UNIVERSIDADE ESTADUAL PAULISTA “JÚLIO DE MESQUITA FILHO”
FACULDADE DE ENGENHARIA
CAMPUS DE ILHA SOLTEIRA

MAXIMILIANO KAWAHATA PAGLIARINI

GENOTYPE BY ENVIRONMENT INTERACTION IN SLASH PINE AND METHODOLOGIES COMPARISON FOR RADIATA PINE WOOD PROPERTIES

Ilha Solteira
2016

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Thesis presented to Faculdade de Engenharia of Ilha Solteira – UNESP as part of the requirements for obtaining the title of Doctor. Speciality: Production System.

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Ilha Solteira
2016

FICHA CATALOGRÁFICA
Desenvolvido pelo Serviço Técnico de Biblioteca e Documentação

Pagliarini, Maximiliano Kawahata.
P138g Genotype by environment interaction in slash pine and methodologies comparison for radiata pine wood properties / Maximiliano Kawahata Pagliarini. -- Ilha Solteira: [s.n.], 2016
135 f. : il.

Tese (doutorado) - Universidade Estadual Paulista. Faculdade de Engenharia de Ilha Solteira. Especialidade: Sistema de Produção, 2016

Orientador: Ananda Virginia de Aguiar
Inclui bibliografia

1. Pinus elliottii Engelm. var. elliottii . 2. Pinus radiata D. Don. 3. Genetic diversity. 4. Genotype by environment interaction. 5. Pilodyn. 6. Silviscan.

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Ilha Solteira

CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: GENOTYPE BY ENVIRONMENT INTERACTION IN SLASH PINE AND METHODOLOGIES COMPARISON FOR RADIATA PINE WOOD PROPERTIES

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Ilha Solteira, 20 de maio de 2016

To God for life and received opportunities.

To my parents Miguel Pagliarini and Antonia Dalva Kawahata Pagliarini that raised me with love, respect and dedication.

In the middle of many difficulties,

They had love, courage,

Persistence and wisdom,

Allowing me to move on forever.

I DEDICATE

OFFERING

To my sister Juliana, my brother-in-law Anderson and my friends: Thiago Marchetti, Adilson Araújo, Alexandre Cardoso, Alfredo Zambrano, Andressa Lopes, Barbara Moreira, Carla Gordin, Carolina Arias, Cleber Barufaldi, Danielle Camara, Erica Moreira, Federica Brunoni, Flávia Mariano-Nasser, Giselle Arnaud, Haleh Hayatgheibi, Heverton Schneider, Juliana Moreira, Juliana Rebequi, Juliana Santos, Kaio Barcelos, Maria Cecília Cavalini, Maurício Nasser, Natália Blaite, Regina Castilho, Sandro Naia, Uéliton Silva e Veridiana Mendonça.

Dear and true friends.

ACKNOWLEDGEMENTS

I wish to thank everyone who helped me complete this thesis. Without their continued efforts and support, I would have not been able to bring my work to a successful completion.

To *Faculdade de Engenharia* of *Universidade Estadual Paulista*, Graduate course in Agronomy, speciality Production Systems, for hosting and learning conditions offered throughout the course.

To Embrapa Forests for internship opportunity and research development.

To Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES – by granting scholarships, in Brazil throughout agreement signed with Embrapa Florestas, and abroad throughout inter-university exchange program (PDSE/CAPES).

To my advisor Profa. Dra. Ananda Virginia de Aguiar, for friendship, trust, opportunity and teachings. Her competence, dedication and love for forest improvement are encouraging and aroused in me curiosity to develop this work. I will bring her into my life as example of researcher and mainly as person.

To my teachers who contributed to my professional formation since bachelor degree in particular teachers of UNESP graduate, research line "Genetics" and "Plant Breeding and Plant Propagation": Prof. Dr. Alexandre Magno Sebbenn, Profa. Dra. Ananda Virginia de Aguiar, Profa. Dra. Heloiza Ferreira Alves do Prado, Prof. Dr. João Antonio da Costa Andrade, Prof. Dr. Mário Luiz Teixeira de Moraes, Prof. Dr. Miguel Luiz Menezes Freitas, Prof. Dr. Pedro Cesar dos Santos who passed all their knowledge about plant breeding and quantitative genetics, essential knowledge to foundation and development of this work.

To Umeå Plant Science Center – Swedish University of Agricultural Science, for the possibility of exchange between universities, host and offered learning.

To supervisors in Sweden, Prof. Dr. Harry X. Wu e Prof. Dr. Anders Fries for teachings and competence.

To graduate program coordinator Prof. Dr. Mário Luiz Teixeira de Moraes, for opportunity and friendship.

To laboratory technician Selma Maria Bozzite Moraes, for teachings and friendship.

To Professors Dra. Daniela Araújo, Dra. Camila Regina Silva Baleroni Recco, Dr. Enes Furlani Junior, Dr. Mário Luís Teixeira de Moraes, Dr. Alan Rodrigo Panosso, Dr. Evandro Vagner Tambarussi and Dr. João Antonio da Costa Andrade for corrections in qualification, thesis and friendship.

To Empresa Pinara Reflorestamento e Administração Ltda. for seedlings production and field trials management.

To Embrapa – Forests technicians for experiment installation and conduction.

To graduate friends: Belisa, Cecília, Danilla, Diego, Francine, Janaina, Kelly, Maiara, Marília (e Mainha), Ricardo, Thaisa, Wanderley, Willian and all those who, directly or indirectly, in a special way, collaborated on another stage of my life.

To Embrapa Forests trainees Wesllen Kieras and Matheus Theodorovicz for their assistance in field data.

To my friends in Sweden: Ainhoa, Alfredo, Biyue, Carolina, Chen, Federica, Francisco (Paquito), Giselle, Haleh, Hong, Ilka, Irena, Jenny, Jhon, Mateusz, Mercedes, Pieter, RuiQui, Tamara, QuiFu, Sonali, Tomas; to my officemates: Delphine, Siamsa, Tomas, Ogona for good welcome and friendship.

To my friends in Brazil by living and support during the doctoral period: Thiago, Adilson, Alberto (Beto), Alexandre, Alexandre Andrade, Barbara Moreira, Carla, Cleber, Daniela, Danielle, Denis, Erica, Fernando Proietti, Fernando Rocha, Flávia, Guilherme, Heverton, Jorge, Kaio, Juliana Moreira, Juliana Rebequi, Juliana Santos, Kaio, Luis Neves, Maria Cecília, Maristela, Maurício, Natália, Regina, Sandro, Uéliton, Veridiana e Vilma.

To my grandmothers Glória and Honir; to my ants Fátima, Regina, Mitiko (in memorian), Ieda and Cenira; to my uncle Roberto (Beto); to my cousins Humberto, Adriano, Fernando, Bárbara, Barbara, Bianca, Bruno, Daniele and Gisele who always believed and cheered for my professional success.

To all who directly or indirectly contributed to this work.

Last but not least, to God for having guided me and always taking me to meet special people and achieve all my purposes.

“My scientific studies have afforded me great gratification; and I am convinced that it will not be long before the whole world acknowledge the results of my work”

Gregor Johann Mendel

ABSTRACT

Exotic forest species have been introduced in Brazil in order to promote improvements in socioeconomic development and help to reduce the pressure caused to native forests. With growing demand for these species, research on genetic improvement has increased to find new, more productive germplasm and preferably in less time. Two species were used in the study: slash pine (*Pinus elliottii* Engelm. var. *elliottii*) and radiata pine (*Pinus radiata* D. Don). The first part of the study had the purpose to identify the stability, adaptability, productivity and genetic parameters, in addition to selection gain and genetic divergence in slash pine open pollinated second generation progenies considering phenotypic trait. Two tests were established, one in Ponta Grossa-PR with 24 progenies and one in Ribeirão Branco-SP with 44 progenies, both in Brazil, to identify the most productive genotypes for commercial planting areas in both sites. There was significant variation ($p<0.01$) among progenies for growth and form traits. The high coefficients of genetic variation for wood volume (14.31% to 16.24% - Ribeirão Branco-SP and 31.78% to 33.77% - Ponta Grossa-PR) and heritability (0.10 to 0.15 – Ribeirão Branco-SP and 0.36 to 0.48 – Ponta Grossa-PR) have shown low environmental influence on phenotypic variation, which is important for the prediction of genetic gain by selecting and confirming genetic potential in both places, especially Ponta Grossa. The effect of genotype x environment interaction is simple. Progenies planted in one site can also be planted in the other. Among these C-197, C-189-1, C-084-2 and C-032-2 progenies are suitable for plantations in both studied region of São Paulo and Paraná. Although larger number of progenies in Ribeirão Branco, it was found the same number of clusters through UPGMA and Tocher methods in both tests. There is genetic diversity among slash pine progenies. For breeding programs, it is recommended to cross progenies between different groups to increase genetic variation, and consequently the genetic gain in subsequent generations, not forgetting to take into account the performance of interest trait. The objective of the study in Radiata pine was relate wood quality traits obtained from two methods Pilodyn and SilviScan to validate an efficient phenotyping methodology for a greater number of samples. A test with 30 progenies of Radiata pine was established in Flynn Australia. The evaluated traits were wood density, microfibril ange and modulus of elasticity. Genetic and phenotypic correlation between traits of wood quality obtained from two methods and narrow-sense individual heritability were estimated. The Pilodyn data showed high heritability and high genetic and phenotypic correlation between wood density and moderate with microfibril angle and modulus of elasticity. The results confirm that the Pylodyn is an effective indirect and rapid method for evaluation of genetic parameters for wood quality traits in Radiata pine.

Keywords: *Pinus elliottii* Engelm. var. *elliottii*. *Pinus radiata* D. Don. Genetic diversity. Genotype by environment interaction. Pilodyn. SilviScan.

RESUMO

Espécies exóticas de *Pinus* foram introduzidas no Brasil para promoverem o crescimento socioeconômico do país e ajudar na redução da pressão causada pelo uso de florestas nativas Com a crescente demanda por essas espécies, pesquisas em melhoramento genético tem aumentado na busca de novos germoplasma mais produtivos em menor tempo. Duas espécies foram utilizadas no presente trabalho: *Pinus elliottii* Engelm. var. *elliottii* e *Pinus radiata* D. Don. A primeira parte do trabalho teve a finalidade de identificar a estabilidade, a adaptabilidade, a produtividade e os parâmetros genéticos, além do ganho de seleção e diversidade genética em progênies de polinização aberta de segunda geração de *P. elliottii var. elliottii* considerando os caracteres fenotípicos. Foram estabelecidos dois testes, um em Ponta Grossa-PR com 24 progênies e outro em Ribeirão Branco-SP com 44 progênies visando identificar os genótipos mais produtivos para áreas de plantio comercial em ambos locais. Foi observada variação significativa ($p<0{,}01$) entre as progênies para os caracteres de crescimento e alguns caracteres de forma. Os altos coeficientes de variação genética para volume de madeira (14,31% a 16,24% - Ribeirão Branco e 31,78% a 33,77% - Ponta Grossa) e herdabilidade (0,10 a 0,15 – Ribeirão Branco e 0,36 a 0,48 – Ponta Grossa) mostraram baixa influência do ambiente na variação fenotípica, o que é importante para a predição do ganho genético mediante a seleção e confirmam potencial genético em ambos os locais, especialmente Ponta Grossa. O efeito da interação genótipo x ambiente é simples. As progênies plantadas em um local poderão também ser plantadas no outro. Dentre essas as C-197, C-189-1, C-084-2 e C-032-2 são indicadas para plantações tanto na região estudada do estado de São Paulo quanto do Paraná. Apesar de um número maior de progênies em Ribeirão Branco, constatou-se o mesmo número de agrupamentos de progênies pelo método UPGMA e de otimização de Tocher em ambos os testes. Existe diversidade genética entre as progênies de *P. elliottii.* Para programas de melhoramento, recomenda-se o cruzamento entre progênies de grupos divergentes para aumentar a variação genética, e consequentemente, o ganho genético nas gerações subsequentes, sem esquecer de se levar em consideração a performance do caráter de interesse. O objetivo do trabalho em *P. radiata* foi relacionar os resultados de características da madeira obtidas a partir de dois métodos Pilodyn e SilviScan visando validar uma metodologia eficiente para fenotipagem de um maior número de amostras. Um teste com 30 progênies de *P. radiata* foi estabelecido em Flynn na Austrália. As características avaliadas foram densidade da madeira, o ângulo microfibrilar e o módulo de elasticidade. A correlação genética e fenotípica entre os caracteres da madeira obtidas a partir dos dois métodos e a herdabilidade individual no sentido restrito foram estimadas. Os dados de Pilodyn apresentaram alta herdabilidade e alta correlação genética e fenotípica entre densidade de madeira e moderada com ângulo microfibrilar e módulo de elasticidade. Os resultados confirmam que o Pylodyn é um efetivo método indireto e rápido para avaliação de parâmetros genéticos para caracteres de qualidade madeira em *P. radiata.*

Palavras-chave: *Pinus elliottii* Engelm. var. *elliottii*. *Pinus radiata* D. Don. Diversidade genética. Interação genótipo x ambiente. Pilodyn. SilviScan.

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1 INTRODUCTION

Forest species are important for Brazilian economy as row material sources for timber and non-timber products used mainly in building, biomass for cellulose pulp and paper production, industrial energy source, as well as material for cosmetics, pharmaceutical and food industries (STUDART-GUIMARÃES et al., 2003). In 2014, planted forest area for industrial use in Brazil achieved 7.74 million hectares, and it has increased 1.8% compared to 2013. Two genus stand out on the national scenarios, first is *Eucalyptus* occupying 5.56 million hectares, which represents 71.9% of total established mainly in Minas Gerais (25.2%), São Paulo (17.6%) and Mato Grosso do Sul (14.5%) states. Second is *Pinus* with 1.59 million hectares concentrated in Paraná (42.4%) and Santa Catarina (34.1%) states (BRAZILIAN TREE INDUSTRY – IBÁ, 2015).

Pine species are widely used in homogeneous reforestation in many parts of the globe due to the large adaptation to climate diversity and wide application of their products (wood, resins, fibres, plates, pulp, paper, etc.) (SEBBENN, 1994). In Brazil, research institutions, universities and forest companies have conducted several researches for decades with this genus in breeding programs and silvicultural management (MOURA; VALE, 2002). The species introduced in Brazil are from many places occurring naturally in North and Central America, northern Europe and Asia (LIMA; JARÁ; ALFONSO, 1988).

Based on forest industry demand, trees with desirable phenotypic traits have been selected over time and incorporated into breeding programs in order to obtain more productive genotypes (GARTLAND, 2003). Although improvement programs have contributed significantly to increase productivity in forestry sector, they have run into consequent limitations of intrinsic characteristics, especially pine species, highlighting the long term needed for plants reach a phenotypic stability, reproductive maturity and difficulty of control in crosses between species (DIOUF, 2003; POUPIN; ARCE-JOHNSON, 2005).

Early pine researches were held in 1936 in the Garden of Cantareira in São Paulo state, at that time called Serviço Florestal of São Paulo (now Instituto Florestal of São Paulo - IFSP). European species were introduced first, however, they failed to succeed due to their poor adaptation to the tested region climate. From 1948, species introduction studies have been intensified, with special attention to "southern yellow pine of United States "(*Pinus elliottii*, *Pinus taeda*, *Pinus palustris* and *Pinus echinata*) (PINTO JÚNIOR et al., 2013).

Initially, in Brazilian forests, subtropical species as *Pinus elliottii* and *Pinus taeda* highlighted in the South and Southeast regions of the country. From 1960s, with expansion of forest sector, tropical species have been brought to other regions such as *Pinus caribaea*, *Pinus oocarpa* and *Pinus patula* (SHIMIZU, 2005a).

Slash pine (*Pinus elliottii* Engelm. var. *elliottii*) has been planted in cold and high regions of São Paulo and Paraná states because of similar environmental characteristics where the species comes from. It is used for timber production aiming mechanical processing and resin extraction. Although this species is not the most productive in volume it produces high-quality physical and mechanical wood. Production begins at age seven or eight with wood for processing and use in structures, carpentry, packaging and building. Good adaptability to different environmental conditions such as shallow, stony and soaked soils; frost tolerance; produces few branches which implies higher production of clean wood (without knots) as well as being slightly attacked by wasp-of-wood (AGUIAR et al., 2011; SHIMIZU, 2008).

With introduction of new species and the demand for more wood it was necessary to produce more in less time, therefore, breeding programs for each species needed to be developed. The main objectives of breeding programs are: increment of productivity, obtainment of higher quality raw materials, improvement in adaptive species conditions and maintenance of genetic variability, fundamental requirement for obtaining genetic gains in long term (ZOBEL; TALBERT, 1984). Although more wood production in a shorter period of time has been essential, the quality of this wood must be taken into consideration to achieve market standards requirement. Among the most important wood quality traits, it is possible to highlight wood density, wood stiffness (measured as its modulus of elasticity – MOE), and microfibril angle (MFA). Nevertheless, a serious problem faced by research is how to measure wood properties. Methods may be expensive, slow and mainly destructive (WU et al., 2010). Therefore, non-destructive methods started to be tested and considerably reduced those limitations (WENG et al., 2015).

Non-destructive methodologies for assessment of wood quality traits have been studied for years in many pine species and two of them may be highlighted. For measuring the density variation on field, a method to be applied may be the use of an instrument called Pilodyn (MICKO et al., 1982). The depth penetration of the pin is indicated on the instrument and readings are correlated with the wood density. Pilodyn does not provide density estimates, but the instrument has already been calibrated for trees wood density measurements in several studies (HÖGBERG et al., 2014; CHEN et al., 2015). The second technique estimate wood quality traits based on data provided by SilviScan (CIESZEWSKI et al., 2013; HONG et al., 2015). The equipment is used to evaluate the properties of wood from pith to the bark quickly using a combination of X-ray densitometry, X-ray diffraction and image analysis (BALL; McCONCHIE; COWN, 2005).

Thus, the objectives of this study were: (*i*) estimate genetic parameters for quantitative traits in slash pine progenies, aiming early selection for wood production, (*ii*) identify more productive genotypes for commercial planting in São Paulo and Paraná states, (*iii*) verify genotypes productivity, stability and adaptability deployed in two different soil and climatic conditions in São Paulo and Paraná states from MHVG (harmonic mean of breeding values) statistical procedures, PRVG (relative performance of breeding values) and MHPRVG (harmonic mean of breeding values relative performance) predicted via BLUP, (*iv*) estimate the genetic distance among progenies through growth and form traits by multivariate procedures in order to guide future hybridization in breeding programs aiming wood production and (*v*) estimate narrow-sense individual heritability for Pilodyn penetration and wood quality traits for radiata pine progenies, (*vi*) verify whether Pilodyn penetration measurements may predict wood density, MFA, MOE according to phenotypic and genotypic correlation with SilviScan measurements for radiata pine progenies.

2 LITERATURE REVIEW

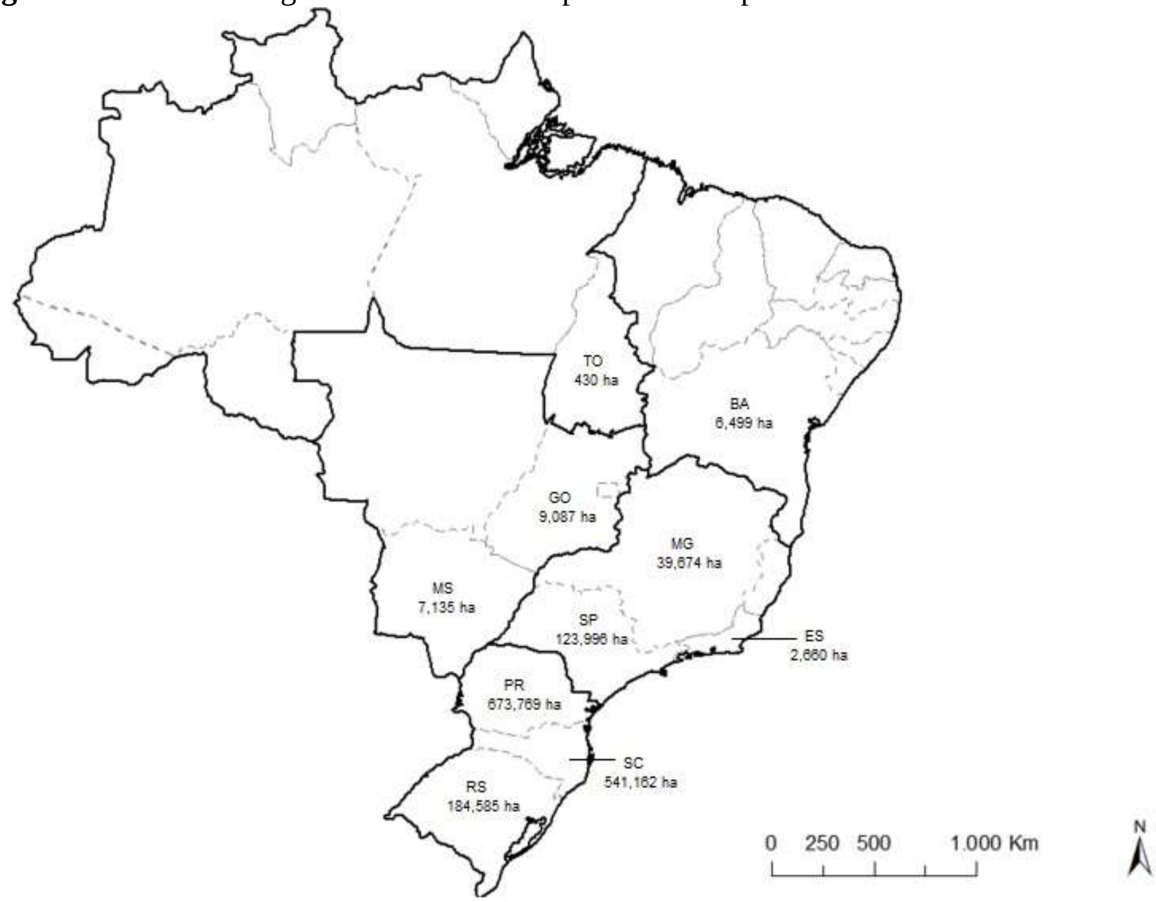
2.1 THE GENUS *Pinus*

Exotic forest species have been introduced in Brazil in order to promote improvements in socioeconomic development and help to reduce the pressure caused to native forests (SAMPAIO et al., 2000). European immigrants introduced *Pinus* genus in decade of 1930s aiming wood production and garnish purpose (FIER; KIKUTI, 1993). However, due to climate differences from origin place, there was no success adaptation for introduction of first species. Several plants originating from United States, Mexico, Central America, Caribbean Islands and Asia were later introduced. But, species from North and Central America have better adaptation and economic importance (SILVA, 2005).

The main use of these species is as a source of raw material for wood, resin, pulp and paper industries. The establishment and management of planted forests with pine have enabled the timber supply, which, previously, was supplied by Brazilian pine (*Araucaria angustifolia* Kuntze) exploration. Thus, this practice has established itself as an important ally (confederate) of native forest ecosystems supplying an increasing necessity of wood (SHIMIZU; MEDRADO, 2005). The potential of pine species as wood production for industrial processing was the main motivation for many species and provenances testing by government agencies and private companies (SHIMIZU; SEBBENN, 2008; PIRES et al., 2013; MOREIRA et al., 2014).

Pine species are valuables by light colour wood, ranging from white to yellowish; softwood, suitable for high-strength paper manufacturing for packaging, newsprint and other kinds of papers; possibility of resin extraction on a commercial scale, in some species; including hardiness and tolerance for agriculture marginal soils aggregating value to the land with wood production (SHIMIZU; MEDRADO, 2005). Large areas, in Brazil, have been planted with this genus taking advantage of tax income to provide the demand of wood, pulp and paper industries. According to the Brazilian Institute for Forest Development - IBDF in 1986 the area planted with this genus, through tax incentives, in South and Southeast regions totalled 1.7 million hectares, 400,000 ha in São Paulo state (AZEREDO, 1988). Currently, the planted area reduced to 1,588,997 ha and it is concentrated in the South region (88.1%), due to environmental conditions and location of processor centres for this type of wood, mainly in Paraná (42.4%) and Santa Catarina (34.1%) states (Figure 1) (IBÁ, 2015).

Figure 1 – Percentage distribution of pine forest plantations in Brazil in 2014.



Source: Ibá (2015) and adapted by the author.

2.2 MAIN GENUS *Pinus* USES

Pine wood is a material with mechanical strength comparable to steel, safeguarding all proportions. However, their use is not widespread, due to some characteristics still considered disadvantageous in relation to other materials (SUN; HAWKE, 1996).

Physical and mechanical properties of wood are important regarding to the applications of each material. Thus, combined with other aspects (economic, aesthetic, durability, workability, etc.), wood can be classified and grouped into more suitable uses as structures, use indoor and outdoor housing, furniture, panels, packaging, etc. (MORAES NETO; TELES; RODRIGUES, 2009). Among main wood physical properties, we can highlighted density (weight per volume) and dimensional stability (shrinkage and swelling due to moisture content). For mechanical properties the most important are resistant to compressive stresses, flexural, tensile, shear and cracking (ARAÚJO, 2002).

Wood chemical components can be grouped in two: cellulose with high molecular weight components and hemicelluloses and lignin with low molecular weight components (PANSHIN; ZEEUW, 1970). According to Fengel and Wegener (1989) proportions and chemical composition differ in conifers and broadleaved trees. While in conifers cellulose and hemicellulose content is 42% and 27%, respectively, in broadleaved trees content is higher than 45% and 30%. Regarding, conifers have higher lignin content than broadleaved trees (28% and 20%, respectively).

Many pine species have advantage of multiple uses, in addition to wood production, it is possible to include the recovery of degraded areas and pulp and paper production (RIGHT; SABOURIN; DVORAK, 1995). In this case the growing demand for pulp and paper, globalization of its markets and environment concernment have been increasing researches to find new appropriate raw materials and less polluting. In Brazil, the pine wood is an example due to the intensity of studies for this genus (BIRKLAND, 1990). Furthermore, secondary wood presents tracheids and resiniferous ducts enabling resin production (SUASSUNA, 1977).

2.3 CHARACTERISTICS OF PINE WOOD

The use of pine wood emerged as an alternative to lack of Brazilian pine wood and other native species from south country. Due to the inherent genus characteristics as good slightly acidic soil adaptation, resistance and easy field deployment, adaptation was possible (COELHO et al., 2010). Therefore, characterize the mechanical behaviour of wood, for example, is necessary to take into account some anatomical, physical and chemical properties to indicate certain species to certain location.

Genetic analysis of conifers wood quality properties were held, mainly in larger scale for economically important species such as *Pinus radiata* (BALTUNIS; WU; POWELL, 2007; WU et al., 2007, 2008), *Pinus taeda* (ISIK; MORA; SCHIMLECK, 2011; ANTONY et al., 2011) and *Pinus sylvestris* (HONG; FRIES; WU, 2015). Most of these studies have focused on evaluating wood density, wood stiffness (by modulus of elasticity – MOE) and microfibril angle (MFA). In *Pinus sylvestris*, genetic parameters for wood mechanical components were examined and the conclusion was that selective breeding for wood quality traits should result in considerable genetic gain (HANNRUP; EKBERG, 1998; FRIES, 2012; HONG; FRIES; WU, 2014).

2.3.1 Wood density

Basic density is closely linked to wood quality and it is well used by researchers and breeders to determine final use of wood. Species, provenances and progenies of pine have shown highly wood density variation. This trait presents high heritability, according to growth traits. In forest genetic improvement, selections are usually directed to growth and form aspects, such as gains in volume and form. In the other hand, less attention has been given to technological aspects and wood quality. These are considered only in the secondary selection processes (BRASIL; NICCOLLETO; VEIGA, 1980).

Wood density is expressed as the ratio between dried green mass and volume of sampling unit (BOWYER; SHMULSKY; HAYGREEN, 2007). It is often referred as "density" or dry weight divided by volume of water-saturated wood. The relationship between mass and volume is one of the simplest and most important functional properties of wood index and reflect different attributes for life story of tree species (MULLER-LANDAU, 2004; WILLIAMSON; WIEMANN, 2011).

Despite of complex nature of this property, variations in wood density may be difficult to interpret because certain density value can be derived from various combinations of components (GASPAR et al., 2008), for instance, precipitation is positively linked to wood density, mainly winter rainfalls. Dry season can cause temporary reduction in growth resulting false rings (MEAD, 2013). In addition to this, silvicultural practices as thinning and fertilizing can interfere in wood density, after thinning, wood density can reduce (WILKES, 1987; COWN, 1999) and after fertilizing deficient sites tends to restore density (GERISCHER; VILLERS, 1963). Moreover, density is different along the stem where pith presents higher values than the bark.

2.3.2 Wood stiffness (Modulus of elasticity)

Modulus of elasticity (MOE) or stiffness is a portion of the distortion that wood undertakes when subject to an applied weight, and it is measured as a ratio between stress and tension. It is a property of particular interest to farmers and processors of structural lumber as it determines the end-use and value of lumber cut from a tree (ANTONY et al., 2012). According to Roth et al. (2007) and Soto, Valenzuela and Lasserre (2012), wood with high MOE is more rigid material, and, therefore, harder to deflect, thus, high MOE values mean better wood quality.

MOE is also a key property for determining quality of laminated veneer wood. Low MOE in radiata pine, for example, restricts exploitation possibilities, and is more predominant when trees are developed on fertile sites over short rotations designed for fast achievement of large piece-size (BURDON; BRITTON; WALFORD, 2001).

Corewood MOE is highly variable and affected by genetic, environmental and silvicultural influences (DUNGEY et al., 2006; WATT et al., 2009; WAGHORN; WATT; MASON, 2007). In softwood of radiata pine and slash pine huge genetic difference in wood stiffness has been found at young trees, with medium to high heritabilities (KUMAR, 2004; DUNGEY et al., 2006; LI et al., 2007). As wood density, corewood MOE may be influenced by site conditions and silvicultural practices ranging widely across environmental gradients (WATT et al., 2009).

The wood stiffness of many pine species is largely correlated upon their density, microfibril angle (MFA) and MOE. According to researches high MFA and low specific gravity are found near the pith (corewood zone) associated with low MOE. The opposite is also true, low MFA, high specific gravity and MOE means stiffer wood, mainly near the bark (outerwood zone) (DOWNES et al., 2002; ANTONY et al., 2012; FUJIMOTO et al., 2015; WESSELS et al., 2015).

2.3.3 Microfibril angle

Microfibril angle (MFA) is the average orientation of the cellulose microfibrils in the S2 layer of the wood cell wall in relation to longitudinal axis. It is one of the key determinants of solid wood performance due to its strong effect on stiffness, strength, and shrinkage properties and dimensional stability of structural wood (CAVE; WALKER, 1994), timber properties improve as far as MFA decreases (WINCK; FASSOLA, ÁREA, 2015). In conifers, values of MFA are commonly higher near the pith (usually 25° to 30° in the first few growth rings) and decline fast in the first 10 to 15 growth rings before forthcoming more stable value (approximately 10° to 12°) in mature wood (BARNETT; BONHAM, 2004; ALTEYRAC; CLOUTIER; ZHANG, 2006). Despite the influence of the MFA in wood properties research is limited because its difficulty to measure, however, some authors used orientation scores and crossing fields on tracheids wall for measuring MFA as it is indicative of microfibrils alignment (WINCK; FASSOLA, ÁREA, 2015).

2.3.4 Methodologies for wood quality traits evaluation

Accurate measurements of wood quality traits require laboratory measurements with destructive samples use, which is not applicable for monitoring purposes, therefore, non-destructive techniques started to be studied. Non-destructive measurements is the evaluation of a material's properties without damaging its end use (ROSS; BRADSHAW; PELLERIN, 1998). The eldest non-destructive evaluation of wood was visual examination, mostly used for load-carrying members classification (BUCUR, 2006). A machine stress assessment system was introduced lately, which is one of the most used methods in lumber grading and has been commercially used since the 1960's (GALLIGAN; McDONALD, 2000). Advances in equipment have made it possible to use scientific non-destructive tools for the last two decades. Vibration and ultrasonic wave velocity are mainly significant in finding, for instance, modulus of elasticity. Ultrasonic wave velocity has more benefits over other methods in practical terms (ESTEBAN; FERNANDEZ; DE PALACIOS, 2009).

Another developed non-destructive technique with many studies is near infrared spectroscopy (NIRS). It has high potential for the fast evaluation of various wood characteristics, including wood stiffness (TSUCHIKAWA; KOBORI, 2015; FUJIMOTO et al., 2015). A distinguished benefit of NIRS is that it can be used to measure multiple traits concurrently. When selection is done to improve economic values of tree growth and wood properties, it is commonly applied to several traits all together and not just one, because economic value depends on more than one trait (FALCONER; MACKAY, 1996).

For measuring density variation range at field level a method to be applied may be the use of spring call Pilodyn (MICKO et al., 1982), which is an instrument originally developed for determining rot degree of telephone poles (HANSEN, 2000). Pilodyn measures the resistance to penetration of wood when a steel pin with a determined force is introduced into it by a spring (SPRAGUE et al., 1983; GREAVES et al., 1996; LEE; CONNOLLY, 2010). Penetration depth of pin is indicated on the instrument and readings are correlated with wood density. The instrument itself does not provide density estimates, however, it was calibrated for wood density measurements in several studies (MICKO et al., 1982; SPRAGUE et al., 1983; HÖGBERG et al., 2014; MURPHY; COWN, 2015). Another way of analysing physical wood properties is through X-rays methodologies. Many equipment has been developed including the SilviScan. It is a set of automated tools for rapid assessment of physical wood properties, using a combination of X-ray densitometry, X-ray diffraction and image analysis. SilviScan is used for its ability to evaluate these properties from pith to bark in a quickly way (BALL; McCONCHIE; COWN, 2005).

Many technologies have been proposed and developed along the years and all of them have their advantages and disadvantages. It is up to each breeder to test the possibilities to find the best way to evaluate wood quality traits either using existing methodologies or creating new ones.

2.4 *Pinus elliottii* ENGELM. var. *elliottii*

This species is commonly known as slash pine or Florida pine. The origin centre is located in the south-eastern North America at Alabama, Florida, Georgia, Louisiana, Mississippi, North Carolina, South Carolina and Texas states (Figure 2).

A special feature of slash pine is the tolerance to wet soils it can be planted in areas where water table is near surface. It is suitable for planting in South and Southeast regions of Brazil, because tolerates frost and a moderate degree of water stress, which can be planted in areas of Dense Ombrophylous Forest, Mixed Ompbrophylous Forest and their transition zones to Cerrado (Brazilian Savannah) and semi-deciduous forests of Paraná and São Paulo states (DORMAN; SQUILLACE, 1974).

Although slash pine is widely used in the manufacture of pulp and paper in the United States, the same does not occur in Brazil. Due to high resin content in the wood, an additional manufacturing process would be necessary to separate that component from raw material. Therefore, the use of this species in Brazil is limited to timber production for mechanical processing and resin extraction of standing trees (SHIMIZU; SEBBENN, 2008).

Figure 2 – Natural geographic distribution of slash pine in United State, highlighted in green.

Source: United States Department of Agriculture (2016).

Slash pine has got fast growth and may live for more than 200 years, its height can achieve 18 to 30 m, stem is straight and cylindrical with diameter ranging from 0.60 m to 0.80 m. Bark is grooved with scaly plaques grey in young plants and brown-red tone in adult. There is natural pruning of branches and canopy opens greater than 3.0 m radius creating a light shade. Needles are sharp and thin, bright green colour and length between 18 cm and 24 cm, branches are grouped into two or three units with constant falling forming thick blanket in under forest. Flowering usually occurs in spring, pinecone weight may be over 46 g with 12.5 cm x 4.5 cm dimensions, producing a mean of 10 g of seeds, usually triangular shape, black and winged (BUBNA, 2013; GILMAN; WATSON, 1994).

Each megasporophyll (carpel scale) carries two eggs and it is protected by a sterile leaf and coverage scale. This scale does not develop after fertilization and incorporated into the base of carpel scale growing and becoming woody. Seeds often winged formed from a portion of the carpel scale easily spread on across landscape, and sometimes may become invasive. Male flowers are formed in dense elongated strobilus and each microsporophyll carries two pollen bags (Figure 3B). Cones or female strobilus are formed by numerous small megasporophyll supported by protective external scales (Figures 3A and 3B). In each megasporophyll two eggs have grown with the upper part thereof (SUASSUNA, 1977).

Root system is very aggressive competing with plants around. It presents some sensitivity to diseases and pests that affect its health with appearance of chlorosis, wood rot, rust and canker (GILMAN; WATSON, 1994).

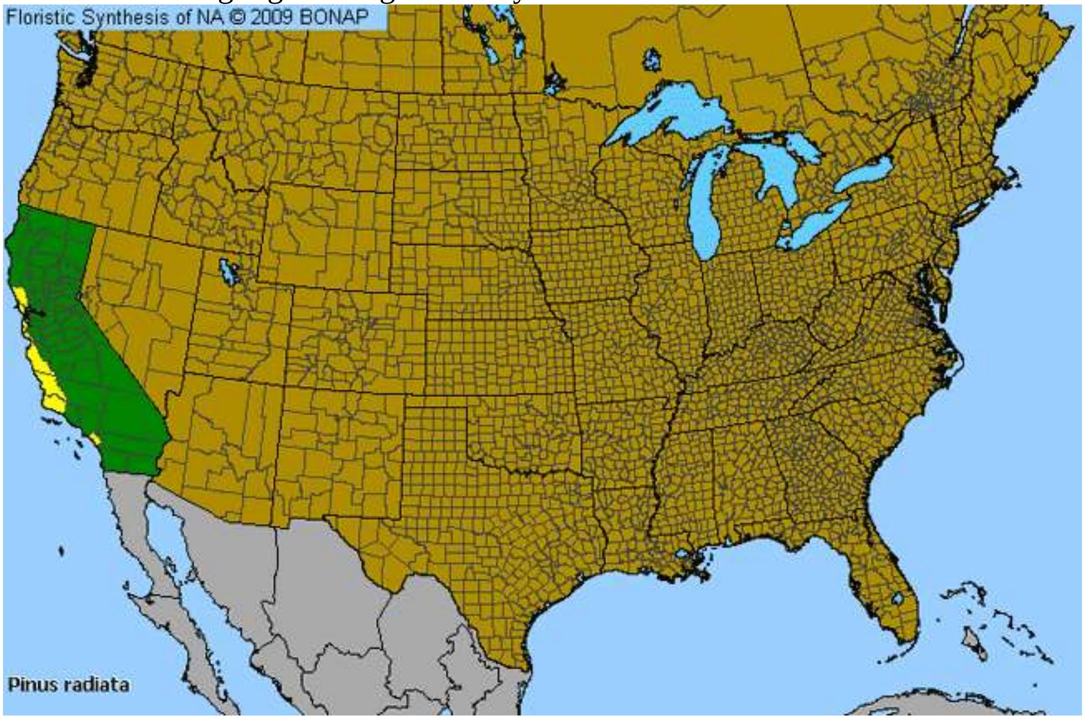
Figure 3 – Slash pine reproductive structures: female strobilus (A), male strobilus (B) and female strobilus already developed, called cone (C).

Source: Ananda Aguiar (A e B) and the author (C).

2.5 *Pinus radiata* D. DON

Radiata pine, also known as Monterey pine, is naturally ranged in a very limited and largest found in the central part of California coast in United State. In this region is possible to find natural portions next to Monterey Peninsula around the city of Cambria and Swanton (Figure 4). This pine species can also be found naturally in Guadalupe Island distant 300 km off the Californian coast. However, radiata pine was introduced and well adapted to climatic conditions of New Zealand, Australia, Chile, South Africa, Spain, Argentina and Uruguay (ROY, 1966).

Figure 4 – Radiata pine natural geographic distribution in the United State highlighted in green and yellow.



Source: Floristic Synthesis of North America (2016).

The species may achieve around 15-30 m of height in natural conditions and more than 60 m in ideal conditions under cultivation, branches pointing up and a rounded top. Needles are light green, in groups of three, slender and 8-15 cm long (ROY, 1966).

Flowering time is in winter and early spring. Female strobilus (Figure 5A) are formed in a spiral or nodal groups from three to seven both on stem or on branches where they can be subterminal or head side (LINDSAY, 1932). This pine is multinodal and normally produces one to three nodes each year, thus one to three groups of cones are also produced. Male strobilus (Figura 5B) is usually produced in secondary branches. Pollination is most effective during first and second week after female flowers opening. Cones are developed only after receiving viable pollen (Figure 5C), in some cases pollen may be unable to produce seeds (PAWSEY, 1961), if it has not happened cones have died within three months or less. In other cases, cones apparently mature may be found empty or only with wings of seed formed. Cones maturity happen in autumn of the second year and open releasing seeds during the first warm days of following spring (ROY, 1966).

Figure 5 – Radiata pine reproductive structures: female strobilus (A), male strobilus (B) and female strobilus already developed, called cone (C).

Source: Carols Cornwall (A), Phil Bendle (B), Ruth Tittherson(C) (2016).

2.6 FOREST BREEDING

Genetics is the science related to two major distinct areas (*i*) heredity whose concept is equal tend to generate equal, explaining the fact that offspring resemble their ancestors and (*ii*) variation as being all environmental or genetic differences between organisms related to descent. Thus, variations can be due to the environment which individual is inserted, and therefore not hereditary, but can also be produced by changes in the genetic constitution, in which case, hereditary (RAMALHO; SANTOS; PINTO, 2004). The principal objective of breeding programs is to detect genotypes whose offspring will grow well in commercial plantations (EGBÄCK et al., 2012). Researches emphasise on volume growth, including height and diameter at breast height (BERLIN et al., 2009a), wood quality properties, resistance to diseases (ROSVALL et al., 2002) and maintenance of genetic variability, fundamental requirement for obtaining long-term genetic gains (MORI, 1993).

According to Ramalho, Santos and Pinto (2004), forest genetic improvement is relatively a new science. It has made great advances since 1950 and the first large-scale improved species were *Pinus elliottii* and *Pinus taeda* in the United States and *Acacia mearnsii* (black wattle) in South Africa. In Brazil, first researches in forest breeding have begun in the early twentieth century, where the main objective was wood production due to process of natural forests destruction and impossibility replacement with native species (FERREIRA; SANTOS, 1997). Assis (1996) explains that the restrictions on native forests wood use combined with the urgent need to supply the domestic market are cited as major factor that led search of fast growing species (*Eucalyptus* and *Pinus*) and the development of appropriate technologies to achieve industries demand.

The introduction of exotic forest species in Brazil provide great benefits for socio-economic development of different regions, especially in areas whose soil characteristics and climate discouraged agricultural activity. The development of improved populations that satisfy the requirements of forest productivity depends on ability to identify genotypes characteristics in under selection population (MORI, 1993). A proven strategy for genotypes selection is the combination of provenance and progeny tests. These tests and estimate genetic parameters have allowed breeders to simultaneously obtain information on the geographic variation and genetic differences between trees of each provenance (SHIMIZU; PINTOJÚNIOR, 1988).

In forest breeding it is possible to mention many types of selection such as recurrent selections, in which, can be separate in recurrent selection intrapopulation and interpopulation. Reciprocal recurrent selection is a breeding scheme developed aiming the improvement of performance of two distinct crossings population heterosis groups. In this method, genotypes of two populations are evaluated in reciprocal crosses (half-sib interpopulation progenies), and the best of each population are selected, and its S1 offspring recombined to lead improved population (COMSTOCK; ROBINSON; HARVEY, 1949; HALLAUER; EBERHART, 1970). In the other hand, in intrapopulation recurrent selection the objective is to improve performance of trees inside the same population (HALLAUER, 1985; SOUZA JÚNIOR, 1993).

The selection based on progeny test, in which genetic parameters is estimated, is always more efficient than simply performed based on the phenotype of plants individually. In this case, both individuals and their progenies are selected (PAIVA; RESENDE; CORDEIRO, 2002). For Robertson (1960) genetic gain depend essentially on genetic variability which is maintained through selection cycles being unconstrained by recombination when the cycle ends. Thus, breeding population should be established on the basis of genetic variability. Assis

(1996) and Resende and Barbosa (2005) include progenies and clones in breeding or selected populations in which breeders can manipulate them for further breeding.

Intense individual selection for a high heritability trait should be used with caution, according to Kageyama and Vencovsky (1983). High intensities can cause early elimination or loss of valuable alleles responsible for other traits. Authors also argued that to reduce risk of loss only (*i*) if pure or adapted species are used, (*ii*) if matrices are selected in normal environmental conditions, (*iii*) if high effective population number (N_e) is held and (*iv*) if short-term programs are used. In case of medium and long-term programs should be used moderate selection intensities. Another important adoption for breeding strategic efficiency is the use of accurate selection methods depending on heritability of trait, individual, between and within progenies, combined methods, etc (RESENDE et al ., 1995). Furthermore, it should also be noted that many factors that affecting final results are out of breeders control, such as, pests and diseases attack, mechanical damage of cultural tract contributing to the reduction of experimental precision (VERONESI et al., 1995).

2.7 GENETIC PARAMETERS ESTIMATES

Provenance and progeny tests are mainly used to distinguish genetically different materials in breeding programs (OTSUBO et al., 2015). Progeny tests are aimed population conservation, determination of genetic structure, production of improved seeds, selection individuals or progenies for recurrent selection and genetic parameters estimates (SHIMIZU; KAGEYAMA; HIGA, 1982). The term parameter is used to specify the constant characteristics of a population, particularly mean and variance. In case of populations used in breeding programs, the parameters of interest are grouped into two: genetic and non-genetic. The estimation of genetic parameters is necessary to (*i*) obtain information about genes action involved in inheritance of characters under investigation and (*ii*) establish basis for breeding methods choice applicable to the population. In discussing of genetic parameters estimation it must consider that obtained estimates are valid only for population from which experimental material was collected and for environments conditions in which the study was conducted. When the objective is estimate experimentally genetic variances, both genotypes the trial environments should be appropriately sampled for population and geographical area of interest (COCKERHAM, 1956; ROBINSON; COCKERHAM, 1965).

According to Falconer and MacKay (1996), selection success basically depends on the degree of correspondence between the phenotypic value and breeding value presented by metric character in circumstances of environment and population where it was evaluated. This degree of correspondence reflected in practical terms in analysed trait heritability. For breeders, the most important is the existence of additive genetic variability which is expressed as a variance and its magnitude relative to the total phenotypic variance. This is because most of desire interest traits for breeders are controlled by multiple genes, so the variation follow an approximately normal distribution, therefore, there is a tendency to rely on quantitative variances studies (ZOBEL; TALBERT, 1984). Thus, the total phenotypic variance (V_p) for a given trait is estimated by genetic variance (V_g), environment variance (V_e) and variance of interaction (V_{ge}): $V_p = V_g + V_e + V_{ge}$. Moreover, proportion of genetic and environmental components may vary widely between traits. Diameter at breast height (DBH) has a large influence of environment, while wood density is largely controlled by genetic components. Thus, density of wood density has relatively high heritability in comparison with diameter (MEAD, 2013).

Among quantitative genetic parameters we can highlight, coefficient of heritability, which expresses genetic heritage present in phenotypic variation. This coefficient, according to Falconer and MacKay (1996), Zobel and Talbert (1984) and Vencovsky and Barriga (1992) is a proper parameter of population in a given environment, it not being property of species and could vary in different environments and age (SEBBENN, 1998).

Significant genetic variation for growth and form traits and resin production in tests of *Pinus caribaea* var. *bahamensis* progenies was reported by Sebbenn (1994), Zheng, Ennos and Wang (1994), Gurgel-Garrido, Romanelli and Garrido (1996), Gurgel-Garrido et al. (1999) and Missio (2004). In addition to this, they found selection gains up to 23.4% for height, 30.6% for DAP (ZHENG; ENNOS; WANG, 1994), 8.26% for real volume (SEBBENN, 1994) and 46.23% for the production of resin (GURGEL-GARRIDO et al., 1999). These results demonstrate the great potential of pine species for improvement of several economic traits to different locations.

Some statistical parameters have been used to assess the accuracy and quality of forest experiment as the coefficient of experimental variation (CV_e) and accuracy ($r_{\hat{a}a}$). The coefficient of experimental variation should be maintained at appropriate levels for each species and trait. CV_e value depends exclusively on residual variations as a ratio of experimental mean (RESENDE; DUARTE, 2007). The accuracy refers to the correlation between the true genotypic value and that predicted or estimated from experimental data. As regards a correlation it can vary from 0 to 1, being the most suitable values closest to unity or 100%. Thus, it is common high accuracy values are estimated in forest improvement experiments, so this parameter is much higher than smaller the absolute differences between the observed and estimated breeding values (RESENDE; DUARTE, 2007).

2.8 EARLY AGE SELECTION

The efficiency of breeding program can be expressed by genetic gain per unit of time. For forest species time to complete a selection cycle is the main obstacle in breeding programs. Pine, for example, takes 15 to 20 years to complete selection cycle. Therefore, the interval of time between generations must be reduced to maximize gains per unit of time and early age selection has an important role in this case (BORRALHO; COTERRILL; KANOWISKI, 1992; PEREIRA et al., 1997; GONÇALVES et al., 1998).

Early age selection aims to assess traits in early age that are seen as predictors and economically important at rotation age (adult). This practice has been advantageous for genetic gain per unit of time. With time reduction it is possible to eliminate quicker lower genetic material, consequently there are earlier recommendations of new individuals for commercial plantations, reduced breeding cycles, and, consequently, to reduce time between generations (REZENDE; BERTOLUCCI; RAMALHO, 1994). This method of selection may increase genetic gain rate of great importance in forest breeding programs. Thus, it is important to remember selection efficiency is closely linked to the existence of genetic correlation between traits in youth or adulthood (FALCONER; MACKAY, 1996).

The use of early selection in forest sector has taken important results seen in studies with *Eucalyptus* spp. (PEREIRA et al., 1997; BELTRAME et al., 2012), *Hevea brasiliensis* (GONÇALVES et al., 1998), *Pinus elliottii* (ROMANELLI; SEBBENN, 2004), *Pinus taeda* (GWAZE et al., 2000; XIANG, LI, MCKEAND, 2000), *Pinus sylvestris* (HAAPANEM, 2001; KROON et al., 2011), *Pinus pinaster* (KUSNANDAR et al., 1998), *Pinus contorta* (XIE; YIANG, 1996; CHEN et al., 2003), *Pinus banksiana* (RIEMENSCHNEIDER, 1988; CARTER et al., 1990; WENG et al., 2007) and *Pinus menziesii* (JONSON; SNIEZKI; MANDEL, 1997; DEAN; STONECYPHER, 1997).

2.9 GENOTYPE X ENVIRONMENT INTERACTION (G X E)

Shelbourne (1972) defines G x E interaction as being the genotype changes in response to different environmental conditions, since metric phenotypic expression is a result of the synchronized effect between specific genotype, environment and the interaction between them (MAIA et al., 2009). Environment can be defined as all intra and extracellular factors that influence the genotype expression (BREWBAKER, 1965). For Cruz, Regazzi and Carneiro (2004), physiological and biochemical factors are the causes of inherent interaction to each individual and genotype development in dynamic systems with constant changes; therefore there is a separate behaviour of each progeny in relation to responses to environmental variations. Furthermore, Robertson (1959), suggest a classification for G x E interaction: simple, when it is provided by the difference in variability between genotypes in environments, and complex when denoting lack of correlation between measurements of the same genotype in different environments and indicates that there is inconsistency in superiority of genotypes to environmental variations causing increased bias to breeders work. For interactions with genotypes occurs, environmental conditions may be predictable as environment variations especially with regard to the type of soil, climate, and systematic characteristics, such as day length, degree of insulation, etc., and unpredictable as rainfall, temperature changes, in case of pine occurrence of frosts (VENCOVSKY; BELLY, 1992).

In Brazil, the most limiting climatic factors for pine cultivation are temperature and precipitation. Based on these effects observation under reforestation areas (experimental tests) it has been established two growing areas for pine species in Brazil, tropical regions with varieties of *Pinus caribaea* (*caribaea, hondurensis* and *bahamensis*), *Pinus tecunumanii* and sub-tropical / temperate zone, whose more adapted species were *Pinus taeda*, *Pinus greggii* and *Pinus patula* (SHIMIZU; SPIR, 1999; FONSECA et al., 1978). In addition to these species, *Pinus elliottii* adapted well to Brazilian environmental conditions making it the second most widely planted species of the genus *Pinus* for wood production and first for resin extraction (ROMANELLI; SEBBENN, 2004).

Despite of exotic species introduced in Brazil, as slash pine, have promoted forest increment production, many researches focused on obtaining higher quality raw materials, improved adaptive conditions, tolerance to pests and diseases and still maintain genetic variability (MORI, 1993), however, more specific issues such as genotype x environment interaction (G x E) were less prioritized, especially for this genus.

The importance of the G x E interaction in radiata pine breeding programs, for instance, is still under debate. Spanish researchers suggest that for most of traits it is possible to select trees showing good combination of capacity, therefore, no need to explore the G x E interactions (CODESIDO; FERNÁNDEZ-LÓPEZ, 2009). For this species, the G x E interaction exploitation would be worthwhile for frost resistance. However, in traditional breeding programs based on seed production in seed orchards, further gains to be made using G x E interactions is often small in relation to the effort needed (CARSON, 1991).

In general, there are few studies for this topic in forest species. Consequently it is indispensable to evaluate the maximum number of environments and the adoption of more rigorous as possible in order to obtain data with high accuracy, which allows you to select the best materials to be multiplied on a commercial scale for various environments. To make this recommendation as reliable as possible a detailed study is required for the temporal stability of genotypes and with regard to their economically important traits (CRUZ, 2006). Nevertheless, despite its importance, the simple analysis of genotype x environment interaction does not give complete and accurate input on the behaviour of each genotype in various environmental conditions. Thus, it is recommended statistical procedures for materials with more stable performance identification that respond predictably to environmental variations through adaptability, stability and productivity analysis (SILVA; DUARTE, 2006).

Adaptability refers to genotypes ability of responding to environmental stimulation and stability is the ability of genotypes maintain the same performance under various environmental conditions (ALWALA et al., 2010). Stability and adaptability analysis allows to identify genotypes with predictable behaviours (production) in various environments (CRUZ; REGAZZI; CARNEIRO, 2004). Considering the range of planting zones and diversity existence in pine material used in commercial plantation, more detailed investigation about its behaviour will generate important information of more productive crops establishment. Whence, selection of methodologies that combine stability and adaptability in a single statistic can be considered superior, compared to those that only use production as selection criteria (RESENDE; DUARTE, 2007; VERARDI et al., 2009).

Harmonic Mean of the Relative Performance of Genetic Values method (MHPRVG) is based on analysis of genetic values using mixed models selecting genotypes through simultaneous consideration of individual productivity, stability and adaptability (RESENDE, 2007). The model considers genotypic as random effects and therefore provides genotypic stability and adaptability, allowing unbalanced data analysis, non-orthogonal designs and heterogeneity of variances. In addition to this, model allows to consider correlated errors within sites as well as individual selection stability and adaptability within progenies; provides breeding values with instability discount; and can be applied to any number of environments. The method generates results in its unit or in evaluated trait scale, interpreted directly as breeding values, an additional factor in relation to other methods. This model allows additional inferences such as: selecting specific genotypes for each site; selection of stable genotypes across sites; selection of responsive genotypes (with high adaptability); and simultaneous selection by three attributes (productivity, stability and adaptability).

The estimated Harmonic Mean for Genetic Values (MHVG), Relative Performance of Genetic Values (PRVG) and the Harmonic Mean of Relative Performance of Genetic Values (MHPRVG) are used to measure genotypic stability and perennials adaptability and are employed as different criteria to productivity, stability and adaptability simulation selection. Another method widely used for stability and adaptability analyses is based on bissegmented regression. The method includes the formation of favourable environment groups, when environmental indices have positive values, and unfavourable environments, when indexes are negative (CRUZ; REGAZZI; CARNEIRO, 2004).

Environmental stratification process consists on subdivision of heterogeneous regions in more uniform subregion, where any significant G x E interaction is excluded or in other cases a significant interaction with predominant single portion, not compromising cultivars recommendation. The aim of this method is to find environments with similar genotypic classification regardless of the environmental quality. However, bissegmented regression has consistency only for G x E interaction and not for genotypes x age, genotype x harvest or genotypes x sites x age interactions (OLIVEIRA et al., 2005). Comparing this two methodologies, MHPRVG method presents more advantages in relation to bissegmented regression and others ones such as: consider phenotypic effects as random; allows to handle with variances heterogeneity; allows to deal with unbalanced data; allows to tackle with non-orthogonal designs; consider correlated errors within sites; provides values with discounted genetic instability and generates results with own magnitude scale or evaluated trait scale (RESENDE, 2004).

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3 CHAPTER I

ESTIMATE OF ADAPTABILITY, STABILITY, PRODUCTIVITY AND GENETIC PARAMETERS IN EARLY AGE SLASH PINE SECOND-GENERATION PROGENIES

ABSTRACT

The study was conducted to estimate the stability, adaptability, productivity and genetic parameters in Slash pine second-generation open pollinated progenies considering phenotypic traits in early age for forty-four progenies from a first generation seed orchard planted in Colombo-PR, Brazil. Two progenies tests were established in a randomized complete block design. The first test was implemented in March 2009 in Ribeirão Branco, São Paulo state, Brazil, containing 40 blocks, one tree per plot, 44 treatments (progenies) and 6 controls. Another test was implemented in Ponta Grossa, Paraná state, Brazil, using the same experimental design and number of plants per plot, and with 24 treatments, 32 blocks, both in 3.0 x 3.0 meters spacing. The growth traits evaluated were total height, diameter at breast height and wood volume, within five years. The form traits evaluated were stem form, branch thickness, branch angle, number of branches, fork and fox tail five years after planting. Deviance analysis and estimates of stability, adaptability, productivity and genetic parameters were performed using the methods of best linear unbiased predictor (BLUP) and residual maximum likelihood (REML). There was significant variation ($p<0.01$) among progenies for growth and form traits. Considerable genetic variation was detected mainly for wood volume as moderate to high coefficients of genetic variation (14.31% to 16.24% - Ribeirão Branco-SP and 31.78% to 33.77% - Ponta Grossa-PR) and heritability (0.10 to 0.15 – Ribeirão Branco-SP and 0.36 to 0.48 – Ponta Grossa-PR) evidencing high genetic control for the traits is significant and the possibility of genetic gains with selection. The effect of genotype x environment interaction is simple which means progenies planted in one site can also be planted in the other. Genotypes such as C-197, C-189-1, C-084-2 and C-032-2 are indicated to plantations in both studied regions in São Paulo and Paraná states according to stability, adaptability and simultaneously productivity, stability and adaptability.

Keywords: *Pinus elliottii* Engelm. var. *elliottii*. Seed production. Selection gain. REML/BLUP.

RESUMO

O objetivo deste trabalho foi estimar a estabilidade, a adaptabilidade, a produtividade e os parâmetros genéticos em progênies de polinização aberta de segunda geração de *Pinus elliottii var. elliottii* considerando os caracteres fenotípicos em idade precoce de quarenta e quatro progênies provenientes de um pomar de sementes de primeira geração instalados em Colombo, PR, Brasil. Dois testes de progênies foram estabelecidos em um delineamento em blocos completos casualizados. O primeiro teste foi implantado em março de 2009 em Ribeirão Branco, SP, contendo 40 repetições, uma planta por parcela e 44 tratamentos (progênies) e 6 testemunhas comerciais. Em Ponta Grossa, PR, foi implantado outro teste utilizando o mesmo delineamento experimental e mesmo número de plantas por parcela com 24 tratamentos, 32 repetições, ambos no espaçamento 3,0 x 3,0 metros. Foram avaliados sobrevivência, caracteres de crescimento como altura total, diâmetro à altura do peito e volume de madeira, nos cinco anos subsequentes e caracteres de forma como forma de fuste, espessura de galho, ângulo de galho, número de galhos, bifurcação e *fox tail* cinco anos após o plantio. Análise de *Deviance* e as estimativas da estabilidade, a adaptabilidade, a produtividade e parâmetros genéticos foram realizadas a partir dos métodos de melhor preditor linear não-viesado (BLUP) e máxima verossimilhança residual (REML). Foi observada variação significativa ($p<0,01$) entre as progênies para os caracteres de crescimento. Variação genética considerável, principalmente para o caráter volume foi constatada. Os altos coeficientes de variação genética para esse caráter (14,31% a 16,24% - Ribeirão Branco e 31,78% a 33,77% - Ponta Grossa) e herdabilidade (0,10 a 0,15 – Ribeirão Branco e 0,36 a 0,48 – Ponta Grossa) mostraram baixa influência do ambiente na variação fenotípica, o que é importante para a predição do ganho genético mediante a seleção. O efeito da interação genótipo x ambiente é simples, ou seja, progênies plantadas em um local podem também ser plantadas no outro. Genótipos como C-197, C-189-1, C-084-2 e C-032-2 são indicados para plantações tanto na região estudada de São Paulo e Paraná de acordo com a estabilidade, adaptabilidade e simultaneamente produtividade, estabilidade e adaptabilidade.

Palavras-chave: *Pinus elliottii* Engelm. var. *elliottii*. Produção de sementes. Ganho de seleção. REML/BLUP.

3.1 INTRODUCTION

The *Pinus* genus species are widely used in homogeneous reforestation in many parts of the globe due to the large adaptation to climate diversity and the wide application of their products (wood, resins, fibers, cellulose, etc.) (MOREIRA et al., 2015). In addition, they present much higher phenotypic plasticity than the other exotic tree species (SEBBENN, 1994).

In Brazil, the most limiting climatic factors for pine cultivation are temperature and precipitation. Based on these effects observation under reforestation areas (experimental tests) it has been established two growing areas for pine species in Brazil, tropical regions with varieties of *Pinus caribaea* (*caribaea, hondurensis* and *bahamensis*), *Pinus tecunumanii* and sub-tropical / temperate zone, whose more adapted species were *Pinus taeda*, *Pinus greggii* and *Pinus patula* (SHIMIZU; SPIR., 1999; FONSECA et al., 1978). In addition to these species, *Pinus elliottii* adapted well to Brazilian environmental conditions making it the second most widely planted species of the genus *Pinus* for wood production and first for resin extraction (ROMANELLI; SEBBENN, 2004).

Slash pine (*Pinus elliottii* Engelm. var. *elliottii*) occurs on coastal plains all over the Indian River Lagoon area, and in freshwater upland areas. It may be found on coastal plains from South Carolina to Central Florida, and west to Louisiana. Slash pine was introduced in Kentucky, Virginia and eastern Texas in the 1900's, and now reproduces naturally within these states (UNITED STATES DEPARTMENT OF AGRICULTURE – USDA, 2004).

The introduction of exotic species in Brazil, as Slash pine, have driven the forest breeding mainly for increasing productivity, obtaining raw materials of higher quality, improving species adaptive conditions, tolerance to pests and diseases and maintaining genetic variability (MORI, 1993). More specific issues such as genotype x environment interaction were less prioritized, especially for this genus. In general, there are few studies about this topic in forest species. Therefore, it is indispensable to evaluate the maximum number of environments and the adoption of more rigorous methods of improvement as possible in order to obtain data with high accuracy, which allows the selection of the best materials to be multiplied on a commercial scale for many environments. To make this recommendation as reliable as possible, a detailed study is required for the temporal stability of genotypes and with regard to their most important economically traits (CRUZ, 2006). Analysis of adaptability, stability and productivity are statistical procedures to identify more stable and performance materials that respond predictably to environmental variations (SILVA; DUARTE, 2006).

Adaptability refers to genotypes ability of responding to environmental stimulation and stability is genotypes ability of maintaining the same performance under various environmental conditions (ALWALA et al., 2010). Adaptability and stability analysis allows, in this way, the identification of predictable genotypes behaviours (production) in different environments (CRUZ; REGAZZI; CARNEIRO, 2004). Considering the range of planting zones and the existence of diversity in the used material in commercial pine plantations more detailed investigation in relation to the behaviour of genotypes will generate important information for establishment of more productive plantings. Furthermore, all intents and purposes to be considered to estimate genetic parameters such as adaptability, stability and productivity for forest species trials, a major impediment faced by forest breeders is the traditional perennial species breeding cycle, which can be very long, 25 years for pine (PEREIRA et al., 1997). The use of biotechnology in many stages of the process and estimation of genetic parameters at early ages, especially genetic correlations, accuracies and heritability are the alternatives to reduce the selection cycle. Thus, silvicultural traits of economic interest in juvenile trees are measured in order to predict the performance of each individual in adulthood (GONCALVES et al., 1998). In this way, in the forest improvement, the early age selection has shown great advantage shortening the interval between generations by reducing the breeding cycle. Thus, it can obtain more genetic gain per unit of time (year) if a strong genetic correlation exists between the initial stages and the end of the production cycle (NANSON, 1970; LAMBETH, 1980; McKEAND, 1988; MATHESON et al., 1994; SILVA et al, 2014).

Then, the study was conducted (*i*) to estimate the stability, adaptability, productivity, genetic diversity and genetic parameters in slash pine second-generation open pollinated progenies considering the phenotypic traits at early age, and (*ii*) to identify more productive genotypes for commercial plantations in the two Pine planting regions in São Paulo and Paraná states.

3.2 MATERIALS AND METHODS

3.2.1 The trials

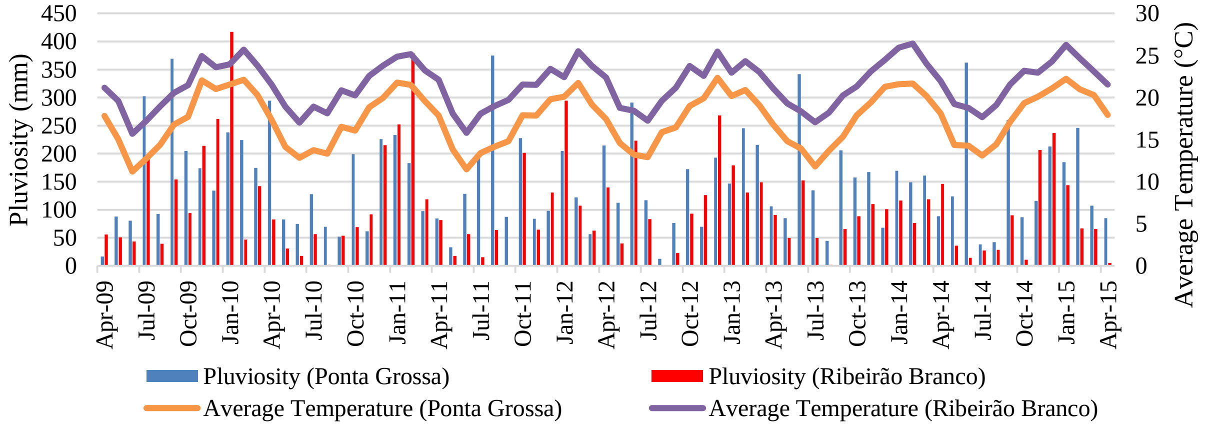
Forty-four progenies originated from a slash pine first generation seed orchard established in Colombo-PR, Brazil, were used in this study. The seedlings were produced in municipality of Ribeirão Branco-SP, Brazil, in 2008. In March of the following year two second generation progeny tests they were installed, one in Ponta Grossa-PR and another one in Ribeirão Branco-SP, Brazil (Table 1, Figures 6 and 7).

Table 1 – Details of slash pine progeny tests deployed in municipalities of Ribeirão Branco-SP and Ponta Grossa-PR, Brazil.

	Ribeirão Branco-SP	Ponta Grossa-PR
Progenies number	44	24
Blocks	40	32
Controls	6	-
Spacing	3m x 3m	
Plating date	March/2009	
Border	2 lines	
Latitude	25° 05' 42" S	24° 13' 15" S
Longitude	50° 09' 43" W	48° 45' 56" W
Altitude	969 m	875 m
Pluviosity	1,346 mm	1,495 mm
Clime	Cfb	Cfb
Average temperature	18 °C	17.5 °C
Soil type	Cambissolo	Cambissolo

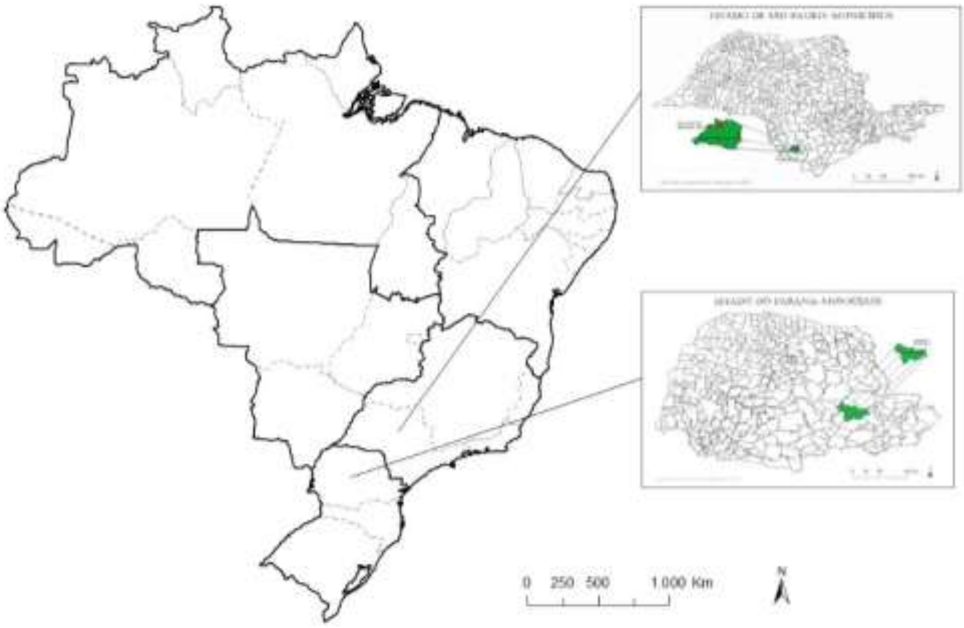
Source: The author.

Figure 6 – Total precipitation and monthly temperature mean for municipalities of Ribeirão Branco-SP and Ponta Grossa-PR, Brazil, from April 2009 to April 2015.



Source: Prepared by the author based on data provided by the National Institute of Meteorology.

Figure 7 – Geographical localization of municipalities of Ribeirão Branco-SP and Ponta Grossa-PR, respectively, in Brazil.



Source: IBGE cartographic base, adapted by Ananda Aguiar and the author.

3.2.2 Traits measurement

Data was obtained within five years after planting and traits were: survival rate; total height, diameter at breast height (dbh) and wood volume, computed based on the total height of data and diameter at breast height, according to the following equation:

$$VOL = \frac{\pi(DBH)^2.FF}{40000}\ TH \quad (1)$$

Where VOL was wood volume, dbh was the diameter at breast height, FF was the stem form factor and TH was total height. The height trait was measured using a telescopic ruler, circumference at breast height was measured with a tape calculating from these values the diameter at breast height and survival was adopted "1" to alive and "0" to dead plants. Form traits were visually scored as follows (Table 2).

Table 2 – Scoring for form traits of slash pine progenies in municipalities of Ribeirão Branco and Ponta Grossa, Brazil.

	Scale		Description	
Traits	Ribeirão Branco	Ponta Grossa	Ribeirão Branco	Ponta Grossa
Stem straightness	1-3	1-5	1 = most crooked steams, 3 = straightest stems	1 = most crooked steams, 5 = straightest stems
Branch thickness	1-3		1 = thicker, 3 = thinner	
Branch angle	1-3		1 = less than 45º, 2 = greater than 45º and 3 = approximately 90º	
Number of branches	1-7		1 = 42 to 48 branches, 7 = 1 to 6 branches	1 = 17 to 18 branches, 7 = 0 to 2 branches
Fork	1 and 2		"1" for absence and "2" for presence	
Fox tail (only for Ponta Grossa trial)	1 and 2		"1" for absence and "2" for presence	

Source: The author.

3.2.3 Estimates of variance components and genetic parameters

Estimates of variance and genetic parameters components were obtained by REML / BLUP method (restricted maximum likelihood/best linear unbiased prediction) from unbalanced data, using genetic-statistical software SELEGEN-REML/BLUP, developed by Resende (2007a). Different models were used to perform statistical analyses and estimate genetic parameters, as described below.

3.2.4 Individual analysis and estimation of genetic parameters

The model applied to perform the deviance analysis of half-sib progenies tests was randomized complete block, one tree per plot, one place:

$$y = \boldsymbol{X_r} + \boldsymbol{Z_a} + \boldsymbol{e} \qquad (2)$$

Where y is the data vector **r** is the vector of replication effects (assumed to be fixed) added to the general average, **a** is the vector of individual additive genetic effects (assumed to be random), **e** is the error vector or residues (random). The capital letters represent the incidence matrices for these purposes. To use this model, it was necessary to assume that the open pollinated progenies of slash pine are half-sibs. Thus, the first step was carried out analysis considering all treatments including the control (for Ribeirão Branco). Later, controls were removed, proceeding further analysis to estimate the parameters and predict genetic values.

The significance test was performed using the likelihood ratio test (LRT), obtained by the difference between deviances for models with and without effect to be tested and using the chi-square test. The genetic parameters estimated were:

a) Narrow sense individual heritability

$$h_a^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_e^2} \tag{3}$$

Where σ_a^2 is additive genetic variance and σ_e^2 is residual variance.

b) Average heritability of progenies

$$h_m^2 = \frac{[1+(n-1)\rho_a]\, h_a^2}{1+(n-1)\,(\rho_a h_a^2)} \tag{4}$$

Where ρ_a is additive genetic correlation intraclass between individuals of considered progeny type, n in number of trees per progeny and h_a^2 narrow sense individual heritability.

c) Accuracy

$$r_{\hat{a}a} = \sqrt{\frac{n}{n+\left(\frac{4-h_a^2}{h_a^2}\right)}} \tag{5}$$

Where n in number of trees per progeny and h_a^2 narrow sense individual heritability.

d) Individual phenotypic variance

$$\hat{\sigma}_f^2 = \hat{\sigma}_a^2 + \hat{\sigma}_e^2 \tag{6}$$

Where σ_a^2 is additive genetic variance and σ_e^2 is residual variance.

e) Coefficient of individual genetic variance

$$CV_{gi} = \frac{100.\sqrt{\widehat{\sigma_a^2}}}{\mu} \tag{7}$$

Where σ_a^2 is additive genetic variance and μ is the overall trait mean.

f) Coefficient of genetic variation between progenies

$$CV_{gp} = 100.\frac{\sqrt{0{,}25\hat{\sigma}_a^2}}{\mu} \tag{8}$$

Where σ_a^2 is additive genetic variance and μ is the overall trait mean.

g) Coefficient of experimental variation

$$CV_e = 100.\frac{\sqrt{SM_{res}}}{\mu} \tag{9}$$

Where SM_{res} is residual square mean and μ is the overall trait mean.

3.2.5 Effective population sizes and expected selection gains

To select superior genotypes for commercial plantations and seed orchards formation were simulated different selection methods from BLUP classification of each tests. Wood volume was chosen due to higher narrow-sense individual heritability and because it is the most important trait to wood industry. After obtaining additive values for selected trees, according to individual classification obtained by BLUP procedure, genetic gain in percentage was estimated by the expression: SG (%) = (mean of the additive genetic values of selected individuals / m) x 100, where m is the overall average of the experiment for a given trait.

Selection strategies were: i) selecting of the 80 best individuals from each site to form a clonal orchard; ii) selecting the two best individuals per progeny for the next breeding cycle and iii) selecting the best 50% individuals per progenies to form a seedling seed orchard.

To guide the selection strategy adopted in order to avoid excessive loss of genetic variability after application of the proposed selection methods, the effective population size (N_e) and genetic diversity ($\hat{D}$) were estimated from the equation 10 and 11.

$$N_e = \frac{4\, N_f\, \overline{k_f}}{[\overline{k_f} + 3 + \left(\frac{\sigma^2_{k_f}}{\overline{k_f}}\right)]} \tag{10}$$

Where N_f is number of selected progenies, $\overline{k_f}$ is the average number of individuals selected per progeny and $\sigma^2_{k_f}$ is variance of selected individuals per progeny.

$$\hat{D} = \frac{N_{ef}}{N_{fo}} \tag{11}$$

Where $0 < \hat{D} \leq 1$, N_{ef} is effective number of selected progenies and N_{fo} is original number of progenies.

3.2.6 Trait-trait and age-age genetic and phenotypic correlations

Genetic and phenotypic correlations between traits and ages were undertaken with genetic-statistical software SELEGEN-REML/BLUP, developed by Resende (2007a) to determine the relationship between them.

3.2.7 Productivity, stability and adaptability

For joint analysis, considering the two sites, was used statistical procedure MHPRVG (harmonic mean of the relative performance of the breeding values) predicted by BLUP, according to Resende (2007b).

The model used was:

$$y = \boldsymbol{X_r} + \boldsymbol{Z_g} + \boldsymbol{W_i} + \boldsymbol{e} \tag{12}$$

Where y is the data vector **r** is the vector of replication effects (assumed to be fixed) added to the general average, **g** is the vector of genotypic effects (assumed to be random), **i** is the vector of the effects of genotype x environment interaction (random) and **e** is the vector of errors or residues (random). The capital letters represent the incidence matrices for these purposes. The vector r includes all measurements in all replications in multiple sites simultaneously and adjusts to all these effects and their interactions (Resende, 2007b). In addition to the narrow sense individual heritability (h_a^2), average heritability of progenies (h_m^2), accuracy ($r_{\hat{a}a}$), coefficient of individual additive genetic variation (CV_{gi}) and coefficient of residual variation ($CV_e - \%$) were estimated:

a) Coefficient of determination for genotype x environment interaction

$$C_{int}^2 = \frac{\sigma_{int}^2}{\sigma_a^2} \tag{13}$$

Where σ_{int}^2 is variance of interaction between genotypes and environment.

b) Genotypic correlation between progenies in different environments (Type b correlation)

$$r_{gloc} = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_{int}^2} \tag{14}$$

3.3 RESULTS AND DISCUSSION

3.3.1 Individual deviance analysis and genetic parameters estimation

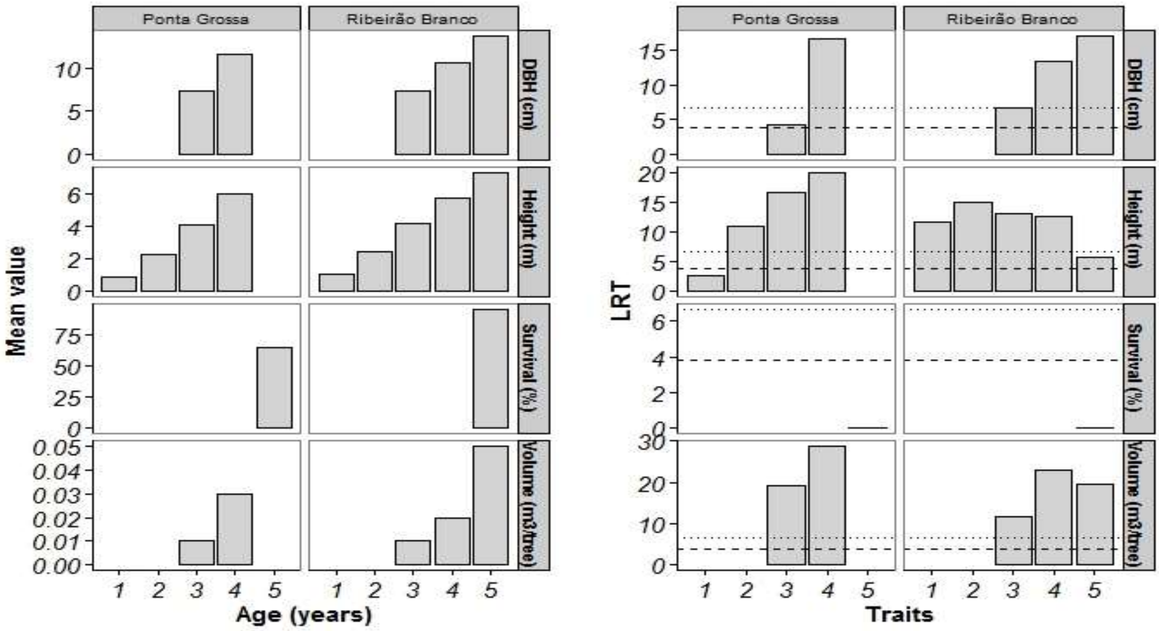
Survival rate was high in Ribeirão Branco-SP five years after planting (95%) while in Ponta Grossa-PR was relatively low, only 65% (Figure 8). Yang et al. (2013) showed the first years are important to plant recover the root system and adapt to the new environment, if this does not occur, mortality rate of seedlings may be high, as can be observed in Ponta Grossa. Furthermore, lack of water in the first weeks after planting may be responsible for plant mortality, the first month after planting there was less rainfall in relation to the normal April month at Ponta Grossa. According to Schultz (1997), pine productivity is positively associated to places with mild temperatures and mostly smaller water deficit.

Total height, diameter at breast height (dbh) and wood volume means ranging from 1.08 m to 5.82 m, 7.38 cm to 10.73 cm and 0.01 $m^3.arv^{-1}$ to 0.03 $m^3.arv^{-1}$ and 0.91 m to 6.04 m, 7.35 cm to 11.52 cm and 0.01 $m^3.arv^{-1}$ to 0.03 $m^3.arv^{-1}$, respectively, in Ribeirão Branco-SP and Ponta Grossa-PR. This shows that there was little difference in relation to second-generation slash pine progenies productive performance in both assays. Significant variation among progenies was observed for most of evaluated growth traits at 5% and 1% of probability, except for the survival trait in both tests and one year after planting in Ponta Grossa trial (Figure 8).

In Ribeirão Branco, commercial controls of slash pine were planted in the experiment aiming to compare performance of established trees with new progenies which can be as adaptable as the previous one. The best commercial control was I123, however, comparing to test progenies it is possible to notice that, for wood volume at age three, only two progenies were better than this control (C-098-1 and C-100-2). Two years later, at age five, the tested progenies were more adapted to new environment and in this case it is possible to highlight five better progenies than I123, such as C-063-2, C-098-1, C-228, C-001-1 and C-100-2 (Appendix 12).

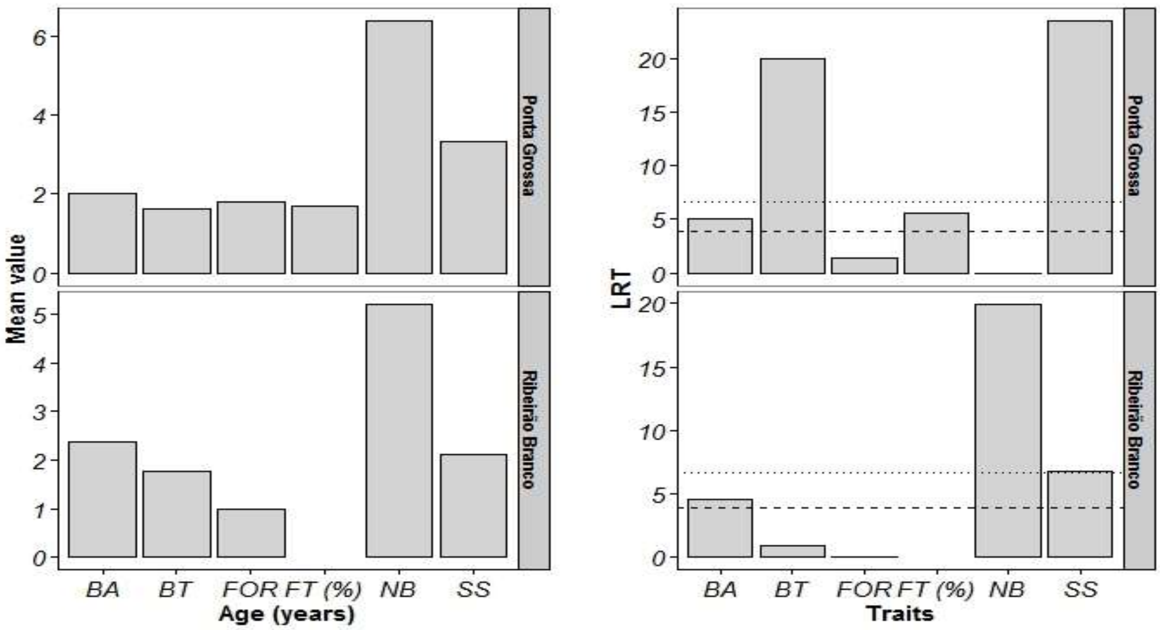
In relation to form traits, there was statistical difference only for stem straightness and number of branches for Ribeirão Branco and stem straightness, branch thickness, branch angle and fox tail to Ponta Grossa (Figure 9). Form traits mean were similar to both sites, except stem straightness and number of branches. Ponta Grossa produced trees with score mean of 3.34 and Ribeirão Branco of 2.11, in other words, greater numbers indicates straighter trees.

Figure 8 – Mean values, likelihood ratio test (LRT) and significance levels for *slash pine* growth traits in municipalities of Ribeirão Branco and Ponta Grossa, Brazil. (LRT above dashed line = p<0.05; above dotted line = p<0.01 and below dashed line = not significant).



Source: The author.

Figure 9 – Mean values, likelihood ratio test (LRT) and significance levels for *slash pine* form traits in municipalities of Ribeirão Branco and Ponta Grossa, Brazil. (LRT above dashed line = p<0.05; above dotted line = p<0.01 and below dashed line = not significant – BA: branch angle, BT: branch thickness, FOR: fork, FT: fox tail, NB: number of branches, SS: stem straightness).



Source: The author.

Experimental design showed good precision for majority growth and form traits (Figure 10 and 11). Coefficient of experimental variation (CV_e) ranging from 8.55% (fork) to 28.94% (stem straightness) for Ribeirão Branco and 8.33% (number of branches) to 34.67% (branch thickness) for Ponta Grossa (Figure 11). Experimental variation between 10% and 20% can be considered low in tests if it occurs plant competition (PIMENTEL-GOMES; GARCIA, 2002). These results may indicate a good experimental control and therefore good accuracy in genetic parameters estimation.

Considerable genetic variation for wood volume may be found across the magnitudes of coefficient of individual genetic variation (CV_{gi}) which are from 14.31% to 16.24% in Ribeirão Branco and from 33.77 to 31.78% in Ponta Grossa (Figure 10), as stem straightness and branch thickness for Ponta Grossa trial (14.74% and 21.81%, respectively (Figure 11). These values have provided high magnitude of individual heritability.

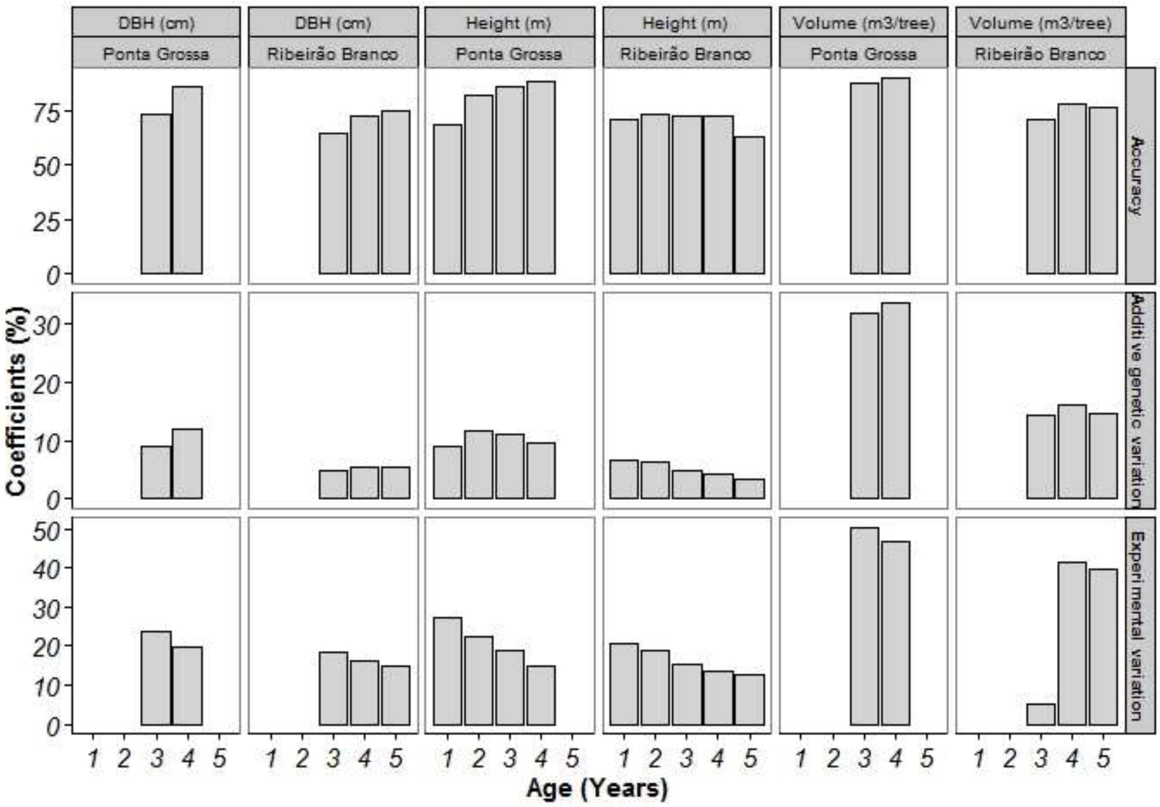
Other traits expressed medium to low magnitude for genetic variation. For height CV_{gi} ranged from 3.29% to 6.55% in Ribeirão Branco and 9.01% to 11.60% in Ponta Grossa. For dbh, estimates ranged between 4.86% and 5.38% in the first site and 9.05% and 11.90% in the second one. Fork presented low CV_{gi} estimation for both sites 1.91% (Ribeirão Branco) and 5.92% (Ponta Grossa).

These estimates were considered high in relation to those reported in other studies of slash pine (ROMANELLI; SEBBENN, 2004; MAX et al., 2008), *Pinus caribaea* var. *bahamensis* (SEBBENN, 1994; SEBBENN et al., 2010), *Pinus tecunumanii* (SEBBENN et al., 2005) and scots pine (FRIES, 2012). In contrast, Gapare at al. (2012) obtained genetic variation for Radiata pine closer to Ponta Grossa trail, but higher than Ribeirão Branco. Thus, the results of this study confirm the genetic potential of this progenies in both tests, especially Ponta Grossa. Consequently, genotypes with the highest genetic values may be selected for commercial plantations and subsequent breeding generations. According to Miranda et al. (2015), as higher the coefficient of genetic variation as greater the chances of finding superior individuals.

Low genetic variation for fork can be related to binary factor. When the trait is binary both phenotypic and genetic variances may be affected by low frequency (WENG et al., 2015). According to quantitative genetic theories, estimation of heritability for binary traits is due to its incidence rate it means as lower the incidence as lower will be the phenotypic and genetic variances (FALCONER; MACKAY, 1996).

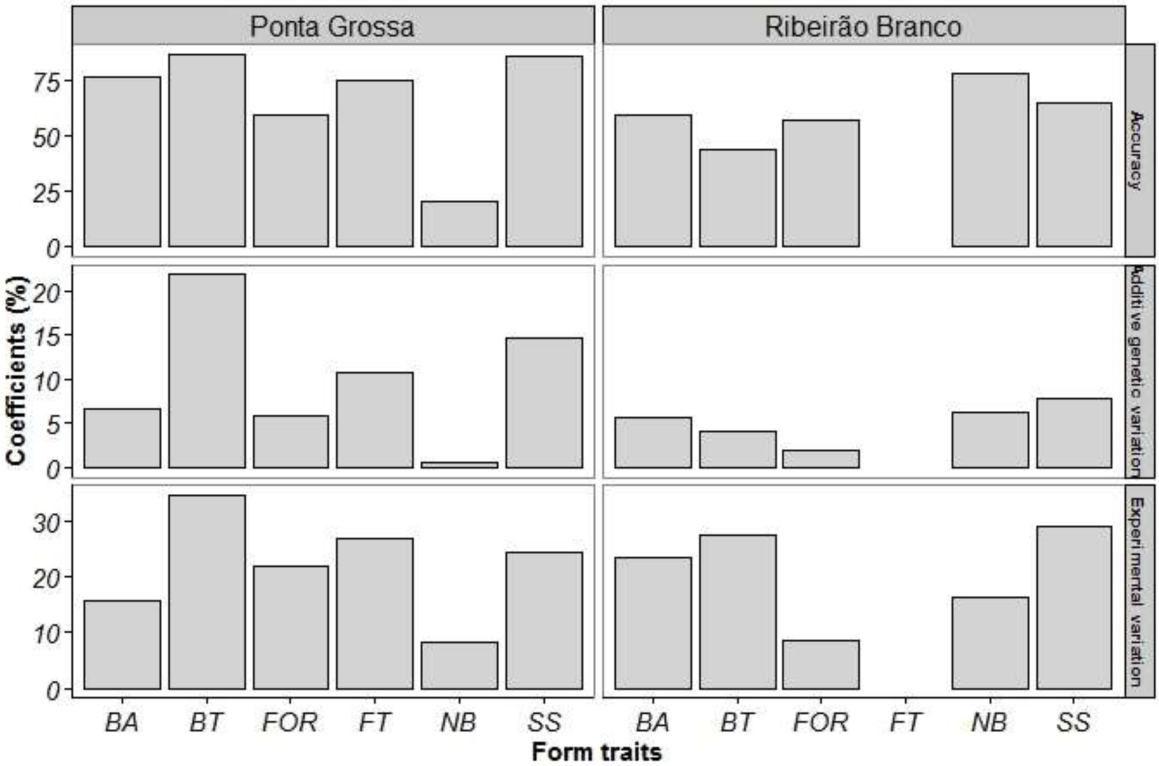
In early and intermediate stages of breeding programs accuracy around 70% or higher are desirable. Most of growth traits in both tests showed values more than 70% (Figure 8). Accuracy is the ratio between true genetic value and the predicted one and as higher the value, as higher the accuracy selection and genetic gain obtained. It can be considered low in the range from 0.10 to 0.40; median from 0.41 to 0.70 and high if major than 0.70 (RESENDE et al., 1995). Consequently, the estimated accuracy for height can be considered median in Ribeirão Branco and mid-high in Ponta Grossa. For dbh and wood volume accuracies were high for both sites. Therefore, there is great expectation to reduce errors in the applied selection, and consequently, the selected material in the test will maintain their potential productive in commercial plantations and experiments.

Figure 10 – Coefficients of experimental variation, individual additive genetic variation and accuracy for growth traits for Slash pine tests in municipalities of Ribeirão Branco and Ponta Grossa, Brazil.



Source: The author.

Figure 11 – Coefficient of experimental variation, individual additive genetic variation and accuracy for Slash pine progenies test in Ribeirão Branco-SP and Ponta Grossa-PR, Brazil. (BA: branch angle, BT: branch thickness, FOR: fork, FT: fox tail, NB: number of branches, SS: stem straightness).



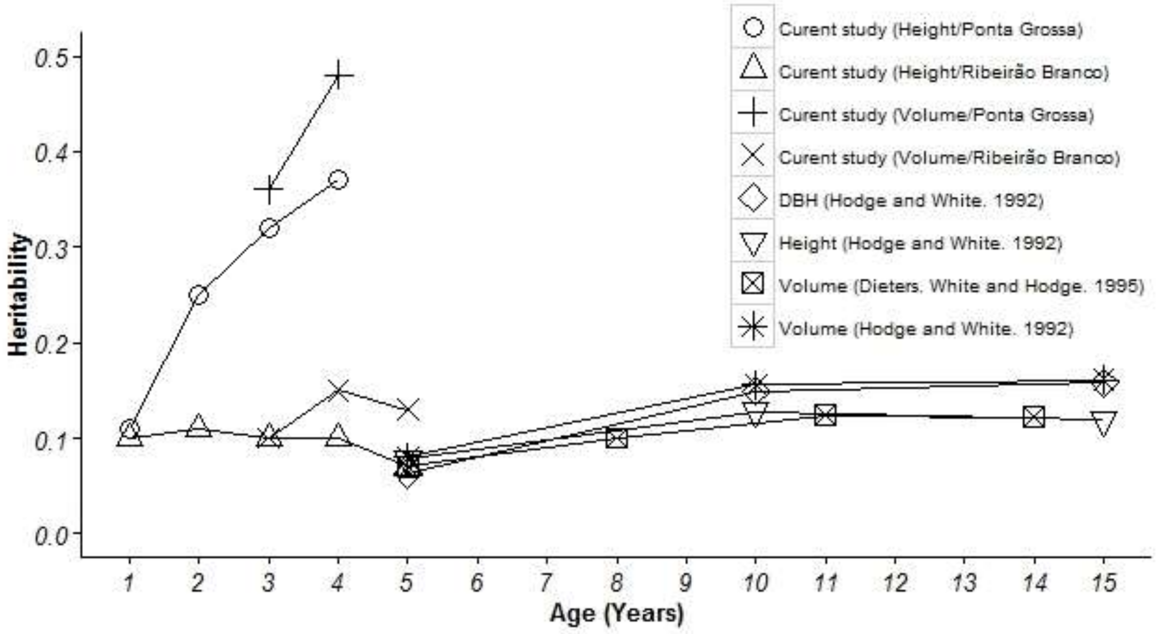
Source: The author.

Estimates of narrow sense individual heritability (h_a^2), for Ribeirão Branco site were considered low for all growth traits and moderate to high for number of branches. Ponta Grossa presented medium values for growth traits and moderate to high for stem straightness, branch thickness and branch angle. Resende et al. (1995) considered values from 0.01 to 0.15 as low, 0.16 to 0.50 as medium and more than 0.50, higher.

Dornan and Squillace (1974), Hodge and White (1992), Dieters, White and Hodge (1995) and Dieters (1996), previously reported low growth traits h_a^2 (Figure 12) as well as in Ribeirão Branco. In the same way, Ettori et al. (2004) found lower h_a^2 values in thin-leaf pine (*Pinus maximinoi* Moore) as Silva et al. (2011) in *Pinus caribaea* var. *caribaea.*

In the other hand, Parayi et al. (1996) and Zobel (1961) estimated median and high values also for *Pinus caribaea* var. *caribaea.* at 5, 8, 14 and 15 years of age, Aguiar, Souza and Shimizu (2010) for Gregg's pine (*Pinus greggii* Engelm.) at 13 years old and Missanjo, Kamanga-Thole and Manda (2013) for Khasi pine (*Pinus kesiya* Royle ex Gordon) at 18 years old in South Africa as in Ponta Grossa.

Figure 12 – Individual narrow-sense heritability estimates for height, dbh and wood volume for Slash pine published works.



Source: The author.

Wood volume is one of the most important trait to analyse if objective is wood production. Individual narrow-sense heritabilities for Ribeirão Branco were around 0.07 to 0.10 (Figure 13), similar results found by Atwood, White and Huber (2002) and Sierra-Lucero (1999) in 3-11 years old Loblolly pine progenies at Florida, Georgia, Mississippi and Alabama states. In Ponta Grossa, h_a^2 were higher between 0.36 and 0.48 (Figure 13). When different works are analysed together the aim is not to compare and conclude one is better than the other, because there are several factors that may cause this differences, the main idea is to infer if the studied material is promising for a region.

Hodge and White (1992) affirm although it may be possible to compare previous estimates for Slash pine with other conifers, the low h_a^2 found in Ribeirão Branco are not due to differences between species. The authors said there is a tendency not to publish very low or negative results of h_a^2 estimates. Furthermore there is different ways to estimate this parameter and also many things may interfere at the results such as number of progenies per test or environmental homogeneity (WHITE; ADAMS; NEALE, 2007).

In general, family heritability means were higher in Ponta Grossa than Ribeirão Branco (Figure 13). Although they have got the same progenies, different weather conditions in Ponta Grossa, such as frost occurrence, may have contributed to a different behaviour of some progenies show their genetic potential. These results indicate that the traits genetic control is high for average level, medium for individual level within progenies. Thus, the results show low environmental influence on phenotypic variation, which is important for the prediction of genetic gain by selection.

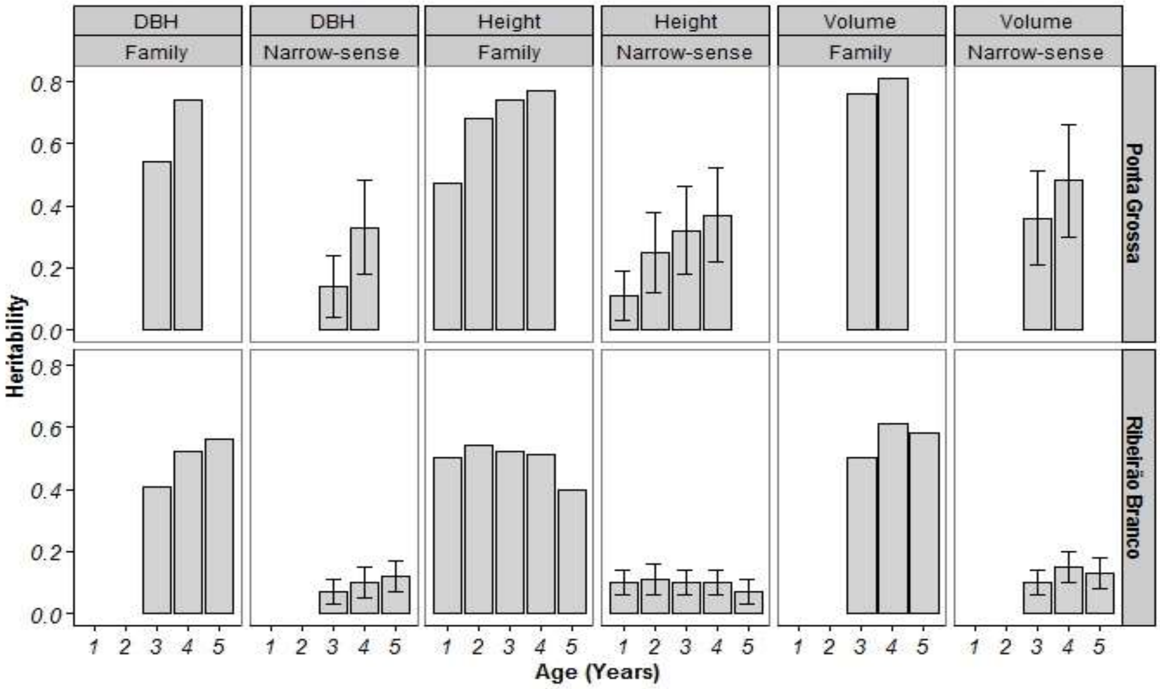
Heritability estimates vary with age, environment and species. Overall, estimates of family heritability mean for growth traits in pine species varies from 0.42 to 0.76 for *P. caribaea* var. *bahamensis* and slash pine (MISSIO et al., 2004; ROMANELLI; SEBBENN, 2004; SEBBENN et al., 2008). Olsson and Ericsson (2002) found low family heritability mean for six years old scots pine progenies in Sweden. They also affirm the estimate of this parameter increases as trial age also increase. As a result, considering the age of the progenies tests, they have a good prediction for traits of economic importance, which is interesting when it aims early age selection.

Deviations in shape, caused by sinuosity, stem malformation, apex loss or damage, and even fork may decrease the volume and the value of economic parts of the stem increasing the price of handling and transportation (Codesido; Fernández-López, 2008). In this case, fork is not easily transmissible to present lower h_a^2 0.05 (Ribeirão Branco) and 0.07 (Ponta Grossa) possibly due to the lower incidence of forked trees (Figure 14). Cotterill and Zed (1980) also found low h_a^2 for fork (0.02-0.04) in radiata pine progenies.

The stem straightness presented h_a^2 of 0.07 and 0.33 in Ribeirão Branco and Ponta Grossa, respectively (Figure 14). The average of this estimate varies considerably in relation to other species of pine as observed by: Wu et al. (2008) for radiata pine progenies - 0.02 to 0.57; Gwaze, Woolliams and Kanowski (1997) for loblolly pine - 0.13 to 0.55 and Weng et al. (2015) for jack pine - 0.11 to 0.22. Narrow-sense heritability for branch angle was 0.06 (Ribeirão White) and 0.17 (Ponta Grossa) (Figure 14), similar results found by Arregui et al. (1999), Espinel and Aragones (1997) and Codesido and Fernández-López (2008) in radiara pine trees.

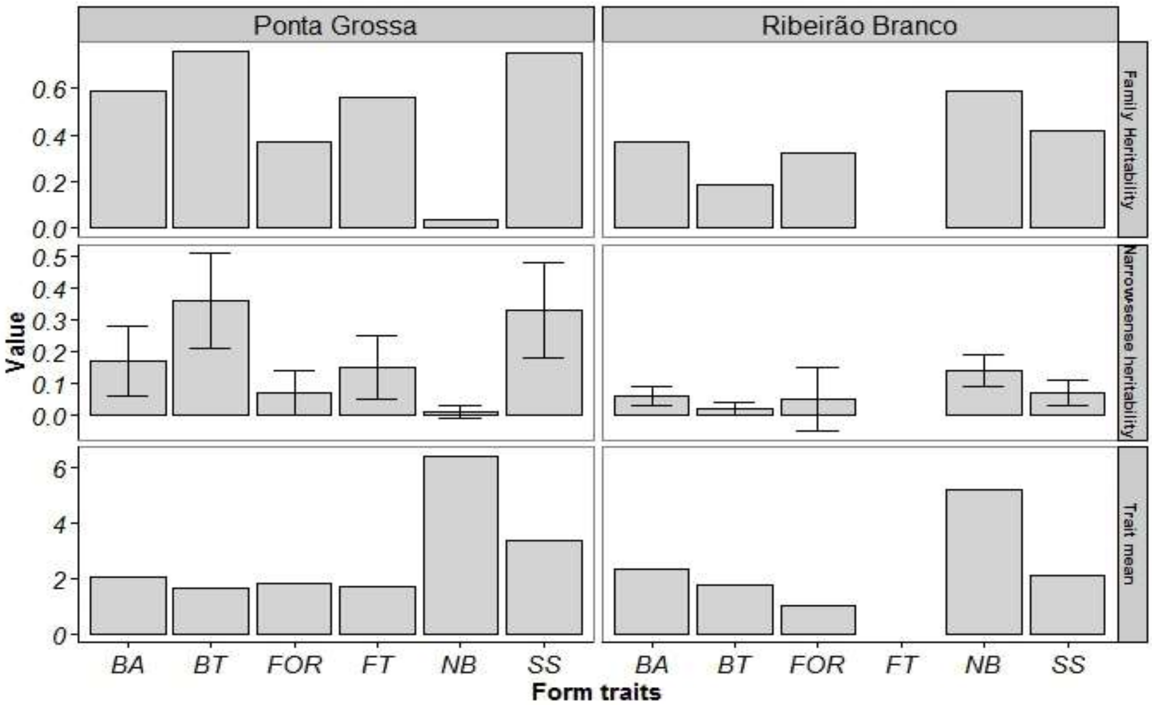
Taking everything into consideration, genetic selection for high heritabilities both individual and family mean levels indicate that these progenies have evolutionary potential to respond to environmental changes, because significant part of genetic variability is from genetic origin.

Figure 13 – Narrow-sense individual heritability and family heritability mean for slash pine growth traits in municipalities of Ribeirão Branco and Ponta Grossa, Brazil.



Source: The author.

Figure 14 – Narrow-sense individual heritability and family heritability mean for slash pine form traits in municipalities of Ribeirão Branco and Ponta Grossa, Brazil. (BA: branch angle, BT: branch thickness, FOR: fork, FT: fox tail, NB: number of branches, SS: stem straightness).



Source: The author.

3.3.2 Effective population sizes and expected selection gains

Three-selection strategies for each site were proposed base on individual BLUP (Table 3). This form of selection, enables, according to Resende and Higa (1994), explore fractions of additive genetic variance that are not considered in the selection among and within progenies, leading to accuracy selection maximization, although the inclusion of plot and blocks effects can change the selection.

Strategy I selected 80 best trees from each site with intention to establish clonal seed orchards. In Ribeirão Branco from 44 progenies, 19 were selected based on volume and eight for stem straightness, creating genetic diversity of 21% and 15%, respectively. In contrast, from 24 progenies in Ponta Grossa trial 15 were selected for both chosen traits, creating genetic diversity of 31% for both (Table 3).

After applying strategy, selection gain (S_g) was 8.65% (wood volume) and 4.04% (stem straightness) in Ribeirão Branco and 23.52% (wood volume) and 10.52% (stem straightness). It is notable percentage of genetic gain was greater in Ponta Grossa in relation to Ribeirão Branco for both traits with the same selected plant number. This superiority is due to greater genetic variation estimated at Ponta Grossa (Figure 10 and 11).

As experiment was planted in one-tree-plot design Strategy II selected two best trees per progeny to compose the next generation of improvement. As all progenies were chosen, genetic diversity was 100% for both local and traits (Table 3).

After thinning, S_g were 4.95% (wood volume) and 1.73% (stem straightness) in Ribeirão Branco while in Ponta Grossa were 16.09% (wood volume) and 7.63% (stem straightness). Adoption of blander selection intensity (strategy II) within progenies decreased the percentage of gain selection in Ribeirão Branco and Ponta Grossa trials. However, Ponta Grossa still showed higher selection gains due to higher genetic variation.

Strategy III selected 50% of best trees per progeny, as previous selection all progenies were selected obtaining 100% of genetic diversity (Table 5). With a bland selection there was S_g decreased for all traits and sites except stem straightness in Ponta Grossa where it is possible to see S_G increment compared to other strategies.

Although intense selection, as Strategy I, of high heritability values may lead to early elimination of important alleles (KAGEYAMA; VENCOVSKY, 1983), and result of genetic drift in combination with selection processes (PEREIRA; VENCOVSKY, 1988), in this case occurred increment of gain percentage. Moreover, estimative of these parameters confirm that for subsequent breeding program cycles and seedling seed orchard formation it is necessary to leave at least one tree per progeny to avoid excessive loss of variability and commitment of genetic gain in the next cycles, as observed in increment of genetic diversity when it happened. To form clonal and commercial plantations (as Strategies I and II) smaller selection intensities may be adopted to avoid loss of good material. The reason, if you consider intrapopulation recurrent selection the proposal is to increase, gradually, the frequency of desirable alleles (HALLAUER, 1992), mainly in early selection. According to Prescher (2007), while genetic gain increase, seed orchard produce genetically better material.

In theory, the selection made by BLUP create greater gains due to progeny effects (PAIVA et al., 2002), but nevertheless possibility of making different selections in different sites should be taking into consideration which would provide greater efficiency of genetic gain maintaining high genetic diversity, fundamental premise for breeding.

Table 3 – Selection strategies based on wood volume (VOL) and stem straightness (SS) for slash pine progenies in municipalities of Ribeirão Branco and Ponta Grossa, Brazil at five and four years old respectively. Strategy: I) Individual selection of 80 best trees; II) best two individuals per progeny and III) best 50% individuals per progeny.

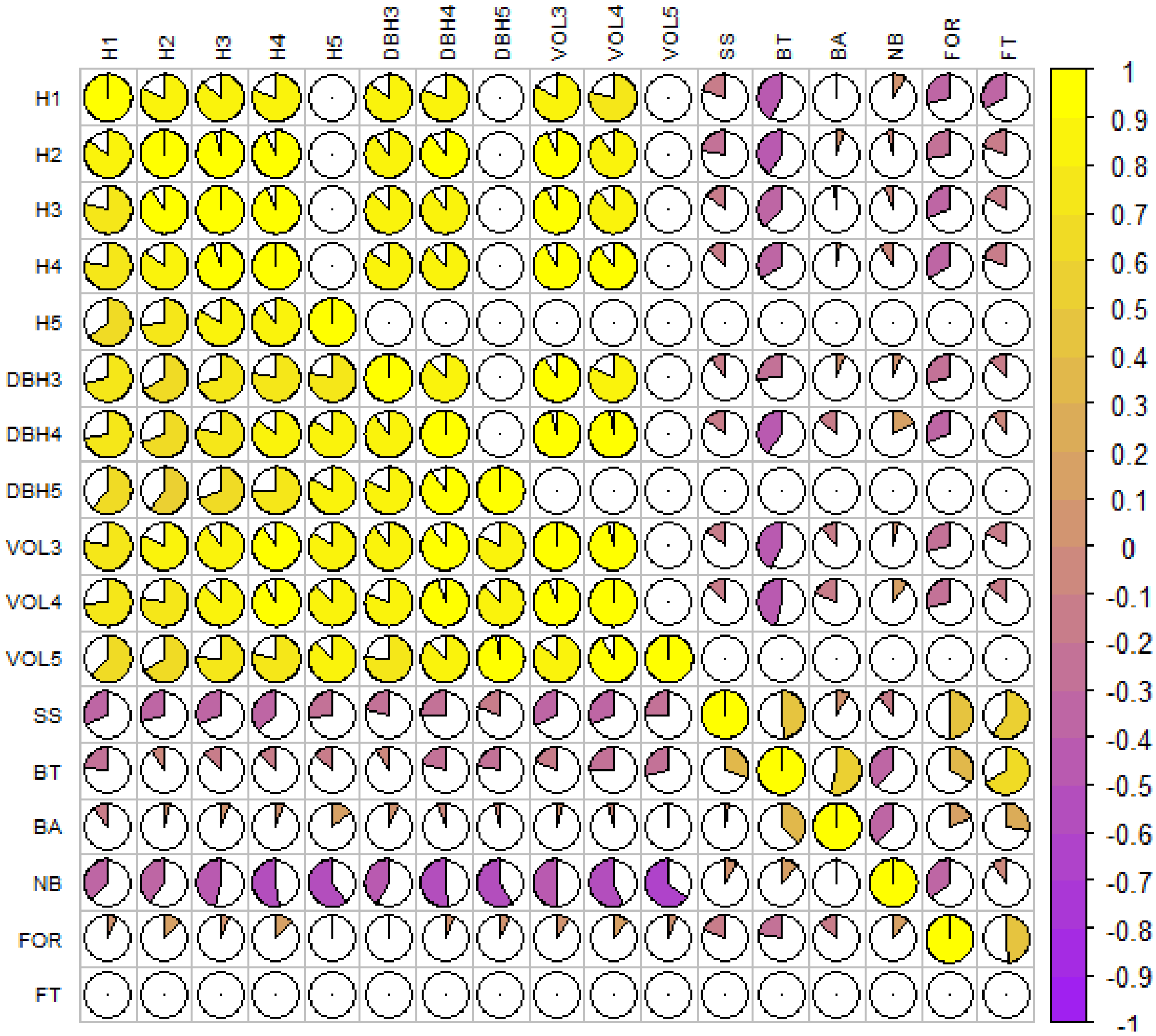
	Site	Traits	N	SI (%)	N_{fo}	N_f	k_f	σ^2_{kf}	N_e	MEI	S_G (%)	$\hat{D}$	$\bar{x}$
I	Ribeirão Branco	Vol	80	4.8	44	19	4.21	19.18	13.43	0.009	8.65	0.21	0.104
		SS	80	4.8	44	8	10.00	21.43	17.80	0.121	4.04	0.15	3.00
	Ponta Grossa	Vol	80	16.1	24	15	5.33	30.52	11.37	0.012	23.52	0.31	0.053
		SS	80	16.1	24	15	5.33	20.52	15.7	0.466	10.52	0.37	4.43
II	Ribeirão Branco	Vol	88	5.2	44	44	2.00	0.00	70.40	0.005	4.95	1.00	0.103
		SS	88	5.2	44	44	2.00	0.00	70.40	0.051	1.73	1.00	2.99
	Ponta Grossa	Vol	48	9.7	24	24	2.00	0.00	38.40	0.009	16.09	1.00	0.055
		SS	48	9.7	24	24	2.00	0.00	38.40	0.356	7.63	1.00	4.67
III	Ribeirão Branco	Vol	828	50.0	44	44	18.82	0.48	151.42	0.004	4.42	1.00	0.10
		SS	828	50.0	44	44	18.82	0.48	151.42	0.029	1.17	1.00	2.50
	Ponta Grossa	Vol	246	50.0	24	24	10.13	2.46	71.07	0.002	5.07	1.00	0.037
		SS	246	50.0	24	24	10.25	2.11	71.75	0.621	13.80	1.00	4.50

Number of selected trees (N); selection intensity (SI); original number of progenies (N_{fo}); number of selected progenies (N_f); number of selected individuals per progeny (k_f); variance of number of selected individuals per progeny (σ^2_{kf}); effective number (N_e); a: additive genetic effect = MEI: multi-effect index; selection gain (S_G (%)); genetic diversity ($\hat{D}$); general mean ($\hat{x}$). Source: The author.

3.3.3 Age-age and trait-trait genetic and phenotypic correlation

Coefficients of genetic correlation (Figure 15) were positive and significant for all evaluated pairs of growth traits ranging from 0.60 to 0.96 for different ages in Ribeirão Branco. However, form traits presented genetic correlation values with low to moderate magnitude, positive and negative, it include non-significant variables. Ponta Grossa presented positive and significant genetic correlation coefficients for all pairs of growth traits with values between 0.80 and 0.97. Most of pairs with form traits showed negative genetic correlation and some not significant among it.

Figure 15 – Estimates of genetic correlations (Ponta Grossa = above the diagonal, Ribeirão Branco = below the diagonal) between growth and forms traits for Slash pine progenies in Brazil.



H1, H2, H3, H4 and H5 = height at one, two, three, four and five years after planting; DBH3, DBH4 and DBH5 = diameter at breast height at three, four and five years after planting; VOL3, VOL4 and VOL5 = wood volume at three, four and five years after planting; SS = stem straightness; BT = branch thickness; BA = branch angle; NB = number of branches; FOR = fork. Source: The author.

High and significant genetic correlation values between different assessments of height and dbh in early age indicate that both traits are controlled by the same group of genes and allows a great reliability in the early selection. According to Phillips et al. (2013) and Sant'Ana et al. (2013), when the selection is made on traits with positive and high magnitude correlation it is possible to achieve high correlated response occurring an advantage on selection.

Favourable genetic correlations were found between the growth traits and stem straightness and fork indicating the selection of trees with high growth rate will result in trees straighter stems and no fork. These results have a practical implication, especially when the purpose is wood for sawing. Opposite result was found by Adams and Bastien (1994) in Douglas fir progenies and Cumbie, Isik and McKeand (2012) and Xiong et al. (2014) for Loblolly pine.

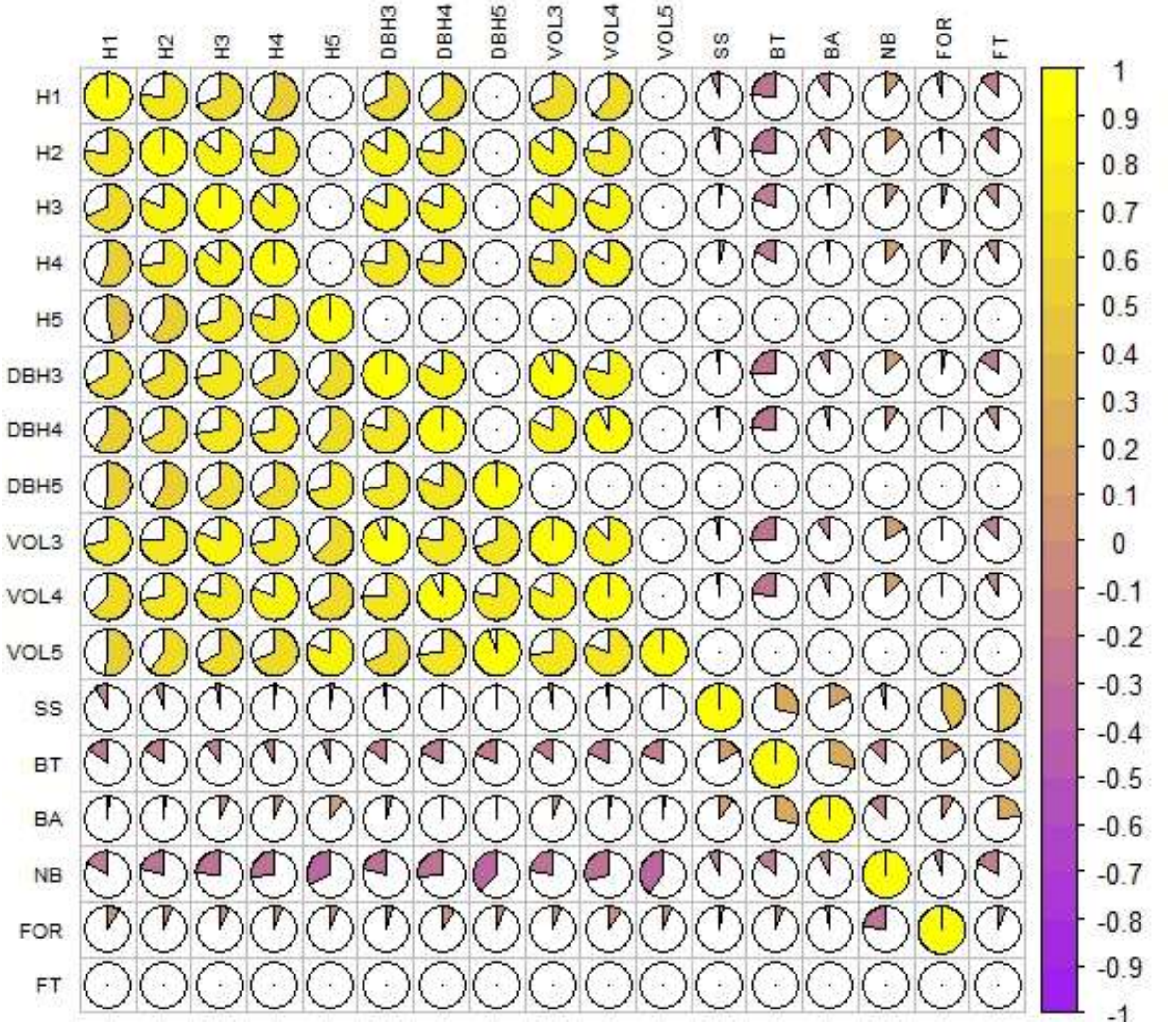
Fork and stem straightness had desirable genetic correlations only for Ponta Grossa. It means straightest trees will have less probability to present fork. Similar results were found in loblolly pine by Xiong et al. (2010). For the same authors, positive genetic correlation between traits imply that improvement of one trait can automatically lead to the improvement of the other one.

Phenotypic correlations were strongly positive and significant between pairs of growth traits to both sites (Figure 16). Exception was noted among fork and growth traits in Ribeirão Branco. Selection on one trait of a pair that is controlled by genetic correlation caused by pleiotropic effects will also stimulate response in unselected trait. Since the correlations we report here are possibly of this nature, truncation selection to improve height or dbh in slash pine is expected to cause increased wood volume (ROBERDS et al., 2003).

Genetic correlations were mostly higher than phenotypic correlations to both sites. According Gwaze (2009), models based on phenotypic correlations will underestimate the genetic progress and unnecessarily delay selection. Similar results have also shown that difference in other species of conifers as Loblolly pine (GWAZE; BRIDGWATER, 2002) and Jack pine (RIEMENSCHNEIDER, 1988).

For that reason, genetic correlations are important for making decision in forest breeding programs because, breeders can know if a trait can affect the improvement of another. Evaluation of genetic correlation between characters is needed to determine whether there will be favourable or unfavourable benefits for breeding program and if these correlations are required for the determination of multi-trait selection index (ATWOOD; WHITE; HUBER, 2002).

Figure 16 – Estimates of phenotypic correlations (Ponta Grossa = above the diagonal, Ribeirão Branco = below the diagonal) between growth and forms traits for Slash pine progenies in Brazil.



H1, H2, H3, H4 and H5 = height at one, two, three, four and five years after planting; DBH3, DBH4 and DBH5 = diameter at breast height at three, four and five years after planting; VOL3, VOL4 and VOL5 = wood volume at three, four and five years after planting; SS = stem straightness; BT = branch thickness; BA = branch angle; NB = number of branches; FOR = fork. Source: The author.

3.3.4 Joint analysis

Likelihood ratio test (LRT) was significant only for height and wood volume for conjoint analysis, indicating that there are genetic differences for slash pine progenies in both sites (Table 4).

Individual narrow-sense heritability (h_a^2) in the combined analysis showed low magnitude for all traits (Table 4), reaffirming the differences between trees at sites. On the other hand, the estimate family heritability mean level (h_m^2) were high (> 0.53 - dbh, and SS) implying that most of total variation observed is from genetic origin leading to high estimates accuracy

($r_{\hat{a}a}$) at conjoint analysis (> 0.73 - dbh and SS). These values show high genetic control and efficiency in a general selection involving two experiments, in case conjoint analysis be adopt as base.

Coefficients of genotype x environment interaction (C_{int}^2), which quantifies the portion of the total variation (phenotypic), which is represented by the variation of G x E interaction, showed low values for all traits. However, led to high magnitude of genetic correlation between the performance of progeny and environments (r_b – type b correlation) (Table 4), suggesting low G x E interaction.

Type b genetic correlations estimate genotypic performance correlation in different environments and provide an indication of the importance of G x E interaction (MCKEAND et al., 2006). Mulder et al. (2006) and Raymond (2011) affirm type b correlation levels should be greater than 0.60 to suggest an improvement level. Therefore, the situation is very favourable for application selection in both sites, in this case, height, dbh and stem. Similarly, Hodge and White (1992) and Dieters et al. (1995) also found type b correlation values greater than 0.60 in five years old slash pine progenies. The same way, Jayawickrama (2001) and Baltunis and Brawner (2010) reported relatively high type-b genetic correlations in New Zealand, for different radiata pine progenies sites, for most important traits demonstrating open-pollinated progenies are well protected against environmental variation. However, authors affirm also that results interpretation should be viewed carefully, mainly, if the experiments cover a representative species plantation area.

Progeny ranking for both sites was similar being able to indicate the same progenies for both growing region. Moraes (2013) also found type b correlation above 0.60 for height and dbh in three progenies tests of *Eucalyptus urophylla* at age 12 and 24 months, concluding that there was simple type G x E interaction. According to the authors, these results infer that although individuals presenting different behaviour in different sites, the environment does not affect productivity.

The coefficient of genetic variation (CV_{gi}) was high only for wood volume (10.04%) compared to the other traits (2.67% - height, 3.04% - dbh and 3.27% - SS) (Table 4). Martinez et al. (2012) also observed this tendency in loblolly pine progenies for height and DBH. The authors assert that the variance components differences between conjoint and individual analysis is due to the fact that conjoint analysis generate average components between sites.

Table 4 – Estimate of genetic parameters and genotype x environment interaction for height, diameter at breast height (DBH), wood volume and stem straightness (SS) in four years old slash pine progenies in Ribeirão Branco-SP and Ponta Grossa-PR, Brazil.

	Height (m)	DBH (cm)	Volume (m^3 $tree^{-1}$)	SS
h_a^2	0.03 (±0.01)	0.02 (±0.01)	0.05 (±0.01)	0.02 (±0.01)
h_m^2	0.60	0.53	0.62	0.53
$r_{âa}$	0.77	0.73	0.79	0.73
C_{int}^2	0.01	0.01	0.04	0.00
r_b	0.72	0.61	0.58	0.89
$CV_{gi}(\%)$	2.67	3.04	10.04	3.27
$CV_e(\%)$	15.57	19.42	41.93	25.23
μ	5.86	10.92	0.03	2.10
LRT	4.24*	3.00^{ns}	5.13*	3.16^{ns}

Narrow sense individual heritability (h_a^2); average heritability of progenies (h_m^2); accuracy ($r_{âa}$); coefficient of determination of genotype x environment interaction (C_{int}^2); genetic correlation between progenies between sites – type b correlation (r_b); coefficient of individual additive genetic variation (CV_{gi}); coefficient of residual variation (CV_e); general mean (μ); likelihood ratio test (LRT); significant to 1% (*) with 1 degree of freedom; (^{ns}) not significant. Source: The author.

3.3.5 Productivity, stability and adaptability

If the four best progenies were taken into consideration (E-197, E-189-1, E-084-2 and E-032-2) there is 100% of coincidence based on the adaptability (PRVG), stability (MHVG) and adaptability, stability and productivity criteria simultaneously (MHPRVG) for wood volume (Table 5). Although the selection order of 24 tested progenies at two sites is not exactly the same, the best progenies agreed for conjoint analysis, revealing that the most productive progenies are the most stable and high adaptability. The PRVG and MHPRVG values indicate the average genotype superiority in relation to the environmental in which it is grown (RESENDE, 2007b; ZENI NETO et al., 2008). For example, the best progeny (E-197) for wood production showed superiority over 1.27 times the average of environment in which it is located. MHPRVG * MH value shows the average genotypic value of the progenies in the two sites, and this amount has already been penalized by instability and capitalized by adaptability (CARBONELL et al. 2007). According to Gonçalves et al. (2003), environmental variations can interfere at G x E interactions, thus it is important and necessary to identify stable genotypes that produce well in various conditions.

In general, there were not many changes in the ordering of progenies (79% of coincidence). This is due to the high correlation of progeny genetic behaviour on sites, therefore, it is suggested that the MHVG, PRVG and MHPRVG methods present ranking agreement for progenies and the use of this selection criteria confer improvement of selection in deducting secure genetic values predictions and concomitantly for productivity, stability and adaptability (PINTO JUNIOR et al, 2006;. RESENDE, 2007b).

For inferences about the expected productivity, the genotypic values should be considered as the characteristics of the planting site. If plantings are carried out in several other sites with varying G x E interaction patterns it should be considered the genotypic values (genetic means) for sites average, penalized by instability and capitalized by the adaptability (MHPRVG). In addition to these, MHVG values should be considered if the planting is done in others unknown sites or with different interaction of experimental network pattern or high heterogeneity within sites. If the sites present the same G x E interaction pattern of planting environment should be based on the sites average in order to capitalize the responsiveness of each progeny in relation to environment improving, PRVG values will be prioritized. For planting in the same sites of the experiments consider the genotypic values (genetic means) of each local (individual analysis).

Taking everything into consideration, it is important to test a major number of progenies in major number of sites as possible to verify adaptability and stability of them in relation to next breeding cycles. With this practice will be possible to create plantations zones which could decreased time and increased productivity.

Table 5 – Slash pine progenies selection based on wood volume for stability (MHVG), adaptability (PRVG) and simultaneously to productivity, stability and adaptability (MHPRVG), at four years of age in municipalities of Ribeirão Branco and Ponta Grossa, Brazil.

Stability		Adaptability			Stability and adaptability		
Pro	MHVG	Pro	PRVG	PRVG*MG	Pro	MHPRVG	MH PRVG*MG
C-197	0.034	C-197	1.268	0.034	C-197	1.259	0.034
C-189-1	0.031	C-189-1	1.165	0.032	C-189-1	1.162	0.032
C-084-2	0.031	C-084-2	1.142	0.031	C-084-2	1.140	0.031
C-032-2	0.029	C-032-2	1.083	0.029	C-032-2	1.083	0.029
C-228	0.029	C-012-3	1.082	0.029	C-012-3	1.082	0.029
C-012-3	0.029	C-228	1.079	0.029	C-228	1.079	0.029
C-128-2	0.029	C-128-2	1.076	0.029	C-128-2	1.076	0.029
C-067-1	0.029	C-067-1	1.054	0.029	C-067-1	1.053	0.029
C-083-1	0.027	C-083-1	1.004	0.027	C-016-2	1.002	0.027
C-016-2	0.027	C-016-2	1.003	0.027	C-083-1	1.000	0.027
C-048-2	0.027	C-048-2	0.995	0.027	C-048-2	0.995	0.027
C-013	0.027	C-013	0.979	0.027	C-013	0.979	0.027
C-068-1	0.027	C-068-1	0.974	0.026	C-068-1	0.973	0.026
C-025-3	0.026	C-025-3	0.970	0.026	C-025-3	0.970	0.026
C-047-1	0.026	C-047-1	0.966	0.026	C-047-1	0.966	0.026
C-019-1	0.026	C-019-1	0.949	0.026	C-019-1	0.949	0.026
C-217-1	0.025	C-217-1	0.935	0.025	C-217-1	0.935	0.025
C-036-1	0.025	C-032-1	0.917	0.025	C-032-1	0.917	0.025
C-032-1	0.025	C-010-1	0.915	0.025	C-010-1	0.915	0.025
C-010-1	0.025	C-036-1	0.914	0.025	C-036-1	0.914	0.025
C-039-2	0.025	C-039-2	0.908	0.025	C-039-2	0.906	0.025
C-023-1	0.024	C-023-1	0.899	0.024	C-023-1	0.898	0.024
C-272-1	0.024	C-272-1	0.892	0.024	C-272-1	0.892	0.024
C-225	0.023	C-225	0.834	0.023	C-225	0.834	0.023

Pro = Progeny. Source: The author.

3.4 CONCLUSIONS

There are significant differences among open-pollinated juvenile progenies in Ribeirão Branco and Ponta Grossa trials for growth traits in Brazil. Considerable genetic variation, especially for wood volume was found in Ribeirão Branco (14.31% to 16.24%) and Ponta Grossa (31.78% to 33.77%) sites as high narrow-sense individual heritability (0.36 to 0.48) for Ponta Grossa evidencing high genetic control for the traits is significant and the possibility of genetic gains with selection.

The percentage of selection gain based on wood volume and tem straightness were 8.65% and 4.04% (Ribeirão Branco) and 23.52% and 10.52% (Ponta Grossa) when higher intensity is applied aiming clonal orchard formation.

Positive and significant genetic correlation were found for all evaluated pairs of growth traits ranging from 0.60 to 0.96 for different ages in Ribeirão Branco and 0.80 and 0.97 in Ponta Grossa. In relation to form traits it was found values with low to moderate magnitude, positive and negative for both site.

The effect of genotype x environment interaction is simple which means progenies planted in one site can also be planted in the other.

Genotypes such as C-197, C-189-1, C-084-2 and C-032-2 are indicated to plantations in both São Paulo and Paraná states according to stability (MHVG), adaptability (PRVG) and simultaneously productivity, stability and adaptability (MHPRVG).

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4 CHAPTER II

GENETIC DIVERGENCE AND TWO DIFFERENT CLUSTERING METHODS MID SLASH PINE SECOND GENERATION PROGENIES IN EARLY AGE

ABSTRACT

The objective of this study was to estimate the genetic divergence and test two different clustering methods in slash pine open-pollinated progenies from growth and form traits at early age. Seeds from 44 parent trees were collected from a first generation seed orchard in Colombo, PR, Brazil. With this material, it was established two progeny tests in a randomized complete block. The first test was implemented in March 2009 in Ribeirão Branco, São Paulo state, Brazil, containing 40 blocks, one tree per plot, 44 treatments (progenies) and 6 controls. The second was established in Ponta Grossa, PR using the same experimental design and number of plants per plot with 24 treatments, 32 repetitions. The 3.0 x 3.0 meters spacing has been adopted in both tests. We evaluated growth traits as total height, diameter at breast height and wood volume in the five years following planting. Form traits as stem straightness, stem thickness, branch angle, number of branches, fork, survival and fox tail were evaluated only for five years after planting. The genetic divergence was estimated from Mahalanobis distance. Based on matrices of distances were applied and compared two methods of hierarchical groupings UPGMA method (Unweighted Pair Group Method using Arithmetical Averages) and Tocher optimization. There was agreement between clustering methods. Five and ten groups were identified by UPGMA and Tocher grouping respectively for Ribeirão Branco and Ponta Grossa. For breeding programs, it is recommended to cross-divergent groups progenies to increase genetic variation and genetic gain in subsequent generations.

Keywords: *Pinus elliottii* Engelm. var. *elliottii*. Mahalanobis distance. UPGMA. Tocher optimization.

RESUMO

O objetivo deste trabalho foi estimar a divergência genética e testar dois diferentes métodos de agrupamento em progênies de polinização aberta de segunda geração de *P. elliottii* var. *elliottii* a partir caracteres de crescimento e forma em idade precoce. Sementes de 44 árvores matrizes foram coletadas de um pomar de sementes de primeira geração em Colombo, PR, Brasil. Com esse material, foram estabelecidos dois testes de progênies em delineamento de blocos completos casualizados. O primeiro teste foi implantado em março de 2009 em Ribeirão Branco, SP, contendo 40 repetições, uma planta por parcela e 44 tratamentos (progênies) e 6 testemunhas comerciais. O segundo foi estabelecido em Ponta Grossa, PR utilizando o mesmo delineamento experimental e número de plantas por parcela, com 24 tratamentos e 32 repetições. O espaçamento 3,0 x 3,0 metros foi adotado em ambos os testes. Foram avaliados os caracteres de crescimento como altura total, diâmetro à altura do peito e volume de madeira nos cinco anos subsequentes ao plantio. Os caracteres de forma de fuste, espessura de galho, ângulo de galho, número de galhos, bifurcação, sobrevivência e *fox tail* foram avaliados somente aos cinco anos após o plantio. A divergência genética foi estimada a partir da distância generalizada de Mahalanobis. Com base nas matrizes de distâncias foram aplicados e comparados dois métodos de agrupamentos métodos hierárquico UPGMA (Unweighted Pair Group Method using Arithmetical Averages) e o otimização Tocher. Existe divergência genética entre as progênies de *P. elliottii* avaliadas em ambos testes. Houve concordância entre os métodos de agrupamentos. Cinco e dez grupos foram discriminados pelo agrupamento UPGMA e Tocher, respectivamente para Ribeirão Branco e Ponta Grossa. Para programas de melhoramento, recomenda-se o cruzamento entre progênies de grupos divergentes para aumentar a variação genética e ganho genético nas gerações subsequentes.

Palavras-chave: *Pinus elliottii* Engelm. var. *elliottii*. Distância de Mahalanobis. UPGMA. Otimização de Tocher.

4.1 INTRODUCTION

Exotic forest species were initially introduced in Brazil in order to improve economy development for being an alternative way to native forest exploration (SAMPAIO; REZENDE; ARAÚJO, 2000). European immigrants introduced the first pine species in the country in the late 1936 for ornamental and wood production purpose (FIER; KIKUTI, 1993). However, this first attempt was unsuccessful by climate difference between the two continents making these species do not adapt well to new habitat. Subsequently, several forest species from the United States, Mexico, Central America, Caribbean Islands and Asia have been introduced in country (SILVA et al., 2012). Nowadays, those pine species are more important economically and more used by forestry sector.

Among the species of this genus we can highlight slash pine (*P. elliottii* Engelm. var. *elliottii*), tree originating from southern United States, economically important and widely cultivated in subtropical regions of Brazil due to its plasticity and ability to adapt in different environments (UNITED STATES DEPARTMENT OF AGRICULTURE – USDA, 2004). Besides the good adaptability, population must present good genetic divergence to increase genetic variability among progenies. In breeding programs, the divergence is one of the most important parameters evaluated, especially in early age. It is possible to identify parents when crossed, enable a higher heterotic effect (CARVALHO et al., 2003).

Genetic divergence may be estimated based on dialelics analysis, however, in perennial plants, tend to be disadvantageous and costly. Another measure method is the predictive nature, which is more feasible and used based on the morphological, agricultural and molecular differences. It is performed from the clustering methods to express the degree of diversity among progenies (CRUZ; REGAZZI; CARNEIRO, 2004). The same authors affirm predictive methods of genetic divergence are widely used, mainly because they are based on morphological and physiological genotypes differences, between these methods we can mention those that quantify the genetic diversity by cluster methods such as Mahalanobis and Euclidean generalized distances. The first offers more advantage than the second, because it takes into account the correlation among traits, however, it is necessary experimental trials with repetition. With distance estimates between each pair of genotype, results can be presented in symmetric matrix, and from it can easily visualize and interpret distances through cluster methods or dispersion graphic (CRUZ; REGAZZI; CARNEIRO, 2004). Several authors demonstrate the feasibility of Mahalanobis distance as a way to parent’s choice (MALUF;

FERREIRA; MIRANDA, 1983; DIAS; KAGEYAMA, 1997; MACHADO et al., 2002; SUINAGA et al., 2003).

Cluster methods tend to separate a group of initial observations in multiple subgroups in order to obtain similarity and distinguish between subgroups, hierarchical and optimization are the most common in plant breeding (BERTAN et al., 2006). In relation to hierarchical methods, progenies are grouped by a series of methods which are reproduced in various levels establishing a dendrograma, without number of groups regardless. The representation of cluster structure can be made by different methods, as average distance between all pairs of progenies, called method of average distance (UPGMA) (CRUZ; REGAZZI; CARNEIRO, 2004). The optimization methods, on the other hand, formed groups by improving certain clustering criteria what differ from the first one by the fact that it creates another specific groups. In the optimization method proposed by Tocher, criteria used is to maintain the average distance always lower intragroup at any distance groups (RAO, 1952; CRUZ; REGAZZI; CARNEIRO, 2004).

The genetic diversity is of great importance for plant breeding, therefore, adequately explored, it can reduce vulnerability to diseases and at the same time accelerate genetic progress for certain trait (CUI et al., 2001). More divergent are the parents, greater is resulting variability at segregating population, and greater the probability to regroup alleles in new favourable combinations (MANFIO et al., 2012). Thus, the objective of this study was to estimate the genetic distance among slash pine progenies and compare two clustering methods through growth and form traits by multivariate procedures in order to guide future control pollination in breeding programs aiming wood production.

4.2 MATERIALS AND METHODS

4.2.1 The trials

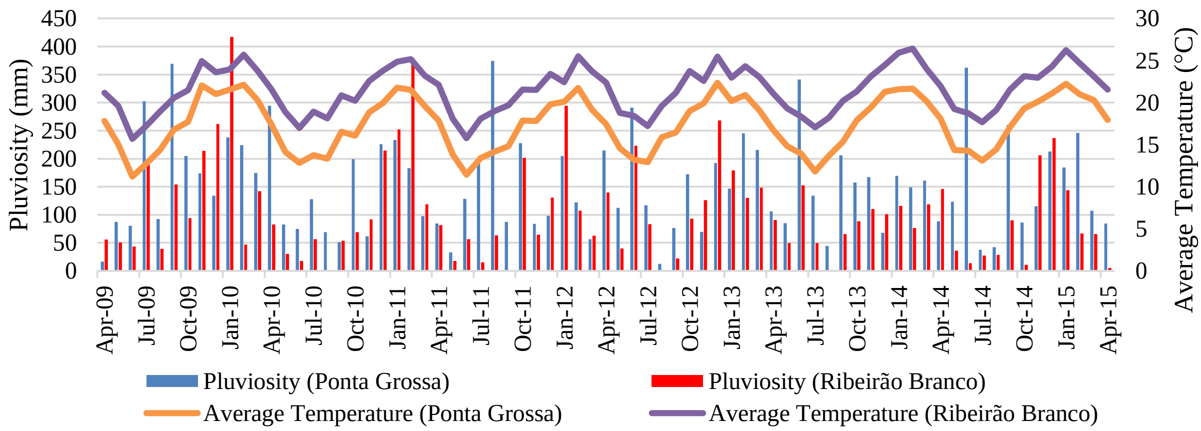
Forty-four progenies originated from a slash pine first generation seed orchard established in Colombo-PR, Brazil, were used in this study. The seedlings were produced in municipality of Ribeirão Branco-SP, Brazil, in 2008. In March of the following year two second generation progeny tests they were installed, one in Ponta Grossa-PR and another one in Ribeirão Branco-SP, Brazil (Table 1, Figures 6 and 7).

Table 6 – Details of slash pine progenies tests deployed in municipalities of Ribeirão Branco-SP and Ponta Grossa-PR, Brazil.

	Ribeirão Branco-SP	Ponta Grossa-PR
Progenies number	44	24
Blocks	40	32
Controls	6	-
Spacing	3m x 3m	
Plating date	March/2009	
Border	2 lines	
Latitude	25° 05' 42" S	24° 13' 15" S
Longitude	50° 09' 43" W	48° 45' 56" W
Altitude	969 m	875 m
Pluviosity	1,346 mm	1,495 mm
Clime	Cfb	Cfb
Average temperature	18 °C	17.5 °C
Soil type	Cambissolo	Cambissolo

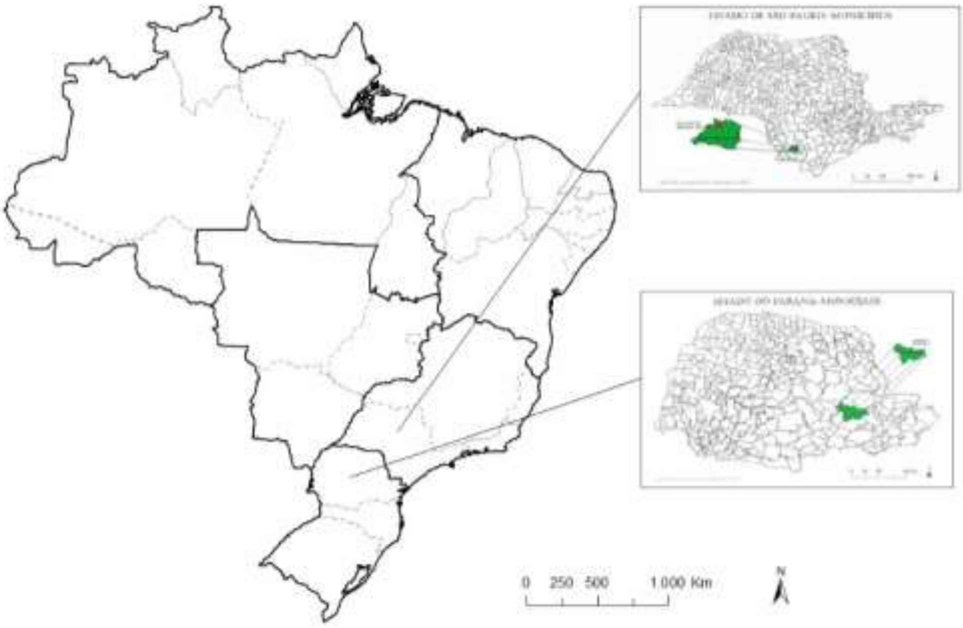
Source: The author.

Figure 17 – Total precipitation and monthly temperature mean for municipalities of Ribeirão Branco-SP and Ponta Grossa-PR, Brazil, from April 2009 to April 2015.



Source: Prepared by the author based on data provided by the National Institute of Meteorology.

Figure 18 – Geographical localization of municipalities of Ribeirão Branco-SP and Ponta Grossa-PR, respectively, in Brazil.



Source: IBGE cartographic base, adapted by Ananda Aguiar and the author.

4.2.2 Traits measurement

Data was obtained within five years after planting and traits were: survival rate; total height, diameter at breast height (dbh) and wood volume, computed based on the total height of data and diameter at breast height, according to the following equation:

$$VOL = \frac{\pi(DBH)^2.FF}{40000}\ TH \tag{15}$$

Where VOL was wood volume, dbh was the diameter at breast height, FF was the stem form factor and TH was total height. The height trait was measured using a telescopic ruler, circumference at breast height was measured with a tape calculating from these values the diameter at breast height and survival was adopted "1" to alive and "0" to dead plants. Form traits were visually scored as follows (Table 2).

Table 7 – Scoring for form traits of slash pine progenies in municipalities of Ribeirão Branco and Ponta Grossa, Brazil.

	Scale		Description	
Traits	Ribeirão Branco	Ponta Grossa	Ribeirão Branco	Ponta Grossa
Stem straightness	1-3	1-5	1 = most crooked steams, 3 = straightest stems	1 = most crooked steams, 5 = straightest stems
Branch thickness	1-3		1 = thicker, 3 = thinner	
Branch angle	1-3		1 = less than 45º, 2 = greater than 45º and 3 = approximately 90º	
Number of branches	1-7		1 = 42 to 48 branches, 7 = 1 to 6 branches	1 = 17 to 18 branches, 7 = 0 to 2 branches
Fork	1 and 2		"1" for absence and "2" for presence	
Fox tail (only for Ponta Grossa trial)	1 and 2		"1" for absence and "2" for presence	

Source: The author.

4.2.3 Estimates of genetic divergence

Genetic diversity among progenies was estimated by Generalized Mahalanobis Distance (D^2), and this methodology suitable for quantitative data analysis by considering the standard deviation and invariant scale. Thus, D^2 is estimated by expression (CRUZ; REGAZZI; CARNEIRO, 2004):

$$D^2_{ii'} = \delta'\psi^{-1}\delta \qquad (16)$$

Where:

$D^2_{ii'}$ = Mahalanobis distance between genotype i and i';

δ' = [d1, d2, ..., dv], where $d_j = Y_{ij} - Y_{i'j}$;

ψ = residual variance and covariance matrices;

Y_{ij} = average of the i^{th} genotype with respect to the i^{th} variable.

In quantitative data analysis, this methodology is appropriate for considering the variances matrices and residual covariances between measured traits.

With Mahalanobis distance matrix estimated by genetic statistical software SELEGEN-REML / BLUP (RESENDE, 2007) we can build a hierarchical structure dendrogram format by the average distance method (UPGMA - Unweighted Pair Group Method using Arithmetical Averages). To carry out this analysis we used the statistical software R version 3.2.0.

With getting D^2, Tocher optimization method was applied, in order to recognize groups of homogeneous genotypes. This method adopt average D^2 values (intracluster) criteria and must be lower than the D^2 values (inter-cluster) (CRUZ; REGAZZI; CARNEIRO, 2004).

4.3 RESULTS AND DISCUSSION

4.3.1 Generalized Mahalanobis distance

Mahalanobis generalized quantitative distances (D_2) between pairs of slash pine progenies varied from 7.59 to 72.38 and from 10.34 to 41.63 for Ribeirão Branco and Ponta Grossa, respectively. The maximum distance between progenies in Ribeirão Branco was observed between C-053-1/C-197 and the minimum between C-084-2/C-112-1 (Appendix 15). For better understanding results, we consider the maximum distance as 100% and the minimum distance as 10.5% of it which means closer individuals (Table 8). In Ponta Grossa, the maximum distance was between progenies C-047-1 and C-023-1 and the minimum distance was between C-084-2 and C-025-3 (Appendix 16). The same way as in Ribeirão Branco, the minimum distance would correspond to 24.8% of it (Table 9). As noticed, progenies in Ribeirão Branco presented bigger distances among them in relation to Ponta Grossa. This difference can be due to the number of progenies in each site, Ribeirão Branco has got 44 progenies and Ponta Grossa 24. Based on these results individuals will be selected according to their superior genetic divergence and production performance and/or greater genotypic value to make controlled crossings. Thus, cross between C-197 x C-053-1 and C-047-1 x C-023-1 could perform better other progeny pairs. These combinations should be exploited for future hybridization, especially for detailed studies of heterosis effects on quantitative traits.

For Martins, Pires and Oliveira (2002), the choice of crossed individuals based only on their genetic divergence, without taking into account their performance, must be avoided. The most appropriate, according to the authors, is to recommend crossings between divergent individuals that exhibit higher performances to main features. Checking progeny rank (Table 10) it is possible to notice that the most distant progenies are not the most productive for wood volume in both sites. In this case it is better to consider the choices between the most distant and the most productive individuals. However, this comparison should be cautious as the genetic divergence takes into account all traits.

Estimates of distance measures, obtained by Mahalanobis generalized quantitative distances for slash pine in both sites were higher than observed by Silva et al. (2012) for *Pinus caribaea* var. *caribaea* at age 14 without and with thinning; Missio, Moraes and Dias (2007) for *Pinus caribaea* var. *bahamensis* at age 13 also without and with thinning; and Santos et al. (2016) for *Pinus caribaea* var. *hondurensis* in relation to Ribeirão Branco. It is possible to note that the current material with five years of age presents more genetic divergence than older materials.

According to Dias and Kageyama (1997), pairs of greater genetic divergence guides the hybridization process. Thus, pairs more divergent must be used for generating more heterosis, while less divergent, differentiated primarily by transferred gene allow recover recurrent parent quickly through backcross. Heterosis is dependent on the presence of directional dominance, epistasis and allele frequency differences. Heterotic groups in cross-pollinated species have been created and enhanced by creating groups and families that differ in allele frequencies in genes affecting target trait (BETRÁN; MORENO-GONZÁLEZ; ROMAGOSA, 2009). In addition to this, hybrid superiority may ascend by heterosis, epistasis and also by trait complementarity. Complementarity is found over additive effects and results from independent traits synergy according to particular environments where parents are less well adapted than their hybrid, in addition to these, it may happen when non divergent individuals are crossed to forest species (NICHOLAS 1987; SEDGLEY; GRIFFIN 1989; MARTIN 1989).

It is important to notice the relative contribution of each trait for the genetic divergence has got great importance to identify the traits with highest contribution and also to assist in the disposal of those which contribute less to genotypes discrimination, reducing, thus, manpower, time and cost spent on experimentation (CORREA; CONÇALVES, 2012).

Table 8 – Generalized Mahalanobis Distance (D^2) in percentage between slash pine progenies in municipality of Ribeirão Branco-SP, Brazil in relation to grater progeny distance.

D^2 Mahalanobis Distance									
Progeny	**Greater (%)**	**Progeny**	**Smaller (%)**	**Progeny**	**Progeny**	**Greater (%)**	**Progeny**	**Smaller (%)**	**Progeny**
C-016-2	72.6	C-098-1	23.0	C-013	C-032-1	65.6	C-081-3	28.6	C-025-3
C-082-1	61.6	C-047-1	19.9	C-084-2	C-068-1	59.6	C-159-1	27.2	C-063-2
C-082-1	73.2	C-071-1	18.3	C-032-2	C-053-1	71.8	C-217-1	30.9	C-048-2
C-074-2	65.2	C-010-1	18.6	C-045-1	C-053-1	75.2	C-048-2	24.9	C-059-1
C-082-1	61.3	C-023-1	21.4	C-112-1	C-053-1	65.2	C-272-1	21.3	C-043-3
C-053-1	70.4	C-100-2	23.7	C-001-1	C-053-1	62.0	C-128-2	62.0	C-053-1
C-197	55.1	C-084-2	10.5	C-112-1	C-053-1	65.5	C-032-1	35.8	C-043-3
C-082-1	59,7	C-120-1	59.7	C-082-1	C-063-2	55.76	C-053-1	48.5	C-032-2
C-053-1	69.3	C-036-1	18.7	C-048-2	C-047-2	77.0	C-068-1	33.5	C-043-3
C-053-1	82.5	C-012-3	26.0	C-045-1	C-019-1	60.4	C-063-2	40.3	C-013
C-082-1	67.3	C-151-1	21.5	C-039-1	C-016-2	64.9	C-042-1	24.4	C-013
C-082-1	69.4	C-045-1	23.3	C-189-1	C-218-1	50.5	C-043-3	25.0	C-032-2
C-082-1	70.2	C-112-1	11.6	C-218-1	C-025-3	49.8	C-047-2	25.0	C-218-1
C-053-1	76.9	C-001-1	35.0	C-189-1	C-067-1	49.1	C-039-1	25.0	C-013
C-197	68.3	C-039-2	12.5	C-272-1	C-025-3	64.4	C-067-1	23.0	C-032-2
C-053-1	66.6	C-225	16.9	C-013	C-016-2	55.8	C-218-1	25.2	C-019-1
C-074-2	83.9	C-082-1	46.7	C-059-1	C-016-2	58.7	C-013	31.8	C-059-1
C-197	68.5	C-189-1	25.2	C-048-2	C-025-3	42.8	C-019-1	16.0	C-059-1
C-053-1	74.6	C-083-1	33.2	C-047-2	C-059-1	39.0	C-025-3	33.8	C-016-2
C-197	86.7	C-074-2	40.7	C-272-1	C-016-2	33.7	C-032-2	30.8	C-059-1
C-068-1	65.8	C-228	22.3	C-047-2	C-059-1	25.9	C-016-2		
C-053-1	100.0	C-197	52.3	C-272-1					
Maximum:	100.0						progenies:	C-053-1 and C-197	
Minimum:	10.5						progenies:	C-084-2 and C-112-1	

Source: The author.

Table 9 – Generalized Mahalanobis Distance (D^2) in percentage between slash pine progenies in municipality of Ponta Grossa-PR, Brazil in relation to grater progeny distance.

D2 Mahalanobis Distance									
Progeny	**Greater (%)**	**Progeny**	**Smaller (%)**	**Progeny**	**Progeny**	**Greater (%)**	**Progeny**	**Smaller (%)**	**Progeny**
C-047-1	80.1	C-084-2	24.8	C-025-3	C-047-1	96.5	C-019-1	36.8	C-012-3
C-197	95.4	C-025-3	52.1	C-083-1	C-047-1	88.9	C-067-1	44,3	C-217-1
C-019-1	89.6	C-010-1	46.5	C-016-2	C-047-1	67.1	C-012-3	32.5	C-083-1
C-039-2	94.2	C-068-1	48.0	C-032-1	C-047-1	83.0	C-225	62.0	C-228
C-047-1	81.7	C-016-2	34.5	C-012-3	C-189-1	85.6	C-036-1	60.9	C-083-1
C-128-2	85.7	C-272-1	37.1	C-083-1	C-047-1	93.0	C-228	62.0	C-083-1
C-036-1	90.7	C-128-2	53.3	C-083-1	C-048-2	93.0	C-189-1	47.0	C-083-1
C-048-2	86.5	C-032-1	30.1	C-012-3	C-047-1	68.6	C-048-2	47.3	C-083-1
C-217-1	91.4	C-013	47.4	C-067-1	C-047-1	94.5	C-197	70.7	C-217-1
C-189-1	86.3	C-032-2	44.2	C-012-3	C-047-1	75.6	C-083-1	60.6	C-217-1
C-023-1	78.3	C-039-2	14.08	C-225	C-217-1	84.4	C-047-1		
C-047-1	100.0	C-023-1	33.8	C-036-1					
Maximum:	100.0						progenies:	C-047-1 and C-023-1	
Minimum:	24.8						progenies:	C-084-2 and C-025-3	

Source: The author.

Table 10 – Progeny rank for wood volume at four years of age at Ribeirão Branco and three years of age for Ponta Grossa, Brazil (a: additive genetic effect).

Site	Rank	Progeny	a	Gain	New mean	Rank	Progeny	a	Gain	New mean
Riberão Branco	1	C-063-2	0.0139	0.0139	0.0642	23	C-120-1	-0.0002	0.0042	0.0545
	2	C-098-1	0.0098	0.0118	0.0622	24	C-012-3	-0.0003	0.0040	0.0543
	3	C-228	0.0092	0.0109	0.0613	25	C-159-1	-0.0008	0.0038	0.0541
	4	C-001-1	0.0090	0.0105	0.0608	26	C-013	-0.0009	0.0036	0.0539
	5	C-100-2	0.0089	0.0101	0.0605	27	C-218-1	-0.0010	0.0034	0.0538
	6	C-071-1	0.0071	0.0096	0.060	28	C-032-1	-0.0015	0.0033	0.0536
	7	C-067-1	0.0056	0.0091	0.0594	29	C-036-1	-0.0017	0.0031	0.0534
	8	C-032-2	0.0045	0.0085	0.0588	30	C-068-1	-0.0027	0.0029	0.0532
	9	C-189-1	0.0042	0.0080	0.0583	31	C-043-3	-0.0028	0.0027	0.053
	10	C-083-1	0.0041	0.0076	0.0580	32	C-039-2	-0.0031	0.0025	0.0529
	11	C-047-2	0.0037	0.0073	0.0576	33	C-019-1	-0.0039	0.0023	0.0527
	12	C-197	0.0035	0.0070	0.0573	34	C-217-1	-0.0043	0.0021	0.0525
	13	C-053-1	0.0030	0.0066	0.0570	35	C-010-1	-0.0046	0.0019	0.0523
	14	C-039-1	0.0028	0.0064	0.0567	36	C-025-3	-0.0050	0.0018	0.0521
	15	C-084-2	0.0018	0.0061	0.0564	37	C-082-1	-0.0055	0.0016	0.0519
	16	C-151-1	0.0015	0.0058	0.0561	38	C-023-1	-0.0058	0.0014	0.0517
	17	C-042-1	0.0013	0.0055	0.0559	39	C-225	-0.0071	0.0011	0.0515
	18	C-081-3	0.0013	0.0053	0.0556	40	C-045-1	-0.0075	0.0009	0.0513
	19	C-128-2	0.0008	0.0051	0.0554	41	C-016-2	-0.0087	0.0007	0.051
	20	C-047-1	0.0001	0.0048	0.0551	42	C-059-1	-0.0089	0.0005	0.0508
	21	C-048-2	0.0000	0.0046	0.0549	43	C-074-2	-0.0096	0.0002	0.0506
	22	C-112-1	-0.0001	0.0044	0.0547	44	C-272-1	-0.0100	0.0000	0.0503
Ponta Grossa	1	C-197	0.0272	0.0272	0.0579	13	C-048-2	-0.0025	0.0054	0.0362
	2	C-189-1	0.0157	0.0214	0.0522	14	C-068-1	-0.0040	0.0047	0.0355
	3	C-084-2	0.0136	0.0188	0.0496	15	C-032-1	-0.0043	0.0041	0.0349
	4	C-128-2	0.0063	0.0157	0.0464	16	C-023-1	-0.0045	0.0036	0.0344
	5	C-012-3	0.0056	0.0137	0.0444	17	C-019-1	-0.0046	0.0031	0.0339
	6	C-032-2	0.0039	0.0120	0.0428	18	C-217-1	-0.0048	0.0027	0.0334
	7	C-016-2	0.0026	0.0107	0.0415	19	C-010-1	-0.0053	0.0023	0.0330
	8	C-228	0.0024	0.0097	0.0404	20	C-083-1	-0.0055	0.0019	0.0326
	9	C-067-1	0.0006	0.0087	0.0394	21	C-272-1	-0.0068	0.0015	0.0322
	10	C-025-3	-0.0010	0.0077	0.0384	22	C-036-1	-0.0080	0.0010	0.0318
	11	C-047-1	-0.0017	0.0068	0.0376	23	C-039-2	-0.0105	0.0005	0.0313
	12	C-013	-0.0023	0.0061	0.0368	24	C-225	-0.0123	0.0000	0.0308

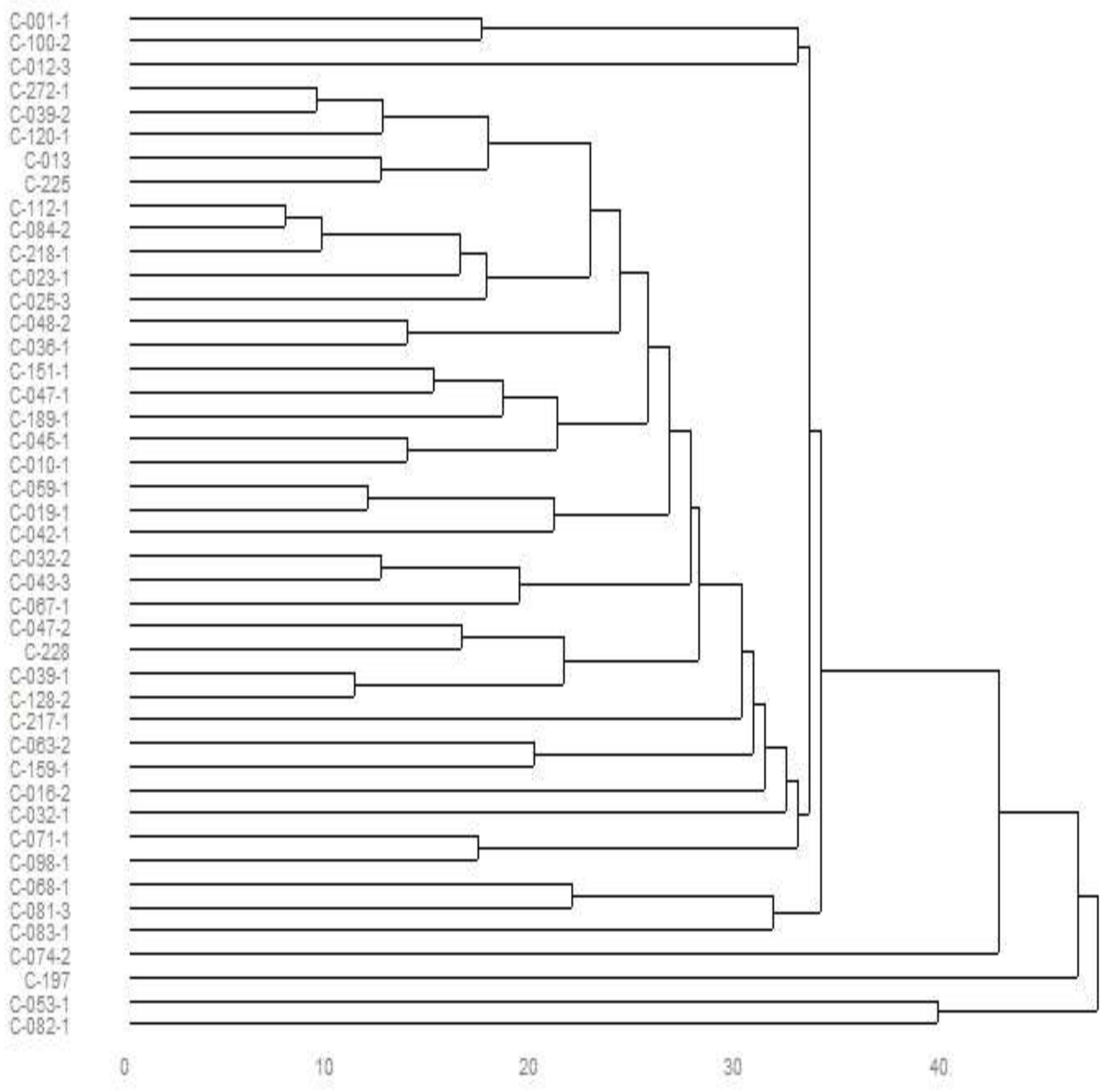
Source: The author.

4.3.2 Comparison between clustering methods

Based on Mahalanobis distance matrix among progenies a dendrogram by UPGMA method was obtained. We observed the formation of five distinct groups in Ribeirão Branco

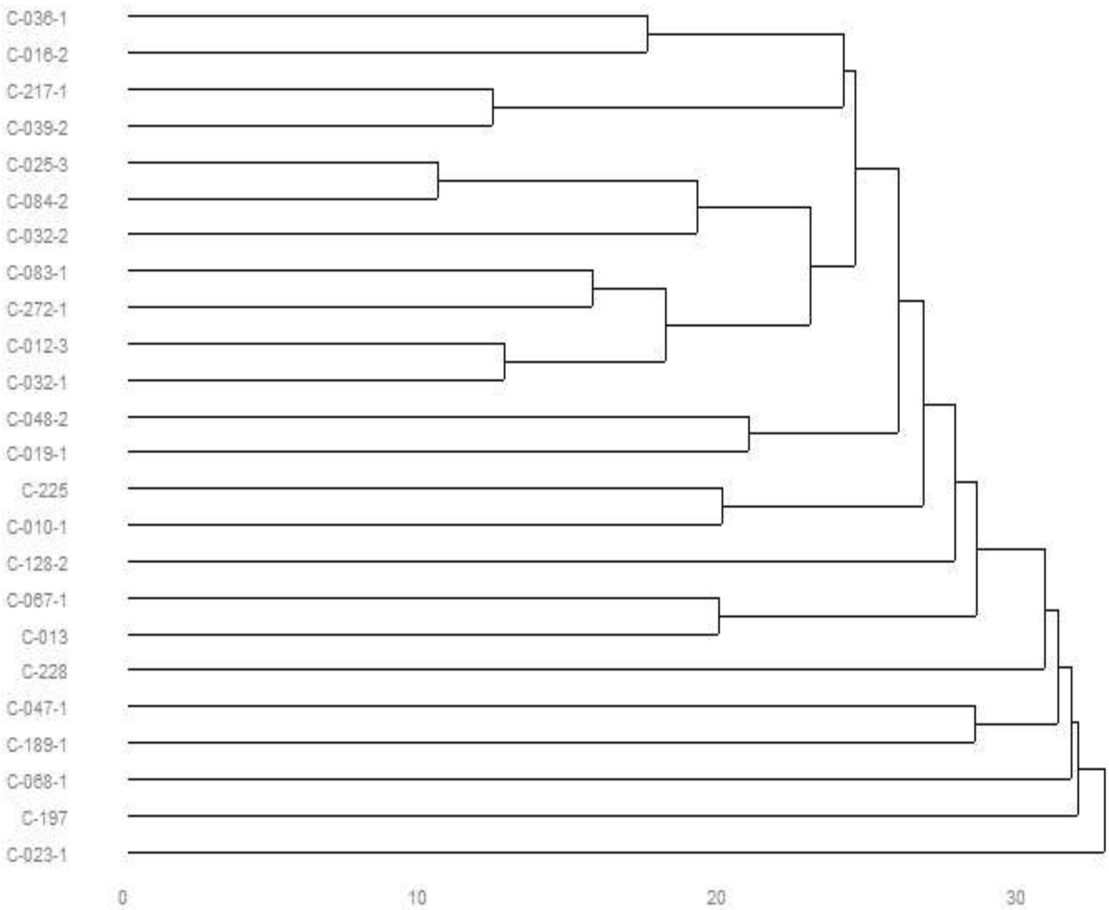
(Figure 19) and 10 groups in Ponta Grossa (Figure 20). In this graph it can check easily the closest and most distant progeny that were provided by Tables 8 and 9. Coefficient of cophenetic correlation were 0.90 and 0.76 in Ribeirão Branco and Ponta Grossa, respectively, making good adequation between distances graphical representation and its new matrix (ROHLF, 2000). For genetic improvement, the hierarchical method of average distance (UPGMA) is higher than the nearest neighbour and farthest neighbour (SNEATH; SOKAL, 1973; DUDLEY, 1994).

Figure 19 – Dendrogram resulting from analysis of 44 progenies of slash pine in municipality of Ribeirão Branco, Brazil, obtained by UPGMA clustering method based on the Mahalanobis distance. The coefficient of cophenetic value (r) is 0.90.



Source: The author.

Figure 20 – Dendrogram resulting from analysis of 24 progenies of slash pine in municipality of Ponta Grossa, Brazil, obtained by UPGMA clustering method based on the Mahalanobis distance. The coefficient of cophenetic value (r) is 0.76.



Source: The author.

Tocher optimization method separated Ribeirão Branco progenies into five groups (Table 11). Group I included 91% of the progenies, while Groups II, III, IV and V contemplated 2.25% of each progeny. Ponta Grossa (Table 12) formed 10 groups, group I contains 58.3% of progenies, while group II contains 8.3% and the groups III to X have 4.2% each. As confirmed genetic divergence among progenies in Ponta Grossa is higher than Ribeirão Branco because formation of more distinct groups. Crosses between more productive materials and divergent groups should be prioritized in order to increase the likelihood of obtaining high specific combining ability and heterosis. For example, more productive trees of genotype C-197 (group III) should be crossed with C-098-1 belonging to the group I, obtaining full-sib. Crosses between trees of Ribeirão Branco and divergent groups of Ponta Grossa test should also be prioritized. This clustering method tends to separate progenies with uniformity within group and heterogeneity among groups, keeping the criterion that intra-group distances are always smaller than the distances between groups. Thus, Cruz, Regazzi and Carneiro (2004) recommend not crossing progenies of same group for not restrict the genetic variability, and avoid loss in gain selection.

The highest distance found by Mahalanobis generalized quantitative distances (Tables 8 and 9) reveals that there is a greater variability possibly between these progenies and comparing the results with obtained by Tocher grouping (Tables 11 and 12). We can note progenies with greater distance are in different group, while progenies with smaller distances belong to the same group. These groups report distances in numerical order, however, not reveals which of arrays really are the most distant, within the same group, comparing them with other groups.

The clustering by UPGMA method showed to be similar to Tocher method for forming groups of more divergent genotypes. It can be observed because of progenies belonging to groups II, III, IV and V of Tocher method in Ribeirão Branco (Table 11) were the same as those groupings of greater distance by UPGMA (Figure 15) with the exception of C-225, C-019-1 and C-048-2 that presented different groups from each other.

Observing the mean value of each group formed by Tocher optimization, in Ribeirão Branco we can cross individuals from group I and IV. These groups presented the greatest means in relation to other groups and bigger than the total mean. In the same way, for Ponta Grossa, it is recommended to cross individuals from groups IV and IX, according to group means. In a breeding program, it is desirable to prioritize crosses between materials with high means ad high genetic divergence among themselves in order to identify productive materials that complement each other to use existing non-additive fraction into genetic variance (NASCIMENTO et al., 2014).

Nikles and Griffin (1992) reported that, in addition to this, high genetic divergence is essential for intraspecific hybrids development, also most productive performance or complementary traits, aiming to obtain more productive hybrids and these hybrid superiority may occur from heterosis per se, epistasis or trait complementarity (additive effect).

However, when breeding program aims to the genes recovery of recurrent parents crosses should be done with the most similar genetically progenies. low genetic divergence, because the use of similar progenitors basically differentiated by the gene to be transferred allows recovery faster the recurrent parent (MORAES, 2001). The use of similar progenitors basically differentiated by the gene to be transferred allows recovery faster the recurrent parent (MORAES, 2001).

Table 11 – Groups formed by Tocher Optimization method and mean value for height (H), diameter at breast height (DBH), wood volume (VOL) and stem straightness (SS) at age five for slash pine progenies in municipality of Ribeirão Branco, Brazil.

GROUP	PROGENIES	TOTAL	H (m)	DBH (cm)	VOL (m^3 $tree^{-1}$)	SS
I	C-098-1 C-047-1 C-071-1 C-010-1 C-023-1 C-100-2 C-084-2 C-120-1 C-036-1 C-012-3 C-151-1 C-045-1 C-112-1 C-001-1 C-039-2 C-225 C-189-1 C-083-1 C-228 C-081-3 C-159-1 C-217-1 C-048-2 C-272-1 C-128-2 C-032-1 C-068-1 C-063-2 C-042-1 C-043-3 C-047-2 C-039-1 C-067-1 C-218-1 C-013 C-019-1 C-025-3 C-032-2 C-016-2 C-059-1	40	7.31	13.64	0.05	2.11
II	C-074-2	1	6.94	12.68	0.04	2.11
III	C-197	1	7.24	13.47	0.05	1.97
IV	C-053-1	1	7.45	13.85	0.05	2.14
V	C-082-1	1	7.13	13.06	0.05	2.31
TOTAL		**44**	**7.25**	**13.34**	**0.05**	**2.15**

Source: The author.

Table 12 – Groups formed by Tocher Optimization method and mean value for height (H), diameter at breast height (DBH), wood volume (VOL) and stem straightness (SS) at age four for slash pine progenies in municipality of Ponta Grossa, Brazil.

GROUP	PROGENIES	TOTAL	H (m)	DBH (cm)	VOL (m^3 $tree^{-1}$)	SS
I	C-084-2 C-025-3 C-016-2 C-272-1 C-032-1 C-032-2 C-039-2 C-019-1 C-012-3 C-225 C-036-1 C-048-2 C-083-1 C-217-1	14	5.95	11.32	0.03	2.06
II	C-013 C-067-1	2	6.02	11.44	0.03	2.10
III	C-010-1	1	6.08	10.80	0.03	2.10
IV	C-189-1	1	6.64	13.19	0.04	2.13
V	C-128-2	1	6.38	11.85	0.03	2.04
VI	C-228	1	6.13	11.96	0.03	2.05
VII	C-068-1	1	5.78	11.20	0.03	1.95
VIII	C-047-1	1	6.18	11.50	0.03	2.07
IX	C-197	1	6.86	13.97	0,05	2.10
X	C-023-1	1	5.41	10.06	0.03	2.05
TOTAL		**24**	**6.14**	**11.73**	**0.03**	**2.07**

Source: The author.

4.4 CONCLUSIONS

There is genetic divergence among slash pine progenies detected by form and growth traits.

The most similar progenies are C-084-2 and C-112-1 in Ribeirão Branco, while in Ponta Grossa are C-084-2 and C-025-3. The most dissimilar are C-053-1 e C-197 in the first and C-047-1 and C-023-1 in the second planting site.

Crossings must be chosen not only by distances between formed groups, but it is necessary to take into account the productivity rank because the most distant individuals/progenies not always be the most productive.

By Tocher grouping method, progenies were separated into five groups in Ribeirão Branco and ten groups in Ponta Grossa. For breeding programs, it is recommended to cross individuals between different and more productive groups to increase genetic diversity, and consequently genetic gain.

The clustering by UPGMA method was concordant to Tocher optimization method for forming groups of more divergent genotypes.

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5 CHAPTER III

COMPARISON BETWEEN METODOLOGIES FOR WOOD QUALITY TRAITS IN RADIATA PINE

ABSTRACT

We compared results of Pilodyn penetration and SilviScan analysis based on wood quality traits aiming to evaluate if Pilodyn penetration measurements may predict wood traits. Thirty *Pinus radiata* progenies were selected from Flynn, Australia, planted in 1969. Pilodyn penetration depth (PP) was measured using a 2.0-mm diameter pin, without removing bark. The measurements were made at approximately 1.3 m above the ground twice for each tree at age 29. For SilviScan measurements 12-mm-thick wood increment cores were collected from each tree at breast height (1.3 m) evaluating wood density (DEN), microfibril angle (MFA) and modulus of elasticity (MOE), beyond diameter at breast height (DBH). Deviance analysis and narrow-sense individual heritability was applied for PP, MFA, MOE, DEN and DBH, as well as genetic and phenotypic correlations between all measured traits and PP. There was a significant difference ($p < 0.10$) for DEN to ages eight, nine, 23, 24 and 27, MFA ($p < 0.05$) from nine to 22 years old tress, PP ($p < 0.01$) at age 29. And there was no significant difference for MOE and DBH. DBH ranged from 2.06 mm to 126.01 mm during the assessment years. DEN decreased at age thee, however, increased in the last year of analysis. MFA had lower mean values at age 29, while MOE ranged from 3.50 Gpa one year after implantation and 14.03 Gpa at last measurement. Individual heritability for Pilodyn penetration was considered high (0.34), as genetic correlation with wood density (-0.99), DBH (0.99) and low to moderate for MOE (-0.61) and MFA (-0.33). Pilodyn penetration measurements showed good heritability and high genetic correlation with wood density and moderate with MOE and MFA. It can be considered as an effective indirect trait to evaluate genetic parameters for Radiata pine wood density.

Keywords: *Pinus radiata* D. Don. Wood properties. MFA. MOE. Wood density.

RESUMO

Comparou-se resultados de penetração do Pilodyn e análises de SilviScan baseados em propriedades de madeira com a finalidade de avaliar se dados de penetração do Pilodyn podem predizer caracteres de qualidade de madeira. Foram selecionadas 30 progênies de *Pinus radiata* em Flynn, Austrália, plantadas em 1969. Penetração do Pilodyn (PP) foi mensurada com um pino de ferro de 2 mm de diâmetro, sem remoção da casca, à altura do peito duas vezes por árvore aos 29 anos de idade. Para análise no SilviScan foram retiradas amostras de 12 mm de espessura à altura do peito, avaliando-se densidade de madeira (DEN), ângulo de microfibrilas (AMF), módulo de elasticidade (MOE) além do diâmetro à altura do peito (DAP) de um a 29 anos. Foram realizadas análise de *deviance* e estimativa de herdabilidade para PP, AMF, MOE, DEN e DAP, além de correlações genéticas e fenotípicas entre todos os caracteres mensurados e PP. Houve diferença significativa ($p<0,10$) para DEN aos oito, nove, 23, 24 e 27 anos, AMF ($p<0,05$) dos nove aos 22 anos, PP ($p<0,01$) aos 29 anos. Não houve diferença significativa para MOE e DAP. DAP variou de 2,06 mm a 126,01 mm durante os anos de avaliação. DEN diminuiu aos 3 anos, porém, aumentou no último ano de análise. AMF apresentou menores médias aos 29 anos, enquanto que MOE variou de 3,50 Gpa um ano após a implantação e 14,03 Gpa aos 29 anos. A herdabilidade individual da PP foi considerada alta (0,34), assim como correlação genética com densidade de madeira (-0,99), DAP (0,99) e baixa a moderada para MOE (-0,61) e AMF (-0,33). A penetração do Pilodyn pode ser considerado como um importante e efetivo caráter de avaliação de parâmetros genéticos para caracteres de qualidade de madeira em *Pinus radiata*.

Palavras-chave: *Pinus radiata* D. Don. Propriedades de madeira. AMF. MOE. Densidade de madeira.

5.1 INTRODUCTION

Analysis of quantitative genetic for coniferous species, mainly for wood properties was mostly lead on fast developing for some important species such as *Pinus taeda* L. (ISIK; MORA; SCHIMLECK, 2011; ANTONY et al., 2011), *Picea abis* K. (CHEN et al., 2014, 2015; PIISPANEN et al., 2014), *Picea sitchensis* Bong. (KENNEDY; CAMERON; LEE, 2013), *Pinus sylvestris* L. (HONG; FRIES; WU, 2014) and *Pinus radiata* D. Don (LI; WU, 2005; BALTUNIS; WU, POWELL, 2007; WU et al., 2008). The last one is extensively grown as a cultivated area tree, especially in New Zealand and Australia (LI; WU, 2005; GAPARE, 2015).

Among most important wood quality traits, it is possible to highlight wood density, wood stiffness (measured as its modulus of elasticity), and microfibril angle. Wood density is intensely correlated to other wood properties and growth traits. Moreover, it may determine the capacity of biomass accumulation for each progeny and consequently affects forest carbon sequestration performance (FUKATSU et al., 2011). Modulus of elastic (MOE) has an important influence on mechanical timber grade. This is mainly determined by the purpose to intensify the recovery of structural and appearance-grade products, which gives a higher economic return (DUNGEY et al., 2006). Microfibril angle (MFA) refers to the angle between the longitudinal wood cell centre line and the cellulose microfibril in the fibres and tracheids secondary wall (BARNETT; BONHAM, 2004).

Nevertheless, a serious problem faced by research is how to measure wood properties. Methods may be expensive, slow and mainly destructive (WU et al., 2010). Therefore, non-destructive methods started to be tested and considerably reduced those limitations (WANG et al., 2010).

Accurate measurements of wood density based on volume and weight of samples require laboratory procedures with destructive methods, which is not applicable for monitoring purposes. For measuring the density variation on field, a method to be applied may be the use of an instrument called Pilodyn (MICKO et al., 1982), which is initially developed for determining the rot degree of telephone poles (HANSEN, 2000). The Pilodyn measures the resistance to penetration when a steel pin is introduced with a determined force through the wood by a spring (SPRAGUE et al., 1983; GREAVES et al., 1996; LEE; CONNOLLY, 2010). The depth penetration of the pin is indicated on the instrument and readings are correlated with the wood density. Pilodyn does not provide density estimates, but the instrument has already been calibrated for trees wood density measurements in several studies (MICKO et al., 1982;

SPRAGUE et al., 1983; WU et al., 2011; FUKATSU et al., 2011; HÖGBERG et al., 2014; CHEN et al., 2015; MURPHY; COWN, 2015).

Another non-destructive technique widely used to estimate wood quality traits is based on data provided by SilviScan (SHELBOURNE et al., 1997; WU et al. 2007; CIESZEWSKI et al., 2013; HONG et al., 2015). The equipment is used to evaluate the properties of wood from pith to the bark quickly using a combination of X-ray densitometry, X-ray diffraction and image analysis (BALL; McCONCHIE; COWN, 2005).

The aim of this work was to evaluate whether Pilodyn penetration measurements may predict wood density, MFA, MOE according to phenotypic and genotypic correlation with SilviScan measurements.

5.2 MATERIALS AND METHODS

5.2.1 The trial

One mature genetic trial with 30 open pollinated progenies was chosen among 11 sites across Australia and sampled for this study. Seeds of the 30 progenies were collected in 1968 from the Tallaganda seed orchard in New South Wales (BROWN, 1971). The trial was planted in 1969 on a sand loam soil at Flynn, Victoria (38° 12’S, 140° 40’E, elevation 100 m) with an average annual rainfall of 760 mm. The field design was completely randomized blocks with nine replicates of 2 x 3 tree plots. This site was not thinned, as a result, competition was severe and some trees died or stopped growing due to suppression. Therefore, only 90 non-supressed trees from three replications (one from each plot) were selected for SilviScan analysis and 615 trees were selected for Pilodyn measurements.

5.2.2 SilviScan and Pilodyn measurements

A 12-mm-thick wood increment core was collected from each tree at breast height (1.3 m). Cores from a total of 90 trees were sampled, and wood properties were analysed by SilviScan®.

Wood density from SilviScan was measured using dry volume and weight at about 7% relative humidity under about 20 °C in contrast with green volume (100% relative humidity) ad oven-dry weight (bone dry) used in WinDENDRO X-ray densitometry. SilviScan predicted dynamic MOE was derived from measured MFA and density according to method described by Evans and Illic, 2001.

Pilodyn penetration depth was measured using a Pilodyn 6J Forest (POCEQ, Zurich, Switzerland) with a 2.0-mm diameter pin, without removing bark. The measurements were made at approximately 1.3 m above the ground twice for each tree.

5.2.3 Statistical analyses

Components of variance and covariance for genetic analyses were estimated using ASREML 3.0 (GILMOUR et al., 2009), and the following linear mixed model for individual tree analysis was fitted as follow:

$$Y_{ijl} = \boldsymbol{\mu} + \boldsymbol{R_i} + \boldsymbol{F_j} + \boldsymbol{e_{ijl}} \tag{17}$$

Where Y_{ijl} is the observation of the l_{th} tree from the j_{th} progeny in i_{th} replication, μ is the overall mean, both R_i (replication) and F_j (progeny) were considered as random effect.

The open-pollinated offspring of progenies were assumed as open pollinated, and additive genetic variance for each trait was calculated as 4 times the progeny variance (FALCONER; MCKAY, 1996). Narrow-sense heritability (h_i^2) was computed for each year assuming half-sib family structure as:

$$h_i^2 = \frac{4\ \sigma_{progeny}^2}{\sigma_{progeny}^2 + \sigma_e^2} \tag{18}$$

Where $\sigma_{progeny}^2$ is progeny variance and σ_e^2 is the residual variance.

Phenotypic and genetic correlation between traits x and y were estimated as:

$$r_{x,y} = \frac{\hat{\sigma}_{x,y}}{\sqrt{\hat{\sigma}_x^2\ \hat{\sigma}_y^2}} \tag{19}$$

where $\hat{\sigma}_x^2$ and $\hat{\sigma}_y^2$ are the estimated phenotypic or genetic variances for traits x and y, respectively, $\hat{\sigma}_{x,y}$ is the estimated phenotypic or genetic covariance between traits x and y.

5.3 RESULTS AND DISCUSSION

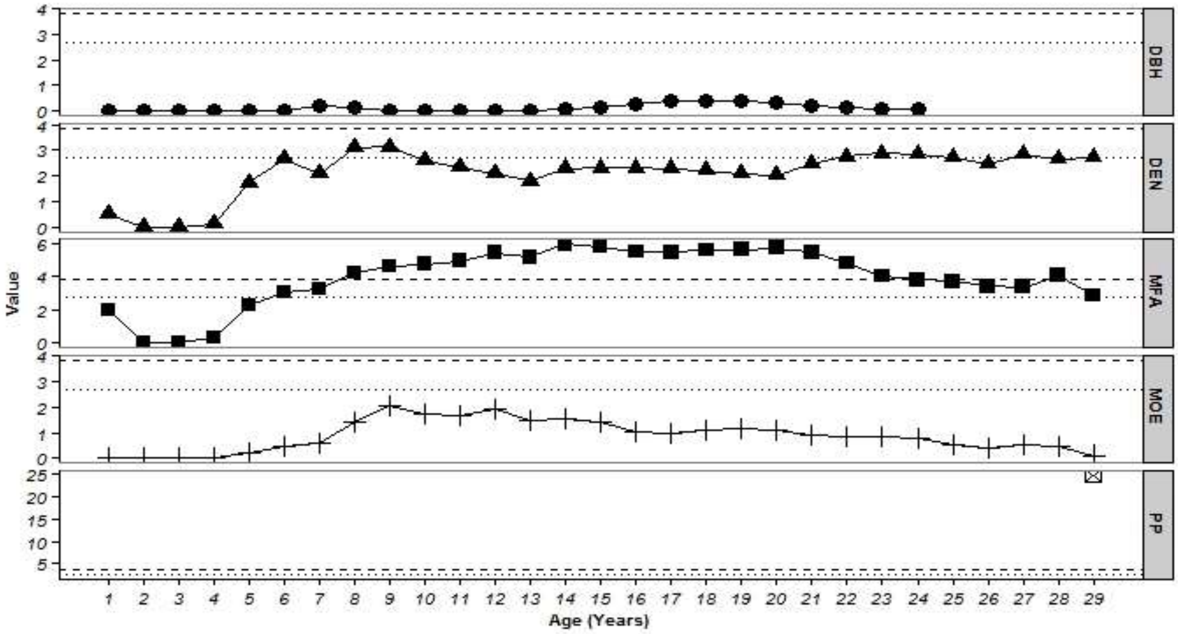
5.3.1 Wood quality traits means

There were not significant differences between progenies for Modulus of elasticity (MOE) and diameter at breast height (DBH) in various ages. Wood density (DEN) had been significant only for 10% of probability at ages eight, nine, 23, 24, 27 and, Pilodyn penetration (PP) and microfibril angle (MFA) for ages nine to 22 had been at 5% and 1% probability, respectively (Figure 21). The lower or absence statistical significance for wood quality traits may be due the small size of samples in the current work.

DBH varied from 2.06 mm to 126.01 mm at age one until 29; DEN decreased at age three (389.92 kg m^{-3}), however, it increased to 541.19 kg m^{-3} at last age. MFA presented smaller mean value at age 29 (17.71°). MOE showed increasing range during the time from 3.50 Gpa with one year old to 14.03 at age 29 and PP presented 11.20 mm at 29 years old (Figure 22).

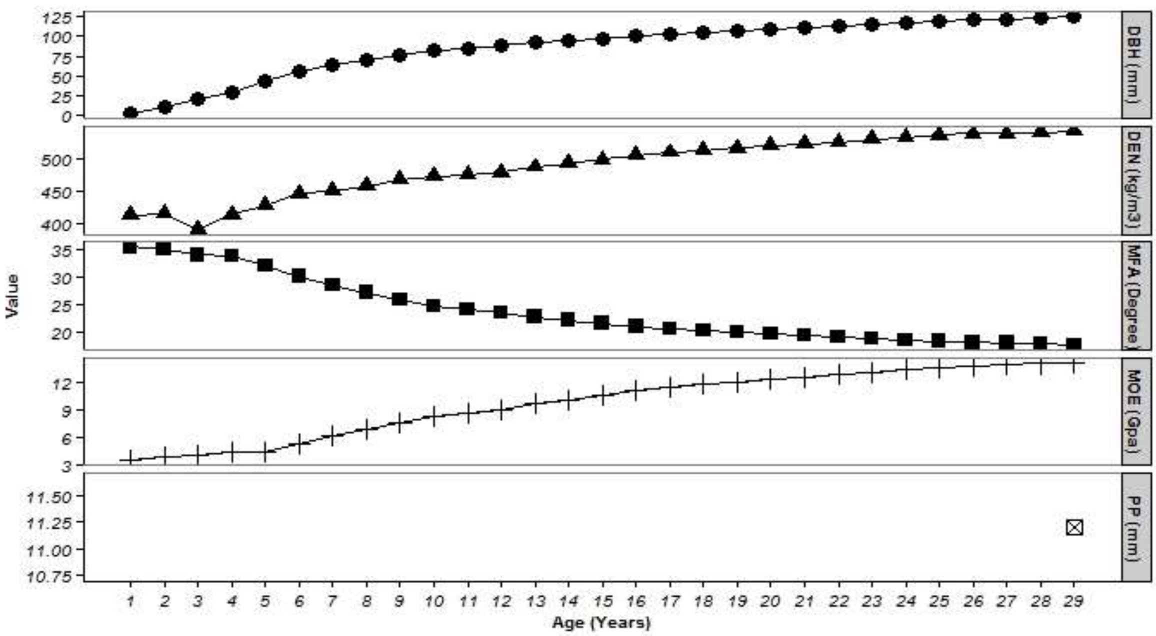
Faster growth rate is directly related to lower densities in Radiata pine, according to Baltunis, Wu and Powell (2007). The authors reported range of wood density from 376.5 to 551.60 kg m^{-3}, with a mean of 459.70 kg m^{-3} for 10 year-old progenies at the same region (Flynn). For the same age the current work presented almost the same average density (471.72 kg m^{-3}). Furthermore, the trial presented MFA and MOE average of 28.6° and 7.40 GPa, respectively, while in the present research the mean for the same age was 24.74° and 8.26 GPa. Low MFA is desirable, it means higher stiffness and it is related to height and DBH in moderately way for genotypic level (LENZ et al., 2013). The results were expected because according to Macdonald and Hubert (2002) and Watt et al. (2010) juvenile wood normally presents low density, thin cell walls, short tracheids, high microfibril angle and low modulus of elasticity. Therefore, juvenile wood has low strength and stiffness if compared with late wood. It is important to highlight that MFA was inversely proportional to MOE, decreasing along the years. The increment in stocking may be related to it, as reported by Waghorn et al. (2007), Lasserre et al. (2008, 2009) for Radiata pine and Chuang and Wang (2001) for Japanese cedar and Zhang et al. (2002) for Black spruce.

Figure 21 - Likelihood ratio test (LRT) and significance level for diameter at breast height (DBH), microfibril angle (MFA), modulus of elasticity (MOE) and wood density (DEN) measured by SilviScan and Pilodyn penetration (PP) for Radiata pine trial, Australia. (LRT above dashed line = p<0,05; above dotted line = p<0,10; PP = p<0,01).



Source: The author.

Figure 22 - Mean values for diameter at breast height (DBH), microfibril angle (MFA), modulus of elasticity (MOE) and wood density (DEN) measured by SilviScan and Pilodyn penetration (PP) for Radiata pine trial, Australia.



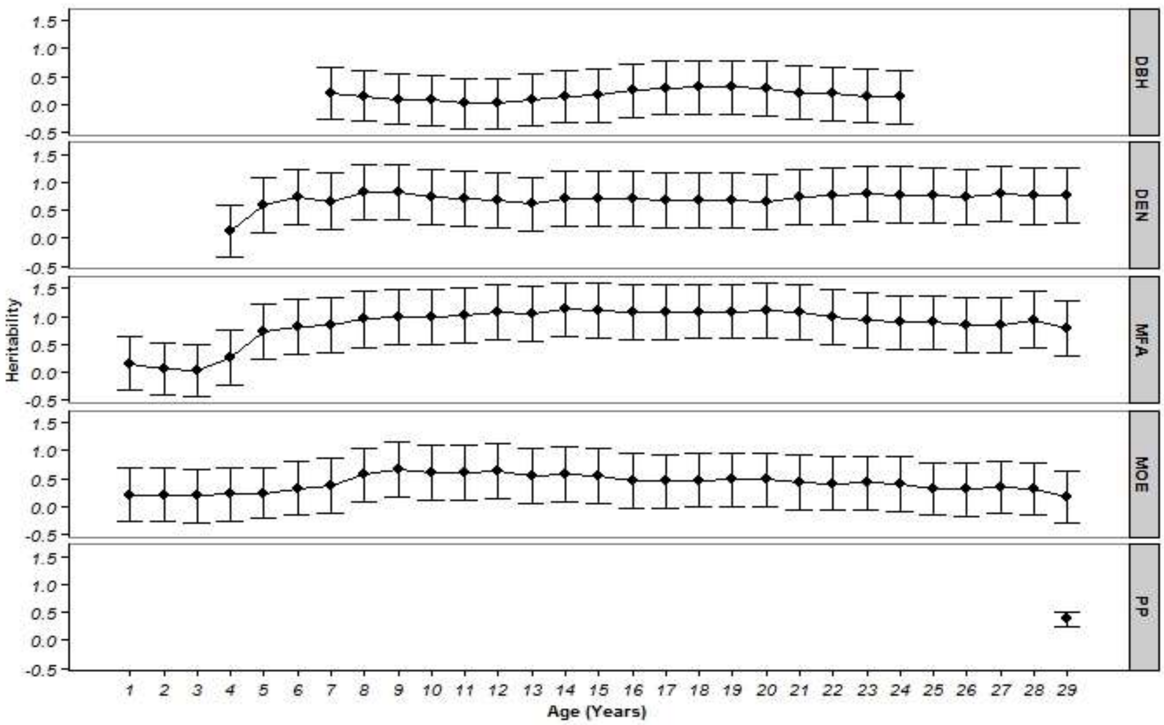
Source: The author.

5.3.2 Narrow-sense individual heritability

Narrow-sense individual heritability (h_i^2) for Pilodyn penetration was 0.39 (Figure 23). In general, the heritability increased according to the ages for all traits. However, it showed less fluctuation for wood density, MOE and MFA. High heritabilities may provide effective selection and indicate that these traits are under moderate to high genetic control. Standard errors were high for SilviScan measurements because the number of samples was small compared to Pilodyn.

Heritability for DBH was similar to reported by Chen et al. (2015) for Norway spruce at 12 and 21 years but higher in relation to wood density, MFA and MOE. Ukrainetz et al. (2008) also reported DBH heritability for Douglas-fir progenies similarly to the current work, Bian et al. (2014) for Chinease-fir, Yang et al. (2013) for Loblolly pine and Egbäck et al. (2012) for Scots pine. For MFA and MOE, Gapare et al. (2012) found heritability coefficients for Radiata pine near to the present work, as well as Hallingbäck, Jansson and Hannrup (2010) for Pilodyn penetration in trials with Norway spruce.

Figure 23 - Narrow-sense individual heritability (h_i^2) for diameter at breast height (DBH), microfibril angle (MFA), modulus of elasticity (MOE) and wood density (DEN) measured by SilviScan and Pilodyn penetration (PP) for Radiata Pine trial, Australia.



Source: The author.

5.3.3 Genetic and phenotypic correlations

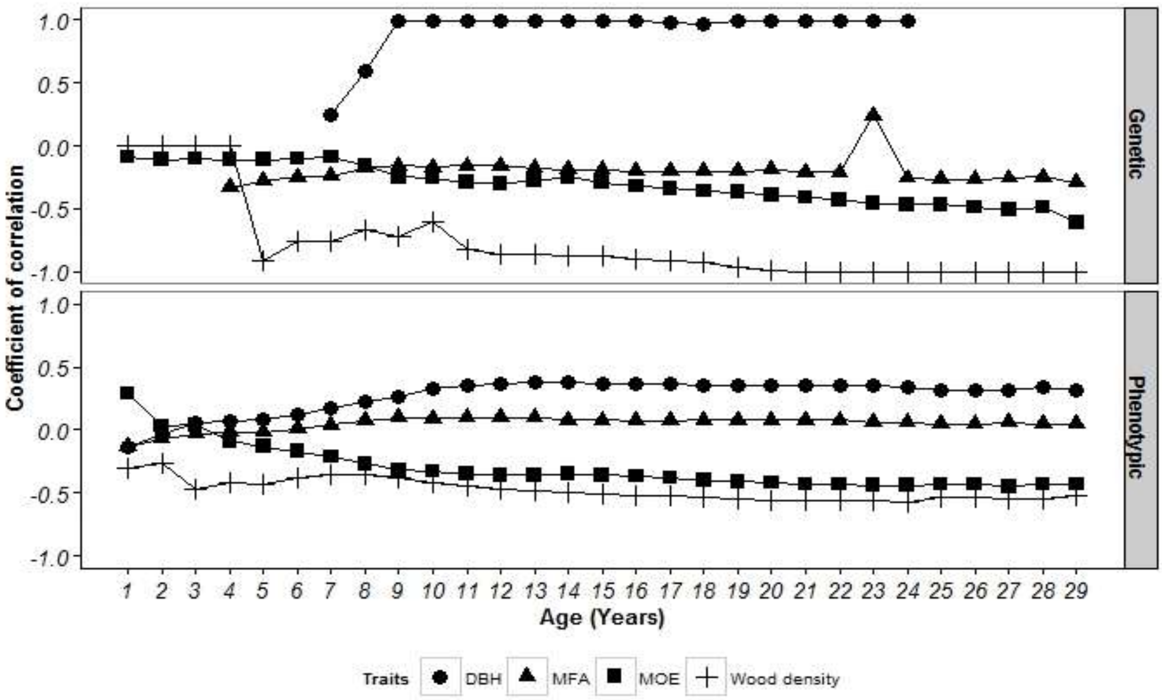
In general, phenotypic correlation between Pilodyn and SilviScan measurements was negatively moderate for DEN and MOE, positively moderate for DBH and very low for MFA as expected (Figure 24).

The genetic correlations were higher than the phenotypic ones, as observed in Figure 24, this is probably because environment interaction. Pilodyn penetration had very high negative genetic correlation with DEN and high positive genetic correlation with DBH. For MOE and MFA the correlation was moderate to low. It is important to note the phenotypic correlation involves environmental and genetic part. If the genetic correlation is greater than the phenotypic, meaning that any relation between traits has more genetic influence. According to Cruz, Regazzi and Carneiro (2004) the environment may becomes a cause of correlation between two traits when they are influenced by variations in environmental conditions.

MOE and DEN correlates well with Pilodyn penetration measurements both genetically and phenotypically. When PP decreases DEN and MOE increase. It means more wood in the same volume and stiffer wood. This proposes that the improvement or control of wood stiffness could be executed with field measurements and Pilodyn can be used to assess the wood MOE. Högberg et al. (2014) and Chen et al. (2015) both for Norway spruce found similar results, as well as, Wu et al. (2011) for Eucalypt clones and Kennedy, Cameron and Lee (2013) for Sitka spruce. For progeny level, Pilodyn penetration also showed high reliability for correlation with wood density in progenies of Lodgepole pine and Douglas-fir as reported by Adams et al. (1993) and Wang et al. (1999), respectively.

We may see wood properties vary according both genetic and environment situations, as age, origin, location source, and spacing, rate of growth among species and even between trees of the same species. The main thing to take into consideration is to determine the finality of the wood. Agreeing with Evans, Stuart and Van Der Touw (1996), for instance, the mechanical properties of the paper are powerfully predisposed by the strength and stiffness of the wood fibres.

Figure 24 - Genetic and phenotypic correlations between Pilodyn penetration and diameter at breast height (DBH), microfibril angle (MFA), modulus of elasticity (MOE) and wood density measured by SilviScan for Radiata pine trial, Australia.



Source: The author.

6.4 CONCLUSIONS

DBH ranged from 2.06 mm to 126.01 mm during the assessment years. DEN decreased at age thee, however, increased in the last year of analysis. MFA had lower mean values at age 29, while MOE ranged from 3.50 Gpa one year after implantation and 14.03 Gpa at last measurement.

Individual heritability for Pilodyn penetration was considered high (0.34) as genetic correlation with Pilodyn and wood density (-0.99), DBH (0.99) and low to moderate correlation between Pilodyn and MOE (-0.61) and MFA (-0.33).

Pilodyn can be considered as an effective indirect trait to evaluate genetic parameters for Radiata pine wood density.

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APPENDIX 1 – Deviance analysis and significant levels for growth and form traits in slash pine progenies in municipality of Ribeirão Branco-SP, Brazil.

Effect	Height (1 year)	
	Deviance	LRT
Progeny	-3110.95	11.80**
Model	-3122.75	

Effect	Height (2 years)	
	Deviance	LRT
Progeny	-688.03	15.04**
Model	-703.07	

Effect	Height (3 years)	
	Deviance	LRT
Progeny	353.38	13.06**
Model	340.32	

Effect	Height (4 years)	
	Deviance	LRT
Progeny	1065.52	12.69**
Model	1052.83	

Effect	Height (5 years)	
	Deviance	LRT
Progeny	1590.77	5.87*
Model	1584.90	

Effect	DBH (3 years)	
	Deviance	LRT
Progeny	2806.86	6.60*
Model	2800.26	

Effect	DBH (4 years)	
	Deviance	LRT
Progeny	3663.03	13.35**
Model	3649.68	

Effect	DBH (5 years)	
	Deviance	LRT
Progeny	4193.45	16.84**
Model	4176.61	

Effect	Volume (3 years)	
	Deviance	LRT
Progeny	-16332.31	11.62**
Model	-16343.93	

Effect	Volume (4 years)	
	Deviance	LRT
Progeny	-13190.52	23.01**
Model	-13213.53	

Effct	Volume (5 years)	
	Deviance	LRT
Progeny	-10962.97	19.37**
Model	-10982.34	

Effect	Survival (5 years)	
	Deviance	LRT
Progeny	-3532.40	0.00^{ns}
Model	-3532.40	

Effect	Stem straightness	
	Deviance	LRT
Progeny	211.90	6.82**
Model	205.08	

Effect	Branch thickness	
	Deviance	LRT
Progeny	-574.64	0.91^{ns}
Model	-575.55	

Effect	Branch angle	
	Deviance	LRT
Progeny	-117.88	4.54*
Model	-122.42	

Effect	Number of branches	
	Deviance	LRT
Progeny	1329.01	19.88**
Model	1309.13	

Effect	Fork	
	Deviance	LRT
Progeny	-371.82	0.00^{ns}
Model	-371.82	

Tabulated chi-square: 3.84 e 6.63 for significant levels of 5% (*) and 1% (**), respectively and ^{ns} not significant. Source: The author.

APPENDIX 2 – Deviance analysis and significant levels for growth and form traits in slash pine progenies in municipality of Ponta Grossa-PR, Brazil.

Effect	Height (1 year)	
	Deviance	LRT
Progeny	-764.87	2.67^{ns}
Model	-767.54	

Effect	Height (2 years)	
	Deviance	LRT
Progeny	-35.86	11.07**
Model	-46.93	

Effect	Height (3 years)	
	Deviance	LRT
Progeny	369.69	16.73**
Model	352.96	

Effect	Height (4 years)	
	Deviance	LRT
Progeny	511.04	20.00**
Model	491.04	

Effect	DBH (3 years)	
	Deviance	LRT
Progeny	1098.11	4.19*
Model	1093.92	

Effect	DBH (3 year)	
	Deviance	LRT
Progeny	1361.99	16.45**
Model	1345.54	

Effect	Volume (3 years)	
	Deviance	LRT
Progeny	-4492.63	19.24**
Model	-4511.87	

Effect	Volume (4 years)	
	Deviance	LRT
Progeny	-3348.67	28.69**
Model	-3377.36	

Effect	Survival (4 years)	
	Deviance	LRT
Progeny	-252.86	0.01^{ns}
Model	-252.87	

Effect	Stem straightness	
	Deviance	LRT
Progeny	-565.27	2.87^{ns}
Model	-568.14	

Effect	Branch thickness	
	Deviance	LRT
Progeny	67.77	20.00**
Model	47.77	

Effect	Branch angle	
	Deviance	LRT
Progeny	-494.32	4.99*
Model	-499.31	

Effect	Number of branches	
	Deviance	LRT
Progeny	-28.97	0.00^{ns}
Model	-28.97	

Effect	Fork	
	Deviance	LRT
Progeny	-306.38	1.39^{ns}
Model	-307.77	

Effect	Fox tail	
	Deviance	LRT
Progeny	-167.81	5.56*
Model	-173.37	

Tabulated chi-square: 3.84 e 6.63 for significant levels of 5% (*) and 1% (**), respectively and ^{ns} not significant. Source: The author.

APPENDIX 3 – Square mean (SM) of block effect for slash pine progenies for growth and form traits in municipalities of Ribeirão Branco-SP and Ponta Grossa-PR, Brazil.

Site	Traits	Age (years)	SM (block)
Ribeirão Branco	Height (m)	1	0.52
		2	1.14
		3	2.36
		4	2.21
		5	6.31
	DBH (cm)	3	16.41
		4	6.40
		5	13.84
	Volume (m^3 $tree^{-1}$)	3	0.0001
		4	0.0002
		5	0.0020
	Survival	5	0.05
	Stem straightness	5	0.64
	Branch thickness	5	0.74
	Branch angle	5	0.92
	Number of branches	5	1.80
	Fork	5	0.04
	Fox tail	5	-
Ponta Grossa	Height (m)	1	0.18
		2	0.52
		3	0.93
		4	1.89
	DBH (cm)	3	3.98
		4	8.79
	Volume (m^3 $tree^{-1}$)	3	0.000
		4	0.001
	Survival	4	0.27
	Stem straightness	4	1.51
	Branch thickness	4	0.61
	Branch angle	4	0.21
	Number of branches	4	45.55
	Fork	4	0.35
	Fox tail	4	0.41

APPENDIX 4 – Variance estimates for slash pine growth traits in municipalities of Ribeirão Branco-SP and Ponta Grossa-PR, Brazil.

Site	Traits	Age (years)	σ_a^2	σ_e^2	σ_f^2
Ribeirão Branco	Height (m)	1	0.005	0.05	0.05
		2	0.025	0.20	0.22
		3	0.044	0.37	0.42
		4	0.066	0.58	0.64
		5	0.057	0.83	0.89
	DBH (cm)	3	0.127	1.73	1.86
		4	0.328	2.80	3.13
		5	0.528	3.82	4.34
	Volume (m^3 $tree^{-1}$)	3	0.000002	0.0000	0.00002
		4	0.000020	0.0001	0.00010
		5	0.000100	0.0004	0.00040
	Survival	5	0.000100	0.0400	0.04000
Ponta Grossa	Height (m)	1	0.007	0.06	0.06
		2	0.070	0.21	0.28
		3	0.124	0.45	0.67
		4	0.338	0.57	0.91
	DBH (cm)	3	0.442	2.70	3.14
		4	1.878	3.82	5.70
	Volume (m^3 $tree^{-1}$)	3	0.00001	0.00001	0.00002
		4	0.00001	0.00001	0.00002
	Survival	4	0.00100	0.23	0.23

Additive genetic variance (σ_a^2); Non-additive environment residual variance (σ_e^2); individual phenotypic variance (σ_f^2). Source: The author

APPENDIX 5 – Variance estimates for slash pine form traits in municipalities of Ribeirão Branco-SP and Ponta Grossa-PR, Brazil.

Site	Traits	σ_a^2	σ_e^2	σ_f^2
Ribeirão Branco	Stem straightness	0.0270	0.355	0.382
	Branch thickness	0.0060	0.231	0.237
	Branch angle	0.0180	0.295	0.313
	Number of branches	0.1030	0.652	0.756
	Fork	0.0004	0.007	0.007
Ponta Grossa	Stem straightness	0.2420	0.482	0.724
	Branch thickness	0.1270	0.225	0.352
	Branch angle	0.0180	0.088	0.106
	Number of branhes	0.0020	0.285	0.287
	Fork	0.0110	0.148	0.160
	Fox tail	0.0330	0.181	0.213

Additive genetic variance (σ_a^2); Non-additive environment residual variance (σ_e^2); individual phenotypic variance (σ_f^2). Source: The author

APPENDIX 6 – Variance estimates for slash pine growth and form traits in joint analysis in municipalities of Ribeirão Branco-SP and Ponta Grossa-PR, Brazil, four years of age.

	Height	DBH	Volume	Stem straightness
σ_a^2	0,025	0,110	0,00001	0,005
σ_{int}^2	0,010	0,069	0,00001	0,001
σ_e^2	0,833	4,499	0,0001	0,282
σ_f^2	0,867	4,678	0,0001	0,287

Additive genetic variance (σ_a^2); genotype x variância da interação genótipo x ambiente (σ_{int}^2) variância residual ambiental não aditiva (σ_e^2); variância fenotípica individual (σ_f^2). Source: The author

APPENDIX 7 – Estimates of genetic parameters for slash pine growth traits in municipalities of Ribeirão Branco-SP and Ponta Grossa-PR, Brazil.

Site	Traits	Age (years)	$\bar{x}$	CV_e (%)	CV_{gi} (%)	CV_{gp} (%)	LRT
Ribeirão Branco	Height (m)	1	1.08	20.79	6.55	2.27	11.80**
		2	2.48	18.66	6.38	3.19	15.04**
		3	4.20	15.15	4.97	2.49	13.06**
		4	5.81	13.63	4.43	2.21	12.69**
		5	7.30	12.80	3.29	1.65	5.87*
	DBH (cm)	3	7.36	18.34	4.86	2.43	6.60*
		4	10.68	16.34	5.38	2.69	13.35**
		5	13.61	15.08	5.34	2.67	16.84**
	Volume (m^3 $tree^{-1}$)	3	0.01	5.32	14.31	7.16	11.62**
		4	0.02	41.29	16.24	8.12	23.01**
		5	0.05	39.87	14.80	7.40	19.37**
	Survival (%)	5	95.00	21.77	1.03	0.52	0.00^{ns}
Ponta Grossa	Height (m)	1	0.91	27.21	9.01	4.50	2.67^{ns}
		2	2.28	22.54	11.60	5.80	11.07**
		3	4.12	19.00	11.24	5.62	16.73**
		4	6.02	15.08	9.64	4.82	20.00**
	DBH (cm)	3	7.34	23.71	9.05	4.53	4.19**
		4	11.51	19.87	11.90	5.95	16.45**
	Volume (m^3 $tree^{-1}$)	3	0.01	50.52	31.78	15.89	19.24**
		4	0.03	46.65	33.77	16.88	28.69**
	Survival (%)	4	65.00	73.79	3.60	1.80	0.01^{ns}

Overall mean ($\bar{x}$); coefficient of experimental variation (CV_e); coefficient of individual additive genetic variation (CV_{gi}); coefficient of progeny genetic variation (CV_{gp}); likelihood ratio test (LRT); **significant to 1%, *significant to 5% with 1 degree of freedon and ^{ns} not significant. Source: The author

APPENDIX 8 – Estimates of genetic parameters for slash pine form traits in municipalities of Ribeirão Branco-SP and Ponta Grossa-PR, Brazil.

Site	Traits	$\bar{x}$	CV_e (%)	CV_{gi} (%)	CV_{gp} (%)	LRT
Ribeirão Branco	Stem straightness	2.11	28.94	7.78	3.89	6.82**
	Branch thickness	1.76	27.52	4.21	2.10	0.91^{ns}
	Branch angle	2.36	23.49	5.64	2.92	4.54*
	Number of branches	5.19	16.45	6.20	3.10	19.88**
	Fork	1.00	8.55	1.91	0.95	0.00^{ns}
Ponta Grossa	Stem straightness	3.34	24.39	14.74	7.37	23.58**
	Branch thickness	1.63	34.67	21.81	10.90	20.00**
	Branch angle	2.02	15.80	6.73	3.36	4.99**
	Number of branches	6.41	8.33	0.62	0.31	0.00^{ns}
	Fork	1.8	22.05	5.92	2.96	1.39^{ns}
	Fox tail	1.68	26.96	10.77	5.39	5.56**

Overall mean ($\bar{x}$); coefficient of experimental variation (CV_e); coefficient of individual additive genetic variation (CV_{gi}); coefficient of progeny genetic variation (CV_{gp}); likelihood ratio test (LRT); **significant to 1%, *significant to 5% with 1 degree of freedon and ^{ns} not significant. Source: The author
.

APPENDIX 9 – Estimates of genetic parameters for slash pine form traits in municipalities of Ribeirão Branco-SP and Ponta Grossa-PR, Brazil.

Site	Traits	h_a^2	h_m^2	h_{ad}^2	$r_{\hat{a}a}$ (%)
Ribeirão Branco	Stem straightness	0.07 (±0.04)	0.42	0.05	0.65
	Branch thickness	0.02 (±0.02)	0.19	0.02	0.44
	Branch angle	0.06 (±0.03)	0.37	0.04	0.60
	Number of branches	0.14 (±0.05)	0.59	0.11	0.78
	Fork	0.05 (±0.10)	0.32	0.04	0.57
Ponta Grossa	Stem straightness	0.33 (±0.15)	0.75	0.27	0.86
	Branch thickness	0.36 (±0.15)	0.76	0.30	0.87
	Branch angle	0.17 (±0.11)	0.59	0.14	0.77
	Number of branches	0.01 (±0.02)	0.04	0.00	0.21
	Fork	0.07 (±0.07)	0.37	0.05	0.60
	Fox tail	0.15 (±0.10)	0.56	0.12	0.75

Narrow-sense individual heritability (h_a^2); family heritability mean (h_m^2); additive heritability within progenies (h_{ad}^2) and accuracy ($r_{\hat{a}a}$). Source: The author
.

APPENDIX 10 – Estimates of genetic parameters for slash pine growth traits in municipalities of Ribeirão Branco-SP and Ponta Grossa-PR, Brazil.

Site	Trait	Age (years)	h_a^2	h_m^2	h_{ad}^2	$r_{âa}$ (%)
Ribeirão Branco	Height (m)	1	0.10 (±0.04)	0.50	0.07	0.71
		2	0.11 (±0.05)	0.54	0.09	0.73
		3	0.10 (±0.04)	0.52	0.08	0.72
		4	0.10 (±0.04)	0.51	0.08	0.72
		5	0.07 (±0.04)	0.40	0.05	0.63
	DBH (cm)	3	0.07 (±0.04)	0.41	0.05	0.64
		4	0.11 (±0.05)	0.52	0.08	0.72
		5	0.12 (±0.05)	0.56	0.09	0.75
	Volume (m^3 $tree^{-1}$)	3	0.10 (±0.04)	0.50	0.07	0.71
		4	0.15 (±0.05)	0.61	0.12	0.78
		5	0.13 (±0.05)	0.58	0.10	0.76
	Survival (%)	5	0.00 (±0.01)	0.02	0.00	0.15
Ponta Grossa	Height (m)	1	0.11 (±0.08)	0.47	0.08	0.68
		2	0.25 (±0.13)	0.68	0.20	0.82
		3	0.32 (±0.14)	0.74	0.26	0.86
		4	0.37 (±0.15)	0.77	0.31	0.88
	DBH (cm)	3	0.14 (±0.10)	0.54	0.11	0.73
		4	0.33 (±0.15)	0.74	0.27	0.86
	Volume (m^3 $tree^{-1}$)	3	0.36 (±0.15)	0.76	0.30	0.87
		4	0.48 (±0.18)	0.81	0.41	0.90
	Survival (%)	4	0.00 (±0.01)	0.02	0.00	0.14

Narrow-sense individual heritability (h_a^2); family heritability mean (h_m^2); additive heritability within progenies (h_{ad}^2) and accuracy ($r_{âa}$). Source: The author.

APPENDIX 11 – Individual selection of 80 best slash pine trees based on wood volume and stem straightness in municipalities of Ribeirão Branco-SP and Ponta Grossa-PR, Brazil, at age five and four, respectively.

MEI - k_f: variable							
Strategy I							
Ribeirão Branco				Ponta Grossa			
Volume 2014		Stem straightness		Volume 2013		Stem straightness	
Progeny	k_f	Progeny	k_f	Progeny	k_f	Progeny	k_f
C-001-1	4	C-010-1	3	C-012-3	6	C-010-1	6
C-032-2	4	C-036-1	13	C-013	1	C-012-3	2
C-036-1	1	C-039-2	4	C-016-2	1	C-013	3
C-047-2	2	C-043-3	15	C-023-1	2	C-019-1	4
C-053-1	3	C-048-2	7	C-025-3	1	C-032-2	2
C-063-2	18	C-082-1	13	C-032-2	5	C-039-2	4
C-067-1	2	C-083-1	12	C-067-1	6	C-047-1	7
C-071-1	4	C-225	13	C-084-2	15	C-048-2	3
C-081-3	1			C-128-2	6	C-067-1	3
C-083-1	3			C-189-1	10	C-083-1	6
C-084-2	2			C-197	19	C-189-1	4
C-098-1	12			C-217-1	1	C-197	5
C-100-2	8			C-225	1	C-217-1	2
C-112-1	1			C-228	5	C-225	9
C-151-1	1			C-272-1	1	C-272-1	20
C-189-1	3						
C-197	3						
C-217-1	1						
C-228	7						
N	80		80		80		80
N_{fo}	44		44		24		24
N_f	19		8		15		15
k_f	4.21		10.00		5.33		5.33
σ^2_{kf}	19.18		21.43		30.52		20.52
N_e	13.43		17.80		11.37		15.7
MEI	0.009		0.121		0.012		0.466
$G_s(\%)$	8.65		4.04		23.52		10.52
$\widehat{D}$	0.21		0.15		0.31		0.31
$\bar{x}$	0.104		3.00		0.053		4.43

Number of selected trees (N); number of original progenies (N_{fo}); number of selected progenies (N_f); number of selected trees per progeny (k_f); variance of the number of selected trees per progeny (σ^2_{kf}); effective population size (N_e); a: additive genetic effect = MEI: Multi-effect index; selection gain ($G_s(\%)$); genetic diversity ($\widehat{D}$); overall mean ($\bar{x}$). Source: The author.

APPENDIX 12– Selection of two best slash pine trees per progeny based on wood volume and stem straightness in municipalities of Ribeirão Branco-SP and Ponta Grossa-PR, Brazil, at age five and four, respectively.

MEI - k_f: variable											
Strategy II											
Ribeirão Branco								Ponta Grossa			
Volume 2014				Stem straightness				Volume 2013		Stem straightness	
Progeny	k_f	Progeny	k_f	Progeny	k_f	Progeny	k_f	Progeny	k_f	Progeny	k_f
C-001-1	2	C-071-1	2	C-001-1	2	C-071-1	2	C-010-1	2	C-010-1	2
C-010-1	2	C-074-2	2	C-010-1	2	C-074-2	2	C-012-3	2	C-012-3	2
C-012-3	2	C-081-3	2	C-012-3	2	C-081-3	2	C-013	2	C-013	2
C-013	2	C-082-1	2	C-013	2	C-082-1	2	C-016-2	2	C-016-2	2
C-016-2	2	C-083-1	2	C-016-2	2	C-083-1	2	C-019-1	2	C-019-1	2
C-019-1	2	C-084-2	2	C-019-1	2	C-084-2	2	C-023-1	2	C-023-1	2
C-023-1	2	C-098-1	2	C-023-1	2	C-098-1	2	C-025-3	2	C-025-3	2
C-025-3	2	C-100-2	2	C-025-3	2	C-100-2	2	C-032-1	2	C-032-1	2
C-032-1	2	C-112-1	2	C-032-1	2	C-112-1	2	C-032-2	2	C-032-2	2
C-032-2	2	C-120-1	2	C-032-2	2	C-120-1	2	C-036-1	2	C-036-1	2
C-036-1	2	C-128-2	2	C-036-1	2	C-128-2	2	C-039-2	2	C-039-2	2
C-039-1	2	C-151-1	2	C-039-1	2	C-151-1	2	C-047-1	2	C-047-1	2
C-039-2	2	C-159-1	2	C-039-2	2	C-159-1	2	C-048-2	2	C-048-2	2
C-042-1	2	C-189-1	2	C-042-1	2	C-189-1	2	C-067-1	2	C-067-1	2
C-043-3	2	C-197	2	C-043-3	2	C-197	2	C-068-1	2	C-068-1	2
C-045-1	2	C-217-1	2	C-045-1	2	C-217-1	2	C-083-1	2	C-083-1	2
C-047-1	2	C-218-1	2	C-047-1	2	C-218-1	2	C-084-2	2	C-084-2	2
C-047-2	2	C-225	2	C-047-2	2	C-225	2	C-128-2	2	C-128-2	2
C-048-2	2	C-228	2	C-048-2	2	C-228	2	C-189-1	2	C-189-1	2
C-053-1	2	C-272-1	2	C-053-1	2	C-272-1	2	C-197	2	C-197	2
C-059-1	2			C-059-1	2			C-217-1	2	C-217-1	2
C-063-2	2			C-063-2	2			C-225	2	C-225	2
C-067-1	2			C-067-1	2			C-228	2	C-228	2
C-068-1	2			C-068-1	2			C-272-1	2	C-272-1	2
N		88				88			48		48
N_{fo}		44				44			24		24
N_f		44				44			24		24
k_f		2.00				2.00			2.00		2.00
σ^2_{kf}		0.00				0.00			0.00		0.00
N_e		70.40				70.40			38.40		38.40
MEI		0.005				0.051			0.009		0.356
$G_s(\%)$		4.95				1.73			16.09		7.63
$\widehat{D}$		1.00				1.00			1.00		1.00
$\bar{x}$		0.103				2.99			0.055		4.67

Number of selected trees (N); number of original progenies (N_{fo}); number of selected progenies (N_f); number of selected trees per progeny (k_f); variance of the number of selected trees per progeny (σ^2_{kf}); effective population size (N_e); a: additive genetic effect = MEI: Multi-effect index; selection gain ($G_s(\%)$); genetic diversity ($\widehat{D}$); overall mean ($\bar{x}$). Source: The author.

APPENDIX 13 - Selection of 50% best slash pine trees per progeny based on wood volume and stem straightness in municipalities of Ribeirão Branco-SP and Ponta Grossa-PR, Brazil, at age five and four, respectively.

MEI - k_f: variable											
Strategy III											
Ribeirão Branco								Ponta Grossa			
Volume 2014				Stem straightness				Volume 2013		Stem straightness	
Progeny	k_f	Progeny	k_f	Progeny	k_f	Progeny	k_f	Progeny	k_f	Progeny	k_f
C-001-1	17	C-071-1	19	C-001-1	17	C-071-1	19	C-010-1	10	C-010-1	10
C-010-1	18	C-074-2	19	C-010-1	18	C-074-2	19	C-012-3	10	C-012-3	10
C-012-3	19	C-081-3	19	C-012-3	19	C-081-3	19	C-013	8	C-013	9
C-013	19	C-082-1	19	C-013	19	C-082-1	19	C-016-2	10	C-016-2	10
C-016-2	19	C-083-1	19	C-016-2	19	C-083-1	19	C-019-1	13	C-019-1	13
C-019-1	19	C-084-2	18	C-019-1	19	C-084-2	18	C-023-1	9	C-023-1	10
C-023-1	20	C-098-1	19	C-023-1	20	C-098-1	19	C-025-3	12	C-025-3	12
C-025-3	19	C-100-2	19	C-025-3	19	C-100-2	19	C-032-1	11	C-032-1	11
C-032-1	19	C-112-1	20	C-032-1	19	C-112-1	20	C-032-2	9	C-032-2	9
C-032-2	18	C-120-1	19	C-032-2	18	C-120-1	19	C-036-1	11	C-036-1	11
C-036-1	18	C-128-2	18	C-036-1	19	C-128-2	18	C-039-2	9	C-039-2	9
C-039-1	18	C-151-1	19	C-039-1	18	C-151-1	19	C-047-1	7	C-047-1	7
C-039-2	19	C-159-1	19	C-039-2	19	C-159-1	19	C-048-2	9	C-048-2	9
C-042-1	18	C-189-1	19	C-042-1	18	C-189-1	19	C-067-1	12	C-067-1	12
C-043-3	19	C-197	19	C-043-3	19	C-197	18	C-068-1	10	C-068-1	10
C-045-1	19	C-217-1	19	C-045-1	19	C-217-1	19	C-083-1	9	C-083-1	9
C-047-1	20	C-218-1	19	C-047-1	20	C-218-1	19	C-084-2	11	C-084-2	11
C-047-2	19	C-225	17	C-047-2	19	C-225	17	C-128-2	13	C-128-2	13
C-048-2	19	C-228	19	C-048-2	19	C-228	19	C-189-1	8	C-189-1	9
C-053-1	18	C-272-1	20	C-053-1	18	C-272-1	20	C-197	10	C-197	10
C-059-1	19			C-059-1	19			C-217-1	9	C-217-1	9
C-063-2	19			C-063-2	19			C-225	12	C-225	12
C-067-1	20			C-067-1	20			C-228	10	C-228	10
C-068-1	18			C-068-1	18			C-272-1	11	C-272-1	11
N		828				828			243		246
N_{fo}		44				44			24		24
N_f		44				44			24		24
k_f		18.82				18.82			10.13		10.25
σ^2_{kf}		0.48				0.48			2.46		2.11
N_e		151.42				151.42			71.07		71.75
MEI		0.004				0.029			0.002		0.621
$G_s(\%)$		4.42				1.17			5.07		13.80
$\widehat{D}$		1.00				1.00			1.00		1.00
$\bar{x}$		0.10				2.50			0.037		4.5

Number of selected trees (N); number of original progenies (N_{fo}); number of selected progenies (N_f); number of selected trees per progeny (k_f); variance of the number of selected trees per progeny (σ^2_{kf}); effective population size (N_e); a: additive genetic effect = MEI: Multi-effect index; selection gain ($G_s(\%)$); genetic diversity ($\widehat{D}$); overall mean ($\bar{x}$). Source: The author.

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APPENDIX 14 – Ranking for slash pine progenies and witnesses (controls) at municipality of Ribeirão Branco-SP, Brazil.

	Trait	Height					DBH			Volume			SS	BT	BA	NB	For
	Age	1	2	3	4	5	3	4	5	3	4	5	5	5	5	5	5
Ranking	1	C-012-3	C-100-2	C-100-2	C-063-2	C-063-2	C-098-1	C-098-1	C-098-1	C-098-1	C-100-2	C-063-2	I73	C-043-3	C-081-3	IR02	C-045-1
	2	C-098-1	C-001-1	C-063-2	C-100-2	C-081-3	C-067-1	C-071-1	C-063-2	C-100-2	C-001-1	C-098-1	TEST	C-053-1	C-053-1	C-074-2	I117
	3	C-039-1	C-039-1	C-039-1	C-039-1	C-001-1	C-228	C-063-2	C-228	I123	C-063-2	C-228	C-043-3	C-067-1	C-010-1	C-225	C-012-3
	4	C-100-2	C-098-1	C-001-1	C-001-1	C-039-1	C-071-1	C-001-1	C-071-1	C-063-2	I123	C-001-1	C-225	C-045-1	C-228	C-016-2	C-189-1
	5	C-001-1	C-012-3	C-098-1	C-067-1	C-100-2	C-039-1	C-100-2	C-067-1	C-197	C-098-1	C-100-2	C-036-1	C-272-1	C-128-2	C-025-3	C-217-1
	6	C-063-2	C-063-2	I123	I123	C-067-1	C-032-2	C-032-2	C-001-1	C-039-1	C-071-1	I123	C-082-1	TEST	C-016-2	C-071-1	C-047-2
	7	C-228	C-128-2	C-012-3	C-098-1	C-228	C-063-2	C-228	C-032-2	C-228	C-228	C-071-1	I85	C-032-2	C-039-1	C-039-2	C-098-1
	8	C-067-1	I123	C-071-1	C-012-3	C-083-1	C-128-2	C-067-1	C-100-2	C-001-1	C-197	C-067-1	C-083-1	C-068-1	C-025-3	I30	C-047-1
	9	C-128-2	C-067-1	C-151-1	C-228	C-098-1	C-218-1	I123	C-047-2	C-071-1	C-032-2	C-032-2	C-039-2	C-010-1	C-083-1	C-217-1	C-100-2
	10	C-197	C-151-1	C-067-1	C-081-3	C-084-2	C-001-1	C-012-3	I123	C-067-1	C-067-1	C-189-1	C-048-2	C-151-1	C-068-1	I117	C-151-1
	11	C-151-1	C-228	C-228	C-128-2	C-053-1	C-120-1	C-039-1	C-083-1	I30	C-083-1	C-083-1	C-010-1	C-083-1	IR02	C-272-1	C-001-1
	12	I123	C-039-2	C-128-2	C-151-1	C-151-1	C-083-1	C-083-1	C-042-1	C-128-2	C-039-1	C-047-2	C-013	C-012-3	C-045-1	C-059-1	C-032-1
	13	C-082-1	C-120-1	C-189-1	C-032-2	C-071-1	TEST	C-081-3	C-189-1	C-032-2	C-189-1	C-197	C-032-2	C-074-2	C-032-2	C-023-1	C-042-1
	14	C-218-1	C-068-1	C-197	C-071-1	C-159-1	C-047-2	C-084-2	TEST	C-083-1	C-081-3	C-053-1	C-272-1	C-081-3	TEST	C-019-1	C-197
	15	C-071-1	C-197	C-032-2	C-197	I123	C-081-3	C-048-2	C-039-1	C-047-2	C-012-3	C-039-1	C-032-1	C-071-1	C-047-2	C-010-1	C-128-2
	16	C-047-2	C-081-3	C-016-2	C-084-2	C-032-2	I123	C-042-1	C-048-2	C-081-3	C-084-2	C-084-2	C-074-2	C-016-2	C-043-3	C-032-2	C-063-2
	17	C-120-1	C-189-1	C-084-2	C-189-1	C-120-1	C-084-2	C-128-2	C-053-1	C-084-2	C-047-2	C-151-1	I30	I85	I85	I85	C-039-1
	18	C-013	C-043-3	C-081-3	C-047-2	C-047-2	C-100-2	C-189-1	C-128-2	C-120-1	C-128-2	C-042-1	I117	C-039-2	C-047-1	C-013	C-067-1
	19	C-084-2	C-032-2	C-043-3	C-120-1	C-128-2	C-112-1	C-120-1	C-084-2	C-012-3	C-120-1	C-081-3	C-047-1	I30	C-151-1	C-098-1	C-019-1
	20	C-039-2	C-071-1	C-039-2	C-016-2	C-189-1	C-012-3	C-047-2	C-012-3	C-218-1	C-151-1	C-128-2	I123	C-225	I123	I123	C-025-3
	21	I30	I30	C-047-2	C-043-3	C-012-3	I30	C-112-1	C-151-1	C-112-1	C-042-1	I30	C-063-2	I123	C-189-1	C-081-3	I30
	22	C-025-3	C-013	C-159-1	C-159-1	C-068-1	C-053-1	TEST	C-218-1	TEST	C-048-2	C-047-1	C-120-1	C-120-1	C-120-1	C-045-1	I123
	23	C-159-1	C-016-2	C-083-1	C-068-1	C-112-1	C-048-2	C-218-1	C-013	C-151-1	C-068-1	C-048-2	C-067-1	C-082-1	C-217-1	C-048-2	C-023-1
	24	C-081-3	C-218-1	C-112-1	C-083-1	C-013	C-197	C-068-1	C-120-1	C-043-3	C-112-1	C-112-1	C-081-3	C-019-1	C-112-1	C-128-2	I73

Continue...

...Continuation

Trait	Height					DBH			Volume			SS	BT	BA	BN	For
Age	1	2	3	4	5	3	4	5	3	4	5	5	5	5	5	5
25	C-048-2	C-159-1	C-068-1	C-039-2	C-042-1	C-025-3	C-013	C-081-3	C-039-2	C-043-3	C-120-1	C-059-1	C-039-1	C-272-1	C-112-1	C-120-1
26	C-032-2	C-084-2	C-120-1	C-042-1	C-218-1	C-019-1	C-197	C-112-1	C-068-1	C-013	C-012-3	C-098-1	C-042-1	C-067-1	C-159-1	C-016-2
27	C-217-1	C-047-2	C-082-1	C-019-1	C-039-2	C-039-2	C-053-1	C-036-1	C-189-1	TEST	TEST	C-053-1	C-047-1	C-001-1	C-151-1	C-036-1
28	C-189-1	C-025-3	C-025-3	C-013	C-047-1	C-043-3	C-043-3	C-197	C-053-1	C-218-1	C-159-1	IR02	I117	C-084-2	C-228	C-059-1
29	C-016-2	C-272-1	C-218-1	C-218-1	C-197	C-042-1	C-151-1	C-159-1	C-019-1	C-053-1	C-218-1	C-071-1	C-218-1	C-074-2	C-218-1	C-039-2
30	C-010-1	C-032-1	TEST	C-112-1	I30	C-159-1	C-019-1	C-047-1	C-048-2	C-039-2	C-013	C-128-2	C-036-1	C-197	TEST	E-074-2
31	C-272-1	C-112-1	C-053-1	C-053-1	C-048-2	C-036-1	C-025-3	C-043-3	C-025-3	C-019-1	C-032-1	C-217-1	C-025-3	C-063-2	C-039-1	C-228
32	C-068-1	C-053-1	C-013	C-048-2	C-019-1	C-013	C-159-1	I30	C-013	I30	C-036-1	C-039-1	C-098-1	C-032-1	C-032-1	C-048-2
33	C-112-1	TEST	I30	E-059-1	C-043-3	C-151-1	C-036-1	C-019-1	C-036-1	C-159-1	C-068-1	C-025-3	C-112-1	C-071-1	C-047-1	C-272-1
34	C-036-1	C-082-1	C-036-1	C-047-1	C-025-3	I73	C-082-1	C-217-1	C-082-1	C-082-1	C-043-3	C-068-1	C-228	C-012-3	C-120-1	C-043-3
35	C-042-1	C-036-1	C-019-1	I30	C-016-2	C-082-1	C-039-2	C-068-1	C-159-1	C-016-2	C-039-2	C-189-1	C-059-1	C-036-1	C-043-3	TEST
36	C-225	C-083-1	C-048-2	C-082-1	C-082-1	C-068-1	C-217-1	C-032-1	C-016-2	C-036-1	I73	C-045-1	C-197	C-048-2	C-084-2	C-068-1
37	C-043-3	C-074-2	C-047-1	TEST	TEST	C-217-1	C-059-1	C-039-2	C-042-1	C-047-1	C-019-1	C-023-1	C-189-1	C-023-1	C-053-1	C-084-2
38	C-083-1	C-042-1	C-272-1	C-025-3	C-036-1	C-272-1	I30	I73	C-047-1	C-025-3	C-217-1	C-047-2	C-013	C-082-1	C-197	C-083-1
39	TEST	C-048-2	C-032-1	C-036-1	C-010-1	C-010-1	C-047-1	C-010-1	C-272-1	C-217-1	C-010-1	C-159-1	C-084-2	C-218-1	C-189-1	C-081-3
40	C-032-1	C-010-1	C-059-1	C-272-1	C-217-1	C-059-1	C-016-2	C-025-3	C-032-1	C-059-1	C-025-3	C-001-1	I73	C-159-1	C-012-3	C-159-1
41	C-059-1	C-047-1	C-042-1	C-217-1	C-225	C-189-1	C-010-1	C-082-1	I73	C-010-1	C-082-1	C-042-1	C-032-1	C-019-1	C-082-1	I85
42	C-053-1	C-019-1	C-074-2	C-032-1	C-059-1	C-016-2	I73	C-225	C-059-1	C-032-1	C-023-1	C-151-1	C-047-2	C-039-2	C-100-2	C-218-1
43	C-045-1	C-225	C-010-1	C-010-1	C-032-1	C-047-1	C-272-1	C-023-1	C-010-1	I73	I117	C-016-2	C-001-1	C-225	C-042-1	C-013
44	I117	C-217-1	C-045-1	C-045-1	C-272-1	C-032-1	C-032-1	C-045-1	C-217-1	C-272-1	C-225	C-084-2	C-128-2	I73	C-047-2	C-071-1
45	C-047-1	C-059-1	C-225	C-225	C-045-1	I85	C-225	I117	C-225	C-045-1	C-045-1	C-197	C-023-1	C-059-1	C-036-1	C-010-1
46	C-019-1	I73	I117	I117	C-074-2	C-225	C-045-1	C-059-1	I85	C-225	C-016-2	C-012-3	IR02	C-013	I73	C-032-2
47	C-023-1	C-045-1	C-217-1	I73	I117	I117	I117	IR02	C-023-1	C-023-1	C-059-1	C-019-1	C-159-1	C-100-2	C-083-1	C-112-1
48	I73	C-023-1	I73	C-074-2	C-023-1	C-023-1	C-023-1	I85	I117	I117	C-074-2	C-218-1	C-217-1	I117	C-068-1	C-225
49	I85	I85	C-023-1	C-023-1	I73	IR02	I85	C-272-1	C-074-2	C-074-2	IR02	C-228	C-063-2	C-042-1	C-067-1	IR02
50	C-074-2	I117	IR02	IR02	IR02	C-045-1	IR02	C-074-2	C-045-1	I85	C-272-1	C-112-1	C-048-2	C-098-1	C-001-1	C-082-1
51	IR02	IR02	I85	I85	I85	C-074-2	C-074-2	C-016-2	IR02	IR02	I85	C-100-2	C-100-2	I30	C-063-2	C-053-1

DBH: diameter at breast height, SS: stem straightness, BT: branch thickness, BA: branch angle, NB: number of branches, For: fork. Witnesses are I30, I73, I117, TEST, I85, IR02, I123.

APPENDIX 15 – Estimates of genetic correlation (r_g = above diagonal) and phenotypic (r_f = below diagonal) between slash pine growth and form traits in municipality of Ribeirão Branco-SP, Brazil.

			Height					DBH			Volume			SS	BT	BA	NB	FOR
Traits		Age	1	2	3	4	5	3	4	5	3	4	5					
Ribeirão Branco	Height	1	-	0.85**	0.80**	0.78**	0.64**	0.72**	0.73**	0.62**	0.79**	0.74**	0.62**	-0.32**	-0.23**	-0.11**	-0.37**	0,07**
		2	0.76**	-	0.91**	0.86**	0.74**	0.67**	0.70**	0.60**	0.83**	0.78**	0.66**	-0.31**	-0.09**	0.03^{ns}	-0.40**	0,12**
		3	0.69**	0.82**	-	0.94**	0.83**	0.72**	0.80**	0.68**	0.90**	0.88**	0.76**	-0.32**	-0.13**	0.07**	-0.47**	0,07**
		4	0.56**	0.73**	0.87**	-	0.89**	0.78**	0.86**	0.75**	0.91**	0.92**	0.79**	-0.36**	-0.13**	0.05*	-0.53**	0,12**
		5	0.47**	0.58**	0.70**	0.78**	-	0.79**	0.85**	0.83**	0.85**	0.87**	0.87**	-0.28**	-0.14**	0.14**	-0.60**	0,02^{ns}
	DBH	3	0.67**	0.68**	0.72**	0.67**	0.61**	-	0.90**	0.82**	0.90**	0.81**	0.76**	-0.22**	-0.09**	0.07**	-0.42**	0,02^{ns}
		4	0.60**	0.67**	0.73**	0.72**	0.60**	0.80**	-	0.91**	0.90**	0.95**	0.88**	-0.26**	-0.20**	-0.06**	-0.53**	0,08**
		5	0.52**	0.58**	0.64**	0.66**	0.71**	0.72**	0.80**	-	0.83**	0.88**	0.96**	-0.20**	-0.22**	-0.05*	-0.58**	0,08**
	Volume	3	0.71**	0.75**	0.82**	0.72**	0.63**	0.93**	0.77**	0.70**	-	0.94**	0.87**	-0.33**	-0.18**	0.04^{ns}	-0.50**	0,09**
		4	0.63**	0.73**	0.79**	0.81**	0.67**	0.75**	0.94**	0.78**	0.83**	-	0.92**	-0.31**	-0.24**	-0.04^{ns}	-0.57**	0,11**
		5	0.52**	0.60**	0.66**	0.69**	0.81**	0.67**	0.73**	0.94**	0.73**	0.80**	-	-0.26**	-0.28**	-0.01^{ns}	-0.63**	0,06*
	SS		-0,09**	-0.05*	-0.03^{ns}	0.01^{ns}	0.02^{ns}	-0.02^{ns}	0.00^{ns}	0.01^{ns}	-0.03^{ns}	-0.01^{ns}	-0.01^{ns}	-	0.30**	0.04^{ns}	0.09**	-0.18**
	BT		0,16**	0.15**	0.10**	0.07**	0.05*	0.14**	0.17**	-0.19**	0.15**	0.18**	0.18**	0.16**	-	0.38**	0.12**	-0.18**
	BA		0,01**	0.01^{ns}	0.08**	0.07**	0.11**	0.05*	0.01^{ns}	0.01^{ns}	0.05*	0.02^{ns}	0.01^{ns}	0.10**	0.29**	-	0.01^{ns}	-0.13**
	NB		-0,17**	-0.22**	-0.24**	-0.27**	-0.33**	-0.22**	-0.27**	-0.38**	-0.22**	-0.29**	-0.39**	-0.07**	-0.13**	-0.09**	-	0.08**
	FOR		0,08**	0.05*	0.08**	0.05^{ns}	0.05*	0.05*	0.10**	0.05*	0.06*	0.09**	0.05^{ns}	0.03^{ns}	0.06*	0.03^{ns}	-0.23**	-

**($p<0,01$); *($p<0,05$); ^{ns} not significant, by t test. SS = stem straightness; BT = branch thickness; BA = branch angle; NB = number of branches; FOR = fork.
Source: The author.

APPENDIX 16 - Estimates of genetic correlation (r_g = above diagonal) and phenotypic (r_f = below diagonal) between slash pine growth and form traits in municipality of Ponta Grossa-PR, Brazil.

	Trait	Age	Height				DBH		Volume		SS	BT	BA	NB	FOR	FT
			1	2	3	4	3	4	3	4						
Ponta Grossa	Height	1	-	0.83**	0.86**	0.80**	0.85**	0.81**	0.84**	0.79**	-0.19**	-0.44**	0.00^{ns}	0.08*	-0.30**	-0,33**
		2	0.76**	-	0.96**	0.93**	0.89**	0.91**	0.94**	0.89**	-0.22**	-0.41**	0.05^{ns}	-0.04^{ns}	-0.28**	-0,19**
		3	0.69**	0.84**	-	0.94**	0.89**	0.90**	0.93**	0.89**	-0.14**	-0.38**	-0.02^{ns}	-0.06^{ns}	-0.31**	-0,16**
		4	0.58**	0.75**	0.87**	-	0.86**	0.88**	0.91**	0.90**	-0.13**	-0.33**	0.03^{ns}	-0.10**	-0.34**	-0,19**
	DBH	3	0.68**	0.83**	0.83**	0.77**	-	0.89**	0.92**	0.83**	-0.10**	-0.26**	0.05^{ns}	0.05^{ns}	-0.29**	-0,11**
		4	0.62**	0.77**	0.81**	0.78**	0.83**	-	0.96**	0.97**	-0.14**	-0.40**	-0.14**	0.18**	-0.31**	-0,10**
	Volume	3	0.68**	0.84**	0.86**	0.78**	0.93**	0.83**	-	0.97**	-0.14**	-0.43**	-0.11**	0.04^{ns}	-0.28**	-0,16**
		4	0.61**	0.77**	0.81**	0.83**	0.80**	0.94**	0.88**	-	-0.13**	-0.46**	-0.20**	0.10**	-0.29**	-0,13**
	SS		-0,06^{ns}	-0.05^{ns}	0.01^{ns}	0.05^{ns}	-0.02^{ns}	-0.02^{ns}	-0.02^{ns}	-0.01^{ns}	-	0.48**	0.08*	-0.11**	0.49**	0.60**
	BT		-0,24**	-0.25^{ns}	-0.18**	-0.16**	-0.24**	-0.23**	-0.24**	-0.23**	0.30^{ns}	-	0.55**	-0.37**	0.33**	0.66**
	BA		-0,10**	-0.08*	-0.02^{ns}	-0.02^{ns}	-0.08*	-0.05^{ns}	-0.09*	-0.06^{ns}	0.16^{ns}	0.29**	-	-0.37**	0.19**	0.28**
	NB		0,11**	0.13**	0.09*	0.11**	0.11**	0.08*	0.16**	0.12**	-0.05^{ns}	-0.12**	-0.13**	-	-0.36**	-0.10**
	FOR		-0,04^{ns}	-0.02^{ns}	0.04^{ns}	0.07^{ns}	0.04^{ns}	0.01^{ns}	0.01^{ns}	0.00^{ns}	0.42**	0.14**	0.09*	-0.06^{ns}	-	0.48**
	FT		-0,13**	-0.11*	-0.09**	-0.08*	-0.13**	-0.08*	-0.12**	-0.08**	0.49**	0.38**	0.24**	-0.16**	0.07^{ns}	-

**($p<0,01$); *($p<0,05$); ^{ns} not significant, by t test. SS = stem straightness; BT = branch thickness; BA = branch angle; NB = number of branches; FOR = fork; FT = fox tail..
Source: The author.

APPENDIX 17 – Generalized Mahalanobis Distance (D^2) between slash pine progenies in municipality of Ribeirão Branco-SP, Brazil.

D^2 Mahalanobis Distance									
Progeny	**Greater**	**Progeny**	**Smaller**	**Progeny**	**Progeny**	**Greater**	**Progeny**	**Smaller**	**Progeny**
C-016-2	52.57	C-098-1	16.63	C-013	C-032-1	47.47	C-081-3	20.67	C-025-3
C-082-1	44.61	C-047-1	14.41	C-084-2	C-068-1	43.17	C-159-1	19.70	C-063-2
C-082-1	53.01	C-071-1	13.24	C-032-2	C-053-1	52.00	C-217-1	22.37	C-048-2
C-074-2	47.19	C-010-1	13.49	C-045-1	C-053-1	54.46	C-048-2	18.02	C-059-1
C-082-1	44.36	C-023-1	15,46	C-112-1	C-053-1	47.18	C-272-1	15.42	C-043-3
C-053-1	50.92	C-100-2	17.18	C-001-1	C-053-1	44.88	C-128-2	44.88	C-053-1
C-197	39.85	C-084-2	7.59	C-112-1	C-053-1	47.39	C-032-1	25.90	C-043-3
C-082-1	43.21	C-120-1	43.21	C-082-1	C-063-2	55.76	C-053-1	35.14	C-032-2
C-053-1	50.16	C-036-1	13.56	C-048-2	C-047-2	47.60	C-068-1	24.27	C-043-3
C-053-1	59.71	C-012-3	18.80	C-045-1	C-019-1	43.73	C-063-2	29.15	C-013
C-082-1	48.72	C-151-1	15.57	C-039-1	C-016-2	46.95	C-042-1	17.64	C-013
C-082-1	50.22	C-045-1	16.88	C-189-1	C-218-1	36.53	C-043-3	12.25	C-032-2
C-082-1	50.82	C-112-1	8.36	C-218-1	C-025-3	36.08	C-047-2	18.10	C-218-1
C-053-1	55.66	C-001-1	25.31	C-189-1	C-067-1	35.54	C-039-1	18.07	C-013
C-197	49.43	C-039-2	9.07	C-272-1	C-025-3	46.79	C-067-1	16.64	C-032-2
C-053-1	48.22	C-225	12.22	C-013	C-016-2	40.42	C-218-1	18.27	C-019-1
C-074-2	60.74	C-082-1	33.82	C-059-1	C-016-2	42.47	C-013	23.04	C-059-1
C-197	49.57	C-189-1	18.24	C-048-2	C-025-3	31.00	C-019-1	11.56	C-059-1
C-053-1	54.02	C-083-1	24.02	C-047-2	C-059-1	28.25	C-025-3	24.48	C-016-2
C-197	62.76	C-074-2	29.43	C-272-1	C-016-2	24.36	C-032-2	22.32	C-059-1
C-068-1	47.61	C-228	16.16	C-047-2	C-059-1	17.78	C-016-2		
C-053-1	72.38	C-197	37.89	C-272-1					
Maximum:	72.38						progenies:	C-053-1 and C-197	
Minimum:	7.59						progenies:	C-084-2 and C-112-1	

Source: The author.

APPENDIX 18 – Generalized Mahalanobis Distance (D^2) between slash pine progenies in municipality of Ponta Grossa-PR, Brazil.

D2 Mahalanobis Distance									
Progeny	**Greater**	**Progeny**	**Smaller**	**Progeny**	**Progeny**	**Greater**	**Progeny**	**Smaller**	**Progeny**
C-047-1	33.36	C-084-2	10.34	C-025-3	C-047-1	40.16	C-019-1	15.33	C-012-3
C-197	39.75	C-025-3	21.71	C-083-1	C-047-1	37.02	C-067-1	18.46	C-217-1
C-019-1	37.31	C-010-1	19.34	C-016-2	C-047-1	27.94	C-012-3	13.53	C-083-1
C-039-2	39.25	C-068-1	19.97	C-032-1	C-047-1	34.55	C-225	25.83	C-228
C-047-1	34.02	C-016-2	14.38	C-012-3	C-189-1	35.63	C-036-1	25.34	C-083-1
C-128-2	35.67	C-272-1	15.46	C-083-1	C-047-1	38.74	C-228	25.80	C-083-1
C-036-1	40.27	C-128-2	22.17	C-083-1	C-048-2	37.81	C-189-1	19.55	C-083-1
C-048-2	36.03	C-032-1	12.54	C-012-3	C-047-1	28.54	C-048-2	19.87	C-083-1
C-217-1	38.07	C-013	19.73	C-067-1	C-047-1	39.34	C-197	29.42	C-217-1
C-189-1	35.93	C-032-2	18.39	C-012-3	C-047-1	31.49	C-083-1	25.21	C-217-1
C-023-1	32.58	C-039-2	14.08	C-225	C-217-1	35.12	C-047-1		
C-047-1	41.63	C-023-1	23.94	C-036-1					
Maximum:	41.63						progenies:	C-047-1 and C-023-1	
Minimum:	10.34						progenies:	C-084-2 and C-025-3	

Source: The author.

APPENDIX 19 – Genetic and phenotypic correlations between Pilodyn penetration and wood density (DEN), modulus of elasticity (MOE), microfibril angle (MFA) and diameter at breast height (DBH) for Radiata pine planted in Flynn, Australia from age one to 29.

	Genetic				Phenotipic			
Age	DEN	MOE	MFA	DBH	DEN	MOE	MFA	DBH
1	-	-0.09	-	-	-0.30	0.29	-0.13	-0.13
2	-	-0.11	-	-	-0.27	0.03	-0.07	-0.03
3	-	-0.1	-	-	-0.47	0.04	-0.03	0.06
4	-	-0.11	-0.33	-	-0.42	-0.09	-0.02	0.07
5	-0.92	-0.11	-0.28	-	-0.43	-0.13	-0.02	0.08
6	-0.76	-0.10	-0.25	-	-0.38	-0.17	0.01	0.12
7	-0.76	-0.09	-0.24	0.24	-0.36	-0.21	0.04	0.18
8	-0.67	-0.16	-0.18	0.59	-0.35	-0.27	0.07	0.23
9	-0.72	-0.25	-0.16	0.99	-0.38	-0.32	0.10	0.27
10	-0.6	-0.26	-0.17	0.99	-0.42	-0.33	0.09	0.33
11	-0.83	-0.29	-0.16	0.99	-0.45	-0.35	0.10	0.35
12	-0.86	-0.30	-0.16	0.99	-0.47	-0.36	0.10	0.37
13	-0.86	-0.28	-0.18	0.99	-0.48	-0.36	0.10	0.38
14	-0.88	-0.25	-0.19	0.99	-0.50	-0.35	0.08	0.38
15	-0.88	-0.29	-0.19	0.99	-0.51	-0.36	0.08	0.37
16	-0.9	-0.32	-0.20	0.99	-0.52	-0.37	0.07	0.37
17	-0.91	-0.34	-0.20	0.98	-0.52	-0.38	0.07	0.37
18	-0.93	-0.36	-0.20	0.97	-0.54	-0.40	0.08	0.36
19	-0.97	-0.37	-0.20	0.99	-0.55	-0.41	0.08	0.36
20	-0.99	-0.39	-0.19	0.99	-0.56	-0.42	0.08	0.36
21	-1.00	-0.41	-0.21	0.99	-0.56	-0.43	0.08	0.35
22	-1.00	-0.43	-0.21	0.99	-0.56	-0.43	0.08	0.35
23	-1.00	-0.46	0.24	0.99	-0.56	-0.44	0.06	0.35
24	-1.00	-0.47	-0.26	0.99	-0.57	-0.44	0.06	0.34
25	-1.00	-0.47	-0.27	-	-0.54	-0.43	0.05	0.32
26	-1.00	-0.49	-0.27	-	-0.54	-0.43	0.05	0.32
27	-1.00	-0.51	-0.26	-	-0.55	-0.45	0.06	0.32
28	-1.00	-0.49	-0.25	-	-0.55	-0.43	0.05	0.34
29	-1.00	-0.61	-0.29	-	-0.52	-0.43	0.05	0.32