CHARACTERIZATION OF GLYCOALKALOID CONTENT AND MOLECULAR MAPPING IN EGGPLANT

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Öyküm KIRSOY

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We approve the thesis of **Öyküm KIRSOY**

Date of Signature

..... 30 November 2006
Assoc.Prof. Anne FRARY
Advisor
Department of Biology
Izmir Institute of Technology

…….. 30 November 2006
Assist.Prof. Rithchie EANES
Co-Advisor
Department of Chemistry
Izmir Institute of Technology

……... 30 November 2006
Assist.Prof. Çağlar KARAKAYA
Department of Biology
Izmir Institute of Technology

…… 30 November 2006
Asist.Prof. Alper ARSLANOĞLU
Department of Biology
Izmir Institute of Technology

…… 30 November 2006
Asist.Prof. Gülşah ŞANLI
Department of Chemistry
Izmir Institute of Technology

……... 30 November 2006
Asist.Prof. Ayten NALBANT
Head of Department
Izmir Institute of Technology

……
Assoc.Prof.Dr. M.Barış ÖZERDEM
Head of the Graduate School

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ABSTRACT

CHARATERIZATION OF GLYCOALKALOID CONTENT AND MOLECULAR MAPPING IN EGGPLANT

In this thesis, solamargine which is a known eggplant glycoalkaloid and has an important place for human health was characterized in eggplant. For characterization, two eggplant lines *S.melongena* MM738 and *S.linnaeanum* MM195 were used. Although, for identification and detection of glycoalkaloid concentration, many different methods have been utilized, for this thesis, high-performance liquid chromatography (HPLC) was used to analyze glycoalkaloid concentration in eggplant. In HPLC, spiking of samples was done using a solamargine standard and it was found that *S. melongena* had an undetectable level of solamargine while *S. linnaeanum* had between 17.6 and 33.4 mg (average 25.5 ± 11) solamargine per gram of freeze dried powder.

In addition to characterization of glycoalkaloids in *S.melongena* MM738 and *S.linnaeanum* MM195, different types of molecular markers were surveyed for polymorphism in *S.melongena* MM738 and *S.linnaeanum* MM195 for mapping. A total of 47 polymorphic markers were then tested on the F2 population and located on the eggplant molecular genetic map.

ÖZET

GLYKOALKALOİD İÇEREĞİNİN KARAKTERİZE EDİLMESİ VE PATLICANDA MOLEKÜLER HARİTALAMA

Bu tezde, patlıcan glykoalkaloid'i olarak bilinen ve insan sağlığı için önemli bir yere sahip olan, solamargine patlıcanda karakterize edilmiştir. Karakterizasyon için, *S.melongena* MM738 ve *S.linnaeanum* MM195 olan iki patlıcan türü kullanılmıştır. Glykoalkaloid'in tanımlanması ve konsantrasyonunun belirlenmesi için bir çok değişik metot kullanılmasına rağmen, bu tezde, patlıcandaki glycoalkaloid konsantrasyonu HPLC kullanılarak analiz edilmiştir. HPLC'de örneklerde solamargine standartları kullanılarak solamargine miktarı arttırılmıştır ve *S.linnaeanum* MM195 için her bir gram kurutulmuş tozda 17.6 ve 33.4 mg değerleri arasında solamargine bulunurken, *S. melongena'* da belirlenebilir bir değer kaydedilmemiştir..

S.melongena MM738 ve *S.linnaeanum* MM195 için glykoalkaloid'in karakterizasyonuna ilaveten, değişik tipteki moleküler işaretleyiciler polimorfizim için *S.melongena* MM738 ve *S.linnaeanum* MM195 'da haritalanmıştır. Toplam 47 adet polimorfik işaretleyici daha sonra F2 populasyonunda test edilmiş ve patlıcan moleküler genetik haritalarına yerleştirilmiştir.

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CHAPTER 1

INTRODUCTION

In Turkey, agriculture has an important place for the people's lives and the Turkish economy. Approximately, 1500 commercial vegetable types such as pepper, tomato, potato, corn, and cucumber are grown in the field or greenhouse in Turkey. In 2005, vegetable production was determined to be 271.100.00 tonnes in Turkey "(WEB_1 2006)". According to this result, Turkey is situated in the first ten countries in the world for vegetable production. Statistics for the vegetables with the highest production in 2005 are shown in table 1.1.

Table 1.1. The most grown vegetables and their production in Turkey, 2005. (Source: WEB_1 2006)

Most grown vegetable	**Production (tonnes)**
Sugar beet	13.800.000
Tomato	9.700.000
Potato	4.170.000
Maize	3.500.000
Cucumber	1.725.000
Eggplant	880.000
Cabbages and other brassicas	701.000

One of vegetables which has a significant place for the Turkish economy, is eggplant (aubergine, brinjal, guinea squash). Eggplant which is in the Solanaceae family and has 24 chromosomes (2n), is commonly produced in China, India, Egypt, Turkey, Spain, and Italy. Turkey accounts for 2.89% of the world production of eggplant "(WEB_1 2006)". In table 1.2., eggplant production according to countries is shown for 2005.

Table 1.2. Eggplant production.
(Source: WEB_1 2006)

Country	**Eggplant production (tonnes) / 2005**
China	17.030.300
India	8.200.000
Egypt	1.000.000
Turkey	880.000
Japan	395.000
Italy	373.635
Spain	60.000
World	30.523.867

In addition to its economic importance, eggplant is generally used to produce pharmaceutical products because of its valuable secondary metabolites such as glycoalkaloids. The glycoalkaloids in eggplant can also be utilized as fungicide, pesticide, insecticide, and bactericide. However, these compounds are toxic for animals and humans. In this thesis, the glycoalkaloid content of eggplant was characterized. In addition, molecular markers were mapped in the eggplant genome in order to develop a higher resolution map of this crop species.

CHAPTER 2

SECONDARY METABOLISM

In all organisms, there are two types of metabolism which synthesize complex molecules from simple molecules with the help of catalysts. These metabolisms are called primary and secondary metabolism "(Edwards and Gatehouse 1999)". Primary metabolism, also known as basic metabolism, is very significant for organisms, because the compounds produced in primary metabolic pathways are used in vital functions "(Nugroho and Verpoorte 2002)". While primary metabolites play roles in development and growth functions, compounds synthesized in secondary metabolism do not enter into these types of reactions "(Croteau et al. 2000, Nugroho and Verpoorte 2002)". However these compounds have important functions including interactions between organisms and their environments "(Nugroho and Verpoorte 2002)". These secondary metabolites are often necessary so that organisms can survive in their ecosystem.

Although primary and secondary metabolites are different, they can have overlapping biosynthetic pathways and primary metabolites often serve as precursors for the production of secondary metabolites "(Edwards and Gatehouse 1999)". When these metabolisms are compared with each another, it is seen that primary metabolites are present in greater quantities than secondary metabolites "(Edwards and Gatehouse 1999)". This feature is important because primary metabolites can serve as substrates in secondary metabolic pathways. As figure 2.1. shows, in the primary metabolic pathway of carbon, different compounds, such as pyruvate and acetyl-CoA, produced in intermediate steps, are used as precursors to produce secondary metabolites. For example, the glucose generated by photosynthesis is converted to pyruvate by glycolysis. The pruvate can then be converted into aliphatic amino acids which are precursors for alkaloid production. Alternatively, the pruvate can be used to make acetyl-CoA which has a role in flavanoid, terpenoid and wax production. If malonic acid is produced by acetyl-CoA, this product can give flavanoids or waxes according to two different pathways. However, if acetyl-CoA turns into mevalonic acid, this product can be utilized as an intermediate compound to synthesize terpenoids. These pathways are shown in Figure 2.1.

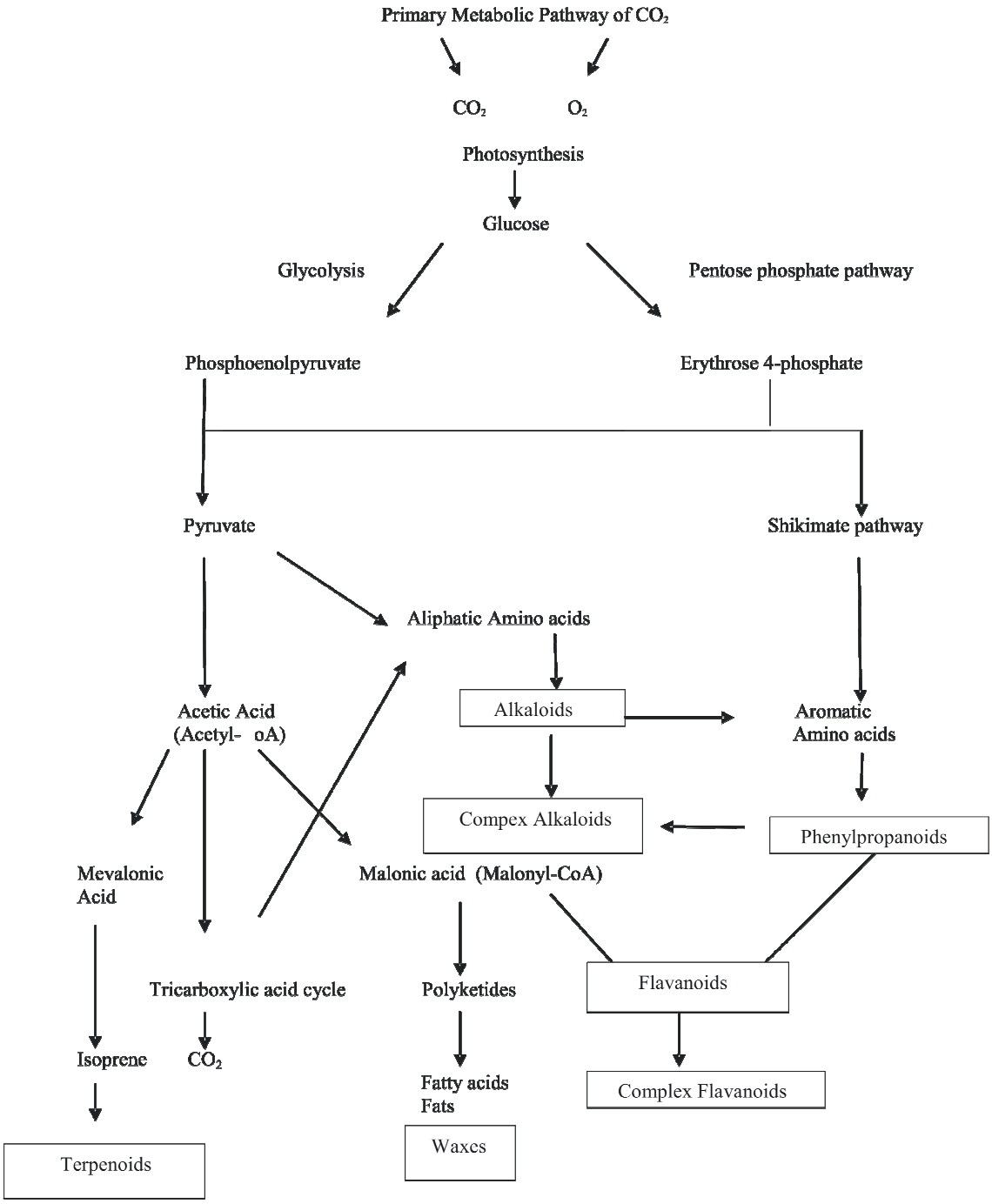

Figure 2.1. Relationships between primary metabolic pathways and secondary metabolic pathways. Secondary metabolites are shown in boxes. (Source: Adapted from Nugroho and Verpoorte 2002, Edwards and Gatehouse 1999)

2.1. Functions of Secondary Metabolites in Plant

For a long time, secondary metabolites have been known as metabolic wastes. However in recent years, researchers have started to research the functions of secondary metabolites. When the pathways of secondary metabolism are studied, it is seen that synthesis of secondary metabolites is very costly for plants and other organisms which have secondary metabolism "(Wink 1999)". 'Costly' means that secondary metabolism requires high concentrations of ATP. Because high levels of ATP are needed during secondary metabolite production, it has been thought that these metabolic compounds are not functionless products "(Wink 1999)".

Research about the functions of secondary metabolites reveals that secondary metabolites have importance for organism survival "(Demain and Fang 2000)". In plants, these metabolic compounds have ecological functions for plants and other organisms related to plants "(Taiz and Zeiger 1998)".

There are many significant functions of secondary metabolites in plants. For example, secondary metabolites protect plants against microbial pathogens and viruses. Plants having secondary metabolites are also protected aganist herbivores. Moreover, secondary compounds provide a defence system aganist abiotic enviromental stresses. Some secondary metabolites attract insects for pollination and seed dispersial. In addition, they are used for interactions between plants and symbiotic microorganisms "(Yazaki 2005 and Wink 1999)". Furthermore, these compounds are important in a plant's relations with other plants.

All of these functions are physiological. In addition, secondary metabolites are used as natural medicines "(Yazaki 2005)". This function will be described in the 'Importance of Plant Secondary Metabolites for Human' section of this thesis.

The most well known feature of secondary metabolites in plants is protection against herbivores (insect and animals) and pathogens (bacteria and fungi). Generally, plants have both constitutive and inducible defence mechanisms against microbial pathogens "(Edwards and Gatehouse 1999)". In these defence systems, secondary metabolites play a very significant role. For example, phenylpropanoids are secondary metabolic compounds which are necessary to form the wall polymers lignin and suberin. During infection, these polymers are used as a barrier to surround infected tissues and protect uninfected tissues from pathogens "(Edwards and Gatehouse 1999)".

Moreover, phenolic compounds in the cell wall provide resistance to fungal hydrolases. Polyketides and terpenoids which are phenolic components, are utilized as antibiotics in the defence system. Generally, secondary metabolites that are used as antibiotics against pathogens are active at all times, because these components are part of the constitutive defence mechanism and are usually localized in the cell wall or in the vacuole "(Edwards and Gatehouse 1999)". However, in the inducible defense system, defence compounds are formed after infection by the pathogen "(Edwards and Gatehouse 1999)". These responses include the production of different secondary metabolites such as steroid glycoalkaloids. Moreover secondary metabolic compounds serve as signal agents during infection. When a plant is infected by a pathogen, it can become resistant against the pathogen that infected the plant. This defense system is called systemic acquired resistance (SAR). SAR is generally induced by secondary metabolites such as salicyclic acid "(Edwards and Gatehouse 1999)".

Apart from defence functions against pathogens, secondary metabolites protect plants from herbivores because of their toxicity effects "(Attardo and Sartori 2003)". Plants also use toxic secondary metabolites to compete with other individuals of the same or different species "(Attardo & Satori 2003 and Edwards & Gatehouse 1999)". This type of toxic compound is called an 'allelopathic substance'"(Yazaki 2005 and Attardo & Sartori 2003)". For instance, two plants which share the same territory, will compete for insufficient water or nutrients. Under these conditions, allelopathic substances play an important role. Thanks to these components, the plant having toxic secondary metabolic compounds can survive and produce progeny.

Moreover plants have to defend themselves against abiotic stresses such as temperature, light level and water level. These abiotic stresses cause oxidative stress in plants "(Slater et al. 2003)". Oxidative stresses can also occur because of air pollutants , UV radiation, and radioactive deposition "(Grassmann et al. 2002)". Generally, oxidative stresses damage plants due to reactive oxygen species (ROS). Hydroxyl radicals (free radicals) which are one type of ROS and have atoms containing unpaired electrons, have an important role in oxidative stress. Free radicals react with other molecules because of their unpaired electrons and can easily cause damage "(WEB_2 2006)". For example, hydroxyl radicals can affect the cell membrane and proteins which are involved in amino acid modification "(Slater et al. 2003)". Also reactive oxygen species can damage bases and the sugar in DNA and so deletions and mutations may occur "(Slater et al. 2003)". These effects can be very dangerous for plants. Antioxidant

compounds are used by the plant to scavenge reactive oxygen species. Some secondary metabolites, such as flavanoids and quercetin, have significant roles as natural antioxidants "(Milbury et al. 2002 and Grassmann et al.2002)". Generally, these compounds provide inhibition of free radicals by scavenging and inhibiting the oxygen activating enzymes "(Grassmann et al. 2002)". Moreover, phenolics can be used to break radical chains. Although it has not yet been proven, it is hypothesized that terpenoids and their precursors can also act as antioxidants "(Grassmann et al. 2002)". Also, these compounds have a important role for the plant in tolerating extreme temperatures. When the temprature increases, terpenes provide increasing thermotolerance "(Grassmann et al. 2002)". In addition to protection against oxidative stresses, some phenolics such as flavanoids act as an UV protection shield "(Martens and Mithöfer 2005 and Grassmann et al. 2002)". They are amassed to form a barrier against UV in epidermal cells "(Yazaki 2005)" and absorb UV radiation "(Martens and Mithöfer 2005 and Grassmann et al. 2002)".

In addition to these functions, some secondary metabolites serve to attract insects or animals for pollination and seed dispersal "(Briskin 2000)". Generally secondary metabolites serve as flower pigments, scent and taste compounds and attract other organisms "(Edwards and Gatehouse 1999)". For example, terpenes and aromatic compounds are known as attractive compounds because of their volatility "(Edwards and Gatehouse 1999)". Also flavanoids are used as colour pigments in flower petals to attract the attention of insects or animals "(Yazaki 2005, Schijlen et al. 2004 and Martens and Mithöfer 2005)".

Another function of secondary compounds is to give signals for symbiotic interaction "(Briskin 2000)". These metabolites serve as signal agents to provide communication between a host plant and symbiotic microorganisms "(Wink 1999)". The interaction between Agrobacterium or Rhizobium and a plant can be given as an example of symbiotic interaction. For instance, some phenolic compounds derived from flavanoids are used to attract Rhizobium bacteria to plant roots for formation of nodules. When the bacterium can form nodules and live on the plant, the plant also benefits by using the soil nitrogen that has been fixed by the bacterium "(Edwards and Gatehouse 1999 and Martens and Mithöfer 2005)".

In addition to all these functions, secondary metabolites are also utilized as plant growth regulators at the cellular level and as regulators of gene expression "(Briskin 2000 and Eastwood 2001)". Especially, monoterpenes, abscisic acid and flavanoids act

as promoters to regulate gene activity in plants "(Eastwood 2001)". It has been shown that gene expression in plant and mammalian cell systems are increased by salicylic acid and phenolic acid "(Eastwood 2001)". It has also been demonstrated that some secondary metabolites such as opiates and abscisic acid act as modulator for activity of transmembrane channel in many mammalian tissue receptors "(Eastwood 2001)". Because of these survival functions, secondary compounds have an important role for plants, other organisms and the balance of nature.

2.2. Importance of Plant Secondary Metabolites for Humans

In section 2.1. 'functions of secondary metabolites in plants', the significance of these compounds for plants was mentioned. Besides these functions, they are very important for humans. Secondary metabolites are used for different purposes in medicine, the building sector, agriculture, the food sector, and the cosmetics, soap and shampoo industries.

In recent years, it has been found that secondary metabolites can have antioxidant, anticarcinogenic, anti-inflammatory, antiallergenic, and antimicrobial effects on human health "(Singh et al. 2003, Kris-Etherton et al. 2002, Martens and Mithöfer 2005, Goff and Klee 2006, Milbury et al. 2002, Briskin 2000, Hoensch and Kirch 2005, Chung et al. 1998, Galati and O'Brien 2004, Ferguson 2001)". Because of these functions, they have been attractive for medicine and used as drugs. Therefore some secondary metabolites are called natural drugs. One of their most important functions is an antioxidant function. Most secondary metabolites such as flavanoids, beta-carotene, and lycopenes have antioxidant functions. This function protects humans from oxidative stress. It is known that oxidative stress damages humans because of ROS (reactive oxygen species). Generally free radicals which are a kind of ROS, can interact with lipids, proteins, low-density lipoproteins, and also DNA "(Slater et al. 2003)". When free radicals join together with one of these biomolecules such as a lipid, some modifications in the biomolecule occur due to the unpaired electrons of ROS. These modifications can cause some diseases in humans such as cardiovascular disease, cancer, and atherogenesis "(Slater et al. 2003 and Rao and Agarwal 2000)".

Secondary metabolites prevent these types of damages to humans, because they can scavenge all elements of ROS due to their antioxidant function "(Slater et al. 2003

and Rao and Agarwal 2000)". For example, it was found that lycopene scavenges nitrogen dioxide, thiyl (RS˙) and sulphonyl effectively and is an effective ROS scavenger because of its lipophilic structure "(Rao and Agarwal 2000)". Moreover, flavanoid is known to have the ability to scavenge endogenous ROS "(Galati and O'Brien 2004)". Some chronic diseases such as cardiovascular disease and cancer have been demonstrated to be prevented by antioxidant compounds "(Arab and Steck 2000, Rao and Agarwal 2000, Agarwal and Rao 2000, Galati and O'Brien 2004, Chung et al.1998)". For example, it was found that lycopene inhibited atherogenesis because of its ability to prevent oxidation of low-density lipoprotein "(Rao and Agarwal 2000)". Atherogenesis, is an important disease for humans and is the formation of a fatty deposit on the walls of the arteries and also affects arterial blood vessels "(WEB_3 2006)". Lycopene is also reported to protect humans against coronary heart disease or cardiovascular disease "(Arab and Steck 2000 and Agarwal and Rao 2000)". In addition, most polyphenols which act as antioxidants, protect humans against genomic instability and cancer thanks to their ability to protect DNA from oxidation "(Ferguson 2001)". In recent years, it has been demonstrated that phenolic antioxidants can be used in cancer therapy due to their antioxidant and pro-oxidant funtions. Pro-oxidant forms are the oxidized forms of phenolics and also antioxidant forms are their reduced forms "(Galati and O'Brien 2004)". It is known that antioxidant forms of phenolics protect humans against cancer thanks to their ability to scavenge ROS. However, it has been found that pro-oxidant forms of flavanoids cause the induction of apoptosis (programmed cell death) in cancer cells because of their ability to cause mitochondrial toxicity by collapsing the mitochondrial membrane "(Galati and O'Brien 2004 and Ferguson 2001)". Flavopiridol is used in medicine as an anticancer drug due its ability to induce apotosis in chronic lymphocytic leukemia cells "(Galati and O'Brien 2004)". Also it has been indicated that lycopene and β-carotene can be used as chemoprotective agents "(Khachik et al. 2002)". Moreover, flavone has been found to trigger apoptosis in colon and lung cancer cells and also it has been shown that lycopene has protective effects against breast, prostate, cervix, gastric and also colon cancers in humans "(Giovannucci 1999, Kris-Etherton et al. 2002, Martens and Mithöfer 2005)". Furthermore, phytoestrogens have been indicated to play an important role as protective agents in breast, prostate, and colon cancers "(Kris-Etherton et al. 2002)". It has also been found that glycoalkaloids, especially solamargine and solasonine, inhibit the growth of human

skin tumors and also colon and liver cancer cells "(Lee et al. 2004)" and it has been proven that solamargine induces apoptosis in human cancer cells "(Kuo et al. 2000)".

Besides the antioxidant and also anticancerogenic functions of secondary metabolites, these compounds have anti-inflammatory, antimicrobial, antibacterial, antiallergic, antihypertensive, and antiarthritic functions which are used in medicine "(Pimiä et al. 2002, Martens and Mithöfer 2005, Ferguson 2001, Singh et al. 2003, Vieira et al. 2003, Delporte et al. 1998, Cowan 1999)". Generally, polyphenols have an important role as anti-inflammatory, antimicrobial, and antibacterial compounds in medicine "(Pimiä et al. 2002, Martens and Mithöfer 2005, Ferguson 2001, Singh et al. 2003)". It is known that cranberry juice has an antibacterial effect aganist *Escherichia coli* adherence to mucosal surfaces because of its flavanoids "(Pimiä et al. 2002)". Especially, flavone has been found to be inhibitory to respiratory syncytial virus "(Cowan 1999)". Also, it has been shown that other phenolic acids inhibit some different viruses, bacteria and fungi "(Cowan 1999)". For example, it has been indicated that alkaloids can be used to inhibit the HIV virus "(Cowan 1999)". In addition to these functions, each different secondary metabolite may have different functions on human health. For example, ginsenosides which are present in the phytomedical plant, *Ginseng*, have an immunostimulatory effect in humans and also are used as an adaptogen to increase physical performance "(Briskin 2000)". Moreover, hyperforin which is extracted from St. John's Wort is utilized as an antidepressant in medicine "(Briskin 2000)". Ginkgolide which is obtained from *Ginkgo biloba*, has different roles in human health. It has an important role in brain blood circulation and also it provides improved circulation in the leg. Thus, this compound has an effect on the blood vessel "(Briskin 2000)". Another secondary metabolite is kawain which is present in *Kava (Piper methysticum)*, and is used for some diseases such as anxiety, nervous tension and insomnia "(Briskin 2000)". Two other interesting compounds are hyoscyamine and scopolamine, tropane alkaloids that have relaxant activity and are utilized as smooth muscle relaxants "(Edwards and Gatehouse 1999)". Vincristine and vinblastine which are extracted from Madagascar periwinkle, are indole alkaloids. These compounds are used to treat Hodgkin's disease and leukaemia "(Edward and Gatehouse 1999)". In addition to these indole alkaloids, the isoquinoline alkaloid morphine which is extracted from opium poppy, has also been utilized as a painkiller for a long time "(Edward and Gatehouse 1999)". Table 2.1., shows some other secondary metabolites that are used in medicine.

Table2.1. Some plant secondary metabolites, their sources and their therapeutic applications (Source: Singh et al.2003).

PSM	Major Sources	Therapeutic application
Tannin	vascular woody plants ferns	Anticancer applications Cardioprotective effects Antimicrobial effects
Saponin	higher plants some marine animals	Anticancer applications Immunomodulatory applications Cardioprotective effects Antidiabetic effects Analgesic Anti-inflammatory effects
Mimosine	*Leucaena leucocephala* *Mimosa pudica*	Anticancer applications Anti-inflammatory effects
Phytoestrogens	leguminous forage seeds foods	Anticancer effects Prevention of prostate cancer Prevention of colon cancer Cardioprotective effects
Phytic acid	cereals nuts oilseeds pollen spores	

Because of all these fuctions, secondary metabolites are referred to as drugs for human health in medicine. However, secondary compounds are also used in different sectors such as in agriculture, building, food, cosmetics, soaps and shampoos "(Edward and Gatehouse 1999)". In agriculture, humans use some secondary metabolites as herbicides. Also in the building sector, two known secondary compounds, lignin and cellulose are utilized in building materials "(Edward and Gatehouse 1999)". Moreover, most secondary compounds are very important for the taste and colour of plant foods

"(Edward and Gatehouse 1999)". Furthermore, several secondary metabolites such as essential oils are used for perfume, soap and shampoo in the cosmetic, soap and shampoo industries "(Edward and Gatehouse 1999)".

Despite these benefical functions of secondary metabolites, they are also studied by scientists because of their toxic effects on humans "(McGehee et al. 2000, Korpan et al. 2004, Chami et al. 2003, Cipollini & Levey 1997, Yencho et al. 1998, Lachman et al. 2001)". Especially, glycoalkaloids which are extracted from the Solanaceae family, have been found to have toxicity due to inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) "(McGehee et al. 2000, Chami et al. 2003, Lachman et al. 2001)". It has been indicated that when these compounds inhibit acetycholinesterase or butyrylcholinesterase, they cause neurological impairment and also some symptoms such as diarrhea, abdominal pain, headache, neurological disorders, fever, delirium and coma "(Lachman et al. 2001)".

Due to all these functions and effects on human health, secondary metabolites have been used for a long time and will continue to be utilized in medicine and other industries by humans. Because of their diverse effects, chemical structures and uses, these metabolites are also very attractive for new research.

2.3. Major Classes of Secondary Metabolites

Plants are generally classified according to the secondary metabolites which they produce. This classification is called ‘chemotaxonomy’ "(Edwards and Gatehouse 1999)". However, secondary metabolites are classified according to their carbon skeletal type or biosynthetic orgins "(Croteau et al. 2000)". Generally, classification of secondary metabolites based on carbon skeletal type is not used because their carbon skeletons are very long. Thus, biosynthetic origin is the more commonly used classification. According to their biogenesis (biosynthetic) origin, secondary metabolites are categorized as terpenoids, nitrogen-containing, nitrogen and sulfur containing and also phenolics "(Edwards and Gatehouse 1999, Croteau et al. 2000)".

Table 2.2. shows the major classifications of plant secondary metabolites with examples of compounds in each class.

Table 2.2. The major classification of secondary metabolites and their subunits. (Source: Croteau et al. 2000 and Edwards and Gatehouse 1999)

Major class of SM	Subunite of SM
Terpenoids	Hemiterpenes
	Monoterpenes
	Sesquiterpenes
	Diterpenes
	Triterpenes
	Tetraterpenes
	Polyterpenes
	Meroterpenes
Phenolics	Phenolic acids
	Lignin
	Lignan
	Flavanoids
	Coumarins
	Furanocoumarins
	Stilbenes
	Tannin
Nitrogen and sulfur containing	Glucosinolates
Nitrogen containing	Alkaloids
	Glycoalkaloids

One major class of secondary metabolites are the terpenoids. Their biosynthetic origin is an isopentane skeleton "(Croteau et al. 2000)". Aromatics, carotenoids, and chlorophylls are significant members of this class. Terpenoids are usually used as essential oils in perfumes, phytopharmaceuticals and insecticides. Also, they include cardiac glycosides and therefore are utilized in medicine "(Croteau et al. 2000)".

Another major class is phenolics. They are derived from phenylalanine. This class has some of the best known members such as flavanoids, lignins, and tannins "(Croteau et al. 2000)". These phenolics have very significant roles for human health and plants. These functions were mentioned in sections 2.1. and 2.2.

In addition to these major classes, there is an important class of secondary metabolites which has several especially important roles in human health. This class of nitrogen containing compounds contains the alkaloids. Because of their structural complexity, significant functions and also toxicity, alkaloids will be described in more detail in section 2.2.1.

2.3.1. Alkaloids

Alkaloids are one of the largest classes of secondary metabolites. They are nitrogen-containing secondary compounds which are found in plants, bacteria, fungi and also animals "(Wink 1997 and Wink 2003a)". Generally, they are synthesized by 20% of higher plants "(Edwards and Gatehouse 1999 and Croteau et al. 2000)". So far, approximately 12.000 alkaloids have been isolated from different plant species "(Croteau et al. 2000)". They are especially found in certain families of higher plants such as Apocynaceae, Asclepiadaceae, Boraginaceae, Berberidaceae, Gnetaceae, Leguminosae, Liliaceae, Solanaceae, Papaveraceae, Ranunculaceae, Rubiaceae, and also Rutaceae "(Wink 1997 and Wink 2003a)". It has been known that alkaloids have been extracted from plants and used for a long time "(Croteau et al. 2000)". The first identified alkaloid was morphine which was extracted from opium poppy, *Papaver somniferum*, in 1806, by German pharmacist Friedrich Sertürner "(Croteau et al. 2000)". Since the discovery of morphine, alkaloids have been attractive for scientists because they have a range of features from beneficial effects to lethal effects on humans "(Wink 1997 and Croteau et al. 2000)".

2.3.1.1. Functions of Alkaloids

Alkaloids have a wide range of functions that are important for plants, animals and humans. Thanks to their different features, they can be utilized for different purposes such as dyes, flavors, fragrances, stimulants, hallucinogens, insecticides, animal and human poisons, and also therapeutic agents "(Wink 2003a)". Like other secondary metabolites, alkaloids also have allelopathic properties "(Wink and Schmeller 1998)". Because of these features, plants synthesizing alkaloids can compete with other plants. According to Wink's research "(Wink 2003a)", many alkaloids including

aconitine, berberine, boldine, caffeine, cinchonine, colchicine, cytisine, ergometrine, gramine, harmaline, hyoscyamine, lobeline, lupanine, narcotine, nicotine, papaverine, quinidine, quinine, salsoline, sanguinarine, sparteine, strychinine, and yohimbine, have been found to have allelopathic effects on other plants "(Wink and Schmeller 1998)". Also it has been shown that these compounds affect different molecular targets in other plants "(Wink and Schmeller 1998)". For example, some alkaloids such as sanguinarine, harmine, berberine, berbamine, quinidine, quinine, cinchonidine, cinchonine, boldine, and norharman, are effective on DNA and related enzymes. These compounds have been demonstrated to cause DNA intercalation which causes mutations in DNA. Another target of alkaloids is protein biosynthesis "(Wink and Schmeller 1998)". Several alkaloids in plants such as papaverine, solasonine, salsonine, yohimbine, ajmaline, boldine, and lobeline, serve as protein biosynthesis inhibitors "(Wink and Schmeller 1998)". Moreover, some alkaloids including steroid saponins, have been found to affect the stability of biomembranes. Especially, solanine which is a glycoalkaloid has been demonstrated to have strong hemolytic properties "(Wink and Schmeller 1998)". In addition it has been shown that colchicine inhibits formation of microtubules "(Wink and Schmeller 1998)". It is known that microtubules have a significant role in cell division during germination "(Wink and Schmeller 1998)". If the formation of microtubules has been inhibited by colchicine, germination will be inhibited. Thanks to this feature of colchicine, plants which synthesize this alkaloid can eliminate other plants easily.

Apart from allelopathic features, alkaloids have effects as chemical defense compounds, against herbivores, insects and other arthropods "(Wink 2003a and Wink 2004)". Also several alkaloids can serve as antimicrobial agents. For example, nicotine is known to have toxic effects against insects and so it has been used as an insecticide for a long time "(Croteau et al. 2000)". Also it was found that caffeine is effective against larvae of the tobacco hornworm (*Manduca sexta*) "(Croteau et al. 2000)". It has been known that alkaloids serving as chemical defense against animals, have generally high affinity for neurotransmitter receptors "(Wink 2003a, Wink and Schmeller 1998)". Therefore, they can bind these receptors easily and affect neuronal signal transduction including muscular and physiological activity "(Wink and Schmeller 1998)". In addition, it has demonstrated that several alkaloids such as aconitine, cytisine, lupanine, and sparteine have high affinity for acetylcholine receptors "(Wink and Schmeller 1998)". Because of this affinity of lupanine for acetylcholine receptors, this compound

is toxic for grazing animals "(Croteau et al. 2000)". Also lupin alkaloids inhibit Na and K channels "(Wink and Schmeller 1998 and Wink 2001)". Furthermore, caffeine, another alkaloid, was found to inhibit phoshodiesterase "(Croteau et al. 2000 and Wink and Schmeller 1998)". Due to phoshodiesterase inhibition, it causes the inhibition of cAMP hydrolyzation "(Croteau et al. 2000)".

Moreover, most alkaloids have toxic effects in humans. Due to both their toxicity and beneficial effects, alkaloids are frequently used in medicine. As mentioned in section 2.2., alkaloids are utilized as cardiac stimulants, relaxants for skeletal muscles, respiratory stimulants, analgesics, local anesthetics, and chemotherapeutics in medicine "(Wink 2003b)". However, several alkaloids cause toxic or pharmacological effects on humans "(Wink 2003b)". For example, some alkaloids such as narcotine cause narcotic and hallucinogenic effects. Several alkaloids such as nicotine, lobeline, muscarine, yohimbine, anabasine, solanine, and other steriod alkaloids can impair sensory ability, muscle activity, and organ functions because of their ability to affect the nervous system "(Wink 2003b)". Also, diarrhea or constipation can result from alkaloids thanks to their ability to inhibit digestive enzymes "(Wink 2003b)".

Due to all these features, alkaloids have been purified from plants for use in medicine. Therefore, the purification and determination of the biosynthetic pathways of alkaloids have been investigated by scientists.

2.3.1.2. Alkaloid Biosynthesis

Alkaloid biosynthesis has been of recent interest by scientists, because these secondary metabolites are usually used in a range of medical applications. To obtain pure alkaloids, their biosynthetic pathways should be known. However, this is not easy. It has been shown that biosynthetic pathways of most alkaloids are very complex. Therefore, little information about alkaloid biosynthesis is known. According to research, alkaloids in plants are usually derived from the decarboxylation of amino acid precursors such as lysine, ornithine, tryptophan, and tryosine "(Luca and Pierre 2000, Luca and Laflamme 2001, Wink 1997)". For example, the large class of alkaloids, monoterpenoid indole alkaloids are derived from both terpenoid and tryptophan precursors "(Luca and Pierre 2000)". In table 2.3, some precursor amino acids and the alkaloids which are synthesized from them are shown.

Table 2.3. Some alkaloids, their precursor amino acids and plants in which these alkaloids are found. (Source: Wink 1997).

Precursor Amino acid	**Alkaloid**	**Main occurrences**
Ornithine	Pyrrolizidine	*Senecio* ssp., *Crotalaria* ssp., *Heliotropium,* Other Boraginaceae
	Tropane	Solanaceae, *Erythoxylum,*
	Nicotine	*Nicotiana* ssp.
Lysine	Punica	*Punica granatum, Duboisia, Sedum*
	Sedum	*Sedum* ssp
	Lobelia	*Lobelia* ssp.
	Quinolizidine	*Lupinus, Cytisus, Bastisia, Thermopsis, Laburnum,* Other Genisteae
Lysine	Lycopodium	*Lycopodium* ssp.
Aspartic acid	Areca	*Areca catechu*
Histidine	Pilocarpine	*Pilocarpus* ssp.
Anthranilic acid	Benzoxazines	Gramineae
	Furoquinoline	Rutaceae ,
	Acridone	*Acrony*chia, *Melicope* Rutaceae
Tryptophan	Indole	*Phosostigma*
	Ergoline	*Claviceps,* Convolvulaceae
	β carboline	Loganiaceae Apocynaceae, Zygophyllaceae
	Monoterpene indole	Apocynaceae, Loganiaceae, Rubiaceae

Table 2.3. Some alkaloids, their precursor amino acids and plants in which these alkaloids are found. (Source: Wink 1997). (con.)

Precursor Amino acid	**Alkaloid**	**Main occurrences**
	Quinoline	*Cinchona, Camptotheca*
Phenylalanine-Tyrosine	Amaryllidaceae	Amaryllidaceae
	Tetrahydroisoquinoline	*Lophophora, Cephaelis*
	Benzylisoquinoline	Papaveraceae,
Phenylalanine-Tyrosine	Protoberberine	Berberidaceae, Papaveraceae
	Benzophenanthridine	Papaveraceae
	Morhinane	Papaveraceea
	Aporphine	Papaveraceae, Monimiaceae, Magnoliaceae, Lauraceae
	Erythrina	*Erythrina*
	Phenylethyl-isoquinoline	*Colchium, Gloriso superba*
	Aristolochia	*Aristlochia, Asarum*
Phenylalanine-Tyrosine	Betacyanin/betaxanthin	Centrospermae
Glycine, Alanine, Arganine(gly, ala, arg)	Steroid	Solanum, Veratrum

Amino acids are converted to amines and different amines come together to produce central intermediates "(Luca and Laflamme 2001)". The biosynthesis of different alkaloid starts with different central intermediates. Because of the variety of different central intermediates, the diversification of alkaloids occurs "(Luca and Pierre 2000, Luca and Laflamme 2001)". For example, although norcoclaurine is used as a central intermediate for isoquinoline alkaloids, homospermidine is used as the intermediate for pyrrolizidine. Another, central intermediate in the biosynthesis of monoterpenoid indole is strictosidine "(Luca and Laflamme 2001)". These different central intermediates are modified by specific enyzmes to obtain alkaloids. Each

enzyme is expressed by a different gene. For instance, in the biosynthesis of nicotine alkaloids, five genes have been cloned so far "(Hughes & Shanks 2001 and Nugroho & Verpoorte)". In figure 2.2, nicotine biosynthesis and cloned genes are shown.

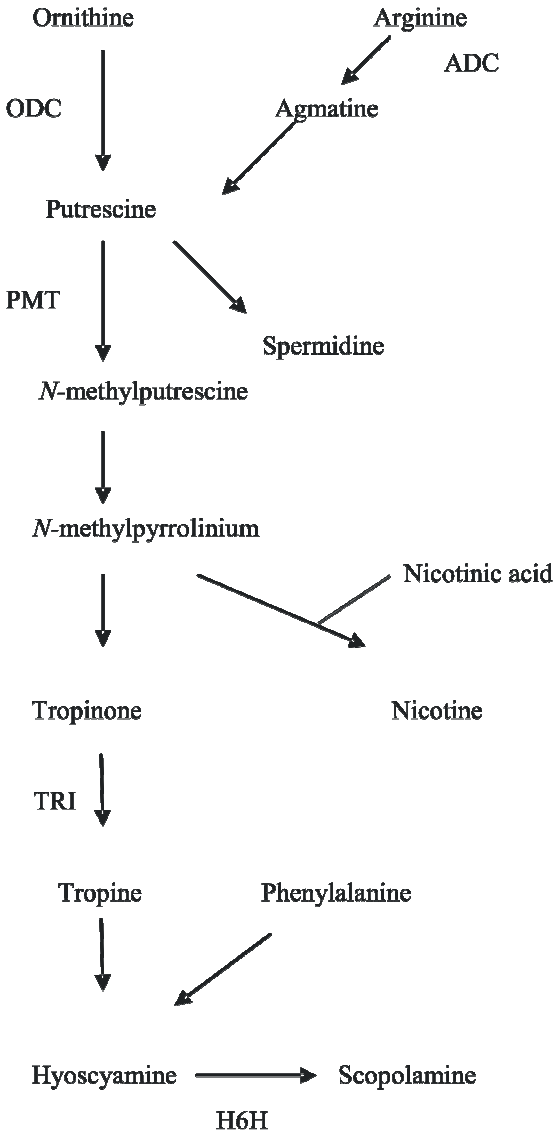

Figure 2.2. Nicotine and tropane alkaloid pathways and cloned genes in these pathways. ODC, ornithine decarboxylase; ADC, arginine decarboxylase; PMT, putrescine *N*-methyl-transferase; TRI tropinone reductase I; H6H, hyoscyamine 6β-hydroxylase (Source: Hughes & Shanks 2001 and Nugroho & Verpoorte 2002)

According to Hughes and Shanks 2001, in the nicotine pathway, ODC catalyzes ornithine to produce putrescine and also ADC catalyzes arginine to produce agmatine, agmatine is then converted to putrescine. PMT is needed to converted putrescine to *N*-

methylputrescine.TRI reduces tropinone to produce tropine. H6H also catalyzes the production of scopolamine from hyoscyamine.

Although each alkaloid has a different biosynthetic pathway, the biosynthetic pathways of most alkaloids are related to each other. In figures 2.3.and 2.4., the biosynthetic pathways of some alkaloids and their relationships are shown.

Simplified biosynthetic patways for tyrosine, morphine, codeine, macarpine, berberine tetrahydropalmatine and palmatine biosynthetic pathways are shown in figure 2.3. In addition, lupinate, calystegine, scopolamine, cocaine, senecionine, nicotine, slaframine biosynthesis are shown in figure 2.4.

For example, in figure 2.3., codeine is formed from codeinone which is converted from neopinone. After codeine formation, morphine can be obtained by converting codeine or alternatively formed from morphinone.

As as shown in figure 2.4., cocaine and scopolamine have the same precusor, L-phenylalanine, but their intermediate steps are different. For instance, L-phenylalanine is needed for the formation of trans-cinnamate. After the formation of trans-cinnamate, this intermediate compound can give cocaine or can be used to form other intermediate compounds such as tropate, L-hyoscyamine, and (6S)-hydroxy hyoscyamine for scopolamine.

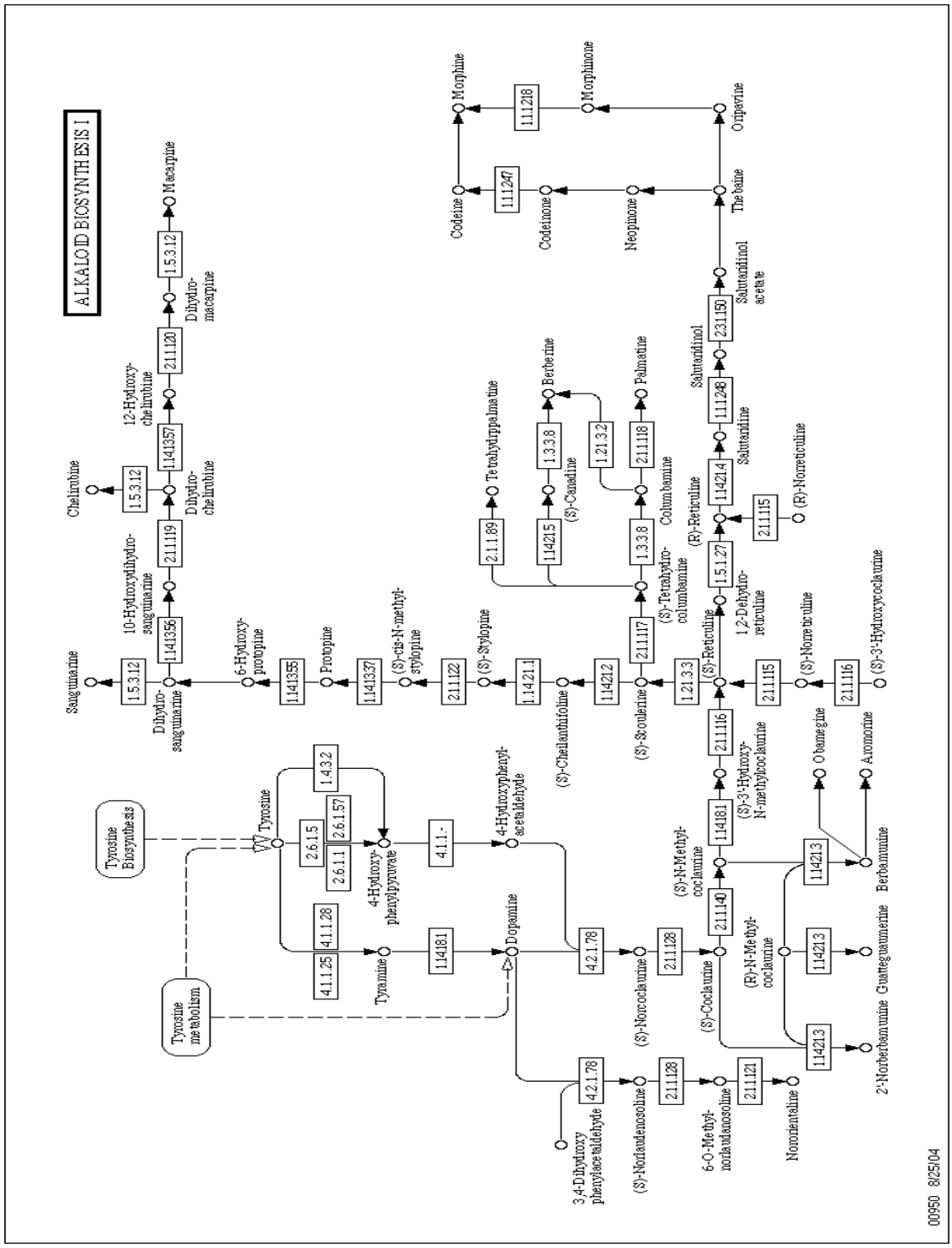

Figure 2.3. Biosynthetic pathways of some known alkaloids
(Source: WEB_4 2006)

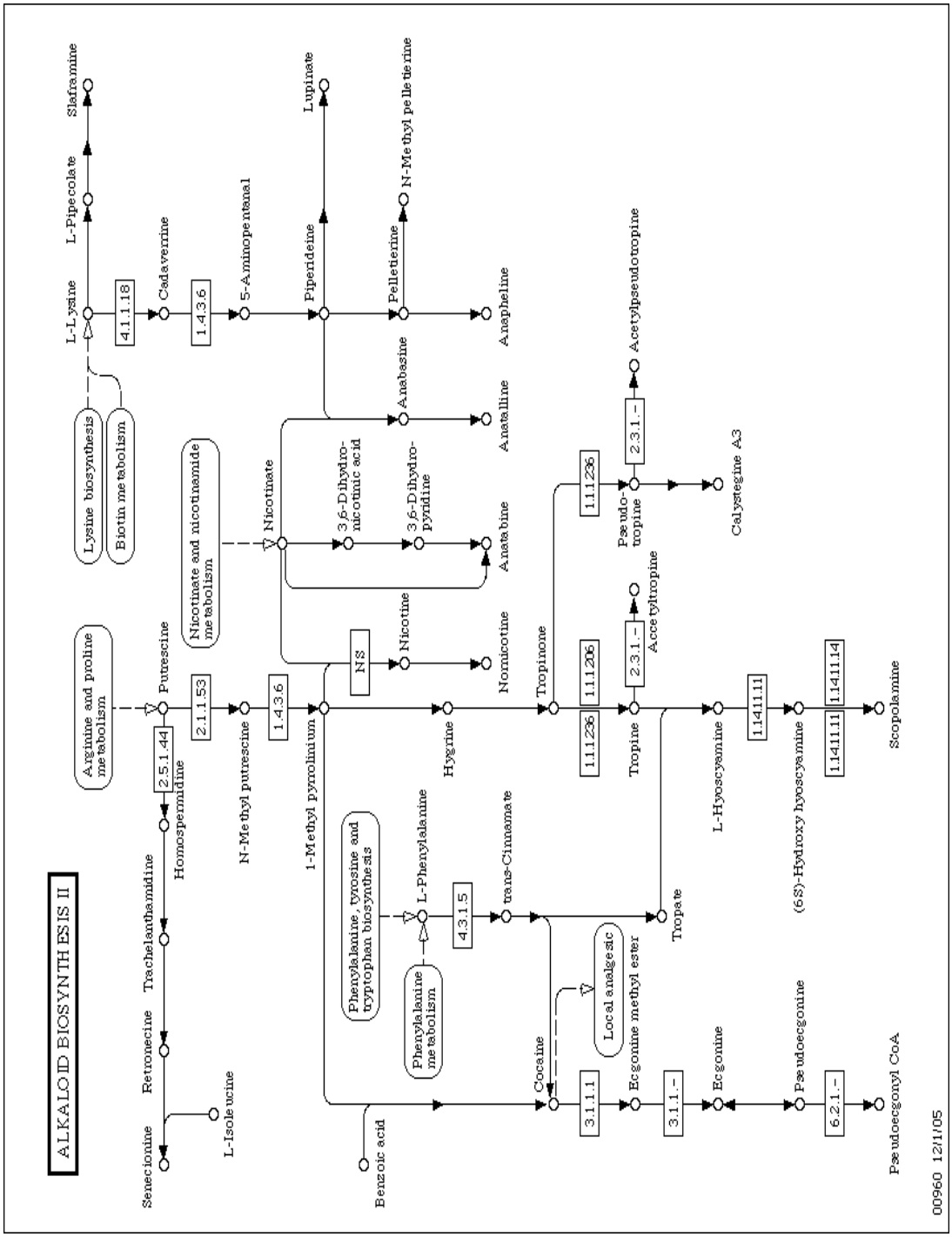

Figure 2.4.Biosynthetic patways of other alkaloids and their relationships with each other (Source: WEB_5 2006).

In recent years, it has been determined that alkaloid biosynthesis is affected by transcription factors "(Endt et al. 2002)". It has been shown that these factors have an important role as sequence specific DNA-binding proteins. Also they have been demonstrated to bind promotors of target genes and affect activity of these genes

depending on tissue type and internal and external signals such as hormones and UV-light, respectively "(Endt et al. 2002)". For example, it was found that methyl-jasmonate (MeJA), a stress hormone, induces terpenoid indole alkaloid biosynthesis "(Endt et al. 2002)". This hormone has been shown to affect gene expression in terpenoid indole alkaloid biosynthesis. In another example, ORCA 2 and ORCA 3 (octadecanoid-derivate-responsive *Catharanthus roseus* Apetala2 domain) transcription factors were found to be induced by MeJA and elicators and then bind to the promotor region of the terpenoid indole alkaloid biosynthetic gene *strictosine synthase* (STR). Because of these abilities of ORCA2 and ORCA3, they have been demonstrated to activate the biosynthetic pathway of terpenoid indole alkaloids "(Endt et al. 2002)". Like internal and external signals, tissue types also affect alkaloid biosynthetic pathways. While many alkaloids can be synthesized in roots, others can not be synthesized in these tissues. Similarly some alkaloids are formed in shoots or leaves, while others can not be synthesized in these organs. For example, it has been demonstrated that quinolizidine alkaloids are synthesized in mesophyll chloroplasts "(Wink and Robert 1998)". In studies with root culture, Wink and Roberts could not obtain quinolizidine alkaloids, however, they showed that small amount of quinolizidine alkaloid formation occured when these roots were exposed to light "(Wink and Robert 1998)". This result shows that both tissue type and external signals can affect alkaloid biosynthesis. Thus, alkaloid biosynthesis is regulated by different regulators and is a very complex process.

2.3.1.3. Transport and Storage of Alkaloids

In section 2.3.1.2., alkaloid biosynthesis was briefly mentioned and it was said that most alkaloids are synthesized in specific tissues. Also, it is known that accumulation and storage of alkaloids can change according to the type of alkaloid. In this section, the accumulation and storage of alkaloids in different tissues and their transport will be discussed.

Generally, alkaloids in plants are synthesized in specific tissues where the biosynthetic genes needed for specific alkaloids are expressed "(Yazaki 2005)". After biosynthesis of alkaloids, either they accumulate at the site of synthesis or they are transported to other specific tissues such as the leaves which are attacked by insects or herbivores "(Yazaki 2005, Wink 1997, Luca and Laflamme 2001)". It has been known

that alkaloids are usually stored in young tissues, roots and stem bark, leaves, seedlings and flowers "(Wink 1997)". For example, it was suggested that the pyrrolidine alkaloid of *Nicotiana* species, nicotine, is synthesized in root tissue, but this alkaloid is transported to the aerial part and leaves of the plant via xylem and accumulates there "(Yazaki 2005 and Tanguy 2001)". It was showed that like pyrrolidine alkaloid, biosynthesis of tropane alkaloid also occurs in the root but after its biosynthesis, it is transported to leaves and accumulated and stored there "(Luca and Laflamme 2001)". In contrast, it was demonstrated that isoquinoline alkaloid biosynthesis in *P. somniferum* occurs in parenchyma cells in the aerial part of the plant and also is stored in this part of the plant "(Weid et al. 2004)". It was exhibited that herbaceous plant alkaloids such as colchicine, aconitine, nicotine, coniine, veratrine, and steroidal alkaloids such as glycoalkaloids, are stored in epidermal tissues so that plants can resist their enemies "(Wink 1997)". For instance, monoterpenoid indole-alkaloids such as secologanin, strictosidine, and morphinan alkaloids such as codeine and morphine, have been displayed to be accumulated and stored in phloem parenchyma, laticifers and epidermis cells "(Kutchan 2005, Weid et al. 2004, Luca & Laflamme 2001)". Laticifers are specialized internal secretory cells "(Weid et al. 2004)". In addition, some alkaloids are stored in another type of specialized cell, the idioblast, in plant, but every plant does not have this type of cell. According to Wink, idioblasts were found in many plants such as *Corydalis, Sanguinaria, Ruta, Macleaya,* and *Catharanthus* for alkaloids such as corydaline, sanguinarine, rutacridones, protopine, and indole alkaloids, respectively "(Wink 1997)".

According to the functions of alkaloids in the plant, they are transported to different target tissue as mentioned above. For this transport, transport systems are needed. There are two transport system for alkaloids according to transport distance "(Wink 1997)". The first system is a long-distance transport system. In this system, alkaloids are transported by xylem and phloem "(Wink 1997)". For example, polyamine-derived alkaloids such as nicotine and tropane, are carried by xylem, but aconitine is transported by phloem "(Wink 1997 and Luca & Pierre 2000)". However the exact mechanism of long-distance transport is not known "(Tanguy 2001)". In Table 2.4, long-distance transport of alkaloids is shown.

Table 2.4. Long-distance transport of Alkaloids. (X: Transported —: Non-transported) (Source: Modified from Wink 1997 and Luca & Pierre 2000)

Alkaloids	Xylem	Phloem
Lupanine	—	X
Cytisine	—	X
Matrine	—	X
Senecionine	—	X
Seneciphylline	—	X
Aconitine	—	X
Nicotine	X	—
Hyoscyamine	X	—
Scopolamine	X	—
Swainsonine	—	X

The second system is a short-distance transport system (intacellular transport) "(Wink 1997)". Recently the short-distance transport system has been researched because it is known that alkaloids are synthesized in the cytosol or in membrane-enclosed vesicles and also accumulate in vacuoles in the cell. Thus alkaloids need to pass the tonoplast (vacuolar membrane) to enter into the vacuole for accumulation "(Wink 1997 and Taiz 1992)". To pass the tonoplast, three different mechanisms are used "(Wink 1997)". The first one is simple diffusion. Some lipophilic alkaloids such as nicotine, ajmalicine, vinblastine, colchicine, can pass the tonoplast by simple diffusion "(Wink 1997)". Another mechanism is carrier-mediated transport. In this mechanism, alkaloids move from one side to another side according to their charge and polarity "(Wink 1997)". The third mechanism is membrane fusion (vesicle-mediated transport) "(Wink 1997 and Yazaki 2005)". In this mechanism, alkaloids are biosynthesized in vesicle-enclosed compartments "(Wink 1997)". For example, it was found that berberine biosynthesis in *Berberis* occurs in specific vesicles which come from the endoplasmic reticulum and then fuse with the central vacuole "(Yazaki 2005)".

In addition to these three mechanisms, it has been shown that some alkaloids can be passed into the vacuole by transporters which are activated by a proton-substrate antiport mechanism "(Wink 1997 and Yazaki 2005)". For instance, the berberine in *C. japonica* has been suggested to be transported by an H+-antiporter "(Yazaki 2005)".

However, this transport is accepted as carrier-mediated transport according to experimental evidence "(Wink 1997 and Yazaki 2005)".

2.3.1.4. Major Classification of Alkaloids

Alkaloids are generally classified according to their precursors. For example, in general, they are divided into two groups: alkaloids derived from amino acids such as indole alkaloids and other alkaloids (alkaloids derived from amino acid and terpenoids such as aconitine, and sterol-derived alkaloids such as solanine) "(Wink 1997)". Sometimes, sterol-derived alkaloids are classified into terpenoid groups. However, the type of classification shown in table 2.5. is usually used.

Table 2.5. Major classification of Alkaloids
(Source: Adapted from WEB_6 2006)

Alkaloid Groups	Their Members
Pyridine	Piperine, Coniine, Trigonelline, Areacaidine, Guvacine, Pilocarpine, Cytisine, Nicotine, Sparteine, Pelletierine
Pyrrolidine	Hygrine, Cuscohygrine, Nicotine
Tropane	Atropine, Cocaine, Ecgonine, Scopolamine
Quinoline	Quinine, Quinidine, dihydroquinine, strychnine, Brucine, Veratrine, Cevadine
Isoquinoline	Morphine, Codeine, Thebaine, Papaverine, Narcotine, Sanguinarine, Narceine, Hydrastine, Berberine
Phenethylamine	Mescaline, Ephedrine, Amphetamine, Methamphetamine
Indole	
Tryptamines	Psilocybin, Serotonin, Melatonin
Ergolines	Ergine, Ergotamine, lysergic acid
Beta-carbolines	Harmine, Yohimbine, Reserpine, Emetine
Purine	
Xanthines	Caffeine, Theobromine, Theophylline
Terpenoids	
Aconite	Aconitine
Steroids	Solanine, Samandarin
Betaines	Muscarine, Choline, Neurine
Pyrazole	Pyrazole, Fomepizole

In addition to this type of classification, alkaloids can be classified according to the plants in which the alkaloids are synthesized such as Solanaceae alkaloids. In this thesis, one type of Solanaceae alkaloid will be mentioned.

2.3.1.5. Alkaloids in Solanaceae

Solanaceae is a family of flowering plants which has 96 genera and 3000 species "(Gemeinholzer and Wink, WEB_7 2006)". This family is important for agriculture, pharmacology, and the economy, because of their use as food, crops and in medicine. One of the special traits of the Solanaceae is alkaloid production. Due to their alkaloids, plants in the Solanaceae family can sometimes be beneficial, or sometimes toxic. It is known that the Solanaceae has a diverse range of alkaloids. For example, one alkaloid group in the Solanaceae, tropane alkaloids, are very known well. It has been found that tropane alkaloids are especially important in *Atropa, Datura, Mandragora, and Brugmansia* genera "(Özcan Seçmen et al. 2000 and WEB_7 2006)". Tropane alkaloids are used as anticholinergics which which have a relexant effect. Because they inhibit neurological signals in humans and animals, the consumption of tropane alkaloids has symptoms such as mouth dryness, ataxia, urinary retention, hallucination, convulsions, coma and death "(WEB_7 2006)". Another alkaloid from the Solanaceae family is nicotine. Nicotine is known to be found in the *Nicotiana* genus. It was displayed that nicotine affects neurological signals similar to tropane alkaloids "(WEB_7 2006)". Moreover, some alkaloids which are obtained from different *Solanum* species such as *S. lycocarpum, S. ligustrinum and S. americanum,* have been found to have anti-inflammatory, antihepatotoxic and antiallergic effects in humans "(Ferreira et al. 2003 and Delporte et al. 1998)". Additionally, it has been shown that alkaloids which are extracted from *S. lycocarpum*, can be used in diabetic and obese patients because of their diuretic, antiepileptic, sedative and antispasmodic effects "(Ferreira et al. 2003)". Alkaloids obtained from both *S. lycocarpum* and *S. tuberosum*, have also been proven to reduce cholesterol levels in humans "(Ferreira et al. 2003 and Arnqvist et al. 2003)".

Another type of alkaloid in the Solanaceae family is steroid alkaloids. It has been suggested that steroid alkaloids generally exist in many family such as Buxaceae (*Buxus*), Liliaceae (*Veratrum, Zigadenus, Notholiron, Schoenocaulon*), Apocynaceae (*Holorrhena, Funtumia, Kibatalia*) and Solanaceae "(Gemeinholzer and Wink)".

Steroid alkaloids in the Solanaceae family are especially found in the Solanum and Lycopersicon genera. Recently, much research has been done about steroid alkaloids in Solanaceae because this type of alkaloid has a vital role for humans and animals. Steroid alkaloids have sometimes toxic, sometimes pharmaceutical effects. In this thesis, one type of steroid alkaloid in Solanum, glycoalkaloids, was be analyzed in eggplant.

2.3.1.5.1. Glycoalkaloids

As mentioned in section 2.3.1.5., there are several alkaloids in the Solanaceae family. One category is glycoalkaloids. These alkaloids are also known as steroidal glycoalkaloids because of thier structure. Generally, the structure of steriodal glycoalkaloids is like steroids or steroid saponins "(WEB_8 2006)". Only, steroidal glycoalkaloids do not have oxygen in the F-ring of sapogenin and also steroid saponins do not have nitrogen "(WEB_8 2006)". Steroidal glycoalkaloids are composed of a hydrophobic C27- carbon skleton of cholestane (aglycone) and various hydrophilic sugar moeities (di, tri,and tetrasaccharide) "(WEB_8 2006 and Lachman et al. 2001)". It has been found that some steriodal glycoalkaloids have the same aglycone "(Arnqvist et al. 2003)". For example, two potato glycoalkaloids, α-chaconine and α-solanine have the same aglycone, solanidine, but they have different sugar moeities "(Arnqvist et al. 2003 and Bejarano et al. 2000)". So far, it has been found that there are five different aglycone groups the solanidanes (solanidine etc.), the spirosolanes (tomatidine etc.), the epiminocholestanes (solacongestidine etc.), solanocapsines (solanocapsine etc.), and the 3-aminospirostanes (jurubidine etc.) "(WEB_8 2006)". Ninety different steroidal glyalkaloids are known to exist in over 350 Solanum species "(WEB_8 2006)". Well known members of the Solanaceae family that produce steroidal glycoalkaloids are potato, tomato, pepper, and eggplant "(WEB_8 2006, Korpan et al. 2004, Tanksley et al. 2002, Lachman et al. 2001, Carman et al. 1986)". In table 2.6., the most common steroidal glycoalkaloids are shown.

Table 2.6. The most common steroid glycoalkaloids, their aglycone and plants producing them (Source: Adapted from WEB_8 2006).

Steroidal glycoalkaloids	**Aglycones**	**Sugar moiety**	**Plant**
α- Solanine	Solanidine	Solatriose	Potato
β- Solanine	Solanidine	Solabiose	Potato
α- Chaconine	Solanidine	Chacotriose	Potato
β- Chaconine	Solanidine	Chacobiose	Potato
α- Solamarine	Tometidenol	Solatriose	Potato
β- Solamarine	Tometidenol	Chacotriose	Potato
Demissine	Demissidine	Lycotetraose	Tomato
α- Tomatine	Tomatidine	Lycotetraose	Tomato
Solamargine	Solasodine	Chacotriose	Eggplant
Solasonine	Solasodine	Solatriose	Eggplant

In table 2.7., sugar moieties found in steroidal glycoalkaloid structures are listed. Also figure 2.5. displays the structures of the most common steroidal glycoalkaloid aglycones.

Table 2.7. Sugar moieties and their glycoside structures
(Source: According to Lachman et al.2001 and WEB_8 2006)

Sugar moiety	**Glycoside structure**
Solatriose	Galactose+ glucose+ rhamnose
Solabiose	Galactose+ glucose
Chacotriose	Glucose+ rhamnose+ rhamnose
Chacobiose	Glucose+ rhamnose
Lycotetraose	Galactose+ glucose+ β-D-xylose+ glucose

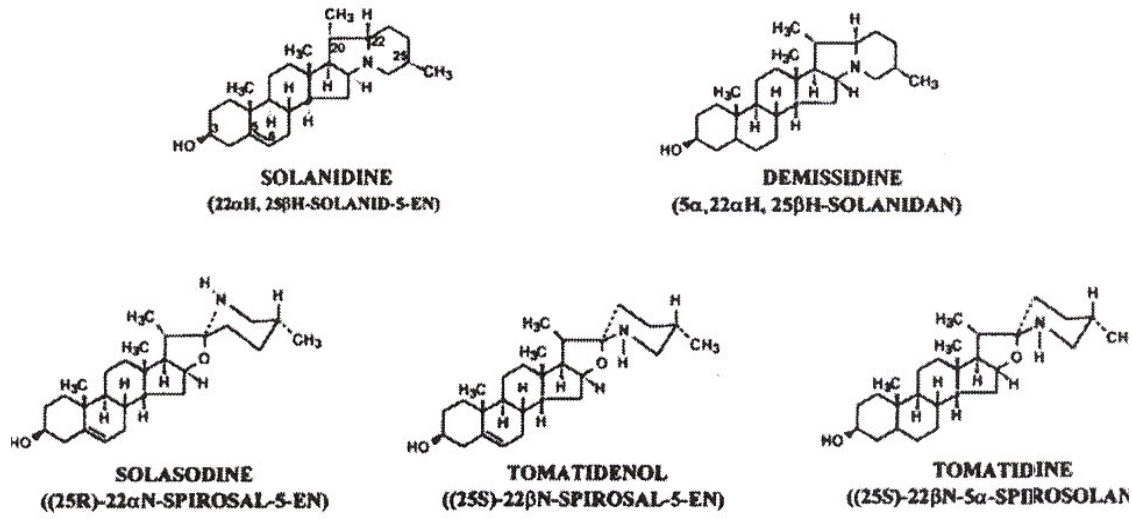

Figure 2.5. The most common steroidal glycoalkaloid aglycones.
(Source: According to WEB_8 2006)

For 186 years, steroidal glycoalkaloids have been investigated especially in potato because of their traits. According to Jaana Laurila (2004), firstly solanine was discovered in 1820 and then α-chaconine and α-solanine were found in 1954. Although steroid glycoalkaloids has been researched for a long time and the biosynthesis of steroidal glycoalkaloids has been studied extensively, the biosynthesis of glycoalkaloids is not yet completely known.

2.3.1.5.2. The Biosynthesis of Glycoalkaloids

In sections 2.3.1.2. and 2.3.1.3., alkaloid biosynthesis, transport and accumulation were mentioned. Like alkaloids, it has been found that glycoalkaloids are firstly synthesized in microsomal organelles or plastids in the cell and then accumulated in the cytoplasm or the vacuoles "(WEB_8 2006)". However, glycoalkaloids are different from alkaloids in point of transport. Glycoalkaloids can not be transported from one part of a plant to another part although alkaloids can be transported "(WEB_8 2006)". Recently, it has been suggested that the biosynthesis of steroidal glycoalkaloid is like steroid biosynthesis. Although the biosynthesis of glycoalkaloids is not known exactly, it has been found that the biosynthetic pathway of glycoalkaloids starts with reaction of acetate and acetyl-CoA "(WEB_8 2006 and Arnqvist et al. 2003)". According to Jaana Laurila, acetate is transformed to mevalonic acid, squalene,

lanosterol and cycloartenol, respectively "(WEB_8 2006)". It was showed that cycloartenol is an important precursor for both cholesterol and glycoalkaloid biosynthesis "(Arnqvist et al. 2003)". Cholesterol was shown to have a role as intermediate in the biosynthesis of aglycones "(WEB_8 2006 and Arnqvist et al. 2003)". According to this work, it has been postulated that several aglycones come from cholestrol, and several others come from saturated cholesteranol. For example, cholesterol is the intermediate for solasodine and soladulcidine while saturated cholesteranol is the intermediate for tomatidenol, tomatidine, solanidine, and demissidine "(WEB_8 2006)". After aglycone biosynthesis, these aglycones are glycosylated immediately by sugar and turned into the α-form of glycoalkaloids by the action of several enzymes such as solanidine glucosyltransferase "(WEB_8 2006 and Arnqvist et al. 2003)". For example, it was proven that solanidine is converted to α-chaconine when solanidine glucosyltransferase, cloned by Moehs et al. (1997), catalyses this aglycone "(WEB_8 2006 and Arnqvist et al. 2003)". According to several experiments done by Arnqvist et al., an antisense solanidine glucosyltransferase gene inhibits the biosynthesis of potato glycoalkaloids "(Arnqvist et al. 2003)". Apart from solanidine glucosyltransferase enzyme, one more enzyme called *SMT1* (a soybean type 1 sterol methyltransferase) which affects the biosynthesis of glycoalkaloids was found "(Arnqvist et al. 2003)". It was shown that overexpression of soybean *SMT1* down-regulated glycoalkaloid levels in potato tuber "(Arnqvist et al. 2003)". In addition to these genes affecting the biosynthesis of glycoalkaloids, several conditions have been found to stimulate or inhibit glycoalkaloid biosynthesis.

2.3.1.5.3. Factors affecting glycoalkaloid biosynthesis

As mentioned in section 2.3.1.5.1., glycoalkaloids were first identified in potato. Since glycoalkaloid compounds were discovered, glycoalkaloids in potato have usually been analyzed. As a result of this research, it was exhibited that factors such as genetic factors, enviromental conditions, and pathogen infection, affect glycoalkaloid level in potato "(Lachman et al.2001, Peksa et al. 2002, Yencho et al.1998, Eltayeb et al. 2003, Bejarano et al.2000, WEB_8 2006)". When glycoalkaloid content was analyzed genetically, it was displayed to be highly heritable "(Eltayeb et al.2003)". According to research, glycoalkaloid level is controlled by several genes and also affected by

dominant and recessive alleles "(Eltayeb 2003, Yencho 1998, WEB_8 2006)". It was shown that a few dominant alleles provide suppression of glycoalkaloid biosynthesis. In addition to these dominant alleles, multiple recessive alleles have been found to be necessary to increase the expression of glycoalkaloids "(Yencho 1998, WEB_8 2006)". Moreover, a study identification QTLs (quantitative trait loci) for glycoalkaloid accumulation in potato was done by Yencho et al.(1998). According to this study, it was found that there are quantitative trait loci on chromosomes-4, 6, and 12 for the accumulation of solasodine and QTL for the accumulation of solanidine on chromosomes 4, 8, and 11 (Yencho 1998). Also, in the same study, other QTLs were identified for both solanidine and solasodine on chromosomes 1 and 4 (Yencho 1998). Furthermore, it was shown that genes having a role in aglycone synthesis segregated from genes having a role in carbohydrate synthesis of glycoalkaloids "(WEB_8 2006)".

In addition to genetic factors, enviromental conditions were displayed to affect glycoalkaloid content in potato "(WEB_8 2006, Lachman et al.2001.,Yencho 1998, Peksa et al.2002)". Enviromental factors can be separated into two different groups for potato: enviroment conditions during the growing season and environmental conditions after harvest of potato "(Bejarano et al. 2000 and WEB_8 2006) ". It is known that during the growing season, extreme conditions stimulate glycoalkaloid production in potato. In extreme conditions, plants are under stress. For example, extended daylength has been found to increase glycoalkaloid contents "(WEB_8 2006 and Lachman et al. 2001)". Also, extreme temperature was shown to stimulate glycoalkaloids "(Eltayeb et al. 2003)". In terms of glycoalkaloid production, 16 ˚C is the optimal temperature for potato. When the temperature decreases or increases, glycoalkaloid level is affected immediately "(WEB_8 2006)". Moreover, it was shown that potatoes growing in a hot and dry climate have more glycoalkaloid content than potatoes growing in a cold climate "(WEB_8 2006)". In addition to climate, it has been found that drought stress or waterlogging cause increasing glycoalkaloid levels in potato "(Bejarano et al. 2000, Eltayeb et al. 2003, WEB_8 2006)". After harvest of potato, when potato tubers were exposed to light, it was showed that glycoalkaloid content increased "(Eltayeb et.al. 2003 and Lachman et al. 2001)". Apart from light, high storage temperature and long storage time have been found to stimulate glycoalkaloid synthesis "(Peksa et al. 2002 and WEB_8 2006)". Also it is known that mechanical damage causes increased glycoalkaloid amounts in potato "(Yencho 1998 and WEB_8 2006)". In addition to mechanical damage, pathogen damage affects glycoalkaloid synthesis in potato "(WEB_8 2006)". For instance, it was shown that

zoospores of *P.infestans* were associated with increasing accumulation of glycoalkaloid in potato leaves "(WEB_8 2006)". Also it has been found that the well-known pest, Colorado potato beetle, has an important role about increasing glycoalkaloid amount in potato tubers. However, infection by peach potato aphids was displayed to decrease glycoalkaloid contents "(WEB_8 2006)".

All these conditions affecting glycoalkaloid level, are most significant for scientists, farmers, and pharmacologists to improve glycoalkaloid content. As mentioned above, glycoalkaloids are very sensitive and so glycoalkaloids level are easily affected by small changes in conditions. Moreover glycoalkaloid level is very important because of their toxic effects on human health.

2.3.1.5.4. Importance of Glycoalkaloid Content

As a result of many studies, alkaloids in the Solanum genus, and glycoalkaloids, have been exhibited to have both benefical and toxic effects on humans and animals "(Korpan et al. 2004, Chami et al. 2003, McGehee et al. 2000, Delporte et al. 1998, Lachman et al. 2001, Arnqvist et al. 2003, Friedman et al. 2003, Nawloka et al. 2003, Karim et al. 1997, Yencho et al. 1998, Bejarano et al. 2000, Edwards & Cobb 1999, Chami et al. 2003, Lee et al. 2004, Kuo et al. 2000, Liu et al. 2004)". In general, total glycoalkaloids between 20-130 mg/kg (fresh weight) are known to be in cultivated potato, but it was shown that this glycoalkaloid level could easily change "(Lachman et al. 2001)". When glycoalkaloid level, especially solanine, increases more than 140 mg/kg, it is known to a give bitter taste to potato "(Lachman et al. 2001)". Also it was shown that when this level continues increasing to more than 200 mg/kg, glycoalkaloid content causes toxic effects in humans "(Benjarano et al. 2000, Lachman et al. 2001)". However, recently, it has been estimated that 1mg of glycoalkaloid per kilo of body weight is a safe level and 1.75 mg/kg body weight can be an acute toxic dose for humans. Moreover, 3-6 mg/kg body weight of glycoalkaloid has been accepted to be a lethal dose for humans "(Friedman et al. 2003 and Karim et al. 1997)". It was suggested that glycoalkaloids are toxic because of their anticholinesterase effect "(Friedman et al. 2003 and Lachman et al. 2001)". They act as cholinesterase inhibitors (especially acetylcholinesterase and butyrylcholieterase inhibitors) and affect the central nervous system "(Friedman et al. 2003, Lachman et al. 2001 and Chami et al. 2003)". It is known that acetylcholinesterase (AChE) is responsible for

terminating cholinergic transmission at the neuromuscular junction because of controlling Na pumps in the nervous system "(McGehee et al. 2000 and Lachman et al. 2001)". Also, it was shown that acetylcholinesterase plays a role in disruption of sterol existing in the membrane "(Karim et al. 1997)".However, it has only been suggested that butyrylcholineterase has a role like acetylcholinesterase in the nervous system, but its function is not exactly known "(McGehee et al. 2000)". So far, some symptoms occuring in cases of glycoalkaloid poisoning have been shown "(Korpan et al.2004, Chami et al.2003 and Lachman et al. 2001)". At low dose, gastrointestinal disturbances such as vomiting and diarrhea were seen; and, at high dose, fever, rapid pulse, low blood pressure, rapid respiration, neurological disorders, and sometimes coma were seen "(Korpan et al. 2004, Chami et al. 2003)". So far, 30 deaths and more than 2000 cases have been registered because of glycoalkaloid poisoning "(Benjarano et al. 2000)".

In contrast to toxic effects, glycoalkaloids are usually used in pharmacology and agriculture because of their beneficial effects. In agriculture, their physiological functions in the plant such as resistance factors against fungi, nematodes, molluscus and insects, are used "(Nawloka et al. 2003)". In table 2.8., several glycoalkaloids and their physiological functions are shown. For example, it was shown that several glycoalkaloids existing in *Solanum ligustrinum* such as solanine, solasodine, tomatilliden, dihydrotomatillidine, inhibited the growth of *Candida albicans, Aspergillus niger, and Achremonium falciforme*.

Table 2.8. Glycoalkaloids and their physiological functions
(Source: Lachman et al. 2001)

Glycoalkaloids	**Physiological functions**
α-chaconine	Antifeedent, fungicide, nematistat, pesticide
α-solanine	Antifeedent, fungicide, pesticide
β-chaconine	Pesticide
Solanidine	Antifeedent, pesticide
Solasodine	Fungicide, pesticide
Tomatine	Bactericide, fungicide, molluscocide, pesticide
Tomatidine	Antifeedent, pesticide
Demissine	Antifeedent, pesticide
Demissidine	Antifeedent, insectifuge

Also it was exhibited that the glycoalkaloid α-tomatidine has toxic properties against *Fusarium* wilt "(Lachman et al. 2001)". In addition to these functions, the glycoalkaloid α-solamargine was suggested to inhibit Herpes simplex, Herpes zoster, genital Herpes and Trypanosoma cruzi "(Chami et al. 2003 and Friedman et al. 2003)".

Apart from these beneficial effects, glycoalkaloids are usually used in medicine for treatment of diabetes, obesity and ulcers. It was shown that they also have diuretic, antiepileptic, sedative and antispasmodic activities "(Vieira et al. 2003)". Moreover, some glycoalkaloids in Solanum such as solasodine have been found to possess anti-inflammatory, antihepatotoxic and hypotensive activities and also to inhibit allergic reactions "(Vieira et al. 2003)".

Another function of glycoalkaloids that is used in medicine is their anticancer activity "(Lee et al. 2004, Liu et al. 2004, Kuo et al. 2000)". According to studies, it has been found that β-solamargine from *Solanum dulcamara* inhibits sarcoma tumors in mice and also solamargine isolated from *Solanum nigrum* shows cytotoxic properties to human solid tumor cell lines "(Lee et al. 2004)". Furthermore, solasonine from *Solanum crinitium* and *Solanum jabrense* was displayed to be cytotoxic against human K562 leukemia cells "(Lee et al. 2004)". It has been suggested that the anticarcinogenic action of glycoalkaloids is the result of cell death, apoptosis "(Lee et al. 2004, Liu et al. 2004, Kuo et al. 2000)". Apoptosis means the process of cell death to prevent uncontrolled cell proliferation "(Liu et al. 2004)". Especially the eggplant glycoalkaloid, solamargine, is used for treatment of cancer because it induces apoptosis "(Lee et al. 2004, Liu et al. 2004, Kuo et al. 2000)". For apoptosis, several elements are necessary such as tumor necrosis factors (TNFs) acting as mediators for both antiproliferative and tumergenic effects in malignant tumors, and their receptors, tumor necrosis factor receptors (TNFR I and TNFR II) "(Liu et al. 2004)". It was indicated that solamargine induced gene and protein expression of TNFRs in human lung cancer cells and in human hepatoma cells (Hep3B) "(Lee et al. 2004, Liu et al. 2004)". Recently, it was demonstrated that solamargine isolated from *Solanum incanum* inhibited the growth of human tumor cells such as breast (T47D and MDA-MB-231), human hepatoma (PLC/PRF/5), prostate (LNCaP, PC-3), and colon (HT-29, HCT-15) cells "(Liu et al. 2004, Kuo et al. 2000)".

2.4. Methods for Glycoalkaloid Analysis

For a long time, much research has been done about glycoalkaloids which are known to have important features for human. When glycoalkaloids are studied, two steps and sometimes three steps are used for extraction, clean-up and analysis of glycoalkaloids. For extraction of glycoalkaloids, freeze-dried materials are utilized to prevent hydrolysis of glycoalkaloids. Also, methanol is usually used to stop enzymatic reactions "(Dao and Friedman 1996)". After extraction, some samples can be dirty and so they need to be cleaned-up. For this step, solid-phase extraction (SPE) is generally applied to clean the sample "(Carman et al. 1986, Edwards and Cobbs 1996)". However, some sample can be lost when SPE method is used. The last step is the analysis of glycoalkaloids. To analyze glycoalkaloids, several methods are reported in literature. One of them is gas chromatograpy (GC). This method was used for determination of the glycoalkaloid aglycones of potato "(Herb et al.1975)", but it cannot be used easily for glycoalkaloids because glycoalkaloids are not good volatile compounds. In addition to GC, thin-layer chromatograpy (TLC) is generally utilized for identification analysis "(Carman et al 1986)". Moreover, for glycoalkaloid analysis, NMR is also used to characterize glycoalkaloid structures "(Lawson et al.1997)", but it needs large amount of glycoalkaloids for detections. Another frequently used method is HPLC (high-performance liqued chromatography). This method is commonly utilized to analyze all glycosides and aglycones "(Edwards and Cobb 1996, Friedman et al. 2003)". It provides both separation nonvolatile species. In HPLC, there are different detectors that can be used such as UV-detectors and fluoroscense. For glycoalkaloid analysis, commonly a UV detector is utilized because of its reproducibility and accuracy. C18 and NH2 columns are used to determine glycoalkaloids. In this thesis, HPLC was used for the characterization of glycoalkaloid because of its many advantages.

2.5. Molecular Mapping

As mentioned in section 2.3.1.5.4., it was demonstrated that glycoalkaloid content is a genetically controlled trait "(WEB_8 2006)". Also, it has been found to be either a qualitative and quantitative trait "(Yencho et al. 1998, Hutvagner et al. 2001 and WEB_8 2006)". Qualitative traits are traits that show segregation in Mendelian ratios.

These traits are controlled by one or two gene (mono or digenic) and are not affected by enviromental conditions. However, quantitative traits do not show discrete segregation, but demonstrate continuous phenotypic distribution and can easily be changed by enviromental effects. This is because quantitative characters are controlled by more than one gene (polygenic).

A QTL is a chromosome site which includes one or more genes controlling a quantitative trait. These sites are generally identified by linkage analysis with molecular markers "(Tanksley 1993)". According to Tanksley (1993), an F2 population is suitable to detect QTLs because F2 populations show the most variation and have all combinations of parental alleles. Thus, when codominant markers are used, more information can be obtained from an F2 population compared to other types of populations (for example, backcross populations) "(Tanksley 1993)". Markers are classified into two group: morphological markers and moleculer markers. Morphological traits/markers can be used to detect segregation of dominant and recessive alleles and are visible as phenotypic changes in the plant "(Tanksley 1993)". Isozymes are protein molecules which have different charges and are detected by gel electrophoresis "(Staub and Serquen 1996)". Although they can be used as codominant markers, post-translational modification cannot be determined "(Staub and Serquen 1996)". In contrast, other types of molecular markers are utilized to examine DNA. These markers are independent of phenotypic changes and also generally codominant (Tanksley 1993). There are many different molecular markers such as RFLPs, RAPDs, AFLPs, SSRs, and SRAP markers "(Staub and Serquen 1996, Jones et al. 1997, Grant and Shoemaker 2001, and Li and Quiros 2001)". RFLP (restriction fragment length polymorphism) analysis is based on restriction enyzmes. DNA fragments cut at specific sites by restriction enzymes can show variation according to their size. By Southern blotting and a labelled DNA probe, these variations can be detected "(Staub & Serquen 1996, Jones et al. 1997, Grant & Shoemaker 2001)". Other molecular markers are PCR-based markers and show variation after PCR amplification. RAPD (random amplified polymorphic DNA) markers are formed by PCR amplification of random DNA segments with single, short primers (generally 10 nucleotides long) "(Grant & Shoemaker 2001 and Jones et al. 1997)". AFLP (amplified fragment length polymorphism) is used to detect changed 2-3 base changes in specific restriction sites by selective amplification "(Grant & Shoemaker 2001 and Jones et al. 1997)".

Another PCR-based marker is SSR (simple sequence repeat or microsatellite). SSRs or microsatellites are short, tandemly head-to-tail repeated DNA sequences (usually 2-6 bases in length) "(Grant & Shoemaker 2001 and Varshaney et al. 2005)". Microsatellites are highly polymorphic. In SSR analysis, specific primers which flank the microsatellite region are used to amplify the repeat "(Grant & Shoemaker 2001 and Varshaney et al. 2005)". After amplification, the size of the amplified products is detected by using gel-electrophoresis using polyacrylamide or normal agarose gels "(Grant & Shoemaker 2001 and Varshaney et al. 2005)". In figure 2.6., microsatellite analysis is shown.

COS II markers are another PCR-based marker. COSII (Conserved Ortholog Set II) markers were developed from a set of single-copy conserved orthologous genes "(WEB_9 2006)". Orthologs mean that the genes are found in different species but are derived from a common ancestor "(WEB_10)". COSII markers allow identification of conserved regions across diverse species. In COSII analysis, genomic DNAs are amplified with primers for single-copy orthologous genes and then the amplified DNA fragments are cut with restriction enzymes. For this reason COSII markers can be said to be analogous to RFLPs and CAPs. After digestion, the size of products can be determined by gel electrophoresis and polymorphism can be detected. In figure 2.7., COSII analysis is demonstrated.

Sequence-related amplified polymorphism (SRAP) markers amplify at open reading frames "(Li & Quiros 2001)". Like COS II and SSR, SRAP markers are based on two –primer amplification (forward and reverse). Each primer is 17-18 nucleotides in length. Also each primer has a core sequence in 10-11 nt length which are followed by three selective nucleotides at the 3'end "(Li & Quiros 2001 and Budak et al. 2004)". In this thesis, SSR, COSII and SRAP markers were tested for polymorphism in eggplant and its wild type relative, *S. linnaeanum*. In addition, SSR and COSII markers were added to the eggplant map (Doganlar et al. 2002).

Simple Sequence Repeat (SSR or Microsatellites)

1. Amplification of microsatellite region in genome by PCR

Primer →

GTGTCGATATATATATATATATAT**CCTGATT**

GTGTCGATATATATATATATATATATAT**CCTGATT**

← Primer

Numbers of repeats can be highly variable among alleles.

2. Electrophoresis to differentiate alleles in F2 individuals.

3. Three genotypes can be seen by from a codominant SSR marker.

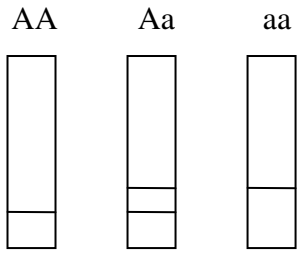

Figure 2.6. Microsatellite (SSR) analysis. AA: homozygote, aa: homozygote, Aa: heterozygote genotype

Conserved Ortholog Set II (COSII) Analysis

1. Amplification of single-copy conserved orthologous genes in genome.

Primer →

ATGCTAATCGCTTAACCGGGTATCCC**CCTAA**

ATGCTAATCGCTTAACCGGCTATCCC**CCTAA**

← Primer

2. Digestion of amplified PCR products with restriction enzyme to detect variation for each allele.

Taq I (digestion at a site in parA, no digestion in parB)

↓ ↓

ParA: **ATGCT**AATCGCTTAACCAAGCTTCCC**CCTAA**

ParB: **ATGCT**AATCGCTTAACCAACCTTCCC**CCTAA**

3. Electrophoresis to measure different fragment sizes of alleles.
4. Variations of alleles and three genotypes can be detect.

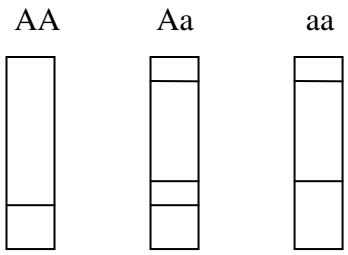

Figure 2.7. Conserved Ortholog Set II (COSII) analysis. AA: homozygote, aa: homozygote, Aa: heterozygote genotype.

CHAPTER 3

MATERIALS AND METHODS

3.1. Plant Material

For this thesis, egglant was analyzed for glycoalkaloids. Two parental lines were used, *S. melongena* MM738 (cultivated) and *S. linnaeanum* MM195 (wild type). These parents were grown in INRA-Unité de Génétique & Amélioration des Fruits et Légumes in France. The plants were grown in the field and fruits for the two genotypes were harvested. After harvesting, 3 fruits for each genotype were cut into three slices about 1.5 cm thick for each fruit, lyophilized and packaged in vacuum conditions. Then samples were sent to Turkey for glycoalkaloid extraction.

3.2. Glycoalkaloid Analysis

3.2.1. Extraction of Glycoalkaloid

There are some established extraction method for glycoalkaloids. In studies which have been done so far, approximately the same method has been used. However, these methods have been generally utilized for potato experiments. For this thesis, many methods were tried for eggplant. Finally, a general method was developed. According to this method, first eggplant powder from lyophilized fruits was prepared.

Fruits were ground with a mortar and pestel in a glove box which contained only nitrogen and the powder was put into packet and zipped to protect from humidity. Then the powder was weighed and stored in a -80°C freezer. For glycoalkaloid extraction, 1 g powder for each parent was mixed with 40 ml methanol- 5% acetic acid solution in a 50mL falcon tube. For each parent, two tubes were prepared. Then the tubes containing powder and methanol-acetic acid solution, were put on a shaker at 400 mot 1/min overnight. The next day, these tubes were shaken at 600 mot 1/min for 10 minutes. After shaking, each extract was filtered through 90mm filter paper using a buchner funnel and vacuum pump. Then each sample was filtered again with 0.45µm polyamide

filter by using vacuum pump. Finally, two tubes for each sample was mixed in a rotary flask and evaporated until the volume was 8-9 ml. Then each sample was filtered with a 0.2µm syringe filter into 10ml volumetric flask and diluted with methanol- 5% Acetic acid to 10 ml. After dilution, the extract was kept on at 4°C until it was measured in HPLC. For each sample, two extractions were done to check reproducibility.

3.2.2. HPLC Analysis

In HPLC analysis, a Shimadzu Class-VP (Kyoto, Japan) single piston high pressure liquid chromatography with photodiode array detection and C18 column were utilized. For glycoalkaloid analysis, UV detection at 205 nm and 208 nm were chosen. Flow rate and temperature were set to 1 ml/min and 50°C respectively. Acetonitrile containing methanol (10%) and 100 mM ammonium dihidrogen phosphate buffer (pH 2.58) were used as mobile phase. While preparing 100mM ammonium dihydrogen phosphate buffer, pH was adjusted with orthophosphoric acid (85%). After pH was adjusted, buffer was filtered with polyamide filter by using vacuum pump. Then both acetonitrile-methanol and ammonium dihydrogen phosphate buffer were put into ultra sonic bath to do away with bubbles in those solutions. When all bubbles were gone away, the filter of A pump was put into ammonium dihidrogen phosphate buffer and also the filter of B pump was inserted into acetonitrile-methanol (10%). In HPLC, 70% for B pump and 30% for A pump were set for glycoalkaloid analysis. Every day, the column was washed with mobile phase for 40 min with a 1mL/min.flow rate. Then, methanol-5%acetic acid as a blank was injected into the HPLC and samples were measured. In addition, standard stock solution (1mg/mL) for solamargine as glycoalkaloids which were prepared in acetonitrile-water (1/1 v/v) containing one drop orthophosphoric acid (85%) to adjust pH to 2 and stored at 4°C, was added into samples for spiking to calculate solamargine amount in samples. Like extraction, for each sample, two repetitions were also done for HPLC. After measuring each sample, the sample containing solamargine was measured to calculated the amount of solamargine in our sample. After running, peak areas of samples and samples with spikes were analyzed and then the amount of glycoalkaloids was calculated.

3.3. Molecular Marker Analysis and Mapping

For molecular marker analysis, tomato SSR (simple sequence repeats) markers and tomato COSII (Conserved orthologous set II) and SRAP (sequence-related amplified polymorphism) markers were used. These markers were amplified in the mapping population parents, *S. melongena* MM738 and *S. linnaeanum* MM195 to polymorphic markers. SSR and COSII markers were then mapped in the F2 population (*S. linnaeanum* MM195 X *S. melongena* MM738).

For this thesis, 75 tomato SSR markers (Frary et al. 2005) were screened for polymorphism between parents. For PCR, 25 µl reaction mixtures contain of ~25 ng template DNA, 1 pmol of each forward and reverse primer, 1x PCR buffer (50mM KCl, 10 mM Tris-HCl, pH 8.3, 1,5 mM $MgCl_2$), 0.2 mM dNTPs, and 0.5 U *Taq* polymerase, were prepared for each sample. PCR was performed in GeneAmp® PCR System 9700 (Applied Biosystem) using the following program: initial denaturation at 94°C for 5 min, denaturation at 94°C for 30 s, annealing at 50°C for 45 s, and extension at 72°C for 45 s (35 cycle), then final extension at 72 °C for 5 min. After amplification, PCR products were run on 3% agarose gels in TAE buffer at 100-120 volt at 4°C for approximately 5 hours. Markers which did not amplify at 50°C, were retested at 55°C or 60°C. In addition to tomato SSRs, 23 eggplant SSR (EM) markers which were developed by Nunome et al. (2003) were tested for polymorphism between parents. For EM markers, the same procedure was used but annealing temperature was increased to 60°C.

In addition, COS II markers being developed at Cornell University (NY, USA), were amplified in *S. melongena* MM738 and *S. linnaeanum* MM195. For PCR, 25 µl reactions for each sample were prepared as described for the SSR markers. Then samples were amplified using the following programme: initial denaturation at 94°C for 5 min, denaturation at 94°C for 30 s, annealing at 55°C for 45 s, and extension at 72°C for 45 s (35 cycle), final cycle at 72 °C for 5 min. After amplification, PCR products were separated on 2% agarose gels in TAE buffer at 100-120 volt at 4°C for approximately 5 hours. COSII marker PCR products which were not polymorphic after amplification were then digested with 27 different restriction enzymes (*AluI, TaqI, HaeIII, EcoRI, DraI, HindIII, EcoRV, BamHI, HinfI, SacI, RsaI, KpnI, MboI, NsiI, HpaII, MspI, MseI, HincII, SspI, BglII, AvaII, AccI, ApaI, ScaI, CfoI, DpnII, DpnI*) to

detect polymorphism between parents. For digestion, 20µl reaction mixtures containing 10µl PCR product, 2 µl restriction enzyme buffer, 0.5µl restriction enzyme and 0.2 µl BSA (bovine serum albumin) were prepared for sample. All restriction enzyme digest, except for *TaqI,* were incubated at 37°C, *TaqI* digests were incubated at 65°C. All polymorphic markers were then tested on the F2 mapping population. After amplification, PCR products were separated on 4% agarose gel in TAE buffer at 4°C to see segregation on mapping population. Each individual in the mapping population was scored according to their marker genotype. Linkage analysis was done with the MAPMAKER computer programme using a LOD of 3.0 (Lander et al. 1987).

Moreover, sequence-related amplified polymorphism (SRAP) markers were amplified in *S. melongena* MM738 and *S. linnaeanum* MM195. For SRAP markers, 15 µl reaction mixture contain of ~37,5-40 ng template DNA, 1pmol of each forward and reverse primer, 0.2 mM dNTPs, and 0.5 U *Taq* polymerase were prepared for each sample. Then samples were put into PCR machine using the following programme: initial denaturation at 94°C for 5 min, 5 cycles of 94°C/1 min, 35°C/1 min, 72°C/1 min then 35 cycles of 94°C/1 min, 50°C/1 min, 72°C/1min, final extension at 72°C for 10 min and hold at 4°C. After PCR, amplified samples were run on 2% agarose gels in TAE buffer at 100-120 volt at 4°C for 3 hours.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Glycoalkaloid Analysis

To characterize glycoalkaloid in eggplant, two parents were used *S.melongena* MM738 and *S.linnaeanum* MM195. Solamargine which is generally an eggplant glycoalkaloid, was analyzed. For characterization of glycoalkaloid, a HPLC-UV detector was used at 205 and 208 nm. Before HPLC analysis, it should be noted that the samples consisted of only freeze-dried powder from the interior fruit that was ground under dry nitrogen (approx. 99.99% purity) conditions. The peel of the eggplant fruit was not included in the grinding process. It is also important to note that MM195 contained seeds which were also ground along with its interior fruit; however, MM738 contained no seeds. For precision of extraction, there were two extractions were done for each parent. After extraction, extracted solutions of *S.melongena* MM738 and *S.linnaeanum* MM195 were analyzed for solamargine. For analysis, each sample was injected three times to check precision of HPLC and also each sample was spiked with solamargine to characterize the solamargine peak. Figure 4.1. shows the *S.melongena* MM738 sample without the solamargine spike and *S.melongena* MM738 with the solamargine spike chromatograms. Figure 4.2. shows the chromatograms for diluted *S.linnaeanum* MM195 without and with the solamargine spike. In figure 4.3., only the chromatogram of *S.linnaeanum* MM195 is shown.

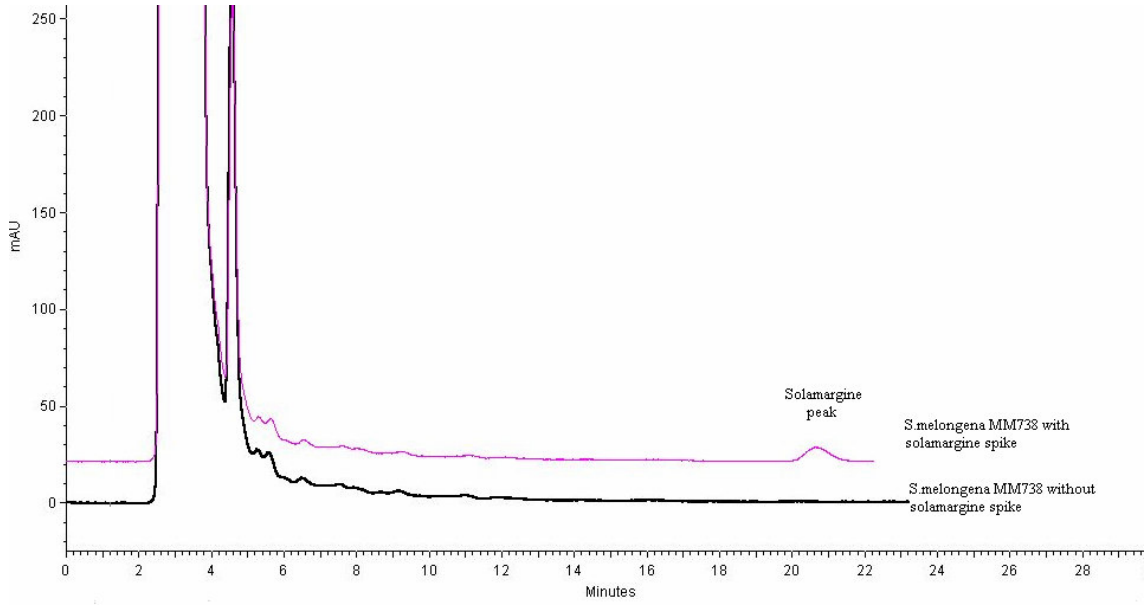

Figure 4.1. *S.melongena* MM738 sample with and without solamargine spike.

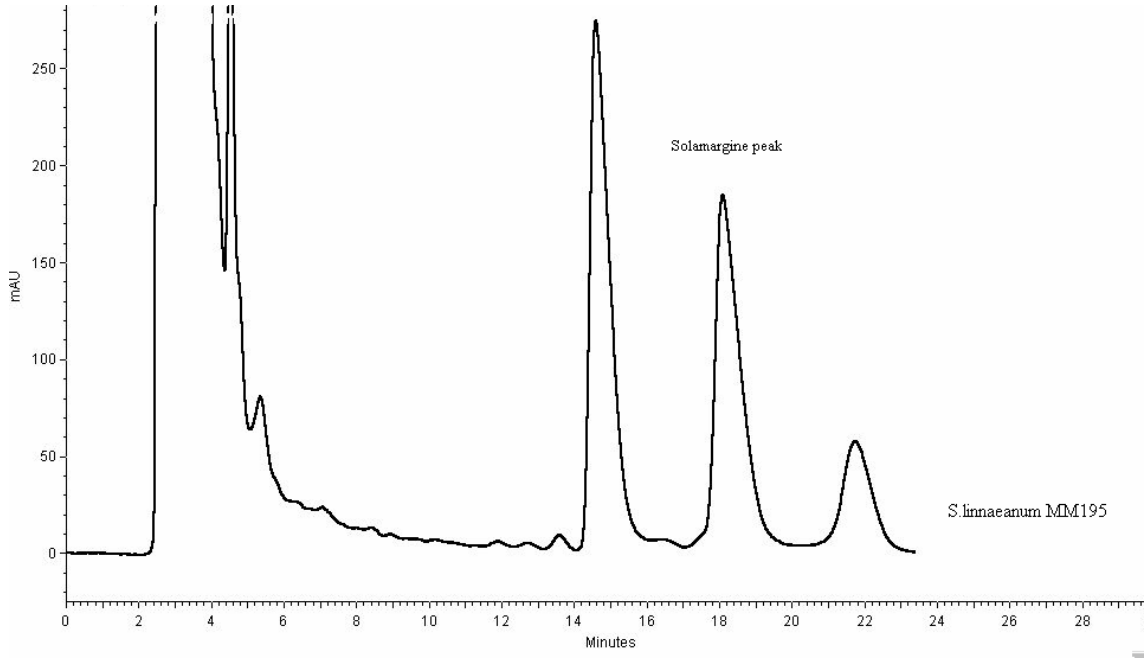

Figure 4.2. The chromatogram of *S.linnaeanum* MM195.

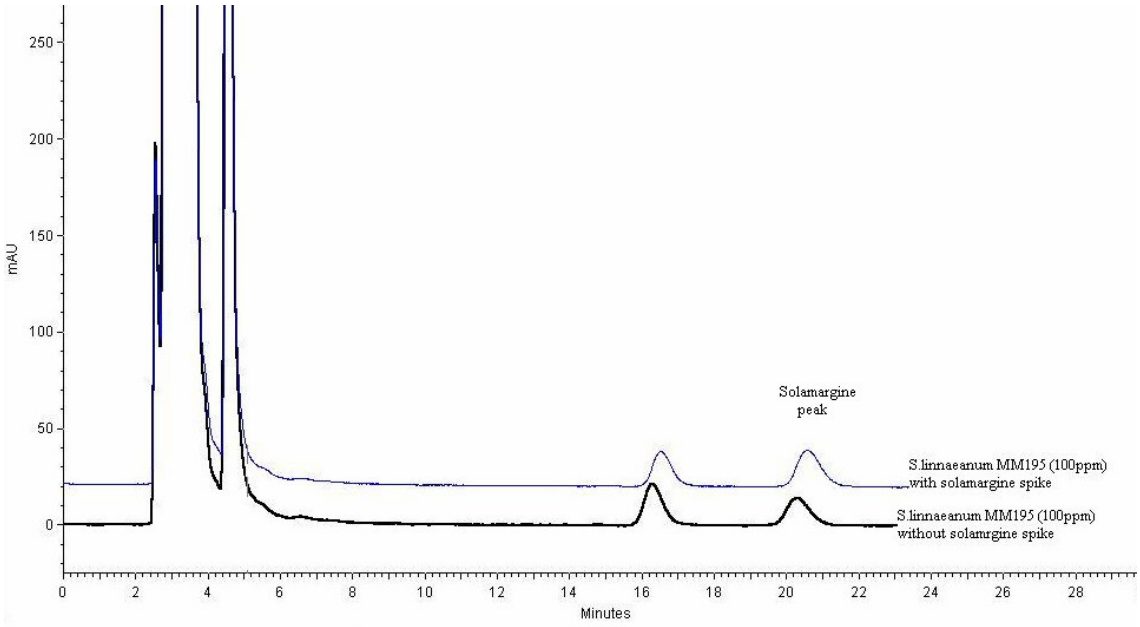

Figure 4.3. The diluted *S.linnaeanum* MM195 (100 ppm) with and without solamargine spike.

According to figure 4.1., no solamargine was detected in *S.melongena* MM738. Based on previous work done with solasodine using a similar HPLC method for analysis "(Tek 2006)", the current expected limit of quantitation for solamargine as determined in real samples is between 10 and 20 ppm (corresponding to 0.1 to 0.2 mg solamargine per gram of freeze-dried eggplant sample). Likewise, the limit of detection should be approximately half of these values. It is well known that the lack of a suitable chromaphore on these types of compounds makes their UV detection particularly difficult.

When *S.linnaeanum* MM195 was analyzed to characterize solamargine, a very large peak for solamargine was seen. Figure 4.2. shows the *S.linnaeanum* MM195 chromatogram. After solamargine was spiked into the *S.linnaeanum* MM195 sample, no difference in the solamargine peak was detected because of the large amount of solamargine in *S.linnaeanum* MM195. Therefore, the extraction solution of *S.linnaeanum* MM195 was diluted to 100 ppm, then solamargine was spiked into the sample. In figure 4.3., a small difference in the solamargine peaks for the diluted *S.linnaeanum* MM195 with and without solamargine spike can be seen.. After obtaining chromatograms, peak areas of solamargine were found and solamargine concentrations in *S.linnaeanum* MM195 was calculated for the two extractions with this formula;

$$C_x = \frac{S_1.C_s.V_s}{(S_2 - S_1).V_x}$$

S_1= mAU for sample (Area)

S_2= mAU for sample spike (Area)

C_s= Concentration of spike

V_s= Volume of solamargine spike before spiking

V_x=Volume of unknown sample before spiking

C_x= Concentration of solamargine in unknown sample

According to this formula, concentrations of solamargine in the diluted *S.linnaeanum* MM195 for two extractions were calculated. It was found that initial duplicated results for *S.linnaeanum* MM195 gave complete extraction and analysis method-replicated values of 19.4 ± 1.8 mg solamargine per gram freeze-dried powder and 29.6 ± 3.8 mg solamargine per gram freeze-dried powder. Although unknown, due to their variable water content, these values would most likely be much less (at least by a factor of 5, 10 or more) for determinations based on fresh fruit weight.

The stated standard deviations reported for *S.linnaeanum* MM195 reflect the approximate precision of measurement by HPLC and do not give information regarding extraction efficiency; therefore, further work is needed for verification. Combining the above extraction replicates and considering their full range, an average value of 25.5 ± 11.2 mg solamargine per gram freeze-dried powder can be taken as the most reliable determined concentration in *S.linnaeanum* MM195 at this time. An initial recent single recovery value of 125 % was obtained for a *S.melongena* MM738 sample spiked with solasodine before extraction using a very similar method. It is therefore reasonably expected that extraction efficiency should be very close to 100% for the initial recovery of solamargine, lending added assurance to the above stated concentrations. Of course, further work is necessary to validate this in which case determined values would have to be adjusted accordingly.

4.2. Mapping of Molecular Markers

For molecular marker analysis, 82 individuals of an F2 population which was developed from *S.melongena* MM738 and *S.linnaeanum* MM195 was used (Doganlar et al. 2002). First, the two parents, *S.melongena* MM738 and *S.linnaeanum* MM195, were tested with tomato SSR, eggplant EM and tomato COSII markers. Then polymorphic markers were amplified in the F2 population. A total of 75 tomato SSR markers was tested on parental lines. It was found that of the tested markers, 17 (23%) were polymorphic for the two parents and 44 (59%) were not polymorphic. S. A few markers (14) did not amplify at 50 or 55 °C. Tested SRR markers and the sizes of their amplification products are shown in table 4.1.

Table 4.1. SSR markers tested with parental lines and their product sizes are indicated. (*) indicates the polymorphic markers.

		Anneal.	**Product Size (bp)**	
Marker	**Repeat Motif**	**Temp.**	***S.melongena***	***S.linnaeanum***
SSR11	$(CAG)_6$	50°C	250	250
SSR111	$(TC)_6(TCTG)_6$	55°C	200	195
SSR117*	$(TC)_{11}$	50°C	295	290
SSR128	$(CAG)_6(CAA)_3(CAG)_7$	50°C	100	100
SSR135	$(ATT)_6$	55°C	130	130
SSR136	$(CAG)_7$	55°C	140	140
SSR14	$(ATA)_9$	50°C	150	150
SSR150	$(CTT)_7$	50°C	220	220
SSR156	$(TCT)_7$	50°C	170	170
SSR192	$(ATC)_6$	50°C	1400+700	700
SSR20*	$(GAA)_8$	50°C	900+110	900+700+210+115
SSR223	$(TCT)_7$	50°C	220	220
SSR248	$(TA)_{21}$	50°C	220	220
SSR26	$(CGG)_6$	50°C	520+180	600+520+180
SSR270	$(GAA)_5(GGAGAA)_7$	50°C	300	300
SSR300	$(TTC)_{10}$	50°C	700	705
SSR301*	$(TTGGT)_2(TA)_{15}$	50°C	500+490	500+290
SSR32	$(TTC)_7$	50°C	190	190
SSR34	$(GA)_7(TCGA)_2$	55°C	190	190
SSR350	$(AT)_{13}$	50°C	200	200

Table 4.1. SSR markers tested with parental lines and their product sizes are indicated. (*) indicates the polymorphic markers.(con.)

		Anneal.	**Product Size (bp)**	
Marker	**Repeat Motif**	**Temp.**	***S.melongena***	***S.linnaeanum***
SSR356*	$(AT)_{20}$	50°C	800	600+150
SSR38	$(TCT)_8$	50°C + Mg	990+700	980+faint
SSR40	$(AC)_7(GC)_7$	55°C	160	160
SSR43	$(TAC)_7$	55°C	250+200	250+200
SSR46*	$(AT)_{14}$	55°C	400+370	380
SSR47*	$(AT)_{14}$	50°C	400+220+faint	350+210+faint
SSR51*	$(ACAA)_6$	50°C	700+650+350	1600+650+350
SSR578	$(AAC)_6(ATC)_5$	50°C	300	300
SSR586	$(AAC)_6$	50°C	280	280
SSR593	$(TAC)_7$	50°C	210	210
SSR594	$(TCT)_8$	50°C	300	295
SSR66	$(ATA)_8$	50°C	180	180
SSR70	$(AT)_{20}$	50°C	800+530+400+ 320+200	530+390
SSR80	$(TTTCAA)_2(GTACAA)_2(CAA)_7$	50°C	180	185
SSR85	$(TAA)_7$	50°C	200	200
SSR96	$(AT)_{12}$	55°C	290	290
SSR4	$(CGG)_7$	50°C	50	50
SSR19	$(AT)_{16}$	50°C	Multible band	Multible band
SSR22	$(AT)_{11}$	50°C	250+70	250+150+70
SSR27	$(TC)_6(ATGT)_2$	50°C	290	290
SSR44	$(GA)_{54}$	50°C	390+410	390+410
SSR45	$(AAT)_{14}$	50°C + Mg	70	70
SSR52	$(AAC)_9$	50°C	60+470	50+60+470
SSR67	$(AGA)_2(AAG)_7$	50°C	650	650
SSR69	$(TAT)_7$	50°C	check again	
SSR76	$(CGG)_7$	50°C	check again	
SSR115	$(AT)_{16}$	50°C	Nothing	50+60
SSR124	$(CACC)_2(GA)_7$	50°C	60+300	60
SSR146	$(AT)_7(CAT)_5$	50°C	100+220	100+130+220
SSR155	$(TAT)_9$	50°C	check again	
SSR188	$(AT)_{11}$	50°C	310+400	Nothing
SSR192*	$(ATC)_6$	50°C	500+280+190	280+250+170
SSR218*	$(TCA)_7$	55°C	500+480	380
SSR222	$(TCT)_7$	50°C	Nothing	50

Table 4.1. SSR markers tested with parental lines and their product sizes are indicated. (*) indicates the polymorphic markers.(con.)

		Anneal.	**Product Size (bp)**	
Marker	**Repeat Motif**	**Temp.**	***S.melongena***	***S.linnaeanum***
SSR231*	$(TA)_{10}$	50°C	550	900+560+540
SSR237	$(AT)_{11}$	50°C	40	40
SSR241	$(AAT)_{13}$	50°C	Multible band	Multible band
SSR244*	$(TA)_{14}$	50°C	510+50	510+300+280
SSR285	$(TTAT)_2(AT)_6$	50°C	300+50	50
SSR306*	$(ATT)_7$	50°C	400	50
SSR308	$(TA)_{12}$	50°C	Nothing	40+170
SSR310	$(TGA)_9$	50°C	200+650	200+650
SSR316	$(AG)_6(TTGCAG)_2$	50°C	40+50+80+400	40+50+80+400
SSR320	$(AT)_{12}$	50°C	50+170	50+170
SSR327	$(AAT)_7$	50°C	280	40+70+280+440
SSR344*	$(AT)_{12}$	50°C	850+820	850+650
SSR349	$(ATAAAA)_2(TA)_{11}$	50°C	100	100
SSR383	$(AT)_{11}$	50°C	50	50
SSR450*	$(AAT)_7$	50°C	400	70
SSR478	$(GGTG)_2(CT)_{16}$	50°C	70	70
SSR557*	$(ATCT)_7$	50°C	600+180	150
SSR590	$(TC)_6(AC)_4$	50°C	510	510
SSR603*	$(GAA)_8$	50°C	600+280	600+400+300+200
SSR605	$(CAA)_6$	50°C	40	40
SSR638*	$(GT)_9(AT)_8(AC)_{13}(GA)_{12}$	50°C	150+50	550+50

In addition to tomato SSRs, 23 EM microsatellite markers were tested on *S.melongena* MM738 and *S.linnaeanum* MM195. Eleven (48%) of the EM markers demonstrated polymorphism on 3%TAE agarose gels and 12 (52%) EM markers were not polymorphic. In table 4.2., EM markers and their product sizes are shown. An example of polymorphic EM markers is shown in figure 4.4. From this gel, it is clear that markers, EM 133, EM 135, EM 145, EM 146 and EM 151 are polymorphic in the two eggplant lines.

Table 4.2. Tested EM markers and their product size. (*) indicates the polymorphic markers.

		Product Size (bp)	
Marker	**Repeat Motif**	***S. melongena***	***S. linnaeanum***
EM104	$(TC)_9(AC)_{38}(AT)_{19}$	240	240
EM107	$(AC)_{13}(AT)_7$	200	200
EM114	$(AC)_{13}$	220	220
EM116	$(AC)_{12}(AT)_8$	240	240
EM117*	$(AC)_{19}(AT)_{11}$	140	120
EM119*	$(GGAGG)_5\ldots(AT)_8(GT)_3AT(GT)_{14}$	200	180
EM120*	$(AC)_{16}$	200	240
EM126	$(AT)_7(GT)_{18}$	210	210
EM127	$(AC)_{13}(AT)_{13}$	215	215
EM128	$(CA)_{26}(TA)_{19}$	220	220
EM131*	$(AT)_5(AC)_3A(AC)_{14}\ldots(TG)_5(TA)_3$	220	220
EM133*	$(AC)_{13}(AT)_4$	180	200
EM134	$(GT)_2GC(GT)_6$	170	170
EM135*	$(CA)_{11}(GA)_{20}$	280	260
EM139	$(AC)_6AT(AC)_{11}(AT)_{10}$	270	270
EM140*	$(AC)_4GC(AC)_5T\ldots(AC)_6(AT)_5G(TA)_{13}$	270	210
EM141*	$(AT)_{16}(GT)_{19}$	200	180
EM145*	$(TACA)_4TA(TACA)_4\ldots(TA)_3(TTAA)_3$	370	350
EM146*	$(AC)_{19}(AT)_{11}AC(AT)_2$	280	220
EM151*	$(TG)_3TA(TG)_8(TA)_6$	160	160
EM155	$(CT)_{38}$	240	240
EM157	$(AC)_{11}(AT)_8$	240	240
EM162	$(CA)_5$	160	160

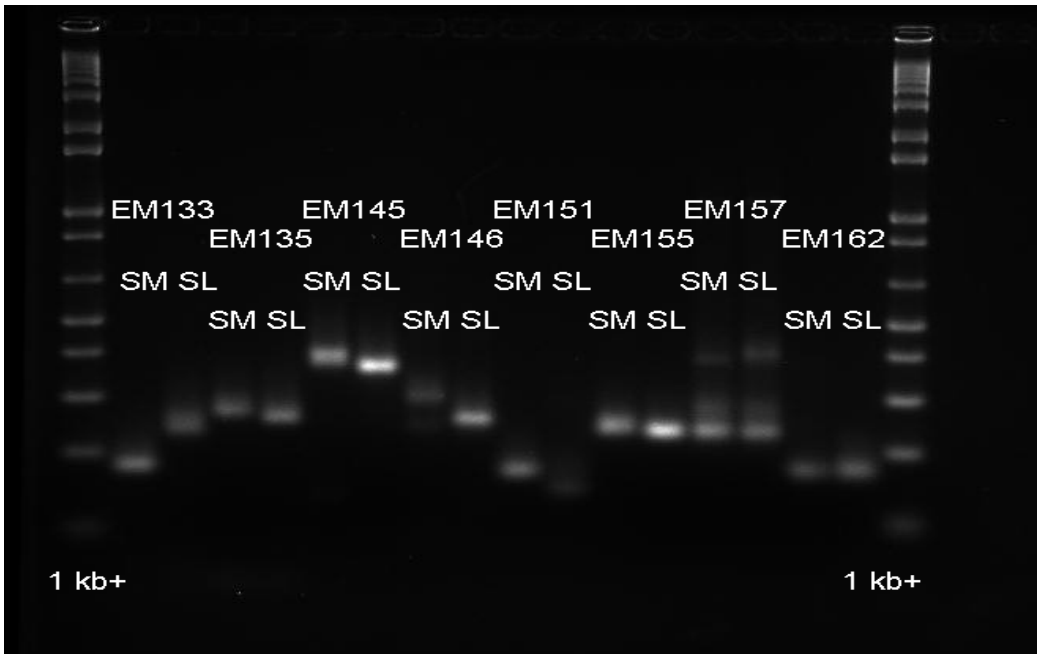

Figure 4.4. Survey of parental DNA for EM marker polymorphism. 1 kb+ : 1 kb size standard DNA ladder. SM : *S.melongena* MM738 , SL: *S.linnaeanum* MM195

COS II markers were also amplified with the eggplant parents. A total of 124 COS II markers were tested and it was found that only 7 (4.61%) demonstrated polymorphism without restriction enzyme digestion and 25 (16.45%) amplified in only one parent. In table 4.3., the tested COS II markers and their product sizes for each parent are shown.

Table 4.3. Tested COS II markers. (*) indicates COS II markers that were polymorphic without digestion. (^) indicates markers that may show polymorphism on polyacrylamide gels.

COSII	**Product Size (bp)**	
Marker	***S. melongena***	***S. linnaeanum***
At1g 02140	100	100
At1g 02560	300	300
At1g 02910	1000+250+150+90	100
At1g 07080	290+130	290+130
At1g 08940	2300	2300
At1g 10500	500	500
At1g 13380	300	300
At1g 14000	800	800
At1g 14300	280	280

Table 4.3. Tested COS II markers. (*) indicates COS II markers that were polymorphic without digestion. (^) indicates markers that may show polymorphism on polyacrylamide gels.(con.)

COSII	**Product Size (bp)**	
Marker	***S. melongena***	***S. linnaeanum***
At1g 14310	80	80
At1g 19140	400	400
At1g 20050*	1500	1000
At1g 20575*	350+220	225+230
At1g 23890	850+150	850+150
At1g 27385	100	100
At1g 29900	1500	1500
At1g 30580	1100	1100
At1g 32070	90	90
At1g 42990	400	400
At1g 43700	1600	1600
At1g 44446	450	450
At1g 44575	280	280
At1g 44760	300	300
At1g 44790	70	70
At1g 47830	600	600
At1g 50020	400	400
At1g 52980	100	100
At1g 53000	1200+80+faint at 150	1200+150
At1g 53670^	400	410
At1g 55170	200	200
At1g 55870	410	410
At1g 60440	350	350
At1g 60640	200	200
At1g 61620	2200	2200+1650 (weak)
At1g 63770	210	210
At1g 63980	600+550	600+550
At1g 67730	300	300
At1g 71810	420	420
At1g 74520	1800	1800
At1g 74970	700	700
At1g 75350	80	80
At1g 76080	800	800
At1g 76150	80	80

Table 4.3. Tested COS II markers. (*) indicates COS II markers that were polymorphic without digestion. (^) indicates markers that may show polymorphism on polyacrylamide gels.(con.)

COSII	**Product Size (bp)**	
Marker	***S. melongena***	***S. linnaeanum***
Atg 77470	1800	1800
At1g 78620	420+180	420
At1g 80360	800	800
At1g 80460	500	No amp
At2g 01110	800	800
At2g 01720	150	150
At2g 15890	350	350
At2g 16920	650	650
At2g 18710	330	330
At2g 20360	80	80
At2g 20860	600	600
At2g 22570	650	650
At2g 24090	220	220
At2g 25950	150	150
At2g 26590	370	370
At2g 27290*	400	650
At2g 27730	420	420
At2g 34470	220	220
At2g 34560	800+390+300+220	800+390+300+220
At2g 35610	140	140
At2g 35920	400	400
At2g 38025	500	500
At2g 38730	400	1500+400+300
At2g 40760	500	500
At2g 42810	400+250	400+250
At2g 43360	420	420
At2g 45730	1700	1700
At2g 46820	Many bands	Many bands
At2g 47580	Many bands	Many bands
At3g 03100	900	900
At3g 04780	2000	2000
At3g 06050	900	900
At3g 06580	300	300
At3g 06730	300	300

Table 4.3. Tested COS II markers. (*) indicates COS II markers that were polymorphic without digestion. (^) indicates markers that may show polymorphism on polyacrylamide gels.(con.)

COSII	**Product Size (bp)**	
Marker	***S. melongena***	***S. linnaeanum***
At3g 09740	100	100
At3g 10920	700	No amp
At3g 13235	750	750
At3g 14910	1000	1000
At3g 15290*	1050	1000
At3g 16150	No amp	300
At3g 17000	950	No amp
At3g 19630	300	No amp
At3g 20390	950	No amp
At3g 24050	800-900	800
At3g 26060	200	200
At3g 44890	1450	No amp
At3g 52220	300	300
At3g 54360	No amp	350
At3g 54470	750	750
At3g 54770	800+1450	No amp
At3g 55360	600	No amp
At3g 58470	1250	1250
At3g 63190	450	450
At4g 00090	1500	1500
At4g 03290	1250	1250
At4g 09010	550	550
At4g 10030	2000	2000
At4g 12230	350	350
At4g 10050	950	950
At4g 12590	100	100
At4g 15520	1100	1100
At4g 18810	300	300
At4g 20410	No amp	1250
At4g 22260^	600	625
At4g 24690	380	No amp.
At4g 24830	2000	2000
At4g 26750	No amp	400

Table 4.3. Tested COS II markers. (*) indicates COS II markers that were polymorphic without digestion. (^) indicates markers that may show polymorphism on polyacrylamide gels.(con.)

COSII	**Product Size (bp)**	
Marker	***S. melongena***	***S. linnaeanum***
At4g 29490	500	500
At4g 30930	750	750
At4g 31130	No amp	900
At4g 33250	375+400	400
At4g 33985	750+800	No amp
At4g 34700	250	No amp
At4g 38240	1450	1450
At4g 38630	1000	1000
At4g 38810	1950	1950
At4g 39660*	500	600
At5g 01990	200	200
At5g 04590	1100	1100
At5g 04910	400	400
At5g 06130	150	150
At5g 06370	2500	2500
At5g 06430	No amp	1500
At5g 07910	No amp	1000
At5g 08420	400	400
At5g 09880	550	550
At5g 11480*	250	350
At5g 12200*	575	100
At5g 12370	100	100
At5g 13450	775+1600+1700	No amp
At5g 13640	800+1000+1200	No amp
At5g 16710	850	850
At5g 19690	500+700+1300+1500	500+1475
At5g 20180	1475	700+1450
At5g 20890	780	780
At5g 25630	950	950
At5g 25760	2000	No amp
At5g 27620	No amp	300
At5g 35360	450	No amp
At5g 37360	600	600
At5g 42740	800	800

Table 4.3. Tested COS II markers. (*) indicates COS II markers that were polymorphic without digestion. (^) indicates markers that may show polymorphism on polyacrylamide gels.(con.)

COSII	**Product Size (bp)**	
Marker	***S. melongena***	***S. linnaeanum***
At5g 49830	500	500
At5g 49970	500+700+750+1250	500+700+750+1250
At5g 50720	No amp	275+1000
At5g 51040	200	200
At5g 51110	400	400
At5g 51840	200	200
At5g 52820	900	900+950
At5g 62390	No amp	1100

The 145 COS II markers which were not polymorphic without restriction enzyme digestion, were digested with 27 restriction enzymes to detect polymorphism. After digestion, 35 COSII markers showed polymorphism. In table 4.4., the COS II markers digested with different restriction enzymes are shown.

Tablo 4.4. COS II markers digested with different restriction enzymes. (*) indicates polymorphic markers.

COSII Marker	**Enzyme Set Tested**[1]	**Polymorphic Enzyme(s)**
At1g 02140	A	
At1g 02560	A	
At1g 02910*	A	*HindIII*
At1g 07080	A	
At1g 08940	C	
At1g 10500*	A	*HinfI*
At1g 13380	A	
At1g 14000	A	
At1g 14300	A	
At1g 14310	A	
At1g 19140*	A	*AluI, RsaI, MboI, SspI, DpnII*
At1g 20050*	D	*TaqI*
At1g 22850	C	
At1g 23890	A	

Tablo 4.4. COS II markers digested with different restriction enzymes. (*) indicates polymorphic markers. (Cont.)

COSII Marker	**Enzyme Set Tested[1]**	**Polymorphic Enzyme(s)**
At1g 27385	A	
At1g 29900	A	
At1g 30580	C	
At1g 32070	A	
At1g 42990	A	
At1g 43700	C	
At1g 44446	A	
At1g 44575*	A	*TaqI*
At1g 44760	A	
At1g 47830*	A	*DraI*
At1g 50020	A	
At1g 52200	C	
At1g 52980	A	
At1g 53000	A	
At1g 53670*	A	*BglII*
At1g 55170	A	
At1g 55870	A	
At1g 60440	A	
At1g 60640*	A	*EcoRI, Taq I*
At1g 61620	E	
At1g 63770	D	
At1g 63980	A	
At1g 67730	A	
At1g 71810*	A	*TaqI*
At1g 74520	C	
At1g 74970*	A	*TaqI*
At1g 75350	A	
At1g 76080*	A	*DraI, HinfI, HincII*
At1g 76150	A	
At1g 77470	C	
At1g 78620	A	
At1g 80360*	A	*TaqI*
At1g 80460	A	
At2g 01110*	C	*HaeIII, EcoRV*
At2g 01720	B	
At2g 15890	B	

Tablo 4.4. COS II markers digested with different restriction enzymes. (*) indicates polymorphic markers. (Cont.)

COSII Marker	**Enzyme Set Tested**[1]	**Polymorphic Enzyme(s)**
At2g 18710*	B	*HindIII, TaqI*
At2g 20360	B	
At2g 20860	B	
At2g 22570*	B	*CfoI*
At2g 24090	B	
At2g 25950	B	
At2g 26590	B	
At2g 27290	B	
At2g 27730	B	
At2g 34470	B	
At2g 34560*	B	*TaqI*
At2g 35610	B	
At2g 35920	B	
At2g 38025	B	
At2g 38730	B	
At2g 40760	B	
At2g 43360	B	
At2g 45730	B	
At2g 46820*	B	*RsaI, SspI*
At2g 47580	B	
At3g 03100	B	
At3g 04780*	B	*RsaI, HinIf, Dra I, EcoRV,Hind III, Hae III, Dpn II,*
At3g 06050	B	
At3g 06580	B	
At3g 06730	B	
At3g 09740	B	
At3g 10920	B	
At3g 13235	B	
At3g 14910	B	
At3g 16150*	B	*BamHI*
At3g 17000	B	
At3g 19630	B	
At3g 20390*	B	*TaqI*
At3g 24050	B	
At3g 26060	B	
At3g 44890	B	

Tablo 4.4. COS II markers digested with different restriction enzymes. (*) indicates polymorphic markers. (Cont.)

COSII Marker	**Enzyme Set Tested[1]**	**Polymorphic Enzyme(s)**
At3g 52220	B	
At3g 52730	B	
At3g 54360*	B	*MspI*
At3g 54470*	B	*BamHI, HinfI,Dpn II*
At3g 54770	B	
At3g 55360	B	
At3g 58470*	B	*DpnII*
At3g 63190	B	
At4g 00090	B	
At4g 03280	B	
At4g 09010*	B	*DpnII*
At4g 10030*	B	*TaqI,CfoI*
At4g 10050	B	
At4g 12590	B	
At4g 15520	B	
At4g 18810	B	
At4g 20410	B	
At4g 22260*	B	*TaqI, HinfI*
At4g 24890	B	
At4g 24830	B	
At4g 26750	B	
At4g 29490	B	
At4g 30930	B	
At4g 31130*	B	*Hinf I*
At4g 33250	B	
At4g 33985*	B	*Rsa I, EcoRI, TaqI*
At4g 34700	B	
At4g 38240	B	
At4g 38630*	B	*TaqI, DraI,Hinf I*
At4g 38810	B	*TaqI*
At4g 39660	B	
At5g 01990	B	
At5g 04590*	B	*KpnI*
At5g 04910*	B	*DpnI*
At5g 06130	B	
At5g 06370*	B	*EcoV, CfoI*

Tablo 4.4. COS II markers digested with different restriction enzymes. (*) indicates polymorphic markers. (Cont.)

COSII Marker	**Enzyme Set Tested**[1]	**Polymorphic Enzyme(s)**
At5g 06430	B	
At5g 07910*	B	*Hae III*
At5g 08420	B	
At5g 09880*	B	*SacI ,KpnI*
At5g 12200	B	
At5g 12370	B	
At5g 13030	B	
At5g 13450	B	
At5g 13640	B	
At5g 16710*	B	*HinfI*
At5g 19690*	B	*CfoI*
At5g 20180	B	
At5g 20890	B	
At5g 25630*	B	*DpnII*
At5g 25760*	B	*RsaI, HinfI*
At5g 27620	B	
At5g 35360	B	
At5g 37360	B	
At5g 39040	B	
At5g 42740	B	
At5g 49830	B	
At5g 49970	B	
At5g 50720*	B	*SspI*
At5g 51040	B	
At5g 51110	B	
At5g 51840	B	
At5g 52820	B	
At5g 62390	B	

[1] Enzyme Sets:

A = *AluI, TaqI, HaeIII, EcoRI, DraI, HindIII, EcoRV, BamHI, HinfI, SacI, RsaI, KnpI, MboI, NsiI, HpaII, MspI, MseI, HincII, SspI, BglII, AvaII, AccI, ApaI, ScaI, CfoI, DpnII, DpnI*

B = *ApaI, RsaI, MspI, BamHI, EcoRI, BglII, TaqI, HinfI, DraI, SacI, SspI, KpnI, EcoRV, HindIII, HaeIII, DpnII, CfoI*

C = *AluI, TaqI, HaeIII, EcoRI, DraI, HindIII, EcoRV, BamHI, HinfI, SacI, RsaI, KnpI*

D = *AluI, TaqI, HaeIII, EcoRI*

E = *AluI, TaqI, HaeIII, EcoRI, DraI, HindIII, EcoRV, BamHI*

In addition to COS II, SSR, EM markers, sequence-related amplified polymorphism (SRAP) markers were tested with parents, *S.melongena* MM738 and *S.linnaeanum* MM195 for polymorphism. A total of 238 combination were tested with 14 forward primers (mE primers) and 17 reverse primers (Em primers) on the eggplant. A total of 137 primer combinations (58%) showed dominant and codominant polymorphism between the two parents. Approximately 20% of the markers did not amplify in one of the two parents (10% did not amplify in *S. melongena* and 9% did not amplify in *S. linnaeanum*). A total of 19% (45) of the markers were not polymorphic and 4% did not amplify in both parents. In table 4.5., the results of testing the SRAP markers are shown. A total of 43 markers showed codominant polymorphism. This represents 18% of all markers and 31% of all polymorphic markers.

After marker polymorphism was determined, the polymorphic markers were mapped on the F2 population. In this way, 49 polymorphic SSR and COSII markers were identified and analyzed on the F2 population. One polymorphic marker which was tested on the F2 population, is shown in Figure 4.5.

Table 4.5. Results of SRAP primer assays on parents of the mapping population. P=polymorphic, NP=not polymorphic, -= no amplification, -/+=no amplification in *S. melongena*, +/-= no amplification in *S. linnaeanum*, * indicates codominant polymorphism

	mE01	**mE02**	**mE03**	**mE04**	**mE05**	**mE06**	**mE07**	**mE08**	**mE09**	**mE10**	**mE11**	**mE12**	**mE13**	**mE14**
Em01	-	-/+	-	-/+	P*	-/+	-/+	P*	P	P	P	+/-	NP	-/+
Em02	P	-/+	NP	P*	NP	-/+	-	NP	P*	+/-	P*	+/-	P*	P
Em03	-/+	-	-/+	NP	NP	-/+	NP	P*	-	-	P	P	+/-	P
Em04	-/+	P	NP	P	NP	NP	P*	P	P	P*	P	P*	P*	P
Em05	P	P	NP	P	-	P*	P	NP	P*	P	-/+	+/-	P	P
Em06	-/+	P*	P	NP	NP	-/+	NP	-/+	P	P	P*	P	P	P*
Em07	P*	NP	-	P	NP	+/-	P	-	P*	P*	P*	-/+	P	P
Em08	+/-	NP	NP	NP	NP	P	P*	P*	NP	P*	P	P	P*	NP
Em09	P	P*	+/-	P*	P*	NP	+/-	P	P	P*	P*	P*	P*	NP
Em10	NP	P	NP	NP	+/-	P	+/-	P	P*	P*	+/-	P	NP	NP
Em11	P*	P	P	P	P	P	P	+/-	P*	P	P	P	P	+/-
Em12	P*	P*	+/-	P*	NP	NP	P	P*	P	NP	-/+	P	P	-/+
Em13	-/+	P	NP	P	P	P	P*	P	NP	P	P	P	P	P
Em14	-/+	+/-	+/-	P	P	P	P	P	P	+/-	P	P	+/-	P
Em15	P	+/-	P	P	P	P	P	-/+	P	P	+/-	P	NP	NP
Em16	P	P	-/+	+/-	P	P*	P	P	P	P*	NP	NP	P	P*
Em17	P	-/+	-/+	NP	NP	P	P	NP	P	NP	NP	-	NP	P

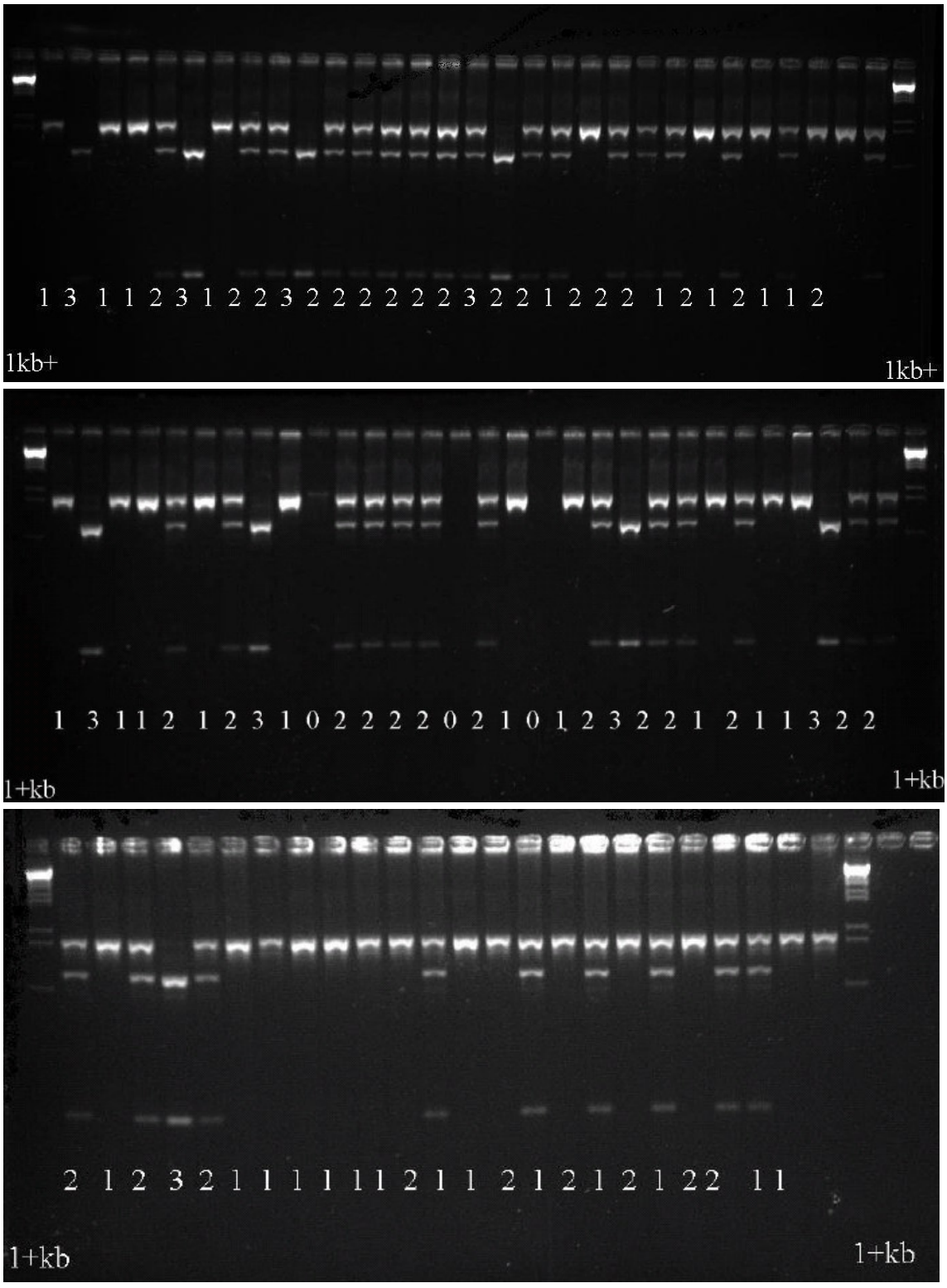

Figure 4.5. Example of a COSII marker tested on the F2 population. For all pictures, Lane 1 shows 1 kb DNA ladder. For first picture, lane 2 shows parent *S.melongena* MM738, lane 3 indicates parent *S.linnaeanum* MM195, remaining lanes are F2 progenies. In these pictures, F2 population and parents were amplified with COS II marker At1g 60440 and digested with Hind III. All individuals are scored as: 1 for the homozygous *S.melongena* MM738 genotype, 3 for the homozygous *S.linnaeanum* MM195 genotype and 2 for the heterozygous genotype.

Chi-square analyses were then performed on the data to check if marker segregation fit the Mendelian ratio (1:2:1) for codominant markers. A total of 49 markers were analyzed and it was found that 30 markers fit the Mendelian ratio (1:2:1) and 17 markers did not fit. Of the markers that which did not fit, 7 markers were skewed to *S.linnaeanum* parent genotype, and 3 markers were skewed to the *S.melongena* parent genotype. The remaining 7 markers didn't fit the Mendelian ratio because of skewing to the heterozygous genotype. Table 4.6. shows the results of Chi-square analysis.

Table 4.6. Chi-square results for the markers assayed on the *S. linnaeanum* x *S.melongena* F_2 population. P-values, number of individuals genotyped (N) ,and number of individuals in each genotypic class are included. AA= homozygous *S. melongena* genotype, Aa= heterozygous genotype, aa= homozygous *S. linnaeanum* genotype. A P-value threshold of 0.05 was used.

Marker	**P-value**	**N**	**AA**	**Aa**	**aa**	**Interpretation**
EM117	0.90	82	22	39	21	Fits 1:2:1
EM120	<0.0001	81	2	29	48	Skew to *S. linnaeanum* parent
EM131	0.67	82	22	43	17	Fits 1:2:1
EM133	<0.0001	82	7	31	44	Skew to *S. linnaeanum* parent
EM135	0.79	70	20	33	17	Fits 1:2:1
EM140	0.06	82	20	50	12	Fits 1:2:1
EM141	0.77	82	21	38	23	Fits 1:2:1
EM145	0.45	81	22	35	24	Fits 1:2:1
EM146	<0.0001	81	2	46	32	Skew to *S. linnaeanum* parent
EM151	<0.0001	82	45	23	12	Skew to *S. melongena* parent
SSR20	0.02	82	10	50	22	Skew to heterozygote
SSR46	0.0008	55	12	38	3	Skew to heterozygote
SSR47	0.46	49	5	26	10	Fits 1:2:1
SSR51	0.001	79	16	55	8	Skew to heterozygote
SSR117	0.007	82	10	42	30	Skew to *S. linnaeanum* parent
SSR192	0.27	80	24	40	14	Fits 1:2:1
SSR218	<0.0001	80	11	57	2	Skew to heterozygote
SSR356	0.01	82	15	53	12	Skew to heterozygote
SSR450	<0.0001	78	5	0	68	Skew to *S. linnaeanum* parent
At2g24270	<0.0001	80	42	31	7	Skew to *S. melongena* parent
SSR 557	0.07	37	4	25	8	Fits 1:2:1
SSR 306	<0.0001	44	4	2	38	Skew to *S. linnaeanum* parent
At1g20575	0.21	47	10	20	17	Fits 1:2:1

Table 4.6. Chi-square results for the markers assayed on the *S. linnaeanum* x *S.melongena* F_2 population. P-values, number of individuals genotyped (N) ,and number of individuals in each genotypic class are included. AA= homozygous *S. melongena* genotype, Aa= heterozygous genotype, aa= homozygous *S. linnaeanum* genotype. A P-value threshold of 0.05 was used.(con.)

Marker	**P-value**	**N**	**AA**	**Aa**	**aa**	**Interpretation**
At1g02910	0.50	37	8	22	7	Fits 1:2:1
At1g10500	0.37	37	12	19	6	Fits 1:2:1
At1g44575	0.30	47	16	19	12	Fits 1:2:1
At1g19140	0.80	43	12	22	9	Fits 1:2:1
At1g60440	0.003	46	19	24	3	Skew to *S. melongena* parent
At1g60640	<0.0001	47	3	17	27	Skew to *S. linnaeanum* parent
At1g71810	0.31	46	13	18	15	Fits 1:2:1
At1g53670	0.15	44	6	23	15	Fits 1:2:1
At1g74970	0.65	42	9	20	13	Fits 1:2:1
At1g83060	0.25	47	7	28	12	Fits 1:2:1
At3g20390	0.47	66	19	28	19	Fits 1:2:1
At3g54360	0.63	81	24	38	19	Fits 1:2:1
At3g54470	<0.0001	78	5	61	12	Skew to heterozygote
At3g58470	0.55	81	21	44	16	Fits 1:2:1
At4g09010	0.69	69	16	38	15	Fits 1:2:1
At4g22260	0.33	78	14	44	20	Fits 1:2:1
At4g31130	0.51	76	15	39	22	Fits 1:2:1
At4g33985	0.37	74	16	43	15	Fits 1:2:1
At4g38630	<0.0001	66	8	55	4	Skew to heterozygote
At4g38810	0.31	76	14	44	18	Fits 1:2:1
At4g39660	0.19	82	14	48	20	Fits 1:2:1
At5g07910	0.11	80	21	47	12	Fits 1:2:1
At5g11480	0.66	81	17	44	20	Fits 1:2:1
At5g16710	0.41	76	14	42	20	Fits 1:2:1

Locations of the polymorphic markers were determined by using the Mapmaker computer program. Some of the markers, SSR 51, SSR218, SSR 450, and also At3g 54470 could not be mapped. However, 26 COS II, 9 SSR, 10 EM markers were mapped in the F2 population. COS II markers were mapped on eleven different chromosomes with the only exceptionas LG12. SSR markers also were mapped on chromosomes 1, 7, 9, 10, and 12. EM markers were mapped on LG2, LG4, LG5, LG9, and LG11. Figure 4.6. shows the eggplant linkage groups with the newly mapped markers (in bold).

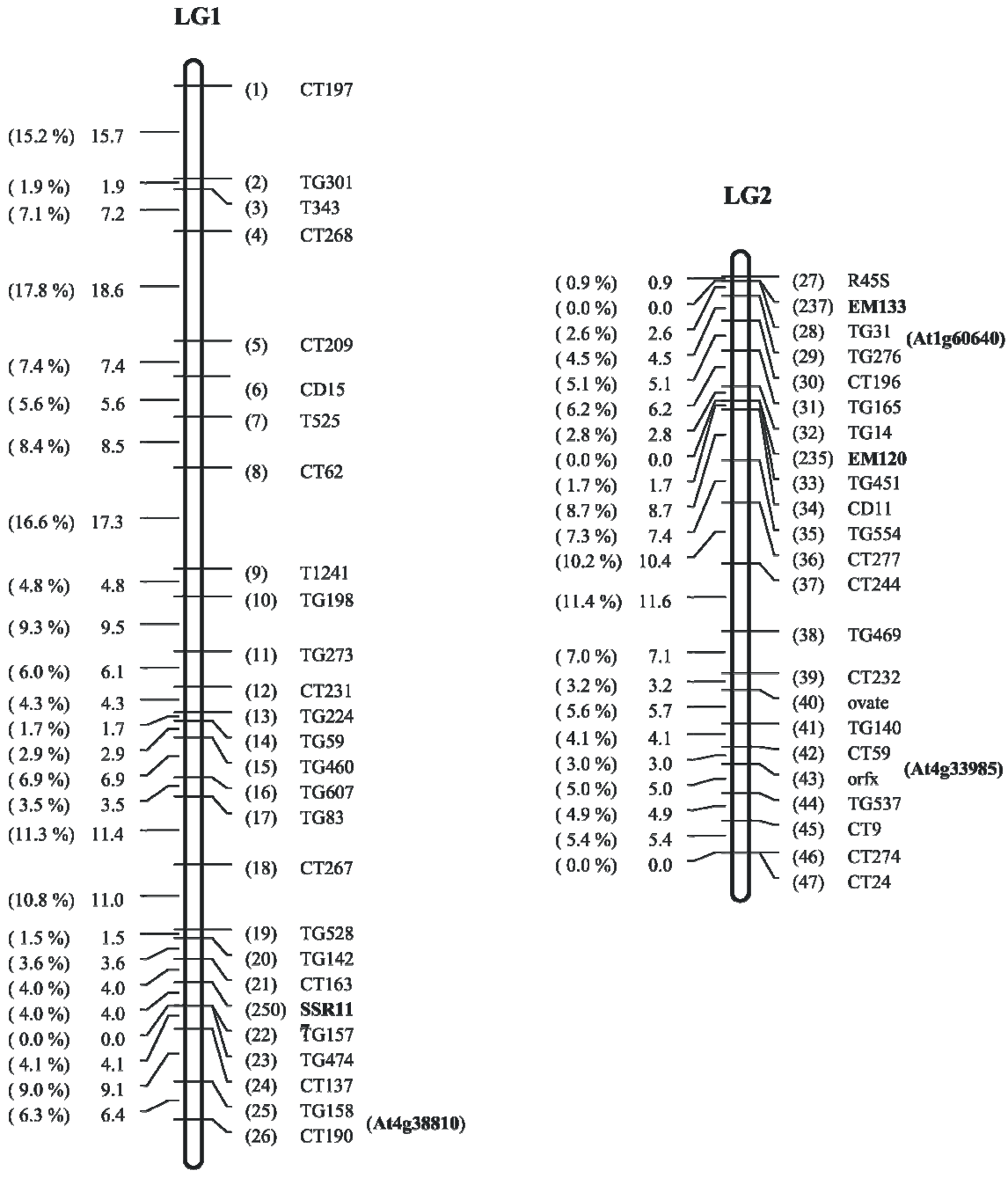

Figure 4.6. Eggplant linkage groups with mapped markers. Markers in parenthesis were mapped at LOD <3.0. Map distance are in centiMorgans (cM).

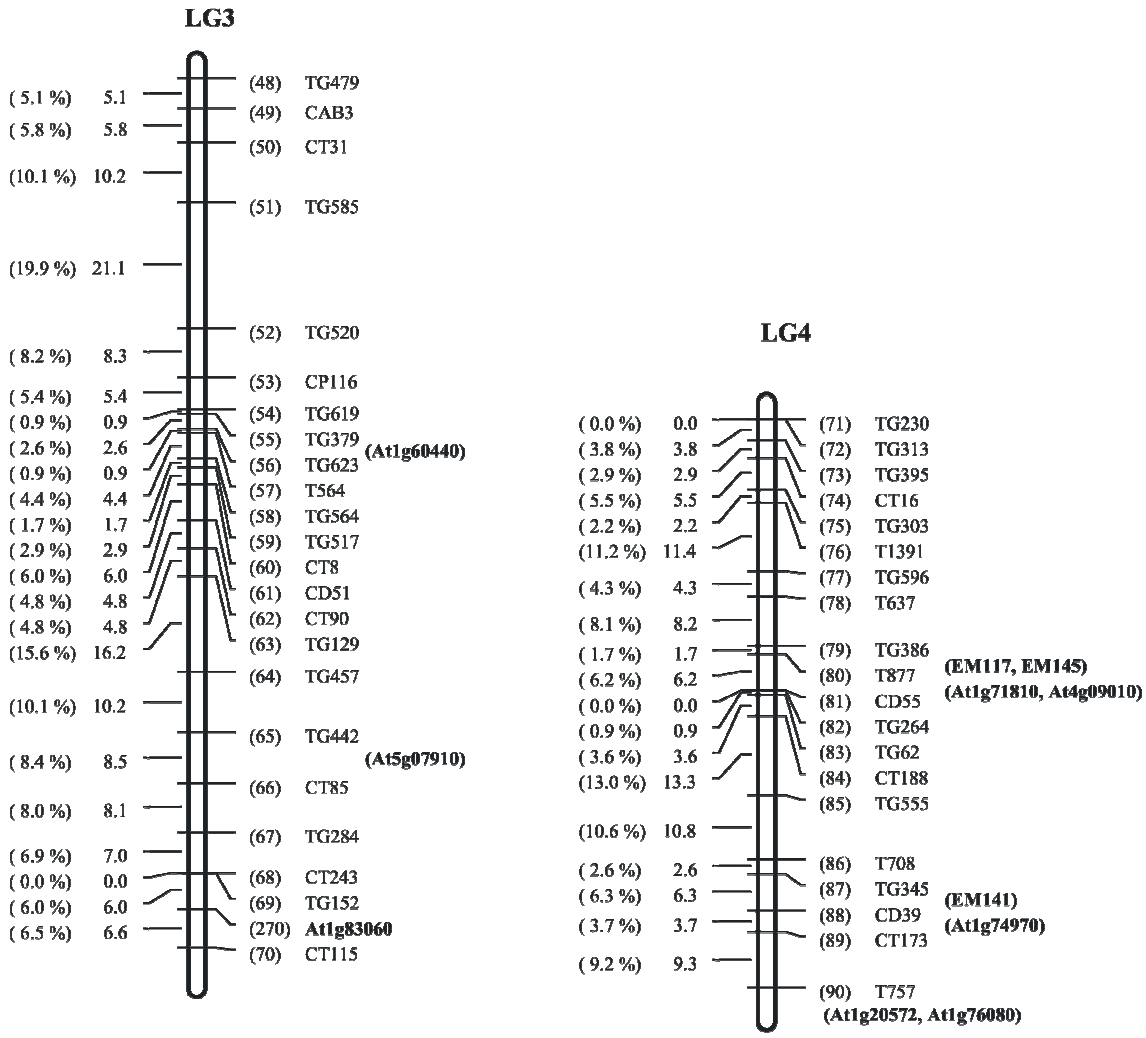

Figure 4.6. Eggplant linkage groups with mapped markers. Markers in parenthesis were mapped at LOD <3.0. Map distance are in centiMorgans (cM).(con.)

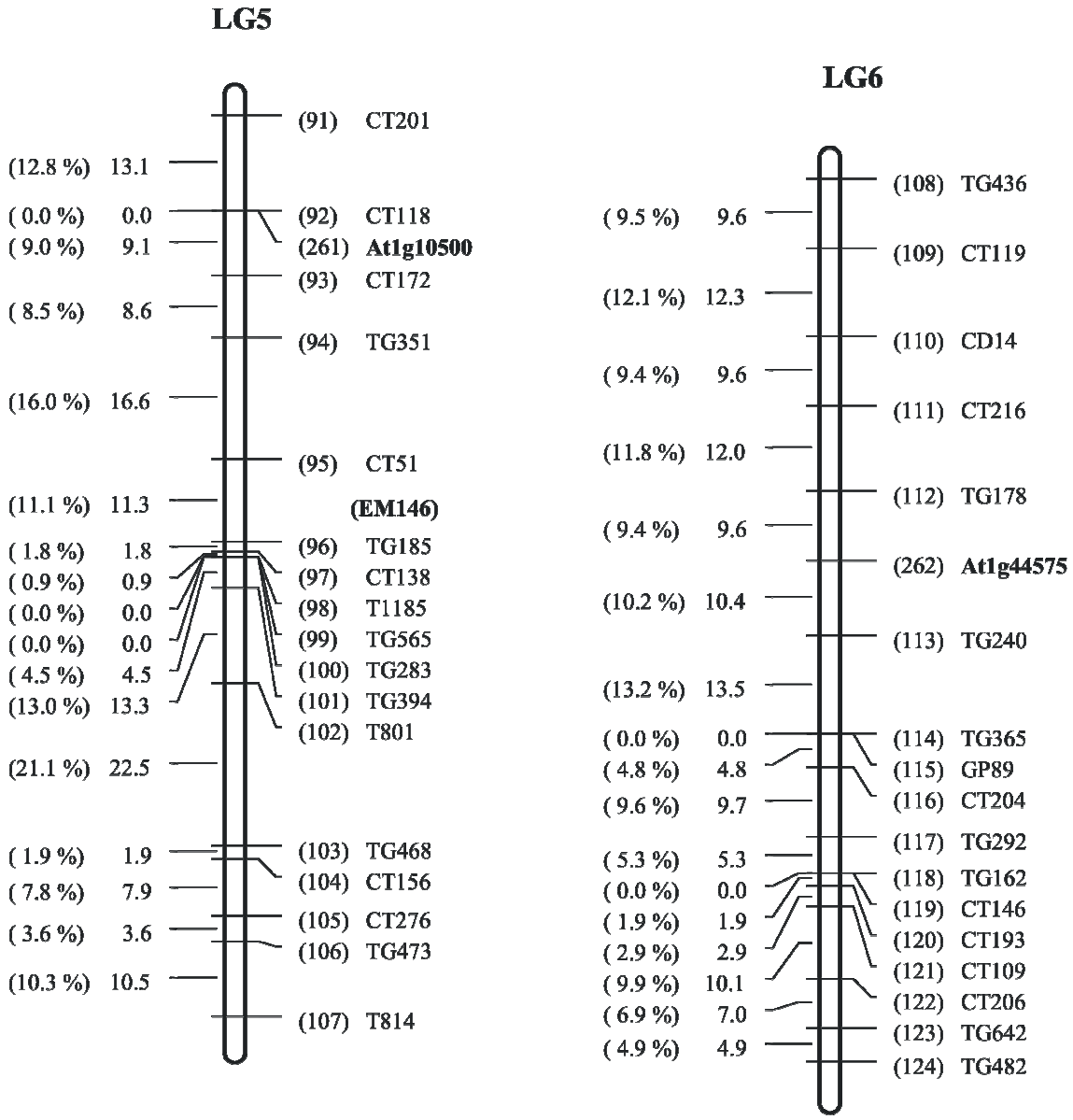

Figure 4.6. Eggplant linkage groups with mapped markers. Markers in parenthesis were mapped at LOD <3.0. Map distance are in centiMorgans (cM).(con.)

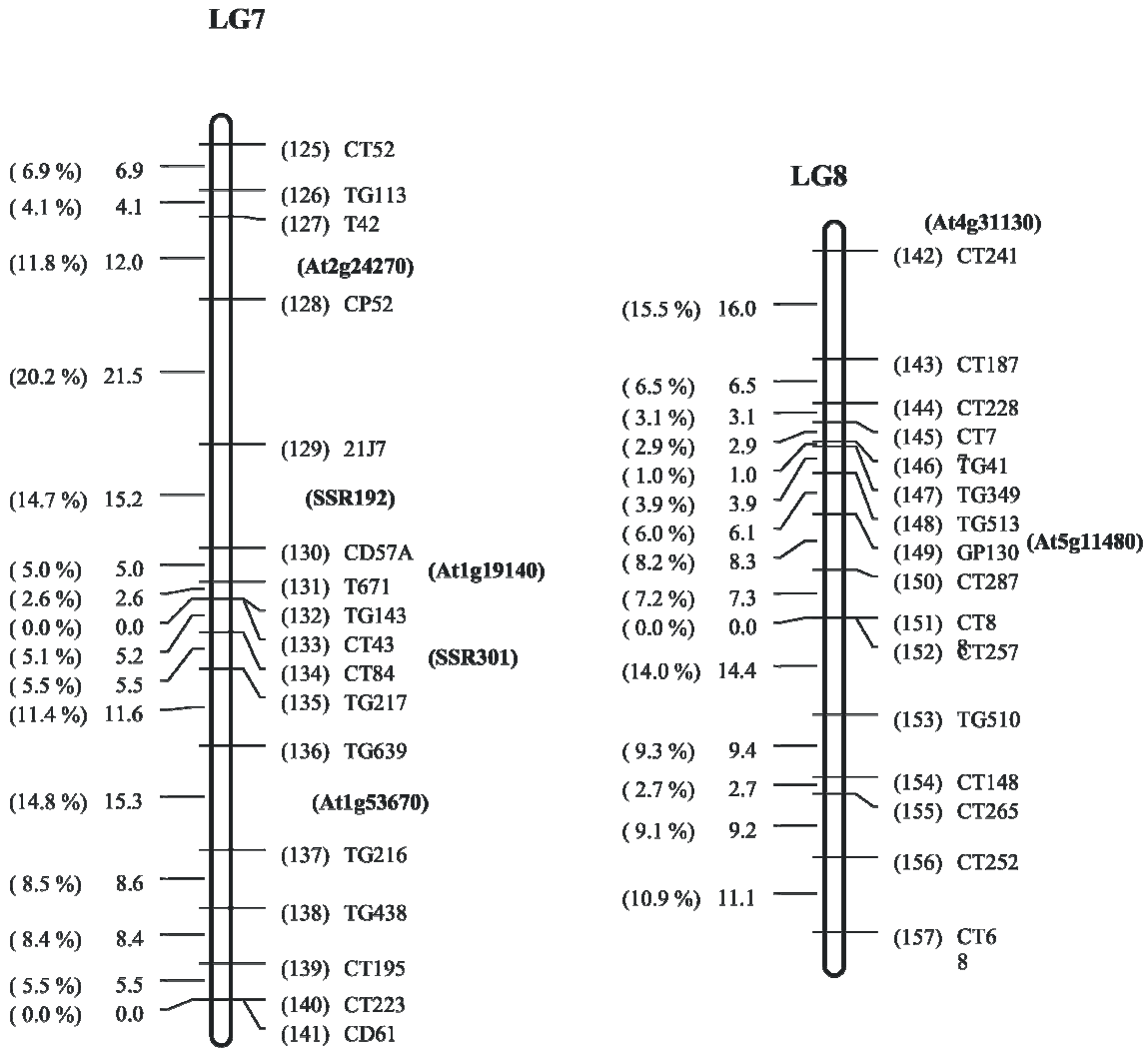

Figure 4.6. Eggplant linkage groups with mapped markers. Markers in parenthesis were mapped at LOD <3.0. Map distance are in centiMorgans (cM).(con.)

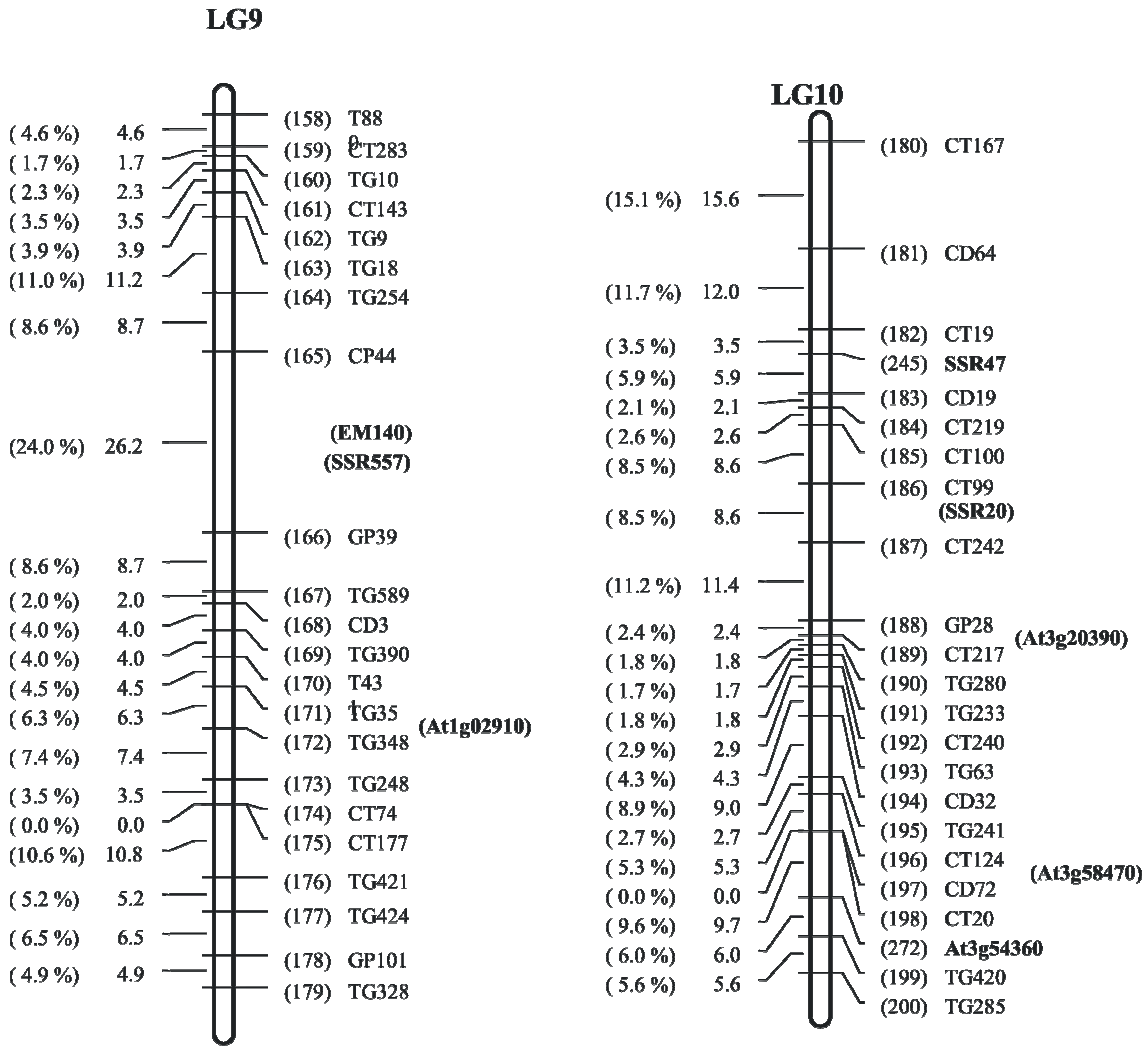

Figure 4.6. Eggplant linkage groups with mapped markers. Markers in parenthesis were mapped at LOD <3.0. Map distance are in centiMorgans (cM).(con.)

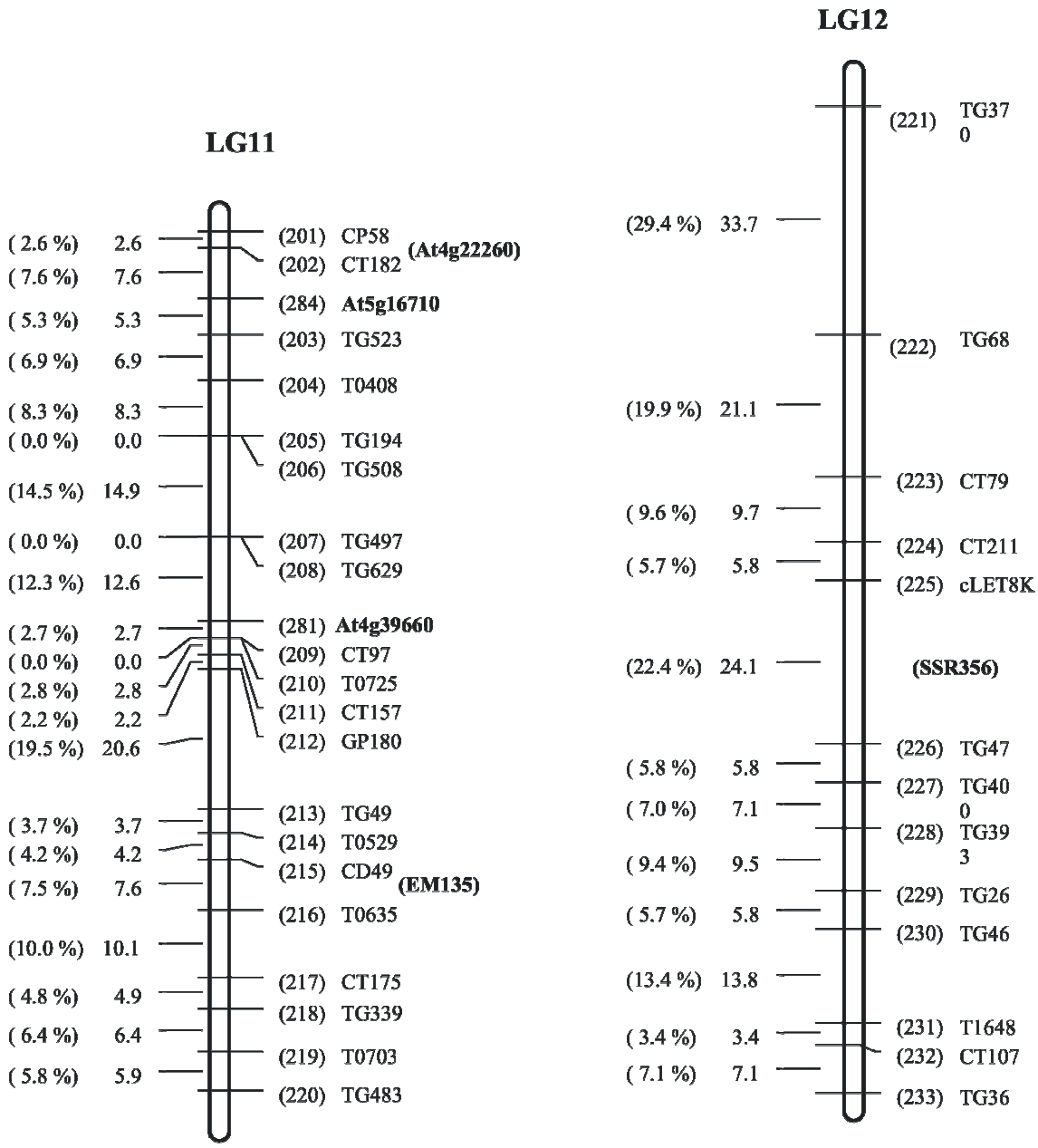

Figure 4.6. Eggplant linkage groups with mapped markers. Markers in parenthesis were mapped at LOD <3.0. Map distance are in centiMorgans (cM).(con.)

Seven COS II, 2 SSR and 2 EM markers gave a LOD scores greater than 3.0 indicating greater confidence in their map positions. Eighteen (75%) of the 24 COS II and 8 (80%) of the EM markers showed synteny with tomato. Thus, the map positions of these markers in eggplant agrees with their positions in tomato such that the order of the markers in both species is the same. In table 4.7., the locations of the analyzed markers are summarized.

Table 4.7. List of mapped markers and their locations.

Marker	Mapping Results	Comments
EM117	LG4	Syntenic in tomato
EM120	LG2	LOD >3.0, Syntenic in tomato
EM131	Didn't map	clean data
EM133	LG2	LOD >3.0, Syntenic in tomato
EM135	LG11	Syntenic in tomato
EM140	LG9	Syntenic in tomato
EM141	LG4	Syntenic in tomato
EM145	LG4	Syntenic in tomato
EM146	LG5	Syntenic in tomato
EM151	Didn't map	difficult to genotype
SSR20	LG10	Not syntenic in tomato
SSR46	Didn't map	difficult to genotype
SSR47	LG10	LOD >3.0
SSR51	Didn't map	difficult to genotype
SSR117	LG1	LOD >3.0, Syntenic in tomato
SSR192	LG7	Not syntenic in tomato
SSR218	Didn't map	multiple bands, difficult
SSR356	LG12	Not syntenic in tomato
SSR450	Didn't map	no heterozygotes
At2g 24270	LG7	Syntenic in tomato
SSR301	LG7	Not syntenic in tomato
SSR557	LG9	Not syntenic in tomato
SSR306		Didn't map
At1g20572	LG4	Not mapped in tomato
At1g02910	LG9	Syntenic in tomato
At1g10500	LG5	LOD>3.0, syntenic in tomato
At1g44575	LG6	LOD>3.0, syntenic in tomato
At1g19140	LG7	Syntenic in tomato
At1g60440	LG3	Syntenic in tomato
At1g60640	LG2	Syntenic in tomato
At1g71810	LG4	Syntenic in tomato
At1g53670	LG7	Syntenic in tomato
At1g76080	LG4	Syntenic in tomato
At1g74970	LG4	Syntenic in tomato
At1g83060	LG3	LOD>3.0, not mapped in tomato
At3g20390	LG10	LOD>3.0,syntenic in tomato

Table 4.7. List of mapped markers and their locations.(con.)

Marker	Mapping Results	Comments
At3g54360	LG10	LOD>3.0,syntenic in tomato
At3g54470	Didn't map	skewed segregation
At3g58470	LG10	Synthenic in tomato
At4g09010	LG4	Not syntenic in tomato
At4g22260	LG11	Not syntenic in tomato
At4g 31130	LG8	Possibly syntenic
At4g 33985	LG2	Syntenic in tomato
At4g 38630	LG2	Not syntenic in tomato
At4g 38810	LG1	Syntenic in tomato
At4g 39660	LG11	LOD>3.0
At5g 07910	LG3	Syntenic in tomato
At5g 11480	LG8	Not syntenic in tomato
At5g 16710	LG11	LOD>3.0, syntenic in tomato

In this thesis, four different marker types (COS II, SSR, EM, SRAP) were tested for polymorphism on the parents of the mapping population, *S.melongena* MM738 and *S.linnaeanum* MM195. COS II markers showed 4.61% polymorphism without digestion and 25.52% polymorphism after digestion. SSR and EM markers gave 23% and 48% polymorphism, respectively. SRAP markers showed 58% polymorphism. When they were compared with each other, it was demonstrated that SRAP markers are the most polymorphic markers. This may be because SRAP markers have the wide genome coverage and provide multibanded PCR products. The other markers types provide much fewer bands. Of the other markers, the eggplant and tomato SSR markers were more polymorphic than COS II markers. This may be expected because these markers correspond to microsatellites which can be highly polymorphic between species. The eggplant (EM) SSR markers were more polymorphic than the tomato SSRs suggesting that further isolation of eggplant SSRs will be a good strategy for increasing the resolution of the eggplant molecular map. COSII markers correspond to expressed portions of the genome (genes) and therefore may be expected to have less polymorphism than the other types of markers. Although SRAP markers have an advantage from the point of view of polymorphism, they are both dominant and codominant markers. COS II, SSR and EM markers have an advantage in that they are generally codominant markers.

CHAPTER 5

CONCLUSION

This thesis consisted of two parts. In the first part, solamargine was characterized in eggplant lines *S.melongena* MM738 and *S.linnaeanum* MM195. According to the results, it was found that solamargine was not detected in *S.melongena* MM738. The limit of quantitation is between 10 and 20 ppm for solamargine in HPLC and it is also expected that limited of detection is between 5 and 10 ppm. When solamargine concentration is less than 10 ppm, it cannot be detected. Thus, it is expected that cultivated eggplant contains less than 10 ppm solamargine. However, solamargine was detected in *S.linnaeanum* MM195. Also Maximum and minimum values were found for solamargine in *S.linnaeanum* MM195 (The average value: 25.5±11.2 mg solamargine per gram freeze-dried powder). In addition, the initial recovery of extraction method was determined. Recovery should be between 80 and 100%, but in this thesis, recovery of extraction method was found to be 125%.

In the second part of the thesis molecular mapping was done in eggplant. In this part, four different types of markers: SSR, EM, COS II, and SRAP markers, were first amplified in *S.melongena* MM738 and *S.linnaeanum* MM195. Polymorphism levels of 23% for SSR, 48% for EM, 4,61% for COS II, and 58% for SRAP markers were found. After identifying polymorphic markers in the parents, 11 SSR, 10 EM, and 26 COS II markers was mapped in 82 F2 individuals. Then each marker was located on the eggplant chromosomes. It was shown that there was synteny between the markers that had been mapped in both tomato and eggplant. Thus, the newly mapped markers will be important for future comparative genetic mapping in these two species. In future work, these markers can be used to map qualitative and quantitative traits in eggplant such as glycoalkaloid content.

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APPENDIX A

DEVELOPMENT OF AB-QTL POPULATION FOR EGGPLANT

It is known that agriculture has been very important for humans since the beginning of human life. For a long time, it has been demonstrated that land which is used for agriculture, is not enough for feeding the increasing human population. In addition to increasing population and insufficient farm land, drought also badly affects agriculture. Therefore, in recent years, much research has been done to improve agricultural plants. Especially, many quantitative traits in plants such as yield, fruit size, salt tolerance etc., which have importance for agriculture, have been examined. For analysis of these popular traits, advanced backcross QTL analysis has been used. Advanced backcross QTL analysis is especially useful for detection of valuable QTL alleles from unadapted wild type lines (Tanksley and Nelson 1996). This technique allows simultaneous QTL detection and transfer of favorable QTL alleles from wild types to established inbred lines (Tanksley and Nelson 1996). Each individual in an advanced backcross population (BC2 or BC3) is more similar to the elite parent from the point of view of both genotype and phenotype than in balanced populations (F2 and BC1) (Tanskley and Nelson 1996). Because of these benefits, advanced backcross populations are utilized for advanced QTL analysis.

During this thesis period, many different hybridizations were done to obtain balanced and advanced backcross populations for future QTL analysis in eggplant.

Materials and Methods:

For hybridization, three cultivated parents, one wild type parent, seven different F1 hybrids and one BC1 population were sown on 30 May 2005. Table appendix I.1. shows these plants and the number of seed sown.

Table A.1. Plants sown for hybridization experiments.

Pedigree Number	**Plant Name**	**Number of seed sown**
05T1235	*S.linneaenum* MM195	100
05T1236	*S.melongena* MM738	250
05T1239	*S.melongena -aydın siyahı*	100
05T1240	*S.melongena Dusky*	7
05T1238	F1(*S.melongena DuskyX S. incanum*)	20
05T1241	F1 (Dusky S002-1)	10
05T1242	F1 (Dusky S002-3)	10
05T1243	F1 (Dusky S000-5)	10
05T1244	F1 (Dusky S000-16)	10
05T1245	F1 (98T649 X *S.melongena cv. Dourga*)	10
05T1246	F1 (*S.dasyphyllum* (00T334)X *S.melongena cv.Black Beauty* (00T311))	10
05T1148	BC1(F1(*S.melongena*MM738*X S.aethiopicum*) X *S.melongena* MM738)	150

During flowering, backcross hybridization was done between BC1 (F1 (*S.melongena*MM738*X S.aethiopicum*) X *S.melongena* MM738) and *S.melongena* MM738 to form BC2 progeny. In this hybridization, *S.melongena* MM738 was used as female and BC1 (F1 (*S.melongena*MM738*X S.aethiopicum*) X *S.melongena* MM738) was used as male. Moreover, F1(*S.melongena DuskyX S. incanum*) was hybridized with *S.melongena Dusky*. These two plants were hybridized reciprocally. Also *S.melongena Dusky* was reciprocally hybridized with F1 (Dusky S002-1), F1 (Dusky S002-3), F1 (Dusky S000-5), and F1 (Dusky S000-16). In addition to these hybridizations, F1 (98T649 X *S.melongena cv. Dourga*) and F1 (*S.dasyphyllum* (00T334)X *S.melongena cv.B.B* (00T311)) were self pollinated.

In 2006, new plants were sown in addition to these plants. These lines are shown in table appendix I.2.

Table A.2. Plants sown on 26 March 2006.

Pedigree Number	Plant Name	Number of seed sown
06T437	*S.melongena cv.Aydın siyahı*	40
06T438	*S.melongena cv.Topan*	40
06T439	*S.melongena cv.Kemer*	40
06T440	*S.melongena cv.Black beuaty*	40
06T441	*S.melongena cv.Dolmalık*	40
06T442	*S.melongena cv.Dusky*	40
06T443	F1 (*S.melongena cv.DuskyX S.incanum* 2413)	20
06T446	F1(*S.incanum* X *S.melongena*MM738)	10
06T445	BC1 *S.incanum*	150

During flowering of these plants, *S.melongena cv.Aydın siyahı, S.melongena cv.Topan, S.melongena cv.Kemer, S.melongena cv.Black beuaty, S.melongena cv.Dolmalık* were reciprocally hybridized with each other to produce F1 hybrids. Furthermore, F1 (*S.melongena cv.DuskyX S.incanum* 2413) which was developed in 2005, was hybridized with *S.melongena cv.Dusky* to obtain a BC1 population. Also *S.melongena cv.Dusky* was crossed with BC1 *S.incanum*. In this hybridization, *S.melongena cv.Dusky* was used as male and BC1 individuals were used as females to develop a BC2 population.

Results of all hybridizations:

From the BC1 (F1 (*S.melongena*MM738*X S.aethiopicum*) X *S.melongena* MM738) individuals which were hybridized with *S.melongena* MM738, 15 BC2 individuals were obtained, but fruits of these individuals did not give any seeds. From backcross hybridization of F1 (*S.melongena DuskyX S. incanum*), 11 BC1 *S.incanum* individuals were developed and seeds of one BC1 individual were sown in 2006 and were used to develop a BC2 population. Hybridization between *S.melongena Dusky* and F1 (Dusky S002-1), F1 (Dusky S002-3), F1 (Dusky S000-5) and F1 (Dusky S000-16) gave 1 BC1 individual. Also, new F1 hybrids were developed: (*S.melongena* MM738 X *S.melongena cv.Dolmalık*) and (*S.melongena* MM738 X *S.melongena cv.Kemer*). In 2006, a BC2 population (44 individuals) was developed by hybridization between BC1 *S.incanum* and *S.melongena cv.Dusky*. In figure appendix I.1., a BC2 hybrid from BC1 (F1 (*S.melongena*MM738*X S.aethiopicum*) X *S.melongena* MM738

and *S.melongena* MM738 is shown. In figure appendix I.2., a BC1 hybrid from F1 (Dusky S002-3) and *S.melongena Dusky* is shown. In figure appendix I.3., a BC1 hybrid from F1(*S.melongena DuskyX S. incanum*) and *S.melongena Dusky* is shown.

Figure A.1. BC2 (BC1 (F1 (*S.melongena*MM738*X S.aethiopicum*) X *S.melongena* MM738)X *S.melongena*MM738)

Obtained BC2 fruit didnot have any seeds.

Figure A.2. BC1 (F1 (Dusky S002-3)X *S.melongena Dusky*)

Only one BC1 fruit developed as a result of hybridization between F1 (Dusky S002-3)X *S.melongena Dusky*, but this fruit did not have any seeds.

Figure A.3. BC1 (F1(*S.melongena DuskyX S. incanum*) X*S.melongena Dusky*)

BC1 individuals had more seed and seeds were sown in 2006. When the seeds were grown, it was seen that BC1 individuals were like F1(*S.melongena DuskyX S. incanum*) from the point of view of phenotypic characters.

APPENDIX B

EXTRACTION AND HPLC METHODS

B.1. Extraction Methods

For this thesis, many extraction and HPLC methods were used to develop the main methods for extraction and HPLC. To develop methods, eggplants from the market and greenhouse were used. In the first method, that was tried, the aim was to obtain precipitation of glycoalkaloids. According to this method, one frozen eggplant was taken from -80 frezeer and thawed at room temperature. After thawing, 100 g eggplant was homogenized with 200 ml distilled water (Purelab Prima ELGA) in Waring commercial blender (before mixing, the blender must be clean and free of eggplant material. To further prevent contamination, the blender may be cleaned with a small amount 50-100 g of the same eggplant material that will be used to prepare the current solution plus 50 ml deionized water). Then 10 g homogenate was homogenized again with 15 ml MeOH/ 5% acetic acid with Heidolph Silent Crusher-Ultra turax (Ultra-Turax should be cleaned in the same manner as the blender). After this step, the homogenate was filtered with nylon mesh into two 15 ml falcon tubes and these tubes were centifuged. Supernatants were removed to a round flask and evaporated with Heidolph Rotary Evaporator to remove MeOH/ 5% acetic acid until 2-3ml supernatant was left. After evaporation, 10 ml ammonium hydroxide was added to supernatant. Then, this solution was centrifuged at 18.000 rpm for 10 min at 1°C. Supernatant and precipitate were separated and then precipitate was dissolved with Methanol. Finally, supernatant and precipitate were measured with UV spectrophotometer between 200-215 nm for the glycoalkaloid peak at this wavelenght range but samples were very dirty so this extraction method was tried again with some differences.

In the second extraction method, the same homogenate was prepared and filtered with nylon mesh into four 15 ml falcon tubes and these tubes were centrifuged. Supernatants of three tubes were combined in a round flask to evaporate until 2-3 ml and the supernatant of one tube was also evaporated until 2-3 ml. After evaporation, ammonium hydroxide (2-3 pasteur pipet fuls) was added to each tube. Then the samples were centrifuged at 18.000 rpm for 10 min. at 1°C again. After centrifugation,

supernatants and pellets were separated for each tube. Pellets were dissolved with 10 ml methanol, then they were filtered with syringe filter (0.2µm) and each was measured in UV-spectrophotometer. When the results of samples were compared with each other, all samples were also dirty. It was thought that chlorophylle gave extra peaks and made the samples dirty. Therefore, a different method was tested.

According to this third method, 145 g thawed eggplant was homogenized with 155 ml water, and 10 g homogenate was also homogenized with 20 ml choloroform: methanol (2:1 v/v) with Ultra Turax. Then it was filtered with nylon mesh into two 15 ml tubes and these tubes were centrifuged at 3000 rpm for 10 min at 4°C. Then methanol and chloroform phase were separated and measured in UV-spectrophotometer for one tube, but again too many peaks were seen. Both chloroform and methanol phases were diluted to 50% with chloroform and methanol, respectively. Another sample was centrifuged, filtered with syringe filter (0.2µm) and evaporated in rotary evaporator until 2-3 ml supernatant was obtained. After evaporation, ammonium hydroxide (10ml) was added to the tube. The sample was kept at room temperature overnight. The following day, some precipitate was seen but it was not too much.

In another extraction method, 145 g thawed eggplant was homogenized with 155 ml water, and 10 g homogenate was again homogenized with 20 ml methanol: 5% acetic acid with the Ultra Turax. Then, it was filtered into two 15 ml falcon tubes with nylon mesh and tubes were centrifuged at 3000 rpm for 10 min at 4°C. After centrifugation, samples were filtered with syringe filter (0.2µm) into 50 ml erlenmayer flask and washed with 30 ml water: 5% acetic acid. Then 10 ml ammonium hydroxide was added to each flask. The flasks were put into water bath at 70°C for 50 min. Then one flask was put at 4°C and another flask was kept in room temperature overnight. Some precipitates were seen in the tube which was kept at room temperature the next day. Then, the evaporation of the supernatant in the tube which was left at 4°C was attempted using nitrogen gas but it could not evaporated. Supernatant in the sample which was kept at room temperature was evaporated by placing the beaker into a water filled beaker that was heated on a hot plate and then 10 ml ammonium hydroxide was added again. The sample was left at room temperature again overnight to increase the amount of precipitation. However, no difference was seen. Finally, a 0.5 ml sample which contained precipitate crystals was taken from the bottom of the sample and mixed with both 2 ml methanol and 25 ml 0.2 N HCl. Then this mixture was used for HPLC. Also, a 2 ml sample with crystals was mixed with both 2 ml acetonitrile and 25 ml 0.2

N HCl and this mixture also was used for HPLC. It was thought that water in eggplant caused hydrolyzation of glycoalklaoids. For this reason, in next extraction method, less water was used. According to the, 200 g thawed eggplant was homogenized with 50 ml water in steel Waring blender. The homogenate was ground with liquid nitrogen by using pestle and mortar. After grinding, the sample was evaporated in rotary evaporator until all water was evaporated, but all water was not evaporated. Then the powder which was obtained in rotary evaporator was ground again with small mortar and pestle. When the grinding was finished, the powder was homogenized with 20 ml methanol: 5% acetic acid in Ultra Turax. After this step, the homogenate was filtered with nylon mesh. into two 15ml falcon tubes. They were centrifuged at 3000 rpm for 10 min at 4°C. Then they were also filtered with syringe into two erlen flasks and washed with 30 ml water: 5% acetic acid. 10 ml ammonium hydroxide was added to each flask and the flasks were put into water bath at 70°C for 50 min. Finally, they were left at room temperature overnight and cloudy particeles were seen in the bottom of the solution the next. In this method, all water in the homogenate could not be evaporated. Therefore, in the next extraction method, the same steps were used except for the evaporation step. Lyophilization was used to obtain freeze-dried samples instead of evaporation and it more brown precipitates were seen. Then this sample was filtered with glass filter and measured with HPLC.

According to another tested extraction method, 200 g thawed eggplant was homogenized with 100 ml distilled water. Two samples of 10g homogenate were into small volumetric cylinders. Theses samples were homogenized with 20 ml methanol: 5% acetic acid by used Ultra Turax. One of them was filtered with nylon mesh, another one was filtered with filter paper using a vacuum pump. Residue on filter paper was washed three times with 20 ml methanol: 5% acetic acid. Then all tubes were centrifuged at 3000 rpm for 10 min at 4°C. After centrifugation, each tube was filtered with syringe into erlenmeyer flask and washed with 30 ml water: 5% acetic acid. Then 20 ml ammonium hydroxide was added to each flask. The flasks were put into water bath at 70°C for 50 min. Finally, they were kept at room temperature. The following day, some white particles like tissue were seen.

In addition to the other methanol-based extraction methods, a butanol extraction method was also tried. In butanol extraction, 200 g thawed eggplant was homogenized with 100 ml distilled water. Then 10 g of solution was homogenized with 20 ml of 5% acetic acid using the Ultra Turax. The slurry was transferred to 50 ml erlenmeyer flask

and stirred with a magnetic stirrer for 45 minutes. After mixing, the sample was filtered through filter paper with vacuum pump. The residue was reextracted for 45 min. with another 20 ml of acetic acid and filtered again through filter paper. The two filtrates were combined and transferred to a 125 ml separator funnel. The pH was adjusted to 10-11 with Ammonium hydroxide. The alkaline extract was partitioned once with 40 ml of water saturated butanol overnight. After one day, some brown particles were seen in the middle. The brown particules were filtered and the two phase were separated from each one. The bottom phase was partitioned 4 times with 10 ml water-saturated butanol. Then the combined butanol extracts were evaporated with air vacuum rotary evaporator. However, any residue was not obtained and butanol could not be evaporated. Because this method was not successful, another method was tried.

According to this method, 200 g wild type, thawed eggplant and 100 ml of water were homogenized in a waring blender for 2 min. Then 10 g of solution was taken and mixed throughly with an Ultra-Turax mixer at 18.000 rpm revolution per minute for 1 minute. The same for all the remain eggplant material was done. Each homogenous solution was put in a watch glass dish. Four watch glasses were put into an oven at 50°C but they burnt. Other samples were placed into a carbon box until samples dried to obtain sun dried samples. Sun dried samples and oven dried samples were turned to powder with mortar and pestle. Then 0.25 g powder of sun dried sample, was extracted within 20 ml methanol: 5% acetic acid and it was stirred with magnetic stirrer for 10 min. Then the upper phase was taken and 20 ml methanol: 5% acetic acid was added and stirred for 10 min. again. This step was done 3 times. Then all upper phases were mixed and filtered through polyamide filter. After this step, 10 ml ammonium hydroxide was added to solution and put into water bath at 70°C for 50 min. However, nothing was seen. Then sun dried powder on polyamide filter was put into rotary evaporator after 10 ml NH_4OH was added to this sample. As the liquid phase evaporated, some brown precipitates were seen. The precipitates were filtered with polyamide filter and evaporator flask was washed with diluted NH_4OH (100 ml distil water: 10 ml NH_4OH). However, those precipitates could not be removed from the polyamide filter. Also the 0.25 g powder of oven dried sample was extracted within 20 ml methanol: 5% acetic acid and it was stirred with magnetic stirrer for 10 min. The upper phase was taken and 20 ml methanol: 5% acetic acid was added and stirred for 10 min. again. This step was done 3 times and all upper phases were mixed and filtered through polyamide filter. Then the residue on polyamide filter was extracted with 40 ml

methanol: 5% acetic acid and mixed with magnetic stirrer for 10 minutes. This solution was filtered with nylın mesh and centrifuged at 4 °C at 3000 rpm for 10 min. Then supernatant was filtered by using syringe filter. After this step, 10 ml NH_4OH was added to flask and it was put into water bath at 70°C for 50 min. Then 10 ml Hexane was added into extract and this mixture was put into separation funnel. After separation, lower phase was taken and put into rotary evaporator to evaporate the little hexane in the solution. Then, one or two drops of water were added to the flask and the solution looked oily. There were stil small drops. It was thought that those drops could be hexane. According to another extraction method, 2.7 g sun dried powder was extracted with 20 ml methanol: 5% acetic acid and stirred with magnetic stirrer for 10 min. The upper phase was taken and 20 ml methanol: 5% acetic acid was added and stirred 10 min again. This step was done three times. Then all upper phases were mixed and filtered through polyamide filter. After this step, 20 ml ammonium hydroxide was added to the solution which was put into water bath at 70°C overnight but nothing was seen the next day. The sample was then put into rotary evaporator to decrease its volume. After evaporation, some precipitates were seen. To obtain these precipitates, sample was filtered with polyamide filter. Because there was too much sample, the filtering was not successful, so the sample was filtered with paper filter again. Then filtered liquid phase was saved. After this extraction method, for another extraction method, 0.2530g eggplant powder was mixed with 20 ml MeOH : 5% acetic acid with magnetic stirrer and liquid phase was removed to a flask. This step was done 3 times and all liquid phases were mixed. Then the total solution was filtered with nylon mesh into 50 ml falcon tubes and these tubes were centrifuged at 4°C at 3000 rpm for 10 min. After centrifugation, liquid phase was filtered with syringe filter into flask and 10 ml NH_4OH was added to flask. Then flask was put into water bath at 70°C for 50 minutes. After water bath, sample was left in hood for 2-3 days. Then it was filtered with polyamide filter. After filtration, the liquid phase was taken into eppendorf tube for HPLC. Also, the residue on the polyamide filter was extracted with 20 ml methanol: 5% acetic acid with magnetic stirrer for 10 min. Then that solution was centrifuged and supernatant was filtered with syringe filter. After this step, 10 ml NH_4OH was added to flask and it was placed in water bath at 70°C for 50 minutes. After 50 min., it was filtered with polyamide filter again, and filtered solution was taken into eppendorf tube for HPLC.

In another stage for extraction, small changes were added to the last extraction method. To obtain powder, 10 g of homogenate which was obtained by homogenizing 200 g fresh eggplant with 100 ml deionized water in the waring blender at 4°C for 2 minutes, was homogenized with 10 ml water using Ultra Turax at 18.000 rpm for 1 minute. Then homogenate was left on flat glass to dry for 2 days. Dried homogenate was scraped off powdered with mortar and pestle. The powder was put in oven at 99°C for 10 minutes and then it was put into dessicator. In the extraction method, 0.2514 g of this powder was used. It was mixed with only 20 ml methanol with magnetic stirrer for 10 minutes, after 3 min, the liquid phase was taken. A total of 12,6 ml liquid phase was taken and left at 4°C. Then 20 ml MeOH: 5% acetic acid was added to solid phase and mixed with Ultra-Turax at 10.000 1/min for 2 minutes. After ultra turax, top phase was filtered with polyamide filter and 20 ml hexane was added to filtered supernatant. After shaking, 20 ml supernatant from bottom phase was taken into 250 ml flask and 10 ml NH_4OH was added to flask. When NH_4OH was added to flask, some physical changes were seen in solution. The color of solution turned from clear to yellow. Crystals in the bottom of the flasks for the two different phase- hexane and NH_4OH were also seen. Also, the sample had an oily appearance. Finally, the solution was put in water bath at 70°C for 50 minutes. In the next extraction method, some differences were made. According to this method, 0.2519 g eggplant powder was mixed with only 20 ml methanol: 5% acetic acid with magnetic stirrer for 10 minutes. After mixing, the liquid phase was taken after 3 min. Then, 15 ml liquid phase was taken and put at 4°C. Then 20 ml MeOH: 5% acetic acid was put into the residue and they were mixed using Ultra Turax at 10.000 1/min for 2 minutes. That step was repeated 3 times and in each time, liquid phase was collected into small flask. After ultra turax, total liquid phase was filtered with polyamide filter. Then 20 ml NH_4OH was added into filtered supernatant. In final, this sample was put into water bath at 70°C for 50 minutes but nothing was seen. The small amount of sample was put into rotary evaporator to see if it would precipitate. However, nothing was seen again. This same method was tested again using naylonmesh filtration instead of polyamide filtration. Despite this different filtration system, no results were obtained. Therefore, another new method was tried. According to that method, both fruit and leaf powders were utilized for extraction. A total of 0.25 g powders were weighed into falcon tubes from both fruit and leaf samples. Then 5 ml methanol: 5% acetic acid was added into each tube and each tube was mixed with vortex for 10 sec. every 30 min. for 3 hours. After 3 hours, the sample were left at room

temperature overnight. In the morning, all tubes was centrifuged for 5 min.Then all tubes were kept at room tempereture at least one week. After one week, their liquid phases were filtered with syringe filter and tested with HPLC. Although some glycoalkaloid peaks were seen in HPLC chromotogram, this method was not used because it took too much time.

Finally, a method which was very close to the extraction method used in this thesis was tested. For this method, 200 g fresh eggplant was homogenized with 100 ml deionized water by using waring blender at 4°C for 2 minutes. Then 10 g eggplant solution was homogenized with 10 ml deionized water by using Ultra Turax at 18.000 1/min. for1 minute. That step was repeated 18 times. Each homogenate was spread out on big, flat pieces of glass and were dried for 2 days under sun. Then the sample was scraped off with razor blade. After scraping off, the pieces of eggplant like paper were powdered with mortar and pestle by using liquid nitrogen. After this step, the eggplant powder was put into lyophilizer for 3 days. Then 2 g eggplant powder was weighed and put into 50 ml falcon tube. Five falcon tubes were prepared like this. Then 40 ml MeOH: 5% was added to each falcon tube. These tubes were put on shaker at 400 mot 1/min for overnight. The Next day, those tubes were put on strong shaker at 600 mot 1/min for 10 minutes. Then each tube was filtered with filter paper by using buchner funnel and vacuum pump. After this filtration, each tube was filtered with polyamide filter. Then all tubes were mixed in circular rotary evaporator flask and evaporated until the solution decreased to approximately 20 ml. Then that solution was filtered again with polyamide filter. Before filtration, polyamide filter was washed with 5ml methanol: 5% acetic acid. After filtration, the solution was poured into a 250 ml volumetric flask and diluted 250 ml with methanol: 5% acetic acid. Then the sample was used for HPLC. In HPLC, no peaks were not seen because the sample was very dilute. In the next extraction, small changes were done and this method was used for the thesis research. In evaporation step, the solution was decreased to 8-9 ml. After evaporation, the solution was filtered with syringe filter. The solution was put into 10 ml volumetric flask and diluted to 10 ml with methanol: 5% acetic acid. This solution was used for HPLC. To check this extraction method, a solasodine standard was used and 0.4 mg solasodine was put into each falcon tube with 2 g eggplant powder and 40 ml methanol: 5% acetic acid. After the extraction finished, qualification and quantification of solasodine was checked and that extraction method was accepted. In addition to extraction method, SPE cleaning was applied for only one sample because

samples were very dirty. However, after SPE cleaning, glycoalkaloids in sample were lost. Therefore that cleaning method was not used for other samples. In SPE cleaning, 1ml SPE-C18 from M-Nagel was utilized. To clean column, it was first washed with 2-3 ml MeOH and then 2-3 ml H_2O. After washing, 1 ml extract was applied. Then SPE column was washed with 3 ml H_2O: MeOH (60:40) and 10 ml MeOH (100%) was applied to elute the SPE column. The obtained solution was then evaporated to almost dryness and all steps were repeated with fresh 1 ml extract. After obtaining 1-2 ml solution, it was used for HPLC.

B.2. HPLC Methods

In HPLC, some methods which were developed by Neslihan TEK were tried in this thesis. For aglycones (especially solasodine) acetonitrile (60%- B pump) and ammonium dihydrogen phosphate-100mM (pH: 2.5)(40%- A pump) were used as mobile phase. The temperature and flow rate were set as 50°C and 1 min/ml respectively. Pressure was between 79 and 82 kgf. For solasonine and solanine analysis, both acetonitrile (30%- B pump) and ammonium dihydrogen phosphate-100 mM (pH: 2.5)(70%-A pump) were utilized as mobile phase. The temperature and flow rate were set as 50°C and 1 min/ml respectively. Under these conditions, the pressure was noted as 96 kgf. In addition to those methods, for solamargine analysis, acetonitrile-10%MeOH (30%- B pump) and ammonium dihydrogen phosphate-100 mM (pH: 2.5)(70%-A pump) were used. When that mobile phase was used, the pressure was determined to be between 97-100 kgf. Also flow rate and temperature were set as 1 min/ml and 50°C, respectively. It was learned that when the pH of the ammonium dihydrogen phosphate buffer changed, the separation of peaks changed. When the pressure increased or decreased suddenly, the HPLC column was washed with MeOH. In washing, column was washed with MeOH (100%) first and then washed with acetonitrile: water (70%-30%). After that step, column was also washed with acetonitrile: ammonium dihydrogen phosphate (30%-70%).