GENETIC CONTROL OF CELLULOSE, LIGNIN AND GLUCOSE CONTENTS IN EUROPEAN BLACK POPLAR (*POPULUS NIGRA* L.) POPULATIONS FROM TURKEY

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

BİRCAN TAŞKIRAN

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
BIOLOGY

FEBRUARY, 2014

Approval of the thesis

GENETIC CONTROL OF CELLULOSE, LIGNIN AND GLUCOSE CONTENTS IN EUROPEAN BLACK POPLAR (*Populus nigra* L.) POPULATIONS FROM TURKEY

submitted by **BİRCAN TAŞKIRAN** in partial fulfillment of the requirements for the degree of **Master of Science in Biology Department, Middle East Technical University** by**,**

Prof. Dr. Canan Özgen _____
Dean, Graduate School of **Natural and Applied Sciences**

Prof. Dr. Gülay Özcengiz _____
Head of the Department, **Biology**

Prof. Dr. Zeki Kaya _____
Supervisor, **Biology Dept., METU**

Examining Committee Members:

Prof. Dr. Tülin Güray _____
Biology Dept., METU

Prof. Dr. Zeki Kaya _____
Biology Dept., METU

Assoc. Prof. Dr. Sertac Önde _____
Biology Dept., METU

Assist.Prof. Dr. Fatih Temel _____
Faculty of Forestry, Artvin Çoruh University

Dr. Gaye Kandemir _____
Gn. Directorate of Forestry, Ministry of Forest and Water Affairs

Date: 07.02.2014

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last name: Bircan TAŞKIRAN

Signature:

ABSTRACT

GENETIC CONTROL OF CELLULOSE, LIGNIN AND GLUCOSE CONTENTS IN EUROPEAN BLACK POPLAR (*Populus nigra* L.) POPULATIONS FROM TURKEY

Taşkıran, Bircan
M.S., Department of Biology
Supervisor: Prof.Dr. Zeki KAYA
February 2014, 86 pages

Populus nigra L. is considered as one of the most economically significant forest tree species with respect to production of wood, biomass, timber, pulp, paper and other wood-based products, besides its ecological and evolutionary importance. Because of the increased wood needs of the world and demands of renewable energy sources, fast-growing poplar has gained importance. While wood quality, pulp mechanical strength, and biomass are directly associated with high cellulose content, lignin emerges as an undesirable polymer for both pulp and biofuel manufacturing industries.

To estimate the contents of the main cell wall polymers, cellulose, lignin, and total D-glucose content of wood cell, and also their proportion to each other, we established a large collection of biological samples from one year old branches of poplar trees (285 clones x 3 replicates x 2 ramets) which were grown for three years in an outdoor nursery (Behiçbey Nursery) in Ankara. These clones were originally collected from all over Turkey. Additionally, five commercially registered clones and six foreign clones were included in the study for comparison.

The mean values for cellulose, lignin and glucose content were 21.8 ± 16.29 µg/ml, 23 ± 4.64 µg/ml, and 35 ± 9.71 µg/ml, respectively. However, particular clones were detected with extraordinary high values for these traits. The components of the total variance due to among clones within regions were 15.11 %, 3.47 %, and 12.94 % for cellulose, lignin and glucose, respectively. Positive correlations were observed between cellulose and height ($r = 0.22$; $p < 0.01$), cellulose and diameter ($r = 0.23$; $p < 0.01$) and also between lignin and glucose ($r = 0.31$, $p < 0.01$). On the other hand, negative correlation was detected between lignin and diameter ($r = -0.121$; $p < 0.05$) as expected.

In the study, all clones were also evaluated with respect to high quality and yield to realize valuable clones with high cellulose to low lignin content. The superior clones were 62172, N91075, 62160, N03377, and 62191, whereas the inferior clones were 641410, N91085, 8511, N03372, and N92169. Also the study by choosing the superior and eliminating the inferior clones pave the way for future investments and applicable new poplar breeding programs.

Key Words: *Populus*, poplar, cellulose, lignin, glucose, genetic variation

ÖZ

TÜRKİYE'DEKİ AVRUPA KARA KAVAĞI (*Populus nigra* L.) POPÜLASYONLARININ SELÜLOZ, LİGNİN VE GLUKOZ İÇERİKLERİNİN GENETİK KONTROLÜ

Taşkıran, Bircan
Yüksek lisans, Biyoloji Bölümü
Tez yöneticisi: Prof. Dr. Zeki KAYA
Şubat 2014, 86 sayfa

Populus nigra L. ekolojik ve evrimsel öneminin yanısıra, odun, biyokütle, kereste, kağıt hamuru, kağıt ve diğer odun kaynaklı ürünlerin üretimi açısından da en önemli ekonomik orman ağaçlarından biri olarak kabul edilmektedir. Dünyanın büyümekte olan odun ihtiyacı ve yenilenebilir enerji kaynağı gereksinimi sebebiyle, hızlı büyüyen kavak türleri önem kazanmıştır. Odun kalitesi, kağıt hamurunun mekanik dayanıklılığı ve biyokütle doğrudan yüksek selüloz içeriği ile ilişkilendirilirken, lignin hem kağıt hemde biyoyakıt üretim endüstrileri tarafından istenmeyen polimer olarak karşımıza çıkmaktadır.

Hücre duvarının ana polimerleri olan selüloz ve ligninin ve ayrıca odun hücresinin toplam D-glukoz içeriğini ve birbirlerine oranlarını tahmin etmek için, Behiçbey Fidanlığı'nda (Ankara) açık havada büyümüş üç yıllık denemedeki ağaçlarım bir yıllık dallarından kapsamlı bir biyolojik örnek koleksiyonu oluşturuldu (285 klon x 3 tekrar x 2 ramet). Bu klonlar daha önce tüm Türkiye'den toplanmıştı. Ayrıca beş tane ticari olarak tescillenmiş klon ile altı yabancı klon karşılaştırma yapabilmek için çalışmaya dahil edildi.

Ortalama selüloz, lignin ve glukoz içerikleri sırasıyla 21,8 ± 16,29 µg/ml, 23 ± 4,64 µg/ml, ve 35 ± 9,71 µg/ml olarak hesaplandı. Öte yandan, belirli klonlar bu karakterlerde gösterdikleri sıradışı içerikler ile tespit edildi. Selüloz, lignin ve glukoz içeriğinde klonlardan kaynaklanan varyans bileşeni bölge içinde sırasıyla 15,11 %, 3,47 %, ve 12,94 % olarak gözlendi. Pozitif korrelasyonlar selüloz ve boy (r = 0,220; p < 0,01), selüloz ve çap (r = 0,235; p < 0,01) ve ayrıca lignin ve glukoz (r = 0,314; p < 0,01) arasında gözlendi. Öte yandan beklediğimiz gibi, lignin ve çap arasında negatif korrelasyon (r = -0,121; p < 0,05) tespit edildi.

Bu güncel çalışmada, kalite ve verim açısından, yüksek selüloz ve düşük lignin içeriği ile kıymetli kabul edilen klonları tespit edebilmek için tüm klonlar değerlendirildi. 62172, N91075, 62160, N03377 ve 62191 kodlu klonlar üstün klonlar olarak gözlemlenirken, 641410, N91085, 8511, N03372 ve N92169 kodlu klonlar düşük kaliteli klonlar olarak gözlemlendi. Ayrıca bu çalışma, üstün kaliteli klonları seçerek ve düşük kaliteli klonları elimine ederek, gelecek yatırımlar ve yeni uygulanabilir kavak melezleme programları için zemin hazırlamıştır.

Anahtar Kelimeler: *Populus*, kavak, selüloz, lignin, glukoz, genetik varyasyon

to my family...

ACKNOWLEDGEMENTS

I am greatly indebted to my supervisor, Prof. Dr. Zeki Kaya for his guidance, supervision and endless patience throughout the study.

I would like to express my thanks to all jury members for their helpful comments and criticisms on the manuscript.

I would like to thank all my colleagues from Department of Biology, Plant Genetics and Tissue Culture Laboratory; especially, Evrim Zeybek, Kubilay Yıldırım, Pelin Acar, Çiğdem Kansu and Asiye Çiftçi for their help and encouragement in every step of this study.

I would like to express my gratitude to dear my cousin Huriye Yener, Bayram Demir, Ercan Taşkıran, Serdar Taşkıran, Sedat Akkaya, and Hicran Karataş for their support during homogenization stages of specimens.

I wish to express my appreciation to my best friends Elif Yetkin, Emine Kork, Fatma Aykaç and Nurcan Çamdereli for their endless love and support.

I like to thank also my homemates Ayten Dizkırıcı, Nehir Yasan, and Betül Yeniterzi for their lovely frienships in Konukevi 1.

I would like to thank specially to my dear mother Sevim TAŞKIRAN, my dear father Mustafa TAŞKIRAN, my precious sister Hicran KARATAŞ, my brothers Ercan and Serdar TAŞKIRAN, and my sister-in-law Tuğba TAŞKIRAN for their continuous love, support, understanding and encouragement through all my life.

TABLE OF CONTENTS

LIST OF TABLES

TABLES

LIST OF FIGURES

FIGURES

LIST OF ABBREVIATIONS

A	Absorbance
ANOVA	Analysis of Variance
EUROFERGEN	European Forest Genetic Resources Program
FAO	Food and Agriculture Organization
FRA	Global Forest Resources Assessment
GLM	General Linear Model
IPC	International Poplar Commission
IUFRO	International Union of Forestry Research Organizations
SSR	Simple Sequence Repeat
TAPPI	Technical Association of Pulp and Paper Industry

CHAPTER 1

INTRODUCTION

1.1 *Populus nigra* L.

1.1.1 Taxonomy

The European black poplar, *Populus nigra* L. is a member of the Salicaceae family (willow family). It is a typical fast-growing tree species of the Genus *Populus* in the Aigeiros section which is also known as cottonwoods (Toplu, 2005). *P.deltoides* and *P. fremantii* are the other species of the section. The other sections of genus *Populus* described by Eckenwalder (1996) based on relative morphological similarity and crossability are: *Populus* (white poplars and aspens), *Tacamahaca* (balsam poplars), *Leucoides*, *Abaso*, and *Turanga*. There are three well-known subspecies of *P. nigra* which are *Populus nigra* subsp. *nigra, Populus nigra* subsp. *betulifolia* (Pursh) W.Wettst, and *Populus nigra* subsp. *caudina* (Ten.) Bugała. These are distributed in Europe and Mediterranean region and southwest Asia, respectively.

According to recent taxonomic studies, the total number of species of genus *Populus* ranges from 29 to 32 (Dickmann and Kuzovkina, 2008). Because of the fact that the members of genus *Populus* can hybridize with each other, classification of the genus is very complicated (Cagelli and Lefevre, 1997). This kind of interspecific hybridization generally occurs between closely related sympatric species (Barnes and Pregitzer, 1985; Dickmann and Stuart, 1983, Eckenwalder, 1984a, b; Keim *et al.*, 1989; Muhle-Larsen, 1970; Ronald *et al.*, 1973a, b; Rood *et al.*, 1986; Whitham *et al.*, 2006). Moreover, it is possible with controlled crosses (Ronald, 1982; Stettler *et al.*, 1980; Willing and Pryor, 1976).

1.1.2 Biology and Ecology

The European black poplar is a single-stemmed tree, which can reach to 30 meters height with a trunk diameter of up to 2.5 meters. They are one of the fastest growing trees and spread clonally by root fragments or through seeds (Figure 1.1.). Because of their excess sunlight need for growth, black poplar generally creates small populations in open areas with alluvial soils. The black poplar has an important role as a key species in riparian ecosystems together with *P. alba* L., willows, alders, maple, elm, and ash (Rathmacher *et al.,* 2010; Arens *et al.,* 1998; Csencsics *et al.,* 2009). Individual trees can live for over 400 years (Vanden Broeck, A. 2003). Diploid chromosome number of the European black poplar is 2n= 38. The black poplar is a dioecious species, having male and female plants.

Figure 1.1 General appearance of *Populus nigra* test plantation in Behiçbey nursery

The leaves generally appear in the late April after the catkins which are yellow-green in females and purplish-red in males (Figure 1.2. A and B). *Populus nigra* depends on wind for pollination. When they are 10-15 years old, they reach at the reproductive age (Braatne *et al.,* 1996). Seeds emerge with a coma of cottony hairs on parietal placentas in thin-walled capsules (Figure 1.2. C) (Rae *et al.*, 2007). Owing to its structure, seeds are dispersed through wind and water in early summer. Besides this, they need very particular requirement of soil and water for germination (Gaudet, 2006).

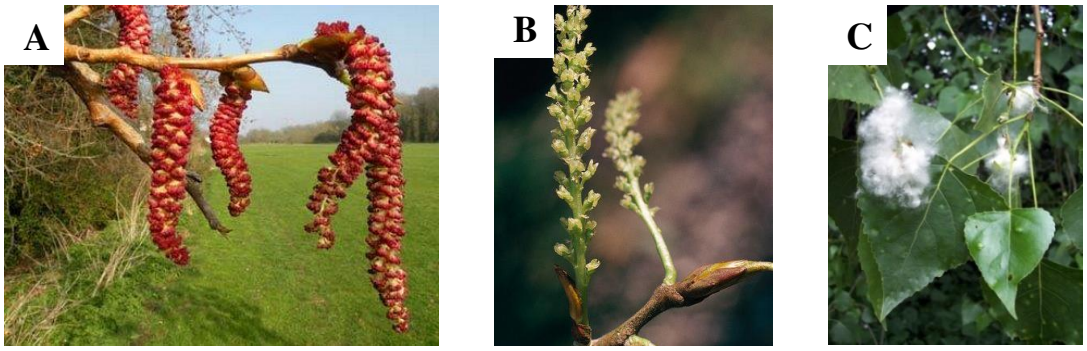


Figure 1.2 Reproductive structures of *Populus nigra* A.Male catkin (http://treeaware.blogspot.com/2009/03/spring-in-bath-black-poplar-catkins.html*),* B.Female catkin *(*http://www.arkive.org/black-poplar/populus-nigra/image-A1424.html) C.Seed (http ://gallery.nen.gov.uk/assets/0607/0000/0093/plants_426_mid.jpg) Retrieved on 19.12.2013

1.1.3 Distribution

The species of the genus *Populus*, collectively known as poplars, are accepted as the most abundant woody plants in forests of the northern hemisphere (Hillier and Coombes, 2002). If the distribution range is examined for *P. nigra*, it is clearly seen that there is no correlation between particular climatic area and distribution of *P. nigra*. In fact, its distribution is connected with the soil moisture (Gaudet, 2006). The distribution area includes a large portion of Europe, northern Africa, Middle East, and central and west Asia (Figure 1.3.) (Rathmacher *et al.,* 2010).

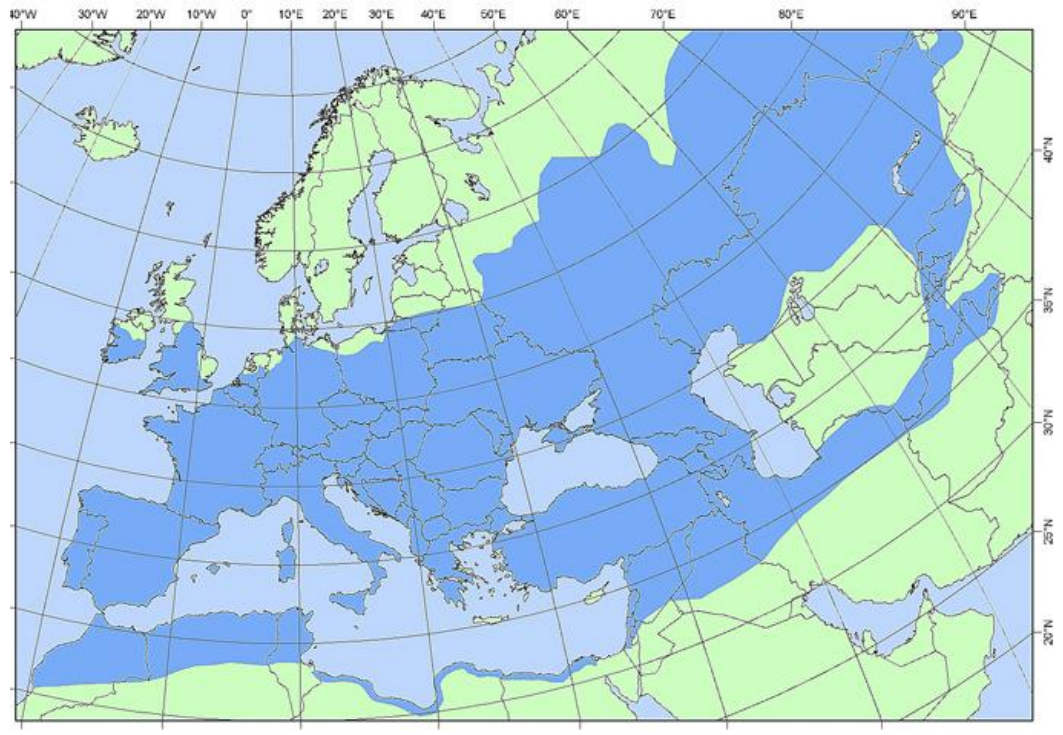


Figure 1.3 The distribution ranges of *Populus nigra* (the blue area) in the world. (http://www.euforgen.org/fileadmin/www.euforgen.org/Documents/Maps/PDF/populus_nigra.pdf) Retrieved on 19.12.2013

1.1.4 Importance and Use

Poplar trees are one of the most important components of any terrestrial and riparian ecosystems because of their unique characteristic features such as their long life span and providing habitats for many living organisms. Carbon sequestration, bioremediation, nutrient cycling, and biofiltration are some of the ecological services provided by poplar trees (Brenner *et al.*, 2004; Taylor, 2002). Black poplar trees have an ecological importance as an indicator and pioneer species of riparian woodlands. In many areas of northern hemisphere, the early successional stage of floodplain woodlands is succeeded by black poplar together with other members of the Salicaceae family. Furthermore, a great number of endangered and common insects like Lepidoptera species and animals are connected with or depended on poplars. *P. nigra* is not only used as a barrier against the wind in Ağrıcultural lands to prevent erosion, but also utilized for ornamental and landscaping purposes (Stettler *et al.,* 1996). Nowadays, the restoration of riparian ecosystems both for natural flood control and protecting the river borders to connect larger forest areas is gained importance. Therefore, conservation of black poplar genetic sources for the benefit of ecosystems is important (Vanden Broeck, 2003; Gaudet *et al.,* 2008). Besides their ecological and evolutional importance, black poplars are accepted as one of the most economically significant forest trees in terms of production of wood, biomass, timber, pulp, paper and other wood-based products (Stettler *et al.*, 1996b; Taylor, 2002). Moreover, it is also used for rural construction and the daily needs of rural people. In addition to all these, since European black poplar wood is lightweighted, white colored and does not have any fragrance, it is widely used to manufacture of fruit boxes (Gaudet *et al.,* 2006).

Domestication of poplar began in Europe in the late eighteenth century. After the Second World War, widespread wood shortages led to their cultivation for timber production (Schreiner, 1959). It was reported by the International Poplar Commission held in Beijing in 2008 that over one hundred and twenty five superior Populus cultivars were in use in the whole world. Over 5.2 million hectares of plantations and 3.8 million hectares of agroforestry and environmental plantings were reported as poplar lands throughout the world (FAO, 2008). The breeding of

improved cultivars has been emphasized by the management of these lands in terms of yield, pathogen resistance and so on (Stanton, 2009).

Many countries have been producing their own national solutions to fulfill their raw wood material demands particularly by focusing on investments of industrial row plantings (Koçer ve Diner, 2003). Based on Global Forest Resources Assessment 2010 Main report, global wood removals were reported as 3.4 billion cubic meters for 172 countries that account for 99.8 % of the global forest area (Table 1)(FRA, 2010). According to the projection of total wood consumption of Turkey, industrial wood need is expected to reach 15.6 million m^3, while the supply of domestic resources is expected to remain 12.3 million m^3 by 2023 (Anon, 2001). In the present circumstances, supplying the 3.3 million m^3 deficit that will emerge will not be obtained from the existing capacity of natural forests. Due to the over exploitation and slow regeneration rate, forest lands are getting reduced day by day. Therefore, poplar has gained importance because of its easy culture, rapid growth rate and wide range usages.

Fuel cost increase and concerns of global warming have led to the rise of interest in renewable energy sources such as wind, water, solar, biofuels, biomass and geothermal (Mason, 2007). Apart from all these usages, *P. nigra* and its hybrids have been accepted as renewable source of energy for net-zero carbon emission lignocellulosic biofuels and biomass (Carroll and Somerville, 2009; Rubin, 2008).

Additionally, poplar is also the first tree species which was completely sequenced (Tuskan *et al.,* 2006; Jansson and Douglas, 2007). The haploid genome size of *Populus* is reported as 550 million base pairs which is only 4 times larger than the genome of *Arabidopsis,* and 40 times smaller than the genomes of conifers like loblolly pine (Bradshaw and Stettler, 1993). By means of their moderate genome size, rapid growth rate, easy propagation via stem cuttings of woody tissues, and economic importance, black poplars have been recognized as an ideal model organism to study biofuels-related traits, molecular mechanisms of trees and their responses to environmental stress (Bradshaw *et al.*, 2000; Tuskan *et al.,* 2006).

Table 1. Wood removals by region and subregion in 2005 (FRA, 2010).

Region/subregion	Industrial roundwood	Fuel wood	Total removals
	million m^3	million m^3	million m^3
Eastern and Southern Africa	39	292	331
Northern Africa	4	24	27
Western and Central Africa	30	301	330
Total Africa	**72**	**616**	**688**
East Asia	86	71	157
South and Southeast Asia	99	464	562
Western and Central Asia	17	13	30
Total Asia	**201**	**548**	**749**
Total Europe	**568**	**167**	**735**
Caribbean	1	5	6
Central America	4	17	22
North America	701	55	756
Total North & Central America	**706**	**77**	**783**
Total Oceania	**55**	**1**	**56**
Total South America	**180**	**167**	**347**
World	**1783**	**1576**	**3359**

1.2 Wood

Wood is a remarkable composite material and is almost totally composed of plant cell walls. The total volume of wood in the world is considered as 4 x 10^{11} cubic meters (FAO, 2010). The terminal differentiation of the xylem tissues of woody plants gives rise to wood production. It is also known as secondary xylem consisting largely of dead cells involved in the transport of water and minerals as well as support. Its strength and energy content are determined by its physical and chemical composition (Lewin *et al.*, 1991). Wood is composed of cellulose, lignin, and hemicellulose with small amounts of minor components such as pectin and inorganic substances. Contents of major structural polymers may vary in different tree species. To illustrate, while lignin content of *Pinus sylvestris* is 26.9 %, *Populus tremuloides* has 18 % lignin content (Sarkanen *et al.*, 1971; Sjostrom, 1993).

Trees are classified as hardwoods and softwoods. Woods of broad-leaved trees like poplar are known as hardwoods and woods of gymnosperms like pines are called softwoods. The terms of hard and soft do not refer to the wood hardness. The cellular structure and chemical contents of the wood make this difference. For example, hardwood species have xylem composed of vessels and tracheids or fibers to carry sap from stem through leaves, whereas softwood species have simpler structures which are mainly longitudinal tracheids and small ray parenchyma cells (Lewin *et al.*, 1991). While the cellulose structure of hardwood and softwood is similar in both, types, the proportions of lignin and hemicellulose are completely different (Sjostrom, 1993).

A major determining factor of the strength and physical characteristics of wood is acknowledged as the morphology of the wood cell wall. Since the primary (P) cell wall is the basic structural unit of the living cell, it exists in all plants. In wood, after the formation of three layers of secondary cell wall towards the lumen of cell, the primary wall remains in the outermost. In primary cell walls, cellulose microfibrils are embedded in a matrix of pectins and hemicelluloses. Together with middle lamella, it is collectively named as compound middle lamella (CML) which is poor in terms of cellulose, but rich in pectin and lignin (> 50%). The properties of the wall are formed mainly by the middle and thickest layer of secondary cell wall, the S2

layer. Arrangements of cellulose microfibrils in these layers are observed in different directions (Figure 1.4.). S layers are rich in cellulose and xylans. At the end of the xylem differentiation, all layers are lignified and lignin-rich middle lamella binds the adjacent cells together (Panshin *et al.*, 1980; Sjostrom, 1993).

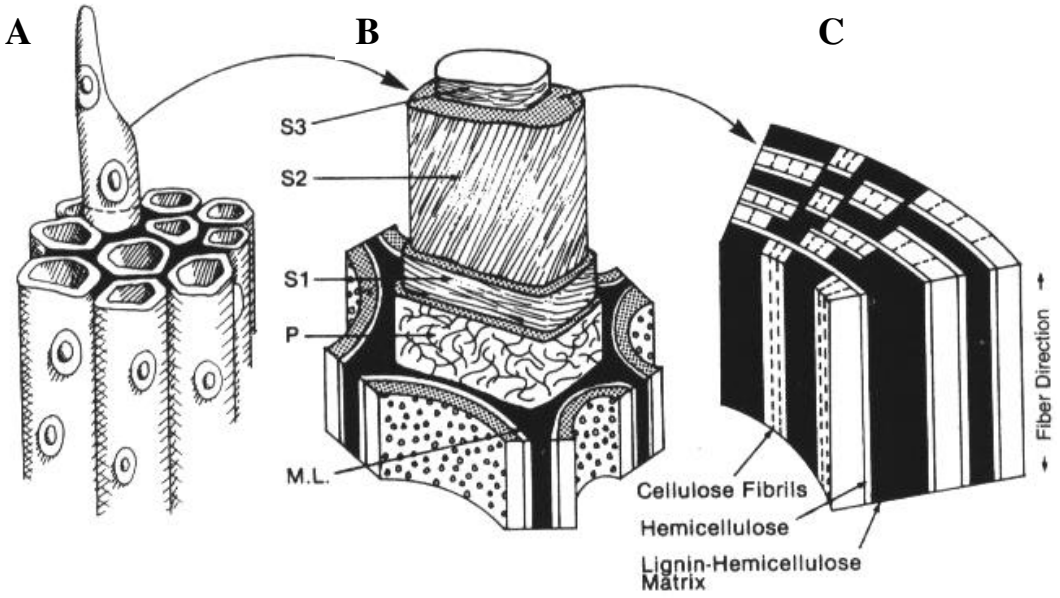


Figure 1.4 (A) The morphology of the tracheids (B) Three dimensional diagram of the cell wall of the tracheid in which the primary, S1, S2, and S3 cell wall layers and the middle lamella (ML) are shown. (C) The relationship of lignin, hemicellulose and cellulose in the secondary wall of a tracheid (After Sjostrom, 1993).

1.2.1 Cellulose

Cellulose is the most stable cell wall polysaccharides and is the main component of wood. It comprises 40-50 % of wood dry matter (Delmer and Haigler, 2002). Usually, cellulose content of primary cell wall is between 20 and 30%, whereas secondary cell walls may contain 50% or much higher amount of it. Cellulose is a highly simple unbranched polysaccharide composed of β (1→4) linked D-glucose residues where every other glucose residue is rotated approximately 180° (Figure 1.5.) (Somerville, 2006). To form a cellulose microfibril of a diameter of 3 to 5 nm, about 30 to 50 β (1→4) linked glucan chains are linked by hydrogen bonds (Brown, 2003). Microfibril size can vary from 36 chains which is called elementary fibril to 1200 chains which found in cellulosic algae (Sugiyama *et al.*, 1985). Microfibrils which include both crystalline and amorphous regions are arranged in bundles to form fibrils. Finally, these fibrils aggregate together to form the backbone of the wood fiber structure (Figure 1.6.) (Delmer and Amor, 1995).

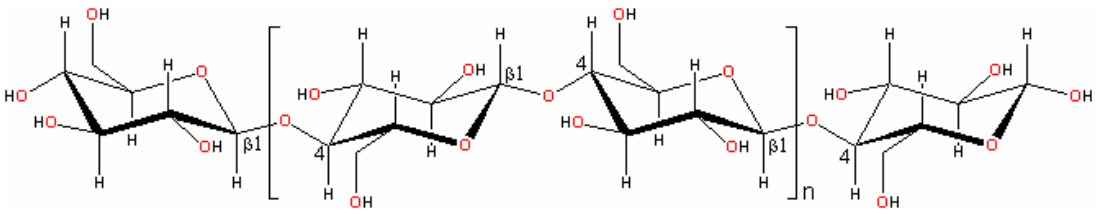


Figure 1.5 Structure of cellulose molecule. The repeating unit, cellobiose, is indicated in brackets.

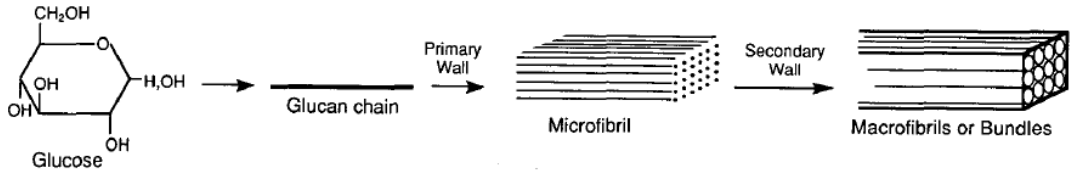


Figure 1.6 Steps in the assembly of native cellulose (After Delmer and Amor, 1995)

Cellulose is synthesized at the plasma membrane by cellulose synthase (CesA) complex, a rosette-like structure and a large cytoplasmic domain (Doblin *et al.*, 2002; Somerville, 2006; Joshi and Mansfield, 2007). Catalytic subunits of CesA complex utilize uridine diphoshoglucose (UDP-Glc), an activated sugar, as a substrate. UDP-Glc can be synthesized by two distinct ways. In the first one, it emerges as a result of the cleavage of sucrose by sucrose synthase (SuSy) which is suggested as a part of catalytic domain of CesA complex (Fujii *et al.*, 2010). In the second one, UDP-glucose pyrophosphorylase (UGPase) converts glucose-1-P to UDP-Gly (Delmer and Haigler, 2002) (Figure 1.7).

In terms of its chemical structures, cellulose is highly stable and extremely insoluble. The crystalline structure of cellulose microfibrils, the hydrogen bonding between cellulose and hemicelluloses and hydrophobic interactions are the main determinants for cell wall strength. Because of that thick-walled cells with small lumens have high wood density and greater strength (Lewin *et al.*, 1991).

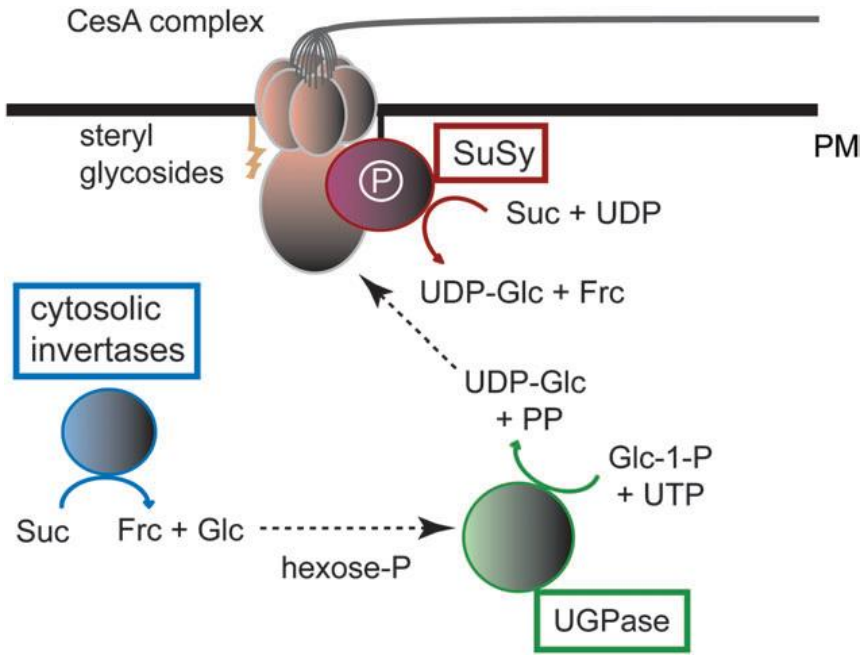


Figure 1.7 Synthesis of UDP-Glucose, an activated sugar donor of CesA complex (After Endler and Staffan, 2011).

1.2.2 Lignin

Lignin is the second most abundant polymer, synthesized by all plants, after cellulose (Figure 1.8). Hydrophobic surface and structural support which are crucial to transport water in the vascular system are provided by lignin (Carder, 1995; Koch *et al.*, 2004). It also serves as a mechanical barrier against pests and pathogens (Vance *et al.*, 1980; Bhuiyan *et al.*, 2009).

Despite the fact that lignin is highly important for plant fitness, it is an undesirable complex compound of pulp, paper, and bioenergy industries (Li *et al.*, 2003; Chen and Dixon, 2007). Following the start of secondary wall formation, lignification begins in the middle lamella, primary wall, and secondary wall, respectively (Donaldson, 2001). Lignification represents the last stage of cell expansion and

elongation before programmed cell death (Timell, 1986). The biosynthesis of lignin begins with the conversion of the amino acid phenylalanine into cinnamic acid by phenylalanine ammonia lyase (PAL) in the cytosol. Lignin is a derivative of phenylpropanoids and heteropolymer of three major p-hydroxycinammyl alcohol precursors (monolignols) which are p-coumaryl, coniferyl and sinapyl alcohols (Sarkanen *et al.*, 1971). Subsequently, this monolignols go through dehydrogenative polymerizations to form p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) lignin, respectively (Weng *et al.*, 2008). While lignins of gymnosperms (softwood) are mainly composed of guaiacyl (G) units which is a derivative of coniferyl alcohol, angiosperm lignins are composed of both guaiacyl (G) and syringyl (S) units in different ratios (Figure 1.9) (Boerjan *et al.*, 2003).

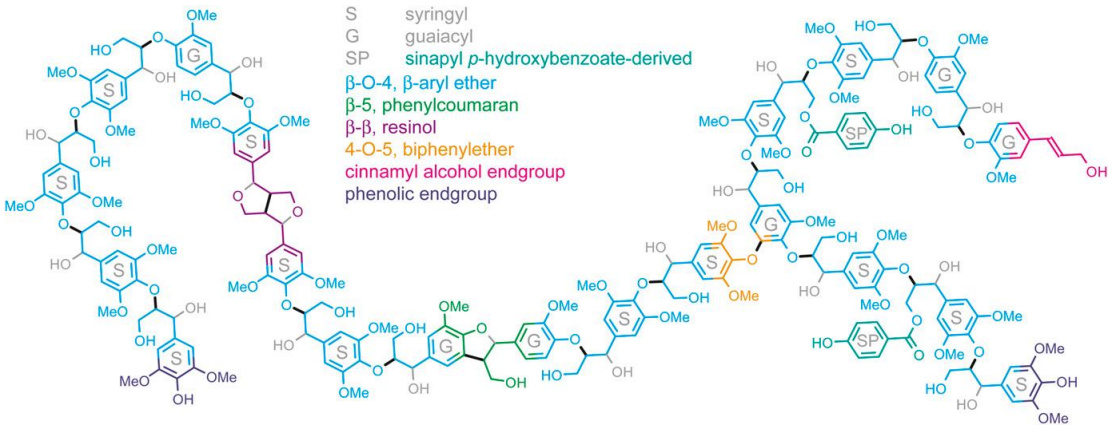


Figure 1.8 Representation of a lignin polymer from poplar, as predicted from NMR-based lignin analysis (After Stewart *et al.*, 2009).

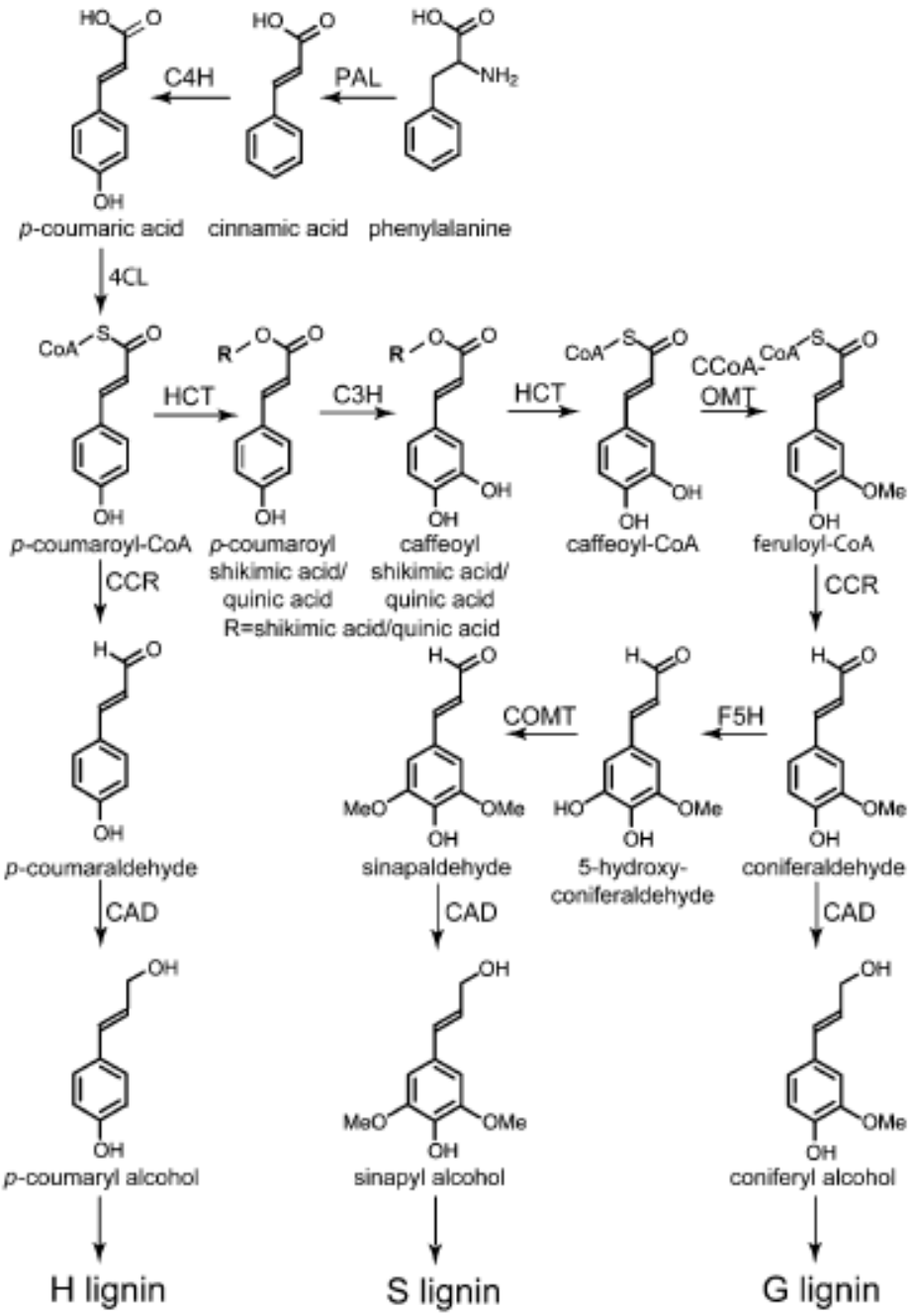


Figure 1.9 The current views of lignin biosynthetic pathway (After Boerjan *et al.*, 2003). The enzymes involved in the pathway; PAL, phenylalanine ammonia lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate-CoA ligase; C3H, coumarate 3-hydroxylase; HCT, hydroxycinnamoyl CoA: shikimate hydroxycinnamoyl transferase; CCoAOMT, caffeoyl CoA 3-O-methyltransferase; CCR, cinnamoyl CoA reductase; F5H, ferulate 5-hydroxylase; COMT, caffeic acid 3-O-methyltransferase; CAD, cinnamylalcohol dehydrogenase.

1.2.3 D-Glucose

D-glucose is one of the main products of photosynthesis and a simple monosaccharide found in plants. It is also known as dextrose and has a general formula of $C_6H_{12}O_6$. All monosaccharides contain one or more asymmetric carbon atoms. Because of this, they occur in optically active isomeric forms. If the hydroxyl group on the asymmetric carbon is on the right in the projection formula, the sugar is the D isomer (Figure 1.10 A). Most of the six carbon sugars (hexoses) of living organisms are D isomers. The ring forms of D-glucose are called as α-D-glucopyranose and β-D-glucopyranose (Figure 1.10 B, C). Glucose is building blocks of sucrose, maltose, and lactose (disaccharides). It is used not only as a precursor of non-structural polysaccharide like starch, but also used as a precursor of important structural cell wall polysaccharides such as cellulose and hemicellulose. While cellulose and starch contain only D-glucose monomers in their structure, hemicellulose contains not only glucose, but also most of D-pentose and D-hexose sugars (Collins and Ferrier, 1995; Aspinall, 1985; Fukuda and Hindsgaul, 1994; Lehmann, 1998; Morrison and Boyd, 1992; Pigman and Horton, 1980; Lehninger, 2008).

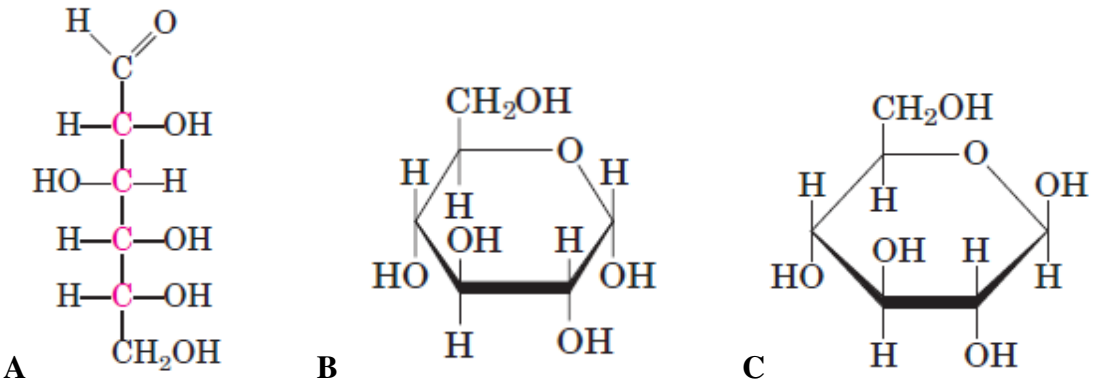


Figure 1.10. A. D-Glucose, B. α–D-glucopyranose, C. β–D-glucopyranose. (After Lehninger, 2008)

1.2.4 Recent Studies

For compensation of significant wood demand and for reducing pressure on natural forests, producing more wood with high quality from plantations has gained importance. Together with public concern related with loss of biodiversity and the increase in the world population, recent studies have emphasized the breeding of improved cultivars of fast-growing trees like poplar (Li *et al.*, 2008; Yuan *et al.*, 2006). The International Poplar Commission (IPC) of the Food and Ağrıculture Organization of the United Nations and the International Union of Forestry Research Organizations (IUFRO) comprise many working groups which aim to promote the cultivation, conservation and utilization of poplars and willows.

Understanding the physiological and morphological components of yield, the inheritance of quantitative and qualitative traits related with quality of wood, fitness and so forth are greatly important for domestication process (Boarjan, 2005; Stanton, 2009). Since the *Populus* genome was sequenced completely, an access of wide range of poplar genomic data like microarray is available today. These shared genetic materials, field measurements, clonal plantation trials and other approaches have been paving the way for more detailed studies like transformation of poplars biogenetically for high yield and so on (Tuskan *et al.*, 2006).

In recent years, scientists and investors have been focusing on the utilization of renewable energy sources because of global warming, decrease in fossil fuels, and severe fluctuation in the global oil market (Asif and Munuer, 2007; Gray *et al.*, 2006; Koonin, 2006; Yuan *et al.*, 2008). There are also increases in national funds for improvement of biomass crops, including *Populus* (Rubin, 2008; Schubert, 2006).

Biofuel production includes three main steps; assembling of biomass, degredation of cell wall polymers, cellulose and hemicellulose into their monomers by pretreatments and saccharification process, and finally obtaining ethanol from these sugars by fermentation. Despite the fact that lignin is one of the most important cell wall polymers, it represents problem for biofuel production since it inhibits saccharification process (Sticklen, 2008; Weng *et al.*, 2008a; Mansfield, 2009). Besides that it is also undesirable polymers in the pulp and paper making industry in

which lignin must be removed from wood with noxious chemicals. Moreover, this removing process pollute the whole process and consumes a large amount of energy (Walker, 1993; Hatfield and Fukushima, 2005). Because of these reasons, many studies aimed to obtain improved genotypes with high cellulose/ low lignin content or to change the structure of lignin to response chemical degradation easily and to remove it with fever chemicals (Baucher *et al.*, 2003; Anterola and Lewis, 2002).

Lignin and cellulose biosynthesis modification by genetic transformation, gene-silencing techniques, and down regulation of certain enzymes like cinnamyl alcohol dehydrogenase (CAD) can be given as examples to certain approaches in *Populus* (Chen and Dixon, 2007; Halpin and Boerhan, 2003; Huntley *et al.*, 2003; Jing *et al.*, 2004; Kirst *et al.*, 2004; Kumar *et al.*, 2001; Li *et al.*, 2003; Novaes *et al.*, 2009; Pilate *et al.*, 2002; Park *et al.*, 2004; Ralph *et al.*, 2006a; Shani *et al.*, 2004; Suziki, 2006). There are also many forward and reverse genetic mutation studies to understand the function of specific genes related with wood and cell wall formation (Dixon and Reddy, 2003; Ralph *et al*., 2006a, b; Davis, 2008). Furthermore, there are some field trials to examine the response of *Populus* to stress conditions in terms of growth and wood composition (Novaes *et al.,* 2009; Meilan *et al.*, 2002; Hawkins *et al.*, 2003).

In Turkey, The Poplar and Fast-Growing Forest Trees Research Institute, İzmit has been carrying out studies mainly on an improvement of the best industrial cultivars, *in situ,* and *ex situ* conservation under the framework of the European Forest Genetic Resources Program (EUFORGEN) (Toplu and Kucukosmanoglu, 2003; Toplu, 2005).

While *in situ* conservation studies have been carried out in eastern Anatolia region, 310 European black poplars chosen from all over Turkey were transferred to Ankara, Erzurum and İzmit to establish clone banks as an *ex situ* conservation effort. From these clone banks, 297 European black poplar trees along five commercially registered clones were chosen in and transferred to the Behiçbey Nursery in Ankara to estimate their growth performance and to characterize them genetically.

In wood processing industry, lignin and cellulose content analysis are routinely carried out to understand wood quality and to estimate chemicals required in pulping and bleaching process. Also, they are performed to characterize the lignocellulosic materials. Beside that, since dissolved lignin present in the waste water can bring about pollution dramatically, there is a great importance of measuring an acid soluble lignin content in aqueous solution.

Our study primarily aims to provide additional data for characterization of clones in terms of cellulose, lignin, and glucose content to evaluate the quality of wood and to choose the best clones for future poplar breeding studies.

CHAPTER 2

MATERIAL AND METHODS

2.1 Plant Material and Sampling

As an *ex situ* conservation program, two hundreds and ninety seven *Populus nigra* clones were collected from all over the Turkey in the past 60 years by foresters. They were planted in the Behiçbey Nursery, Ankara with the collaboration of the Central Anatolia Forest Research Institute (Ankara) and the Institute for Poplar and Fast Growing Forest Tree Species (İzmit) of the Ministry of Forestry and Water Affairs. Clones were grouped according to their origins with respect to the seven geographical regions of Turkey. The geographic information on the studied European black poplar clones are provided in detail in Appendix A. All clones were grown together under the same environmental conditions in the Behiçbey Nursery, Ankara and were harvested at the same time period and physiological stage (Yıldırım, 2013). The Sampling was carried out between 27.06.2012 and 29.06.2012 from new branches of three years old trees. Branches were chosen above 150 cm from the ground. About 30 cm below the tip of a new branch, 15 cm long pieces were cut. All branch pieces had approximately 2 cm diameter. Each clone was represented in the experiment with six individual trees (ramet), collected from three replications (two ramets per clone in each replication). Number of clones used for sampling was presented in Table 2.1. Samples were placed in an ultra-low temperature freezer (New Brunswick Scientific, model U410) for measuring enzyme activity related with cellulose and lignin biosynthesis for subsequent studies later. Homogenization of specimens was performed by use of liquid nitrogen inside a chilled mortar. After grinding, the samples were stored at -80 ^{o}C. To estimate lignin and glucose content, wood meals were also oven dried for an hour.

Table 2.1. Distribution of European black poplar clones with respect to geographic regions that were in The Behiçbey Clone Bank in Ankara

Regions and their codes	Number of clones	Latitude (range)	Longitude (range)	Altitude (m)
Central Anatolia (Region 1)	81	37˚52'- 39˚57'N	32˚35'- 32˚54'E	1205
Eastern Anatolia (Region 2)	57	38˚25'- 39˚57'N	38˚20'- 41˚15'E	1829
Aegean (Region 3)	20	37˚42'- 38˚45'N	29˚02'- 30˚33'E	715
Black Sea (Region 4)	34	40˚15'- 40˚40'N	36˚30'- 35˚50'E	1163
Mediterranean (Region 5)	11	37˚05'- 37˚37'N	36˚10'- 36˚53'E	1027
Southeastern Anatolia (Region 6)	10	37˚06'- 37˚46'N	27˚23'- 38˚17'E	748
Marmara (Region 7)	20	37˚47'- 40˚05'N	30˚30'- 30˚05'E	280
Foreign (Region 8)	18	Unknown	Unknown	Unknown
Open Pollinated (Region 9)	20	Unknown	Unknown	Unknown
Unknown (Region 10)	24	Unknown	Unknown	Unknown
Total	297			

2.2 Estimation of Cellulose Content

Cellulose content of the branches was determined from 0.125 g freeze-dried samples according to the standard procedure described by Uppdegraf (1969). All chemicals were obtained from Sigma-Aldrich (Germany). The powders were digested by 750 µl acetic acid/nitric acid reagent to remove lignin, hemicellulose and xylosans inside of 15 ml falcon tubes. Acetic/nitric reagent was prepared by mixing 150 ml 80% acetic acid and 15 ml concentrated nitric acid. After stirring 2 minutes, loosely capped tubes were placed in a boiling water bath for 30 minutes. Following that they were centrifuged for 10 minutes at 5000 rpm. Supernatants were discarded carefully to prevent disruption of the solid cellulose found at the bottom of the tube. The remaining residue was washed with distilled water to remove acetic/nitric reagent completely and centrifuged again for 5 minutes at 5000 rpm. After discarding supernatant, 2.5 ml 67% sulphuric acid was added to the remaining residue and allowed to stand for an hour at room temperature. During this time, tubes were vortexed twice to assure complete degradation of cellulose. From these tubes, 250 µl of solutions were taken and transferred to 50 ml falcon tubes which include 25 ml distilled water and they vortexed for a minute. Inside of 15 ml falcon tubes, 250 µl of this diluted solution, 1 ml distilled water, and 2.5 ml cool anthrone reagent were mixed well. Anthrone reagent was prepared by dissolving 0.2 g anthrone with 100 ml concentrated 95% H_2SO_4. It was chilled 2 hours in refrigerator prior to use so that it could be utilized freshly. Finally, loosely capped tubes were heated in boiling water bath for 10 minutes and cooled rapidly. By using 96 well Epoch Elisa Micro Plate Reader (Biotech, France), the color range was measured at 630 nm in triplicate by using cold anthrone reagent and distilled water as blanks.

2.2.1 Preparation of Cellulose Standard

To prepare the stock standard of cellulose, 25 mg pure cellulose (Sigma-Aldrich, Germany) was added into 2.5 ml 67% sulphuric acid and allowed to stand for an hour. Tubes were vortexed twice during this time. 250 µl of solution was diluted to 25 ml with distilled water to contain 100 µg cellulose / ml. Volume of series of 25 µl,

50 µl, 100 µl, 200 µl, 400 µl, 600 µl, 800 µl, 1000 µl, and 1250 µl were taken from this diluted stock solution. Final volumes were brought to 1250 µl with distilled water. Finally 2.5 ml cool anthrone reagent was added and vortexed for a minute. The tubes were placed in boling water bath for 10 minutes. After cooling rapidly, the color range was measured at 630 nm in triplicate. Anthrone reagent was used as a blank.

2.3 Estimation of Acid - Soluble Lignin Content

Acid soluble lignin content was determined from 0.2 g oven-dried samples according to the standard TAPPI Test Method T250 "Acid-Soluble Lignin in Wood and Pulp" which is a modification of the classic "Klason Lignin" determination (Dence, 1992). All chemicals were obtained from Sigma-Aldrich (Germany). Samples were first treated with 3 ml 72% cold H_2SO_4, added gradually. After stirring the test tubes for a minute, they were allowed to stand for 2 hours at room temperature and vortexed once an hour. Having transferred the content of the test tubes to baby jars which include 50 ml distilled water, the remaining residue at the test tube was rinsed with extra distilled water and transferred to baby jar. Final volume of solution was brought to 115 ml to dilute the sulphuric acid 3%. Baby jars were loosely capped and autoclaved for 30 minutes to hydrolyze the polysaccharides to soluble fragments. Baby jars were allowed to cool down to room temperature before removing the caps and settle the solution until it is clear. The supernatant solution which includes acid soluble lignin was filtered through Whatman number three filter paper to 100 ml Erlenmeyer flask. To obtain the absorbance range between 0.2 and 0.7, a sample of the clear filtrate was diluted as needed. Inside of 96 well plate, 30 µl sample and 240 µl 3% H_2SO_4 were mixed by pipetting. The absorbance at 205 nm was measured in triplicate by using the same solvent (3 % H_2SO_4) as a blank.

2.4 Estimation of D-Glucose Content

The determination of D-Glucose content was carried out by some modification and combination of TAPPI Test Method T250 “Acid-Soluble Lignin in Wood and Pulp” and the standard procedure which was described by Hedge and Hofreiter (1962). All chemicals were obtained from Sigma-Aldrich (Germany). The supernatant obtained from 0.2 g sample by hydrolysis with 3 ml 72% H_2SO_4, dilution to 115 ml with distilled water, autoclaving for 30 minutes, and filtering through Whatman number three filter paper, respectively was used to measure total carbohydrate content. Exactly one hour later after autoclave stage, , 4 ml cold anthrone reagent above 1 ml of supernatant solution was added and vortexed for a minute inside the 15 ml falcon tube. Afterwards, falcon tubes were placed inside boiling water bath for 8 minutes. Having cooled rapidly, the color range was measured at 630 nm in triplicate by using 96 well Epoch Elisa Micro Reader plate by using cold anthrone reagent as blanks.

2.4.1 Preparation of D-Glucose Standard

To prepare the stock standard, 0.2 g D-glucose was dissolved inside of 115 ml distilled water. Next, to prepare working standard, 10 ml of stock solution was completed to 100 ml with distilled water to contain 174 µg glucose / ml. Volume series of 50 µl, 100 µl, 200 µl, 400 µl, 600 µl, 800 µl, and 1000 µl were taken from this solution. Final volumes were brought to 1000 µl with distilled water. Finally, 4 ml cool anthrone reagent was added and vortexed for a minute. The tubes were placed in a boling water bath for 10 minutes. After cooling rapidly, the color range was measured at 630 nm in triplicate. Anthrone reagent was used as a blank.

2.5 Analysis of Data

2.5.1 Determination of Cellulose Content

To calculate the quantity of cellulose, a standard graph was developed by plotting concentration of the cellulose standard on the X-axis versus absorbance at 630 nm on the Y-axis. The amount of cellulose present in the sample was calculated from the standard. Standard conditions, amount of cellulose, and absorbance read at 630 nm were given at Table 2.2. Also, the standard graph was provided at Figure 2.1.

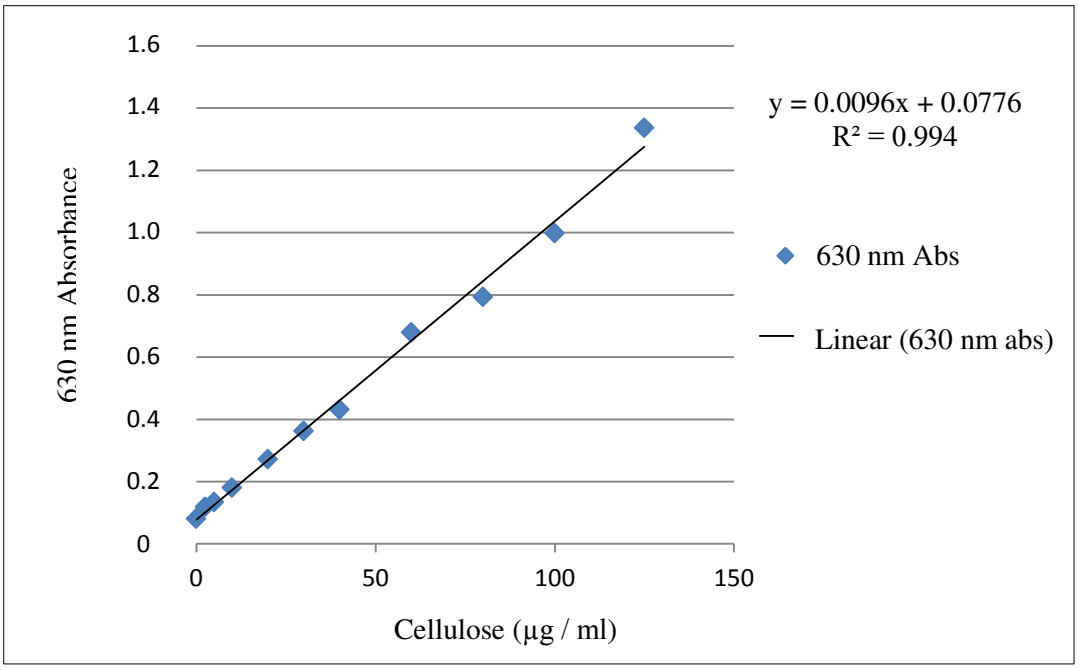


Figure 2.1 The standard calibration graph for the cellulose content

Table 2.2. Standard conditions, amount of cellulose, and absorbance values

Stock Solution (µl)	dH_2O (µl)	Cellulose (µg/ml)	A 630 nm
0	1250	0	0.0803
25	1225	2.5	0.1185
50	1200	5	0.1342
100	1150	10	0.1801
200	1050	20	0.2717
300	950	30	0.3622
400	850	40	0.4304
600	650	60	0.6784
800	450	80	0.7930
1000	250	100	0.9972
1250	0	125	1.3361

2.5.2 Determination of Acid-Soluble Lignin Content

The acid-soluble lignin content of the black poplar samples was calculated from the following expression of Beer-Lambert Law:

$$A = \varepsilon \times l \times C \qquad (1)$$

A = Absorbance (at 205 nm)

l = light path in cm = 1 cm

ε = absorptivity in $1g^{-1}cm^{-1}$ = 110 $1g^{-1}cm^{-1}$

C = lignin concentration in g / L.

Therefore,

$$Lignin\ Concentration = C = {}^{A}/_{(\varepsilon \times l)} \times D \qquad (2)$$

D = dilution factor

Where

$$D = {}^{Vd}/_{Vo}$$

V_d = volume of the diluted filtrate

V_o = volume of original filtrate taken

The amount of acid- soluble lignin content in the dry wood samples was calculated from the expression below:

$$Lignin,\ (\%) = {}^{C\ x\ V\ x\ 100}/_{1000\ x\ W} \qquad (3)$$

V= total volume of the filtrate, 115 ml

W= oven-dry weight of wood sample, g.

C = lignin concentration in g / ml.

2.5.3 Determination of D-Glucose Content

A standard calibration graph was developed by plotting concentration of the D-glucose standard on the X-axis versus absorbance at 630 nm on the Y-axis. The standard graph was provided at figure 2.2. From the standard glucose graph, the quantity of glucose in the sample was calculated. Standard preparation conditions and amount of D-glucose versus absorbance read at 630 nm were given at Table 2.3.

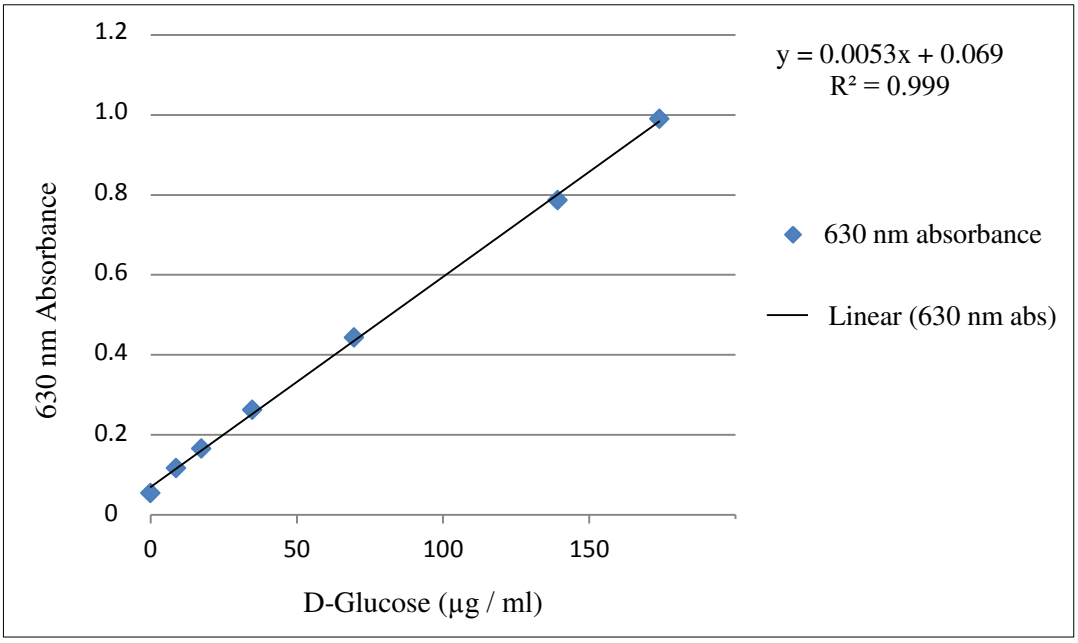


Figure 2.2. The standard calibration graph for the D-glucose content

Table 2.3. Standard conditions, amount of D-glucose, and absorbance values

Stock Solution (µl)	dH_2O (µl)	D-Glucose (µg/ml)	A 630 nm
0	1000	0	0.0543
50	950	8.7	0.1167
100	900	17.4	0.1653
200	800	34.8	0.2623
400	600	69.6	0.4435
800	200	139.2	0.7863
1000	0	174	0.9897

2.5.4 Statistical Analysis

Univariate analyses of each trait (cellulose, acid-soluble lignin, and D-glucose content) were performed using the SAS® System for Mixed Models (Littell *et al.*, 1996) to separately account for the different sources of variation from our experiment.

In a first stage, the normal distribution of the three chemical phenotypes was evaluated. No transformations are needed for lignin and glucose content since these trait were distributed approximately normal. Log transformation was performed for cellulose trait. An ANOVA was carried out for each trait using the following model

$$y_{ijk} = \mu + b_k + c_i + r_{j(i)} + e_{ijk}, \qquad (1)$$

where y_{ijk} is the response measured to the *j*-th ramet of the *i*-th clone within the k-th block, μ is the overall mean, c_i is the random effect of *i* th clone, r_{ji} is the random effect of *j*-th ramet in clone i, b_k is the random effect of block *k*, e_{ijk} is the random error in block *k* on clone *i and ramet j*. Additionally, variance components were

estimated by REML using the GLM and Varcomp procedures in the software SAS 9.2 (SAS Institute, Cary, NC, USA).

Individual broad sense heritability (H^2) was estimated by the equation (2) based on the method developed by Zamudio *et al.* (2008)

$$H^2 = \sigma^2_c / (\sigma^2_c + (\sigma^2_e/r)) \qquad (2)$$

where, r = 3, σ^2_c, and σ^2_e represents the variance due the clone, and residual, respectively.

Also, the height and diameter data were used to estimate correlation among cellulose, lignin, glucose and growth traits. Multiple comparisons of clones weighted by region were carried out one way ANOVA, Post-Hoc tests using Dunnett's T3 method. Statistical tests were considered significant at $P < 0.05$. Error bars in all graphs refer to 95 % significant confidence intervals. Clones with maximum and minimum mean values of traits were determined by the data analysis tools of Excel (Microsoft, WA).

CHAPTER 3

RESULTS

In early summer, we established a large collection of biological samples from one year old branches of 1782 trees (3 replicates x 297 European black poplar genotypes/clones x 2 ramets) which were grown in an outdoor forest nursery in Behiçbey, Ankara. However, twelve clones (72 trees) were excluded from the study. Since cellulose and lignin are accepted as main constituents in the plant cell wall, the clones were analyzed mainly for cellulose and lignin contents (Hajaligol *et al.*, 2001). Also, D- Glucose content was measured to understand carbon allocation. The study was also combined with data of height and diameter which were measured in previous studies of our laboratory (Yıldırım, 2013). Except cellulose, data of all traits followed a normal distribution (Figures 3.1.A, B and Figures 3.2.A, B). Because of that reason, log transformation of cellulose data was performed before statistical analyses.

3.1 Cellulose Content

According to the standard procedure described by Uppdegraf (1969), sample hydrolyzates were analyzed by Elisa Microplate Reader (Biotech, France). The 630 nm absorbance varied from 0.17 to 0.83 which were widely variable among the 285 tested clones. Cellulose content was satisfactory for all clones, although a small overestimation might have occurred because of possible hindrances of the colorimetric method and weighing error. The cellulose content ranged from 9.14 µg / ml to 123.49 µg / ml. The average cellulose content of clones was 21.8 µg / ml ± 16.29.

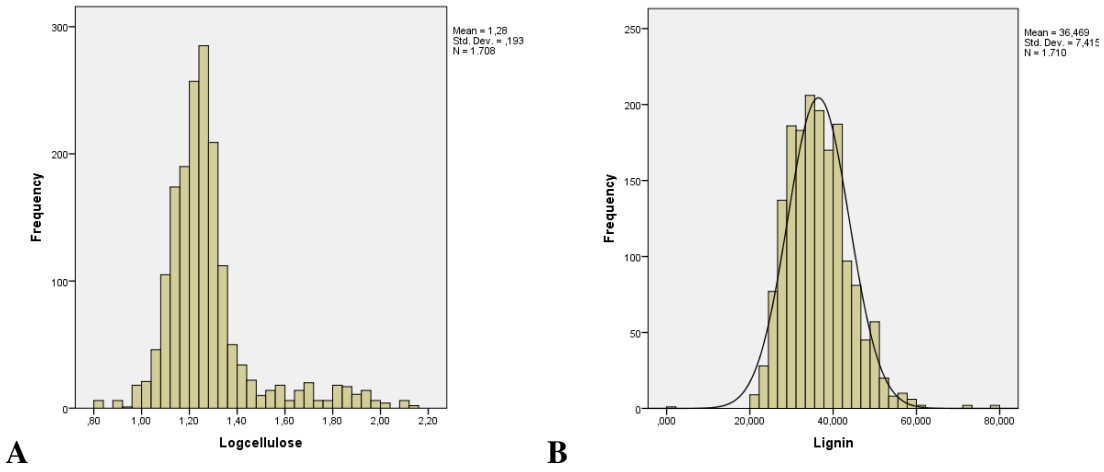


Figure 3.1 Frequency distributions of cellulose (A), and lignin (B) traits in studied European black poplar clones

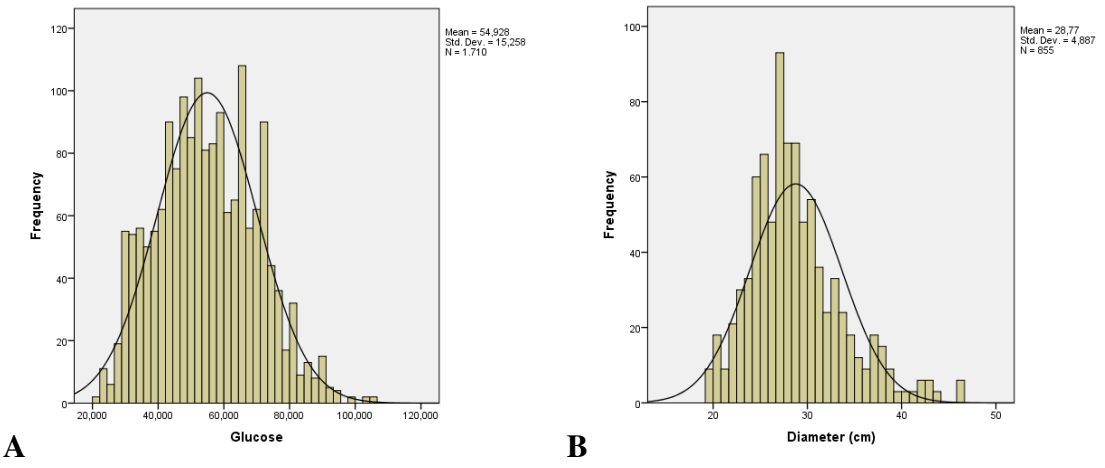


Figure 3.2 Frequency distributions of glucose (A) and diameter (B) traits in studied European black poplar clones

According to mean values, we identified individual clones that exhibited unusually high and low cellulose contents (Figures 3.3 and 3.4). Also, the clones were evaluated in terms of their cellulose to lignin ratio. The clones with the greatest cellulose to lignin ratio were the same with the clones which had the highest cellulose contents. These clones as a whole had a very high cellulose content (56.292 µg / ml ±13.38) when we compare them with the average of 285 clones. Seven of them were observed among the twenty clones which were also with high height and diameter growth values (62172, N03377, N90062, N90102, N91075, N92132, and N92217). Furthermore, three clones with lowest cellulose content were 641410, N92169, and N03366. These were also among the clones with low height and diameter growths.

Pairwise correlations among traits using Pearson correlation coefficient approach were estimated. The correlations between cellulose content and two growth traits (diameter and height) were positive $r = 0.220$. $p < 0.01$ between cellulose and diameter growth and $r = 0.235$, $p < 0.01$ between cellulose and height growth. However, we could not detect any significant correlation between cellulose and lignin (Table 3.1).

Table 3.1. Pearson correlations between traits ($N = 855$)

	Diameter	*Lignin*	*Glucose*	*Height*	*Cellulose*
Cel/Lig	**0.23****	**-0.25****	-0.01	**0.20****	**0.95****
Diameter		**-0.12***	0.02	**0.79****	**0.22****
Lignin			**0.31****	-0.04	0.03
Glucose				0.04	0.05
Height					**0.23****

* Correlation is significant at P ≤ 0.05 level (2-tailed).
** Correlation is significant at P ≤ 0.01 level (2-tailed).

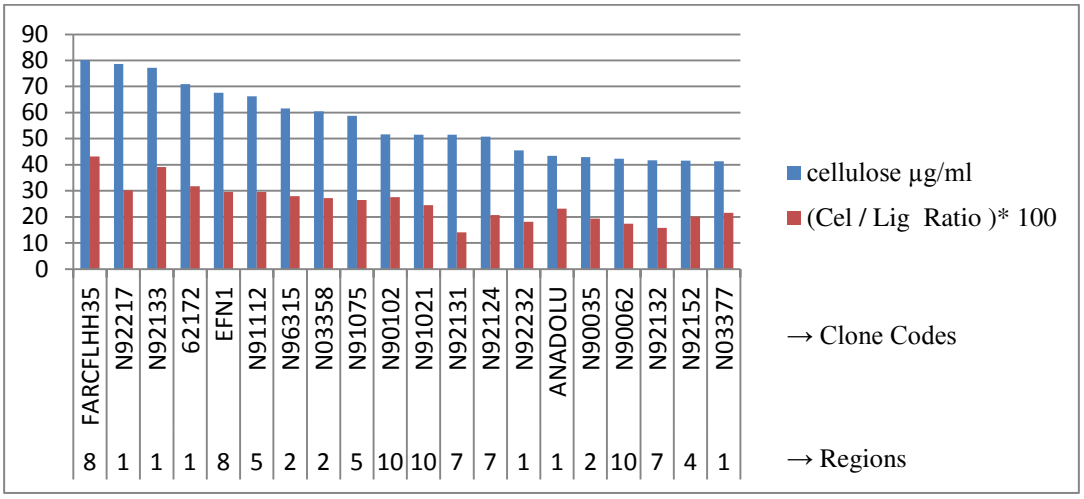


Figure 3.3 The clones with the highest cellulose contents and cellulose / lignin ratio.

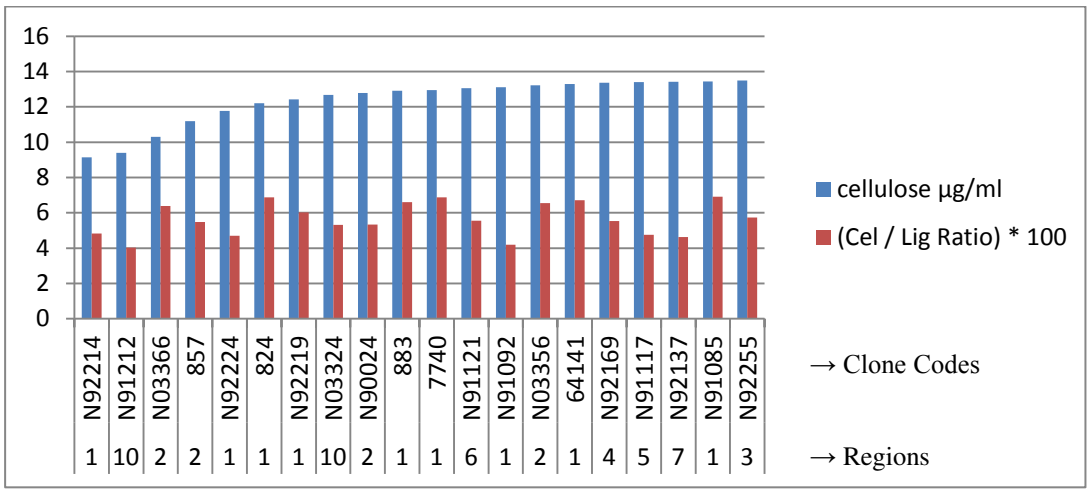


Figure 3.4 The clones with the lowest cellulose contents and cellulose / lignin ratios.

A nested analysis of variance was performed to test the effects of each factor (region, clones) on variation of cellulose content. The effect of region on cellulose contents was nonsignificant at the $p < 0.05$ level. On the other hand, a statistically significant difference was detected among clones within regions ($p < 0.001$). According to estimation of components variances, great portion of the total variance was due to errors in cellulose trait. There was also considerable variation among clones within regions (15.11 %). Only 0.09 % of variance came from regions (Table 3.2). Estimated broad sense heritability for cellulose content for the studied clones was calculated as 0.34. This indicates that there is a considerable genetic control of cellulose content in *P. nigra* populations.

Table 3.2. Analysis of variance for cellulose content.

Source	*Degrees of freedom*	*Mean squares*	*Components of variance*
Replicate	2	457.88 ^{ns}	- ^{a}
Region	9	344.80^{ns}	0.09 %
Clone within region	274	326.68*	15.11 %
Error	530	217.07	84.80 %

* Significant at $p \leq 0.001$

ns. not significant at $p < 0.05$.

a not estimated

Multiple comparisons using the Dunnett's T3 test indicated that the mean scores for the clones from Aegean, Mediterranean, foreign, and open pollinated sources (17.15 ± 5.77; 26.46 ± 20.57; 35.67 ± 27.35, and 18.68 ± 7, respectively) were significantly different from other six sources with an average of 21.7 ± 16.17 at 95 % confidence interval. On the other hand, many clones collected from Central Anatolia and Eastern Anatolia regions were observed as outliers in boxplot graph after weighted by region because of standard deviation range (Figure 3.5).

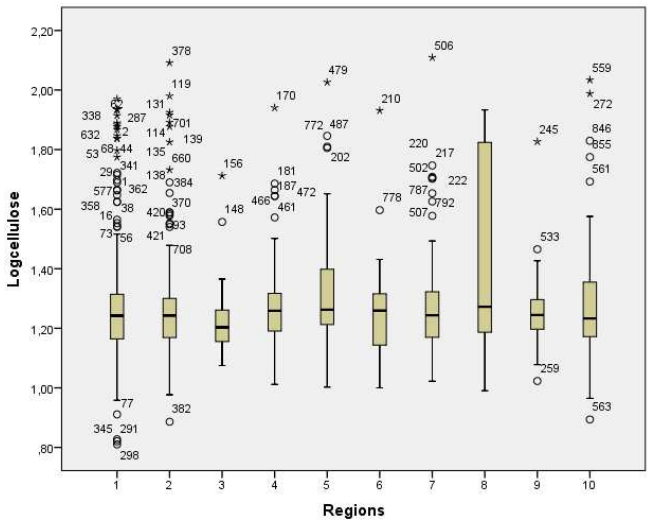


Figure 3.5 The boxplot graph cellulose content in studied *P. nigra* clones

3.2 Acid Soluble Lignin Content

The autoclaved hydrolyzates were diluted by a factor of 9 to bring the final concentration of H_2SO_4 to 3% and analyzed by Elisa Microplate Reader with UV plate. The concentration of lignin was derived from equation (2) with absorptivity

values of 110 $lg^{-1}cm^{-1}$ (ε) for absorbance at 205 nm (Tappi Method UM250). Lignin contents were highly variable among the 285 tested clones and showed normal distribution.

Acid soluble lignin ranged from 13.24 µg / ml (1.33 % of dry weight) to 48.86 µg / ml (3.36 % of dry weight) as expected. The average lignin content of clones was 23 µg / ml ± 4.6. Based on these results, we recognized that particular clones manifested high and low acid soluble lignin content (Figures 3.6-3.7). Among the twenty clones with the highest lignin contents, only one of them were observed among the clones which had the lowest height and diameter values (N92167). On the other hand, seven clones with the lowest lignin contents were observed among the clones with highest height and diameter values (821, 62160, 62191, Ata1, Çubuk 1, Çubuk 2, and Geyve). Twenty clones with the lowest lignin content as a whole had an average of 17.68 µg / ml ± 1.02.

Pairwise Pearson correlations were estimated among the studied traits. A low negative correlation (r = -0.121. $p < 0.05$) between lignin content and diameter growth was observed. However, we observed positive correlation between lignin and glucose contents (r = 0.314, $p < 0.01$). Surprisingly, we could not detect any correlation between cellulose and lignin contents as explained earlier (Table 3.1).

To control the additive effect of each factor on lignin content variation of *P. nigra* clones, we performed a nested analysis of variance. Statistically significant main effects for lignin content were observed among ten regions ($p < 0.001$). On the other hand, there was no statistically significant difference among clones within regions ($p < 0.05$). Only 3.47% of total variance came from clone and region interactions. The large component of total variance was due to error (93.2 %). The component of variance due to regions was small and made up 3.33% (Table 3.3). The broad sense heritability for lignin content was 0.1.

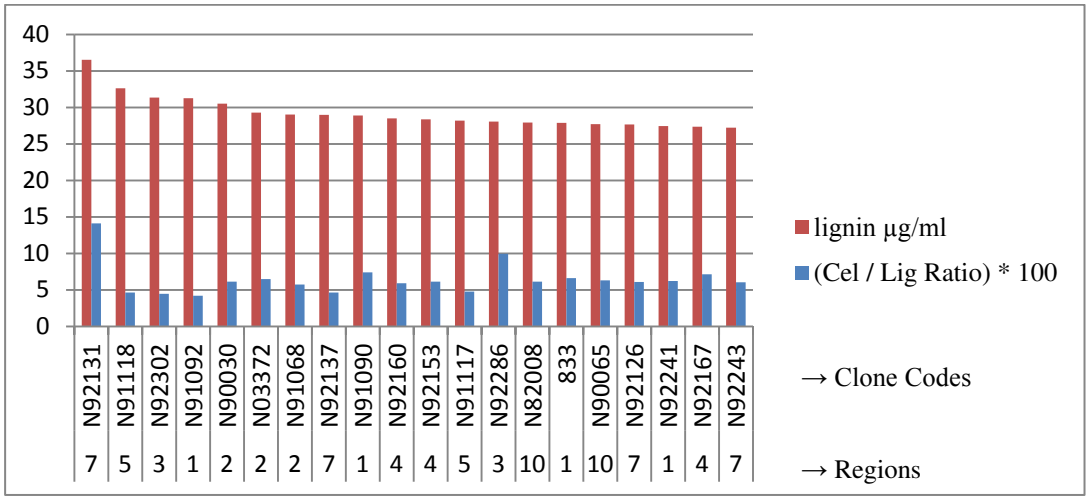


Figure 3.6 The clones with the highest lignin contents and the cellulose/lignin ratio.

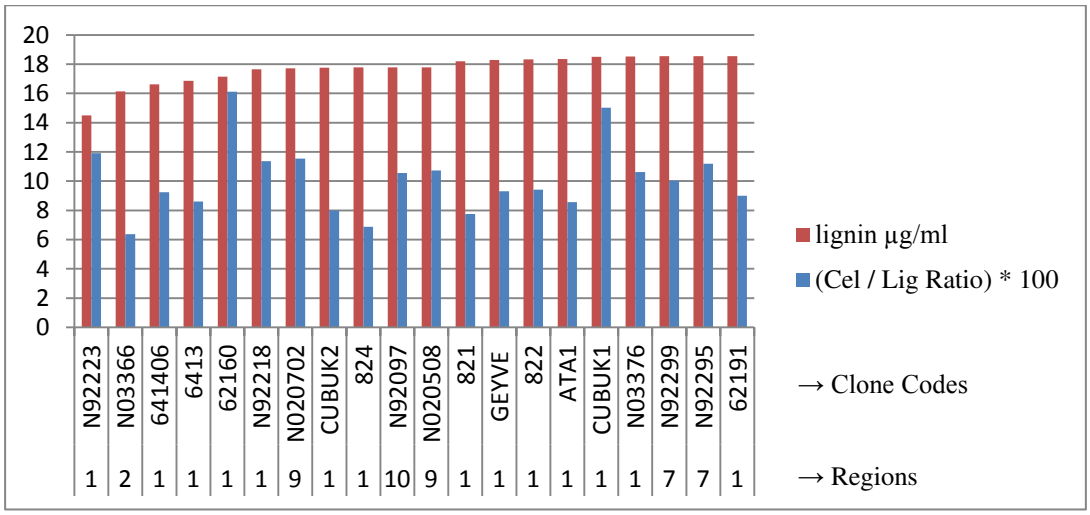


Figure 3.7 The clones with the lowest lignin contents and the cellulose / lignin ratio.

Table 3.3. Analysis of variance for lignin content.

Source	*Degrees of freedom*	*Mean squares*	*Components of variance*
Replicate	2	265.18 ^{ns}	- ^{a}
Region	9	72.07*	3.33 %
Clone within region	275	21.09^{ns}	3.47 %
Error	530	19.06	93.20 %

* Significant at $p \leq 0.001$

ns. not significant at $p < 0.05$.

a not estimated

Post-hoc comparisons using the Dunnett's T3 test indicated that the mean lignin values for Eastern Anatolia, Aegean, and Black Sea regions were almost same with an average of 23.289 ± 4.36 at 95 % confidence interval. Similarly, the same results were detected between foreign and open pollinated clones with an average of 21.24 ± 3.83 and also between Central Anatolia and Southeastern Anatolia regions 21.91 ± 4.38. On the other hand, Mediterranean and Marmara regions had the mean score of 24.59 ± 4.89 which was significantly different from the average of all other regions (22.79 ± 4.6) (Figure 3.8).

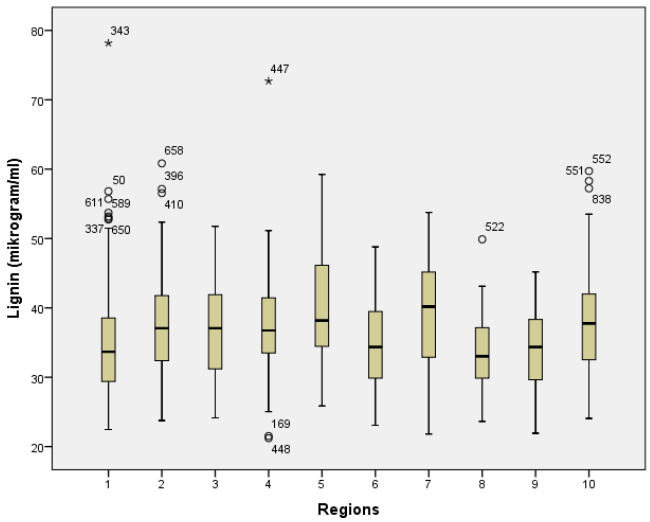


Figure 3.8 The boxplot graph of lignin content of the studied *P. nigra* clones.

3.3 D-Glucose Content

The D-glucose content ranged from 13.32 µg/ml to 65.43 µg/ml in the studied clones. The average glucose content of clones was 35.02 µg/ml ± 9.7. Based on these results, we determined distinctive clones that showed high and low glucose contents (Figures 3.9 and 3.10). Three clones with the highest glucose content was observed among clones which also had the highest height and diameter growth as well (881, 62160, and N91075). Similarly, only one clone with low glucose content (8511) was detected among the clones with lowest height and diameter growths. Particularly, there was a positive correlation between glucose and lignin content which was r = 0.314 ($p < 0.01$). We did not observe any correlation between glucose and cellulose as well as other growth traits (Table 3.1)

The same nested analysis of variance, multiple comparisons and pairwise correlations were performed to test glucose content of *P. nigra* clones. Significantly different main effect for glucose content was monitored among clones within regions ($p < 0.001$) (Table 3.4). According to estimation of variance components, great portion of the total variance was due to error (86.78 %). However, there was

considerable variation due to clones within regions (12.94 %). The broad sense heritability for D-glucose content was estimated as 0.3 which was very promising for a selection program based on D-glucose content of clones.

Table 3.4. Analysis of variance for D-Glucose content.

Source	*Degrees of freedom*	*Mean squares*	*Components of variance*
Replicate	2	1315.76	- [a]
Region	9	126.99[ns]	0.29 %
Clone within region	275	107.86[*]	12.94 %
Error	530	75.58	86.78 %

* Significant at $p \leq 0.001$.

ns. not significant at $p < 0.05$.

a not estimated

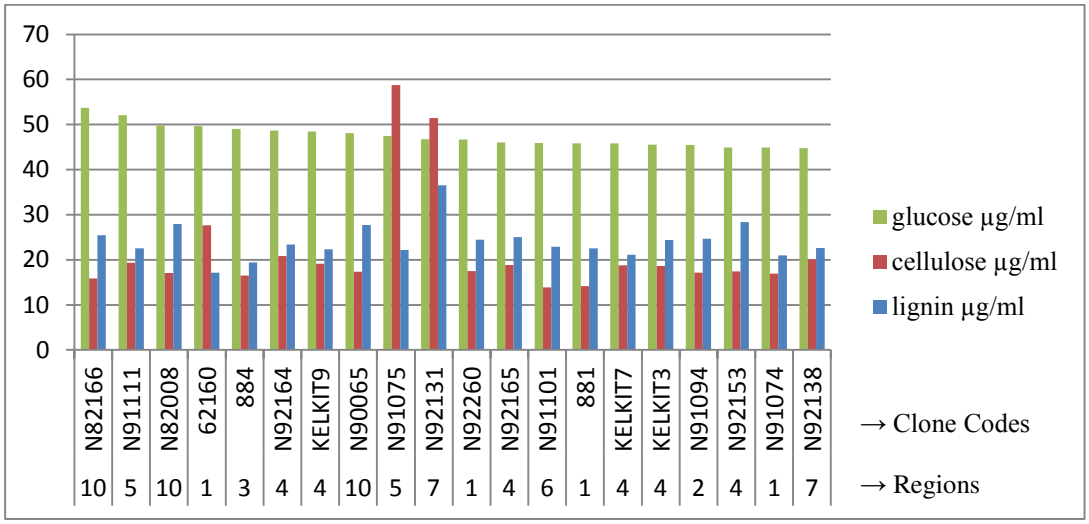


Figure 3.9 The clones with the highest glucose contents and cellulose and lignin contents.

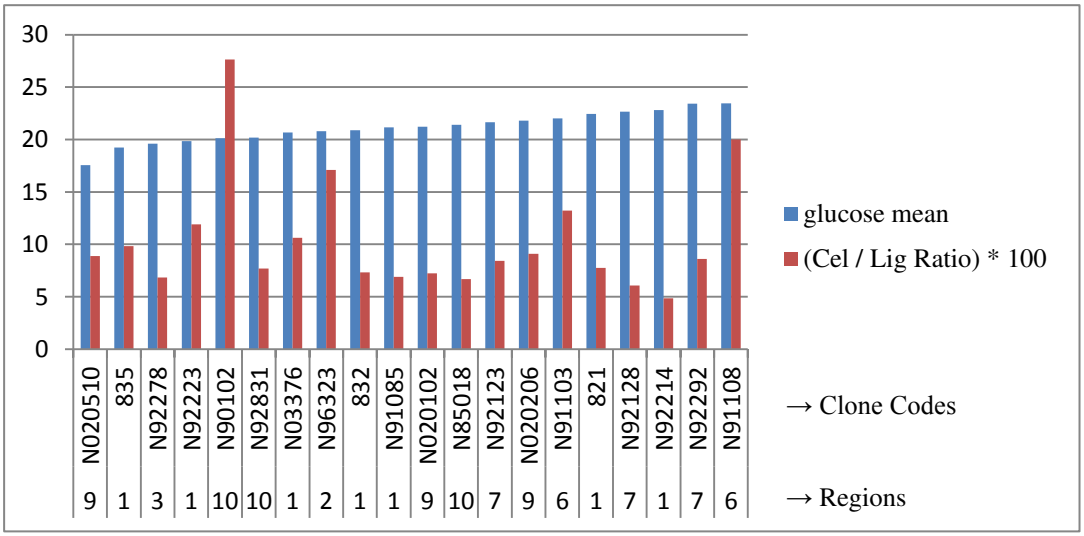


Figure 3.10 The clones with the lowest glucuse contents and the cellulose/lignin ratio.

Post-hoc comparisons using the the Dunnett's T3 test indicated that the mean scores for Eastern Anatolia, Aegean, Southeastern Anatolia and Marmara regions were almost same with an average of 34.81 ± 9.23 at 95 % confidence interval. On the other hand, Mediterranean and foreign regions with the mean score of 39.83 ± 8.0 were observed that was significantly different from the mean all other regions, 33.8 ± 10 (Figure 3.11).

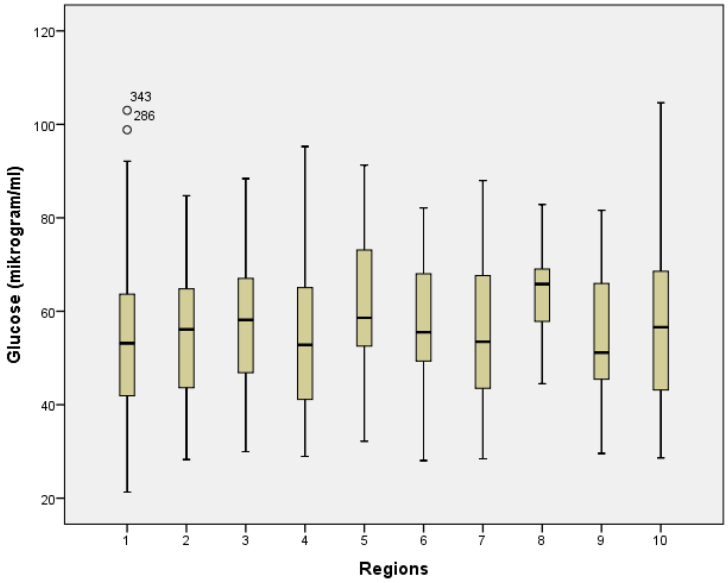


Figure 3.11 The boxplot graph of D-glucose content of *P. nigra* clones

3.4 Height and Diameter

Height and diameter data were obtained from a previous study carried out in our laboratory (Yıldırım, 2013). The height measurements ranged from 273.66 cm to 552.62 cm. The average height measurement of clones was 397.4 cm ± 44.45. Also, diameter traits for clones were observed as widely variable ranging from 19.7 cm to

46.15 cm with average of 28.77 cm ± 4.9. Moreover, two variables were strongly correlated (r = 0.796, p < .01) as expected. By using these data, we chose the best and the worst clones in terms of diameter and height and compared them with wood traits in the current study (Figures 3.12 and 3.13). As mentioned earlier, seven clones with high cellulose content and other seven clones with low lignin content were identified. These were also among the top twenty clones with respect to the highest height and diameter growths.

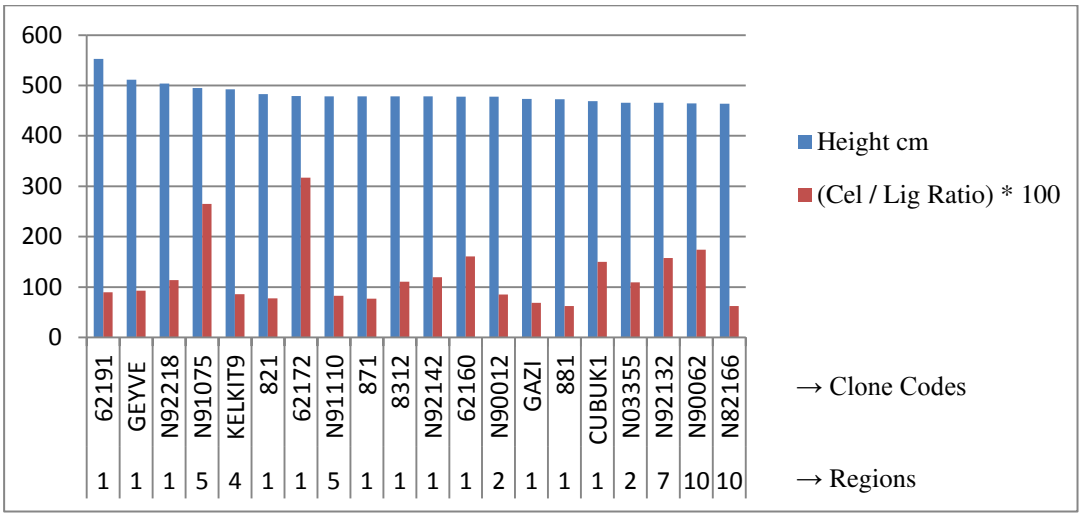


Figure 3.12 The clones with the highest height growths and the cellulose / lignin ratio.

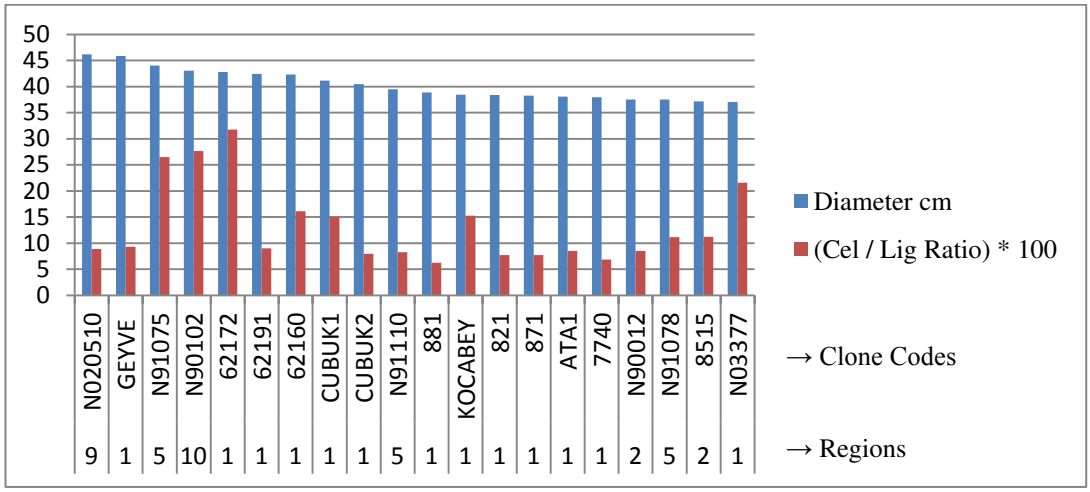
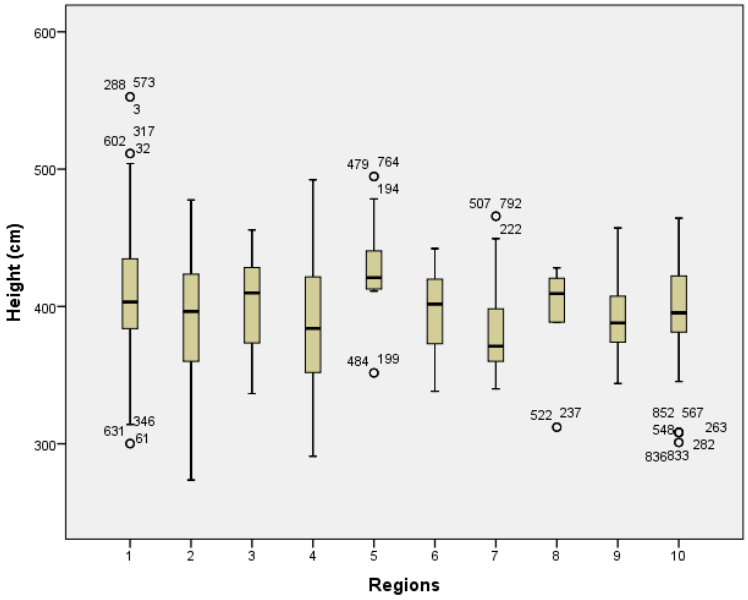
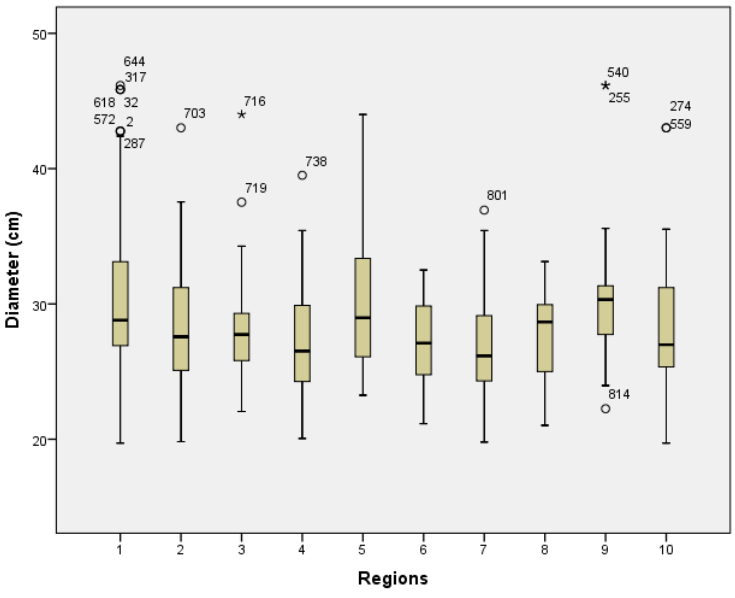


Figure 3.13 The clones with the highest diameter measurements and the cellulose / lignin ratio.

Post-hoc comparisons of height variables using the the Dunnett's T3 test indicated that the mean for the Mediterranean region (394.09 ± 45.31) was significantly different from the mean of all other regions (427.23 ± 36.0; Figure 3.14 A). In terms of diameter, post-hoc comparisons showed that the mean scores for the Central Anatolia, Mediterranean, and open pollinated regions were high (of 30.26 ± 5.2) and significantly different from the mean of all other regions (28.13 ± 4.47; Figure 3.14 B).



A



B

Figure 3.14 The boxplot graph of height (A) and diameter (B) traits of the studied clones

CHAPTER 4

DISCUSSION

This study is, to our knowledge, the first which combines analyses of the content of the two main cell wall components, cellulose and lignin along D-glucose content of wood cells in relation to growth paramaters of *Populus nigra* L. clones which were part of the large collection made in Turkey. The wide-scale sampling strategy was adopted with 285 clones x 3 replication x 2 ramets to ensure physiological comparability between genotypes and also to obtain normal distribution of traits. All poplar trees were grown under common environmental conditions at the Behiçbey Forest Nursery, Ankara.

4.1 Genetic variation

Analysis of variance allowed us an accurate estimation of the desired effects of factors such as clones and regions by controlling the source of variance due to. replicates and ramets within clones. Variations among replications as expected were nonsignificant since they shared a common environment which was quite uniform with respect to soil conditions. Moreover, when region effect was tested, it was detected as nonsignificant for cellulose and D-glucose traits. The variance components due to clones within region were significant for both cellulose (15.11%) and D-glucose (12.94%) traits illustrating there are genetic components in both traits. The study of Novaes *et al.* (2009), deailing with cellulose and lignin traits controlled for two nitrogen levels in different environments, also reported with similar results. On the other hand, only for lignin trait, statistically significant region main effects

were observed ($p < 0.001$), indicating that lignin is sensitive to environmental conditions of geographical locations. Also, only 3.47% of total variance was due to clones within region for lignin trait in the current study. Moreover Post-Hoc comparisons based on lignin trait gave more clear resolution than other traits while differentiationg regions. Additionally, estimated broad sense heritabilities which were 0.34 for cellulose, 0.10 for lignin traits, and 0.30 for glucose traits were also supported our results and gave us a clue about genetic basis of three traits. Based on these results, the selection program for high cellulose and D-glucose contents is possible on a clonal basis over regions. However, with low estimated broad sense heritability, a selection program for low lignin content does not seem feasible.

Eventhough variation within species is generally considered as relatively low in terms of morphological and physiological traits, we observed significant variation among clones within region. Recent research conducted by Barsoum *et al.,* (2004), showed that in *P. nigra,* significant variations in the occurrence of clonity can exist from one location to the next on the floodplain, based on histories of local disturbance. Also, significant differences among populations of *P. nigra* based on morphological leaf characters were reported by several other research groups (Ballian *et al.*, 2006; Gebhardt *et al.*, 2002; Kajba and Romanic, 2002). Actually, the usefulness of morphological and wood traits to evaluate genetic diversity within populations of different regions is not clear yet. There are some studies illustrated that estimating of the genetic diversity based on morphological (e.g. leaf characters) and wood traits is possible (Alba *et al.*, 2002; Storme *et al.*, 2002). On the other hand, Van Dom *et al.* (2002) argued that to estimate genetic diversity of clones which were grown in different field trials by using morphological traits is not applicable because they concluded that the differences in morphological variables are largely due to clone and environment interactions. Alternatively, significant clonal variation in three physiological components was reported by Ridge *et al.* (1986). Moreover, Ceulemans *et al.* (1988) stated that clonal variation in all three variables was observed among poplar clones. At the same time, many studies which were performed to estimate response of poplar clones to different stress conditions such as nitrogen levels, ozone, drought, etc. showed that there were significant variations among clones (Novaes *et al.*, 2009; Sherosha *et al.*, 2011; Wilkins *et al.*, 2009).

To understand region and trait interactions, we also performed Post-hoc comparisons using the Dunnett's T3 test. The most remarkable difference was found that regions five (mediterranean) and eight (foreign) formed separate groups apart from other regions for all traits. In the region eight, the highest mean values were observed for cellulose and glucose contents. Likewise, we observed the highest mean values in terms of lignin and again for glucose contents in the region five. The sixth (southeastern Anatolia) and the second (eastern Anatolia) regions were included into the same group with almost similar mean scores in terms of cellulose and glucose contents. Seperately, the first (black sea) and forth (central Anatolia) regions were grouped in the same manner. These results all together strongly supports poplar distribution hypothesis of Barsoum *et al.* (2004). It argued that distribution of poplar trees was possible consequences of asexual reproduction of same clones which were formed after floods throughout the riparian zones.

As we know, the sampling procedure is carried out randomly in long distances, but generally from the riverside. To illustrate, Fırat and Dicle rivers provide connection between Eastern and Southeastern Anatolia which were categorized in the same groups based on cellulose and glucose content comparisons of ten regions. Moreover, Smulders (2008) illustrated that duplicated neighbor clones may arise from root suckers of mature trees in close areas. Also, this grouping can be accepted as a result of human assisted migration and selection activities. This hypothesis is also supported by SSR studies (Çiftçi, 2013) in which approximately one hundred clones were observed as the same clones based on allelic combination of 12 microsatellite loci.

4.2 Correlations between traits

In our study, a negative correlation was found between lignin content and diameter, but any direct correlation between contents of cellulose and lignin in poplar clones could not detected, contrary to the results of Novaes *et.al.* (2009). However, a positive correlation was observed between cellulose and growth traits (height and diameter). Actually, comparing studies on the same clonally propagated species is

very difficult because of different sampling strategies and sample size. But, nevertheless, the study of Novaes *et al.* (2009) supports our results with respect to observing positive correlation of cellulose with growth traits like height, and negative correlation of lignin content with diameter. Similar results were also observed between cellulose to lignin ratio as well as growth traits (height and diameter). Besides, genetically modified plants showed the similar positive correlation between growth and increased cellulose content when their genes up regulated that are responsible for the production of particular enzymes related with cellulose biosynthesis (Hu *et al.*, 1999; Wu *et al.*, 1999; Yu *et al.*, 2006; Kirst *et al.*, 2004).

The negative correlation between lignin content and diameter can be evaluated as a competition for carbon allocation to cellulose and hemicellulose versus carbon allocation to lignin because these molecules are the major carbon sinks in the formation of the cell wall (Higuchi, 1997; Novaes *et al.*, 2010; Sjostrom, 1993). Whereas the monolignols are synthesized in the cytosol, lignin biosyntesis occurs in the apoplast (Whetten and Sederoff, 1995). Furthermore, monolignols are unstable and higly toxic for plants, since glycosylation is used to stabilize these precursors and to decrease their toxicity. On the other hand, this stabilization process requires extra amount of carbon source and any transport of their stable forms from cytoplasm to vacuole needs energy (Amthor, 2002). The only way to compensate these energy and carbon source requirement is shifting of carbon partitioning between lignin synthesis and biosynthesis of other cell components required for growth in sink tissues (Coleman *et al.*, 2009; Koch, 2004; Winter and Huber, 2000). As a result of increased carbon needs for lignin biosynthesis, the decrease in diameter growth can be accepted as a normal consequence of photoassimilate-partition.

A highly significant positive correlation ($r = 0.314$, $p < 0.01$) was observed between D-glucose and lignin contents. The similar positive correlations between lignin content, starch increase, and decreased growth rates in poplar hybrids (*Populus frenontii* x *angustifolia*) was reported by Harding *et al.* in 2009. Eventhough we did not perform any analysis to estimate hemicellulose and starch content, positive correlation between lignin and glucose contents might be due to a result of high

hemicellulose content or increased starch accumulation since modified and combined procedures of acid soluble lignin and D-glucose estimation allowed us to obtain whole D-glucose derived from both structural and non-structural carbohydrates. To put it differently, there was no particular treatment to remove starch from wood samples. Actually, starch contents in wood cells are incredibly low in contrast to hemicellulose and cellulose. In general, dry plants include 40-50 % cellulose, 15-25 % hemicellulose, 5-10 % other components like ash and 20-25 % lignin (Faik, 2013). On the other hand, the ray parenchyma cells of poplar wood show extraordinary changes in their carbohydrate levels during different seasons of a year (Sauter and van Cleve, 1994). In Spring, starch is synthesized and then mobilized during bud break. At the beginning of the dormant season, this starch is hydrolysed (Sauter and van Cleve, 1991). In the same way, Zeng *et al.* (2014) found that maize has almost no lignin during the vegetative growth phase, but has high lignin content during the reproductive growth phase depend on tissue maturation. There are many studies which illustrate the interaction between lignin metabolism and biosynthesis of other cell wall polymers (Abdulrazzak *et al.*, 2006; Dauwe *et al.,* 2007; Rohde *et al.,* 2004; Sibout *et al.*, 2005). To illustrate, transcriptome analysis by Andersson *et al.* (2006) indicated that there was a significant increase in both lignin and hemicellulose biosynthesis during normal wood formation. In the same manner, there was a decline in both of their biosynthesis during tension wood formation. To sum up, our sampling time, late June with moderate temperature and sufficient water status gives us a clue about correlation between increased starch accumulation, lignin and hemicellulose contents, in turn, positive correlation between D-glucose and lignin contents.

4.3 Extraordinary Clones

The average mean values of clones with respect to cellulose, lignin and glucose contents were calculated as 21.8 ± 16.29 µg/ml, 23 ± 4.64 µg/ml, and 35 ± 9.71 µg/ml, respectively. However, particular clones were identified with extraordinary high values for these traits. As mentioned previously, high cellulose and low lignin contents are correlated with increased growth parameters. Following the analysis

which gave the clones with the highest and lowest contents of the studied traits, comparison were carried out with clones which were chosen based on their superior phenotypic characteristic regarding diameter and height growth performances. Among the studies clones, seven clones were determined with the highest cellulose content and with the highest diameter and height measurements. These were 62172, N03377, N90062, N90102, N91075, N92132, and N92217. Moreover, there were also seven clones among the clones with lowest lignin contents and highest diameter and height growths. These clones were 821, 62160, 62191, Geyve, Ata 1, Çubuk 1, and Çubuk 2. In the results, especially observing Geyve and Ata drew our attention, since there are only five registered commercial clones for *Populus nigra* which are Gazi, Anadolu, Kocabey, Geyve, and Ata in Turkey. Propagation of these clones is strongly suggested by the Poplar and Fast-Growing Forest Trees Research Institute. Actually other registered clones were also observed with low lignin and high cellulose content, but not among the superior clones. Another remarkable result was the detection of FARCFLHH 35 and EFN1 which were foreign-origin clones ranked the first and fifth with respect to high cellulose contents, respectively. The other foreign-origin clones, Lvubaka, Alterra, Uriffmh, and Efn2 were with moderate cellulose content. On the other hand, Lvubaka and Alterra had high height growth performances. At the same time, all foreign clones were detected with low lignin and high glucose contents so that we can seperate all these foreign clones from the Turkish clones.

4.4 Consequences of high cellulose to low lignin ratio

In general, plant breeders try not to choose plants with inferior genotypes because they slow down the breeding progress. Similarly, they try to eliminate environmental factors to select the plant on its genetic basis. Besides commercially registered clones, choosing the superior clones and eliminating inferior ones illustrated in our study can be suggested as useful approaches to create alternative poplar breeding programmes with increased wood quality and yield. Wood quality and pulp mechanical strength are directly associated with cellulose content, while lignin emerges as a undesirable polymer (Madakadze *et al.*, 1999).

Moreover, choosing clones with low lignin content would be beneficial to reduce the amount of chemicals and energy required in pulping process and also to reduce the pollution of air and water. On the other hand, low lignin content is widely correlated with reduced fitness of plants, so it is critically important to perform extra analysis to evaluate their fitness after choosing these clones.

Another manufacturing field which demands high cellulose to low lignin content is the renewable energy production industry. As noted before, the strong association of cellulose and hemicellulose with lignin affects negatively cellulosic ethanol production. Lignin also inhibits the release of polysaccharides while pretreatment process and saccharification (Keating *et al.*, 2006). Usually, scientists prefer down-regulation of lignin pathway enzymes, instead of up-regulation of cellulose and hemicellulose pathway enzymes as genetic engineering approaches (Ziegelhoffer *et al.*, 1999; Ericksson *et al.*, 2000; Biswas *et al.*, 2006; Oraby *et al*., 2007; Ransom *et al.*, 2007; Ralph *et al.*, 2006; Chapple *et al.*, 2007; Chen and Dixon, 2007). Generally, a long time requirement to achieve the aim with breeding strategies is seen as disadvantages (Bouton, 2007). Actually, breeding strategies to obtain valuable lignocellulosic clones, and clones with high quality and yield will provide a real solution for these problems and may relieve concerns of both industries. This study provides valuable and necessary information for these purposes. Also, for the first time, all clones grown as part of the *ex situ* conservation in clone banks, five commercially registered clones and also particularly chosen six foreign clones were evaluated with respect to their cell wall polymers. Instead of dealing with all clones, choosing the superior clones may be considered as an applicable plan to adapt new poplar breeding programmes. For instance, some superior characters were observed as common among clones with high cellulose content, increased height, and diameter growths, and with low lignin content such as 62172, N91075, 62160, N03377, and 62191. By using these clones beside Anadolu, Geyve, Ata, Kocabey, and Gazi, poplar breeders may establish a new breeding programme as a long term strategy.

CHAPTER 5

CONCLUSION

The results of this study indicated that three traits - cellulose, lignin and D-glucose content- provided almost adequate resolution to characterize *Populus nigra* L. clones and to study interactions between clones and regions in Turkey.

The clones with high cellulose to low lignin contents obtained from this study by comparing wood traits with growth traits can be used for making new plantations beside commercially registered clones to obtain valuable amount of wood with high quality. This study also supplied valuable data to establish new breeding strategies to create new clone with high cellulose to low lignin content which is the desire of both paper manufacturing and renewable energy production industry.

On the other hand, to check the reliability of undetectable direct correlation between cellulose and lignin contents, additional studies should be performed. Eventhough our study provided a good resolution to characterize the clones with respect to cellulose, lignin and glucose contents, there would be some overestimations because of possible hindrances of the colorimetric method. Therefore, supporting our results with specific enzyme assays and microarray studies may provide more accurate results. Also, measuring the resistance of clones with high cellulose to low lignin contents to certain stress conditions can be considered as a good approach before establishing any breeding strategies.

REFERENCES

Acquaah, G. (2004). *Horticulture: Principles and practices*, 3rd edn. Prentice Hall, Upper Saddle River, NJ.

Alba N.C., Maestro D., Gundez, M.D., Notivol, E., (2002). Advances in the preservation of genetic resources in *Populus nigra* L. in Spain. In: van Dam B.C., Bor dacs S., (eds.), Towards the: *Generic diversity in river populations of european black poplar: implications for riparian ecosystem management*. Proceedings of an international symposium held Csiszàr Nyomda, Hungary, pp. 125-136.

Allan, G.J., DiFazio, SP, Potts B.M., Fischer, D.G., Gehring, C.A., Lindroth, R.L., Marks, J.C., Hart, S.C., Wimp, G.N., Wooley, S.C. (2006). A framework for community and ecosystem genetics: From genes to ecosystems. *Nat Rev Genet* 7: 510–523.

Anon. (2001). Orman ürünleri arz-talep ilişkileri (Bölüm-3). *Sekizinci Beş Yıllık Kalkınma Planı, Ormancılık Özel İhtisas Komisyonu Raporu*, s. 56 - 115, DPT Yayını, No. 2531 - 547, Ankara.

Anterola, A.M., Lewis, N.G. (2002). Trends in lignin modification: a comprehensive analysis of the effects of genetic manipulations/mutations on lignification and vascular integrity. *Phytochemistry* Oct; 61(3): 221-94.

Arens, P., Coops, H., Jansen, J., Vosman, B. (1998). Molecular genetic analysis of black poplar (*Populus nigra* L.). along Dutch rivers. *Molecular Ecology* 7: 11–18.

Asif, M., Muneer, T. (2007). Energy supply, its demand and security issues for developed and emerging economies. *Renewable and Sustainable Energy Reviews* 11: 1388–1413.

Aspinall, G.O., (1985) *The Polysaccharides,* Vols 1-3, Academic Press, Inc., New York.

Baucher, M., Petit-Conil M., Boerjan, W. (2003). Lignin: genetic engineering and impact on pulping. Crit Rev *Biochem Mol Biol* 38:305-350.

Ballian, D., Kajba, D., Idzojtic, M., (2006). Morphological diversity of hairy european black poplar (*Populus nigra* subsp. caudina) in Bosnia and Herzegovina. *Оригинални научни рад* 5: 13-22.

Barnes, B.V., Pregitzer K.S., (1985). Occurrence of hybrids between big tooth and trembling aspen in Michigan. *Canadian Journal of Botany* 63: 1888–1890.

Bhuiyan, N.H., Selvaraj, G., Wei, Y., King, J., (2009). Role of lignification in plant defense. *Plant Signal Behav 4:* 158–159

Biswas, G., Ransom, C., Sticklen, M. (2006). Expression of biologically active Acidothermus cellulolyticus endoglucanase in transgenic maize. *Plant Sci* 171: 617–623.

Boerjan, W., Ralph, J., Baucher, M., (2003). Lignin biosynthesis. Annu Rev *Plant Biol 54:* 519–546

Boerjan, W. (2005) Biotechnology and the domestication of forest trees. *Curr Opin Biotechnol* **16**: 159–166

Braatne, J. H., Hincky, T. M. Stettler, R. F. (1992). Influence of soil water supply on the physiological and morphological components of plant water balance in *Populus trichocarpa*, *Populus deltoides* and their F1 hybrids. *Tree Physiology 11:* 325–340.

Braatne, J.H., Rood, S.B., Heilman, P.E. (1996). Life history, ecology, and conservation of riparian cottonwoods in North America. Pages 57-85 *in* Stettler, R.F., Bradshaw, H.D., Heilman, P.E., Hinckley, T.M., editors. *Biology of Populus and its implications for management and conservation.* NRC Research Press, Ottawa, Canada.

Bradshaw, H.D., Jr, Stettler, R.F. (1993). Molecular genetics of growth and development in Populus. I. Triploidy in hybrid poplars. *Theor Appl Genet* 86: 301-307

Bradshaw, E.G., Jones, V.J., Birks, H.J.B., Birks, H.H. (2000). Diatom responses to Late–Glacial and Early–Holocene environmental changes at Krakenes, Western Norway. *Journal of Paleolimnology 23*: 21–34.

Brenner, A.M., Busov, V.B., Strauss, S.H., (2004). Poplar genome sequence: functional genomics in an ecologically dominant plant species. *Trends in Plant Sciences* 9: 49-56.

Brown, R.M. Jr. (2003). Cellulose structure and biosynthesis: what is in store for the 21st century? *J.Polymer Science* 42, 487-495

Cagelli, L., Lefevre, F. (1997). The conservation of *Populus nigra* L. and gene flow within cultivated poplars in Europe. *Bocconea* 7: 63–75.

Carder, A. (1995). *Forest Giants of the World: Past and Present*. Fitzhenry and Whiteside, Markham, Canada

Carroll, A, Somerville, C., (2009). Annu Rev Cellulosic biofuels. *Plant Biol.* ;60:165-82.

Chapple, C., Ladisch, M., Melian, R. (2007). Loosening lignin's grip on biofuel production. *Nat Biotechnol* 25: 746–747.

Chen, F., Dixon, R.A. (2007). Lignin modification improves fermentable sugar yields for biofuel production. *Nat Biotechnol 25:* 759–761

Coleman, H.D., Yan, J., Mansfield, S.D. (2009) Sucrose synthase affects carbon partitioning to increase cellulose production and altered cell wall ultrastructure. *Proc Natl Acad Sci* USA 106: 13118–13123

Collins, P.M. & Ferrier, R.J. (1995) *Monosaccharides: Their Chemistry and Their Roles in Natural Products,* John Wiley & Sons, Chichester, England. A comprehensive text at the graduate level.

Csencsics, D., Angelonea, S., Paniga, M., Rotach, P., Rudow, A., Sabioteb, E., Schwab, P., Wohlhauser, P., Holderegger, R. (2009). A large scale survey of *Populus nigra* presence and genetic introgression from nonnative poplars in Switzerland based on molecular identification. *Journal for Nature Conservation 17(3).*: 142–149.

Çiftçi, A. (2013) Genetic diversity of european black poplar (*Populus nigra*) populations from Turkey assessed by microsatellite DNA markers, Ms Thesis, METU, Ankara.

Davis, J.M. (2008). Genetic improvement of poplar (*Populus* spp.) as a bioenergy crop. In: Vermerris W, ed. *Genetic improvement of bioenergy crops*. New York, NY, USA: Springer New York, 397–419.

Delmer, D.P., Amor, Y., (1995) Cellulose Biosynthesis, *The Plant Cell*, Vol. 7, 987-1000

Delmer, D.P., Haigler, C.H. (2002). The regulation of metabolic flux to cellulose, a major sink for carbon in plants. *Metabolic Engineering*, 4, 22–28.

Dence, C. (1992). The determination of lignin. In: Lin S, Dence C, eds. *Methods in lignin chemistry.* Berlin: Springer-Verlag, 33–62.

Dickmann, D.I., Stuart, K., (1983). *The culture of poplars in Eastern North America*. Department of Forestry, Michigan State University, East Lansing, Michigan, USA

Dickmann, D.I., Kuzovkina, J. (2008). Poplars and willows of the world, with emphasis on silvicuturally important species. *Poplar and Willows in the World*, FAO, Rome, Italy.

Dixon, R.A., Reddy, M.S.S. (2003). Biosynthesis of monolignols. Genomic and reverse genetic approaches. *Phytochemistry Reviews* **2**:289–306.

Doblin, M.S., Kurek, I., Jacob-Wilk, D., Delmer, D.P.. (2002). Cellulose biosynthesis in plants: from genes to rosettes. *Plant and Cell Physiology* 43, 1407–1420

Donaldson, L.A. (2001). Lignification and lignin topochemistry - an ultrastructural view. *Phytochemistry 57,* 859–873.

Eckenwalder, J. E. (1984a). Natural intersectional hybridization between North American species of *Populus* in sections *Aigeiros* and *Tacamahaca*. I. Population studies of *P. parryi*. *Canadian Journal of Botany 62*: 317–324.

Eckenwalder, J. E. (1984b). Natural intersectional hybridization between North American species of *Populus* in sections *Aigeiros* and *Tacamahaca*. II. Taxonomy. *Canadian Journal of Botany* 62: 325–335.

Eckenwalder, J.E. (1996). Systematics and evolution of Populus. In: Stettler, R.F., Bradshaw,H.D., Jr., Heilman, P.E., and Hinckley., T.M. (eds). Biology of Populus and its implications for management and conservation. NRC Research Press, National Research Council of Canada, Ottawa, Ontario, Canada, 7-32.

Endler, A., Persson, S. (2011). Cellulose Synthases and Synthesis in Arabidopsis, *Molecular Plant* pp 1–13

Ericksson, M. E., Israelsson, M., Olsson, O., Moritiz, T. (2000). Increased gibberellin biosynthesis in transgenic trees promotes growth, biomass production and xylem fiber length. *Nat biotechnol* 18: 784–788.

Faik, A. (2013) "Plant Cell Wall Structure-Pretreatment" the Critical Relationship in Biomass Conversion to Fermentable Sugars. Chapter 1 In: *Green Biomass Pretreatment for Biofuels Production*. Tingyue Gu (Ed.), Springer Berlin-New York, pp 1-30.

FAO (2008). Synthesis of Country Progress Reports received, prepared for the 23rd Session of the International Poplar Commission, jointly hosted by FAO and by the Beijing Forestry University, the State Forest Administration of China and the Chinese Academy of Forestry; Beijing, China, 27–30 October 2008. International Poplar Commission, *Working Paper IPC/6.Forest Management Division*, FAO, Rome

Falconer, D.S. (1981). *Introduction to quantitative genetics*, 2nd edn. Longman, London

Fujii, S., Hayashi, T., and Mizuno, K. (2010). Sucrose synthase is an integral component of the cellulose synthesis machinery. *Plant Cell Physiol*. 51. 294–301

Fukuda, M. & Hindsgaul, O. (1994) *Molecular Glycobiology,*IRL Press at Oxford University Press, Inc., New York. Thorough, advanced treatment of the chemistry and biology of cell surface carbohydrates. Good chapters on lectins, carbohydrate recognition in cell-cell interactions, and chemical synthesis of oligosaccharides.

Gaudet, M., (2006). Molecular approach to dissect adaptive traits in native European *Populus nigra* L.: Construction of a genetic linkage map based on AFLP, SSR, and SNP markers. PHD Thesis. The Department of Forest Environment and Resources in University of Tuscia, Italia.

Gaudet, M., Jorge, V., Paolucci, I., Beritognolo, I., Scarascia Mugnozza, G., Sabatti, M. (2008). Genetic linkage maps of *Populus nigra* L. including AFLPs, SSRs, SNPs, and sex trait. *Tree Genetics and Genomes*, 4:25–36.

Gebhardt, K., Pohland, A., Vornam, B., (2002). Genetic inventory of black poplar population in the Upper Rhine Floodplains: conclution for conservation of an endangered plant species. In: van Dam B.C., Bordacs S.,(eds.), *Towards the generic diversity in river populations of european black poplar: implications for riparian ecosystem management*. Proceedings of an international symposium held Csiszàr Nyomda, Hungary, pp. 145-156.

Gray, K.A., Zhao, L., Emptage, M. (2006). Bioethanol. *Current Opinion in Chemical Biology* 10: 141–146

Hajaligol, M., Waymack, B., Kellog, D. (2001). Low temparature formation of aromatic hydrocarbon from pyrolysis of cellulosic materials. *Fuel* 80: 1799-1807.

Halpin, C., Boerjan, W. (2003). Stacking transgenes in forest trees. *Trends Plant Sci* 8:363-365.

Hatfield, R. & Fukushima, R.S. (2005). Can lignin be accurately measured? *Crop Science* 45: 832–839.

Hawkins, S., Leple,´ J-C., Cornu, D., Jouanin, L., Pilate, G. (2003). Stability of transgene expression in poplar: a model forest tree species. *Ann For Sci* 60:427-438.

Hedge, J.E. and Hofreiter, B.T. (1962). *Carbohydrate Chemistry*, 17 (Eds). Whistler R.L. and Be Miller, J.N.), Academic Press, New York.

Heyer, A.G., Raap, M., Schroeer, B., Marty, B., Willmitzer, L. (2004) Cell wall invertase expression at the apical meristem alters Xoral, architectural, and reproductive traits in *Arabidopsis thaliana*. *Plant J* 39:161–169

Hilliers, J. & Coombes, A. eds. (2002). *The Hilliers Manual of Trees and Shrubs*. David & Charles, Devon.

Huntley, S.K., Ellis, D., Gilbert, M., Chapple, C., Mansfield, S.D. (2003). Significant increases in pulping efficiency in C4H-F5Htransformed poplars: improved

chemical savings and reduced environmental toxins. *J Ağrıc Food Chem* 51:6178-6183.

Jansson, S., Douglas, C.J. (2007). *Populus*: A model system for plant biology. *Annu Rev Plant Biol* 58: 435–458.

Jing, Z.P., Gallardo, F., Pascual, M.B., Sampalo, R., Romero, J., Torres de Navarra, A., Ca´ novas, D.M. (2004). Improved growth in a field trial of transgenic hybrid poplar overexpressing glutamine synthetase. *New Phytol* 164:137-145.

Joshi, C.P., Mansfield, S.D. (2007). The cellulose paradox-simple molecule, complex biosynthesis. *Current Opinion in Plant Biology* 10. 220–226.

Kajba, D., Romanic, B., (2002). Morphological leaf variability of the european black poplar (*Populus nigra* L.) in natural populations in the Drava River Basin in Croatia. In: van Dam B.C., Bordacs S., (eds.), *Towards the: Generic diversity in river populations of european black poplar: implications for riparian ecosystem management.* Proceedings of an international symposium held Csiszàr Nyomda, Hungary, pp. 221-227

Keating, J.D., Panganiban, C., Mansfield, S.D. (2006). Tolerance and adaptation of ethanologenic yeasts to lignocellulosic inhibitory compounds. *Biotechnol Bioeng* 93: 1196–1206.

Keim, P., Paige, K.N., Whitham, T.G., Lark, K.G. (1989). Genetic analysis of an interspecific hybrid swarm of *Populus*: occurrence of unidirectional introgression. *Genetics* 123: 557–565. Rood SB, Campbell JS, Despins T (1986). Natural poplar hybrids from southern Alberta. I. Continuous variation for foliar characteristics. *Can J Bot* 64: 1382–1388.

Klug, W.S., and M.R. Cummings. (1997). *Concepts of genetics*, 5th edn. Prentice Hall, Upper Saddle, NJ.

Koch, G.W., Sillett, S.C., Jennings, G.M., Davis, S.D., (2004). The limits to tree height. *Nat Biotechnol 428:* 851–854

Koçer, S., Diner, A. (2003). Ülkemizde Kavakçılığın Ekonomik Görünümü. *Türkiye Milli Kavak Komisyonu VII. Olağan Kurulu Tebliğleri.* Kavak ve Hızlı Gelişen Orman Ağaçları Araştırma Enstitüsü, s. 60-83

Koonin, S.E. (2006) Getting serious about biofuels. *Science* 311: 435.

Kumar, S. and Fladung, M. (2001). Gene stability in transgenic aspen (Populus). II. Molecular characterization of variable expression of transgene in wild and hybrid aspen. *Planta* 213, 731–740

Lehmann, J. (Haines, A.H., trans.) (1998) *Carbohydrates: Structure and Biology,* G. Thieme Verlag, New York. The fundamentals of carbohydrate chemistry and biology, presented at a level suitable for advanced undergraduates and graduate students.

Lehninger, A. (2008). *Principles of biochemistry* (5 ed., draft, Freeman).

Lewin, M., Goldstein, I.S. (1991). *Wood Structure and Composition.* New York:Markel Dekker

Li, L., Zhou, Y., Cheng, X., Sun, J., Marita, J., Ralph, J., Chiang, V., (2003). Combinatorial modification of multiple lignin traits in trees through multigene cotransformation. *Proc Natl Acad Sci USA 100:* 4939–4944

Li, X., Weng, J.K., Chapple, C. (2008) Improvement of biomass through lignin modification. *Plant J* **54**: 569–581.

Littell, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, R.D. (1996). *SAS® system for mixed models*. Cary, NC, USA: SAS Institute Inc.

Mansfield, S.D. (2009) Solutions for dissolution-engineering cell walls for deconstruction. *Curr Opin Biotechnol* **20**: 286–294

Mason, A. (2007). The Rise of Consultant Forecasting in Liberalized Natural Gas Markets. *Public Culture: Interdisciplinary Journal of Transnational Cultural Studies* Vol. 19. No. 2, pp. 367-379.

Meilan, R., Auerbach, D.J., Ma, C., DiFazio, S.P., Strauss, S.H. (2002). Stability of herbicide resistance and GUS expression in transgenic hybrid poplars (*Populus* sp.) during four years of field trials and vegetative propagation. *HortScience* 37:277-280.

Morrison, R.T., Boyd, R.N. (1992) *Organic Chemistry,* 6th edn, Benjamin Cummings, San Francisco. Chapters 34 and 35 cover the structure, stereochemistry, nomenclature, and chemical reactions of carbohydrates.

Muhle-Larsen, C. (1970).: Recent advances in poplar breeding. International review of *Forestry Research* 3: 1-67.

Novaes, E., Osorio, L., Drost, D.R., Miles, B.L., Boaventura-Novaes, C.R., Benedict, C., Dervinis, C., Yu, Q., Sykes, R., Davis, M. *et al.* (2009). Quantitative genetic analysis of biomass and wood chemistry of Populus under different nitrogen levels. *New Phytol* **182**: 878–890.

Oraby, H., Venkatesh, B., Dale, B., Ahamd, R., Ransome, C., Oehmke, J., Sticklen, M. B. (2007). Enhanced conversion of plant biomass into glucose using transgenic rice-produced endoglucanase for cellulosic ethanol.*Trans Res* 16: 739–749.

Panshin, A.M., deZeeuw, C.H. (1980). *Textbook of wood technology*, 4th ed. New York: McGraw-Hill

Park, Y.W., Baba, K., Furuta, Y., Iida, I., Sameshima, K., Arai, M., Hayashi, T. (2004). Enhancement of growth and cellulose accumulation by overexpression of xyloglucanase in poplar. *FEBS Lett*, 564:183-187.

Pigman, W., Horton, D. (eds) (1980) *The Carbohydrates: Chemistry and Biochemistry,* Vols IA, IB, IIA, and IIB, Academic Press, Inc., New York. Comprehensive treatise on carbohydrate chemistry.

Pilate, G., Guiney, E., Holt, K., Petit-Conil, M., Lapierre, C., Leple,´ J-C., Pollet, B., Mila, I., Webster, E.A., Marstorp, H.G. *et al.* (2002). Field and pulping performances of transgenic trees with altered lignification. *Nat Biotechnol* 20:607-612.

Rae, A.M., Street, N.R., Rodriguez–Acosta, M. (2007). *Populus* trees. Chittaranjan, K., (Ed.). *Forest trees genome mapping and molecular breeding in plants*, Heidelberg, Berlin, 7: 1–28.

Ralph, J., Akiyama, T., Kim, H., Lu, F.C., Schatz, P.F., Marita, J.M., Ralph, S.A., Reddy, M.S.S., Chen, F., Dixon, R.A. (2006a). Effects of coumarate 3-hydroxylase down-regulation on lignin structure. *Journal of Biological Chemistry* 281: 8843–8853.

Ralph, S., Oddy, C., Cooper, D., Yueh, H., Jancsik, S., Kolosova, N., Philippe, R.N., Aeschliman, D., White, R., Huber, D. *et al. (*2006b). Genomics of hybrid poplar (*Populus trichocarpax deltoides*) interacting with forest tent caterpillars

(*Malacosoma disstria*): normalized and full-length cDNA libraries, expressed sequence tags, and a cDNA microarray for the study of insect-induced defences in poplar.*Molecular Ecology* 15: 1275–1297.

Ransom, C., Venkatesh, B., Dale, B., Biswas, G., Sticklen, M. B. (2007). Heterologous Acidothermus cellulolyticus 1.4-β-endoglucanase E1 produced within the corn biomass converts corn stover into glucose. *Applied Biochem Biotech* 140: 137–219.

Rathmacher, G., Niggemann, M., Kohnen, M., Ziegenhagen, B., Bialozyt, R. (2010). Short distance gene flow in *Populus nigra* L. accounts for small scale spatial genetic structures: Implications for in situ conservation measures. *Conserv. Genet*. 11: 1327–1338.

Ronald, W.G., Lenz, L.M., Cumming, W.A. (1973a).: Biosystematics of the genus *Populus* L. Distribution and morphology of native Manitoba species and variants. *Canadian Journal of Botany 51(12).*: 2431-2442.

Ronald, W.G., Lenz, L.M., Cumming, W.A. (1973b). Biosystematics of the genus *Populus* L. II - Chemotaxonomy, phenology, fertility and segregates of native Manitoba species and variants. *Canadian Journal of Botany 51(12).:* 2443-2450.

Ronald, W.G. (1982). Intersectional hybridization of *Populus* sections, *Leuce-Aigeiros* and *Leuce-Tacamahaca*. *Silvae Genetica* 31: 94–99.

Rubin, E.M., (2008). Genomics of cellulosic biofuels. *Nature* 454: 841-845.

Safavi, S.M., (2011). Estimation of genetic parameters related to morphological traits in poplar clones. *American Journal of Scientific Research* 27: 105-110.

Sarkanen, K.V., Ludwig, C.G. (1971). *Lignins:Occurrence, formation, structure and reactions*. New York: Wiley Interscience

Schreiner, E.J. (1959). Production of poplar timber in Europe and its significance and application in the United States. *United States Department of Ağrıculture Forest Service Ağrıculture Handbook Number* 150:124pp.

Schubert, C. (2006). Can biofuels finally take centre stage. *Nat. Biotechnol.* 24:777–784.

Shani, Z., Dekel, M., Tsabary, G., Goren, R., Shoseyov, O. (2004). Growth enhancement of transgenic poplar plants by overexpression of *Arabidopsis thaliana* endo-1.4-b-glucanase (Cel1). *Mol Breed* 14:321-330.

Sjostrom, E. (1993). *Wood chemistry, fundamentals and applications*. Orlando, USA: Academic Press

Somerville, C. (2006). Cellulose synthesis in higher plants. Annu. Rev. *Cell and Developmental Biology* 22, 53–78.

Stanton, B.J. (2009). The domestication and conservation of *Populus* genetic resources. *FAO Forestry Division Working Paper* IPC/9-4a, Rome, Italy. 92pp.

Stettler, R.F, Koster, R., Steenackers, V. (1980). Interspecific Crossability Studies in Poplars. *Theor. Appl. Genet.* 58: 273-282.

Stettler, R.F., Bradshaw, H.D., Heilman, P.E., T.M. (1996). *Biology of Populus and its implications for management and conservation.* NRC Research Press, National Research Council of Canada, Ottawa, Ontario, Canada, 539 pp.

Stettler, R. F., Zsuffa, L., Wu, R. (1996b). The role of hybridization in the genetic manipulation of *Populus*. *In* R. F. Stettler, H. D. Bradshaw, Jr., P. E. Heilman, and T. M. Hinckley [eds.], *Biology of Populus and its implications for management and conservation,* 87–112. NRC Research Press, National Research Council of Canada, Ottawa, Ontario, Canada.

Stewart, J.J., Akiyama, T., Chapple, C., Ralph, J., Mansfield, S.D. (2009). The effects on lignin structure of overexpression of ferulate 5-hydroxylase in hybrid poplar. *Plant Physiol 150:* 621–635

Sticklen, M.B. (2008) Plant genetic engineering for biofuel production: towards affordable cellulosic ethanol. *Nat Rev Genet* **9**: 433–443.

Storme, V., Vanden Broeck, A.H., Ivens, B., Smulders, M.J.M., Halfmaerten, D., Van Slycken, J., Boerjan, W. (2002). Ex situ conservation of black poplar in Belgium,the margin of the geographical distribution area of the species. In: van Dam B.C., Bordacs S., (eds.), Towards the genetic diversity in river populations of european black poplar: implications for riparian ecosystem management. Proceedings of an international symposium held Csiszàr Nyomda, Hungary, pp. 61-72.

Sugiyama, J., Harada, H., Fujiyoshi, Y., Uyeda, N. 1985. Lattice images from ultrathin sections of cellulose microfibrils in the cell wall of *Valonia macrophysa* Kutz. *Planta* 166: 161–168.

Suzuki, S., Li, L.G., Sun, Y.H., Chiang, V.L. 2006. The cellulose synthase gene superfamily and biochemical functions of xylem-specific cellulose synthase-like genes in *Populus trichocarpa*. *Plant Physiology* **142**: 1233–1245.

TAPPI Standard (1991) T250 um-91. Acid-soluble lignin in wood and pulp. Technical Association of the Pulp and Paper Industry, Atlanta, GA, USA.

Taylor, G. (2002). *Populus. Arabidopsis* for forestry. Do we need a model tree? *Annuals of Botany* 90: 681–689.

Timell, T.E. (1986). *Compression Wood in Gymnosperms*. Vol 3. Springer-Verlag, Berlin

Toplu, F., Kucukosmanoglu, F. (2003). Two natural distributions of *Populus nigra* L. discovered in Turkey. *Journal of Poplar and Fast–Growing Forest Trees Research Institute,* 29: 23–32.

Toplu, F., (2005). Breeding and conservation of black poplar (*Populus nigra*). gene resources in Turkey , *Unasylva* 221. 56: 26-30.

Tuskan, G.A., DiFazio, S., Jansson, S., Bohlmann, J., Grigoriev, I., Hellsten, U., Putnam, N., Ralph, S., Rombauts S, Salamov A, Schein J, Sterck L, Aerts A, Bhalerao RR, Bhalerao RP, Blaudez D, Boerjan W, Brun A, Brunner A, Busov V, Campbell M, Carlson J, Chalot M, Chapman J, Chen G-L, Cooper D, Coutinho PM, Couturier J, Covert S, Cronk Q, Cunningham R, Davis J, Degroeve S, Dejardin A, dePamphilis C, Detter J, Dirks B, Dubchak I, Duplessis S, Ehlting J,Ellis B, Gendler K, Goodstein D, Gribskov M, Grimwood J, Groover A, Gunter L, Hamberger B, Heinze B, Helariutta Y, Henrissat B, Holligan D, Holt R, HuangW, Islam-Faridi N, Jones S, Jones-Rhoades M, Jorgensen R, Joshi C, Kangasjarvi J, Karlsson J, Kelleher C, Kirkpatrick R, KirstM, Kohler A, Kalluri U, Larimer F, Leebens-Mack J, Leple J-C, Locascio P, Lou Y, Lucas S, Martin F, Montanini B, Napoli C, Nelson DR, Nelson C, Nieminen K, Nilsson O, Pereda V, Peter G, Philippe R, Pilate G, Poliakov A, Razumovskaya J, Richardson P, Rinaldi C, Ritland K, Rouze P, Ryaboy D, Schmutz J, Schrader J, Segerman B, Shin H, Siddiqui A, Sterky F, Terry A, Tsai C-J, Uberbacher E, Unneberg P, Vahala J, Wall K, Wessler S, Yang G, Yin T, Douglas C, Marra M, Sandberg G, Van de Peer Y, Rokhsar D

(2006). The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). *Science* 313:1596–1604.

Uppdegraf, D.M. (1969). Semimicro determination of cellulose in biological materials. *Analytical Biochemistry* 32,420-424

Vance, C.P., Kirk, T.K., Sherwood, R.T., (1980). Lignification as a mechanism of disease resistance. Annu Rev *Phytopathol 18*: 259–288

Vanden Broeck, A.H., Storme, V., Cox, K., Quataert, P., Ivens, B., Hostyn, V., Halfmaerten, D., Boerjan, W., Van Slycken, J., (2002). Mating system of *Populus nigra* in a mixed poplar sand composed of *P. nigra*, *P. deltoids* and *P.*x *euramericana*. In: van Dam B.C., Bordacs S., (eds.), Towards the genetic diversity in river populations of european black poplar: implications for riparian ecosystem management. Proceedings of an international symposium held Csiszàr Nyomda, Hungary, pp. 53-60

Vanden Broeck, A. (2003). Genetic conservation and use for European black poplar (*Populus nigra*). *EUFORGEN Technical Guidelines International Plant Genetic Resources Institute*, Rome, Italy, 1–6.

Walker, J.C.F. (1993). Basic wood chemistry and cell wall ultrastructure. In: Walker, J.C.F. (Ed.). *Primary wood processing, principles and practice*. Chapman & Hall, London. p. 23–67.

Website of European Forest Genetic Resources Program available at http://www.euforgen.org/fileadmin/www.euforgen.org/Documents/Maps/JPG/populus_nigra.jpg. Last access date December, (2013).

Website of Food and Ağrıculture Organization of the United Nations available at http://faostat3.fao.org/faostat-gateway/go/to/download/F/FO/E. Last access date December, (2013).

Weng, J. K., Li, X., Bonawitz, N. D., Chapple, C. (2008). Emerging strategies of lignin engineering and degradation for cellulosic biofuel production. *Curr Opin Biotechnol 19:* 66–172

Whitham, T.G., Bailey, J.K., Schweitzer, J.A., Shuster, S.M., Bangert, R.K., LeRoy, C.J., Lons-dorf, E.V., Allan, G.J., DiFazio, S.P., Potts, B.M., Fischer, D.G., Gehring, C.A., Lindroth, R.L., Marks, J.C., Hart, S.C., Wimp, G.M., Wooley,

S.C. (2006) A framework for community and ecosystem genetics: from genes to ecosystems. *Nat. Genet.* 7: 510-523.

Willing, R.R., Pryor, L.D. (1976). Interspecific Hybridization in Poplar. *Theor Appl Genet* 47: 141–151.

Yıldırım, K. (2013) Transcriptional and physiological responses of black poplar to drought stress. PhD thesis METU, Ankara

Yu, Q., Li, B., Nelson, C.D., McKeand, S.E., Batista, V.B., Mullin, T.J. (2006) Asso ciation of the *cad-n1* allele with increased stem growth and wood density in full-sib families of loblolly pine. *Tree Genet Genomes* **2**: 98–108.

Ziegelhoffer, T., Wil, J., Austin-Phillips, S. (1999). Expression of bacterial cellulase gene in transgenic alfalfa (Medicago sativa L), potato (Solanum tuberosum L) and tobacco (Nicotiana tobacum). *Mol Breed* 5: 309–318.

APPENDIX A

The Origin of European Black Poplar Trees in Behiçbey Clone Bank in Turkey

Table A.1.

Clone Identity	Region	City
N.92.282	Aegean	Afyon
62/160	Central Anatolia	Ankara
62/172	Central Anatolia	Ankara
62/191	Central Anatolia	Ankara
64/13	Central Anatolia	Ankara
64/14	Central Anatolia	Ankara
64/14.06.1	Central Anatolia	Ankara
64/14.10	Central Anatolia	Ankara
64/14.12	Central Anatolia	Ankara
77/40	Central Anatolia	Ankara
82/1	Central Anatolia	Kırşehir
82/2	Central Anatolia	Kırşehir
82/3	Central Anatolia	Kırşehir
82/4	Central Anatolia	Kırşehir
83/1	Central Anatolia	Yozgat
83/10	Central Anatolia	Kayseri
83/12	Central Anatolia	Kayseri
83/13	Central Anatolia	Kayseri
83/3	Central Anatolia	Kayseri
83/5	Central Anatolia	Kırşehir
83/6	Central Anatolia	Kırşehir
83/8	Central Anatolia	Kırşehir
83/9	Central Anatolia	Kayseri
85/1	Eastern Anatolia	Ağrı
85/11	Eastern Anatolia	Erzurum

Table A.1 (Continued)

85/14	Eastern Anatolia	Erzurum
85/15	Eastern Anatolia	Erzurum
85/16	Eastern Anatolia	Ağrı
85/4	Eastern Anatolia	Ağrı
85/6	Eastern Anatolia	Ağrı
85/7	Eastern Anatolia	Ağrı
87/1	Central Anatolia	Kırşehir
88/1	Central Anatolia	Ankara
88/3	Central Anatolia	Ankara
88/4	Aegean	Aydin
88/5	Aegean	Denizli
88/6	Aegean	Denizli
88/8	Aegean	Denizli
ALTERRA	Foreign	Foreign
ANADOLU	Central Anatolia	Ankara
ATA 1	Central Anatolia	Ankara
ÇUBUK 1	Central Anatolia	Ankara
ÇUBUK 2	Central Anatolia	Ankara
EFN.1	Foreign	Foreign
EFN.2	Foreign	Foreign
FARCFLHHZ 35	Foreign	Foreign
GAZİ	Central Anatolia	Ankara
GEYVE	Central Anatolia	Ankara
KAE N.92	Unknown	Unknown
KELKİT 1	Blacksea	Gümüşhane
KELKİT 11	Blacksea	Gümüşhane
KELKİT 3	Blacksea	Gümüşhane
KELKİT 4	Blacksea	Gümüşhane
KELKİT 6	Blacksea	Gümüşhane
KELKİT 7	Blacksea	Gümüşhane
KELKİT 8	Blacksea	Gümüşhane
KELKİT 9	Blacksea	Gümüşhane
KOCABEY	Mediterranean	Adana
LVUBAKA	Foreign	Foreign
N.02.01.02	Open pollination	Open pollination
N.02.01.04	Open pollination	Open pollination
N.02.01.05	Open pollination	Open pollination

Table A.1 (Continued)

N.02.02.01	Open pollination	Open pollination
N.02.02.03	Open pollination	Open pollination
N.02.02.06	Open pollination	Open pollination
N.02.05.01	Open pollination	Open pollination
N.02.05.013	Open pollination	Open pollination
N.02.05.02	Open pollination	Open pollination
N.02.05.03	Open pollination	Open pollination
N.02.05.06	Open pollination	Open pollination
N.02.05.07	Open pollination	Open pollination
N.02.05.08	Open pollination	Open pollination
N.02.05.09	Open pollination	Open pollination
N.02.05.10	Open pollination	Open pollination
N.02.05.12	Open pollination	Open pollination
N.02.07.02	Open pollination	Open pollination
N.02.07.03	Open pollination	Open pollination
N.02.07.04	Open pollination	Open pollination
N.02.07.05	Open pollination	Open pollination
N.02.08.01	Open pollination	Open pollination
N.02.338	Unknown	Unknown
N.03.324	Unknown	Unknown
N.03.333	Unknown	Unknown
N.03.355	Eastern Anatolia	Erzincan
N.03.356	Eastern Anatolia	Erzincan
N.03.357	Eastern Anatolia	Erzincan
N.03.358	Eastern Anatolia	Erzincan
N.03.364	Eastern Anatolia	Erzurum
N.03.365	Eastern Anatolia	Erzurum
N.03.365	Eastern Anatolia	Erzurum
N.03.366	Eastern Anatolia	Erzurum
N.03.367	Eastern Anatolia	Erzurum
N.03.368	Eastern Anatolia	Erzurum
N.03.368.1	Eastern Anatolia	Erzurum
N.03.368.A	Eastern Anatolia	Erzurum
N.03.371	Eastern Anatolia	Elazığ
N.03.372	Eastern Anatolia	Elazığ
N.03.373	Central Anatolia	Kırşehir

Table A.1 (Continued)

N.03.375	Central Anatolia	Kırşehir
N.03.376	Central Anatolia	Kırşehir
N.03.377	Central Anatolia	Ankara
N.03.378	Central Anatolia	Ankara
N.03.399	Unknown	Unknown
N.62.164	Unknown	Unknown
N.82.008	Unknown	Unknown
N.82.166	Unknown	Unknown
N.85.010	Unknown	Unknown
N.85.018	Unknown	Unknown
N.90.008	Eastern Anatolia	Kars
N.90.010	Eastern Anatolia	Van
N.90.011	Eastern Anatolia	Van
N.90.012	Eastern Anatolia	Van
N.90.013	Eastern Anatolia	Muş
N.90.014	Eastern Anatolia	Bingol
N.90.016	Eastern Anatolia	Malatya
N.90.020	Eastern Anatolia	Sivas
N.90.024	Eastern Anatolia	Sivas
N.90.027	Eastern Anatolia	Sivas
N.90.028	Eastern Anatolia	Sivas
N.90.030	Eastern Anatolia	Sivas
N.90.032	Eastern Anatolia	Erzurum
N.90.034	Eastern Anatolia	Erzurum
N.90.035	Eastern Anatolia	Erzurum
N.90.036	Eastern Anatolia	Erzurum
N.90.038	Eastern Anatolia	Erzincan
N.90.039	Eastern Anatolia	Erzincan
N.90.045	Eastern Anatolia	Erzurum
N.90.046	Eastern Anatolia	Erzurum
N.90.050	Central Anatolia	Ankara
N.90.062	Unknown	Unknown
N.90.065	Unknown	Unknown
N.90.102	Unknown	Unknown
N.91.002	Unknown	Unknown
N.91.021	Unknown	Unknown
N.91.052	Blacksea	corum

Table A.1 (Continued)

N.91.054	Unknown	Unknown
N.91.058	Central Anatolia	Yozgat
N.91.059	Central Anatolia	Yozgat
N.91.063	Central Anatolia	Yozgat
N.91.067	Eastern Anatolia	Sivas
N.91.068	Eastern Anatolia	Malatya
N.91.071	Eastern Anatolia	Malatya
N.91.073	Eastern Anatolia	Malatya
N.91.074	Central Anatolia	Ankara
N.91.075	Mediterranean	Kahramanmaraş
N.91.076	Mediterranean	Kahramanmaraş
N.91.077	Mediterranean	Kahramanmaraş
N.91.078	Mediterranean	Kahramanmaraş
N.91.080	Mediterranean	Kahramanmaraş
N.91.081	Central Anatolia	Kayseri
N.91.083	Central Anatolia	Kayseri
N.91.084	Central Anatolia	Kayseri
N.91.085	Central Anatolia	Kayseri
N.91.088	Central Anatolia	Niğde
N.91.089	Central Anatolia	Niğde
N.91.090	Central Anatolia	Niğde
N.91.091	Central Anatolia	Aksaray
N.91.092	Central Anatolia	Aksaray
N.91.094	Eastern Anatolia	Malatya
N.91.095	Southeastern	Adiyaman
N.91.101	Southeastern	Gaziantep
N.91.102	Southeastern	Gaziantep
N.91.103	Southeastern	Gaziantep
N.91.105	Southeastern	Gaziantep
N.91.108	Southeastern	Gaziantep
N.91.109	Mediterranean	Osmaniye
N.91.110	Mediterranean	Osmaniye
N.91.111	Mediterranean	Osmaniye
N.91.112	Mediterranean	Hatay
N.91.118	Mediterranean	Antalya
N.91.119	Southeastern	Gaziantep

Table A.1 (Continued)

N.91.120	Southeastern	Gaziantep
N.91.122	Marmara	Sakarya
N.91.212	Unknown	Unknown
N.92.058	Unknown	Unknown
N.92.060	Unknown	Unknown
N.92.073	Unknown	Unknown
N.92.097	Unknown	Unknown
N.92.114	Unknown	Unknown
N.92.123	Marmara	Sakarya
N.92.124	Marmara	Sakarya
N.92.126	Marmara	Bilecik
N.92.128	Marmara	Bilecik
N.92.130	Marmara	Bilecik
N.92.131	Marmara	Bilecik
N.92.132	Marmara	Bilecik
N.92.133	Central Anatolia	Eskişehir
N.92.134	Central Anatolia	Eskişehir
N.92.137	Marmara	Yalova
N.92.138	Marmara	Yalova
N.92.140	Marmara	Kocaeli
N.92.142	Central Anatolia	Çankırı
N.92.144	Central Anatolia	Çankırı
N.92.148	Central Anatolia	Çankırı
N.92.149	Blacksea	Kastamonu
N.92.152	Blacksea	Kastamonu
N.92.153	Blacksea	Kastamonu
N.92.154	Blacksea	Kastamonu
N.92.156	Blacksea	Kastamonu
N.92.159	Blacksea	Sinop
N.92.160	Blacksea	Samsun
N.92.162	Blacksea	Samsun
N.92.164	Blacksea	Amasya
N.92.165	Blacksea	Amasya
N.92.166	Blacksea	Tokat
N.92.167	Blacksea	Tokat
N.92.168	Blacksea	Tokat

Table A.1 (Continued)

N.92.169	Blacksea	Tokat
N.92.170	Blacksea	Amasya
N.92.171	Blacksea	Amasya
N.92.176	Blacksea	Tokat
N.92.179	Blacksea	Tokat
N.92.182	Blacksea	Tokat
N.92.185	Blacksea	Amasya
N.92.187	Blacksea	Amasya
N.92.195	Blacksea	Amasya
N.92.200	Blacksea	Çorum
N.92.202	Central Anatolia	Çankırı
N.92.204	Central Anatolia	Çankırı
N.92.206	Aegean	Kütahya
N.92.208	Aegean	Kütahya
N.92.209	Aegean	Afyon
N.92.211	Aegean	Afyon
N.92.213	Aegean	Afyon
N.92.214	Central Anatolia	Konya
N.92.215	Central Anatolia	Konya
N.92.217	Central Anatolia	Konya
N.92.218	Central Anatolia	Konya
N.92.219	Central Anatolia	Konya
N.92.223	Central Anatolia	Ankara
N.92.224	Central Anatolia	Konya
N.92.230	Central Anatolia	Konya
N.92.232	Central Anatolia	Konya
N.92.233	Central Anatolia	Karaman
N.92.236	Central Anatolia	Konya
N.92.237	Central Anatolia	Konya
N.92.239	Central Anatolia	Niğde
N.92.240	Central Anatolia	Niğde
N.92.243	Mediterranean	Adana
N.92.245	Marmara	İçel
N.92.247	Central Anatolia	Karaman
N.92.250	Central Anatolia	Konya
N.92.252	Central Anatolia	Konya

Table A.1 (Continued)

N.92.254	Aegean	Afyon
N.92.255	Aegean	Afyon
N.92.256	Central Anatolia	Eskişehir
N.92.258	Central Anatolia	Eskişehir
N.92.260	Mediterranean	Adana
N.92.269	Blacksea	Zonguldak
N.92.271	Aegean	Kütahya
N.92.276	Aegean	Uşak
N.92.278	Aegean	Uşak
N.92.284	Aegean	Denizli
N.92.286	Aegean	Denizli
N.92.289	Aegean	Denizli
N.92.292	Marmara	Isparta
N.92.293	Marmara	Isparta
N.92.295	Marmara	Isparta
N.92.297	Marmara	Isparta
N.92.298	Marmara	Isparta
N.92.299	Marmara	Isparta
N.92.301	Aegean	Afyon
N.92.302	Aegean	Afyon
N.92.831	Unknown	Unknown
N.93.304	Southeastern	Gaziantep
N.93.306	Marmara	Bursa
N.93.309	Central Anatolia	Sivas
N.95.045	Unknown	Unknown
N.96.310	Eastern Anatolia	Malatya
N.96.315	Eastern Anatolia	Malatya
N.96.316	Eastern Anatolia	Malatya
N.96.317	Eastern Anatolia	Malatya
N.96.319	Eastern Anatolia	Elazığ
N.96.320	Eastern Anatolia	Bitlis
N.96.321	Eastern Anatolia	Van
N.96.322	Eastern Anatolia	Van
N.96.323	Eastern Anatolia	Van
N.96.325	Eastern Anatolia	Van
URIFFMH	Foreign	Foreign

APPENDIX B

Mean values of three replicates of clones for five traits

Table A.2

Region	Clones	Cellulose µg/ml	Lignin µg/ml	Glucose µg/ml	Height cm	Diameter cm
1	821	14.1	18.2	22.44	482.92	38.41
1	822	17.27	18.32	39.73	373.12	26.99
1	823	14.69	22.45	34.07	319.51	20.18
1	824	12.21	17.77	28.65	423.5	27.68
1	831	21.27	20.67	37.96	393.02	30.42
1	832	15.95	21.79	20.89	422.92	29.02
1	833	18.47	27.88	39.29	442.08	30.38
1	835	18.34	18.67	19.24	396.92	29.07
1	836	19.7	25.81	30.57	396.86	26.18
1	838	16.12	22.02	37.44	362.5	23.7
1	839	15.14	19.25	32.9	411.94	27.96
1	871	19.2	24.89	34.89	478.21	38.29
1	881	14.12	22.51	45.83	472.52	38.9
1	883	12.92	19.55	28.19	386.02	27.95
1	6413	14.5	16.86	40.69	409.81	35.94
1	7740	12.94	18.82	27.32	463.71	37.93
1	8310	14.1	22.96	27.73	414.17	29.07
1	8312	24.89	22.48	29.49	478.13	33.5
1	8313	19.93	18.75	34.57	379.94	26.91
1	62160	27.62	17.14	49.65	477.83	42.28
1	62172	70.92	22.35	35.24	479.27	42.77
1	62191	16.71	18.55	38.82	552.62	42.41
1	64141	13.29	19.82	37.15	399.65	32.96
1	641406	15.36	16.61	24.53	347.07	28.19
1	641410	27.34	21.26	42.91	329.54	24.61
1	641412	18.3	18.93	43.46	402.28	29.79
1	ANADOLU	43.44	18.8	25.8	435.93	29.72
1	ATA1	15.69	18.35	34.17	437.86	38.05
1	CUBUK1	27.79	18.49	42.39	468.74	41.15

Table A.2 (Continued)

1	CUBUK2	14.2	17.76	24.26	437.08	40.47
1	GAZI	16.85	24.53	28.61	473.42	36.97
1	GEYVE	17.01	18.28	34.72	511.33	45.85
1	KOCABEY	30.78	20.12	31.74	443	38.44
1	N03373	19.02	22.81	39.35	389.06	28.8
1	N03375	17.6	21.57	42.61	344.51	24.93
1	N03376	19.67	18.52	20.68	368.69	25.35
1	N03377	41.31	19.12	34.51	438.28	37.02
1	N03378	23.04	25.01	32.96	396.61	26.94
1	N90050	16.3	21.82	39.49	395.94	26.18
1	N91058	18.97	21.01	23.76	383.84	25.35
1	N91059	16.81	24.84	41.18	314.06	19.82
1	N91063	19.09	22.44	33.59	373.92	26.78
1	N91074	16.92	20.98	44.92	365.97	23.2
1	N91081	34.18	24.07	35.56	430.69	30.98
1	N91083	20.47	24.98	30.66	345.01	28.52
1	N91084	13.74	21.76	29.73	425.38	27.6
1	N91085	13.44	19.46	21.17	344.9	22.05
1	N91088	18.42	21.81	24.51	380.67	24.61
1	N91089	18.03	25.4	29.5	399.79	27.01
1	N91090	21.3	28.88	26.13	383.92	29.63
1	N91091	15.25	22.37	36.33	402.53	27.12
1	N91092	13.11	31.25	40.41	432.5	34.27
1	N92133	77.11	19.74	39.25	367.01	28.4
1	N92134	16.12	20.48	25.13	413.68	30.59
1	N92142	26.38	22.08	37.22	478.11	32.68
1	N92144	38.03	24.76	41.37	361.61	23.5
1	N92148	19.13	22.46	26.38	403.82	26.83
1	N92202	15.2	19.25	43.94	409.34	34.46
1	N92204	14.2	24.11	38.65	403.22	27.31
1	N92214	9.14	18.89	22.81	394.97	26.26
1	N92215	17.15	20.24	31.41	300.13	19.79
1	N92217	78.7	26.06	36.77	363.69	28.04
1	N92218	20.07	17.66	33.33	504.04	36.93
1	N92219	12.42	20.62	27.28	406.27	30.01
1	N92223	17.26	14.5	19.84	434.69	30.48
1	N92224	11.77	25.01	30.81	396.81	28.63
1	N92230	16.71	22.46	35.43	361.51	22.22
1	N92232	45.52	25.05	37.88	416.46	27.72
1	N92233	13.84	21.63	35.68	390.69	27.5
1	N92236	38.23	21.26	31.51	430.63	33.12

Table A.2 (Continued)

1	N92237	17.63	24.2	25.11	409.31	27.87
1	N92239	30.15	25.5	38.39	444.25	28.6
1	N92240	38.21	18.7	27.21	397.56	25.42
1	N92241	17.05	27.44	30.71	394.92	29.62
1	N92247	14.65	22.9	37.73	401.27	25.8
1	N92250	15.93	23.38	40.29	375.13	24.16
1	N92252	22.82	19.79	30.46	406.66	31.06
1	N92256	18.47	20.34	37.14	398.08	25.81
1	N92258	18.79	22.52	36.21	408.48	27.57
1	N92260	17.51	24.48	46.7	406.25	27.16
1	N93309	20.57	26.02	33.73	406.58	31.78
2	851	14.73	23.56	31.91	412.42	27.86
2	854	16.51	19.99	36.65	405.52	27.35
2	856	18.54	25.48	33.74	396.35	27.03
2	857	11.19	20.43	32.2	342.43	24.96
2	8511	13.52	25.26	41.29	295.85	24.96
2	8514	23.76	25.02	37.54	441.32	31.56
2	8515	24.82	22.07	32.08	442.33	37.18
2	8516	18.49	20.77	40.63	443.6	36.81
2	N03355	27.94	25.48	40.1	465.94	36.81
2	N03356	13.22	20.17	37.4	409.99	25.64
2	N03357	15.79	24.74	35.9	331.36	21.78
2	N03358	60.48	22.22	27.22	456.13	36.04
2	N03364	20.87	21.75	27.36	325.19	23.72
2	N03365	17.49	19.33	38.99	375.99	24.86
2	N033655	16.46	20.04	37.8	396.3	31.36
2	N03366	10.3	16.14	26.42	341.04	25.99
2	N03367	16	22.77	41.88	355.49	24.23
2	N03368	27.48	24.59	32.71	442.83	29.42
2	N033681	17.17	25.37	23.45	318.33	20.4
2	N03368A	15.42	23.91	35.04	378.29	34.2
2	N03371	18.56	25.48	35.39	276.13	24.18
2	N03372	18.94	29.26	40.82	273.65	22.6
2	N90001	23.33	27.06	33.74	408.52	29.69
2	N90008	14.93	20.77	26.85	368.68	24.66
2	N90010	18.74	19.66	35.21	325.61	23.48
2	N90011	19.14	25.73	42.15	389.51	29.07
2	N90012	19.77	23.19	29.29	477.52	37.54
2	N90013	19.9	23.75	30.16	415.58	30.57
2	N90014	15.13	22.88	29.38	355.03	23.16
2	N90016	13.91	19.76	34.2	418.44	27.58

Table A.2 (Continued)

2	N90020	16.19	25.91	42.57	410.08	27.08
2	N90024	12.79	23.97	40.97	442.29	32.87
2	N90027	34.56	23.57	27.31	447.04	31.85
2	N90028	14.19	24.46	35.05	372.76	28.3
2	N90030	18.63	30.52	41.15	397.83	29.13
2	N90032	28.23	22.82	31.07	431.49	30.31
2	N90034	19.6	22.03	27.27	360.06	24.82
2	N90035	42.97	22.23	38.38	453.85	35.98
2	N90036	16.08	23.98	43.11	341.7	30.66
2	N90038	18.17	23.21	33.88	391.49	26.7
2	N90039	15.42	23.49	32.39	354.36	26.81
2	N90045	17.51	19.89	30.34	367.81	27.03
2	N90046	16.38	22.58	39.96	444.39	32.34
2	N91067	18.67	24.72	39.6	410.06	25.86
2	N91068	16.58	29.01	34.54	423.58	28.2
2	N91071	16.92	25.74	25.57	419.52	28.24
2	N91073	15.46	26.32	38.05	373.98	24.52
2	N91094	17.14	24.63	45.44	363.99	26.01
2	N96310	17.77	25.81	40.08	384	25.13
2	N96315	61.61	22.03	32.66	363.37	28.36
2	N96316	17.18	26.1	40.87	417.78	28.83
2	N96317	15.33	22.83	32.56	386.25	26.69
2	N96319	25.22	20.56	32.17	423.78	31.99
2	N96320	40.06	24.6	38.87	446.21	34.86
2	N96321	26.64	23.72	43.54	355.31	25.09
2	N96322	16.62	21.06	25.7	402.64	30.74
2	N96323	39.57	23.14	20.8	378.42	26.08
2	N96325	37.86	23.4	37.56	420.56	29.21
3	884	16.52	19.38	49.04	423.79	28.58
3	885	15.25	26.77	37.53	405.07	27.07
3	886	17.18	25.94	36.13	411.71	28.6
3	888	15.62	19.98	29.96	455.7	29.79
3	N92206	20.55	25.81	32.46	443.89	31.65
3	N92208	16.92	22.82	23.68	409.97	28.98
3	N92209	14.88	24.18	32.47	366.49	23.21
3	N92211	17.74	20.15	31.19	348.83	23.31
3	N92213	20.83	24.95	38.53	437.95	30.4
3	N92254	15.4	26.63	39.7	348.52	24.03
3	N92255	13.48	23.53	27.62	394.8	26.65
3	N92271	17.79	21.64	31.83	380.4	25.68
3	N92276	17.18	22.13	35.41	393.67	24.63

Table A.2 (Continued)

3	N92278	15.77	23.03	19.61	411.38	27.89
3	N92282	14.38	20.72	35.46	350.31	26.53
3	N92284	18.98	24.34	34.13	451.93	31.56
3	N92286	27.86	28.05	44.27	432.1	28.4
3	N92289	20.3	20.84	44.34	409.63	27.95
3	N92301	14.94	21.25	37.24	336.59	25.81
3	N92302	13.92	31.35	40.21	424.44	31.54
4	KELKIT1	17.47	24.97	37.99	351.9	24.27
4	KELKIT11	19.92	23.96	35.97	329	22.5
4	KELKIT3	18.64	24.36	45.57	293.69	20.28
4	KELKIT4	15.25	22.28	29.68	290.83	20.06
4	KELKIT6	22.48	19.03	26.31	295.22	24.3
4	KELKIT7	18.74	21.15	45.79	304.42	20.5
4	KELKIT8	18.45	19.03	31.04	351.92	20.46
4	KELKIT9	19.15	22.35	48.48	492.22	32.6
4	N91052	16.26	21.74	27.82	372.44	23.5
4	N92149	18.36	20.91	37.79	382.74	27.3
4	N92152	41.59	20.71	33.42	448.29	33.66
4	N92153	17.41	28.36	44.93	442.05	32.89
4	N92154	16.86	24.98	42.79	451.02	32.62
4	N92156	17.51	25.94	41.89	405.46	33.48
4	N92159	16.82	23.54	27.7	367.58	25.13
4	N92160	16.86	28.49	25.22	442.67	31.84
4	N92162	22.25	21.1	23.89	396.56	24.76
4	N92164	20.8	23.36	48.68	413.16	26.75
4	N92165	18.85	25.02	46.02	370.26	24.01
4	N92166	27.43	25.82	29.33	386.9	25.11
4	N92167	19.5	27.35	39.76	327.77	21.15
4	N92168	38.22	23.16	31.44	408.33	27.38
4	N92169	13.36	24.16	33.54	341.88	21.53
4	N92170	15.76	22.34	28.71	354.16	29.84
4	N92171	14.11	23.8	36.95	429.69	32.27
4	N92176	19.94	25.32	40.98	405.53	26.9
4	N92179	18.45	22.21	24.81	363.4	27.69
4	N92182	36.65	24.01	29.58	421.57	26.52
4	N92185	13.58	24.73	32.8	385.19	25.82
4	N92187	16.99	25.07	37.62	361.28	24.65
4	N92195	27.63	22.49	23.61	430.35	28.02
4	N92200	17.87	22.97	36.95	352.85	25.19
4	N92264	15.3	26.19	24.26	427.7	27.3
4	N92269	15.82	24.06	28.38	387.31	25.28

Table A.2 (Continued)

5	N91075	58.73	22.15	47.43	494.65	44.01
5	N91076	18.59	24.65	34.35	412.71	27.39
5	N91077	16.4	22.12	33.13	440.42	26.94
5	N91078	29.41	26.33	38.66	430.3	37.52
5	N91080	18.9	23.65	36.49	413.83	25.92
5	N91109	17.38	21.23	31.11	351.6	23.25
5	N91110	17.77	21.47	28.09	478.38	39.51
5	N91111	19.34	22.55	52.1	431.31	33.37
5	N91112	66.22	22.42	37.45	421	26.09
5	N91117	13.4	28.2	42.9	411.15	29.32
5	N91118	15.09	32.61	40.1	414.17	28.98
6	N91095	19.89	23.17	27.67	396.92	31.88
6	N91101	13.91	22.93	45.88	372.84	25.32
6	N91102	19.43	25.26	44.66	442.08	32.51
6	N91103	29.12	22.04	22.03	406.52	27.31
6	N91105	18.87	18.72	26.51	346.49	23.19
6	N91108	39.01	19.51	23.45	433.23	30.17
6	N91119	18.31	22.48	36.47	419.86	29.38
6	N91120	20.14	21	39.62	338.19	22.35
6	N91121	13.05	23.48	32.47	392.97	25.16
6	N93304	14.58	21.87	35.12	410.03	29.39
7	N91122	23.02	26.76	42.86	449.38	35.42
7	N92123	18.1	21.46	21.66	340.52	22.96
7	N92124	50.74	24.49	35.48	345.61	22.53
7	N92126	16.78	27.66	43.42	398.96	28.49
7	N92128	14.31	23.58	22.67	397.46	25.53
7	N92130	26.38	23.05	42.43	437.11	32.2
7	N92131	51.47	36.53	46.78	442.54	30.74
7	N92132	41.71	26.42	36.78	465.75	33.69
7	N92137	13.43	28.99	30.14	366.49	29.14
7	N92138	20.05	22.61	44.79	347.33	23.88
7	N92140	15.42	26.44	43.09	357.79	24.55
7	N92243	16.41	27.23	36.6	362.89	23.96
7	N92245	14.53	22.93	37.48	366.08	22.26
7	N92292	20.14	23.41	23.42	390.08	26.76
7	N92293	15.88	21.62	33.54	371.11	24.65
7	N92295	20.77	18.55	27	362.22	26.06
7	N92297	17.23	20.31	29.88	371.04	24.07
7	N92298	18.22	24.24	39.7	378.81	25.14
7	N92299	18.58	18.54	23.62	340.03	26.89
7	N93306	20.47	22.83	30.38	376.22	24.97

Table A.2 (Continued)

8	ALTERRA	14.56	18.89	39.09	415	29.94
8	EFN1	67.65	22.95	43.1	420.47	28.73
8	EFN2	18.02	21.79	29.98	312.08	21.02
8	FARCFLHH 35	80.16	18.56	41.78	388.56	25
8	LVUBAKA	17.13	22.55	35.37	428.13	29.84
8	URIFFMH	17.15	20.75	31.42	403.68	33.13
9	N020101	14.05	22.44	31.92	348.64	28.57
9	N020102	16.15	22.36	21.21	379.38	28.6
9	N020104	14.41	22.27	29.27	427.64	35.58
9	N020201	19.62	18.56	40.35	450.42	33.7
9	N020203	35.08	22.19	35.68	441.54	30.52
9	N020206	19.06	20.97	21.81	401.52	31.35
9	N020501	15.32	23.56	36.41	408.02	34.69
9	N0205013	27.5	25.34	28.79	403.94	30.75
9	N020502	17.18	22.53	30.91	371.25	30.92
9	N020503	18.06	19.31	35.41	374.01	30.45
9	N020506	16.12	21.87	35.1	381.94	28.1
9	N020507	18.55	22.97	38.13	366.84	30.7
9	N020508	19.1	17.78	30.24	393.23	31.34
9	N020509	20.91	22.58	37.18	387.6	32.83
9	N020510	18.73	21.12	17.55	457.13	46.15
9	N020512	16.85	21.41	30.94	343.94	27.74
9	N020702	20.47	17.72	27.16	407.46	28.71
9	N020703	17.86	22.09	37.84	405.08	35.17
9	N020704	14.57	21.32	39.86	388.01	31.32
9	N020705	15.58	22.81	30.19	368.33	30.33
9	N020801	17.28	21.11	36.85	377.83	28.26
10	KAEN92	24.64	23.63	38.25	389.2	32.8
10	N02338	18.9	27	37.84	300.95	19.71
10	N03324	12.68	23.86	38.71	389.32	26.59
10	N03333	23.27	18.66	27.31	431.77	33.43
10	N03399	14.08	22.27	31.6	308.35	25.2
10	N62164	15.65	23.56	29.84	451.25	29.42
10	N82008	17.07	27.93	49.79	373.12	26.99
10	N82166	15.86	25.42	53.7	463.72	34.87
10	N85010	15.98	22.97	26.53	394.69	26.84
10	N85018	14.82	22.17	21.41	383.4	25.84
10	N90062	42.35	24.32	27.06	464.33	31.21
10	N90065	17.38	27.69	48.1	416.42	28.11
10	N90102	51.64	18.69	20.12	428.06	43.02
10	N91002	14.17	23.62	40.57	387.94	33.68

Table A.2 (Continued)

10	N91021	51.52	21.01	37.34	443.97	35.53
10	N91054	19.09	24.8	23.65	345.33	22.91
10	N91212	9.39	23.27	36.72	395.63	25.35
10	N92058	23.35	21.15	39.65	412	26.88
10	N92060	24.29	26.17	39.68	409.31	29.9
10	N92073	16.18	21.16	37.73	415.4	30.06
10	N92097	18.76	17.78	29.83	308.15	22.11
10	N92114	18.52	24.43	38.76	397.81	26.39
10	N92831	15.37	20.01	20.17	394.97	27.89
10	N95045	35.4	23.45	38.47	379.33	24.37