A Review On Defence Responses And Resistance Breeding In Forest Tree Species

Progya Dasgupta

M.Sc. Forestry, Forest Research Institute, Dehradun,
Uttarakhand

Abstract: Forest trees play an important role not only for mitigating the effects of climate change but also for their considerable economic and ecological value and maintenance of proper ecosystem stability. They are a vital source of alternative bioenergy source and play important roles in pollution abatement and maintenance of biodiversity. Timber and its associated products obtained from forest trees contribute to the revenue generation of many countries of the world. They also serve as a natural habitat to numerous organisms. Thus, it is essential that the proper growth and health of forest trees should be monitored and maintained regularly. However, forest trees are susceptible to major threats due to pests and diseases. Diseases can be either due to infestations of insects or various fungal and bacterial pathogens. Trees have certain innate resistance mechanisms to resist insects and pathogens. But in certain cases, these mechanisms are breached and successful infestation of pests and pathogens occur. In such cases, defence mechanisms are induced in trees, which can be either by gene manipulation, production of transgenic tree species or by inducing other physical and chemical barriers. The induced defence mechanisms are generally based on modifications of techniques applied for resistance breeding in agricultural crops. This paper is a review of the various types of insect pests and fungal pathogens that infect forest tree species, and the innate and induced defence mechanisms adapted to produce disease resistant species.

Keywords: biotic resistance; disease resistance; resistance breeding; pathogens; tree improvement

I. INTRODUCTION

Forest tree species are generally perennial ones and during their long life span, they encounter the attack of various pests and pathogens which leads to a number of abnormal conditions or diseases. Insect pests and pathogens reduce forest productivity by destroying trees, increasing woody debris (with consequent fire damage), and destroying wildlife habitat, impairing water quality, and diminishing recreation and amenity values (Strauss et al., 1990). Tree breeding is mainly focused on improvement of efficiency, quality and robustness, involving manipulation of their genotype, thus having direct bearing on the genotypic component of the reaction of forest trees to attacks of pests and pathogens. An important feature of forest trees is their longevity compared to agricultural crops. The long-time spans involved in breeding generations also necessitate greater care and conservatism in

the choice and use of selection traits, since there is less opportunity to change selection traits to suit changing circumstances (Carson and Carson, 1989). Following crosses between selected individuals, it is important to select robust and diverse populations as well as novel trait combinations and elite genotypes (Flachowsky, 2008).

For forest trees, breeding efforts are slowed by the long periods of evaluation and delayed onset of reproduction (Fladung, 1998). Breeding for disease resistance in forest trees is encouraged by the promising results with breeding for resistance in agricultural and horticultural crops (Heimburger, 1961). Varieties of agriculturally important species such as maize and wheat have been modified by traditional breeding techniques for thousands of generations (Harlan, 1992; Smartt and Simmonds, 1995). Forest trees on the other hand, because they have traditionally been less valuable to human societies due to their abundance in natural forests and because of their

long generation time were not subjected to traditional breeding methods (Wright, 1976). Rapid global deforestation rates along with huge amount of population growth have focused scientific and commercial attention on improving genetic stock of forest trees in order to improve quality and productivity (Mathews and Campbell, 2000). This included production of disease resistant forest tree species, with increased yield. While resistance against pests and pathogens in forest trees can be ascribed to a combination of stochastic genetic variation (Yanchuk et al., 1988), evolved immunity (Liu and Ekramoddoullah, 2004), plasticity and interaction with environmental conditions (Cruickshank et al., 2010), climate change is predicted to make environments more favorable for pests and pathogens in future (Sturrock et al., 2011).

Tree resistance can be enhanced by a variety of biotic and abiotic inducers, including nonpathogenic and pathogenic microbes, and herbivores, resulting in enhanced protection against further biotic injury (Eyles et al., 2009). Plant defences may involve preformed barriers or induced resistance mechanisms based on recognition of the invader, complex signaling cascades, hormone signaling, activation of transcription factors and production of pathogenesis-related (PR) proteins with direct antimicrobial or anti-insect activity (Naidoo et al., 2014). Induced resistance (IR) could be a valuable tool in sustainable pest management (Eyles et al., 2009). IR can be performed based on aspects like study and application of inducible defence mechanisms in trees, systemic induction of resistance, phytohormone signaling networks, etc. Plants' resistance mechanisms can be categorized under various levels. These include basal resistance, parasite and race-specific resistance (Jones & Dangl, 2006; Kiraly et al., 2007), age-related (ontogenetic) resistance (Develey-Rivie`re & Galiana, 2007), organ-specific resistance (Blodgett et al., 2007) and acquired or induced resistance (IR) (Agrawal et al., 1999). In its broadest sense, IR is a form of resistance caused by activation of the host plant's own genetically programmed defence pathways, resulting in changes that diminish the effects of subsequent biotic attack (Agrawal et al., 1999; Hammerschmidt, 2007). The antimicrobial defense mechanisms comprise preformed physical and chemical barriers restricting microbial invasion, also induced defense reactions expressed by living cells, leading to altered gene expression and metabolic activity (Bonello et al., 2006).

Genetic resistance potentially provides an invaluable management tool for restoring these species or using them in plantations (Sniezko, 2006). Most forest tree breeding activities are concerned with so-called forest tree improvement. This is mainly a selection process based on presumed good correlations between phenotypic expression and genetic basis of several economically important forest tree characteristics (Heimburger, 1961). The genetic background of resistance in the host can be polygenic or be governed by a smaller number of major genes, although in most cases resistance to disease in plants has been found to be based on a combination of polygenes and major genes. Gene stacking is an interesting approach that has the potential to produce trees with enhanced resistance to various pests and pathogens (Chan et al., 2005) while retaining their valuable wood properties.

However, long regeneration cycles pose a serious limitation to multigene approaches with serial transformation, suggesting that multigene constructs will be a requirement. A novel approach to gene manipulation in trees involves the use of transcription activator-like effectors (TALEs) combined with nucleases (TALENs) to knock out a gene or modify its sequence (Pennisi, 2013). This approach to genome editing is expected to be more acceptable to regulatory bodies and society than conventional methods of genetic modification and is comparable to radiation mutagens in plants. The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system from bacteria can also be used to edit host-specific targets and requires the attachment of nucleases to target-specific RNAs (Jinek et al., 2012; Belhaj et al., 2013).

Pathogenesis-related proteins are another important part of the plant immune system. There are 17 currently known PR protein families (PR-1 through -17) in plants (van Loon, 2009). The expression of genes encoding the PR-families PR-1, PR-2, PR-3, PR-5, PR-9, PR-10 and PR-12 are induced by pathogens in different forest tree species (Veluthakkal and Dasgupta, 2010). Plant defence is costly to the host and therefore the plant invests in mechanisms to fine-tune its responses to effectively control the spread of pests and pathogens while conserving cellular resources.

II. DEFENCE SYSTEMS IN PLANTS

Plants and forest trees defend themselves against a variety of pathogens and pests during their lifetime. Pathogens may be viral, bacterial, oomycete or fungal and can adopt a biotrophic, hemibiotrophic or necrotrophic lifestyle. Biotrophs feed on living cells, maintaining host cell viability, while necrotrophs rely on dead tissues as a source of nutrients. Hemibiotrophs have an early, transient biotrophic phase followed by a necrotrophic phase. Pests may be specialists (small host range) or generalists (broad host range), and include chewing, piercing and sucking, mining, boring and galling insects (Wylie and Speight, 2012). In addition to the virulence determinants of the invading agent and environmental factors, the outcome of the host–pest or host–pathogen interaction also depends on the plant's constitutive and induced defences (Naidoo et al., 2014).

The first line of defence against biotic invaders in plants is pre-formed. Plants can possess anatomical variants correlated with levels of disease resistance (Fahn, 1988). Some of these anatomical features include mechanical barriers to pest or pathogen invasion, such as the bark, the pectin and lignin components of plant cell walls, and the leaf cuticle. Other anatomical features associated with defence include secretory cells, glands and ducts that produce and transport defensive substances. These anatomical characteristics can be constitutive or induced by injury or exposure to invading agents (Fahn, 1988; Eyles et al., 2004; Franceschi et al., 2005; Kovalchuk et al., 2013). Other preformed defences include the production of antimicrobial peptides and toxic secondary metabolites that are released upon insect or pathogen attack (recently reviewed by Kovalchuk et al., (2013)). Plant cell walls are actively modified at the sites of interaction with fungi and bacteria, and become reinforced by the deposition of

cell wall appositions, referred to as papillae (Naidoo et al., 2014). In the event of successful fungal penetration, cell wall-associated structures, such as haustorial encasements, collars or neck bands, are formed to halt pathogen spread (Micali et al., 2011; Underwood, 2012). In the event of oviposition by insects, some host plants are able to produce neoplasms (tumor-like growth of undifferentiated cells) beneath the egg, halting larval entry (Doss et al., 2000). Other preformed defences may involve stored chemicals that are released upon attack. If preformed defences are breached, a pathogen or pest would encounter inducible defence responses. Induced responses rely on the plant's ability to distinguish self from non-self, which is analogous to that seen in animal immunity (Jones and Dangl, 2006). However, in contrast to animals, plants lack an adaptive immune system involving somatic recombination of genes, and have no circulating immune cells. Therefore, they rely on the innate defences of each cell and induced defence mechanisms to respond to microbial or pest attack.

Insect herbivores and pathogens must overcome the diverse defence strategies that trees have evolved. This includes multiple constitutive and inducible defences that impede access to, deter or kill insects and inhibit or exclude pathogens physically and/or chemically (Pearce, 1996; Franceschi et al., 2005). Constitutive defences, both below and above ground, are present at all times and represent the first lines of defence. When these barriers are breached, induced defences are triggered (Eyles et al., 2009). Induced defence mechanisms in trees can be grouped under two broad categories:

✓ *INDUCIBLE CHEMICAL DEFENCE:* Toxic, antimicrobial, antinutritive and antidigestive activity via low-molecular weight (LMW) compounds such as phenolic compounds, terpenoids and alkaloids. These are generally secondary metabolites that are classified according to their biosynthetic pathways. In plant–pathogen interactions, LMW antimicrobial compounds that are synthesized de novo upon infection are described as phytoalexins (Hammerschmidt, 1999), while pre-existing LMW antimicrobial compounds are called phytoanticipins (van Etten et al., 1994).

✓ *INDUCIBLE PROTEIN-BASED DEFENCES:* Toxic, antimicrobial, antinutritive and antidigestive activity via proteins and peptides, for example, oxidative and hydrolytic enzymes, and proteinase inhibitors. Families of soluble pathogenesis-related proteins (PR proteins) include proteins (molecular mass < 100 kDa) involved in inducible protein-based defence. Of the 17 PR protein families that are now classified, the majorities have been shown to be rapidly induced, both locally and systemically (Eyles et al., 2009). Members of the PR-3 family (chitinases) exhibit antimicrobial activities in vitro by affecting fungal cell wall or membrane integrity. Other proteins, such as PR-6 proteins (proteinase inhibitors), may target nematodes and herbivorous insects as well as pathogens by impairing their digestive enzyme activity (Jongsma & Beekwilder, 2008). The larval growth rate of *Malacosoma disstria* (forest tent caterpillars) was shown to decrease on transgenic Populus overexpressing the induced leaf PPO (polyphenol oxidases) gene compared

with larvae feeding on control leaves (Wang & Constabel, 2004).

PR-1 gene, originally described in tobacco, has antifungal activity against *P. infestans* (Niderman et al., 1995) and is often used as a diagnostic marker. PR-5 proteins, which are part of the large thaumatin-like protein family, have previously been shown to have activity against fungal and oomycete pathogens. PR-9 proteins are peroxidases, which are involved in the cross-linking of polysaccharides and extension of phenylpropanoid monomers during cell wall reinforcement (Passardi et al., 2004). While no PR-7, PR-8, PR-15 and PR-17 orthologs have been identified in *P. trichocarpa* or *A. thaliana*, putative homologs of their type members (Sels et al., 2008) were identified in the *E. grandis* genome. PR-7 proteins are similar to those within the PR-6 family and are considered proteinase inhibitors, which are important for defence against insects (Ryan, 1990). PR-8 proteins (like PR-3, PR-4 and PR-11 proteins) are chitinases that hydrolyze the β-1, 4 linkages between N-acetyl glucosamine residues of fungal chitin (van Loon, 2009). PR-15 proteins are involved in the production of hydrogen peroxide (H_2O_2), which is toxic to pests and pathogens (van Loon, 2009).

Induced resistance can occur at the site of the initial attack (local defence) or be functional in distant parts of the plant or throughout the entire plant (systemic defence). Systemic host responses are thought to be activated through the plant via one or more signaling molecules and may result in systemic induced resistance (SIR) (Kuc, 1983). To date, at least seven types of SIR have been described. In the case of necrotic lesion-inducing pathogens that cause a hypersensitive response and the systemic expression of PR genes, the phenomenon is known as systemic acquired resistance, or SAR (Durrant & Dong, 2004). In trees, SIR also develops in response to necrogenic pathogens, but given that nothing is known about the signaling system involved, this type of SIR is viewed differently from SAR (Bonello et al. 2001, 2006). SIR can also be induced by rhizosphere microorganisms, in which case it is known as induced systemic resistance (ISR), but unlike SAR, ISR is not associated with induction of PR genes (van Loon, 2007). ISR is activated by colonization of plant roots by selected strains of free-living, nonpathogenic, plant growth promoting rhizobacteria (PGPR) (van Loon, 2007). Different forms of systemic induced resistance (SIR) in plant–pathogen and plant–insect interactions are depicted in Table 1.

Inducing agent	Types of SIR	Plant type	Major endogenous signaling molecules	References
Pathogens causing HR	SAR	Herbaceous species	SA	van Loon et al. (1998); Durrant & Dong (2004).
Necrotizing pathogens	SIR	Conifer species	Unknown	Bonello et al. (2001).
Plant growth-promoting rhizobacteria	ISR	Herbaceous species	JA and ET	van Loon (2007).
Plant growth-promoting fungi	ISR	Herbaceous species	JA and ET	van Wees et al. (2008).
Mycorrhizosphere/ actinomycete	Unknown	Herbaceous species	Unknown	Lehr et al. (2008).
Wounding	Wound-	Herbaceous	Unknown	Chassot et al.

	induced IR	species		(2008)
Herbivores	Herbivore-induced direct and indirect resistance	Herbaceous and tree species	JA and ET	Kessler & Baldwin (2002)

Table 1: Different forms of systemic induced resistance (SIR) in plant–pathogen and plant–insect interactions (After, Eyles et al. 2009) [HR: Hypersensitive Response; SAR: Systemic Acquired Resistance; ISR: Induced Systemic Resistance; IR: Induced Resistance; SA: Salicylic Acid; JA: Jasmonic Acid; ET: Ethylene]

The characterization of SIR against stem and branch pathogens in trees has been largely based on coniferous forest tree species. Systemic induced resistance differs from ISR mainly because it is induced by both biotic wounding (for example, by herbivores) and abiotic (mechanical) wounding, while ISR is not induced by abiotic wounding (Gurr and Rushton, 2005; van Loon, 2007). Epigenetic modifications add another level of complexity to the regulation of host defences (Berr et al., 2012). Epigenetic regulation of gene expression during various tree physiological processes has been reported in pine (Boyko and Kovalchuk, 2011) as well as in poplar (Conde et al., 2013) and was recently reviewed (Bräutigam et al., 2013). However, the majority of studies pertaining to epigenetic gene regulation in plants have focused on model organisms, herbaceous plants (Holeski et al., 2012) or abiotic stress responses (Bräutigam et al., 2013).

III. DEFENCE AGAINST INSECT PESTS

Forest trees show different types of defense responses against pests. Defense response can be induced genetically, when the innate preformed defense mechanisms of trees are breached. Genetically engineered insect pest resistance is often environment friendly as it reduces the use of chemical and synthetic insecticides. Plants present a large spectrum of resistance mechanisms against insect herbivores. The defense mechanism used is largely dependent upon many factors, but primarily the physiological status of the plant, timing of attack, damage level, type of tissue removed, and intimacy of the relationship between plant and herbivore (Fernandes, 1990).

One strategy for producing insect-resistant trees through genetic engineering was the development of trees that could produce the insecticidal toxin from the bacterium Bt (*Bacillus thuringiensis*). Bt produces a protein that is toxic when ingested by certain species of insects. The toxins are found in large crystals in mature sporulating cells of the bacterium. After Bt is eaten by larvae of susceptible insects, the crystals dissolve and the proteins (called delta-endotoxins) are released into the insects' midgut, where digestive enzymes cleave the proteins into smaller toxic fragments (Dulmage and Aizawa, 1982; Schnepf and Whiteley, 1985). The insecticidal toxins in Bt are highly specific; they affect a number of related insect species, but are not toxic to humans or other organisms. There are many varieties of Bt with different insecticidal spectra. Some are toxic specifically to larvae of lepidopterans (moths and butterflies) (Knowles and Ellar, 1988), whereas others are

specific for coleopterans (beetles) (Herrnstadt et al., 1986; McPherson et al., 1988) or dipterans (flies and mosquitoes) (Tyrell et al., 1979). In 2002, insect-resistant black poplar (*P. nigra*) containing Bt genes were approved for commercialization by the Chinese Gene Security Committee (Su et al., 2003). Hybrid triploid poplars [(*Populus tomentosa* x *P. bolleana*) x *P. tomentosa*] transformed with a cowpea trypsin inhibitor gene (CpTI) exhibited resistance to three defoliating insects: forest tent caterpillar (*Malacosoma disstria*), gypsy moth (*Lymantria dispar*) and willow moth (*Stilpnotia candida*) (Zhang et al., 2005). Bt genes were also used in resistance against predation by a beetle *Chrysomela tremulae,* in Poplars Conifers have several resistance mechanisms that repel, kill, inhibit, or otherwise reduce the success of invading pathogens (Zhang et al., 2005). The oleoresin of most conifers contains approximately equal amounts of monoterpenes and diterpenes and smaller amounts of sesquiterpenes. The volatile monoterpenes and sesquiterpenes in oleoresin evaporate over time to leave nonvolatile diterpenoid acids which form a hardened mass upon polymerization (Langenheim, 2003). Conifers produce terpenoids that are toxic to insects or that negatively affect the physiology of the invading insect or offspring (Keeling and Bohlmann, 2006).

Evidence of existence of Proteinase inhibitors (PIs), proteins which inhibit the actions of digestive enzymes, has been reported in *Populus* species (Bradshaw, 1991). They adversely affect growth and development of insects when eaten with plant material and work by binding tightly to proteinases and inhibiting their function, without being cleaved themselves. This reduces the effective concentration of digestive enzymes. The insect is further stressed because overproduction of digestive enzymes is induced, without increase in nutrition (Broadway and Duffey, 1986). When leaves, stems or roots of trees are infected by insects, they are triggered to produce PIs (Green and Ryan, 1972; Brown et al., 1985; Graham et al., 1986; Ryan and An, 1988). A wide variety of insect pests of forest trees could be controlled by means of PIs. Because the target enzymes of PIs are common and critical to the physiology of insects, almost any insect which consumes tree tissues should be susceptible to PIs (Strauss et al., 1990).

The insect pest *Leptocybe invasa*, in case of *Eucalyptus* species, poses a great threat. These threats are managed by planting tolerant *Eucalyptus* genotypes. The genome sequence of *Eucalyptus grandis* which has recently become available, gives us a valuable resource as to understand the defence mechanisms in large woody perennials against insect pests. Based on this genome sequence, various putative pathogenic-related (PR) proteins were identified based on sequence identity to the previously described plant PR proteins. The survey of PR genes in *Eucalyptus* provides a first step in identifying defence gene targets that may be employed for protection of the species in future (Naidoo et al., 2014). Induced defence responses primarily require the identification of self and non-self-substances in the plant body. Recognition of non-self relies on the perception of general elicitors called, in the case of insect pests, damage-associated molecular patterns (DAMPs) (Heil, 2009). Transcription factors (TFs) play an important role in these mechanisms by coordinating

the expression of defence-related genes in response to invasion. Functional studies on TFs that mediate defence responses in *Eucalyptus* are limited, but future research could be modelled on recent studies of TF function during wood formation (Hussey et al., 2011; Creux et al., 2013).

Genes of insects are also sometimes turned against insect pests. Insect pheromones from conifer-feeding bark beetles are often simple derivatives of tree terpenoids which when inserted was found that trees produced pheromones or related compounds themselves, potentially disrupting insect mating and colonization behaviour. Constitutive and inducible anatomically based defense responses help protect conifers against insect and pathogen attacks (Franceschi et al., 2005). Constitutive defenses have been studied in forest entomology for a long time, and in particular secondary compounds have received much attention. Defense is usually credited to phenolics or terpenoids, the main groups of secondary compounds in the foliage of deciduous and coniferous trees, respectively. Sugars and proteins may also be important contributors to variance in the success of forest pests (Schwenke, 1968). Different foliar sugars may have different effects on insects. High levels of galactose (Zou and Cates, 1994) and sucrose (Clancy, 1992) may retard insect growth on artificial diets.

When a tree is attacked or injured, the constitutive PP (polyphenolic parenchyma) cells are activated, new PP cells are produced in the phloem and a tangential row of traumatic resin ducts (TDs) is induced in the xylem (Franceschi et al., 2000; Nagy et al., 2000, 2004). These induced defense reactions increase tree resistance to further attacks and help repair injured sites (Franceschi et al., 2000, 2002; Krokene et al., 2003). Polyphenolic parenchyma cell activation and TD formation can be induced by various stimuli, including bark beetle attack (Franceschi et al., 2002; Martin et al., 2002; Hudgins et al., 2003, 2004; Heijari et al., 2005; Luchi et al., 2005). Jasmonates are phytohormones that serve as important elicitors and signalling molecules in plant defense responses (Koda, 1992; Creelman and Mullet, 1997; Seo et al., 1997; Thaler et al., 2001). Methyl jasmonate elicits anatomically based defense responses in many conifers including members of the Pinaceae, Cupressaceae, Araucariaceae, Podocarpaceae and Taxaceae (Hudgins et al., 2003, 2004). Methyl jasmonate induced resistance is likely mediated by ethylene, acting as a downstream signalling agent (Hudgins and Franceschi, 2004; Hudgins et al., 2006; Ralph et al., 2007). This leads to enhanced resistance to the pathogenic bark beetle-associate *Ceratocystis polonica* large trees (Franceschi et al., 2002; Zeneli et al., 2006).

Plant hypersensitivity is a term primarily used to describe a response to infection by pathogens as well as to many non-pathogenic stimuli (Matta, 1971; Misaghi, 1982). Hypersensitivity has been recognized as an important defense mechanism used by plants against pathogens. The hypersensitive reaction encompasses all morphological and histological changes that, when produced by an injurious agent, elicit the premature dying, or necrosis, of the infected tissue, as well as inactivation and localization of the infectious agent (Miiller, 1959; Maclean et al., 1974; Agrios, 1988). Growth of wood wasps larvae of the genus *Sirex* is drastically impaired on host plants that elicit a hypersensitive response.

Hosts belong to the genera *Pinus, Abies, Picea, Larix, Pseudotsuga*, and *Auricaria* (Morgan, 1968; Madden, 1988). Wood wasps are attracted to physiologically stressed trees (Madden, 1977, 1988). During oviposition, the female injects mucus of unknown chemical nature and species-specific symbiotic fungal spores into the host plant tissue. The mucus alters the water balance of plant needles causing tissue desiccation and collapse of the phloem elements (Fong & Crowden, 1973) and resulting in inhibition of translocation (Madden, 1988). The combination of these processes, plus plant tissue laceration during wood wasp oviposition favours fungus establishment and growth. Host resistance to *Sirex* and its symbiotic fungus is primarily due to a hypersensitive reaction by the invaded host plant (Coutts & Dolezal, 1966; Coutts, 1969). Polyphenols are produced as a specific response to the wood wasp symbionts fungus (Coutts & Dolezal, 1966; Hillis & Inoue, 1968).

IV. DEFENCE AGAINST FUNGAL PATHOGENS

Forest trees are a host to a wide range of fungal pathogens. Trees engineered for disease resistance can provide both environmental and commercial benefits. Many fungal pathogens are responsible for causing diseases like foliage rusts, cankers, vascular wilt diseases and root rots in forest trees. The degree of a fungus attack depends on the genetic background of the host, governing resistance and the response of the host to any given set of environmental factors. It is also influenced by the genetic background of the parasite concerned, governing virulence and response to environment (Mode, 1958). Disease resistance can be conferred through hypersensitive reactions, phytoalexins, host-pathogen specific toxins, barriers to pathogen invasion, and several other mechanisms. These resistance mechanisms often operate at different stages in disease development, and are likely to operate independently of each other (Carson and Carson, 1989).

Hypersensitive reaction is the primary event in resistance to fungal parasites (Maclean et al., 1974; Agrios, 1988). This reaction by the host leads to a disruption of nutrient supplies to the invading microorganism (Wong and Berryman, 1977) and the production of many toxic metabolites, such as phytoalexins (Bayley and Mansfield, 1982; Smith and Banks, 1986) resulting in the cessation of microorganism growth (Maclean et al., 1974; Johal & Rahe, 1988). Furthermore, water and oxygen also are reduced, thus further decreasing the probabilities of establishment and success for the invading organism (Wong and Berryman, 1977). Pathogenesis-related (PR) proteins are a group of diverse proteins whose accumulation is triggered by pathogen attack, abiotic stress, hypersensitive response (HR), and systemic acquired resistance (SAR). They play a major role in natural defense against pests and pathogens. PR proteins form an intersection point for various response networks by reacting with different inducers such as salicylic acid, jasmonic acid, systemin, and ethylene (Herna´ndez et al., 2005). The recognized PRs have been extensively reviewed and currently comprise 17 families of induced proteins (van Loon et al., 2006).

Enhanced disease resistance has been achieved using a variety of genes derived from plants and microorganisms, with varying degrees of success. Chinese white poplar (*P. tomentosa*) expressing a chitinase gene from *Beauveria bassiana* (Bbchit1) exhibited increased resistance to a pathogenic fungus (*Cytospora chrysosperma*) (Zia et al., 2010). Moreover, 2-year old poplars expressing anti-microbial peptides have shown high resistance in leafdisc assays and *Septoria musiva* cankers have been less frequent on field-grown transgenic trees (Powell et al., 2006).

Fusarium circinatum, the causal agent of pine pitch canker (PPC), is an emergent risk that threatens *Pinus* forests worldwide, with potential production and sustainability losses. Its symptoms include damping-off and wilting of seedlings and, on mature trees, branch dieback, stem cankers, pitch formation and mortality (Wingfield et al., 2008). This pathogen is dealt by assessing the physiology, hormones and the primary metabolites of pine as well as studying the gene expression regulation of target primary and pathogenesis-related genes using either high-throughput mRNA sequencing (Carrasco et al., 2017) or targeted approaches with special focus on secondary metabolism and phenylpropanoid pathway induction (Davis et al., 2002; Morse et al., 2004; Fitza et al., 2011, 2013; Donoso et al., 2015).

The pathogens that are found posing a threat to *Eucalyptus* include the myrtle rust pathogen *Puccinia psidii*, the stem canker pathogen *Chrysoporthe austroafricana*, the root rot pathogen *Phytophthora cinnamomi* (Wingfield et al., 2008). The pathogen-associated molecular patterns, microbe-associated molecular patterns (PAMPs) are perceived by pattern recognition receptors (PRRs) (Dardick and Ronald, 2006), and recognition leads to the relatively weak, non-specific immune response termed pattern-triggered immunity (PTI). Following recognition, a MAPK signaling cascade is initiated and various hormones are also involved in amplifying the defence signal. Perception of the pathogen also leads to the activation of nucleotidyl cyclase, giving rise to an increase in cyclic nucleotide concentration. Cyclic nucleotide-gated ion channels (CNGCs) are activated, leading to an increase in cytosolic calcium levels. More calcium binds to calmodulin, and this interaction leads to the regulation of NO and H_2O_2. Secondary metabolites are produced that may result in volatile production, alerting neighboring cells to the threat. Various TFs are produced that activate PR genes which may have direct antibacterial, antifungal or anti-insect activity. Systemic signals prime neighboring cells and distal tissue for subsequent attack (Naidoo et al., 2014). These general elicitors are usually molecules that are essential for the invader's life cycle (Nurnberger and Lipka, 2005; van Loon, 2009). It was observed in several cases that cross species PRRs could potentially be used in improving the plant's resistance to pathogens and could also be attractive targets for gene manipulation in *Eucalyptus* species. *Chrysoporthe austroafricana* is a fungal pathogen which causes stem cankers in *Eucalyptus grandis*. This pathogen can be controlled by the vegetative propagation of *E. grandis* x *E. urophylla* hybrids (Van Heerden et al., 2005).

Oxalic acid (OxA) is a virulence factor of several phytopathogenic fungi, including the model species *Sclerotinia sclerotiorum* (Godoy et al., 1990), and induces localized and systemic resistance against pathogens in a variety of angiosperm crop species (Doubrava et al., 1988; Reglinski et al., 1997; Toal and Jones, 1999). Plant proteins with extracellular leucine-rich repeats (eLRR) play a crucial role in the recognition of pathogens in race-cultivar-specific resistance and non-host general resistance. Polygalacturonase-inhibiting proteins (PGIPs) are eLRR proteins that recognize and inhibit fungal polygalacturonases (PGs). Chimwamurombe et al. (2001) cloned and analyzed the partial sequences of the *pgip* genes from five commercially important Eucalyptus species (Veluthakkal and Dasgupta, 2010). Many plant phenolics are thought to serve as phytoalexins, induced structural barriers, modulators of pathogenicity, and signalling molecules and are related to induced defense of forest trees against fungi. Among the conifer phenolics, stilbenes have been most frequently used in the context of induced resistance to pathogens (Hammerschmidt, 2005).

Spruces and poplars are important forest tree species in various regions, but they also are the target of a wide range of fungal pathogens. Armillaria root rot and Fomes root rot are two examples out of a long list of diseases affecting their root systems (Woodward, 1998). Melampsora leaf rust and Septoria leaf spot and canker are fungal pathogens that specifically affect poplar (Newcombe et al., 2001) while in spruce, needle rust caused by *Chrysomyxa* spp. and root rot caused by *Cylindrocladium* spp. or *Fusarium* spp have been described (Sutherland, 1991; Barnes and Linderman, 2001; Juzwik et al., 1988). Plant chitinases are used as defence against fungal pathogens. An endochitinase gene from *Trichoderma harzianum* (ech42) was shown to encode a potent endochitinase with a stronger antifungal activity by comparison with other chitinolytic enzymes (Lorito et al., 1998). The ech42 gene from *T. harzianum*, under the control of a duplicated enhancer 35SCaMV promoter and containing the AMV (Alfalfa Mosaic Virus) leader sequence, was introduced via *A. tumefaciens* transformation into black spruce and hybrid poplar. Disease development was almost completely abolished in all transgenic lines tested and there were almost no necrotic spots.

V. CONCLUSION

Trees growing in urban and agricultural landscapes or in natural forest systems provide large number of goods and services that are vital to human well-being, such as timber products, non-timber products, biodiversity, balance in ecosystem, watershed services, emissions reduction, carbon storage, scenic landscapes, recreation and aesthetics. Just as breeding for disease resistance in agricultural and horticultural crops is important for the economy of the world; resistance breeding in forest trees has become increasingly necessary. With rapid global change, the provision of such ecosystem services by forest trees is increasingly subject to threats such as pollution, drought and damage from both native and invasive alien pests and pathogens. Also, the increased use of chemicals as insecticides and pesticides to keep trees disease resistant has led to various environmental hazards. Hence, use of biotic resistance for the disease free growth of tree species was very much essential.

Non-native and native pathogens pose a serious threat to a number of forest trees and their associated ecosystems, and in many cases there are few effective management tools as far as conventional breeding measures are concerned. Disease resistance breeding may hold the key to restoration of these species. Disease resistance using high levels of genetic resistance are available to pests and pathogens, either within the host species or through hybridization with related species. With advancement in techniques in molecular genetics, there are chances of increased efficiency of breeding efforts in the future. Although genetic resistance is the keystone to future restoration or reforestation with various tree species, other management activities such as site hazard rating, branch pruning, and use of biocontrol (e.g., hypo-virulence, hyper-parasites, and endophytes) also aids in the process of disease resistance.

Numerous tree protection strategies have been made, as reviewed, which included use of induced resistance in trees by the modification of genetic makeup of the tree species, use of chemicals like secondary metabolites, use of genetically transformed disease causing bacteria and virus, etc. These tree-improvement strategies form an essential part of a multidisciplinary approach to make up for the losses brought about by existing pests and pathogens. Most of the "improved" forest trees used today do not differ much from their wild ancestral forms.

From all the above, it is clear that resistance breeding work on forest trees, with respect to induced resistance, is specifically difficult since they have a long life span and working at a molecular level with these trees is a tedious and long term process. Another major challenge is to develop proper pest management options for forest tree systems that are effective, environmentally sustainable and adaptable to the needs of an uncertain environment, since the environmental conditions in a forest is always dynamic. Induced resistance mechanisms do not always provide complete pest control. There are many environmental factors, such as nutrient supply, water availability and temperature, that influence the efficacy and effectiveness of the induced responses, regardless of whether IR is induced by elicitors, synthetic compounds, pest or pathogens or other microorganisms. Other factors that are likely to be critical include: the timing of application, the risk of rendering the treated plant more susceptible to other pathogens or insect herbivores, and the duration of the induced resistance. Such risks need to be dealt with in the future by proper investigation and research works and also will require the knowledge of the physiology of evolving pests and pathogens.

Breeding programs in trees are long term in nature, and requires a continuous supply of skilled labour and funding to progress. Funding challenges could slow or prevent further development in some of the programs. Public support is also very essential in ensuring the future success of the program, especially in forest lands which are surrounded by local tribes. But, in spite of all the existing challenges, induced resistance has gained considerable popularity in the fields of genetic research and future research on induced resistance in forest systems may provide opportunities to explore mechanisms of local and systemic host defence that may be unique to large and long-lived trees. Results from recent studies of induced resistance in trees have shown that IR can be used as an alternative, eco-friendly solution for mitigating pest impacts in trees, including those arising from biological invasions.

REFERENCES

[1] Agrawal, A.A., Tuzun, S., & Bent, E. (1999). Induced plant defenses against pathogens and herbivores: biochemistry, ecology, and agriculture. (pp. 403). American Phytopathological Society Press: St Paul, MN, USA.

[2] Belhaj, K., Chaparro-Garcia, A., Kamoun, S., & Nekrasov, V. (2013). Plant genome editing made easy: targeted mutagenesis in model and crop plants using the CRISPR/Cas system. Plant Methods,9: 39.

[3] Berr, A., Menard, R., Heitz, T., Shen, W.H. (2012). Chromatin modification and remodelling: a regulatory landscape for the control of Arabidopsis defence responses upon pathogen attack. Cell Microbiol, 14: 829–839.

[4] Blodgett, J.T., Eyles, A., Bonello, P. (2007). Organ-dependent induction of systemic resistance and systemic susceptibility in Pinus nigra inoculated with Sphaeropsis sapinea and Diplodia scrobiculata. Tree Physiology, 27: 511–517.

[5] Bonello, P., Gordon, T.R., Herms, D.A., Wood, D.L., & Erbilgin, N. (2006). Nature and ecological implications of pathogen-induced systemic resistance in conifers: a novel hypothesis. Physiological and Molecular Plant Pathology, 68: 95–104.

[6] Boyko, A., Kovalchuk, I. (2011). Genome instability and epigenetic modification-heritable responses to environmental stress. Curr Opin Plant, Biol 14: 260–266.

[7] Bräutigam, K., Vining, K.J., Lafon-Placette, C. et al. (2013). Epigenetic regulation of adaptive responses of forest tree species to the environment. Ecol Evol, 3: 399–415.

[8] Carson, S.D., Carson, M.J., (1986). A breed of radiata pine resistant to Dothistroma needle blight. Proc. Plant Breeding Symp. Dept. Sci. Indus. Res., Lincoln, New Zealand. Agron. Soc. NZ Spec. Pub, 5: 202-7 14

[9] Chan, Y.L., Lin, K.H., Liao, L.J., Chen, W.H., & Chan, M.T. (2005). Gene stacking in Phalaenopsis orchid enhances dual tolerance to pathogen attack. Transgenic Res, 14: 279–288.

[10] Chassot, C., Buchala, A., Schoonbeek, H., Metraux, J.P., & Lamotte, O. (2008). Wounding of Arabidopsis leaves causes a powerful but transient protection against Botrytis infection. Plant Journal, 55: 555–567.

[11] Chimwamurombe, P.M., Botha, A.M., Wingfield, M.J., & Wingfield, B.D. (2001). Molecular relatedness of the polygalacturonase-inhibiting protein genes in Eucalyptus species. Theor Appl Genet, 102(4): 645–650

[12] Conde, D., González-Melendi, P., & Allona, I. (2013). Poplar stems show opposite epigenetic patterns during winter dormancy and vegetative growth. Trees, 27: 311–320.

[13] Creux, N.M., Bossinger, G., Myburg, A.A., & Spokevicius, A.V. (2013). Induced somatic sector

analysis of cellulose synthase (CesA) promoter regions in woody stem tissues. Planta, 237: 799–812.

[14] Cruickshank, M., Jaquish, B., & Nemec, A. (2010). Resistance of half-sib interior Douglas-fir families to Armillaria ostoyae in British Columbia following artificial inoculation. Can J For Res, 40: 155–166.

[15] Davis, J.M., Wu, H., Cooke, J.E., Reed, J.M., Luce, K.S., & Michler, C.H. (2002). Pathogen challenge, salicylic acid, and jasmonic acid regulate expression of chitinase gene homologs in pine. Mol. Plant Microbe Interact, 15: 380–387.

[16] Develey-Rivie`re, M.P., & Galiana, E., (2007). Resistance to pathogens and host developmental stage: a multifaceted relationship within the plant kingdom. New Phytologist, 175: 405–416.

[17] Donoso, A., Rodriguez, V., Carrasco, A., Ahumada, R., Sanfuentes, E., Valenzuela, S. (2015). Relative expression of seven candidate genes for pathogen resistance on Pinus radiata infected with Fusarium circinatum. Physiol. Mol. Plant Pathol, 92: 42–50.

[18] Doss, R.P., Oliver, J.E., Proebsting, W.M., Potter, S.W., Kuy, S., Clement, S.L., Williamson, R.T., Carney, J.R., DeVilbiss, E.D. (2000). Bruchins: insectderived plant regulators that stimulate neoplasm formation. Proc Natl Acad Sci USA, 97: 6218–6223.

[19] Doubrava, N.S., Dean, R.A., & Kuc, J. (1988). Induction of systemic resistance to anthracnose caused by Colletotrichum lagenarium in cucumber by oxalate and extracts from spinach and rhubarb leaves. Physiol. Mol. Plant Pathol, 33: 69–79.

[20] Dulmage, H.T., & Aizawa, K., (1982). Distribution of Bacillus thuringiensis in nature. In: Kurstak, E. (Ed.), (pp. 209-237). Microbial and Viral Pesticides. Marcel Dekker, New York.

[21] Durrant, W.E., Dong, X. (2004). Systemic acquired resistance. Annual Review of Phytopathology, 42: 185–209.

[22] Eyles, A., Bonello, P., Ganley, R., & Mohammed, C. (2010). Induced resistance to pests and pathogens in trees. New Phytologist, 185: 893-908.

[23] Eyles, A., Davies, N.W., Mohammed, C.M. (2004). Traumatic oil glands induced by pruning in the wound-associated phloem of Eucalyptus globulus: chemistry and histology. Trees, 18: 204–210.

[24] Fahn, A. (1988). Secretory tissues in vascular plants. New Phytol, 108: 229–257.

[25] Fernandes, G.W. (1990). Hypersensitivity: A Neglected Plant Resistance Mechanism Against Insect Herbivores. Environ. Entomol, 19(5): 1173-1182

[26] Flachowsky, H., Hanke, M.V., Peil, A., Strauss, S.H., & Fladung, M. (2009). A review on transgenic approaches to accelerate breeding of woody plants. Plant Breeding, 128: 217-226.

[27] Fladung, M. (1998). Die Bedeutung bio- und gentechnologischer Verfahren fu¨r die Forstpflanzenzu¨chtung. Vortr. Pflanzenzu¨chtg, 43: 124-133.

[28] Franceschi, V.R., Krokene, P., Christiansen, E., & Krekling, T. (2005). Anatomical and chemical defenses of conifer bark against bark beetles and other pests. New Phytologist, 167: 353–375.

[29] Gurr, S.J., & Rushton, P.J. (2005) Engineering plants with increased disease resistance: what are we going to express. Trends Biotechnol, 23: 275–282.

[30] Hammerschmidt, R. (1999). Phytoalexins: what have we learned after 60 years? Annual Review of Phytopathlogy, 37: 285–306.

[31] Hammerschmidt, R., & Nicholson, R.L. (1999). A survey of plant defense responses to pathogens. In: Agrawal, A.A., Tuzun, S., Bent, E. (Eds.), Induced plant defenses against pathogens and herbivores. (pp. 55-71), American Phytopathological Society Press: St Paul, MN, USA.

[32] Harlan, J.R. (1992). Crops and man. American Society of Agronomy, Madison, WI, USA.

[33] Heimburger, C. (1962). Breeding for disease resistance in Fores Trees. The forestry chronical, 38: 356-362.

[34] Herna´ndez, I., Portieles, R., Chaco´n, O., & Borra´s-Hidalgo, O. (2005). Proteins and peptides for the control of phytopathogenic fungi. Biotecnol Aplicada, 22: 256–260

[35] Hilker, M., Kobs, C., Varma, M., & Schrank, K. (2002). Insect egg deposition induces Pinus sylvestris to attract egg parasitoids. Journal of Experimental Biology, 205: 455–461.

[36] Holeski, L.M., Jander, G., & Agrawal, A.A. (2012). Transgenerational defense induction and epigenetic inheritance in plants. Trends Ecol Evol, 27: 618–626.

[37] Hussey, S.G., Mizrachi, E., Spokevicius, A.V., Bossinger, G., Berger, D.K., & Myburg, A.A. (2011). SND2, a NAC transcription factor gene, regulates genes involved in secondary cell wall development in Arabidopsis fibres and increases fibre cell area in Eucalyptus. BMC Plant Biol, 11: 173.

[38] Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A., & Charpentier, E. (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science, 337: 816–821.

[39] Jones, J.D., & Dangl, J.L. (2006). The plant immune system. Nature, 444: 323–329.

[40] Jongsma, M.A., & Beekwilder, J. (2008). Plant protease inhibitors: functional evolution for defence. In: Schaller, A. (Ed.). (pp. 235-253), Induced plant resistance to herbivory. New York: Springer.

[41] Kessler, A., & Baldwin, I.T. (2002). Plant responses to insect herbivory: the emerging molecular analysis. Annual Review of Plant Biology, 53: 299–328.

[42] Kiraly, L., Barnaz, B., & Kiralyz, Z. (2007). Plant resistance to pathogen infection: forms and mechanisms of innate and acquired resistance. Journal of Phytopathology, 155: 385–396.

[43] Knowles, B.H., & Ellar, D.J. (1988). Differential specificity of two insecticidal toxins from Bacillus thuringiensis var. aizawai. Mol. Microbiol., 2(1): 153-157.

[44] Kovalchuk, A., Keriö, S., Oghenekaro, A.O., Jaber, E., Raffaello, T., & Asiegbu, F.O. (2013). Antimicrobial defenses and resistance in forest trees: challenges and perspectives in a genomic era. Annu Rev Phytopathol, 51: 221–244.

[45] Kuc, J. (1983). Induced systemic resistance in plants to diseases caused by fungi and bacteria. In: Bailey, J.A.,

Deverall, B.J. (Eds.), (pp. 191-221). The dynamics of host defence. Sydney, Australia: Academic Press.

[46] Lehr, N.A., Schrey, S.D., Hampp, R., Tarkka, M.T. (2008). Root inoculation with a forest soil streptomycete leads to locally and systemically increased resistance against phytopathogens in Norway spruce. New Phytologist, 177: 965–976.

[47] Liu, J.J., & Ekramoddoullah, A. (2004). Isolation, genetic variation and expression of TIR-NBS-LRR resistance gene analogs from western white pine (Pinus monticola Dougl. ex. D. Don.). Mol Genet Genomics, 270: 432–441.

[48] Morse, A. M., Nelson, C.D., Covert, S.F., Holliday, A.G., Smith, K.E., & Davis, J.M. (2004). Pine genes regulated by the necrotrophic pathogen Fusarium circinatum. Theor. Appl. Genet, 109: 922–932.

[49] Nagy, N.E., Fossdal, C.G., Krokene, P., Krekling, T., Lønneborg, A., & Solheim, H. (2004). Induced responses to pathogen infection in Norway spruce phloem: changes in polyphenolic parenchyma cells, chalcone synthase transcript levels and peroxidase activity. Tree Physiology, 24: 505–515.

[50] Naidoo, S., Kulheim, C., Zwart, L., Mangwanda, R., Oates, C.N., Visser, E.A., et al. (2014). Uncovering the defence responses of Eucalyptus to pests and pathogens in the genomics age. Tree Physiol, 34: 931–43.

[51] Newcombe, G. (1998). Association of Mmd1, a major gene for resistance to Melampsora medusae f. sp. deltoidae, with quantitative traits in poplar rust. Phytopathol, 88: 114–121

[52] Niderman, T., Genetet, I., Bruyere, T., Gees, R., Stintzi, A., Legrand, M., Fritig, B., & Mosinger, E. (1995). Pathogenesis-related PR-1 proteins are antifungal (isolation and characterization of three 14-kilodalton proteins of tomato and of a basic PR-1 of tobacco with inhibitory activity against Phytophthora infestans). Plant Physiol, 108: 17–27.

[53] Nurnberger, T., & Lipka, V. (2005). Non-host resistance in plants: new insights into an old phenomenon. Mol Plant Pathol, 6: 335–345.

[54] Passardi, F., Penel, C., & Dunand, C. (2004). Performing the paradoxical: how plant peroxidases modify the cell wall. Trends Plant Sci, 9: 534–540.

[55] Pearce, R.B. (1996). Antimicrobial defences in the wood of living trees. New Phytologist, 132: 203–233.

[56] Pennisi, E. (2013). The Tale of the TALEs. Science, 338: 1408–1411.

[57] Powell, P.A., Stark, D.M., Sanders, P.R., & Beachy, R.N. (1989). Protection against tobacco mosaic virus in transgenic plants that express tobacco mosaic virus antisense RNA. Proc. Natl. Acad. Sci. USA, 86: 6949-6952.

[58] Ryan, C.A. (1990). Protease inhibitors in plants: genes for improving defenses against insects and pathogens. Annu Rev Phytopathol, 28: 425–449.

[59] Sels, J., Mathys, J., De Coninck, B., Cammue, B., & De Bolle, M.F. (2008). Plant pathogenesis-related (PR) proteins: a focus on PR peptides. Plant Physiol Biochem, 46: 941–950.

[60] Smartt, J., & Simmonds, N.W. (1995). Evolution of crop plants. Longman. Harlow.

[61] Strauss, S.H., Howe, G.T., & Goldfarb, B. (1991). Prospects for genetic engineering of insect resistance in forest trees. For. Ecol. Manage., 43: 181-209.

[62] Sturrock, R.N., Frankel, S.J., Brown, A.V., Hennon, P.E., Kliejunas, J.T., Lewis, K.J., Worrall, J.J., & Woods, A.J. (2011). Climate change and forest diseases. Plant Pathol, 60: 133–149.

[63] Su, X.H., et al. (2003). Advances in tree genetic engineering in China. In: Proceedings of the XIIth World Forestry Congress, Quebec City, Canada, Paper 0280-B2 (http://www.fao.org/docrep/article/wfc/xii/ 0280-b2.htm)

[64] Tyrell, D.J., Davidson, L.I., Bulla, Jr., L.A. & Ramoska, W.A., (1979). Toxicity of parasporal crystals of Bacillus thuringiensis subsp, israelensis to mosquitoes. Appl. Environ. Microbiol., 38: 656-658.

[65] Underwood, W. (2012). The plant cell wall: a dynamic barrier against pathogen invasion. Front Plant Sci, 3: 85.

[66] van Etten, H.D., Mansfield, J.W., Bailey, J.A., Farmer, E. (1994). Two classes of plant antibiotics: phytoalexins versus ''phytoanticipins''. Plant Cell, 6: 1191–1192.

[67] van Loon, L.C. (2007). Plant responses to plant growth-promoting rhizobacteria. European Journal of Plant Pathology, 119: 243–254.

[68] van Loon, L.C. (2009). Advances in botanical research—plant innate immunity. Elsevier, Oxford, UK.

[69] Veluthakkal, R., & Dasgupta, M.G. (2010). Pathogenesis-related genes and proteins in forest tree species. Trees, 24: 993–1006.

[70] Wang, J., Constabel, C.P. (2004). Polyphenol oxidase overexpression in transgenic Populus enhances resistance to herbivory by forest tent caterpillar (Malacosoma disstria). Planta, 220: 87–96.

[71] Wingfield, M., Hammerbacher, A., Ganley, R., Steenkamp, E., Gordon, T., & Wingfield, B., et al. (2008). Pitch canker caused by Fusarium circinatum–a growing threat to pine plantations and forests worldwide. Australas. Plant Pathol, 37: 319–334.

[72] Wright, J.W., (1976). Introduction to Forest Genetics. Academic press. London.

[73] Wylie, F.R., & Speight, M.R. (2012). Insect pests in tropical forestry. CABI Publishing, Wallingford, UK.

[74] Yanchuk, A.D., Yeh, F.C., & Dancik, B.P. (1988). Variation of stem rust resistance in a lodgepole pine provenance-family plantation. For Sci, 34: 1067–1075.

[75] Zhang, J., Li, W., & Xiang, T. et al. (2010). Receptor-like cytoplasmic kinases integrate signaling from multiple plant immune receptors and are targeted by a Pseudomonas syringae effector. Cell Host Microbe, 7: 290–301.