- 155
gi|1403136|thale_cress       H----- 151
SLP60                        HTKSKR 156
                             *
```

Figure 53. Alignment of amino acid sequences of SLP60 with secretory peroxidase-like proteins from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SLP60 used in the alignments were deduced from a loblolly pine EST (accession number: 66976703).

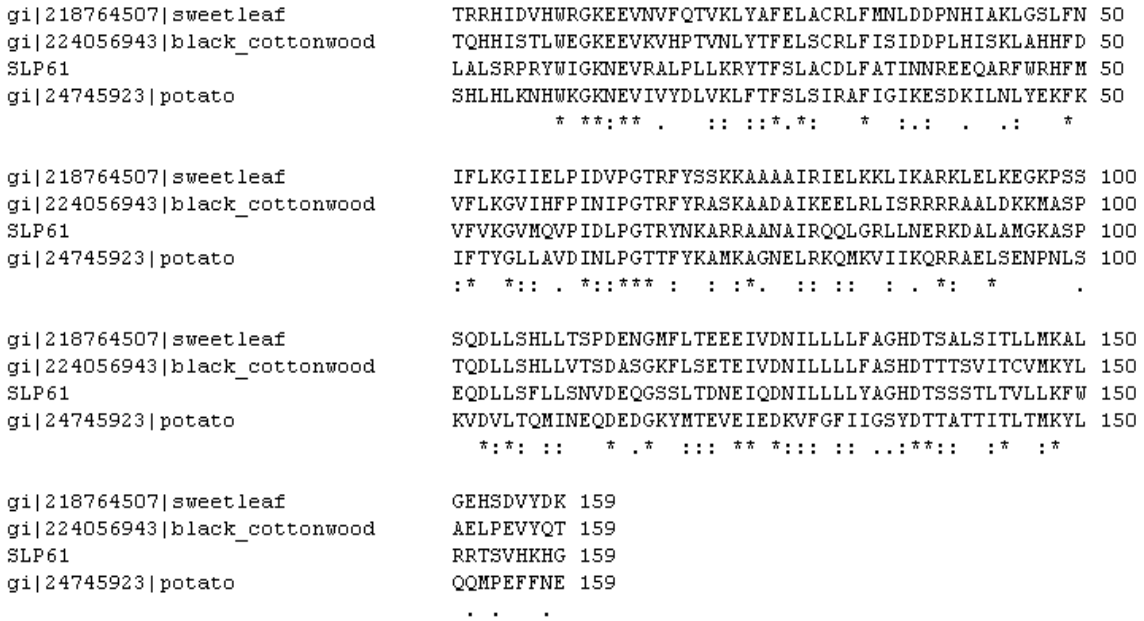

Figure 54. Alignment of amino acid sequences of SLP61 with cytochrome P450 from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SLP61 used in the alignments were deduced from nucleotide sequences.

```
gi|224069573|black_cottonwood      VPIEATMGELKKLVEEGKIKYIGLSEASASTIRRAHAVHPITAVQLEWSL 50
gi|223542204|castorbean            IPIEITMGELKKLVEEGKIKYIGLSEASASTIRRAHAVHPITAIQLEWSL 50
gi|225433674|grape                 VPIEVTIGELKKLVEEGKIKYIGLSEASASTIRRAHAVHPITAVQLEWSL 50
gi|30696459|thale_cress            VPIEITIGELKKLVEEGKIKYIGLSEASASTIRRAHAVHPITALQIEWSL 50
SLP62                              LALGDAFGIQGLEVSALGLGCVGMSDFYG-PPKPEQEMISLIHYAVSRGV 49
                                   :.:  ::*     *.   :  :*:*:  . . :  : : .:    :. .:

gi|224069573|black_cottonwood      WSRDVEEEIVPTCRELGIGIVVYSPLGRGFFSTGPKLVESFSEGDYRKDM 100
gi|223542204|castorbean            WSKDIEEEIVPTCRELGIGIVAYSPLGQGFLSLGTKLVETFKEGDVRKYL 100
gi|225433674|grape                 WTRDVEEEIVPTCRELGIGIVAYSPLGRGFFSSGTKLIENLSNNDFRKNL 100
gi|30696459|thale_cress            WSRDVEEDIIPTCRELGIGIVAYSPLGRGFFASGPKLVENLDNNDVRKTL 100
SLP62                              TFLDTSDIYGPFTNEILIGKAIKG------IREKVQLATKFG-------I 86
                                      * .:   *  .*: ** .  .      :    :*  .:        :

gi|224069573|black_cottonwood      SRFRPENLDHNRQLFERVNEIAARKQCTSSQLALAWLHHQGDDVCPIPGT 150
gi|223542204|castorbean            PKFQPENVEHNKHLFERVNKMAARKQCTPSQLALAWVHHQGDDVCPIPGT 150
gi|225433674|grape                 PRFQPENLGHNKILYERVSEIATRKGCTPSQLALAWVHHQGDDVCPIPGT 150
gi|30696459|thale_cress            PRFQQENLDHNKILFEKVSAMSEKKGCTPAQLALAWVHHQGDDVCPIPGT 150
SLP62                              AYVDGKPEARGDPAYVRAACEASLQRLEVDFIDLYYQHRIDTKVP----- 131
                                   . .  :   :.   : :.   :  :      : * : *: . .*

gi|224069573|black_cottonwood      TKIENFNQNVGALSVRLTLEEMAELE 176
gi|223542204|castorbean            TKIENFNQNIGTLSVKLTPEEMAELE 176
gi|225433674|grape                 TKIENLKQNIGALSVKLTPEEMAELE 176
gi|30696459|thale_cress            TKIENLNQNIGALSVKLTPEEMSELE 176
SLP62                              --------------IEVTIGELKKLV 143
                                             :.:*  *: :*
```

Figure 55. Alignment of amino acid sequences of SLP62 with aldo/keto reductase-like proteins from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SLP62 used in the alignments were deduced from nucleotide sequences.

```
gi|224135577|black_cottonwood      VPKEVVLYQYEACPFCNKVKAYLDYYDIPYKVVEVNPISKKEIKWSD--Y 48
gi|15238304|thale_cress            NPKEVVLYQYEACPFCNKVKAFLDYNKIPYKVVEVNPISKKEIKWSD--Y 48
SLP63                              IPKDLVLYQYEACPFCNKVKAYLDYHHLPYKMVEVNPISKKEIKWSD--Y 48
gi|115457404|rice                  LPQNVVLYQYQACPFCNKVRAFLDYHDIPYKVVEVNPLSKKEIKWSE--Y 48
gi|116061708|green_alga            GGQRVTLYQYDVCPFCNKVKAFLDYHRVPYDVVEVNPLTKGELGWVEDGY 50
                                     : :.****:.*******:*:***  :**.:*****::* *: * :  *

gi|224135577|black_cottonwood      KKVPILLVDGEQLVDSSAIIDKLGNKIH 76
gi|15238304|thale_cress            KKVPILTVDGEQMVDSSVIIDSLFQKMH 76
SLP63                              KKVPILMVDGKQLNDSSAIINQLDSQIH 76
gi|115457404|rice                  KKVPILMVDGEQLVDSSDIINILQQRVR 76
gi|116061708|green_alga            KKVPIVTVGDEKLNDSKHIIAELTKRFD 78
                                   *****: *..::: **. **  * .:.
```

Figure 56. Alignment of amino acid sequences of SLP63 with glutathione S-transferase from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SLP63 used in the alignments were deduced from a loblolly pine EST sequence (accession number: 34490708).

```
SLP_LLP4                    AGREFPEVSKAGRGGGNNKGTVFWMRDPATGNWIPEDHFGETDTAALRQK 50
gi|52788395|aleppo_pine     AGREFPEVSKPGRGGGNNKGTVFWMRDPATGNWIPEDHFGETDTAELRQK 50
gi|1350528|white_spruce     AAREFPEISKAGGGGGNNKRTVFWMRDPATGNWIPEDHFGETDTAELRQK 50
gi|2981167|tobacco          GVRGSGVNIMMKKWEESSKKTTSWVPDPVTGYYRPESHAKEIDAAELRQM 50
                            . *             ..* *. *: **.** : **.*  * *:* ***

SLP_LLP4                    LLSSRK 56
gi|52788395|aleppo_pine     LLSSRK 56
gi|1350528|white_spruce     LLSSRK 56
gi|2981167|tobacco          LLNHKP 56
                            **. :
```

Figure 57. Alignment of amino acid sequences of SLP_LLP4 with LEA from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SLP_LLP4 used in the alignments were deduced from nucleotide sequence.

```
gi|5608497|ajuga              SKPWRYTGQEANMDREDIKMLVKKWWDVYNDESLDFKAEDSIAGEET--F 48
gi|146230136|showy_mullein    SKPWRYTGVEANMDREDIKMLVKKWWDVYDDESLDFKANETIVEDET--F 48
gi|15219093|thale_cress       SKPWRYTGEEANMDREDIKMLVDKWWDVYNDESLDFKSKIPADAEET--V 48
gi|212004612|rape             SKPWRYTGKEANMEREDIKMLVNKWWDIYNDDSLDYKKSVGDLVEESDVV 50
LLP13                         RRPGRYAGKEENMQREDIKVLVKKWWDIYNDESLDYKAEEHSIPEAETLS 50
                               :* **:* * **:*****:**.****:*:*:***:* .     :

gi|5608497|ajuga              SMPSFIAS---LPEP-AVSYIPAPSAA 71
gi|146230136|showy_mullein    SRPSIMAA---MPEP-AISYIPAPSAA 71
gi|15219093|thale_cress       TKSSILAS---VLEP-EMTYFPAPSAA 71
gi|212004612|rape             NLKPFISA---LTEAGPVKYVTAPSAA 74
LLP13                         NLQQITANSLLATIPTAAGFIPAPSAA 77
                              .   : :       .    :..*****
```

Figure 58. Alignment of amino acid sequences of LLP13 with galactinol synthase from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of LLP13 used in the alignments were deduced from nucleotide sequence.

```
SLP36                       EDQTSEYEKALKEEKHHKRMEHVGELGTVAAGGYALYEKHEAKKDPENAR 50
gi|56481789|douglasfir      QTQDDEYEKARKEEKHHKRMEHVGELGTVAAGAYALYEKHEAKKDPEHAG 50
gi|38532363|ginkgo          DSSQDDYEKAMKEEKHHKRMEHVGELGTMAAGAYAMYEKHEAKKDPEHAH 50
gi|1401234|loblolly_pine    NVNSDEYEKARKEEKHHKHMEEVGGLGXMATGAFALHEKHAEKKDPEHAH 50
                            : . .:**** *******:**.** ** :*:*.:*::***  *****:*

SLP36                       RHKIEEEIAAAAAGKW 66
gi|56481789|douglasfir      RHKIEEEIAAAAAVGS 66
gi|38532363|ginkgo          RHKIEEEVAAAAAVGA 66
gi|1401234|loblolly_pine    RHKIEEEIAAAAAVGE 66
                            *******:*****
```

Figure 59. Alignment of amino acid sequences of SLP36 with water deficit inducible proteins from several other tree species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SLP36 used in the alignments were deduced from nucleotide sequence.

```
gi|62701653|rice            ANLVTPAFFTPPSSSSTSMVPPASSMMPTAPPLHPTSASAQR-------- 42
gi|18390886|thale_cress     TDLVTPSFFGPPRMMAQPHLIPGVS-MPTAPPLNPNNASHQQ-------- 41
SLP70                       PPLVSPALMAPP-----------ISSTLTAPPVQP--PSQLQ-------- 29
gi|195622276|corn           TSLVYPSLFSPLTS-SQTKMVHTNSAVPTAPPQHPRIAQQPQSAPLLQPF 49
                            . ** *::: *             *   **** :*  ..  :

gi|62701653|rice            -------ATYGTPLLQPFPPPTPPPSLTPSYNE--GPIISRDKVKEALLR 83
gi|18390886|thale_cress     -------RSYGTPVLQPFPPPTPPPSLAPAPT---GPVISRDKVKEALLS 81
SLP70                       -------PSHGSPLLQPFPPPTPPPSLAPASSMSHGPIITRDGIRDALVK 72
gi|195622276|corn           PLPTASSPPYGTPLLQPFPPPNPSPSLASAPVL--SPALTRDKVRDALLR 97
                                    .:*:*:*******.*.***:.:     .* ::** :::**:

gi|62701653|rice            LVQNDQFIDLVYRELQNAHM- 103
gi|18390886|thale_cress     LLQEDEFIDKITRTLQNALQQ 102
SLP70                       LIQNEHFIDMVYREMMNAHLS 93
gi|195622276|corn           LVENDEFVDLVYREIMNRQ-- 116
                            *:::::*:* : * : *
```

Figure 60. Alignment of amino acid sequences of SLP70 with DCP1-like decapping proteins from diverse species. Residues identical in all compared proteins are marked by asterisks, and residues showing similarity are denoted by colons and periods, with colons meaning higher similarity. The GenBank accession numbers assigned to the sequences analyzed are showing at the left side along with species name. The amino acid sequences of SLP70 used in the alignments were deduced from nucleotide sequence.

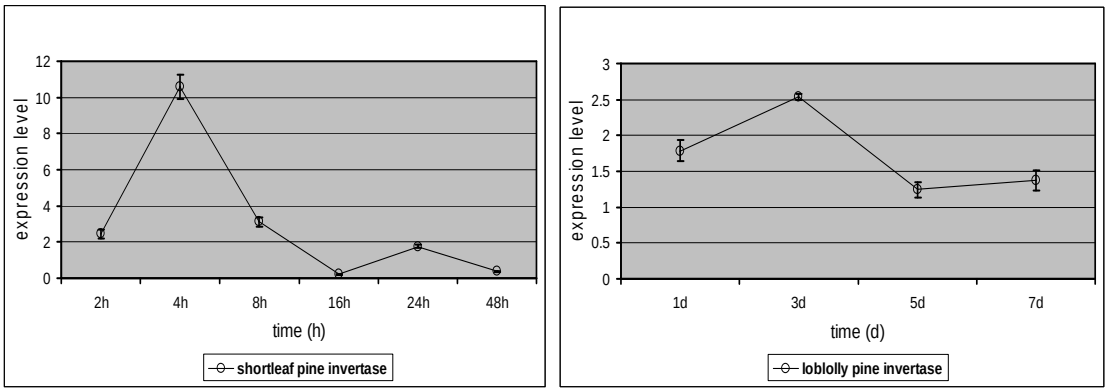

Figure 61. qRT-PCR results for an invertase-like gene (SLP21) in shortleaf pine and loblolly pine. The relative transcription levels in shortleaf pine and loblolly pine were measured using actin as an internal control. Tissues used on shortleaf pine were collected at two, four, eight, 16, 24, and 48 hours after top-killing. Tissues used on loblolly pine were collected at one, three, five, and seven days after top-killing. Expression level units are values linearized with the $2(^{-\Delta\Delta C_T})$ method, where C_T is the threshold cycle.

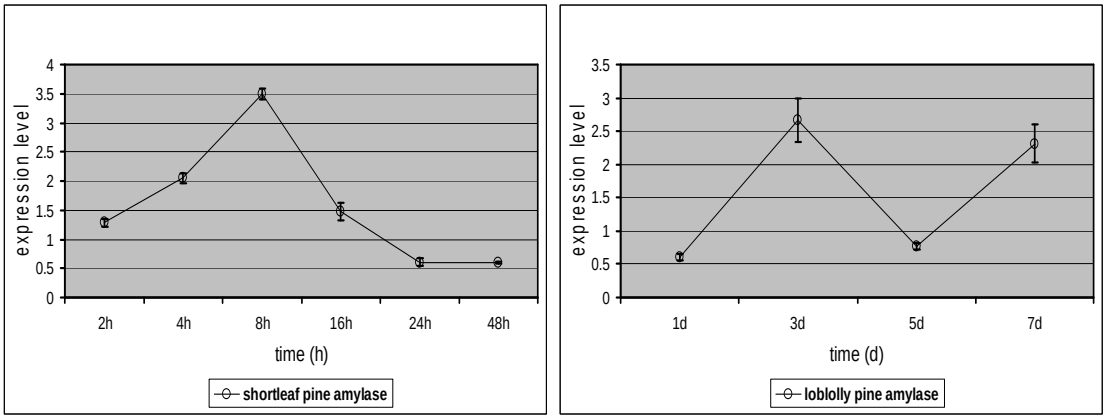

Figure 62. qRT-PCR results for an amylase-like gene (SLP22) in shortleaf pine and loblolly pine. The relative transcription levels in shortleaf pine and loblolly pine were measured using actin as an internal control. Tissues used on shortleaf pine were collected at two, four, eight, 16, 24, and 48 hours after top-killing. Tissues used on loblolly pine were collected at one, three, five, and seven days after top-killing. Expression level units are values linearized with the $2(^{-\Delta\Delta C_T})$ method, where C_T is the threshold cycle.

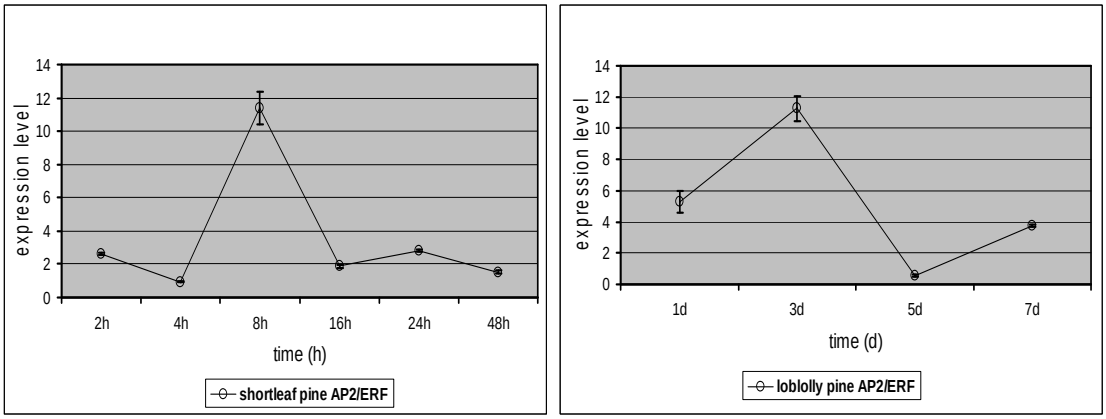

Figure 63. qRT-PCR results for an AP2/ERF transcription factor-like gene (SLP34) in shortleaf pine and loblolly pine. The relative transcription levels in shortleaf pine and loblolly pine were measured using actin as an internal control. Tissues used on shortleaf pine were collected at two, four, eight, 16, 24, and 48 hours after top-killing. Tissues used on loblolly pine were collected at one, three, five, and seven days after top-killing. Expression level units are values linearized with the $2(^{-\Delta\Delta C_T})$ method, where C_T is the threshold cycle.

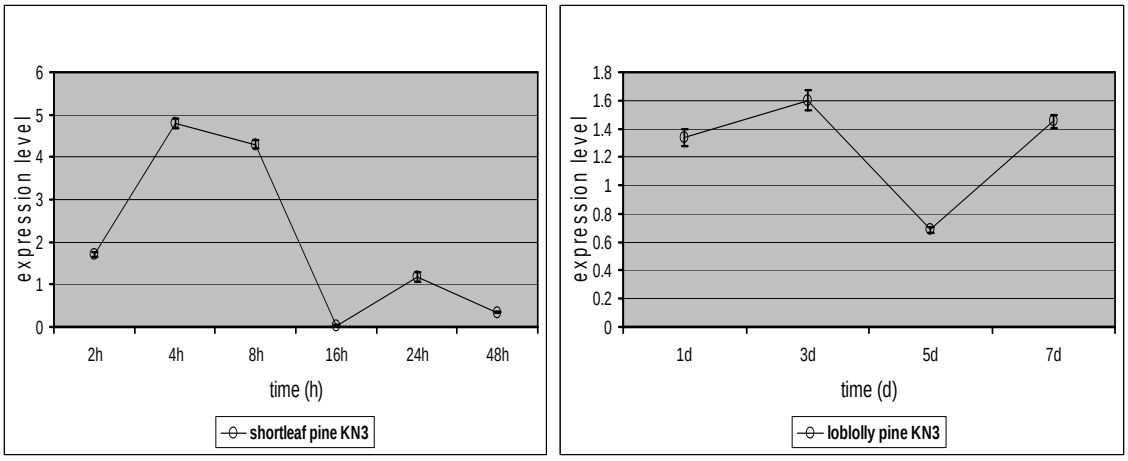

Figure 64. qRT-PCR results for a KN3-like gene (SLP2) in shortleaf pine and loblolly pine. The relative transcription levels in shortleaf pine and loblolly pine were measured using actin as an internal control. Tissues used on shortleaf pine were collected at two, four, eight, 16, 24, and 48 hours after top-killing. Tissues used on loblolly pine were collected at one, three, five, and seven days after top-killing. Expression level units are values linearized with the $2(^{-\Delta\Delta C_T})$ method, where C_T is the threshold cycle.

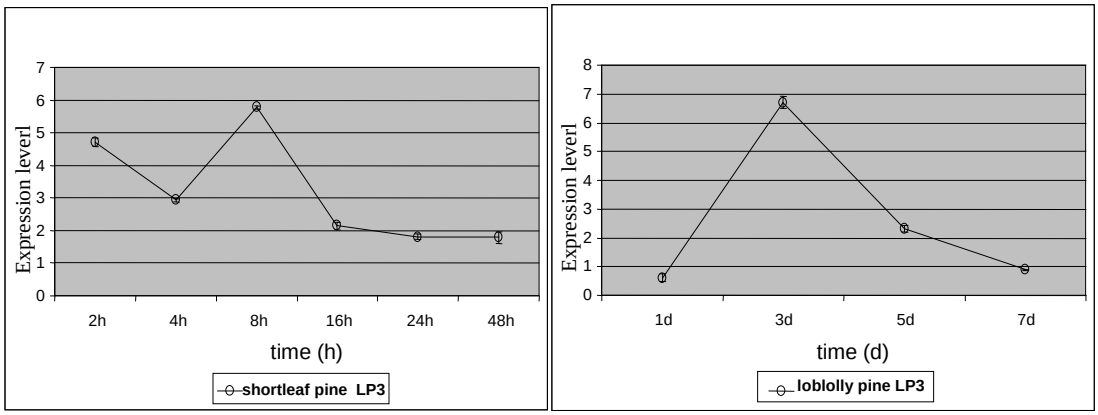

Figure 65. qRT-PCR results for a water deficit inducible protein (SLP36) in shortleaf pine and loblolly pine. The relative transcription levels in shortleaf pine and loblolly pine were measured using actin as an internal control. Tissues used on shortleaf pine were collected at two, four, eight, 16, 24, and 48 hours after top-killing. Tissues used on loblolly pine were collected at one, three, five, and seven days after top-killing. Expression level units are values linearized with the $2(^{-\Delta\Delta C_T})$ method, where C_T is the threshold cycle.

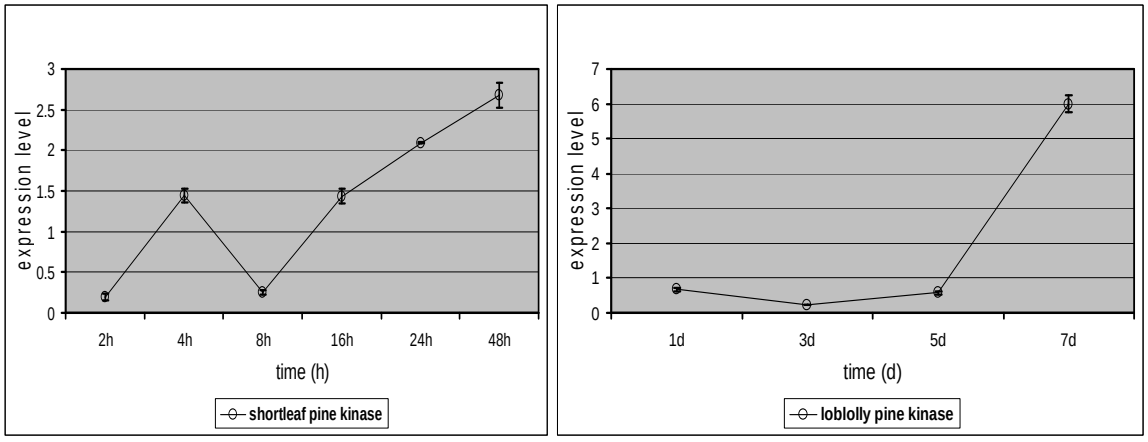

Figure 66. qRT-PCR results for a receptor-like kinase (SLP_LLP1) in shortleaf pine and loblolly pine. The relative transcription levels in shortleaf pine and loblolly pine were measured using actin as an internal control. Tissues used on shortleaf pine were collected at two, four, eight, 16, 24, and 48 hours after top-killing. Tissues used on loblolly pine were collected at one, three, five, and seven days after top-killing. Expression level units are values linearized with the $2(^{-\Delta\Delta C_T})$ method, where C_T is the threshold cycle.

APPENDIX I

cDNA sequences

>SLP1_GO479090
CACTTAGATCGTGCAGATACAGTATTCACCCAATCTCCCCCTCACAGACAAAT
TACAATTCAACAGAGTTAATCTCAGGATTGCATGATGATTTCAGTCGTTCCAA
GGCATCATCATCTTCAGAACCCATCTGGGAGAAAGAAGCTGAGAGCAGCCCC
AGAACGGAAAATCCCTCGCAGAAGCAGCAACAATCATTATTTAATATGGATC
TGGAAGGTCTACAAAGTTCCTTCCCTCATCTAGACCAAATATCTTTTAGCGAT
GCTTATCAAGACTGGCTTTTACTCTAGCGAGCCATGACCCAGTGGAAGGTCTA
TTGACCTCTGGGTTTTTTAATTGTTGAAAGACTACCTCTAATTCGATTGAATTT
ATGGACTTAC

>SLP2_GO479091
TCTTCATATCTGCCGTTTGAAAAGGCCATTTTAGAGATCAGCTATACATATAA
AAGATGAGTCAGACAAATCTACAGCATTGCACTAAAACTGGTGTTTGCCTCA
AAAATGGGTTTAACCTCAACAATCCATTGAAAAGGCAGTTCCATCTGTCCTG
AGATGTCCCTCCAGGAAAAAAGCAGCGTTGTGAGGATTAGGACTATCCATAA
CCACGAACTGCATCTCTTCAGATGGTTTCCAATGTCGCTTGCGCTGATTTATA
AACCAGTTATTTATTTGCTTCTGATCCAGCCCGGTAGATTCTGCCAATGCTAT
CTTTTGACTTTCCGAAGGATATGGCCACTTATAGTTTCTGGTCCACCAATCCA
ACAACTTTTGCCTTGCTTCCTTGGGGAGTTTGCCTTTCCTTTTCTTCTTAAGAA
ATTCTTGCTTGAGACTGCTCAAGTACCTCGGCCGCGACACGCTA

>SLP3_GO479092
CCTATGCAGCTTATTACAGTTTGACTGTGCTCTCTAGTTTTTCAGCTGTGTAAT
TTTTACTAGTTTACAGCTTCACTGCAGTATGAAAGCTTGCACTAGAATGAGAG
GATTTAGCAAATAATCCTAAATGTTGCAAGTGCGGCATCCGAGGCACCTATT
ATTTCCCTTAAGATTCCAATGCCTTACGCAGTATATAAACTGCAGAATGAAAA
AGCAGTCCAATCACTCTTCTCAACTTCTCTCTTCCCAAAACTAATCATTTTACT
GTATGACATGGTTTCTCATAAATATATATATGGGCCCTTTCTGCCTGGGAAAT
GACAGAAGATGAGATAATGGCTACATCTCAAATCAGAAACATCGCATTCAAA
TCCTTGTAAATGCTTTGCTGGTTGCAATCCTATAATAATACACTAGAACCTAC
ATGGAAAGAAATGCTCTTCCATTCAGTCTGCAGGTAACATAGGCATGCGGTT
GCTGT

>SLP4_GO479093
CCTGGGACGGACTTGGATTCATCCGCAGCATTGAGCACAGCTCTGTTCACAG
CGGGCTAAGCTGTGGAGCCTGCTTTGAGATCAATGTGTGAATGACCCAGAAT
GGTGTCACCCGGGTAACCCTTCGATTTTGGTCACGGCCACCAATTTCTGCCCT
CCCAACTATGCTCTGCCTAACGACAATGGCGGCTGGTGCAATCCTCCTCGCCC
CCATTTTGATCTCTCCATGCCAATTTTCCTCAAGATGGCCGTGTACCTCGGCC
GCGCCACGCT

>SLP5_GO479094
CCGATAGCTGGGGTATTCGCCTGCGGGCTGGCTGGAATGGGATGGAGCTTTT
GCTTTGAAAACTTTGTATTATGGAGAGTATATGAATACAGGGCCAGGCTCTG
CTACTGGAAACCGTGTGAAATGGCCTGGCTATCGGGTGATCAAAAGTTCTCA
AGAGGCGAGCAAATTTACAGTGGGAGAATTCATACAAGGAAATTCCTGGTTG
CAGTCCACTGACATCGACTACATCGACGGATTGACAAATTAGCGACGTCGCC
AAACTTTTGAAGAAGTTCGTCTGTGTCTGTAGTTCGCTTTCAACTTTTGAAGA
AGATTGTCTGTGTCTGTAGTGTCTGTATTTCGCTTTCTATCGTTTTTGTAGTGT
ATCCGTAGTTCGCTTTCAACTTTTGAAGGAGATCGTTTGTGTCTGTAGTGTCT
GTATTTCGCTTTCTATCGTTTTTGTAGTGTATCCATAGAACGTTCTATGGAGTA
ATGATAAAATAAAATCATATTGAACTTT

>SLP6_GO479095
CTAGGGTGTCTATGGCTCCTGCAGCTTCATCCAATTAAAGAAGGCTGGAACA
ATACTCCAGGGCATCTAAAACGTTCAGCACCAATGGTGATCACCTGCCCATC
AGGGAGCTCGTAACTTTTGTCAATGGACGAGCTGCTTTTAGCTGTCTCAAGCT
CCTGTTCATAGTCAAGAGCCACATATGCAAGCTTTTCCTTCACATCACGGACA
ATTTCACGCTCTGCAGTGGTGGTGAATGAATACCCTCTTTCTGTCAAGATCTT
CATTAATGCATCTGTCAAATCTCTCCCTGCAAGATCCAACCTAAGTATAGCAT
GAGGCAGGGCATATCCTTCATAAATTGGCACTGTGTGACTAACACCATCCCC
AGAATCCAGTACCTCGGCCGCGACACGCATA

>SLP7_GO479096
CTTTATATTCAGCCTGGCATGCTTAAAAGTATGTTTATGCCCGTTGGTAATCG
TGCTTTCGCTCATGTAAAGACTTTGCAAGT

>SLP8_GO479097
CCATGGGGCGTCTCTGGCTCGGCAGTGGCTTGGCAGTGGCTCTGGTTCGGCTA
CGGAAGTGGTTCTGGTAGCGGCAGTGGCTACGGAAGCGGCTCCGGTGCTGGC
TCGGGCTACGGTTCTGGCCCTGGTTCCGGTTACGGCGCTGGAAATGGTGGCA
GTAATGGAGGAGCTCCTGGTGGTGGATACTAAATTCAAAGAGGAAGGGAAA
CAGAAAAAACCTTCTAGCTTTGCCAAAATAGTTGCGTCGTTTGCATTATAATA
TGGTTTGCATCGTAATATAATAATGCGTACCTCGGCCGCGCCACGC

>SLP9_GO479098
CAAATGCTGGCCCTTATTACCCTCCCGAATCGAACCCGCAACCTCATCGGCTA
AGGAGAAGCGCGAATAATCTGGTCCTAAATCGACCAACCGCACATCTCTTAT
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VITA

Yanyan Liu

Candidate for the Degree of

Doctor of Philosophy

Dissertation: GENE EXPRESSION DIFFERENCES BETWEEN SHORTLEAF PINE AND LOBLOLLY PINE AFTER TOP-KILLING

Major Field: Plant Science, Cell and Molecular

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Experience: Graduate Research Assistant, Department of Natural Resource Ecology and Management, Oklahoma State University, August 2005 to May 2009. Graduate Research Assistant, Department of Agronomy and Biotechnology, Beijing, China, September 2002 to July 2005.

Professional Memberships: The American Association for the Advancement of Science.

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Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: GENE EXPRESSION DIFFERENCES BETWEEN SHORTLEAF PINE AND LOBLOLLY PINE AFTER TOP-KILLING

Pages in Study: 223 Candidate for the Degree of Doctor of Philosophy

Major Field: Plant Science, Cell and Molecular

Scope and Method of Study: Shortleaf pine sprouts prolifically after disturbance such as fire. Much attention has been paid to its restoration due to its increasingly declining population. Shortleaf pine's strong sprouting ability has huge potential in promoting its regeneration. However, little is known about its sprouting mechanism at the molecular level. A microarray experiment was designed to study genes responsible for this sprouting ability.

Findings and Conclusions: In this study, one year old shortleaf pine and loblolly pine seedlings are top-killed, and tissues collected just before sprouting were used. As in the natural environment, shortleaf pine showed extraordinary strong sprouting ability and large amounts of sprouts were seen two days after top-killing. However, on loblolly pine only a few sprouts were seen one week after top-killing. By microarray gene profiling with about 2400 cDNA clones obtained from suppression subtractive hybridization, 139 differentially expressed genes were found to be associated with sprouting, including genes functioning in reserve (carbohydrates and fatty acid) mobilization, transcriptional regulation, stress response, plant development, signal transduction and hormone regulation. 130 differentially expressed genes were found to be responsible for the dormancy release of axillary buds of shortleaf pine after top-killing. In contrast, only 32 differentially expressed genes were detected for loblolly pine. Shortleaf pine responds actively to top-killing at the molecular level. As reported for dormancy release of buds of other perennial plants, oxidative stress might be the major factor in dormancy release of axillary buds of shortleaf pine. It is apparent that cross talking between plant hormones (especially gibberellins, ethylene and auxins), carbohydrates, and other players of signal transduction work cooperatively to promote sprouting of shortleaf pine after top-killing.

ADVISER'S APPROVAL: Charles G. Tauer