Genetic and biotechnological breeding for disease resistance in forest species: A review

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ABSTRACT

There have been important research results recently in the area of genetic breeding for disease resistance in forest species. Most studies have been carried out on families, but there are also some on individual trees within families. Induced resistance depends on factors present only after the contact of the pathogen with the host. By definition, it is the opposite of constitutive resistance, which depends on pre-formed factors. Plants disease resistance, which involve induced defense mechanisms, include the accumulation of phytoalexins, deposit of material similar to lignin and increase in the activity of certain hydrolytic enzymes. In response to the growing impact of disease in forest plantations, some programs have emphasized breeding for disease resistance. New techniques and technologies have become important tools in disease resistant plant selection. While they do not replace conventional plant breeding, they search for ways of reaching objectives not attained by conventional techniques. The tissue culture technique has become an important tool in plant breeding to obtain disease resistant plants through micropropagation or cell culture in selective medium, thus informing how to use genetic markers to study resistance mechanisms. The breeding cycle in trees can be accelerated by genetic engineering techniques that allow the transfer, in a tissue culture cycle, of characteristics that are monogenic or oligogenic controlled. The introduction of genes of interest in plants, by genetic engineering, is becoming an additional strategy to be included in plant breeding. Genes from distinct plant species or from genetic distant organisms may be introduced in the plant genome by genetic engineering.

KEY WORDS: Breeding, biotechnology, resistance mechanisms, patogenicity.

INTRODUCTION

There have been important research results recently in the area of genetic breeding for disease resistance in forest species. Most studies have been carried out on families, but there are also some on individual trees within families (Zobel and Talbert, 1992). The efficiency of a selection process for disease resistance depends initially on finding genetic variability that provides this resistance.

Resistant genotype selection is one of the most economic forms of reducing the effects caused by disease in forest species (Resende et al., 1991).

Research to obtain disease resistant plants has been performed on several *Pinus* species (Jang and Tainter, 1990; 1991; Bronson et al., 1992; Ragazzi et al., 1995), *Eucalyptus* (Cahill et al., 1986; 1992; 1993; Cahill and McComb, 1992; Tippett et al., 1995), and *Acacia* (Tippett and Malajczuk, 1979; Cahill and Weste, 1983; Cahill et al., 1989).

Acacia mearnsii De Wild. has been threatened and limited by damage from gummosis attack. This disease reduces bark use and even causes death to the most susceptible individuals (Auer and Sotta, 1995). The pathology presents external symptoms (depression, necrosis and bark rupture and goma exudation) and internal symptoms (dark stripes in the wood) (Santos et al., 1997a). Trees with gummosis present lesions which extend from the base to the upper portions of the trunk (Santos et al., 1998). Several studies have been carried out to determine the etiology of the disease, subsidizing its control (Auer, 1997). Zeijlemaker (1971) showed, in etiological studies conducted in South Africa, that the *Phytophthora nicotianae* var.

parasitica fungus is one of the gummosis pathogens. Some authors also found gomose association with *Cylindrocladium* sp. and *Fusarium* sp. genera fungi (Auer and Sotta, 1995; Santos et al., 1997a; 1997b; Roux and Wingfield, 1997). Studies for the genetic improvement of *A. mearnsii* have been carried out to increase the quality and quantity of bark and wood by selecting superior trees in progeny tests. Although some experiments have been carried out in Bloemendal (South Africa) to test the resistance of genetic material selected against some pathogens, studies for selection of genotypes resistant to gummosis are only beginning (Dunlop, 1997).

The mass and family selection methods are much used in self pollinating plants to accumulate resistance genes. In bulk selection, plants are selected based on their individual reactions to the disease. In family (progeny) selection the plants are selected based on their progeny response (Camargo and Bergamin Filho, 1995).

Data on forest species genetic breeding are incipient due to the restricted number of researchers in the area and the difficulties inherent to the life cycle of perennial plants. This chapter will present an overview to the types of resistance, resistance mechanisms, aspects of classic genetic breeding and the use of biotechnology to accelerate breeding programs for resistance in trees.

BIBLIOGRAPHIC REVIEW

Vertical and Horizontal resistance

Van Der Plank (1963) defined vertical resistance, also known as perpendicular resistance, as that which is effective against one or some physiological races of a given pathogen; while horizontal resistance, also known as lateral resistance, is defined as resistance to many races. In vertical resistance, there is a differential interaction between the host plant varieties and the races of the same phytopathogenic organism. There is evidence that vertical resistance is governed by few genes, generally by only one or a few in interaction with the environment. In horizontal resistance, there is no manifestation of differential interaction; it is generally polygenic, that is, controlled by many genes which are not specific for disease resistance, but they occur in healthy plants, regulating normal processes that combined express resistance (Van Der Plank, 1968).

A significant interaction may happen when different isolates of a pathogen are inoculated in different individuals of a host. In the absence of interaction, resistance is of horizontal type and isolates differ in virulence. However, if there is differential interaction, the resistance is vertical and the pathogens also differ in virulence. The presence of interaction indicates that there is specialization of the pathogen at an intra-specific level of the host and, therefore, the isolates are classified as races, according to their virulence spectrum and a series of differential hosts, as reported by Camargo and Bergamin Filho (1995) in an extensive review.

Nelson (1978) suggested that horizontal resistance may be caused by a set of vertical resistance genes overcome by the pathogen. However, Eskes (1980) reported that high horizontal resistance levels can be found regardless of the existence of vertical resistance genes.

Varieties showing vertical resistance remain resistant in the field until a race of pathogen arises with the complementary virulence gene. The loss of this resistance is associated with host resistance mechanisms (Van Der Plank, 1968).

Bergamin Filho et al. (1995) reported that studies of H. H. Flor (1942) on linen rust (*Melampsora lini*) resulted in a gene to gene theory, where each virulence gene of a pathogen corresponds to a resistance or susceptibility gene in the host genotype. This theory seems to be valid when the pathogen-host interaction involves vertical resistance and virulence. However, it does not apply to horizontal resistance, when the host defense mechanisms cannot be overcome by the pathogen.

There are several studies on pathogen-host interaction for each gene to gene relationship. Although much research has been directed to the identification of the genes and genetic products involved, elucidation at molecular level is recent

(Boller and Meins, 1992).

The concepts that vertical resistance is monogenic and horizontal resistance is oligo-polygenic are frequently found in the literature. Although there are many examples where this correlation is true, it should not be generalized (Bergamin Filho et al., 1995). Sorghum resistance to *Periconia circinata* is monogenic and horizontal, and rye resistance to *Puccinia hordei* is polygenic, but presents a differential interaction with the pathogen races (Parlevliet, 1977). Monogenic vertical and polygenic horizontal resistance can occur in the same genotype. According to Parleviet et al. (1989) the selection of horizontal resistance in the presence of monogenic vertical resistance can produce an undesired effect, resulting in high frequencies of genes with vertical resistance.

Horizontal resistance was considered partial, due to mechanisms that partially hinder pathogen growth in the host tissues. This resistance may be an expression of near immunity or of a resistance reaction similar to that of vertical resistance (Eskes, 1980).

The hypersensitivity reaction is an extreme cell response by the plant which may lead to a high degree of resistance to the disease resulting in the death of a limited number of host cells close to the points of infection and is considered an induced defense response which impedes pathogen growth in the plant tissues. This response occurs when the host recognizes the infection as a consequence of the incompatibility between the plant and the pathogen (Camargo, 1995).

Horizontal resistance decreases the size of the lesions produced, increases the latent period, and decreases the number of spores produced per lesion. Its effects are partial and quantitative. In plants with horizontal infection, the efficiency of the infection is less than in susceptible plants; the lesions develop more slowly and the pathogen spores are produced later and in smaller quantity (Bergamin Filho et al., 1995).

Resistance with polygenic inheritance generally presents greater durability than monogenic resistance (Van Der Plank, 1968; Parlevliet, 1977). The concepts of vertical and horizontal resistance durability did not only arise from field results. Theoretical considerations indicate that polygenic resistance systems are more able to tolerate genetic changes in the pathogen than monogenic systems. Thus a poligenic resistance will be more stable than a monogenic resistance; genetic changes in several pathogenicity loci are required contrary to the monogenic system where the changes occur in one locus only (Bergamin Filho et al., 1995).

The use of mass and recurrent selection methods in susceptible plant populations increased the probability of selecting plants for horizontal resistance. In self pollinating plants, the mass and family selection methods are much used to bring together resistance genes. In mass selection, the plants are selected by criteria based on the individual responses to the disease. In family selection, the plants are selected according to the response of their progenies where the plant seeds of the more resistant progenies are used in the next selection cycle (Camargo and Bergamin Filho, 1995).

Resistance mechanisms

According to Galli et al. (1968), resistance may be considered as a host defense mechanism, resulting from the sum of the factors which tend to decrease the pathogenicity and virulence of the pathogen once in contact with the host. As it is the result of variable factors, the defense reaction and therefore the resistance are also variable, giving rise to immune plants at one extreme and totally susceptible plants at the other.

Induced resistance depends on factors present only after the contact of pathogen with the host. By definition, it is the opposite of constitutive resistance, which depends on pre-formed factors. Plants with disease resistance which involve induced defense mechanisms show the accumulation of phytoalexins, deposit of material similar to lignin and an increase in the activity of certain hydrolytic enzymes (Sequeira, 1983).

Induced lignification during the penetration of the cell wall has been detected in grass resistance to fungi. In some systems, the lignin is produced in

response to the pathogens. This lignin can differ in chemical composition from normal lignin. Lignin suppression may induce susceptibility, that is, the receptor sites of induce lignification may be blocked by products of a compatible pathogen. Fungicide products may link to cell wall polymers which supply the matrix for lignin deposition and block lignification (Vance et al. 1980).

Sherwood and Berg (1991) studied vessel anatomy and lignin content in *Dactylis glomerata* plants resistant and susceptible to *Stagonospora arenaria* and found high content of compounds, similar to lignin after inoculating the leaves, which were synthesized during infection by the fungus. However, according to these authors, resistance was not related to the constitutive lignin content of non inoculated plants.

Vance et al. (1980) reported that when pathogens are compatible with a host plant induce lignification, they can either metabolize or tolerate intermediary lignin compounds at fungus growth points.

Casares et al. (1986) compared the capacity of *Phytophthora cambivora* and *P. cinnamomi* to remove phenols from lignin in *Castanea sativa* and found that the two fungi differed in their capacity to oxidize phenols. *P. cambivora* did not oxidize one of the 21 phenols tested, while *P.cinnamomi* was able to remove phenols from the lignin and oxidize them.

Weste and Marks (1987) reported that the fungus penetration process in *P. cinnamomi* infected roots was similar in all the species tested. When an infection occurs, a small lesion is formed which increases in three to six days while root growth stops. However, in resistant species, the fungus colonization is limited to a small lesion. Resistance is manifested after penetration and colonization limitation after the primary symptoms arise. This resistance is characterized by the formation of anatomic barriers to the fungus growth, such as callous deposits. Thus secondary symptoms are not found in the shoots, and physiological changes do not occur. Cahill et al. (1992) observed, in roots inoculated with *P. cinnamomi*, that 8% of the primary roots of micropropagated *Eucalyptus marginata* resistant clones could restrict and limit colonization of this pathogen. However, no root of susceptible plants of this species restricted the fungus growth. The authors also observed that while there were few lesions in the *E. marginata* clones, their length was similar to that found on the roots of susceptible plants.

Plant root resistance to *P. cinnamomi* does not depend exclusively on morphological barriers but also on biochemical mechanisms (Cahill and McComb, 1992).

A reduction in the mineral concentration was detected after infection in *E. marginata* roots susceptible to *P. cinnamomi*. However, *E. calophylla* roots resistant in the field were also inoculated with this pathogen and did not present modification at these concentrations (Cahill et al., 1986b). According to Cahill and McComb (1992), plant response to this pathogen varied in degrees of tolerance, however, few species are resistant to it. Resistance was demonstrated in some Eucalyptus species only, mainly in those of the *Symphyomyrtus* subgenus (Cahill et al., 1986b). According to Tippett et al. (1985) , many species of this subgenus expressed resistance, with complete inhibition of the fungus growth. These authors found that phenol compounds in the phloem could have contributed to the resistance. Changes in the phytohormone concentration were also found in a study carried out by Cahill et al. (1986a), where there was a reduction in the concentration of cytokinins (zeatin and isopentenyladenine) in the xylem of susceptible *Eucalyptus* sp. cuttings infected by *P. cinnamomi*. This fungus, penetrated and destroyed the root extremities and consequently the cytokinin synthesis sites.

Cahill and McComb (1992) found an increase in the phenilalanine ammonia liase (PAL) activity, a key-enzyme in phenol propanoid, lignin and phenol product biosynthesis. This increase was detected 48 hours after infection by *P. cinnamomi* in *E. calophylla* roots which were resistant in the field. However, these changes did not occur in roots of susceptible *E. marginata* species. *E.calophylla* resistance to *P. cinnamomi* was associated with phenol production.

Roots of resistant clones (RR), susceptible clones (SS) and individual descendents from resistant plants in a susceptible family (RS) of *E. marginata* were inoculated with *P. cinnamomi.* The activity of the PAL enzyme increased in RR clone roots 48 hours after inoculation with the fungus. The constitutive phenol levels in RR clone roots were over 94% and increased even further after inoculation. Increase in the activity of this enzyme and in lignin and phenol synthesis are associated with lesion restriction and with the resistance in selected *E. marginata* clones (Cahill et al., 1993). Matern and Kneusel (1988) reported that infusions of flavanolics from the cell wall may be an active and coordinated response of cells submitted to stress in the primary root cortex of *Pseudotsuga menziessi*. The exposure of primary *P. menziessi* roots to *Laccaria bicolor* did not inhibit intercell colonization by *Fusarium oxysporum* but induced resistance to intracell colonization by the pathogen. In this process, *L. bicolor* prevented *F. oxysporum* from degrading the host cell wall, due to production of flavanolic infusion in the cell wall. *L. bicolor* stimulated the accumulation of tanine condensates between cortex cells, which indicated that these phenolics were the root protection base (Sylvia and Sinclair 1983; Strobel and Sinclair, 1991).

The most resistant species to *P. cinnamomi* may be the *Acacia pulchella* found in Australian forests, which showed cell reactions similar to those of hypersensitivity response. However, the pathogen penetrated the xylem of this species. Secondary symptoms were not observed in the sprouts, and there was no formation of sporangios in the roots that produced inhibitors (Tippett and Malajczuk, 1979).

Cahill and Weste (1983) observed the formation of callous on roots of some species resistant to *P. cinnamomi,* such as *E. calophylla, A. melanoxylon, A. pulchella, Zea mays, Triticum aestivum* (Gramineae), *Gahnia raducula* (Cyperaceae) and *Juncus bufonius* (Juncaceae), which either delayed or prevented pathogen growth. This formation was not observed in susceptible hosts, such as *E. sieberi, E. marginata, Themeda australis, Xanthorrhoea australis* and *X. resinosa*. Callous formation in *A. pulchella* occurred only in the cortex, along the cell wall and at penetration points. In *A. melanoxylon,* there were deposits in the epidermis, on the edge, around the penetration points, in the cortex, in invaginated deposits in new cells, and in the endodermis.

Cahill et al. (1989) detected cell and histological changes in plants susceptible and resistant to *P. cinnamomi*. Cell wall lignification, phenol deposition and callused papillar formation were observed more frequently in the resistant species (*A. pulchella, E. calophylla, E. maculata, Gahnia raducula, Juncus bufonius, Zea mays* e *Triticum aestivum*), but they also occurred in the susceptible species(*E. sieberi, E. marginata, A. melanoxylon, Xanthorrhoea australis, X. resinosa* e *Themeda australis*). Although the authors found necrotic cells in the epidermis and cortex of roots infected by *A. pulchella*, some cells remained healthy and did not develop large lesions. *A. melanoxylon* roots were quickly invaded, resulting in cytoplasm granulation and interruption. Lesions stopped growing after 48 to 72 hours in resistant species, and their size remained limited.

Induced resistance mechanisms, including phytoalexins accumulation, have recently received special attention from the biochemical, pathological and molecular point of view. These mechanisms contrast with the constitutive resistance mechanisms, such as thick cuticle and trichomas, which offer protection throughout the plant life cycle. Induced resistance mechanisms, which act after plant infection by the pathogen, are either absent in healthy plant tissues or are found at low concentrations detected during the resistance expression (Smith, 1996).

Genetic breeding for disease resistance in forest species: The state of the art of the classic plant breeding

The main objectives of forest genetic breeding are to reduce damage caused by disease and pests and produce trees adapted to grow in adverse environments. Breeding for genetic resistance to disease in trees is complex and is not determined by a simple Mendelian system (Zobel and Talbert, 1992).

In response to the growing impact of disease in forest plantations, some programs have emphasized breeding for disease resistance since, in beginning, the main concern was to improve the growth rate, trunk form and wood properties respectively (Carson and Carson, 1989).

Pathogens which cause diseases in trees are generally difficult to combat, and little is known about their genetic control. Lack of knowledge on the genetic interaction in tree/pathogen systems may be the main reason for the slow progress in research (Nance et al., 1992).

Breeding for disease resistance is most important in breeding programs for forest species. According to Carson and Carson (1989), greater attention has been paid recently to conifer breeding, including several *Pinus* species, although many leafy species such as *Eucalyptus* and *Gmelina* have also been studied.

Butcher et al. (1984) after detecting *P. cinnamomi* resistance genes in *P. radiata* populations, analyzed 49 elite families to localize genotypes resistant to this pathogen. The authors observed great variation in disease resistance among families and found nine resistant families among those studied. This consistency in the greenhouse and field results indicated that *P. cinnamomi* resistance in *P. radiata* was submitted to strong genetic control and was transmitted in response to the high heritability estimates at family level, 0.90 and 0.8% for the two greenhouse tests and 0.86% for the field tests. The authors concluded from these results that the great genetic variation for resistance to this pathogen in *P. radiata* enabled selection to start a new resistant population.

Van Der Kamp and Tait (1990) established a variation model for *P. contorta* susceptibility to *Endocronartium harknessii*. This model promoted an additional source of variation to the number of infections per tree, which was attributed to the random placement of spores in individual trees. Van Der Kamp (1993) analyzed the influence of the model in selection efficiency for resistant trees in natural populations that showed several severity levels of the disease, paying particular attention to the random deposit effect of the inoculum on the host on the selection efficiency. The author found that the variation model in relative resistance can be used to assess selection efficiency for resistance.

Ades et al. (1992) studied, in a series of experiments in Australia, the levels of disease severity caused by *Dothistroma septospora*, characterized by burnt aciculas, among *P. muricata* families and offspring, and compared them with the severity levels in several *P. radiata* origins. The authors verified 0.29 heritability for infected aciculas in a six year old progeny from trees of *P.muricata* derived species, and 0.29 heritability for infected aciculas, which was similar to the estimates found for some characteristics in *P. radiata*. This inheritance that, although this population is less susceptible than the more resistant *P. radiata* derived species, there was great variation in the inheritance and resistance obtained by the selection among derived species.

Tippett et al. (1989) analyzed the effect of sites and seasons of the year on *E. marginata* susceptibility to *P. cinnamomi*. The effect of tissue water content, temperature, and carbohydrate phenol concentrations on the invasion rate of the fungus in the secondary phloem was assessed. The authors found that trees that grew in sites with water shortage were less vulnerable to the pathogen than those which grew in wet sites in the summer. The carbohydrate and phenol concentration changed throughout the different seasons of the year and differed among sites,however, no evidence was found that these changes influenced the fungus growth in the plant tissues directly.

Recently, new techniques and technologies have become important tools in disease resistant plant selection. While they do not replace conventional plant breeding, they help to look for new ways to reach objectives not attained by conventional techniques, reducing the time needed to obtain resistant genotypes.

Initial studies on *A. mearnsii* genetic material selection for gomose were carried out by Resende et al. (1993) and Santos et al., (1998). These authors discussed the genetic variability in *A. mearnsii* and the possibility of selecting resistant

material.

Sobrosa (2001) studied the genetic control of resistance to gomose in Black Wattle individuals (*A. mearnsii* De Wild.), and set up open pollination progeny tests which were designated susceptible or resistant depending on the symptoms manifested in the following maternal trees: PS1, PS2, PS3, PS4, PS5, PS6, PS7, PS8, PS9 and PS10. *Two Cylindrocladium* sp. (I1 and I2), two *Fusarium* sp. isolates and a *Pestalotia* sp (I5) isolate were used. The heritability estimates at progeny mean levels showed high values for the first (h^2p=0.54) and for the second test (h^2p=0.53), indicating that this characteristic has a high degree of genetic control. Highly susceptible individuals were identified among the resistant and the susceptible progeny.

Use of biotechnology as a tool for genetic breeding towards disease resistance in forest species.

Tissue culture and disease resistance

Tissue culture has contributed significantly to plant pathology to elucidate basic pathogen virulence mechanisms and host resistance. Studies have focused on virus infection and replication, but important contributions have also been made concerning the toxins action, the resistance response and physiological changes that occur in plant cells infected by fungi and bacteria (Daub, 1986).

Bronson et al. (1992) studied interactions among organisms to assess disease resistance using selection agents such as pathogen propagules, purified or partially purified phytotoxins, and in some cases, pathogen culture filtrates.

Systems of pathogen co-culture with host plant tissues are used in the study of the relationship between the two organisms under controlled conditions to investigate pathogenicity mechanisms and resistance at cell level (Duval et al., 1998).

Daub (1986) reported on some studies using pathogens to select disease resistant plants. Most of these selections involved virus and, generally, protoplasts were infected. However, the protoplasts were not completely infected and susceptible protoplasts did not die. According to the author, the use of pathogen toxins solved one of the problems found in pathogen selection. Cell cultures were easily and uniformly exposed to toxins by either cell dispersion in a toxin solution or culture filtrate containing toxins.

Both callous and protoplast culture can be used to find disease resistance. Several strategies have been used to obtain plants resistant to fungi, bacteria and virus. These strategies differ in the resistance source and in the mutant selection and/or detection methodologies (Duval et al., 1998).

Jang and Tainter (1990) described the expression of differential resistance in callous tissues of *Pinus* species in response to *P. cinnamomi*. *P. taeda*, *P. echinata*, *P. virginiana* and a hybrid of *P.taeda* x *P. echinata* were inoculated with *P. cinnamomi*. *P. taeda* and the hybrid were more resistant to infection and invasion by the fungus than *P. echinata* or *P. virginiana*. The authors identified two types of reaction among the clones of each species. The resistance reaction showed sparse hypha growth on the callous surface with little or no visible injury in the cells. Some morphological changes in the host cell wall were also detected. The susceptibility reaction showed extensive hypha growth of the fungus and superficial cell collapse. Jang and Tainter (1991) compared the best nutritional medium temperature and growth regulators for callous induction in *P.taeda*, *P.echinata*, *P. virginiana* and a *P. taeda* x *P. echinata* hybrid and assessed their effect on *P.cinnamomi* growth and development in the tissues of these callous. The modified Murashige and Skoog medium (1962) containing 10-5M 2.4-diclorphenoxiacetic acid (2.4-D) at 26°C in the dark, caused differential resistance expression after inoculation. The authors observed that high 2.4 - D concentration inhibited the pathogen growth in the callous tissues.

Ragazzi et al. (1995) used the callous inoculation method to test the response of *Pinus nigra* var. *laricio* to *Cronartium flacidum*. The callous tissues were inoculated with fungus cultures obtained by basidiospore incubation in modified Schenk and Hildebrandt culture (1972). Colony growth was faster in *P. nigra* var. *laricio*, but sparse in *P. sylvestris*. Hypha growth areas were

abundant in *P. nigra* but less frequent in *P. sylvestris*. Hypha branching began after 18 hours in *P.nigra* and after 45 hours in *P. sylvestris*. According to the authors, these results were consistent with the resistance of the two species in whole plants.

Somaclonal variation was proposed as a variability source to be exploited in breeding programs, and in developing disease resistant plants. The spontaneous and random genetic changes may be further detected *in vitro*, or selected after planting in the field (Daub, 1986).

Hammerschlang et al. (1995) reported the possibility of selecting somaclonal variants of peach (*Prunis persica*) by tissue culture techniques. The authors detected an increase in resistance levels to bacterial leaf spots caused by *Xantomonas campestris* in *in vitro* peach regenerates, when submitted to selected and non-selected toxins, in a greenhouse and in the field. Peach regenerates also increased resistance levels to *Pseudomonas syringae* and *Meloidogyne incognita.*

The tissue culture technique has become an important tool in plant breeding to develop disease resistant plants through either micropropagation or cell culture in selective medium, in the presence of fungi phyptotoxins, thus contributing to the *in vitro* selection of genetic variants resistant to certain diseases.

Genetic markers in disease resistance

Molecular genetics explains fungus pathogenicity mechanisms and studies variation in pathogenic fungi (Leong and Holden, 1989).

Genetic markers are fundamentally important in the study of genes or groups of genes associated with disease resistance (Vega, 1997).

According to Klopfenstein et al. (1993) markers are even more important in forest species for selecting individuals in the first stages of development.

The use of different markers, as a tool in pathogen-host relationship studies, depends on the objective of the study. Thus isoenzymes were successfully used in the study of variability in *Colletitrichum orbiculare* and in the identification of the nematode species *Meloidogyne* spp. (Rego et al., 1994; Alonso et al., 1995).

The *Populus* genus is propagated to create monoclonal forests and has presented fragility to pathogen attack (Cervera et al., 1996). The most important diseases found in central and northern Europe were leaf rust, caused by the *Melampsor laricci* fungus, a bacterial canker caused by *Xantomonas populi* and leaf spot, caused by the *Marssonini brunnea* fungi. The authors report the use of AFLP type markers (Amplified Fragment Length Polymorphism) in the study of *M. laricii* resistance mechanisms, which caused premature defoliation and reduced growth by more than 20%. Crosses among resistant species, such as *Populus nigra*, and susceptible species, such as *P. deltoides*, were analyzed. Fifty percent of the variation for resistance was explained by one gene with a large effect, and 50% by additive effect genes. The BSA (Bulked Segregant Analysis) technique was also used for *Populus* to detect resistance genes along with the *pseudo-tesscross* strategy for mapping.

The use of molecular markers, such as RFLP (Restriction Fragment Length Polymorphism) helped in the development of more advanced statistical methods to detect genes responsible for quantitative characteristics, denominated QTL (Quantitative Trait Loci) which permit locate genomic regions with QTLs. According to Bergamin Filho et al. (1995), in an extensive review, the use of these methods in quantitative resistance gene mapping in some pathosystems did not reveal a great number of genes.

Dirlewanger et al. (1996) analyzed *Sphaerotheca pannosa* resistance in hybrid *Prunis persica* x *P. davidiana* progeny. The mapping consisted of 15 RAPD markers (Random Amplified Polymorphic DNA) distributed in four linkage groups. A QTL with greater effect was detected in *P. davidiana*, and five QTLs with lesser effect in different linkage groups.

Genomic mapping was used to identify the region of the *P. taeda* genome which determines resistance to rust caused by *Cronartium quercuum*. One resistance locus behaving as a simple dominant gene was mapped by association with RAPD genetic markers. The importance of genomic mapping was emphasized as an instrument to characterize the genetic base of the pathogen-host interactions in forest species (Wilcox et al., 1996).

According to Yang and Kruger (1994), apple tree resistance to the *Venturia inaequalis* fungus is regulated by the Vf gene. The BSA strategy and RAPD markers were used to study and map this gene. The DNA from five resistant individuals and from ten susceptible varieties was used. The markers obtained were used in the assisted selection in a program of resistance gene introgression. Tartarini (1995) reported the existence of eight larger genes in the apple tree that control resistance to *Venturia inaequalis*. The allelic forms of these resistance genes have been identified in several species. Variation in resistance observed in segregant progeny indicated the presence of genes with smaller effect. According to the author, the progeny used to map the resistance genes came from the *Prima x Golden delicious* cross. Both the RAPD molecular marker and BSA strategy were used.

Introgression of interesting genes is of fundamental importance, although it presents some difficulties such as (i) the use of populations or species that contain the genes of interest that present low quality for the other commercially important characteristics; (ii) the need for many backcross generations to recuperate the characteristics of the required parent and (iii) the high cost of the selection of individuals containing the gene of interest and the characteristics of the required parental (Nance et al., 1992).

For *Castanea dentata*, susceptible to the *Cryphonetria parasitica* fungus, an introgression strategy of resistance genes from the *Castanea mollissima* species was used, which are resistant but of lower commercial quality. RFLP markers were used in the assisted selection of genotypes with the resistance genes in an introgression program (Bernatzky and Mulcahy, 1992).

The use of genetic markers in forest species to study disease resistance mechanisms is an important tool. Thus mapping of larger genes linked to resistance, study of genetic variability in fungus isolates, assisted selection of resistant genotypes, monitoring of genes of interest in introgression studies and QTL mapping have been successfully accomplished.

Genetic Engineering in disease resistance

The breeding cycle in trees can be accelerated by genetic engineering techniques that allow the transfer, in a tissue culture cycle, of characteristics that are mongenic or oligogenic. This solved the problem of transferring a set of chromosomes in conventional plant breeding which can result in undesirable genotypes in a single generation (Seguin et al., 1998).

Research to obtain transgenic trees has been carried out. Several studies focus on the development of protocols to construct plasmideum containing the gene of interest (Hooykaas and Schilperoort, 1992; Leple et al., 1992; Macrae and Staden, 1993; Aronen et al., 1995; Confalonieri et al., 1995; Ebinuma et al., 1997); lignin modification (Boudet and Pettenati, 1996); herbicide and insecticide tolerance (Shin et al., 1994; Donahue et al., 1994; Bauer, 1997); alteration in wood characteristics (Tuominen et al., 1995) and the development of tissue culture protocols to regenerate the transformed plants (Block, 1990; Robertson et al., 1992; Chupeau et al., 1994). Studies were carried out with angiosperms and gimnosperms to obtain disease resistant trees, but gene transfer procedures were only frequent with certain *Populus* hybrids (McCown et al.,1991; Jouanin et al., 1993; Schuerman and Dandekar, 1993; Klopfenstein, 1993).

In the search for fungus resistance, genes have been introduced in plants that code for enzymes that act in the hydrolysis of cell wall components, such as the quitinases and glucanases that act on the cytoplasmatic membrane, modifying its permeability. Transgenic plants that express these enzymes are expected to present resistance to pathogenic fungi (Brasileiro and Dusi, 1999).
Studies have been carried out at the molecular level to explain host resistance genetic mechanisms that may facilitate pest resistance in trees in two

ways: resistance gene characterization in plants and regulatory systems that generate information for rapid selection of resistance traits, (ii) resistance systems in trees that may be based on genetic transformation with identification of pest resistance genes and regulatory sequences (Klopfenstein et al., 1993).

McCown et al. (1991) report that DNA incorporation in trees and the subsequent regeneration of the transformed plants was detected only in some genera, using Agrobacterium as vector to transfer the gene of interest. The authors were successful in direct gene transference with acceleration of particles by electrical discharge in hybrid genotypes, such as *Populus Alba X Populus gradidentata* and *P. Nigra* x *P. trichocarpa*. Plasmideum containing neomicine phophotranferase (NOS-NPT), the constitutive promoter of the cauliflower mosaic virus containing b-glucuronidase (CaMV35S-GUS) and *Bacilus turingensis* (CaMV35S-BT) were used in the transformation. Four transformed *P. alba X P. gradndidentata* hybrid plants containing all the three genes were recuperated and analyzed. Two expressed b - glucuronidase (GUS) and one was highly resistant to two lepidopteras (*Malacosoma disstria* and *Lymantria dispar*).

In a study on breeding for pest resistance, Klopfenstein et al. (1993) analyzed two *Populus* hybrids, which were transformed with defense genes from quimeric plants developed based on the pin2 gene (potato proteinase inhibitor II). A binary vector system of Agrobacterium was used in the transformation of these hybrids. The expression of genes was conformed in *Populus* transformed with Nos PIN2 or 35S-PIN2. *Populus* transgenics expressing PIN2 showed resistance to insects (*Plagioder versicolora* and *Chrysomela scripta*) and fungi pathogens (*Septoria musiva* and *Melampsora medusae*).

Genes were transferred to apricot (*Prunis armeniaca*) and plum (*Prunus domestica*) cultivars in different systems, using material from juvenile and adult plants. The transformation of these cultivars with *Agrobacterium tumefasciens* was reported, containing several binary pBINGUSINT plasmideum carrying the marker gene b-glucuronidase (GUS) and pBINPPVm, carrying the gene of the protein coat of the plum smallpox virus (PPCV), the sharka disease pathogen (Machado et al., 1995).

The introduction of genes of interest in plants, by genetic engineering, is becoming an additional strategy to be included in plant breeding. Genes from distinct plant species or from organisms which present genetic distance may be introduced in the plant genome by genetic engineering (Brasileiro and Dusi, 1999).

RESUMO

Melhoramento genético e biotecnológico para resistência a doenças em espécies florestais: Uma revisão

As pesquisas na área de melhoramento genético para resistência a doenças em espécies florestais têm apresentado importantes resultados, nos últimos anos. A maioria dos estudos tem sido realizados em famílias e em árvores individuais dentro de famílias. A resistência induzida depende de fatores presentes somente após o contato do patógeno com o hospedeiro. Por definição, é o oposto da resistência constitutiva, que depende de fatores pré-formados. Plantas com resistência a doenças, que envolvem mecanismos de defesa induzida, incluem acúmulo de fitoalexinas, deposição de material semelhante à lignina e aumento na atividade de certas enzimas hidrolíticas. Em resposta ao impacto crescente de doenças em florestas plantadas, alguns programas têm enfatizado mais o melhoramento para resistência a doenças. Novas técnicas e tecnologias tornaram-se importantes ferramentas na seleção de plantas resistentes a doenças, certamente não visando a substituir o melhoramento genético convencional, mas buscando formas de alcançar objetivos não atingidos pelas técnicas convencionais. A técnica de cultura de tecidos tornou-se uma importante ferramenta no melhoramento para obtenção de plantas resistentes a doenças através da micropropagação ou pelo cultivo de células em meio seletivo, assim como o uso de marcadores genéticos para estudar mecanismos de resistência. Para acelerar o ciclo de melhoramento em árvores, técnicas de engenharia genética possibilitaram transferir, em um ciclo de cultura de tecidos, características que são monogênicas ou

oligogênicas. A introdução de genes de interesse em plantas, através da engenharia genética, está se tornando uma estratégia adicional a ser incluída no melhoramento genético de plantas. Genes provenientes de espécies vegetais distintas ou mesmo de organismos que apresentam distância genética entre si podem ser introduzidos no genoma vegetal através da engenharia genética.

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