UNIVERSIDADE ESTADUAL PAULISTA

POSTGRADUATE PROGRAM IN AGRONOMY

DIEGO GABRIEL TORRES DINI

BIOLOGIST

DETECTION OF QTLs ASSOCIATED TO DBH IN A *EUCALYPTUS GRANDIS* x *EUCALYPTUS GLOBULUS* MONOPROGENY

Ilha Solteira
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Thesis presented on Engineering University – UNESP –Ilha Solteira Campus, as part of the requirements to obtain a Doctorate Degree in Agronomy. (Production Systems)

Mentor: Professor Dr. Alexandre Magno Sebbenn

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DEDICATION

I dedicate this work to my grandfather Hector Higinio Dini (in memoriam), who since childhood taught me about the infinite value of books to forge thought, character, intellect and personality, helping us to know ourselves and discover how does the world functions. I thank him too for teaching me the complexity and implications through the way.

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ABSTRACT

In Uruguay, reforestation with *Eucalyptus* sp. is of fundamental importance to produce paper, pulp and wood. The productivity of these continually grows due to application of breeding techniques, such as hybridization. This study aimed to investigate genetic parameters, productivity, stability, adaptability and to identify SNP markers associated with the diameter breast height (DBH) for to select *Eucalypts grandis* x *Eucalyptus globulus* full-sibs hybrid clones. The study was conducted in a clonal test, repeated at two different soils, in the state of Rio Negro, Uruguay. The population was phenotypically characterized to the DBH at 48 months of age and cambium tissues of each individual were sampled for genotyping with EuCHIP60K chip. The mean growth in DBH was similar between both places. The genotype-environment interaction was the simple type, with high genotype correlation in clones' performance between environments (0.708), indicating the possibility of the same clones being selected for both places. Mean heritability between clones (0.724), coefficient of individual genetic variation (10.9%) and relative variation (0.916), showed the possibility of obtaining gains by selecting clones with higher growth, which was estimated in 3.1% for both sites together. A total of 15,196 markers SNPs were used in the genomic selection for the DBH, but after cleaning of SNPs data, the number was reduced for 15,196 (23.5%). The predictive capacity was expected to be low or negative (-0.15) for this population given the population size (78 individuals). We used the model rrBLUP with a validation of Jackknife. The model do not showed precision to predict the DBH. These results were consistent with theoretical expectations, which indicate that it is necessary to have an improvement population of at least 1,000 phenotyped and genotyped individuals. The DBH is the most important trait in the breeding of the genus Eucalyptus. However its quantitative nature added to the time necessary for this phenotype to develop makes the early detection of this trait are difficult. The identification of molecular markers associated with quantitative phenotypes is a good choice for the identification of QTLs that will help the early detection of individuals with high DBH. Significant markers associated to DBH , were indentificated into the chromosome 6, suggesting the presence of a QTL in this chromosome. Since they are clones originated from vegetative propagation and a full-sibs single-progeny, they should preferably be used for reforestation based on their cloning, since mating between clones can generate endogamy

by biparental inbreeding. The utilization of SNPs helped to confirm the degree of parentage between the clones as well as clonal identity control.

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1 GENERAL INTRODUCTION

The worldwide reforestation of *Eucalyptus* sp exceeds 20 million hectares, distributed in more than 90 countries with the most diverse climates. South America is responsible for 55% of global production, with the main producers being Brazil, Uruguay, Chile, Argentina and Peru. Asia, China and India together reach the 23% of global production, while Europe reaches 7% of the total together with productions in Spain, Portugal and a smaller quantity in Italy. On the African continent, South Africa is the main producer with 3% of the total, the percentage remaining are shared between countries of the rest of the world with productions of less than 3% (BOOTH, 2013; IGLESIAS TRABADO, 2009).

Uruguay is the second smallest country in South America and has a total of approximately 1.7 million hectares of native forest and commercial plantations. The forest industry is a relatively new economic sector in the history of Uruguay, which emerged about three decades ago and has grown exponentially since. In the year 2015, the total forested area reached 1.1 million hectares (POU, 2011), representing 6.5% of the total area of the country. Of this total, 890.000 hectares correspond to plantations with the genus Eucalyptus, and 260.000 hectares correspond to plantations with the genus Pinus. Of the cultivated Eucalyptus species, the main ones are: *E. globulus, E. grandis, E. maidenii, E. dunnii* and different interspecific hybrids that are evaluated in the local breeding programs. These species are cultivated to produce wood, pulp, paper, etc. They are used in different breeding programs to maintain and increase productivity in the forestry sector and to increase the quality of the derived products, satisfying the final demand of the industries (BALMELLI; RESQUIN, 2008; GRIFFIN et al., 2000; PASEYRO, 2015; RESQUIN; BALMELLI, 1999)

The process of tree genetic improvement involves a series of techniques aimed at both the management of genetic variability and the development of selection tools and models. In the case of clones, it is necessary to evaluate many promising clones from year to year in different environments before making a final recommendation and subsequent multiplication (SUDARIC et al., 2006; ROSADO et al., 2012). In most cases, there is interaction genotype environment (GxE), which affects the gain with the selection which makes it necessary to estimate the magnitude and nature of this interaction. These estimates make it possible to evaluate the actual selection impact by providing a high

degree of reliability in the recommendation of clones for a determined place or group of environments (ROSADO et al., 2012)

Despite the undeniable importance for genetic improvement, simple GXE analysis does not provide complete and accurate information. Therefore, it is necessary to carry out analyses on adaptability and stability (PUPIN, 2014). The adaptability of the genetic material can be defined as the potential that genotypes must respond favourably to positive environmental changes, whereas stability represents the ability of genotypes to be consistent in their general behaviour as a function of changes in environmental quality (RESENDE, 2002; NUNES, 2015).

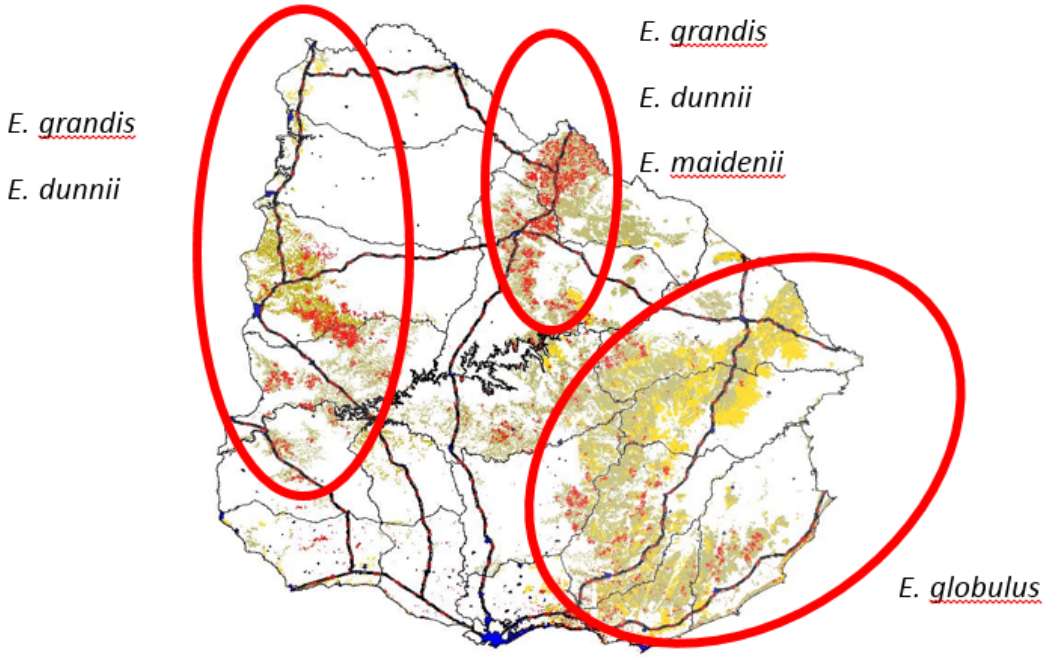
According to Resende (2004) a more complete model may allow additional inferences, such as: selection of genotypes specific to each site; selection of stable genotypes across sites; selection of responsive genotypes (with high adaptability) to the improvement of the environment; and selection by the three attributes (productivity, stability and adaptability), simultaneously. This type of selection can be performed by the Harmonic Mean of Relative Performance of Genotypic Predicted Value (HMRPGV), which classifies genotypic effects as random and therefore provides genotypic stability and adaptability but not phenotypic (MAIA et al., 2009).

The predicted impact is selection (G_s) gain depends on the heritability (h^2) of the trait to be improved and of the differential selection (d_s) that is, $G_s = h^2 d_s$ in which is the product of the standardized selection intensity, in units of phenotypic standard deviation, of the variable being selected, divided by phenotypic standard deviation. Thus, the selection intensity is of great importance in the estimation of these gains, since higher intensity of selection is expected to result in higher gains (VENCOVSKY; BARRIGA, 1992). In addition, selection in forest improvement programs based on multi-effects index (MEI) allows to explore fractions of additive genetic variance that are not considered in the classical selection among and within progenies, leading to a maximization of precision in selection, in many cases, the inclusion of plot and block effects may change the selection in a non-significantly manner (RESENDE; HIGA, 1994).

Genetic markers are used to quantify and monitor levels of genetic diversity, inbreeding, and parentage, to explore genetic variability adequately within breeding populations (ALVES et al., 2007; GRATTAPAGLIA et al., 2011). With the advancement of new genotyping techniques, it is possible to cover the whole genome by reducing the distance between molecular genetics and quantitative genetic techniques (LIMA, 2014). Techniques such as DArT (Diversity Arrays Technology) and SNPs (Single Nucleotide Polymorphisms), are routinely performed by several companies and laboratories in the world that provide massive scale genotyping services, reducing the time of analysis and making them more accessible (GRATTAPAGLIA et al., 2011; AGUIAR et al., 2015; SILVA-JUNIOR et al., 2015).

The production of hybrid clones originated from interspecific crosses is also a routine technique in forest breeding, which allows the creation of new genetic combinations and the exploration of heterosis effects. These hybrids are evaluated in the field in clonal tests for production, interaction of genotypes with the environment, seeking to obtain genetic gains by selection, as well as adaptability and stabilities that improve their development in different environmental conditions (ASSIS, 1975; GRIFFIN et al., 2000; GRATTAPAGLIA; KIRST, 2008). Thus, is tried to gather in the hybrids new genetic mixtures that surpass the comparative performance with the parental species. This information is used to make decisions about the multiple practical objectives of breeding programs, reducing selection times by optimizing the choice of parents (ASSIS et al., 1993; ASSIS, 2014).

Figure 1 Mains species cultivated in Uruguay.



Source: Based on Ministry of Livestock Agriculture and Fisheries from Uruguay.

2 BIBLIOGRAPHICAL REVIEW

The environmental diversity of the countries and of each continent where these are found, requires that in addition to prioritizing the productivity objectives in the Eucalyptus crop, adaptability and stability must be considered since they are key factors for the good utilization of the species (CAPPA et al., 2010; STACKPOLE et al., 2013: PUPIN et al., 2014). Therefore, the good choice of the species and the provenances to be used in the development of the forest sectors of each country, is a step of transcendental importance before starting a program of genetic improvement (BOOTH et al., 2002). This is the first phase of a species domestication program, where the greatest gains in productivity are obtained. Most of the successful Eucalyptus introductions began with a good knowledge of species growth in their natural habitat and comparison of environmental conditions with the destination place (BOOTH et al., 1987; BOOTH et al., 1988). From the study of climate and soil conditions it is determined which are the regions where productivity is profitable (BOOTH and PRYOR, 1991). The proper choice of this genetic basis is what determines the potential success of the breeding program.

2.1 Main commercial species

The genus *Eucalyptus* has a wide diversity of species, being composed of approximately 800 (COPPEN, 2004) and the *sub-genus Symphyomyrtus* contains approximately 300 species (PRYOR; JHONSON, 1971), within which are the most important species of the *E. urophyla, E.grandis, E. globulus, E. dunnii, E. maidenii, E. viminalis,* and more recently *E. benthamii* as a promising one. The wide diversity of this genus allows the selection of species of great plasticity for the adaptation and improvement of the genetic resource. In the case of Uruguay, Eucalyptus species represent 70% of the reforested area, which translates into 890 thousand hectares, leaving the remaining 30% for species of the genus *Pinus* (POU, 2011). The main eucalyptus species planted in Uruguay along with some important species of the others countries are described below.

2.1.1 Eucalyptus globulus

It is the main cultivated specie in Uruguay, Spain and Portugal (BALMELLI et al., 2016; IGLESIAS TRABADO, 2009). This specie contains four major sub-species: *Eucalyptus globulus* itself, *E. pseudoglobulus, E. bicostata and E. maidenii*. The highest growth rate has been observed in *E. globulus and E. pseudoglobulus*, which have a similar growth rate. Some sources of *E. bicostata* and *E. maidenii* compensate for the low growth rate with a better resistance to frost. All these subspecies are susceptible to hybridization and generation of hybrids by controlled crosses. The *E. globulus* sub-species has its natural habitat mainly on Tasmania Island and at the southernmost end of the territory of Vitoria (Figure 2). *E. pseudoglobulus* is distributed only on the southern continent of the territory in Vitoria and on the border with New South Wales. *E.bicostata* is limited to the south, with the populations *of E. pseudoglobulus* located in Vitoria and New South Wales. The subspecies *E. maidenii* is found on the south coast of New South Wales (ELDRIDGE et al., 1993).

The high density of wood, low content of extracts of *E. globulus*, as well as its high yield makes this species one of the most demanded in the international market (BALMELLI, 2016; VILLENA, 2003). The high price of its wood, associated with its high growth rate, encourages plantations of *E. globulus* in Uruguay and is currently the most planted species in the country. However, the appearance of pests and diseases in recent years has strongly affected the plantations of this specie (SIMETO et al., 2010; PÉREZ et al., 2013)

2.1.2 Eucalyptus grandis

This species is native to Australia and the lower bound of distribution near Newcastle, New South Wales, at 32 ° 52'S (Figure 2). It is the second most cultivated specie Uruguay and one of the most important in the world. The predilection for this specie is due to the excellent traits that it presents, such as ease of care in the nursery, rapid growth, being greater than observed in other species of the genus, presenting good stem shape and viable wood properties of different final uses. Unlike *E. globulus, E. grandis* presents low density wood and is therefore more suitable for use as solid wood for sawing. However, in Uruguay it is used in the production of cellulose pulp. In Brazil,

E. grandis is widely used in the formation of hybrids with other species such as *E. urophylla* crosses (ELDRIDGE et al., 1993; GOMINHO et al., 2007).

2.1.3 Eucalyptus maidenii

It is a specie related to *E. globulus* and has been frequently used as an alternative specie since it presents a better resistance to cold and sanity. *E. maidenii* and *E. globulus* occupy an area of more than 350,000 hectares in Uruguay (ELDRIDGE et al., 1993; BALMELLI et al., 2016).

2.1.4 Eucalyptus dunnii

This specie has revealed its economic importance in recent years and is important in breeding programs in Uruguay. It has a high growth rate and frost tolerance. It has aptitude for both cellulose pulp and solid wood production (JOVANOVIC et al., 2000). Its natural distribution (Figure 2) is relatively restricted in a small region in eastern Australia (RESQUIN, 2003). Field evaluations in South America showed superior growth and survival capacity compared to their behaviour in the original habitat (BOOTH et al., 2002). This specie has gained space from *E. grandis*, mainly in low places where frosts are stronger. Frosts in their region of origin can occur from 30 to 60 days a year with temperatures between 2 and 5 ° C (FONSECA et al., 2010). In addition, it showed a superior behaviour against drought in South Africa (NIXON; HAGEDORN, 1983). In China was also selected for its good frost tolerance beyond the versatility to final wood destinations (ARNOLD; CLARKE, 2004). For this reason, it is also an option for southern Brazil (PEREIRA et al., 1986; CLARA et al., 2000). In Uruguay, the cultivation of *E. dunnii* progresses year by year, consolidating itself as one of the main species planted in the country. In Argentina, it is also seen as a viable option (DE LA PEÑA et al., 2012) for the entire pampas region.

2.1.5 Eucalyptus nitens

This specie develops in regions of mild weather, restricted to south-eastern Australia (Figure 2) and presents a wide genetic variability between provenances (FONSECA et al., 2010). In Australia, it is the main specie cultivated for its good aptitude to produce cellulose pulp and fast growth, and at present the genetic breeding programs are already in the second selection cycle (HAMILTON et al., 2008). It is also cultivated

in Chile in the Bio-Bio region, where the altitude varies from 0 to 830 m, a temperature of 8 to 32 ° C in summer and 3 to 13 ° C in winter. It is also used in South Africa and New Zealand besides presenting frost tolerance and better resistance than *Teratosphaeria*, compared to other species of Eucalyptus (HAMILTON; POTTS, 2007).

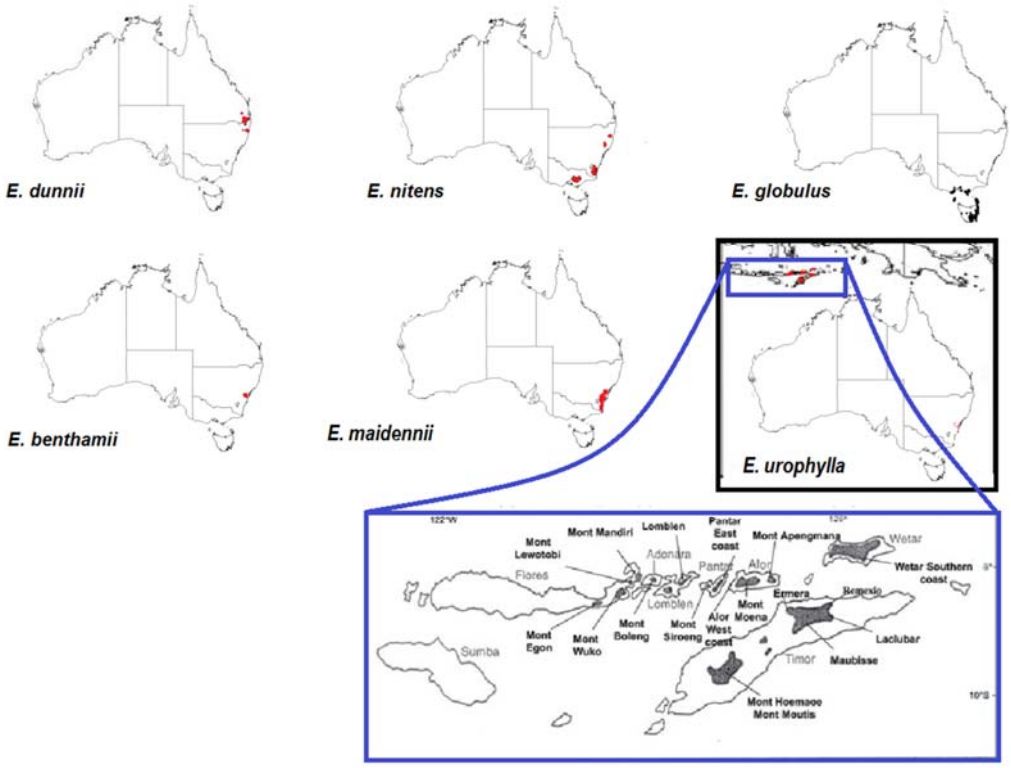
2.1.6 Eucalyptus benthami

This specie is just beginning to gain importance in terms of suitable properties for pulp cellulose production, rapid growth, frost tolerance and adaptation to even summer climates. Uruguay and Brazil were among the first countries to study this specie (HARWOOD, 2011; PALUDZYSZYN FILHO et al., 2006). Its natural distribution is restricted to a small area on the west of Sydney (Figure 2) and is listed as an endangered species (BUTCHER et al., 2005). This has motivated Australia to restrict the sale of its seed, which makes the genetic resources of the ex situ collections of producing countries, the main sources of genetic variability for both improvement and conservation.

2.1.7 Eucalyptus urophylla

This is one of the exceptions of the genus *Eucalyptus* that does not originate in Australia. Its area of occurrence is in Timor, Flores and other islands of the Indonesian archipelago (Figure 2). It grows between 400 and 3000 m altitude, with annual rainfall between 1000 and 1500 mm, mainly concentrated in the summer. The dry season is approximately 29 ° C and the minimum temperatures of the year fluctuate from 8 to 12 ° C. Frost can occur in exceptional cases and especially in zones of higher altitude. In Brazil, it is a transcendental specie and is often harnessed through the production of hybrids with *E. grandis* (ELDRIDGE et al., 1993; GOMINHO et al., 2007).

Figure 2. Centre of origin of some of the main species of Eucalyptus in the world.



Source: based on ELDRIDGE et al., 1993.

2.2 Hybridization and cloning

Hybridization is the cross between different species (ZOBEL; TALBERT, 1984). This can occur naturally in species such as in the case of *E. Benthamii* and *E. vilminalis* (BUTCHER et al., 2005). But in the context of tree genetic improvement, the possibility of assembling into hybrid individuals, specific and valuable characteristics of each species in new and unique combinations that can be maintained by cloning, results in a highly attractive option. This possibility, in important differentiated species as well as the heterosis manifestation observed in the hybrids of several species, has led the Eucalyptus breeders to seek in hybridization a faster mean of capitalizing genetic gains to desirable characters. The viability of heterosis commercial use, verified in several hybrids, as well as the perpetuation and multiplication of superior hybrid combinations through clonal

propagation, allowed the adoption of hybridization as an important tool in the production of higher quality forests (ASSIS, 1975).

In the forest area, for many years, hybridization was a method used only in species whose large-scale vegetative propagation was possible, or that mass production of F1 hybrids was easy, since the repetition of the crosses to obtain the hybrids proved productive are more difficult, especially in hermaphrodite individuals such as eucalyptus (Assis, 1975). With mastery of the techniques of asexual multiplication in Eucalyptus, hybridization has become a useful tool to increase Eucalyptus forest productivity and to improve wood properties, with a great impact on the efficiency of its use in the various processes (Eucalyptus), (ASSIS, 1975; FONSECA et al., 2010).

2.3 Interspecific crosses

Despite the large number of hybrid combinations among Eucalyptus species already known, there are still many that have not yet been tested and which may present productive combinations. Pairs that produce heterotic hybrids and that can be perpetuated and multiplied by vegetative propagation, should be looked for in the collection of introduced species. The heterosis observed at the intersection between several species of Eucalyptus has been of great use in the genetic improvement of desirable traits, especially when its commercial use is viable by large scale cloning. In this way, new combinations can be selected and incorporated into recurrent selection programs (ASSIS, 1975).

The association of traits of interest that are differentiated between pairs of compatible species through the production of hybrids, offers a far-reaching alternative in obtaining superior individuals in a short period. Obtaining such individuals by conventional tree genetic improvement is viable, but several generations of recurrent selection are required for this to occur. Since hybrid propagation techniques on a commercial scale are of full scope, the production of hybrids among species possessing attributes of interest, assumes significant importance within genetic improvement programs (FONSECA et al., 2010).

Differently from the prospective crosses, where it is sought to obtain new combinations between aptitudes or potentially adaptabilities species, in the objective crossings it seeks to transfer important traits, perfectly identified in some species, to

another that is already adapted but that is deficient in relation to this trait. A typical example of this cross is the profit of the growth traits of *E. grandis*, but which has problems of sprouting, density, susceptibility to canker and drought, which receives from *E. urophylla* good budding ability, resistance to canker, resistance to drought and higher density of wood. In this sense, hybridizations with other species such as *E. globulus* and *E. dunnii* can also be tested, for example, to increase wood density and cellulose productivity, reduce lignin and extractive contents, and improve performance of the forest in a broader sense.

In the case of Uruguay, *E. globulus* is the main commercially reforested species with high-priced wood in the fibre market. However, the specie does not adapt to many regions of the world (ELDRIDGE et al., 1993). In Uruguay, its planted area is restricted in the southern region of the country (Figure 1). Planting outside the latitude and appropriate locations causes species development to fail due to cold winters, drought and diseases. *E. globulus* exhibit a excellent quality for pulp and cellulose production, restricted by limited planting potential (GRIFFIN et al., 2000). In contrast, *E. grandis* has a greater capacity for adaptation, rooting, cloning, ease of care in the nursery besides an excellent growth speed and shape of the stem, but wood quality inferior to *E. globulus* when it comes to pulp of cellulose (Table 1). For these reasons, to evaluate interspecific hybrids of *E. globulus* x *E. grandis,* is important as an alternative to increase the productive potential of Uruguay.

Table 1 - Comparative table of the biological characteristics of *E. globulus* and *E. grandis*

Traits	*E.globulus*	*E. grandis*
Potential for seed production	HIGH	HIGH
Early flowering	MID	HIGH
Flowering fecundity	HIGH	HIGH
Number of seeds per 100 pollinated flowers	1507	1717
Rooting	MID	HIGH
Cold tolerance	LOW	LOW
Growth	MID	HIGH
Density	HIGH	LOW
Pulp yield	HIGH	LOW

Source: GRIFFIN et al., 2000

3 OBJETIVES

The main objective of this thesis was integrates molecular approaches for evaluate hybrids of a cross *E. grandis x E. globulus,* in a monoprogeny.

3.1 The specific objectives were:

(i) To estimate the genetic parameters of eucalyptus hybrid clones (*E. grandis x E. globulus*) based on productivity, stability and adaptability, to select the best clones based in technical of traditional breeding and supported by SNPs genotyping

(ii) To study the predictive response of the Genomic wide prediction GWP model rrBLUP, with Jackknife validation in the population of hybrids and comparing with model 52 of Selegen.

(iii) Use the approach GWSA to search QTLs associated to DBH.

Chapter 1: Quantitave Genetics

Estimation of genetic parameters of eucalyptus hybrid clones (*E. grandis x E. globulus*) based on productivity, stability and adaptability, to select the best clones based in traditional breeding and selection supported by SNPs genotyping.

CHAPTER 1: QUANTITAVE GENETICS

4 INTRODUCTION

The phenotypic expression of the quantitative traits is a result of the joint effect between the genotype (G), environment (E) and the interaction between these factors, GxE (MAIA et al., 2009). By environment is meant all intra and extracellular factors that influence the expression of the genotype. Environmental conditions that influence the expression of genotypes can be classified as predictable and unpredictable. In the first one, it refers to those environmental variations that occur from region to region, such as soil, climate, altitude, etc. traits. The unpredictable variations are those climatic conditions, in the same region as for example, the quantity and rainfall distribution, temperature fluctuations, among others that are not possible to be predicted with certainty (VENCOVSKY; BARRIGA, 1992).

One of the main problems in the selection and recommendation of genotypes is the interaction genotype x environment (GxE). There are some traits that do not undergo major changes with environmental variations, but quantitative traits such as wood volume, which can undergo significant phenotypic changes with small changes in the environment, affect the final profitability of plantations (REHFELDT et al., 1999). An alternative to minimize the effects of the interaction is the simultaneous selection of genotypes of high productivity, stability and adaptability (PUPIN, 2014). Although the interaction GxE should be considered as a biological phenomenon that is part of the evolution of the species, this has its implications in genetic improvement (PUPIN et al., 2014). Its effect allows the appearance of stable genotypes adapted to a specific environment, as well as genotypes of general behaviour, which are adapted to several environments (BOURDON, 1977). The interaction GxE can be of the simple type, when it is provided by the difference of variability between genotypes in the environments, and of the complex type, when it denotes lack of correlation between measurements of the same genotype in different environments, indicating variations in the superiorities of the genotypes with the environmental changes conditions (BASFORD et al., 1991). It is important to know this phenomenon to be able to differentiate between genotypes with broad or specific adaptation, to choose selection places and to determine the ideal number of environments and genotypes to be evaluated in each selection phase (PUPIN et al., 2015). Normally, the recommendation of superior genotypes is performed considering

two strategies: the first is the identification of the genotypes of wide adaptability, aiming at the recommendation to a set of heterogeneous environments; and the second is the recommendation of individuals adapted to specific environments, seeking to capitalize the effect of this interaction (ROCHA et al., 2005).

Simultaneous selection for productivity, stability and adaptability can be performed by the Harmonic Mean of Relative Performance of Genetic Values (HMRPGV) prediction method, which presents the following advantages when compared to other methods: a) considers the genotypic effects as random and thus provides genotypic stability and adaptability but not phenotypic; B) it allows to deal with imbalances of the field tests, like tests of progenies; C) allows to deal with heterogeneity of variances; D) allows to consider correlated errors within locations; E) provides already discounted (penalized) genetic values of instability; F) can be applied with any number of environments; G) does not depend on the estimation of other parameters, such as regression coefficients; H) generates results on the own greatness or scale of the evaluated character; I) allows to compute the genetic gain with the selection by the attributes growth, adaptability and stability, simultaneously (RESENDE, 2004).

The molecular markers technology are routinely used to characterize and organize levels of neutral genetic variability in breeding populations of breeding programs. These databases are used for various objectives within breeding programs. The knowledge of the genetic diversity that emerges from them allows to estimate the level of kinship between individuals as well as to characterize the identity of the same in clonal traps avoiding traceability errors guaranteed the clonal identity (GRATTAPAGLIA, KIRST 2008, TORRES-DINI et al. 2011).

Currently the SSR markers and more recently the SNPs have gained space over the others, because they present the following advantages and characteristics: i) they have a high degree of polymorphism; ii) segregate as Mendelian form and are codominant; iii) the presence of a single SSR allows the reading of the bands in a clearer and easier way of interpretation; (GOLSTEIN; POLLOCK 1994; VENDRAMIN et al., 1996) The aim of present study was select the top clones based on DBH and interaction GxE in two places. The molecular tools allow to complement the approaches of the traditional improvement optimizing the decision making allowing a more efficient management of the germplasm by the breeders.

5 MATERIALS AND METHODS

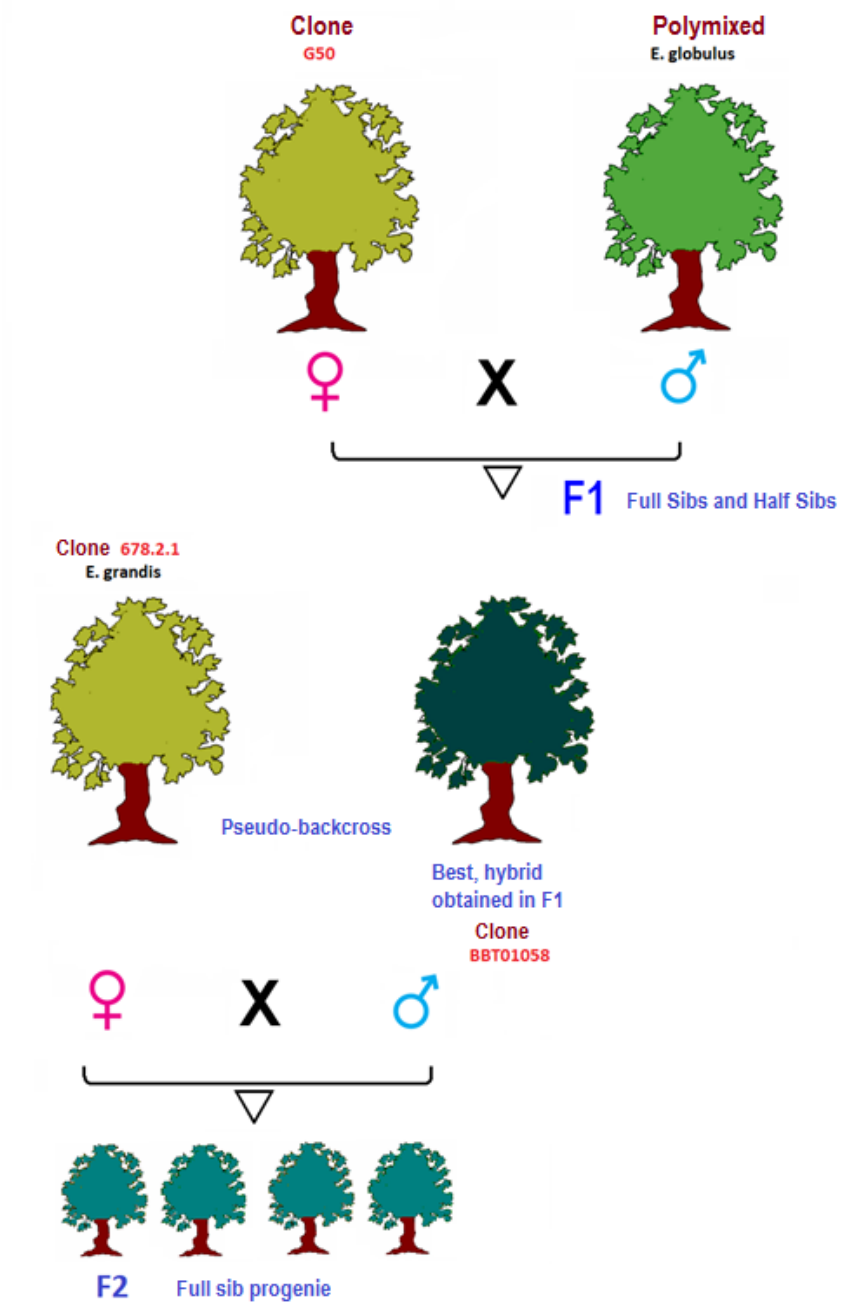
5.1 Clones production

The crossbreeding strategy consisted of a first cross product of the controlled pollination between *E. grandis* G50 (CSIR, South Africa), used as the mother tree and the pollen combination of ten individuals belonging to *E. globulus* species (Figure 3). The best individual at this junction (F1), called clone BBT01058 (Forestal Oriental SA, Uruguay) was backcrossed with clone 678.2.1 (Forestal Oriental SA, Uruguay) of the *E. grandis* species, thus producing an F2 progeny of complete siblings, known as 2162 (MYBURG et al., 2003). Many seeds (approximately 6637) were obtained in the F2 progeny, but only 292 seeds (4.4%) germinated and resulted in seedlings. These seedlings were then subjected to selection in the nursery stage, based on the discarding of weak and abnormal phenotypes, resulting in only 99 seedlings that were cloned by micro-cuttings (GRIFFIN et al., 2000) and used to establish the clonal test. The clones were produced and maintained by UPM Forestal Oriental Uruguay.

5.2 Clonal test installation

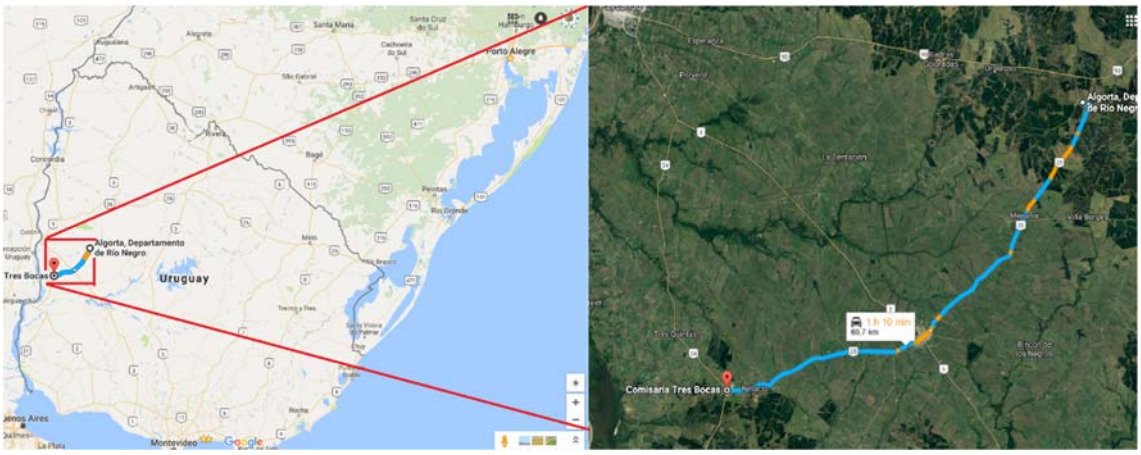
Eucalyptus hybrid clones belonging to F2 were used for the establishment of a clonal test repeated in two locations with contrasting soils of the company UPM Forestal Oriental; One was established in the municipality of Tres Bocas and the other in Algorta "La Unión", both localities in the state of Rio Negro, Uruguay (Figure 4). The soils of Tres Bocas are poorer in drainage, with a clayey Bt horizon that is often superficial, because of the almost total loss of horizon A, the result of decades of poor agricultural management. This causes that in Tres Bocas, the productivities are substantially lower than those of the area of Algorta. The clonal test was planted in January 2000, in the alpha latice delimitation with five replications and one plant per plot at a spacing of 3 x 2 m. Clone 3523 of *E. grandis* was used as a control genotype. The clones were evaluated at 48 months of age for diameter at breast height (DAP,cm)

Figure 3. Representation of the crossover scheme used to produce interspecific clones. The first interspecific cross was produced by controlled pollination of one *E. grandis* mother with a polymix of ten E. globulus individuals. The best individual from F1 was selected as a mother and backcrossed with the *E. grandis* 678.2.1 clone.



Source: based on MYBURG et al., 2003

Figure 4. Geographic location of the test. It presents the localities of Algorta and Tres Bocas



Source: original production

5.3 SNP Genotyping

Leaf tissue was collected and 500 ng of DNA from each sample was purified by standard CTAB DNA extraction methods. The DNA samples were genotyped with the Eucalyptus EuCHIP60K chip, based on the Illumina Infinium technology that counts a total of 64,639 markers (SILVA-JUNIOR et al., 2015). The Geneseek genotyping service (Neogene company, Lincoln, NE, USA) was used. To select SNPs, a 95% call rate was used, a 5% Minor Allele Frequency (MAF). In addition, the Hardy-Weinberg equilibrium test was performed and loci.

The SNPs markers were used to construct a plot of allele effect, a dendrogram based on heterogeneity and one based on genetic similarity among genotypes, which was associated with the top 10 clones. In addition, a heatmap was constructed to schematically represent the parentage coefficient.

5.4 Determination of genetic parameters

Estimates of the genetic parameters were obtained based on the REML / BLUP procedure, using the Selegen statistical model 52 (Resende, 2007b), given by:

Equation 1 $y = Xr + Zg + Wb + Ti + e,$

In which *y* is the data vector, r is the vector of the effects of repetition (assumed to be fixed) plus the general mean, *g* is the vector of genotypic effects (assumed to be random), *b* is the effect vector of the blocks (assumed to be random), *i* is the vector of effects of genotype interaction by environment (random), and *e* is the vector of errors or residuals (random). The capital letters X, Z, W and T represent the incidence matrixes for the mentioned effects. From this analysis, the following components of variance were estimated: genetic variance between clones (σ_c^2), environmental variance between plots (σ_p^2), residual variance (σ_e^2), individual phenotypic variance ($\sigma_f^2 = \sigma_c^2 + \sigma_p^2 + \sigma_e^2$). The following parameters were estimated from the variance components:

a) Individual heritability in the narrow sense: $H_i^2 = \sigma_a^2 / \sigma_f^2$;

b) Heritability of the average between clones, assuming complete survival for:

$$H_c^2 = \frac{\sigma_c^2}{\sigma_c^2 + \frac{\sigma_e^2}{r}}$$

In which, r is the number of repetitions;

c) Accuracy of clone selection, assuming complete survival: $r_c = \sqrt{H_c^2}$;

d) Coefficient of individual additive genetic variation: $CV_{gi} = 100\sqrt{\sigma_a^2 / m}$; in which m is the average of DAP;

e) Coefficient of experimental variation: $CV_e = 100\sqrt{\sigma_e^2 / m}$

f) Coefficient of determination of plot effects: $C_p^2 = \sigma_c^2 / \sigma_f^2$

g) Coeficiente de variação relativa: $CV_r = CV_{gi} / CV_e$

5.5 Gains in selection by multi-effects index method

Selection gain was estimated for a high selection intensity (10%), with only ten of the 99 clones tested in both sites that presented the highest growth in DAP using the multi-effects index (RESENDE, 2002a). The selection was made based on the average performance of the clones in the two experimental sites, as well as within each site.

6 RESULTS AND DISCUSSIONS

6.1 Growth of clones

The average growth in DAP of the clones was similar between Tres Bocas (16.91 cm) and Algorta (16.64 cm). No significant differences were detected between the clones for the deviance chi-square test set for the two study sites, confirming that the growth in DAP was similar between these forest areas (Table 2). These results indicate a simple correlation GxE.

6.2 Environment control and genotype environment interaction

The coefficient of determination of the effects of blocks (C_b^2 = 0.043) and determination of the effects of genotype and environment interaction (C_{ga}^2 = 0.151) were, according to Resende et al. (1995), indicating environmental homogeneity within blocks and the good environmental control of the experimental design alpha latice in the tests. In addition, a low GxE interaction was detected, with the genotype correlation in the performance of the clones between the two environments (r_{gloc}) high (0.708), which shows that the GxE is a simple type interaction (VENCOVSKY; BARRIGA, 1992) The classification of the clones per their growth performance in the two environments presents around 70% of similarity. In other words, the growth in DAP of the clones with higher and lower performance was generally similar between the two environments. This indicates that there is the possibility that some clones may be selected simultaneously for both environments, even these sites presenting soils with different physical and fertility characteristics (Table 2).

6.3 Heritability

The heritability coefficient plays a key role in the selection process because it indicates how much of the phenotypic variation is of genetic origin, so it is inheritable and can be useful in the selection process. The estimated individual heritability (H_i^2) for the two environments was 0.367 (Table 2), indicating a significant degree of genetic control for DBH. Thus, inheritable genetic variability among clones can be explored in selection. On the other hand, it must be considered that these clones are complete siblings. The consideration of these genetic parameters is important to ensure the correct targeting of genetic improvement programs, facilitating decision making in the selection of genotypes, thus avoiding crosses that generate endogamy.

The average heritability between clones (H_i^2 = 0.724) in the two localities evaluated (Table 2). This indicates a high degree of genetic control for DBH at the clone medium level and a high probability of obtaining genetic gains from clone selection. The selective accuracy for mean clones (r_c) was thus equally high (0.851), according to Resende et al. (1995), which defines values between the range of 0.1 to 0.4 as low, from 0.4 to 0.7 as medium and greater than 0.7 as high. Therefore, the greatest the accuracy, the greatest is the precision of the selection and, consequently, the greater the genetic gains. In the present study, high accuracy indicates a strong association between true genetic value and phenotype, which is highly favourable to selection (Table 2).

6.4 Variation coefficients

The higher the value of the coefficient of individual genetic variation (CV_{gi}), the greater the chances of obtaining genetic gains in selection (RESENDE, 2002). The coefficient of individual genetic variation (CV_{gi}= 10.9%) was like the coefficient of experimental variation (CVe= 11.9%), which resulted in a high coefficient of relative variation (CV_r = 0.916). Values CV_r close to unity (1.0) indicate a situation highly favourable to selection (VENCOVSKY; BARRIGA, 1992). Therefore, the value of CV_r in the present study indicates the possibility of obtaining gains by the selection of clones with higher growth for DBH (Table 2).

Table 2 - Estimates of genetic parameters for DBH in both environments.

Parameter	Estimates
Coefficient of determination of block effects: C_b^2	0,043
Coefficient of determination of the effects of the GxE: C_{ga}^2	0,151
Genotype correlation between performances in various environments: r_{gloc}	0,708
Individual plot inheritance: H_i^2	0,367 ± 0,083
Adjusted heritability of the genotype mean: H_c^2	0,724
Accuracy of genotype selection: r_c	0,851
Coefficient of genotypic variation: CV_{gi} (%)	10,9
Residual variation coefficient: CV_e (%)	11,9
Relative variation coefficient: CV_r	0,916
Mean at Tres bocas (cm)	16,91
Mean at Algorta (cm)	16,64
General mean (cm)	16,76

Source: original production

6.5 SNPs markers

Of the 99 hybrid clones, 78 survived and were used for DNA extraction and subsequent analysis of Genomic Wide Prediction. After quality control with a MAF, call rate and Hardy-Weinberg equilibrium test, the number of SNPs was reduced from 64639 to 15196 (23.5%). These results, when compared to those obtained by Silva-Junior et al. (2015), who evaluated the EUChip60K chip in 12 *Eucalyptus* spp included *E. grandis* and *E. globulus* obtained 30,040 SNPs for *E. grandis* and *E. globulus* 19,299, In both cases SNPs "Specie-specific cluster". The results of the present study resemble those obtained by Silva-Junior et al. (2015) for *E. globulus* since it is hybrids it is reasonable to have a reduction in the number of marks when crossing with *E. globulus*. The lowest number of loci selected here is related to the study by Silva-Junior et al. (2015) is also because these authors employed a call rate greater than 98% and an MAF> 0.01,

associated with the fact that in the present study only Hardy-Weinberg equilibrium loci were selected.

The use of genotyping by SNPs was employed in this case study to certify the parentage. This was confirmed by the analysis of the heatmap of figure 5, where a traceability error can be observed in the identification clones 1751 and 1758 are the same genotype. In the graph of figure 6 we can observe the similarity dendogram constructed with genotyping information with SNPs, clones 1778, 1770, 1744 and 1756 are grouped close, by being part of the same node, supporting the fact that the best observed phenotypes share a similar genotyping. On the other hand, clone 1796 is completely outside the main group. This information is useful for the correct targeting of crosses in breeding programs, helping to develop strategies that avoid inbreeding, keeping the effective sizes of the populations high, also helping to avoid errors in the traceability, optimizing germplasm management by breeders (GRATTAPAGLIA; KIRST, 2008; FONSECA et al., 2010) The reduction in the price of genotyping with SNPs added to the codominant trait, makes them gradually replacing the use of microsatellite markers, considering the dollar invested ratio as a function of the number of brands generated, these results are largely favourable to the use of SNPs. When genomic studies are concerned, this is due to their wide genomic coverage, while still the SSR have a well consolidated position in the area of population genetics since they have a higher number of alleles per locus than the SNP. The strategies of tradicional breeding associated with genotyping increase the level of knowledge of the improvement population, making possible a better selection of clones as well as to prevent common errors of the traditional genetic improvement increasing the efficiency of the decisions on the part of the breeders.

The gradual construction of databases based on the use of SNPs or SSR markers by breeding programs allows access to a new era in germplasm management. This allows access to methodologies such as Genomic Wide Prediction (GWP), Marked Assisted Selection (MAS). The enrichment of these databases with phenotypic and genotypic information allows for more efficient choices and better maintenance of the improvement programs. Allowing to reduce the times of the improvement increasing the efficiency of the same ones.

Table 3 - Estimates of genetic gain predicted for DBH simultaneously for the two environments. The control genotype is the clone E. grandis number 3523.

Ranking	Genotype	G	u + g	u+g+ge	Gain (%)	New mean (cm)
1	1744	3,1083	19,8657	20,5072	3,1	19,87
2	1769	2,5302	19,2877	19,8098	2,8	19,58
3	1670	2,4407	19,1981	19,7018	2,7	19,45
4	1796	2,3931	19,1505	19,6444	2,6	19,37
5	1778	2,2618	19,0192	19,4859	2,5	19,30
6	1756	2,2562	19,0136	19,4792	2,5	19,26
7	3523	2,216	18,9734	19,4307	2,5	19,21
8	1783	1,8732	18,6307	19,0172	2,4	19,14
9	1716	1,7733	18,5308	18,8967	2,3	19,07

Note:g is the genotype effect: u+g is the predicted genotypic value; u+g+ge is the mean genotypic value in the environments.
Source: original production

Table 4 - Estimates of predicted genetic gain for DBH in the Tres bocas and Algorta environments. The control genotype and clone *E. grandis* number 3523

Ranking	Genotype	g+ge	u+g+ge	Gain (%)	New mean (cm)
Três bocas (A)					
1	1744	3,3805	20,2890	3,4	20,29
2	1670	3,2752	20,1837	3,3	20,24
3	1778	3,0919	20,0004	3,2	20,16
4	**3523**	3,0261	19,9346	3,2	20,10
5	1769	2,7789	19,6874	3,1	20,02
6	1756	2,7411	19,6496	3,0	19,96
7	1796	2,3509	19,2594	2,9	19,86
8	1684	2,2792	19,1877	2,9	19,77
9	1783	2,2720	19,1805	2,8	19,71
10	1671	2,2080	19,1166	2,7	19,65
Algorta (B)					
1	1744	4,1189	20,7555	4,1	20,75
2	1796	3,4229	20,0595	3,8	20,41
3	1769	3,3258	19,9623	3,6	20,26
4	1756	2,7024	19,3390	3,4	20,03
5	1670	2,6135	19,2501	3,3	19,87
6	1787	2,5012	19,1378	3,1	19,75
7	1716	2,4149	19,0514	3,0	19,65
8	1778	2,3650	19,0016	2,9	19,57
9	1770	2,3622	18,9988	2,9	19,51
10	**3523**	2,3204	18,9570	2,8	19,45

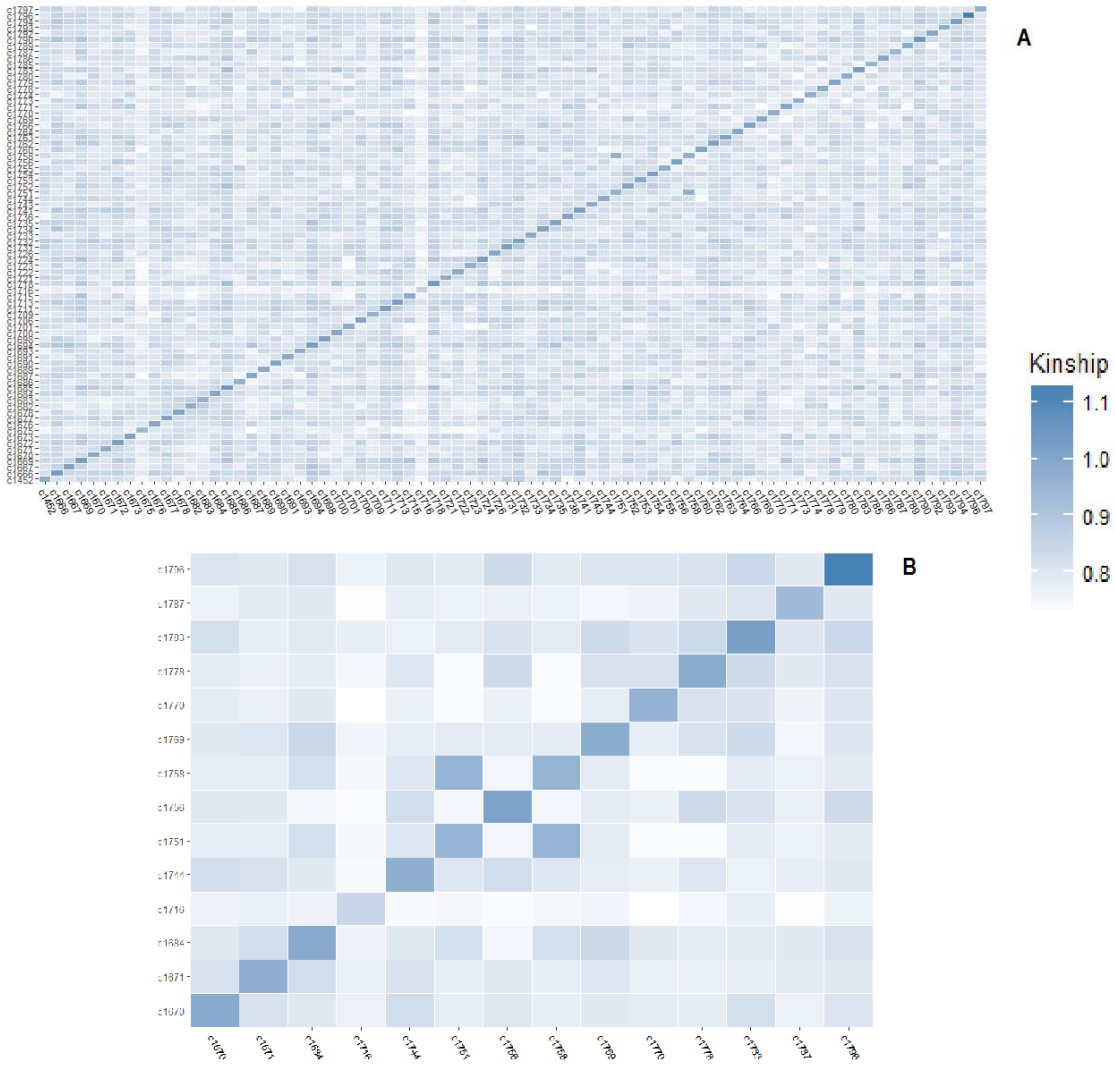
Note: g is the genotype effect; u+g is the predicted genotype value; u+g+ge is the mean genotypic value in the environments.
Source: original production

Table 5 - Stability and adaptability (HMRPGV and HMRPGVxMG) of the clone's genetic values for DBH.

Ranking	Genotype	HMRPGV	HMRPGV*MG
1	1744	1,22	20,46
2	1769	1,18	19,80
3	1670	1,17	19,69
4	1796	1,17	19,63
5	1756	1,16	19,48
6	1778	1,16	19,47
7	**3523**	1,16	19,42
8	1783	1,13	19,01
9	1716	1,13	18,89
10	1770	1,13	18,88

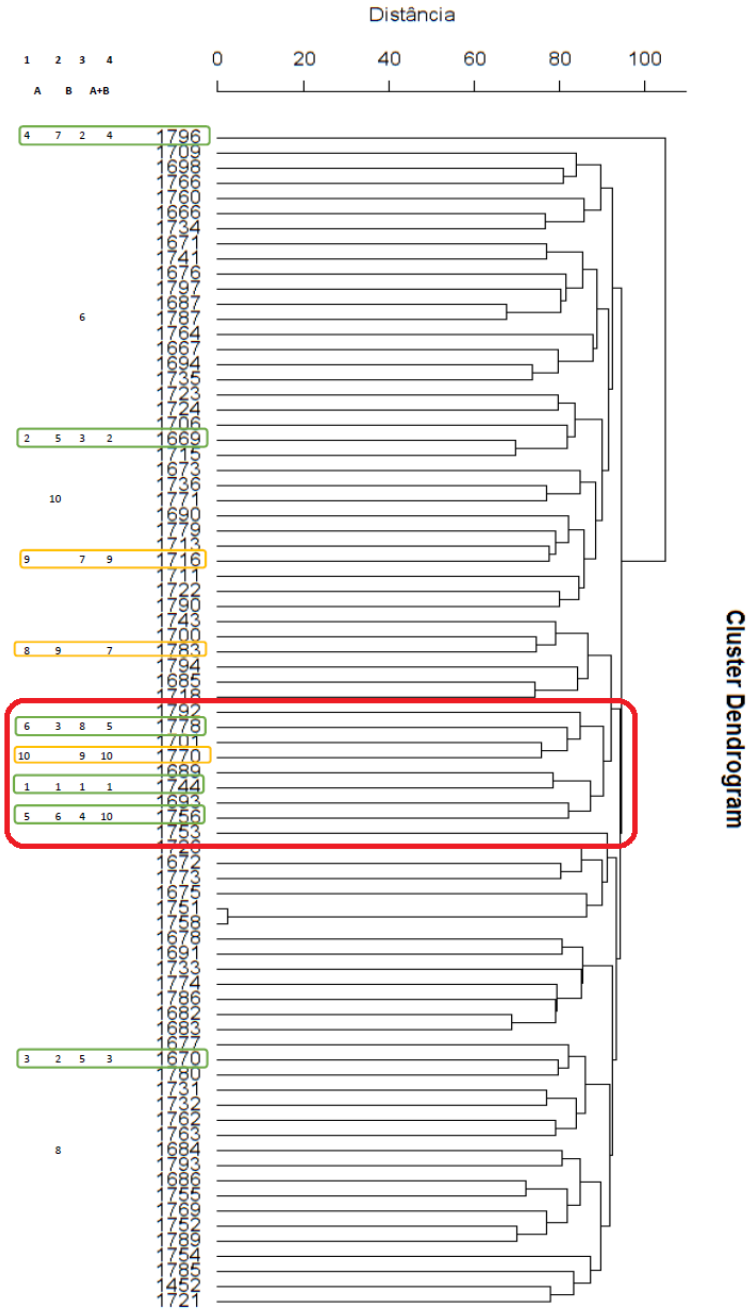
Source: original production

Figure 5. HeatMap, schematic representation of kinship. The genotyping of all clones were compared for the 15,196 SNPs based on one-to-one genotyping, as can be seen on the x- and y-axes. The kinship coefficient was represented by colors indicating the degree of parentage in the progeny nearest to 1 is identified as the same clone. In this case, since these are full siblings and the degree of kinship is high, the scale is between 0.8 and 1. (A) represents the entire population studied, (B) individuals selected and those who merit more discussion.The more blue the parentage coefficient is close to 1. It can be appreciated that the clones named as 1751 and 1758 are the same clone, this is evidencing an error of traceability.



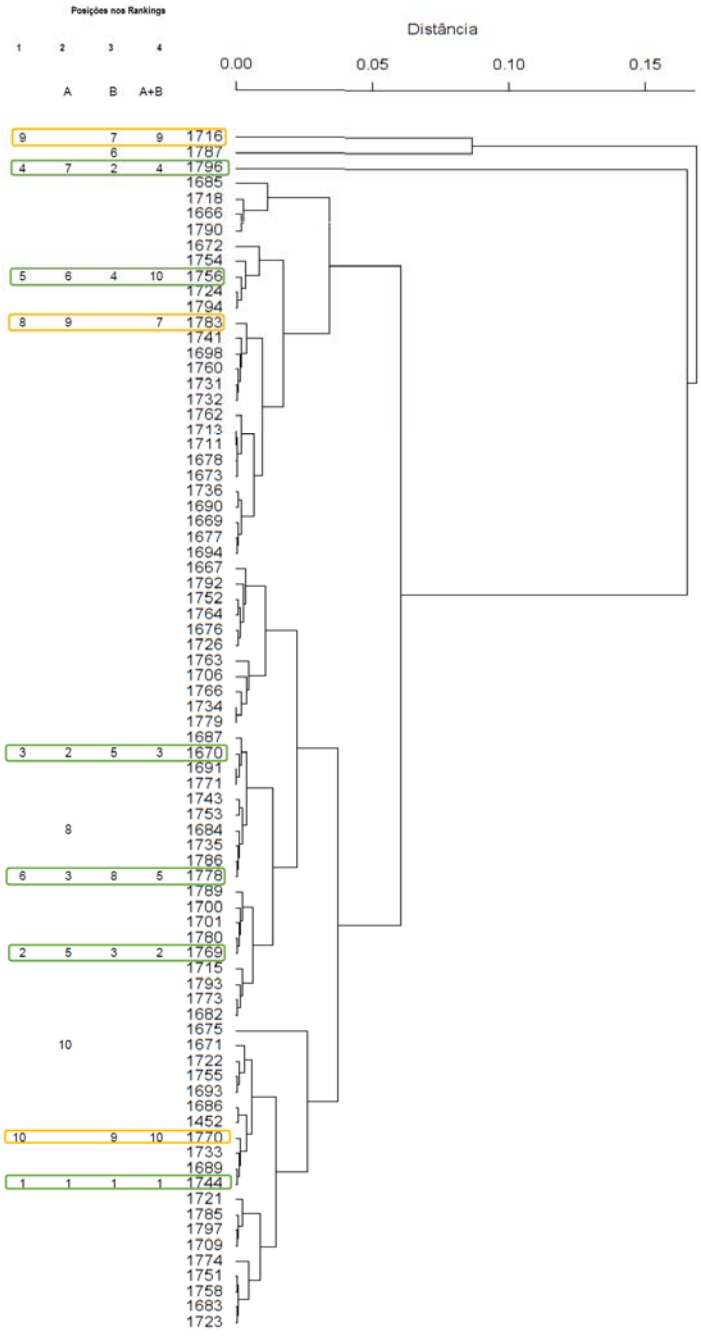
Source: original production

Figure 6. Similarity Dendogram clustering. Constructed with genotyping data with SNPs. The same is associated with the first 10 positions in the rankings obtained with the selegen model 52 for the environments A (Tres Bocas), B (Algorta) and the two environments together A + B (1) Stability and Adaptability of Genetic Values (HMRPGV), (2, 3 and 4) represent the citations in the ranking for the predicted genetic gain of the DAP trait. It is also observed that clones 1778, 1770, 1744 and 1756 group closely by being part of the same node. On the other hand, clone 1796 is completely outside the main group.



Source: original production

Figure 7. Dendrogram. based on heterozygosity, together with the first ten positions in the classifications obtained for environments A, B and the two environments together, A+B (1): Stability and adaptability of genetic values (HMRPGV); 2, 3 and 4 represent the predicted DBH genetic gain positions, as presented in Tables 5 and 6. the information of genotyping clones could be used in controlled crosses with other elite clones from unrelated populations. In such crosses the comparison of the genotypes based on SNP Genotyping will allow to monitor the levels of homozygotes while maintaining high the levels of heterozygosity.



Source: Original Production

6.6 Clones selection

The selection of the best clones was performed for a high selection intensity (10%), that is, with the selection of only ten of the 99 clones tested in both sites for three different selection strategies (Tables 2, 3, 4 and 5; Figure 5 and 6): i) selection of the ten top clones based on the average performance in the two environments; ii) selection of the ten best clones within each site; iii) selection of the ten best clones based on heterozygosity, associated with those clones that were in the top ten positions for A (Tres Bocas), B (Algorta) and A + B environments. In the selection of the top ten clones based on the mean performance in both environments (Table 5), six clones were ranked above the control clone 3523. Clone 1744 was ranked in the first position for all three selection strategies (Tables 2, 3 and 4) for the Algorta and Tres Bocas experiments. The genetic gain of clone 1744 was 3.1% for the two sites together, 3.4% in Tres Bocas, and 4.1% in Algorta, against values of 2.5, 3.0 and 2.8% for these estimates, respectively.

In the locality of Tres Bocas, three clones showed gains superior to those of the genotype control, whereas in Algorta, nine clones surpassed the control. In Figure 7 we can observe the clustering based on heterozygosity, associated with those clones that were in the first ten positions for environments A, B, A + B and the values of adaptability. The clones that were always in the top ten positions are presented in green, and in yellow the clones that were among the top ten in two of the three selection strategies. It is evident that an ordering of the selected materials is maintained for stability, adaptability and production. This is also clearly shown in Table 2, which indicates a positive and high genotype correlation in the performance of clones in both environments (= 0.708). According to these results, those clones that show productivity and wide adaptation can be selected (marked in green and yellow in Figure 5), as well as some clones that represent good performance in specific environments such as clone 1787, sixth classification in environment A (Tres Bocas), and clones 1684 and 1671 in classification 8 and 10 for environment B (Algorta), although the last two were classified under the control clone. The analysis of the interaction GxE had already indicated that it would be possible to select some same clones for the two environments. However, studies of the GxE interaction must be completed by the estimation of adaptability and phenotypic stability, meaning the level of response to environmental stimulus and maintenance of productivity against environmental variation (MAIA et al., 2009; ROSADO et al., 2012). Clones that appear in all environments as the most productive, do not suffer strong influence of the

environment, meaning that they present a small variation in the interaction GxE (ROSADO et al., 2012).

Finally, it is important to emphasize that the selected clones, because they are clones originated from the artificial propagation of full sibs, should be preferentially used for reforestation based on their cloning, since the crossing of these clones is expected to generate inbreeding by relatives. As the endogamy originated between the crosses between relatives and equal to the coancestry coefficient among the parents, which in the present case of complete siblings and 0.25, one can expect at least 25% inbreeding in the descent of the cross between those clones suggested for the selection. Inbreeding in Eucalyptus spp produces depression by inbreeding, resulting in mortality, plant breeding, infertility, etc., as has been documented in the literature (ELDRIDGE, GRIFFIN, 1983; GRIFFIN, COTTERILL, 1988; HARDNER, POTTS, 1995). In addition, the information of genotyping clones could be used in controlled crosses with other elite clones from unrelated populations. In such crosses the comparison of the genotypes based on SNP genotyping will allow to monitor the levels of homozygotes while maintaining high the levels of heterozygosity (Figure 6) avoiding the inbreeding of the populations produced.

CHAPTER 2 - GENOMIC WIDE PREDICTION (GWP) AND GENOMIC WIDE ASSOCIATION (GWAS)

STUDY OF THE PREDICTIVE RESPONSE OF THE GENOMIC WIDE PREDICTION (GWP) AND GENOMIC WIDE ASSOCIATION (GWAS).

CHAPTER 2 - GENOMIC WIDE PREDICTION (GWP) AND GENOMIC WIDE ASSOCIATION (GWA)

7 INTRODUCTION

7.1 Genomic Wide Prediction (GWP)

GWP is a method that integrates quantitative genetics with new genome genotyping technologies. The use of high-density markers is one of the advances that makes this approach possible (MEUWISSEN et al., 2001). Therefore, each QTL is likely to be in linkage imbalance for at least one marker in the entire population. GWP eliminates the need to look for QTLs that are individually associated with markers. Instead, GWP simultaneously counts many predictor markers and is characterized by restricting random estimates to zero (DESTA; ORTIZ, 2014). In addition, GWP can accelerate breeding cycles in such a way that the rate of genetic gain per unit of time can be increased and the cost of selecting superior genotypes reduced (HEFFNER et al., 2010). For this, GWP focuses on the simultaneous prediction of the genetic effects of thousands of dispersed markers in the genome, making it possible to obtain the effects of most of the loci from large to small effects, thus explaining almost the entire quantitative trait (MEUWISSEN et al., 2001).

To implement GWP, three populations can be defined: i) training population (EP); ii) validation population (VP) and; iii) selection population (SP). Based on the three population strategies may be employed, depending on the nature of the breeding program: (i) three physically different populations; (ii) a single population used for estimation and validation; and (iii) only one population used for training, validation and selection (MORAIS JÚNIOR, 2013). In the estimation of the GWP model, the EP population is phenotyped under normal conditions of cultivation of the species, that is, at the field level and is genotyped to estimate the effects of the markers. The estimates associated with each marker or allele are used to calculate the estimated genomic genetic value (GEBV) of each candidate for selection. The estimate of the GEBV corresponds to the sum of all the effects of the markers included in the model for a given genotype. This way the selection of genotypes candidates for the selection carried out using GEBV as one of the criteria. Thus, the GWP selection works by capturing the additive genetic variance, because of the genes effects / QTLs that affect the quantitative trait in the analysis (MORAIS JÚNIOR, 2013; HEFFNER et al., 2011). As for predictive models, several

types of models respond differently because they vary in their assumptions when it comes to complex trait variance. The standard linear model can be formulated as follows

Equation 2

$$y = \mu + \sum_k X_k \beta_k + e,$$

(i)

In which y is the vector of the phenotypic trait, μ is the global phenotypic mean, k represents the locus, Xk is the allelic state of locus k, is the locus marker effect k, and $(e \sim N(0, \sigma e2))$, Where e is the residual random effects vector and $\sigma e2$ is the residue of the variance, where Xk and the allelic status of individuals, coded as a matrix of 1, 0, or -1 for the value of diploid genotypes of AA, AB or BB, respectively (DESTA, ORTIZ, 2014). The advances generated in GWP provide an opportunity to confront alternative models, such as regression of the complete genome (Table 6). Parametric and non-parametric models can group entire genome regression methods (DESTA; ORTIZ, 2014).

Table 6 - Main features of genome-wide prediction models (source, DESTA; ORTIZ, 2014).

Model	Features
RR-BLUP	Assumes that all markers have equal variances with small but non-zero effect. Applies homogeneous shrinkage of predictors towards zero, but allows for markers to have uneven effects. Computed from a realized-relation matrix based on markers. Some QTL are in LD to marker loci, whereas others are not.
LASSO	Combines both shrinkage and variable selection methods. RR-BLUP does not use variable selection, but outsmarts LASSO when there is multicolinearity between the predictors.
EN	Double regularization using ‘1 and ‘2 penalty norms combines the merited features of these norms to confront the challenge of high-dimensional data
BRR	Induces homogeneous shrinkage of all marker effects towards zero and yields a Gaussian distribution of marker effects. Like RR-BLUP, there is a problem of QTL linkages to the marker loci
BL	- Aplica ambos métodos, encolhimento e seleção variável. - Tem um exponencial prévia as variâncias dos marcadores resultando numa exponencial de dupla distribuição DE. - A distribuição DE tem uma massa de alta densidade em zero e mais pesada prévio as caudas comparado com distribuição Gaussiana.
Bayes A	Utilizes an inverse chi-square (x2) on marker variances yielding a scaled t-distribution for marker effects. Similar to BL and in contrast to BRR, it shrinks tiny marker effects towards zero and larger values survive. Has a higher peak of mass density zero compared with the DE distribution.
Bayes B	Similar to Bayes A, uses an inverse x2 resulting in a scaled t-distribution. Unlike Bayes A, utilizes both shrinkage and variable selection methods. When p = 0, then it is similar to Bayes A
Bayes C	Applies both shrinkage and variable selection methods. Characterized by a Gaussian distribution. Bayes B and Bayes C consist of point of mass at zero in their slab priors
Bayes C ****	A modified variant of Bayes B. Used to alleviate the shortcomings of Bayes A and Bayes B Unlike Bayes B, p is not fixed, but estimated from the data
RKHS	Uses the regression model rooted in bootstrapping sample observations. Takes the average of all tree nodes to find the best prediction model. Captures the interactions between markers
RF	- Utiliza o modelo de regressão enraizado (ou baseado) em observação de amostras por *bootstrapping*. - Toma a média de todos os nós de árvore para encontrar o melhor modelo de previsão. - Captura as interações entre os marcadores.

The performance of GWP depends on the accuracy of the prediction to select individuals whose phenotype is unknown. In GWP, the GEBV can be estimated from Equation 2 as:

Equation 3

$$\boldsymbol{GEBV = x_{new}\widehat{\beta}_{k,}}$$

(ii)

In what x_{new} is the compound matrix with the allelic states of the individuals of the breeding population and (BP), and ($\hat{\beta}_k$), is the estimated regression coefficient of β_k.

Cross-validation is used to train and develop the predictive model in the training population. Then, the model that best fits will be employed in the evaluation of the GEBV in the improved population. The accuracy of prediction (r_A) is the Pearson correlation between the selection criterion (GEBV) and the true breeding value (TBV). The expected accuracy prediction (r_A) can be estimated in (DAETWYLER et al., 2010)

Equation 4

$$\boldsymbol{r_A = \sqrt{\frac{h^2}{h^2 + \frac{M_e}{N_p}}},}$$

In which $h^2$is heritability in the strict sense, N_p is the number of individuals in the training population, and $M_e$is the number of independent chromosomal segments, which depends on both the effective size of the population (*Ne*) and the estimated length of the genome in Morgan (L) Which was derived according to (GODDARD, 2009) as, $M_e \approx 2N_eL$ M_e is related to the effective number of QTLs. The combined use of *Np* and h^2, rather than their individual assessment, is fundamental to regulate the expected accuracy (COMBS; BERNARDO, 2013, DESTA, ORTIZ, 2014, DAETWYLER et al., 2010).

7.2 Genomic Wide Association (GWAS)

In countries with a tropical climate like Brazil, tree logging approach 5 years, obtaining IMA values of 100 hectares per year for highly selective clones, while in countries such as Uruguay with subtropical climate the logging exceed 10 years with IMA of between 40 - 60 m3 hectares per year for highly selective clones. The slow growth of Eucalyptus genus in subtropical climates makes tree breeders consider fundamental the need for an early DBH evaluation.

The earliest maps based on molecular markers for the genus Eucalyptus were created in the 1990s, using the RAPD technique. This work could identify approximately 250 RAPD markers to cover an estimated genomic distance of around 1500 cM, including 11 ligament groups (GRATTAPAGLIA; SEDEROFF, 1994; VERHAEGEN; PLOMION, 1996). The technique continued to evolve and by the year 2006, 234 SSRs had already been mapped into 11 ligament groups, due to the advantage of using a codominant type marker, covering an average distance between each marker of 8.4 cM (BRONDANI et al., 2006). An advantage of SSRs was the possibility of transferability of trademarks between species of the same genus, as well as the demonstration of collinearity among SSRs, which facilitated studies and the creation of new link maps, exchange of results between laboratories and mainly the association of markers with QTLs (GRATTAPAGLIA; KIRST, 2008).

The utilization of molecular markers has been performed in Eucalypt breeding population for early identification of plant disease resistance. It is significant that in the selection process of QTLs with larger effects, advances were achieved such as the RAPD marker AT9/917, associated with the *PPr1* gene which resists to the fungal pathogen *Puccinia Psidii*. Subsequently this locus was flanked with EMBRA125 SSR markers at 9.5 cM *PPr1* and 7 cM EMBRA1071 (MAMANI et al., 2010). It was also found regions of the genome that influence the characteristics of wood and fibre in *E. globulus,* identified in two complete siblings: three QTLs that affect wood density, a QTL affects pulp yield and another one that affects the Microfibers angle (THAMARUS et al., 2004).

Recent advances in the genomics field have made revolutionary the opportunities for Genomic Wide Association studies, such as the EuCHIP60K chip, based on the Illumina Infinium technology that counts a total of 64,639 Single Nucleotide Polimorphism (SNP) (SILVA-JUNIOR et al., 2015). The high level of homology that the

genomes of *Eucalyptus* genus present, is visible on markers' behavior developed on this genus. These markers maintain a good level of functionality, when there are SNP type markers or single sequence repeat (SSR) (BRONDANI et al. 2006; TORRES-DINI et al. 2011b; RESENDE ET AL. 2017), apart from the studied species. The GWAS reduce the identification time of QTLs, and its high density and wide genomic coverage trait is an optimal tool to identify loci associated to traits genetically complex at DBH (RESENDE et al., 2017; da SILVA et al 2016).

8 MATERIALS AND METHODS

Plant material and genotyping strategy were described in the Chapter 1

8.1 Predictive model for GWP

The predictive model used for the GWP analyses was the random regression-best linear unbiased prediction (rrBLUP)(MEUWISEN et al, 2001), used BLUP predictors considering the markers genotypes as random effect covariates. Based on Resende (2008), we used the following linear mixed model to estimate the marker effects:

$$y = Xb + Zm + e$$

(y) = is the vector of phenotypic observations, (b) is the vector od fixed effects, (m) is the vector of random effects of markers, and (e) refers to vector of random to the vector of random residuals. X and Z are the incidence matrices for b and m respectively. Under this model the following assumptions must be adopted:

$m \sim N(0, I\sigma_m^2)$;
$E(y) = Xb; e \sim N(0, R = I\sigma_e^2)$;

$Var(y) = ZI\sigma_m^2 Z' + R$

The mixed model equations for predicting m via the rrBLUP method is the following:

$$\begin{bmatrix} X'X & X'Z \\ Z'X & Z'Z + I\,\dfrac{\sigma_e^2}{\sigma_g^2/n} \end{bmatrix} \begin{bmatrix} \hat{b} \\ \hat{m} \end{bmatrix} = \begin{bmatrix} X'y \\ Z'y \end{bmatrix}$$

a) Total genetic variance of the trait: (σ_g^2)

b) Residual variance: (σ_e^2)

The cross-validation was performed by resampling from a group of individuals using Jackknife procedure, which is based on dividing the N set from the sample data into groups with sizes equal to k, where $N = gk$. Thus, the clones were divided into 13 groups of 6 individuals. In each of the 13 analyses, a group was removed from the population,

and the other 72 individuals were used to estimate the effect of the markers in the estimation population. These groupings were chosen with the aim of training in the learning and the execution of models, since the size of the studied population is reduced to generate high accuracies and the genotyping populations of thousands of individuals as recommended in the bibliographies. This is beyond the possibilities of this thesis project. The groups were formed randomly.

All of the estimated marker effects were applied to the validation population to predict the GEBV of the individuals. Their marker incidence matrix (Zv), which corresponds to the marker genotypes for the validation population, was multiplied by the estimated effects of each marker and summed the general estimated average. Because the phenotypic value is known when validating the results, it is possible to evaluate the correlation of the genetic value predicted by the phenotype observed in all individuals. This correlation is known as the predictive capacity ($ry\hat{y}$) of genomic selection for estimating the pnehotypes, and it is theoretically determined by selection accuracy, and it is theoretically determined by selection accuracy ($rg\hat{g}$) multiplied by the square root of individual heritability (h), i.e., (RESENDE 2008; OLIVEIRA et al 2012)

9 RESULTS AND DISCUSSION

9.1 Genomic Wide Prediction model rrBLUP, Jacknife

The GEBV was estimated for a total population of 15,196 markers (Table 7), SNPs was used in the genomic selection for the DAP trait. The predictive capacity was expected to be low or negative (-0.15) for this population given its size (78 individuals). Our model did not show precision in predicting the DAP trait. These results were consistent with theoretical expectations. According to simulations performed by Grattapaglia and Resende (2011), for forest spp it is necessary to have an improvement population (training) of at least 1,000 phenotyped and genotyped individuals. With 1,000 individuals, an accuracy of more than 0.80 was observed with high marker density (Table 8) (GRATTAPAGLIA; RESENDE, 2011). However, little impact was observed for a population of over 2,000 individuals. The results confirm the underlying assumptions of the simulations performed by the authors. On the other hand, this is the first result obtained with the Genomic wide prediction application in Uruguay's genetic improvement program. With advances in technology and cost reduction on genotyping, new populations may be genotyped and considered along with this database, and more accurate results can be obtained with Genomic wide prediction. In this case, the breeding time can be reduced by half and the gain can exceed 100% with the wide genomic prediction (GRATTAPAGLIA 2014). According to the authors, with the reduction of flowering time from 12 to 3 years this gain may be greater than 300%.

The heritability estimated from the phenotype DAP is considered the upper limit that could be explained by the GWP model (RESENDE et al., 2012). In the present study, the model captured only 17% of molecular heritability (Table 8). This result is not consistent when using all markers to estimate the model for complex characters (MEUWISSEN et al., 2001). With 200 large effect markers, it can be obtained 80% of the phenotypic heritability (RESENDE et al., 2012). Higher percentages (around 97%) can be achieved with only 300 to 500 markers (RESENDE et al., 2012). Despite the hybrid origin of the population that could be contributing to a high linkage instability, the effect required to capture the maximum genetic variation was not enough, due to the number of individuals in the population. As well as markers, the number of individuals in genomic selection is crucial in estimating genetic parameters more accurately.

Another effect that negatively affects the transferability of GWP is the genotype x environment interaction (RESENDE et al., 2011). As already mentioned by Resende et

al. (2012) and Resende et al. (2011) the GS prediction model must be specific for each population. This information should be considered in future work.

9.2 Ranking Comparison

A total of 77 individuals (the control genotype was excluded) were compared for both selection strategies described (HMRPGV and rrBLUP) by the produced ranking alignment. The comparison was made with the GEBV ranking position (table 9) along with those obtained by selegen model 52, while in fact the rrBLUP model accuracies were low, some coincidences (showed in green) were observed in the clusters. Eight of the top twenty clones appear in both methodologies. Similar results are observed in the best second and third 20 clones (6 clones in each group of 20), in the last 17 only one coincidence is observed. The 1744 Clone that ranks first in Model 52 of selegen appears at position 46 in the rrBLUP model (Table 9).

Table 7 - GEBV genomic genetic values determined for each clone.

Clone	gbv+u	Clone	gbv+u	Clone	gbv+u
1701	15,24873	1693	14,27186	1786	14,12099
1794	14,56757	1683	14,26072	1735	14,11038
1689	14,52619	1685	14,25215	1773	14,10597
1676	14,47565	1667	14,2469	1726	14,10574
1793	14,45356	1734	14,23118	1771	14,10528
1752	14,44641	1671	14,2275	1687	14,10088
1780	14,42663	1670	14,22409	1673	14,09698
1778	14,42455	1694	14,22051	1684	14,09054
1783	14,42394	1706	14,21857	1713	14,07823
1755	14,40269	1666	14,21804	1762	14,07597
1716	14,38565	1678	14,21494	1711	14,05826
1770	14,38483	1733	14,20987	1753	14,05593
1769	14,37986	1686	14,20303	1722	14,04046
1789	14,37911	1774	14,1876	1751	14,03018
1700	14,36128	1760	14,18467	1797	14,02656
1754	14,35882	1792	14,17846	1721	14,0137
1741	14,33761	1731	14,17806	1763	14,00339
1779	14,33662	1677	14,16286	1785	13,94262
1743	14,33229	1724	14,16173	1690	13,91221
1796	14,30969	1744	14,15857	1764	13,9094
1682	14,30861	1732	14,15811	1758	13,85753
1709	14,30575	1790	14,14264	1766	13,85062
1669	14,30155	1672	14,14105	1787	13,75704
1718	14,29021	1736	14,1331	1756	13,53977
1691	14,28671	1723	14,12835	1698	13,39012
1675	14,27661	1715	14,12299		

Source:Original Production.

Table 8 - Prediction of parameters from genomic wide prediction in a population of Eucalyptus.

Parameters	Estimations
Genotypic heritability	0,37
Accuracy	0,81
Number of markers	15.196
Genotyped and phenotyped individuals	78
Molecular heritability	0,17
Predictive capacity	-0,15
GWP accuracy	-0,25

Source: Original Production.

Table 9 - Ranking order comparison obtained with the SELEGEN model 52 for both environments (Tres Bocas and Algorta) and the SGA rrBLUP model. While in fact the rrBLUP model accuracies were low, some coincidences (showed in green) were observed in the clusters. Eight of the top twenty clones appear in both methodologies. Similar results are observed in the second and third best 20 clones (6 clones in each group of the 20). In the last best 17 only one coincidence observed. The 1744 Clone that ranks first in Model 52 selegen rankings, appears at position 46 in the rrBLUP model.

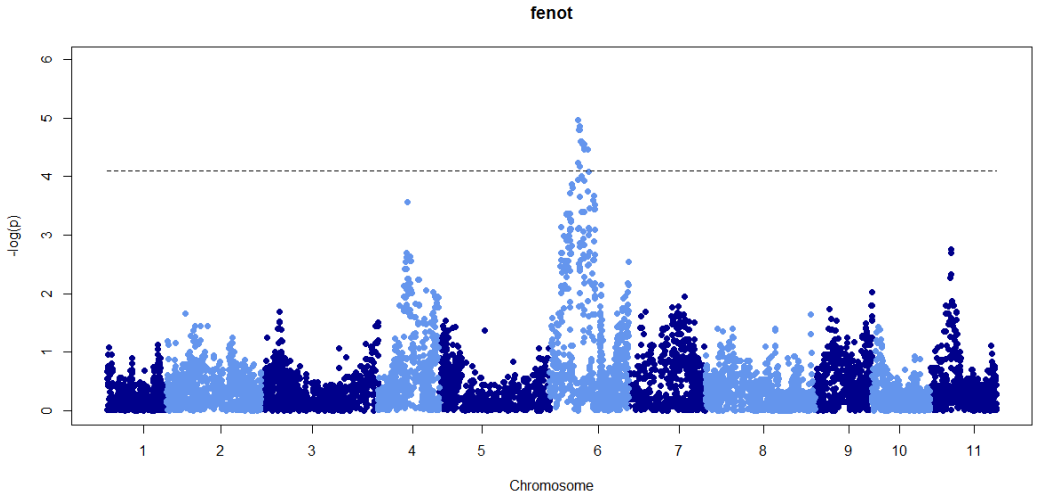
Ranking	MOD 52	GEVB	Ranking	MOD 52	GEVB	Ranking	MOD 52	GEVB	Ranking	MOD 52	GEVB
1	1744	1701	21	1789	1682	41	1693	1760	61	1694	1713
2	1769	1794	22	1785	1709	42	1762	1792	62	1733	1762
3	1670	1689	23	1766	1669	43	1731	1731	63	1709	1711
4	1796	1676	24	1690	1718	44	1721	1677	64	1706	1753
5	1778	1793	25	1669	1691	45	1741	1724	65	1683	1722
6	1756	1752	26	1718	1675	46	1687	1744	66	1771	1751
7	1783	1780	27	1686	1693	47	1724	1732	67	1780	1797
8	1716	1778	28	1726	1683	48	1667	1790	68	1732	1721
9	1770	1783	29	1698	1685	49	1773	1672	69	1755	1763
10	1722	1755	30	1790	1667	50	1751	1736	70	1701	1785
11	1787	1716	31	1793	1734	51	1713	1723	71	1760	1690
12	1684	1770	32	1666	1671	52	1735	1715	72	1672	1764
13	1764	1769	33	1723	1670	53	1752	1786	73	1786	1758
14	1685	1789	34	1682	1694	54	1754	1735	74	1758	1766
15	1671	1700	35	1734	1706	55	1794	1773	75	1675	1787
16	1700	1754	36	1715	1666	56	1779	1726	76	1676	1756
17	1711	1741	37	1797	1678	57	1753	1771	77	1691	1698
18	1792	1779	38	1763	1733	58	1774	1687			
19	1736	1743	39	1673	1686	59	1678	1673			
20	1689	1796	40	1743	1774	60	1677	1684			

Source:Original Production

9.3 Genomic Wide association

In genome-wide association 15196 SNPs analyses were performed using R software. These markers are distributed throughout the Eucalyptus genome by 11 chromosomes as shown in Figure 8. By integrating the phenotypic information from DAP with the chromosomal position, a QTL associated to DAP on chromosome 6 was evidenced (RESENDE et al., 2017; DA SILVA et al. 2016)

Figure 8. Manhattan plot of the SNP effects, and significance levels (−log10 p values) for associations between SNPs and DBH. sexual compatibility along the 11. *Eucalypt* chromosomes. A QTL associated to DBH is observed into the chromosome 6 and the result are statistical significate



Source: Original Production

10 CONCLUSIONS

- There is genetic variation for the DAP trait that can be explored by clones' selection. Genotypes 1744, 1796, 1756, 1670, 1778 and 1769 present greater potential for plantations in both locations due to higher productivity, stability, and adaptability. Clone 1744 is most suitable for reforestation in both locations because it has ranked first in all rankings.

- The use of SNP markers helped confirm the degree of parentage between the clones as well as control of clonal identity. Also, the use of SNP genoyping in controlled crosses with other elite clones from unrelated populations contributes to decision making which maintains optimal heterozygosis levels.

- Genotyping data were also used to test the rrBLUP genomic selection model, followed by Jackknife with very low accuracy, this is a consequence of the population's small size.

- The GWAS assay identified a QTL into the chromosome 6 associated to trait DBH.

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