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Molecular and functional characterization of genes involved in saponin biosynthesis in *Medicago* spp.

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A.A.

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Abbreviations:

CDS: coding sequence

DTT: dithiothreitol

EDTA: ethylendiaminetetraacetic acid

EMS: ethyl methane sulfonate

ER: endoplasmatic reticulum

EST: express sequence tag

GC: gas chromatography

GC-FID: gas chromatography-flame ionization detector

GC-MS: gas chromatography-mass spectrometry

HPLC: high performance liquid chromatography

NADPH: reduced form of Nicotinamide adenine dinucleotide phosphate

ORF: open reading frame

PMSF: phenylmethylsulfonyl fluoride

TLC: thin layer chromatography

Summary

Saponins are a large group of plant secondary metabolites including triterpenoids and steroids glycosylated with one or more sugar chains (Hostettmann and Marston, 1995). In the *Medicago* genus saponins are a complex mixture of triterpenic glycosides showing a broad spectrum of biological (antifungal, insecticidal, phytotoxic, allelopathic and hemolytic) and pharmacological properties (anticholesterolemic, anti-cancer adjuvant) (Tava and Avato, 2006).

In spite of their role in plant defence mechanisms, their importance as antimicrobial compounds and their possible benefits for human health, knowledge of the genetic control of saponin biosynthesis is still lagging behind.

Triterpenic saponins are derived from β-amyrin that is converted to various aglycones by a series of oxidative reactions catalyzed by cytochromes P450; finally glycosyltransferases (GTs) convert aglycones into saponins. In *M. truncatula* at least three genes encoding early enzymes of the β-amyrin skeleton formation have been functionally characterized (Suzuki et al., 2002). However, in *M. truncatula* no gene involved in the oxidative reactions of β-amyrin has yet been isolated.

In our laboratory, we have used *M. truncatula* mutant collections to search mutations affecting saponin biosynthesis. In an activation-tagging mutant collection of *M. truncatula* (Porceddu et al., 2008), one plant lacking hemolytic saponins was evidenced (*lha-1* mutant); the T-DNA tagged locus was identified by inverse-PCR and the putative gene implied in this mutation turned out to be a cytochrome P450 named CYP716A12. A second collection, obtained by EMS-mutagenesis (Porceddu et al., 2008), was screened by TILLING analysis and two mutants for the CYP716A12 gene were identified (*lha*-2 and *lha*-3). Both these mutants lacked hemolytic saponins, although they accumulated soyasaponins. In addition, all *lha* mutants lacked sapogenin precursors of hemolytic saponins suggesting that the biosynthetic pathway was blocked at an early and common step. In order to validate the correspondence between the CYP716A12 gene and the *lha* phenotype, a vector carrying a full-length CDS of CYP716A12 was stably transformed into *lha-1* mutant plants and restoration of the biosynthetic pathway of hemolytic saponins was confirmed by GC-MS analyses. This result confirmed the knock-out of CYP716A12 was responsible for the block in hemolytic sapogenin pathway.

Objectives of this *Ph.D* project were: i) investigating the role of cytochrome CYP716A12 in saponin biosynthesis by heterologous expression in yeast (*Saccharomyces cerevisiae*), ii) studying gene expression levels and sapogenin content in *M. truncatula* plants at different developmental stages and iii) identifying the orthologue of CYP716A12 in *M. sativa*.

To elucidate the function of CYP716A12, the gene was expressed in yeast (*S. cerevisiae*); first, the CYP716A12 coding sequence was introduced in a commercial yeast strain. *In vivo* and *in vitro* microsome assays were performed using β-amyrin and oleanolic acid as possible substrates of the cytochrome, but no modifications of the supplemented substrates were evidenced. These negative results may be explained by the low efficiency of yeast NADPH-P450 reductase (Pompon et al.,

1996) that has been suggested to be important for efficient electron transfer to cytochromes P450. For this reason a new experiment was performed using yeast strains WR and WAT11, overexpressing *S. cerevisiae* and *Arabidopsis thaliana* P450 reductase respectively. An *in vivo* assay was performed, but GC analysis did not show any additional peak except those of the substrates. Enzymatic activities in microsomes (*in vitro* assay) were then tested by supplying β-amyrin, and erythrodiol as substrates; GC-MS analysis revealed the substrate transformation to yield oleanolic acid. These results indicate that CYP716A12 mainly catalyzes the sequential three-step oxidation at the C-28 position necessary to transform β-amyrin into oleanolic acid.

To identify which substrate could restore the saponin pathway in *lha*-1 mutant, plant microsomal fractions were used. Using β-amyrin and oleanolic acid as substrate, wild-type microsomes produced all sapogenins, while mutant microsomes yielded non-hemolytic sapogenins only. In contrast, when supplemented with hederagenin, neither wild-type nor *lha* mutant microsomes restored the saponin pathway. These contrasting results may reflect the difficulty in setting up an efficient and stable plant microsome preparation.

To investigate the expression profiles of the CYP716A12 gene, quantitative PCR analyses were performed on a wild-type line. Leaf stem and root samples were collected at three biological stages (vegetative growth, early flowering, and early pod setting). Roots displayed highest and most stable expression across stages, while in leaves a significant increase in expression appeared in the reproductive stages with a maximum at flowering. The same plants were examined for sapogenin content by GC/FID analysis. Hemolytic sapogenin content was significantly influenced by both phenological stages and plant organs.

Finally, in order to transfer the new findings in hemolytic saponin biosynthesis in crop species, the ORF of the orthologous gene in *M. sativa* was isolated using the same primers utilized in *M. truncatula*.

In summary, results of this *Ph.D* work revealed that CYP716A12 is a multifunctional oxidase catalyzing the sequential three-step oxidation at C-28 position necessary to transform β-amyrin into oleanolic acid. CYP716A12 can use different substrates and plays a key role in hemolytic saponin biosynthesis as its disruption prevents the formation of any aglycone with an oxygenated group in C-28 and consequently the presence of triterpenes glycosylated in this position. In contrast to other transcripts involved in triterpenic saponin pathway, generally restricted to root system, CYP716A12 is expressed in different plant organs and developmental stages.

Building upon this work, we plan to use the WAT11 yeast expression system to investigate the role of CYP716A12 cytochrome from *M. sativa*, and perform TILLING analysis on other candidate P450 genes in order to identify and characterize new functions possibly involved in saponin biosynthesis.

Chapter 1.
Introduction

1. *Medicago* genus

1.1. *Leguminosae* family

The *Fabaceae*, or legumes, constitute the third largest family of flowering plants, comprising more than 650 genera and 8,000 species. Economically, legumes represent the second most important family of crop plants after *Poaceae* (grass family), accounting for approximately 27% of the world's crop production (Graham and Vance, 2003).

On a worldwide basis, legumes contribute about one third of humankind's protein intake, while also serving as an important source of fodder and forage for animals and of edible and industrial oils. One of the most important attributes of legumes is their unique capacity for symbiotic nitrogen fixation, underlying their importance as a source of nitrogen in both natural and agricultural ecosystems. Legumes also accumulate natural products (secondary metabolites) that are beneficial to human health through anticancer and other health-promoting activities (Dixon and Sumner, 2003).

The legumes are highly diverse and can be divided into three subfamilies: *Mimosoideae*, *Caesalpinioideae*, and *Papilionoideae*. Of these, the *Papilionoideae* subfamily contains nearly all the economically important crop legumes, including soybean (*Glycine max*), peanut (*Arachis hypogaea*), mungbean (*Vigna radiata*), chickpea (*Cicer arietinum*), lentil (*Lens culinaris*), common bean (*Phaseolus vulgaris*), pea (*Pisum sativum*), and alfalfa (*Medicago sativa*). With the notable exception of peanut, all these important crop legumes fall into two *Papilionoid* clades, namely, *Galegoid* and *Phaseoloid*, which are often referred to as cool season and tropical season legumes, respectively (Fig. 1) (Zhu et al., 2005). Despite their close phylogenetic relationships, crop legumes differ greatly in their genome size, chromosome number, ploidy level, and self-compatibility. To establish a unified genetic system for legumes, two legume species in the *Galegoid* clade, *Medicago truncatula* and *Lotus japonicus*, which belong to the tribes *Trifolieae* and *Loteae*, respectively, were selected as model systems for studying legume genomics and biology (Cook, 1999).

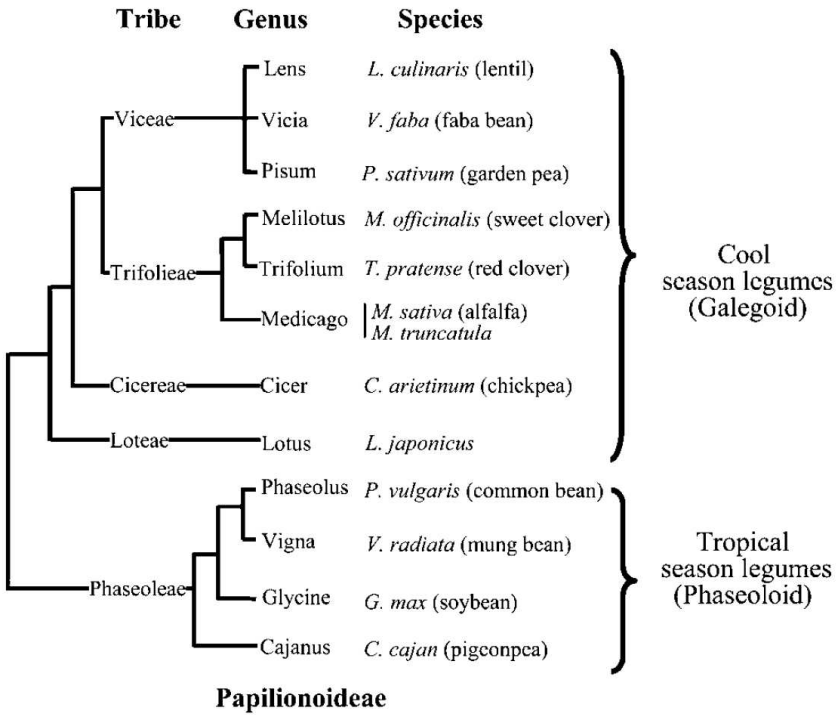

Figure 1. Dendrogram depicting phylogenetic relationships of *Papilionoideae* legumes (Zhu et al., 2005).

1.2. The genus *Medicago*

The genus *Medicago* comprises approximately 87 different species of herbs and shrubs widespread from the Mediterranean to central Asia, including the widely cultivated forage crop and weedy species *M. sativa* L. (commonly named alfalfa or Lucerne) and the legume model species *M. truncatula* Gaertn (Steele et al., 2010).

M. sativa is a perennial, autotetraploid (4x=32 chromosomes) and allogamous plant, native from Near East and Central Asia but considerably adaptable to a large range of environments (D'addabbo et al., 2010).

Annual *Medicago* spp., including *M. truncatula*, play an important agronomic role in dryland farming regions of the world where they are often an integral component of cropping systems, particularly in regions with a Mediterranean or Mediterranean-type climate where they grow as winter annuals providing both nitrogen and disease breaks for rotational crops (Tivoli et al., 2006).

Burr medic (*M. polymorpha* L.) is mainly used for pastures. Originating from the Mediterranean basin, where it occurs naturally, it spread worldwide and became a cultivated species in Australia. In recent decades, an agronomic interest also arose in the Mediterranean region, where this species shows a wide range of adaptation to pedoclimatic conditions (Tava et al., 2011).

Among the medics, spotted medic [*M. arabica* (L.) Huds.] is considered a minor species in agronomic terms and no improved varieties have been selected so far, despite its wide distribution in Eurasia and Africa and its naturalisation in Australia. However, variation for agronomically useful traits exists within the species germplasm, and promising material for breeding programmes can be identified (Pecetti et al., 2010).

M. arborea L., commonly known as tree medic, is a woody shrub growing in many Mediterranean areas, showing good regrowth ability after summer stasis. It represents one of the most interesting plants used to prevent soil erosion in semiarid locations and to increase forage availability (Tava et al., 2005).

1.3. *Medicago truncatula* as a model plant

M. truncatula (commonly know as barrel medic) is a galegoid legume, closely related to alfalfa (*M. sativa*). It originated in the Mediterranean basin and is now cultivated as an annual forage, especially in Australia.

Interest in *M. truncatula* as a model legume began with research into its symbiotic relationship with *Sinorhizobium meliloti* in the early 1990s. This rhizobial symbiont has the capacity to invade and differentiate inside its host plant alfalfa (*M. sativa*) and also the model host plant *M. truncatula*. The scope of *M. truncatula* research later expanded to the study of mycorrhizal symbioses, root development, secondary metabolism, disease resistance, and ultimately to genomics, transcriptomics, proteomics, and metabolomics.

M. truncatula presents many advantages as a model plant, eg a small, diploid genome (2x=16 chromosomes), rapid generation time, self-fertility and ease of seed production (Cook, 1999). The close phylogenetic relationship of *M. truncatula* to perennial cultivated *Medicago* spp. such as alfalfa, and other legumes such as pea, lentil, chickpea and faba bean increases the attractiveness of utilizing *M. truncatula* to improve our understanding of important agronomic traits in related grain and forage legume species (Tivoli et al., 2006). In addition, being a field crop species (unlike *Arabidopsis*) it could be readily used in the field where appropriate.

The genome of *M. truncatula* is being sequenced by international consortiums and high-quality genome sequence data, covering most euchromatic regions, have recently become available (Branca et el., 2011). *M. truncatula* databases and tools comprising whole genome sequencing and annotation, expressed sequence tags (ESTs), structural genomics and comparative mapping, bacterial artificial chromosomes (BACs) libraries and physical maps, gene expression, metabolic profiling and bioinformatic tools are accessible through dedicated websites (www.medicago.org, http://mtgea.noble.org/; www.plantgdb.org/MtGDB; www.tigr.org/tdb/mtgi).

2. Saponins

2.1. General introduction

Saponins are a group of secondary metabolites that consist of an isoprenoidal-derived aglycone, designated genin or sapogenin, linked to one or more sugar moieties.

The name in deduced from the Latin word *sapo* (soap) reflecting their widespread ability to form stable soap-like foams in aqueous solutions. In fact, some saponin-containing plants have been employed for hundreds of years as soap as reflected in their common names: soapwort (*Saponaria officinalis*), soaproot (*Chlorogalum pomeridianum*), soapbark (*Quillaja saponaria*), soapberry (*Sapindus saponaria*) and soapnut (*Sapindus mukurossi*) (Hostettmann and Marston, 1995). This characteristic trait is caused by the amphiphilic nature of saponins due to linkage of the lipophilic sapogenin to hydrophilic saccharide side chains.

The most common sources of saponins are higher plants, but increasingly these compounds are being found in lower marine animals. So far, they have only been found in the marine phylum *Echinodermata* and particularly in species of the classes *Holothuroidea* (sea cucumbers) and *Asteroidea* (starfishes) (Hostettmann and Marston, 1995).

The ability to synthesize saponins is rather widespread among plants belonging to the division of *Magnoliophyta*, covering both monocotyledons and dicotyledons (Augustin et al., 2011). Saponins can be classified into two groups based on the nature of their aglycone skeleton: steroidal, based on C_{27} skeleton, or triterpenoid, based on C_{30} skeleton (Fig. 2) (Sparg et al., 2004). Their distribution in the plant kingdom seems to be correlated with the structural type: triterpenoid saponins are found principally in Dicotyledons, such as *Leguminosae, Araliaceae and Caryophyllaceae* and steroidal saponins are synthesized in Monocotyledons such as members of the *Liliaceae*, *Dioscoraceae* and *Agavaceae* families.

Saponins are also classified based on the number of attached saccharide side chains. Most known saponins are monodesmosides, ie only one position of the aglycone, mainly (both for triterpenoid and steroidal saponins) the C-3 hydroxy group, is glycosilated (Fig. 2); saponins with two sugar moietys are designated bidesmosides. Finally, in the rather rare tridesmosidic saponins, three sugar chains are attached to the aglycone at different positions (Augustin et al., 2011).

While the main human dietary sources of saponins are legumes (soybeans, chickpeas, peanuts, beans and lentils), species such as Soap bark tree (*Quillaja saponaria*), fenugreek *(Trigonella foenum-graceum)*, alfalfa (*Medicago sativa*), horse chestnut (*Aesculus hippocastanum*), liquorice (*Glycyrrhiza glabra*), soapwort (*Saponaria officinalis)*, and ginseng (Panax genus) are the main non-food sources of saponins used in health and industrial applications (Güçlü-

Üstündağ and Mazza, 2007). Otherwise, cereals and grasses appear to be generally deficient in these secondary metabolites with the exception of oat (*Avena* ssp.) that produces two different families of saponins: the steroidal avenacoides accumulating in the leaves, and the triterpenoid avenacins in the roots (Osbourn, 2003).

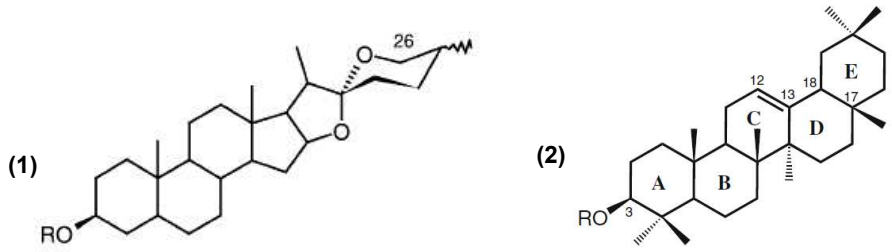

Figure 2. Aglycone skeletons of (1) steroidal, (2) triterpenoid saponins. R = sugar moiety in case of monodesmosidic saponins (Sparg et al., 2004).

2.2. Role and properties

The biological role of saponins is not completely understood. In plants, they are generally considered to be part of defence systems due to anti-microbial, fungicidal, allelopathic, insecticidal and molluscicidal, etc. activities (Sparg et al., 2004).

Plants generally accumulate saponins as part of their normal development. However, saponin accumulation is also influenced by several environmental factors such as nutrient and water availability or light irradiation (Augustin et al., 2011). In addition, saponin distribution has been found to vary greatly in individual plant tissues during ontogenesis or to show seasonal fluctuations (Pecetti et al., 2010). Variations in saponin distribution and levels have been suggested to represent varying needs for protection and to target specific herbivores and pests, respectively. For example, accumulation of glycosides in the root epidermis of oat has been demonstrated to counteract soil-borne fungi (Papadopoulou et al., 1999). Saponin levels are often increased in response to treatment with elicitors such as yeast extract or jasmonate derivatives, known for triggering plant defence responses to herbivory (Augustin et al., 2011).

Beside their role in plant defence, these compounds are of growing interest for drug research or their valuable pharmacological and biological properties. In traditional Chinese medicine, saponins are key ingredients: for instance, the ginseng root (*Panax ginseng*), one of the most important traditional oriental medicines now used worldwide, contain Ginsenoside and oleanolic acid glycosides showed anti-inflammatory activity (Sparg et al., 2004). Moreover the seed of *Saponaria vaccaria,* contained triterpene saponins, are used for the treatment of amenorrhea, breast infections and the stimulation of lactation (Meesapyodsuk et al., 2007).

Saponins have been associated with a wide range of biological properties such as fungicidal, molluscicidal, nematocidal, antiparasitic, insecticidal, antibacterial, antiviral and allelopathic activities (Sparg et al., 2004; Tava and Avato, 2006; D'addabbo et al., 2010; Augustin et al., 2011). In addition, recent studies have reported beneficial anti-inflammatory, anti-cholesterolemic, anti-cancer and cytotoxic properties of saponins (Wu and Yang, 2004; Tava and Avato, 2006; Balestrazzi et al., 2011).

Furthermore, hemolytic properties are generally attributed to the saponins due to their ability to rupture erythrocytes, causing an increase in membrane permeability and a loss of haemoglobin; consequently saponins can also be toxic to monogastric animals, act as anti-palatability factors, or negatively impact forage digestibility in ruminants (Suzuki et al., 2002).

Due to their chemical properties and abilities as foaming agents, saponins are exploited by industry as additives to foods and cosmetics; for example saponins are ingredients of shampoos, toothpaste and lipsticks. Due to their ability to form complexes with cholesterol, saponins have been used for the removal of cholesterol from dairy products such as butter oil (Güçlü-Üstündağ and Mazza, 2007).

2.3. Saponins in *Medicago* ssp.

The *Leguminosae* have been extensively investigated for their saponin content and within this family of plants, *Medicago* spp. represent a particularly rich source of bioactive saponins (Tava and Avato, 2006).

In the genus *Medicago,* saponins are generally complex mixtures of high-molecular weight triterpene glycosides with medicagenic acid, hederagenin, zanhic acid, bayogenin and soyasapogenols A and B as the dominant aglycones; sugar chains are generally linked at C-3 position of the aglycone (monodesmosides) and additionally at C-28 position (bidesmosides). The most abundant monosaccharide units found in the *Medicago* saponins are: arabinose, rhamnose, glucose, xylose and glucuronic acid.

Previous studies (Tava and Avato, 2006) have focused on elucidating the relationship between the biological activities of these compounds and their chemical structure; in fact the type of aglycone moiety and the nature and position of the sugar chains appear to correlate with the different biological properties. Hemolytic activity provides an example being related to the nature of the aglycone moieties. No hemolytic activity was detected for soyasapogenol saponins, while the other aglycones showed from high (hederagenin and medicagenic acid glycosides) to moderate (zahnic acid glycosides) hemolytic activity (Carelli et al., 2011).

Antimicrobial activity of saponins from *M. sativa, M. Arabica* and *M. arborea* against the most medically important yeast and Gram-positive and Gram-negative bacteria was investigated by

Avato et al. (2006). In addition, saponins and sapogenins (medicagenic acid) from alfalfa shoots and roots are able to induce nematode mortality; therefore these compounds could be hypothesized as a valuable option for environmentally safe nematode control (D'addabbo et al., 2010).

Saponins provide plant protection from insect predation and the level of these compounds increases in leaves of damaged plants; saponins from *M. sativa, M. arabica*, *M. hybrida* and *M. murex* have been tested as insecticides against several classes of insects and pests (reviewed in Tava and Avato, 2006).

Citotoxicity of triterpenoid saponins is known: although this aspect was not extensively investigated in *Medicago* spp., saponins from *M. sativa* leaves showed growth inhibition *in vitro* against human leukemic cell line K562 (Tava and Odoardi, 1996). Recently Balestrazzi et al. (2011) reported that alfalfa root saponins induced cell death and nitric oxide (regulator of programmed cell death) production in white poplar (*Populus alba*) cell suspensions.

Non-human primates (*Macaca fascicularis*) fed for up to 78 weeks on alfalfa saponins show no adverse reactions: intestinal adsorption of cholesterol is reduced and excretion of neutral steroids and bile acids are increased. As a result, a possible application of *Medicago* saponins in human pathologies can be hypothesized, although potential toxicity of saponins for human consumption requires detailed investigation (Malinow et al., 1981).

Due to the usefulness of saponins in agro-industry, human health and as food and cosmetic additives, transgenic *Medicago* plants have potential applications as bioreactors for efficient production of these compounds,.

3. Biosynthesis of triterpenoid saponins in *Medicago* genus

Due to the biological activities of saponins and their possible uses in several fields, there is an obvious interest to understand and control the saponin pathway.

The triterpenoid saponin pathway can be divided in three main series of reactions: I) the synthesis of the β-amyrin skeleton (C_{30}), II) the formation of sapogenins possibly mediated by cytochrome P450 monooxygenases and III) the addition of the sugar moyety by glycosyltransferases (GTs) to yield saponins (Fig. 3).

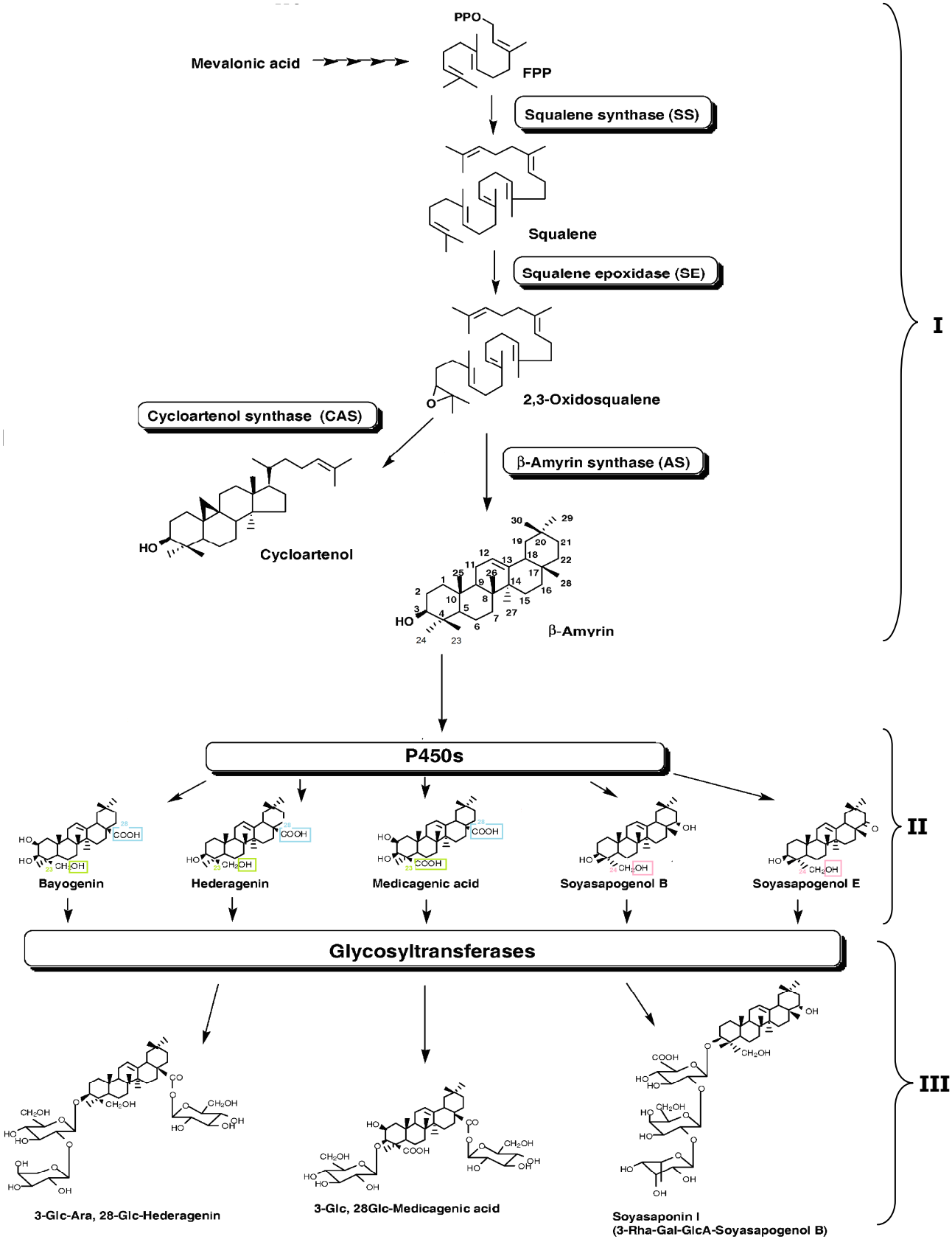

Figure 3. The three parts of the biosynthesis of triterpenoid saponins in *M. truncatula*: I) β-amyrin skeleton synthesis, II) formation of sapogenins and III) glycosyl transfer reactions to obtain saponins (based on figure 1 of Suzuki et al., 2002).

3.1. β-amyrin skeleton formation

The common precursor of all triterpenic aglycones is β-amyrin that is originated from the isoprenoid pathway via the cyclization of 2,3-oxidosqualene (Suzuki et al., 2002). In plants, 2,3-oxidosqualene is mainly cyclized into cycloartenol to yield phytosterols, via the "chair-boat-chair" conformation, while β-amyrin branch off the phytosterol pathway by alternative cyclization via the "chair-chair-chair" conformation (Fig 3) (Tava et al., 2010).

In *Medicago,* the cyclization cascade that forms the β-amyrin pentacyclic skeleton is catalyzed by a specific oxidosqualene cyclase named β-amyrin synthase (β-AS), whereas the two enzymes preceding this step are squalene synthase (SS) and squalene epoxidase (SE) (Fig. 3 I). In *M. truncatula,* these three genes have been functionally characterized by Suzuki et al (2002).

Expression of a cDNA encoding a β-AS from *Aster sedifolius* resulted in a significant increase of total saponins in *M. truncatula* (Confalonieri et al., 2009). This study confirmed that the increased production of β-amyrin promote the biosynthesis of the other sapogenins, suggesting that β-amyrin synthesis is a limiting step in the saponin pathway.

M. truncatula possesses two squalene epoxidase SE1 and SE2 (sharing 82% amino acid identity) encoded by two paralogous genes. These enzymes may have different functions in relation to sterol (SE1) and triterpene (SE2) biosynthesis. This idea is supported by the co-induction of SE2, but not SE1, with β-AS in cell cultures treated with methyl jasmonate (MeJA), an important stress signalling molecules known to be a good elicitor for secondary metabolites (Suzuki et al., 2002). MeJA is a wound signal produced in response to insect attack and many studies reported that saponin biosynthesis seem to be stimulated by MeJA. For instance, high expression of *M. truncatula* β-AS appears to be associated with insect herbivory. In addition, in *Medicago* cell suspension cultures, exposure to MeJA induced the appearance of triterpenoid glycosides (Suzuki et al., 2002).

3.2. β-amyrin oxidations

The β-amyrin skeleton is transformed in triterpenoid aglycones by means of oxidative reactions mediated by membrane-bound cytochromes P450 monooxigenases (Hayashi et al., 1993).

Cytochromes P450, named for their absorption at 450nm, are one of the largest superfamilies of enzymatic proteins and play important roles in the metabolism of a series of physiologically important compounds (e.g. hormones, fatty acids, lignins and secondary metabolites). P450s (also called CYP) are heme-thiolate proteins that use electrons, provided by NAD(P)H-P450 reductase, to catalyze activation of molecular oxygen, leading to specific oxidative attack of substrates (Wreck-Reichhart and Feyereisen, 2000).

Oxidative modifications of β-amyrin give origin to the different sapogenins, characterized by the presence of hydroxyl or carboxyl groups in specific positions of the triterpenic pentacyclic structure (Tava et al., 2010). Based on the position of these functional groups, sapogenins can be classified into two groups: (1) sapogenins with an OH group on C-24 (soyasapogenols A, B and E) without any oxidation at C-28 (Fig. 4A), and (2) sapogenins with a COOH group on C-28 associated to a different oxidation degree (zero, OH, CHO, COOH) of C-23 (Fig. 4B). This suggests that the oxidation at C-24 and the carboxylation at C-28 are mutually exclusive (Tava et al., 2010).

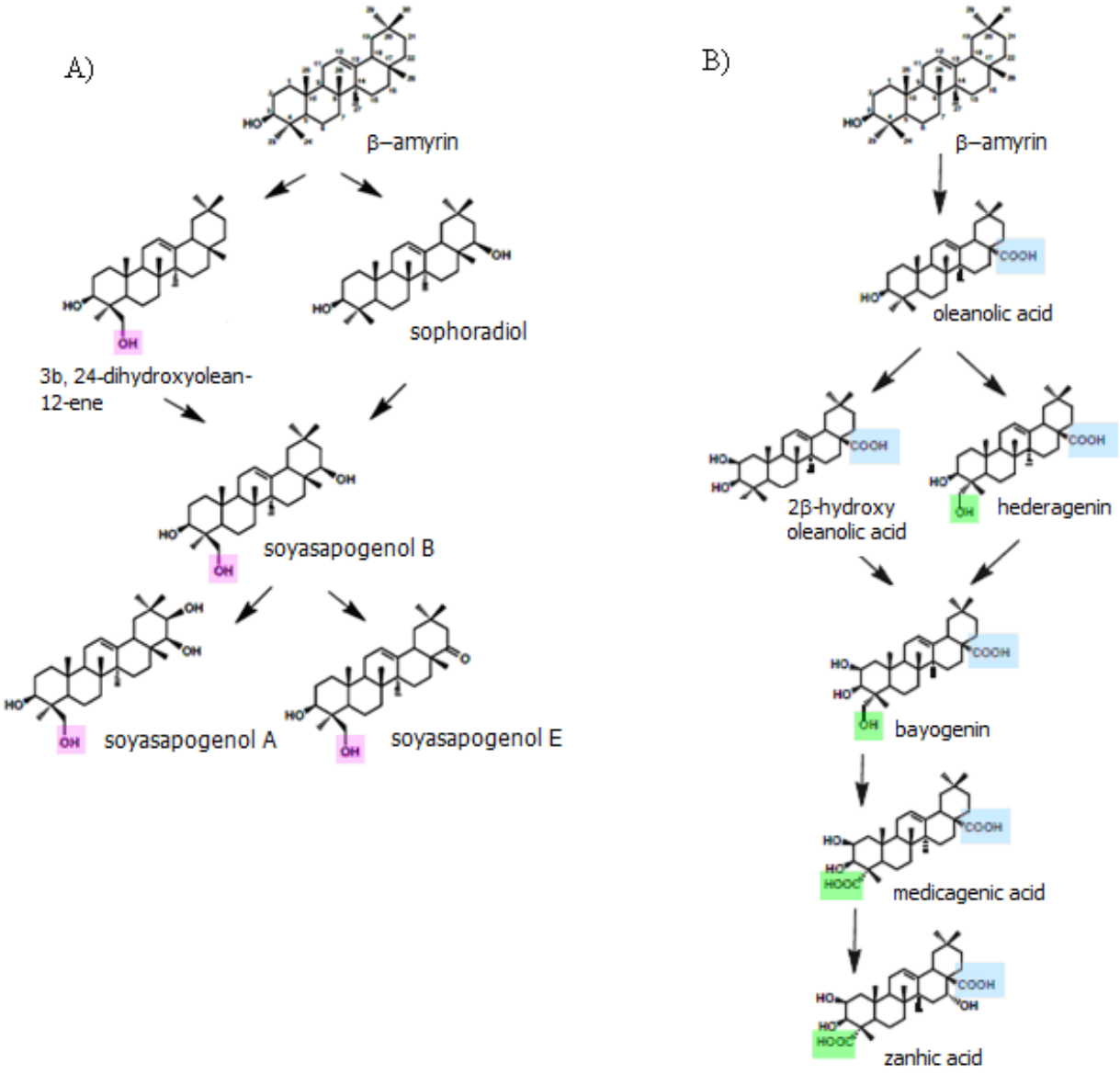

Figure 4. Proposed biosynthetic pathways: A) for soyasapogenols (not-hemolytic sapogenins) and B) for hemolytic sapogenins in *Medicago* spp. (based on figures 5 and 6 of Tava et al., 2010). The colors indicated the position of oxidative reactions: pink at C-24, blue at C-28 and green at C-23.

These differences seem to be correlated with the hemolytic activity of the corresponding saponins: sapogenins (soyasapogenol) of group 1 do not promote hemolysis, while the others have hemolytic properties (Tava et al., 2010). Based on this subdivision two possible biosynthetic pathways can be hypothesized: the not-hemolytic sapogenin pathway characterized by hydroxylation at C-24 (Fig. 4A) and the hemolytic sapogenin pathway characterized by a carboxylation at C-28 and an hydroxylation at C-23 (Fig. 4B). The order of oxidation between C-28 and C-23 is not evident.

Plant P450s are generally classified into two major sequence classes, A-type and non-A-type, containing 10 clans and 62 families. Most of the known P450 families in Dicotiledons exist in the model legume plant *M. truncatula*: 151 putative P450s, compared to P450s in *Arabidopsis* and rice, have been identified by Li et al. (2007) but the functions of most remain elusive. Although Shibuya et al. (2006) identified in soybean (*Glycine max*) a cytochrome P450 CYP93E1 encoding a β-amyrin and sophoradiol 24-hydroxylase, no gene involved in the oxidative reactions of the β-amyrin skeleton has yet been characterized in *M. truncatula*.

3.3. Glycosyl transfer reactions

Several glycosyl transfer reactions, mediated by glycosyltransferases (GTs), are responsible for the addition of the sugar moiety on sapogenins in order to form saponins. Plant GTs are members of a multigene superfamily that can typically transfer single or multiple activated sugars from nucleotide sugar donors to a wide range of metabolites (Townsend et al., 2006).

Saponin sugar chains are generally linked at the 3-*O* position of the triterpene aglycone, giving the corresponding monodesmosides, and additionally at the 28-*O* position, giving the corresponding bidesmosides, only in hemolytic saponins (Fig. 3 III). In the *Medicago* genus, the non-hemolytic saponins (soyasapogenol saponins) mainly occur as monodesmosides, while hemolytic saponins can occur as mono- and bidesmosides with variable sugar chains (Tava et al., 2010). Thus, hemolytic saponins seem to display a wider potential range of biological plasticity in consideration of the variation in the nature and position of the sugar moiety (Tava et al., 2010).

The nature of the saccharide units, their position in the molecule and the similarity of the sugar chains in saponins from the different *Medicago* species, suggest that GTs have enzymatic specificity for the sugar and regiospecificity for the sugar position (Tava et al., 2010). High specificity was demonstrated for two uridine diphosphate glucosyltransferase (UGTs) involved in the biosynthesis of saponins in *M. truncatula:* UGT73K1, specific for hederagenin and soyasapogenols B and E, and UGT71G1, specific for medicagenic acid (Achnine et al., 2005).

A recent study established that *M. truncatula* UGT73F3 shows specificity for multiple sapogenins *in vivo* mediating the glycosylation both at C-28 position of hederagenin, bayogenin and medicagenic acid and at C-3 position of soyasapogenols A and B. This is consistent with the idea that the enzyme is not strictly regiospecific for triterpene glycosylation (Naoumkina et al., 2010).

3.4. Use of M. truncatula mutant collections for identification of sapogenin defective mutants

At CRA-FLC Lodi in cooperation with IGV-CNR Perugia, *Medicago truncatula* mutant collections have been established for functional genomic purposes (Porceddu et al., 2008): a population including 128 Activation Tagging lines obtained via *Agrobacterium* transformation of the R108-1 genotype and ethyl methane sulfonate (EMS)-mutagenized population suitable for TILLING approaches (about 2500 M1 plants).

In our laboratory, 61 lines from the activation tagging collection were grown and screened using a micro-hemolytic test in search for mutants in the hemolytic saponin pathway: a mutant plant lacking hemolytic activity (*lha*-1) was identified. GC-FID analysis showed that *lha*-1 plants did not accumulate hemolytic saponins (hederagenin, bayogenin, medicagenic and zanhic acid saponins) but only non-hemolytic soyasaponins. The T-DNA tagged locus was identified by inverse-PCR and the putative gene implied in this mutation had 97% identity with the CYP716A12 sequence, a member of a cytochrome P450 family belonging to non-A-type class (clan 85), found in *M. truncatula* by Li et al. (2007). These results suggested that the gene was possibly involved in the hemolytic sapogenin synthesis but the function of the gene remained unknown.

The TILLING mutant collection was screened by a reverse genetic approach resulting in identification of two mutants in CYP716A12: *lha-2,* carrying an amino acid change and *lha-3,* carrying a stop codon, both lacking hemolytic activity.

3.5. Aim of the PhD project

The objectives of my PhD project were:

- ✓ performing a detailed biochemical characterization of mutant (Activation Tagging and TILLING) and transformed complemented lines (obtained at CNR-IGV in Perugia, our partner Institute in the project) to demonstrate that loss of CYP716A12 function was responsible for the absence of the hemolytic saponins and that CYP716A12 complete sequence is able to restore the hemolytic saponin pathway;

- ✓ understanding the role of the CYP716A12 gene in the hemolytic saponin pathway by means of an *in vitro* enzymatic activity assay, using plant microsomes and heterologous expression of the CYP716A12 in yeast;
- ✓ analyzing CYP716A12 expression patterns and a sapogenin content in different plant tissues and growth stages.

3.6. Project scheme

Fig. 5 Schematic representation of workflow and results reported in Carelli et al. (2011).

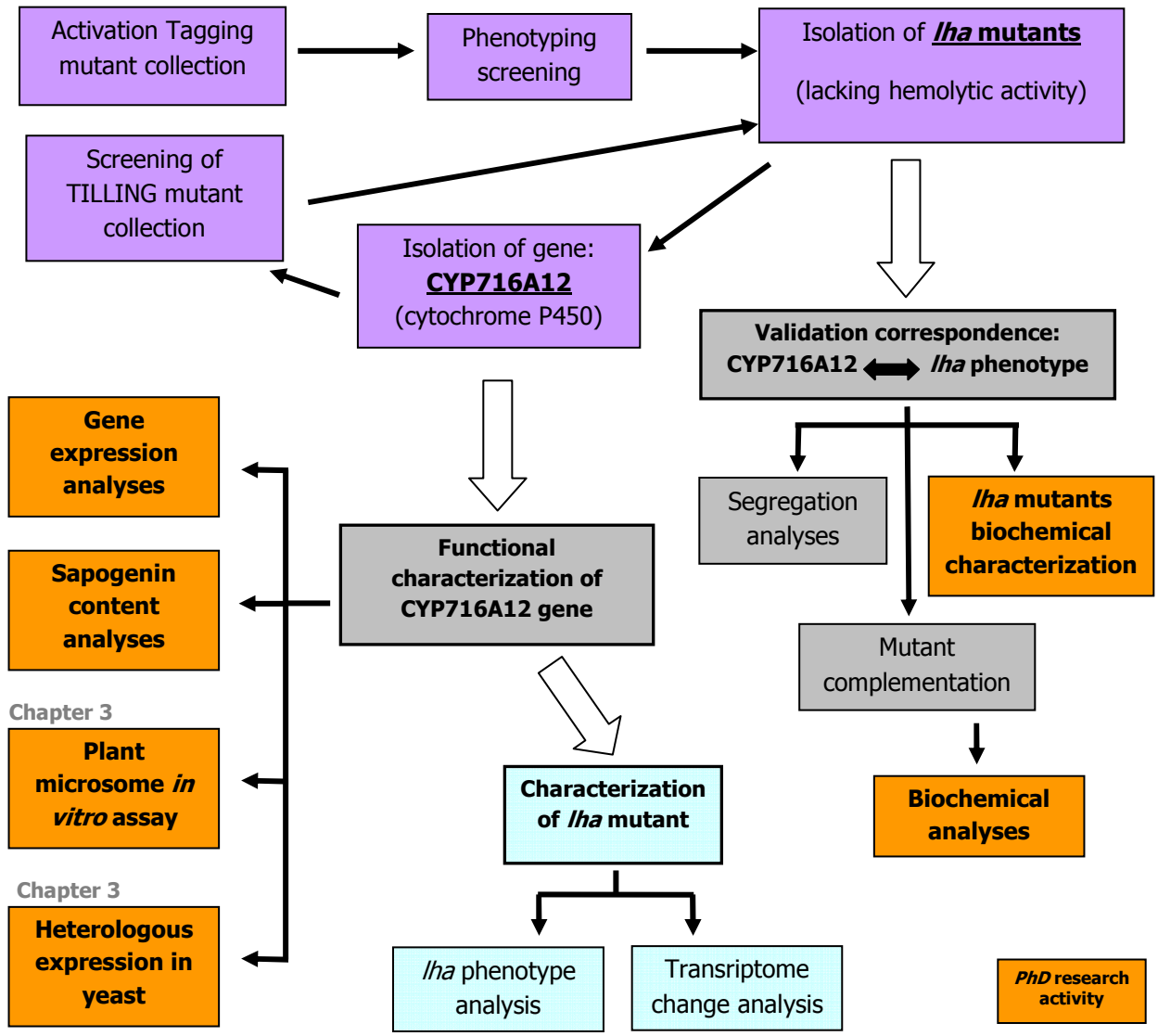

Figure 5. Scheme of the whole project reported in Carelli et al. (2011). My research activity is reported in the orange boxes.

The scheme can be divided in 3 parts: the violet sector, that summarize the identification of *lha* mutants and CYP716A12 gene reported in paragraph 3.4, the grey sectors, that report the objectives described in paragraph 3.5 and finally the pale blue sector reporting the characterization of the *lha*-1 mutant by means of the transcriptome change study and the phenotypic analysis. The plant microsome assay and the heterologous expression in yeast system are extensively explained in Chapter 3.

In the orange boxes is reported my contribution in this project.

Chapter 2.
Carelli et al., 2011

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Medicago truncatula CYP716A12 Is a Multifunctional Oxidase Involved in the Biosynthesis of Hemolytic Saponins [W]

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Saponins, a group of glycosidic compounds present in several plant species, have aglycone moieties that are formed using triterpenoid or steroidal skeletons. In spite of their importance as antimicrobial compounds and their possible benefits for human health, knowledge of the genetic control of saponin biosynthesis is still poorly understood. In the *Medicago* genus, the hemolytic activity of saponins is related to the nature of their aglycone moieties. We have identified a cytochrome P450 gene (CYP716A12) involved in saponin synthesis in *Medicago truncatula* using a combined genetic and biochemical approach. Genetic loss-of-function analysis and complementation studies showed that CYP716A12 is responsible for an early step in the saponin biosynthetic pathway. Mutants in CYP716A12 were unable to produce hemolytic saponins and only synthetized soyasaponins, and were thus named *lacking hemolytic activity* (*lha*). In vitro enzymatic activity assays indicate that CYP716A12 catalyzes the oxidation of β-amyrin and erythrodiol at the C-28 position, yielding oleanolic acid. Transcriptome changes in the *lha* mutant showed a modulation in the main steps of triterpenic saponin biosynthetic pathway: squalene cyclization, β-amyrin oxidation, and glycosylation. The analysis of CYP716A12 expression in planta is reported together with the sapogenin content in different tissues and stages. This article provides evidence for CYP716A12 being a key gene in hemolytic saponin biosynthesis.

INTRODUCTION

Saponins are a class of secondary metabolites present in several plant species, including members of the genus *Medicago* (Jenner et al., 2005). In this genus, saponins are a complex mixture of triterpenic glycosides and have been shown to possess a broad spectrum of biological properties such as antifungal, insecticidal, phytotoxic, allelopathic, and hemolytic (Tava and Avato, 2006). Because of these properties, saponins are thought to participate in plant defense mechanisms (Papadopoulou et al., 1999). The triterpenic pathway is induced by methyl jasmonate (MJ), a signal component in the induction of many defense-responsive plant metabolites (Suzuki et al., 2005). Saponin pharmacological properties have been exploited in herbal medicines and, more recently, evaluated for their anticholesterolemic and anticancer adjuvant activities (Haridas et al., 2001; Shibata, 2001). In the *Medicago* genus, recent studies (reviewed in Tava and Avato, 2006) have focused on elucidating the relationship between the biological activities of these compounds and their chemical structure. The type of aglycone moiety and the nature and position of the sugar chains (sugar moiety) appear to correlate with the different biological properties. The hemolytic activity of saponins, resulting from their affinity for membrane sterols, is related to the nature of the aglycone moieties. No hemolytic activity was detected for soyasapogenol saponins (Yoshiki et al., 1998), while the other aglycones showed from high (hederagenin and medicagenic acid glycosides) to moderate (zanhic acid glycosides) hemolytic activity (Oleszek, 1996).

All of these triterpenic compounds are synthesized from the isoprenoid pathway via the cyclization of 2,3-oxidosqualene to β-amyrin. The β-amyrin skeleton is then transformed in the reported aglycones by means of oxidative modifications possibly mediated by cytochrome P450 monooxygenases (P450s; Tava et al., 2010). Several glycosyl transfer reactions, mediated by a number of glycosyltransferases (GTs), are responsible for the addition of the sugar moiety. In *Medicago truncatula*, at least three genes encoding early enzymes of triterpene aglycone formation—squalene synthase, squalene epoxidase, and β-amyrin synthase (β-AS)—have been functionally characterized (Suzuki et al., 2002; Iturbe-Ormaetxe et al., 2003). In addition, two UDP-GTs have been shown to be active in the transfer of the Glc unit from UDP-Glc to *Medicago* triterpene aglycone mixtures (Achnine et al., 2005). An additional UDP-GT, UGT73F3, acts in the in vivo glucosylation of multiple sapogenins in *M. truncatula* (Naoumkina et al., 2010). A series of P450s that are MJ-induced and coexpressed with β-AS were selected as potential candidates involved in saponin biosynthesis (Naoumkina et al., 2010).

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However, in *M. truncatula* no gene involved in the oxidative reactions of the β-amyrin skeleton leading to triterpene aglycones has yet been characterized.

Here, we report the identification of a P450 gene involved in the biosynthetic pathway of hemolytic saponins in *M. truncatula* by combining an activation tagging method (Weigel et al., 2000) and a reverse genetic TILLING approach (McCallum et al., 2000). A functional characterization of the gene is also reported by expression in a yeast system.

RESULTS

Identification of the Mutant Lacking Hemolytic Activity

T1 plants (770) derived from 61 lines of an activation tagging mutant collection (Porceddu et al., 2008) were grown and evaluated for different traits. In particular, a subset of four plants/line were screened using a microhemolytic test on leaf tissue extracts to identify putative mutants in hemolytic saponin content. One individual plant in line E25 (E25-10) was found to lack hemolytic activity in leaves (thus named *lacking hemolytic activity*, the [*lha-1*] mutant; Figure 1A). All of the T2 progeny of E25-10 (30 plants) showed the same Lha phenotype, suggesting a homozygous condition of the T1 mother plant. Leaves from the T2 progeny of E25-10 were collected, pooled, and used for chemical analyses; the escape line E113, which was untransformed after regeneration, was used as control. The crude saponin content in the *lha-1* mutant was lower than in control plants (0.40 and 1.24% of defatted leaf dry matter, respectively). The crude saponin mixtures were evaluated by thin-layer chromatography (TLC) (Figure 1B) and HPLC (Figure 1C) analysis, and in both cases the *lha-1* mutant showed the absence of spots (TLC) and peaks (HPLC) present in the control line, indicating a loss of specific saponins in the mutant line. Total sapogenins in plant material were evaluated based on their aglycone moieties, obtained after acid hydrolysis of the saponins. After identification by gas chromatography–mass spectrometry (GC-MS) (Figure 2A), aglycones were quantified by GC–flame ionization detection (FID) (Figures 2B and 2C), which showed that no oleanolic acid, hederagenin, bayogenin, medicagenic, or zanhic acid were detected in leaves or roots of the *lha-1* mutant, but only soyasapogenols. All the aglycones were present in the control line.

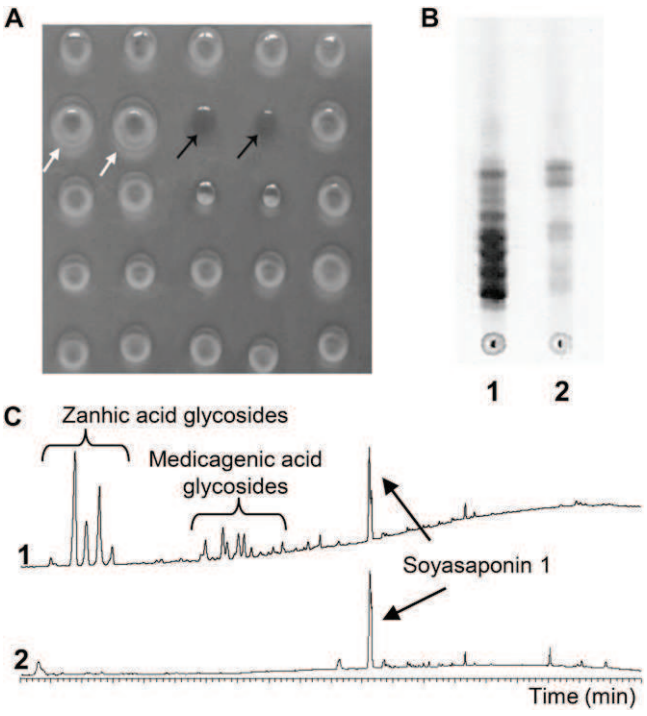

Figure 1. Comparison between *lha-1* Mutant Line and Control Line.

(A) Microhemolytic test on a blood agar plate: Black arrows show the absence of hemolysis in *lha-1* mutant; white arrows show the positive controls; the spots without indications are extracts of lines from the activation tagging collection.
(B) TLC analysis of purified saponins from control (1) and *lha-1* mutant (2).
(C) HPLC analysis of purified saponins from control (1) and *lha-1* mutant (2).

Cloning of the Mutant Gene

DNA gel blot analysis on genomic DNA of T2 plants deriving from the *lha-1* mutant showed two bands, indicating a double insertion. The two insertions were cloned by inverse PCR, generating fragments of 1200 bp (A fragment) and 1900 bp (B fragment), respectively. Sequencing of these fragments and alignment with the *M. truncatula* sequences in The Institute for Genomic Research and GenBank databases showed that fragment A had 97% identity (BLASTn analysis) with the CYP716A12 sequence, a member of a cytochrome P450 family found in *M. truncatula* Jemalong A17 and expressed in root tissue (Li et al., 2007). No significant homologies with known genes were found for fragment B. Cytochrome P450s have been reported to be involved in triterpene saponin biosynthesis (Qi et al., 2006; Shibuya et al., 2006); therefore, CYP716A12 was retained as a candidate gene potentially involved in the *lha* mutation. The complete genomic sequence obtained by genome walking (Figure 3A) showed four exons separated by three introns; the exact site of integration of the T-DNA was inside the first exon (Figure 3A) and the T-DNA integration created a deletion of 72 bp in the first exon and 55 bp in the first intron. The full-length cDNA sequence of CYP716A12 was obtained by rapid amplification of cDNA ends (RACE); during these experiments it became evident that in addition to the full-length transcript, the gene also produced a splicing variant lacking the first exon (see Supplemental Figure 1 online). RT-PCR analysis revealed that the longer transcript of CYP716A12 was expressed in control plants but was absent in *lha-1* (Figure 3B); conversely, the shorter splicing variant was expressed in mutant and wild-type plants.

Cosegregation Analysis

To demonstrate the involvement of CYP716A12 gene in the Lha phenotype, the T2 progenies (30 plants/line) derived from the original 10 T1 E25 plants were analyzed. In five segregant progenies, a 3:1 ratio (wild type:mutant) was found for the Lha phenotype, a ratio consistent with this trait being determined by

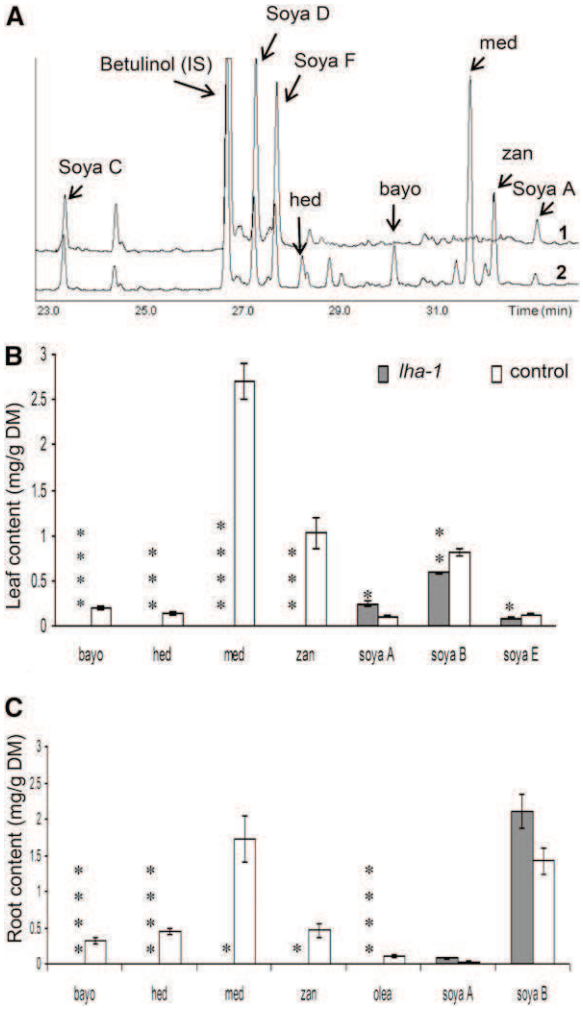

Figure 2. Analysis of Sapogenin Content in Leaves and Roots of *lha-1* Mutant Line and Control Line.

(A) GC-MS chromatograms of sapogenins in roots: *lha-1* mutant (1) and control line (E113) (2).
(B) and **(C)** Sapogenin content obtained by GC-FID analysis in leaves and roots, respectively; values for *lha-1* (gray bars) and the control line (white bars) are means ± SE of three biological replicates (three to 10 plants/replicate). Identification of soyasapogenol B was achieved considering all the artifactual compounds detected (soyasapogenols C, D, and F). *P < 0.05, **P < 0.01, ***P < 0.005, ****P < 0.001 with F test).
bayo, bayogenin; DM, dry matter; hed, hederagenin; IS, internal standard; med, medicagenic acid; olea, oleanolic acid; soya, soyasapogenol; zan, zanhic acid.

a single recessive allele (see Supplemental Table 1 online). All the progenies showed a strict cosegregation between the Lha phenotype and T-DNA insertion in CYP716A12 gene in the homozygous state (tested by PCR); one *lha-1* mutant line was found carrying only T-DNA insertion in CYP716A12 in the homozygous state. Homozygous *lha* mutant plants showed severe reduction in root and shoot size (Figure 4 and see Supplemental Table 2 online). No correlation was found between the Lha phenotype and insertion B; in particular, the E25-08 progeny showed only insertion B in the homozygous state and had no alteration in hemolytic activity.

Identification of CYP716A12 Loss-of-Function Alleles in a TILLING Collection

M2 plants (2300) of an ethyl methanesulfonate (EMS)-mutagenized collection (Porceddu et al., 2008) were screened by TILLING analysis and two mutants for CYP716A12 were identified (see Supplemental Figure 2 online): *CYP2768* (*lha-2*) and *CYP3201* (*lha-3*). In the *lha-3* M2 plant, a Trp to stop codon (Trp449 → stop) change was identified in a heterozygous state that caused the loss of the last 30 amino acids of the peptide. This substitution was 22 amino acids after the highly conserved heme Cys ligand. The *lha-3* mutant segregated in a Mendelian fashion in the M3 progeny and a retarded-in-growth phenotype was observed for the homozygous mutant individuals. The *lha-3* homozygous mutants lacked medicagenic and zanhic acid; as for zanhic acid, however, the null value in the mutant and the trace amounts in control plants were not statistically different (Figure 5B). In *lha-2* M2 plant, the mutation in the homozygous state caused a

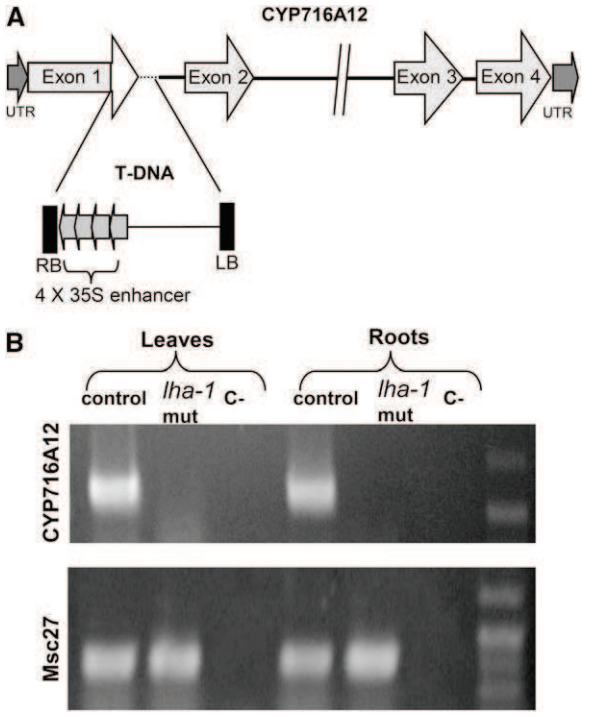

Figure 3. Identification of the T-DNA–Tagged Locus and Expression of the CYP716A12-Tagged Gene in the *lha-1* Mutant.

(A) Location of the T-DNA insertion in CYP716A12 gene (not drawn to scale). The relative location and orientation of the T-DNA are shown. The T-DNA right border (RB) with four copies of the 35S enhancer was inserted at the end of the first exon producing the loss of small parts of the exon (in white) and the intron (hatched). LB, left border; UTR, untranslated region.
(B) RT-PCR analysis of transcript level of CYP716A12 gene in leaves and roots of the control line (E113) and the *lha-1* mutant. C-, negative control; Msc27, reference gene.

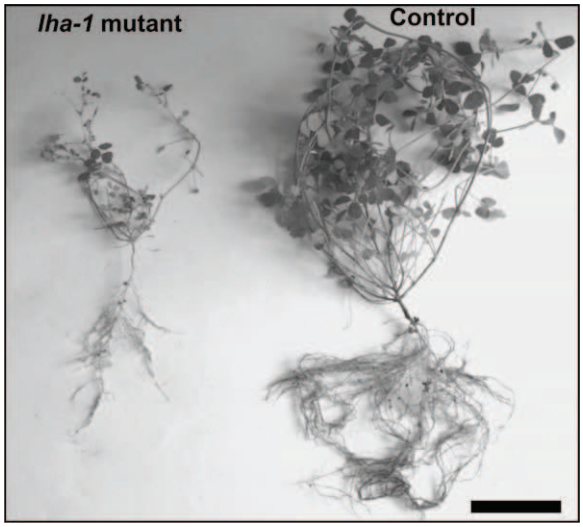

Figure 4. Plants of *lha-1* (E25-10) and Control Line (E113).

Ten-week-old *lha-1* mutant plant is reduced in growth compared with the contemporary control (E113) plant. Bar = 10 cm.

Pro to Leu (Pro355→Leu) amino acid change. The evaluation of the M3 generation for sapogenin content in leaves was performed by GC-MS analysis. Interestingly, the *lha-2* mutation resulted in a loss-of-function allele because no bayogenin, medicagenic, or zanhic acid (hemolytic sapogenins) were found, whereas soyasapogenols A and B were significantly higher than in control plants Jemalong 2HA10-9-3 (Figure 5A). The *lha-2* homozygous mutant plants did not show a retarded-in-growth phenotype.

Genetic Complementation of the *lha-1* Mutant

Several independent transgenic lines were obtained for the construct 35S:CYP716A12 in the *lha-1* mutant and were confirmed by PCR analysis. Ten randomly chosen lines expressing the transgene were examined for sapogenin content in the leaves by GC analysis. Five independent transformants showed detectable levels of bayogenin, medicagenic, and zanhic acid, confirming that disruption of the CYP716A12 gene is responsible for the lack of hemolytic saponins in the *lha* mutant (see Supplemental Figure 3 online).

Functional Expression of CYP716A12

To elucidate the function of CYP716A12 in the hemolytic saponin pathway, the gene was expressed in a yeast (*Saccharomyces cerevisiae*) system. Enzymatic characterization was performed in an in vitro system employing microsomes from the GAL-induced yeast strains WAT11 and WR (Pompon et al., 1996) transformed with the pESC-HIS expression vector containing the CYP716A12 coding sequence with a C-terminal FLAG epitope tag. The same strains transformed with an empty vector were used as control. WAT11 and WR strains, overexpressing an *Arabidopsis thaliana* and a yeast P450 reductase, respectively, were used to optimize electron transfer during catalysis. After microsomal membrane isolation, immunoblot analysis confirmed the presence of an ~57 KDa protein in the CYP716A12-harboring strains as expected (see Supplemental Figure 4 online). In the mutant *lha*, none of the sapogenin forming the hemolytic saponins was detected, suggesting that the biosynthetic pathway is blocked at an early step. Enzymatic activities in microsomes were then tested by supplying β-amyrin, which is the carbon skeleton common to all the sapogenins, and erythrodiol, a derivative of β-amyrin carrying a hydroxylic group at the C-28 position. GC-MS analysis of the reaction products (Figure 6A) showed the CYP716A12-dependent formation of the same detectable compound from β-amyrin and erythrodiol; the retention time and mass spectrum of the product (Figure 6B) showed an excellent match with those of oleanolic acid (see Supplemental Figure 5 online). No enzymatic activity on the tested substrates was detected in microsomes from the control strains. These results indicate that CYP716A12 mainly catalyzes the sequential three-step oxidation at the C-28 position necessary to transform β-amyrin into oleanolic acid.

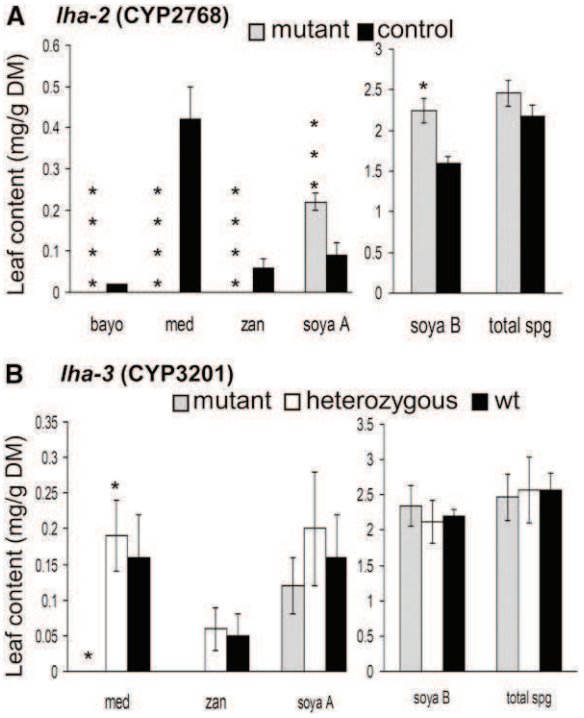

Figure 5. Sapogenin Content in Mutant and Control Plants.

Data were obtained by GC-FID analysis of *lha-2* (CYP2768) and *lha-3* (CYP3201) mutant lines (M3 generation) from a TILLING collection and the respective control plants.

(A) *lha-2*: Mutant (gray bars, nine plants) and control Jemalong 2HA10-9-3 (black bars, three plants).

(B) *lha-3:* Homozygous mutant (gray bars, three plants), heterozygous mutant (white bars, three plants), and wild type (black bars, three plants).

Values are means ± SE. (*P < 0.05, **P < 0.01, ***P < 0.005, ****P < 0.001 with F test and linear contrasts).

bayo, bayogenin; DM, dry matter; med, medicagenic acid; soya, soyasapogenol; spg, sapogenins; wt, wild type; zan, zanhic acid.

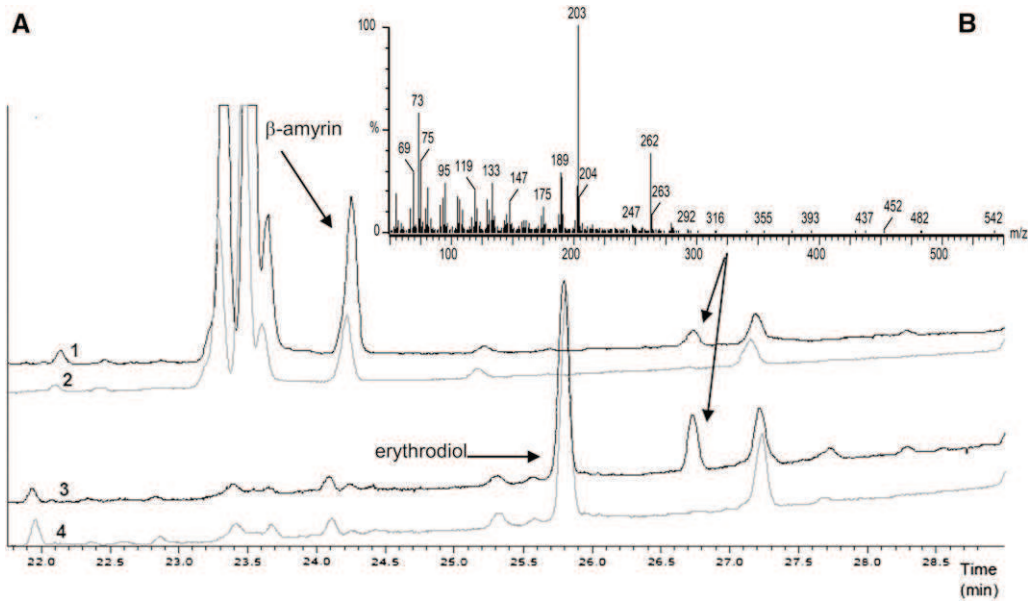

Figure 6. In Vitro Oxidation of β-Amyrin and Erythrodiol by CYP716A12 in Microsomes of the WAT11 Strain.

(A) GC-MS analysis of the reaction products resulting from in vitro assay containing β-amyrin (1 and 2) and erythrodiol (3 and 4) as substrate on strains expressing CYP716A12 (black) and control (gray).

(B) Mass spectrum of the peaks at 26.75 min indicated by the arrows in **(A)**: The retention time and mass spectra of these peaks compare well with those of oleanolic acid.

Expression of CYP716A12

To investigate the expression profiles of the CYP716A12 gene, total RNA was extracted from different organs (leaves, stems, and roots) of the E113 control line at three different developmental stages (preflowering, early flowering, and early pod setting). RNA from flowers, pods, and nodules was also obtained in the last stage. Expression of the gene, analyzed by quantitative real-time PCR, occurred in all the organs and the developmental stages (Figure 7). Higher and more stable expression through stages was found in roots: the gene expression coefficient of variation among stages was 30.44% in roots, 91.35% in leaves, and 87.02% in stems. In leaves, a significant increase in expression was observed in the reproductive stages, with a maximum expression at flowering. Flowers and pods showed the highest and lowest expression of the gene, respectively.

The same plants used for gene expression were examined for sapogenin content by GC-FID analysis. The hemolytic sapogenin content was significantly influenced by developmental stages (increase from vegetative to reproductive stages) and plant organs (leaves > roots > stems), while variation of nonhemolytic sapogenin content was attributable mainly to plant organs (roots > leaves, stems). In particular, hederagenin and bayogenin (hemolytic) and soyasapogenol B (nonhemolytic) were prevalent in roots at all the stages; zanhic acid was prevalent in leaves at all the stages (Figure 8 and see Supplemental Table 3 online). Medicagenic acid, the major component of *M. truncatula* total sapogenin (33, 40, and 43%, respectively, averaged on organs, in the three developmental stages), showed a change in the ranking of organs from the vegetative stage (leaf, stem > root) to the flowering stage (root > leaf, stem). Flowers, pods, and nodules had to be pooled over plants and blocks to give sufficient amount for sapogenin analysis; their variation was then tested using the error term relative to leaves, stems, and roots. Flowers showed the highest content of medicagenic acid, while

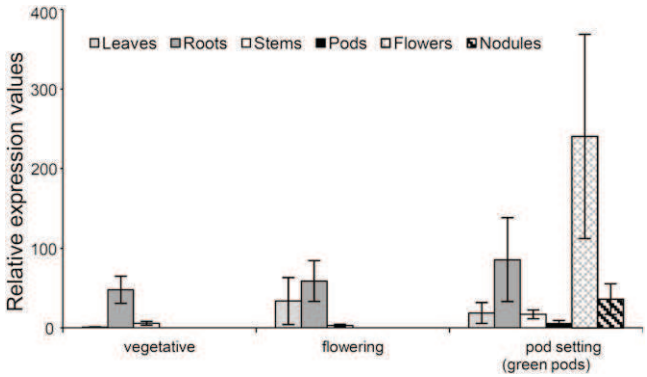

Figure 7. Expression Analysis of CYP716A12 Gene in Control Line (E113) by Quantitative RT-PCR: Different Organs at Different Phenological Stages Were Considered.

Data are shown for leaves (light gray bars), roots (dark gray bars), stems (white bars), pods (black bars), flowers (light cross-hatched bars), and nodules (dark cross-hatched bars).

Values are means ± SE of three biological replicates (three plants/replicate) and are expressed relative to leaves in the vegetative stage.

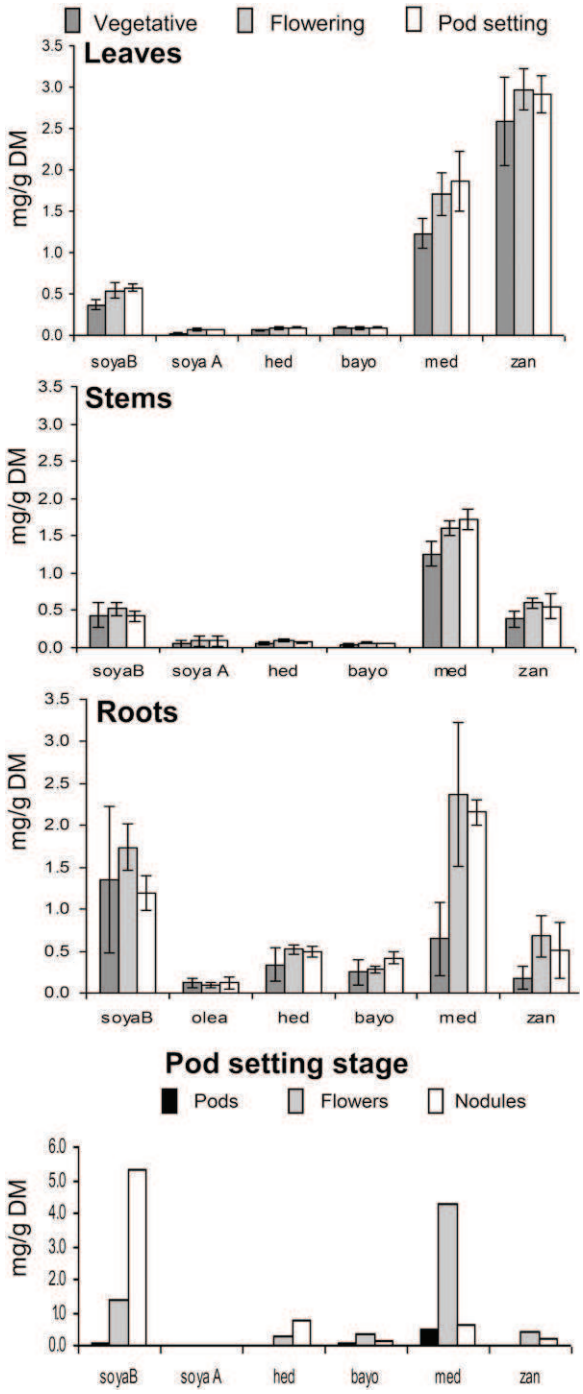

Figure 8. Sapogenin Content Obtained by GC-FID Analysis in Control Line (E113): Different Organs at Different Phenological Stages Were Reported.

Values are means ± SE of three biological replicates (three plants/replicate). bayo, bayogenin; DM, dry matter; hed, hederagenin; med, medicagenic acid; olea, oleanolic acid; soya A, soyasapogenol A; soyaB, soyasapogenol B; zan, zanhic acid.

pods had less hederagenin, bayogenin, medicagenic acid and soyasapogenol B than flowers and roots. Nodules had the highest content of soyasapogenol B and hederagenin (Figure 8).

Transcriptome Changes in the *lha* Mutant

Genome-wide transcript profiling of the *lha-1* mutant identified ~700 genes that were differentially expressed compared with wild-type R-108 ($P < 0.05$, >1.5-fold change). A total of 428 were upregulated and 290 were downregulated in the mutant (see Supplemental Data Set 1 and Supplemental Figure 6 online). The *lha-1* mutant underwent modulation of expression in the saponin biosynthetic pathway at three different steps: First, the cyclization of 2,3-oxidosqualene was altered as evidenced by the downregulation of Medtr8g018540.1, Medtr8g018550, and Medtr8g018610 (see Supplemental Table 4 online). These three genes show high homology (92% in the case of Medtr8g018610) with the β-AS gene (GenBank accession number CAD23247; Suzuki et al., 2002) and with α/β-AS situated on chromosome 8 of *M. truncatula* (Naoumkina et al., 2010). Their consistent modulation and physical proximity suggest that they could act as a cluster of paralogous genes. Second, the CYP450-mediated modifications of β-amyrin were affected as three P450s, putatively involved in saponin biosynthesis on the basis of induction by MJ and coexpression with β-AS (Naoumkina et al., 2010), showed 1.6- to 2.6-fold changes in the *lha-1* mutant. In particular, CYP93E2, a homolog of CYP93E1 responsible for β-amyrin 24-hydroxylase activity in *Glycine max* (Shibuya et al., 2006) and in the legume *Glycyrrhiza uralensis* (Seki et al., 2008), was downregulated in the *lha-1* mutant, while CYP72A67 and CYP72A68 were upregulated (see Supplemental Table 4 online).

Seven other differentially modulated P450s with unknown function were phylogenetically related to those putatively involved in saponin biosynthesis, as they were distributed in the same types and clans (Li et al., 2007). Four of these genes (Medtr5g102050, Medtr8g050160, TC107626, and TC93934) belonged to the A-type clan 71 as CYP93E2 and three (BQ139200, Medtr8g045080, and AW127462) to non-A-type clan 72 (see Supplemental Table 4 online). The CYP716A12 gene, represented by two distinct probe sets (Mtr.43018.1.S1 and Mtr.31199.1.S1), showed no significant differential expression in the mutant. This finding is attributable to the presence of the short splicing variant still transcribed in the *lha* mutant and containing the regions matching the two probe sets. A further P450 (Medtr8g090600) belonging to the same non-A-type clan 85 as CYP716A12 was upregulated in the mutant (see Supplemental Table 4 online). Finally, the glycosylation step was also modulated, as evidenced by the downregulation of UGT73F3, shown to function in saponin biosynthesis in planta (Naoumkina et al., 2010), and GTs UGT91H5 and UGT91H6 also reported to be strongly coexpressed with β-AS (Naoumkina et al., 2010). Three other GTs (Medtr7g076790, Medtr5g076110, and Medtr2g008380) differentially modulated in the mutant showed high similarity with those putatively involved in saponin biosynthesis. Twelve further GTs with unknown function showed differential expression in the *lha-1* mutant (see Supplemental Table 4 online).

DISCUSSION

Involvement of CYP716A12 in Hemolytic Saponin Pathway

Several lines of evidence indicate that the disruption of the CYP716A12 gene is involved in the determination of the *lha* mutant phenotype. The segregation ratio of the T2 heterozygous progenies E-25 from the R108 genotype showed that a single recessive gene was responsible for the mutant phenotype; in the same T2 generation, cosegregation was found between the Lha phenotype and the disruption of the CYP716A12 gene. Additionally, two independent lines from another genotype (Jemalong 2HA10-9-3) carrying different point mutations in the same gene displayed the Lha phenotype. Finally, the complementation of the *lha-1* mutant plants with a vector carrying a full-length coding sequence (CDS) of CYP716A12 restored the biosynthetic pathway of hemolytic saponins. We concluded that the knock-out of CYP716A12 was responsible for the block in this pathway. Interestingly, CYP716A12 was one of the cytochrome P450 genes upregulated by MJ, a wound signal that triggers saponin accumulation in plants, and was coexpressed with β-AS (Naoumkina et al., 2010).

Role of CYP716A12 in Hemolytic Saponin Pathway

In the *lha* mutants, none of the sapogenins—hederagenin, bayogenin, medicagenic, or zanhic acid—forming the hemolytic saponins was detected by GC-MS analysis, suggesting that the biosynthetic pathway is blocked at an early and common step. The aglycones of hemolytic saponins differ from those of nonhemolytic saponins (soyasapogenols) by the hydroxylation at C-23 instead of at the C-24 position, by the presence of the carboxylic group at C-28, and by the absence of oxidation at C-22 (Figure 9B). Hydroxylation at C-24 seems to be the key step for soyasapogenol formation, while carboxylation at C-28 and hydroxylation at C-23 for aglycones of hemolytic saponins (Tava et al., 2010); the order of oxidation between C-23 and C-28 is not evident. In the wild type, E113 oleanolic acid, carrying only the carboxyl group at the C-28 position, was present in trace amounts in roots (Figure 2C), whereas it was never detected in the *lha* mutants. This suggests that the C-28 carboxylation was the most likely step blocked in *lha*. The in vitro enzymatic activity assay confirmed that CYP716A12 is a multifunctional P450 as it catalyzes the sequential three-step oxidation at C-28 of β-amyrin to yield oleanolic acid (Figure 9A) and it can use different substrates (β-amyrin and erythrodiol). As no intermediates (erythrodiol and the corresponding aldehyde) nor oleanolic acid were detected in the *lha* mutants, it is likely that CYP716A12 catalyzes the same three steps in plants. Other P450s involved in the terpenoid pathway were reported to catalyze sequential two- or three-step oxidations in legumes (Seki et al., 2008) and in other species (Helliwell et al., 1999, 2001; Ro et al., 2005).

The *lha-1* mutant displayed differential expression of several P450 monooxygenases and GTs; as two key steps in the synthesis of saponins are represented by oxidation and glycosylation, the results of the microarray experiment appear consistent with the nature of the mutation. Strengthening of the results of the present microarray experiments comes from the data shown by Naoumkina et al. (2010). There is a consistent overlap between the P450 and GT genes found by the analysis of MJ induction and coexpression with β-AS and the genes revealed by transcriptomic analysis of the *lha-1* mutant.

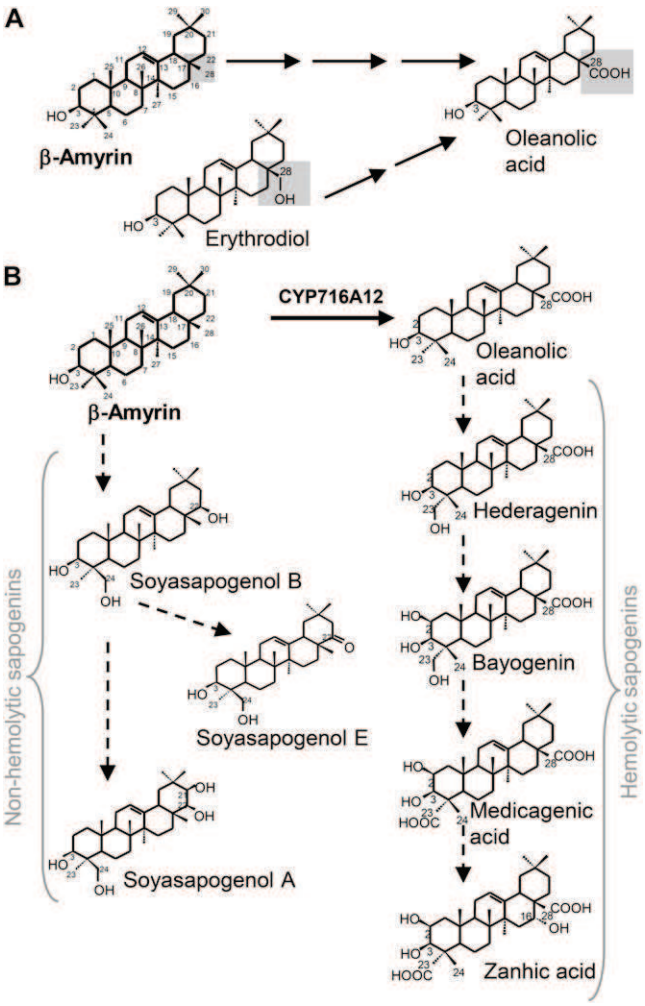

Figure 9. Role of CYP716A12 in Sapogenin Biosynthesis.

(A) Oxidation steps catalyzed by CYP716A12.
(B) Hypothetical sapogenin biosynthetic pathway in *M. truncatula*.

The analysis of the two sets of data could contribute to the identification of other candidate genes involved in the saponin biosynthetic pathway.

Effect of CYP716A12 Loss-of-Function in the *lha* Retarded-in-Growth Mutant Phenotype

The blocking of carboxylation at the C-28 position in the *lha* mutant implies the absence of oxygenated groups in C-28 and consequently the lack of triterpenes glycosylated in this position. In fact, only soyasaponins that are glycosylated at the C-3 position have been found in the *lha* mutants (Figure 1C). The *lha-1* and *lha-3* mutant lines showed a retarded growth phenotype compared with their genetically closest counterparts: E25-08,

wild type for CYP716A12 and homozygous mutant for insertion B, in the case of *lha-1* (see Supplemental Table 2 online) and the wild-type individuals of the same line (full sib) for *lha-3*. The *lha-2* mutant line did not display a retarded growth phenotype, but the homozygous state of the mutation hampered a comparison in the same genetic background, i.e., in the presence of the other unknown point mutations induced by the mutagen EMS treatment. A phenotype with a strong decrease in growth has been described in the *M. truncatula Tnt1* mutant for the UGT73F3 gene responsible for the glycosylation of hederagenin at the C-28 position in root (Naoumkina et al., 2010). All of these findings suggest that the synthesis of hemolytic sapogenins and their consequent 28-glycosylation can play a role in plant growth processes.

Interestingly, among the genes differentially expressed in the mutant, the importance of the biodegradation of xenobiotics (8.4%) and the biosynthesis of secondary metabolites (8.8%) classes are relevant (see Supplemental Figure 6 online). Changes in secondary metabolites and hormone levels in the mutant are suggested by the differential expression of P450s reported to be involved in the respective biosynthetic pathways in *M. truncatula* and other plant species: CYP74B (Medtr2g104500), competing with allene oxide synthase for 13-hydroperoxylinoleic acid, an intermediate of oxylipin and jasmonic acid biosynthesis (Howe et al., 2000); CYP81E9 (TC95424), involved in downstream steps of isoflavonoid pathway (Liu et al., 2003); CYP90B (TC102428, TC102429), acting in early steps of brassinosteroid pathway (Sekimata et al., 2008); and TC110889, showing 68% homology with CYP707A3 responsible for the major abscisic acid catabolic pathway in plants (Okamoto et al., 2009). The *Tnt1* mutant of *M. truncatula* for UGT73F3 also showed a higher isoflavone content than controls (Naoumkina et al., 2010).

As for xenobiotics, the endogenous (metabolome imbalance) or exogenous (biotic/abiotic stresses) origin of these compounds is questionable. A possible alteration of the glycosylation pattern with the formation of an abnormal pool of glycosides in the *lha-1* mutant could be envisaged by the downregulation of GTs specific for hemolytic sapogenins, as UGT73F3, and the enhanced expression of other GTs of unknown function (see Supplemental Table 4 online). With respect to the exogenous origin of xenobiotics, it can be observed that three genes encoding proteins involved in plant resistance responses were differently expressed in *lha-1*: a polygalacturonase inhibiting protein (probe set Mtr.47298.1.S1_at), involved in defense processes against fungi (Ferrari et al., 2003), was downregulated; and a hevein-like protein homolog to the pathogenesis-related antifungal chitin binding PRP4 of pea (*Pisum sativum*; probe set Mtr.42876.1.S1_at) and a protein (probe set Mtr.41478.1.S1_at) showing 65% homology with *Mt*PR10-1 abscisic acid-responsive ABR18 were upregulated. *Mt*PR10-1 was found to be strongly induced in *M. truncatula* leaves in response to bacterial infections and was moderately induced after wounding (Gamas et al., 1998). In conclusion, such a pattern of transcriptome changes is not sufficient to highlight the origin of the *lha* retarded-in-growth mutant phenotype. However, a direct toxicity of intermediates of the saponin pathway is unlikely as in the mutant no accumulation of intermediates was found by chemical analyses but only the loss of an entire class of metabolites, i.e., the hemolytic saponins.

CYP716A12 is expressed in all the plant organs analyzed both aerial (flowers, leaves, stems, and pods) and subterranean (roots and nodules), though roots had the higher and the more stable expression level. In addition, its expression appears finely tuned according to plant organ (e.g., a sharp difference of CYP716A12 expression and hemolytic sapogenin content between flowers and pods) and developmental stage, the reproductive phase involving an increase in expression level and hemolytic sapogenin content. This pattern of expression of CYP716A12 is different from those of the GT UGT73F3 of *M. truncatula* and *Sad3* and *Sad4* from the *Avena* genus (Mylona et al., 2008) mainly expressed in root system. These findings, together with the severe phenotypic effect of the mutation in the *lha-1* and *lha-3* lines and the transcriptome changes exceeding triterpenic biosynthetic pathway, raise the question of a possible dual function, in defense responses and in plant developmental processes, for the entire class of hemolytic saponins or for particular members among them.

METHODS

Plant Material and Growth Conditions

Plants were grown in a greenhouse that was heated during winter (10°C) and was not heated in other months except for the experiments concerning microarray analysis, quantitative PCR, and saponin content determination in different tissues. In those cases, plants were kept in a growth chamber under 16 h of light at 22°C and 8 h of dark at 18°C.

Mutant Collections Used in the Present Study

Activation Tagging Collection

Sixty-one T1 lines (10–25 plants/line) generated in the R108-1 genotype as described in Porceddu et al. (2008) were screened. An escape line (E113) was used as a control. The generations T2, T3, and T4 of mutant and control lines were produced and used in the subsequent experiments.

TILLING Collection

The DNA of the M2 generation (2300 plants) of an EMS-mutagenized collection obtained in Jemalong genotype 2HA10-9-3 (Porceddu et al., 2008) was screened by the TILLING technique (McCallum et al., 2000) to identify mutants in the gene of interest. The M3 generation of the mutant plants identified was used for phenotypic characterization.

Analysis of the Saponin and Sapogenin Content

For chemical analyses, all samples were previously ground in liquid nitrogen and dried at 50°C.

Microhemolytic Method

Blood plates were prepared according to the method reported by Jurzysta (1979). An agar suspension was obtained by mixing 75 mL of sterile 0.9% NaCl solution, in which 4.5 g of agar had been previously dissolved, with 20 mL of blood suspension obtained by mixing 90 mL of fresh bovine blood with 9.9 mL of sterile 3.65% sodium citrate as an anticoagulant. The agar-blood suspension was then plated into glass vessels at 0.2 mm film thickness, and allowed to solidify. Dried and milled

leaf samples (100 mg) were treated with 1 mL of isotonic solution (0.9% NaCl) and extracted at 80°C for 2 h. After cooling and centrifugation, 10 μL of the supernatant was pipetted onto the agar blood plate. After 24 h of dark incubation in a glass box with controlled humidity (80%) and temperature (22°C), hemolysis was visually evaluated. A 1% solution of previously purified saponins from *Medicago sativa* was used as a positive control.

Extraction and Purification of Saponins

Before saponin extraction, the samples were defatted with $CHCl_3$ in a Soxhlet apparatus. One hundred milligrams of defatted material was treated with 5 mL of 30% MeOH in a stoppered tube, heated for 30 min at 50°C, and sonicated for 10 min. The sample was then centrifuged at 3000*g*, the supernatant was removed, and the precipitate was extracted. This procedure was repeated twice under the same conditions. The combined solutions were then run through a LiChroprep RP-18 column (400 mg, Merck, Darmstadt), preconditioned with 30% MeOH. Elution was performed with 35% MeOH (5 mL) to remove sugars and some phenolics; crude saponins were then eluted with 90% MeOH (3 mL) and dried under vacuum (Tava et al., 2005, 2011; Pecetti et al., 2010).

Saponin Analysis

The saponin mixtures were checked using TLC silica gel plates (Tava et al., 2005) and eluted with ethylacetate/water/acetic acid (7:2:2, v/v). Spots were visualized by Liebermann-Burckard reactive (MeOH/acetic anhydride/sulfuric acid, 10:1:1, v/v) followed by heating at 120°C. HPLC analysis was performed using a Perkin Elmer chromatograph equipped with a LC250 binary pump and a DAD 235 detector. Separation was obtained on a Discovery HS C18 column (5 μm, 4.6 × 250 mm, Supelco) with a mobile phase of solvent A: CH_3CN 0.05% CF_3COOH; solvent B: H_2O 1% MeOH 0.05% CF_3COOH. Chromatographic runs were performed under gradient elution from 20% (5-min isocratic condition) to 30% of solvent A for 70 min, increased to 40% of solvent A for 20 min, and then increased to 100% of solvent A for 70 min; 20 μL of methanolic solutions (1 mg/mL) of all samples was injected. Saponins were eluted at 1.0 mL/min and were detected by UV monitoring at 215 nm.

Sapogenin Analysis

Sapogenins were evaluated after the acid hydrolysis of the corresponding saponins (Pecetti et al., 2006). Sapogenins were compared with previously identified sapogenins from *Medicago* spp by TLC (Tava et al., 2005; Bialy et al., 2006). Sapogenins were also identified by GC-MS and quantified by GC-FID, as described in Tava et al. (1993) and Tava and Pecetti (1998).

Identification of the T-DNA–Tagged Loci

The T2 progeny of a single T1 plant (E25-10) homozygous for the Lha phenotype was used to identify the T-DNA insertion sites. The insertion sites were cloned using inverse PCR (Ochman et al., 1988). Briefly, genomic DNA was isolated from leaves of 10-week-old plants using a genomic extraction kit following the manufacturer's protocols (Sigma); 200 ng of genomic DNA was cut with *Pfl*23II and *Bsp*1407I restriction enzymes, incubated overnight at 16°C with T4 DNA ligase, and amplified in a PCR reaction using primers designed for the right (Right 1) and the left (Left 1) borders of the T-DNA; the PCR products were then amplified in a nested PCR to increase the concentration of the desired fragments using Right 2 and Left 2 primers. The amplified DNA fragments were gel purified, cloned in Xl1Blue cells using GenJet cloning system (Fermentas), and sequenced using the Big Dye Terminator, version 3.1, sequencing kit (Applied Biosystems) and an ABI 310 analyzer. The sequences found were used to search the GenBank and The Institute for Genomic Research databases by applying BLASTn (Altschul et al., 1997) to identify putative genes. To obtain the full-length cDNA and genomic sequence for wild-type Mt CYP716A12, 5′ and 3′ RACE experiments (SMART RACE cDNA Amplification Kit, Clontech) and genome walking (GenomeWalker Universal Kit, Clontech) were performed starting from the insertion flanking sequences. All primers used in this research are listed (see Supplemental Table 5 online).

Isolation of Total RNA and RT-PCR Analysis

Total RNA was isolated using the RNeasy plant mini kit (Qiagen) according to the manufacturer's protocol. First-strand cDNA was synthetized with Superscript III reverse transcriptase (Invitrogen) using a polyT 16mer primer.

The mRNA level of CYP716A12 was determined by RT-PCR: 2.5 μL of first-strand cDNA was amplified using primers ATG2fw and TAA2rw with TaKaRa Ex-Taq according to the manufacturer's protocol. The Msc27 gene was used as control (Pay et al., 1992). PCR products were separated on 1% agarose gel.

Genetic Analysis

Cosegregation analysis of the Lha phenotype and the T-DNA insertion was performed on the T2 segregating population. Plants were genotyped by PCR on genomic DNA using one gene-specific primer (CYPfw for the insertion in CYP716A12 gene and InsBrw for insertion B) and a T-DNA right-border primer (PSKI). The wild-type allele was detected using primer pairs CYPfw and CYPrw for CYP716A12 and InsBfw and InsBrw for the genomic sequence related to the insertion B.

Identification of TILLING Alleles

The M2 generation of the EMS-mutagenized collection was screened by the TILLING technique using primers P450fw and P450rw to find mutations in the CYP716A12 gene. DNA from mutant plants was sequenced to confirm the mutation. The M3 generation of the identified mutant plants was analyzed for saponin and sapogenin content with the protocols described above.

Complementation

The full-length CDS for CYP716A12 was amplified with primers ATG2fw and TAA2rw from cDNA produced from total leaf RNA, cloned in pCR8GW (Invitrogen) and sequenced. The CDS was subsequently shuttled by *att*L × *att*R reaction into pH2GW7 (Karimi et al., 2002); manufacturer's protocols were followed for TOPOTA and Gateway cloning (Invitrogen). The construct was electroporated into EHA105 *Agrobacterium tumefaciens* cells. Transformation of the *lha* leaf explants was performed according to Trinh et al. (2001).

Expression of CYP716A12 Gene in *Saccharomyces cerevisiae*

The full-length CYP716A12 CDS fragment was amplified with primers CypEcoRIfw and CypClaIrev, cloned into the pGEM-Teasy vector, and sequenced. The CYP716A12 CDS fragment was subsequently excised with the restriction enzymes *Eco*RI and *Cla*I and subcloned into the pESC-HIS vector (Agilent Technologies) to give an in-frame C-terminal fusion with the FLAG epitope. The expression vector was transformed into yeast (*S. cerevisiae*) strains WAT11 and WR (Pompon et al., 1996) by the lithium acetate procedure (Gietz et al., 1992). For CYP716A12 expression, the recombinant strains were cultured according to low

density procedure (Pompon et al.,1996) with the addition of 13 μg/mL hemin (Sigma-Aldrich) to the medium in the last induction step. WAT11 and WR clones transformed with pESC-HIS empty vector were used as control. Microsome preparation was performed as described by Pompon et al. (1996) except that ultracentrifugation at 100,000*g* was performed for 60 min. The presence of CYP716A12 protein in microsome was tested by immunoblot analysis using an anti-FLAG antibody (Sigma-Aldrich).

In Vitro Enzymatic Activity Assay

The activity of the CYP716A12 protein was tested in a 500-μL reaction mixture consisting of 100 mM potassium phosphate buffer, pH 7.4, containing 20 mM Glc-6-phosphate, 2.5 U of Glc-6-phosphate dehydrogenase, 30 μg of substrate (β-amyrin purchased from Sigma-Aldrich or erythrodiol from Extrasynthese), and 2 mg of microsomal fraction protein. After incubating the reaction mixture for 5 min at 30°C, the reaction was started by adding NADPH to a final concentration of 2mM and then was stopped with 500 μL of 37% HCl after 6 h. The reaction products were subjected to acid hydrolysis, and sapogenin content was evaluated as described above.

Quantitative RT-PCR Analysis

Real-time quantitative PCR analysis was performed on the E113 control line (T4 generation). Total RNA was extracted from three biological replicates, each comprising three individual plants. Sampling was performed in three biological stages (vegetative growth, early flowering, and early pod setting) on leaves, stems, and roots. In the third sampling, flowers, pods, and root nodules were also processed. A Nucleospin RNA plant kit with DNase (Macherey-Nagel) was used according to the manufacturer's protocol. cDNA was synthesized by priming with oligo-dT_{23} anchored using MMLV reverse transcriptase (Sigma-Aldrich) starting from 3 μg of RNA. cDNA was diluted 1:25 and 6 μL was used as template in a 20-μL reaction containing 10 μL of SsoFast EvaGreen supermix (Bio-Rad) and 0.75 μM primers CYP-38FW and CYP-220RW. Thermal cycling conditions were: 3′ of initial denaturation at 95°C, 40 cycles of denaturation (95°C, 25"), annealing and extension (59.5°C, 30"), and a final melting analyses from 55°C to 95°C with 1 degree for each step. The Msc27 control gene was amplified in the same condition using Msc27 269FW and Msc27 424rev primers at 1 μM each. All PCR reactions were performed on three replicates each in a RotorGene 6000 (Corbett). Data analysis was performed with Rotor-Gene 6000 series Software 1.7 (Corbett). To compare data from different PCR runs and different cDNA samples, cycle threshold (Ct) value was normalized against the reference gene Msc27. The value for the expression level of the CYP716A12 gene was calculated by the comparative Ct method using equation $E = 2^{-\Delta\Delta Ct}$ ($\Delta\Delta Ct$ being the differences in ΔCt between a given tissue and leaves in vegetative stage). PCR conditions for each primer combination were optimized for efficiency = 1 and PCR products were verified by melting curve analysis and agarose gel.

Microarray Analysis

RNA was extracted from 2-month-old leaves for *lha* and control plants with the Qiagen RNeasy Mini Kit according to manufacturer's instruction. A total of six hybridizations were performed (two samples per three replicates) at the NASC's Affymetrix service (Nottingham Arabidopsis Stock Centre, University of Nottingham, UK). Total RNA samples were labeled, hybridized, and scanned as per manufacturer's instructions as described in the technical manual [GeneChip Expression Analysis, Affymetrix (www.affymetrix.com)], using the Medicago Genome array (Affymetrix). The raw and processed data have been donated to the Gene Expression Omnibus database at the National Center for Biotechnology Information (http://www.ncbi.nih.gov/geo/), with the accession number GSE22835. The nonscaled RNA CEL files were loaded into the Genespring GX11 (Agilent Technologies) using the robust multichip average prenormalization algorithm (Irizarry et al., 2003). Per-gene normalization was applied to the probe set signal values (i.e., the values for given genes) as follows. For each replicate, probe set signals were standardized to the median probe set signal value for all arrays in the experiment.

Differentially expressed genes were identified using a two-step process: 1) a *t* test was performed to identify genes that were differentially expressed between mutant and control samples ($P \leq 0.05$); and 2) genes that were ≥1.5-fold up- or downregulated between the mutant and control samples.

The significant Gene Ontology (GO) terms for the *Medicago* and *Arabidopsis thaliana* annotation were identified using the GO Analysis function in Genespring GX11. The program Genebins at http://bioinfoserver.rsbs.anu.edu.au/utils/GeneBins/ (Goffard and Weiller, 2007) was also used for annotation analysis.

Statistical Analyses

Analysis of variance was performed using General Linear Models procedure of the SAS software, version 8 (SAS Institute Inc.), with linear contrasts for comparison of specific means.

Accession Numbers

Sequence data from this article can be found in the GenBank/EMBL databases under the following accession numbers FN 995112 (CYP716A12 gene from *M. truncatula* R-108) and FN 995113 (CYP716A12 mRNA from *M. truncatula* R-108). The raw and processed data have been deposited in the Gene Expression Omnibus database at the National Center for Biotechnology Information (http://www.ncbi.nih.gov/geo/), with the accession number GSE22835.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure 1. CYP716A12 Splicing Variant.

Supplemental Figure 2. Mutant Alleles for CYP716A12 from a TILLING Collection.

Supplemental Figure 3. GC-FID Chromatograms of Sapogenins of Control Line (E113; Red), *lha* Mutant (Green), and One Transformed Line Derived from Complementation of the *lha-1* Mutant (Black).

Supplemental Figure 4. Immunoblot Analysis of the CYP716A12-Harboring Strains.

Supplemental Figure 5. GC-MS Analysis (A) and Mass Spectrum (B) of the Standard Oleanolic Acid.

Supplemental Figure 6. Bar Charts Showing GO Annotations of the *M. truncatula* Probe Sets Modulated in the *lha* Mutant.

Supplemental Table 1. T_2 Lines Segregating for the Lha Phenotype: Segregation Ratio and χ^2 Value.

Supplemental Table 2. Comparison of the *lha* Mutant Line (E25-10) and the Full Sib Control Line (E25-08).

Supplemental Table 3. Analysis of Variance of Sapogenin Content (GC-FID) in Control Plants Sampled in Different Organs at Different Phenological Stages: Test F and Significance.

Supplemental Table 4. List of Selected Genes/TC Modulated in the *lha* Mutant and Cited in the Text.

Supplemental Table 5. List of Primers Used in This Research.

Supplemental Data Set 1. Differentially Expressed Genes in the *lha* Mutant Compared with Wild-Type R-108 ($P < 0.05$, 1.5-fold change).

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AUTHOR CONTRIBUTIONS

M.C., E.B., F.P., L.S., A.P., O.C., and C.S. designed and performed genetic and molecular experiments; E.B., A.T., M.C., and O.C. designed and performed biochemical analysis; N.G. and S.M. performed microarray data analysis; M.C., C.S., and O.C. wrote the article; and C.S., A.T., O.C., M.O., E.P., and S.A. supervised research and edited the article.

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Medicago truncatula CYP716A12 Is a Multifunctional Oxidase Involved in the Biosynthesis of Hemolytic Saponins

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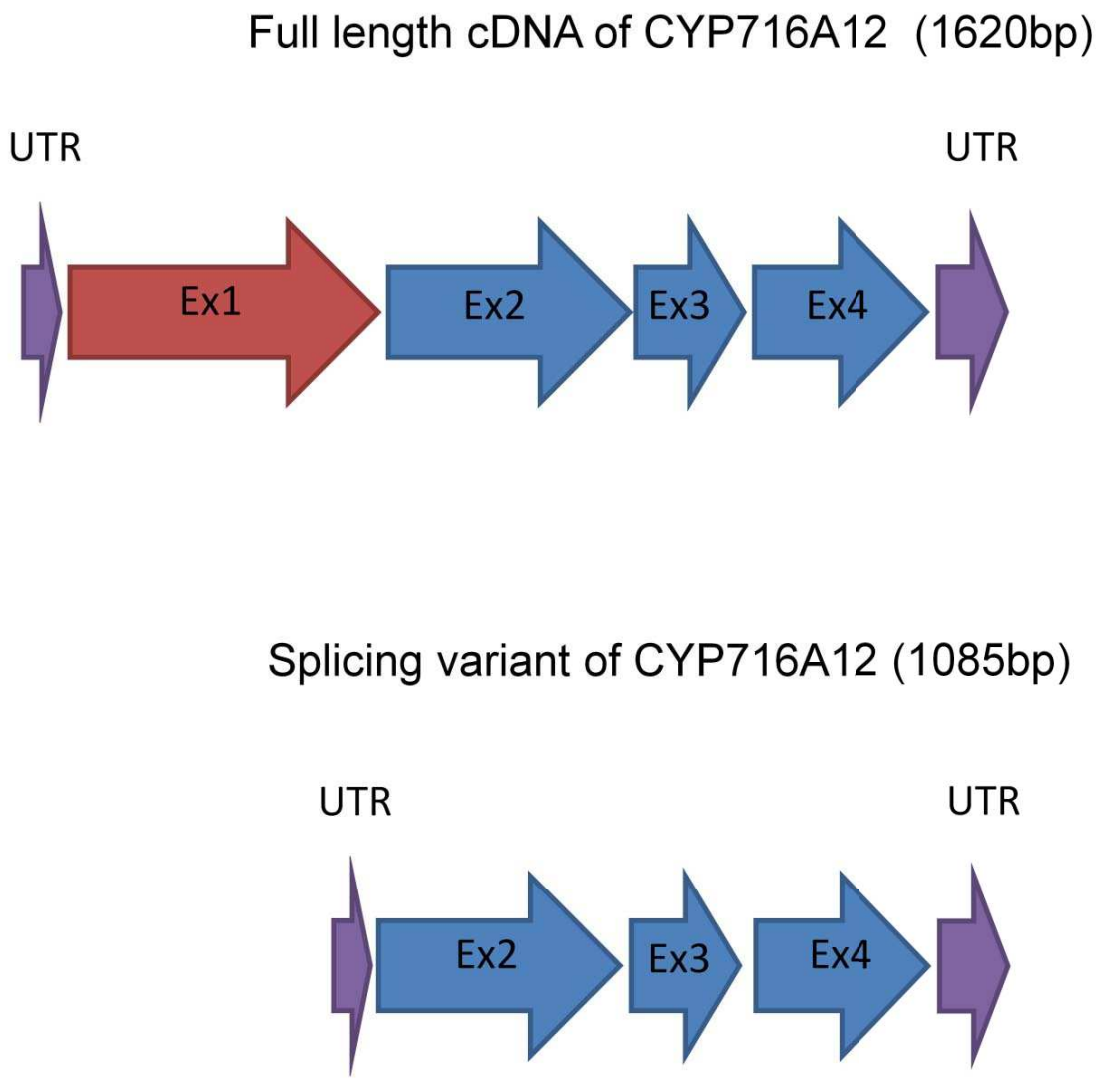
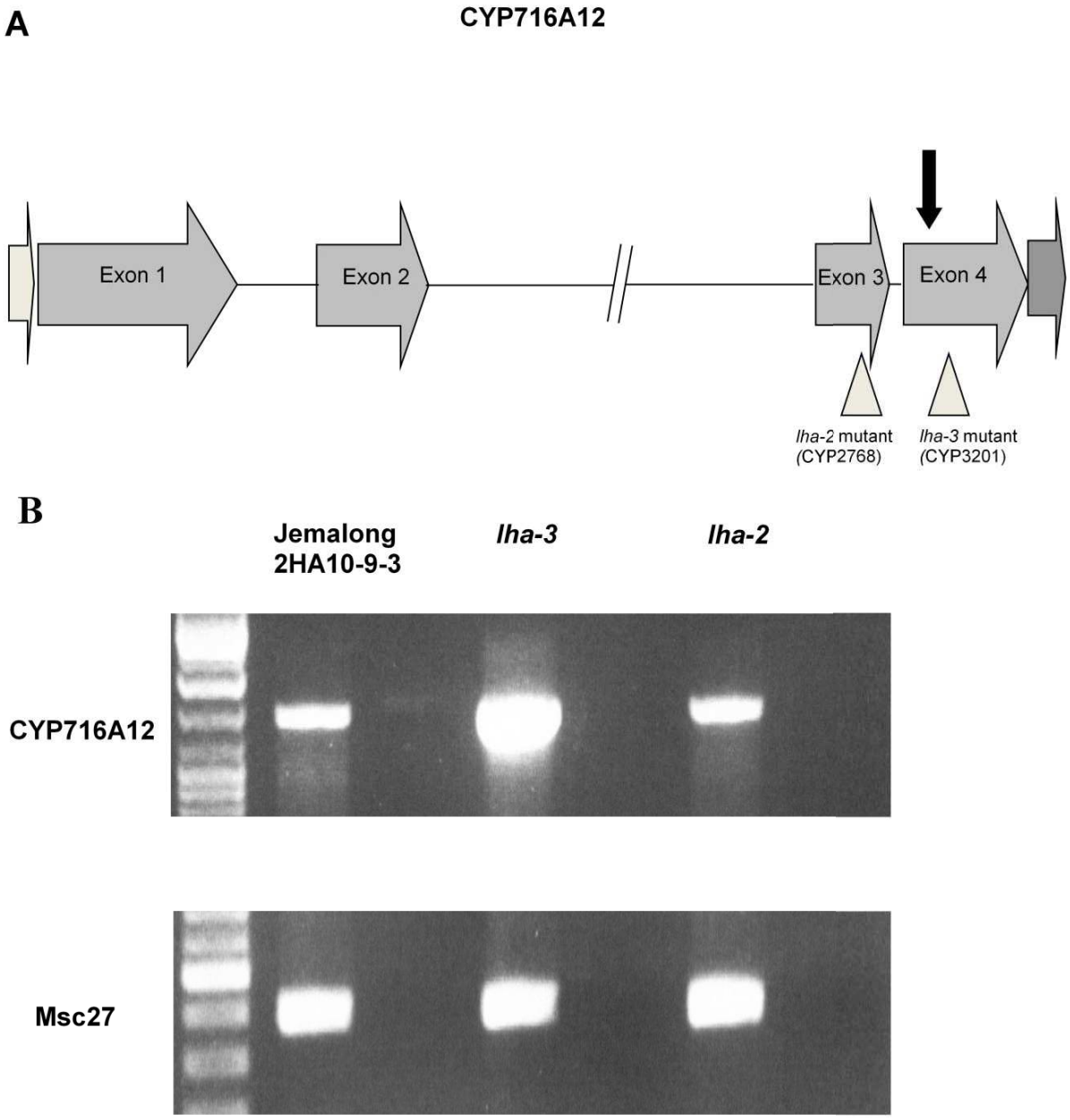
Appendix 1.

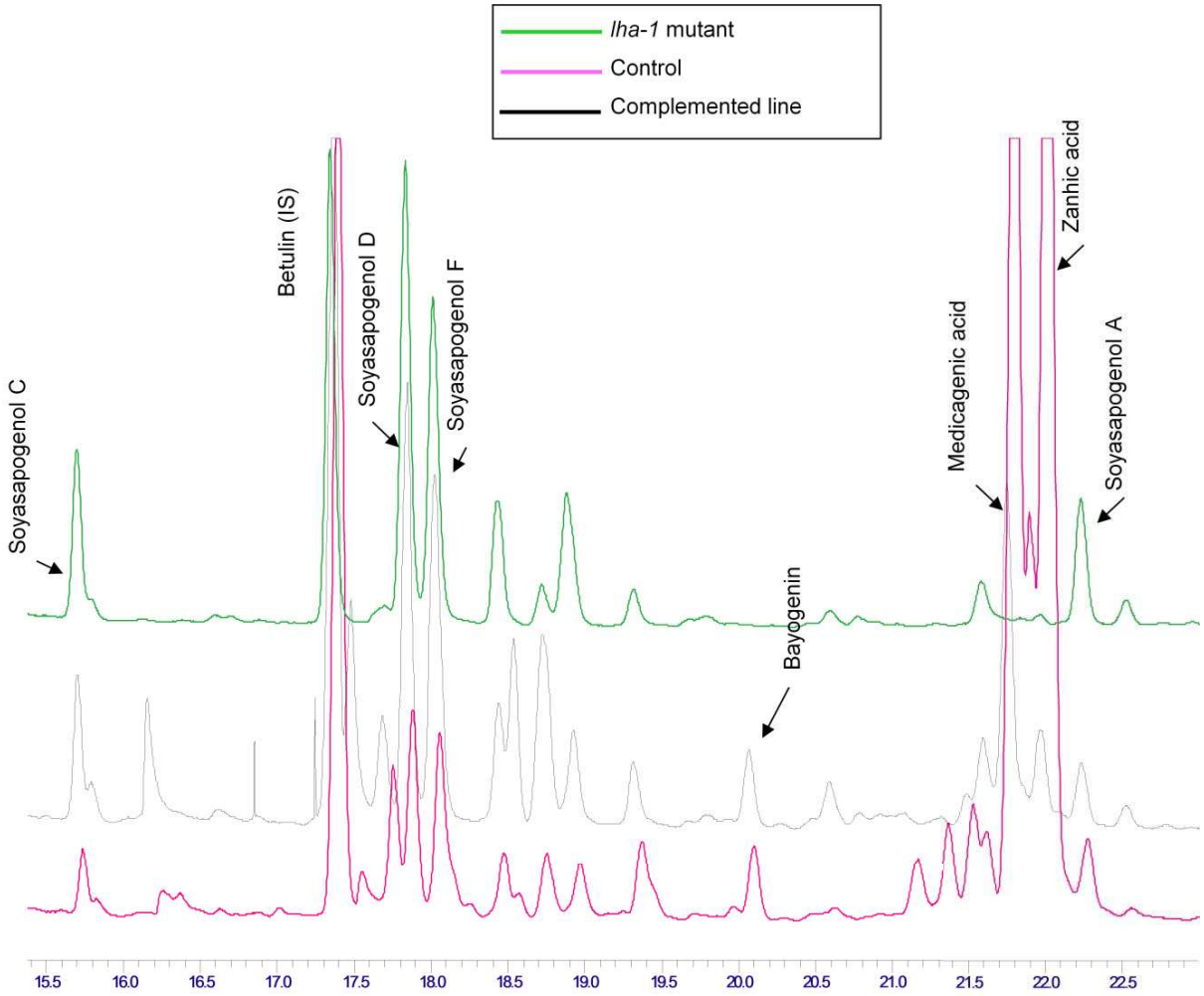
Supplemental Data



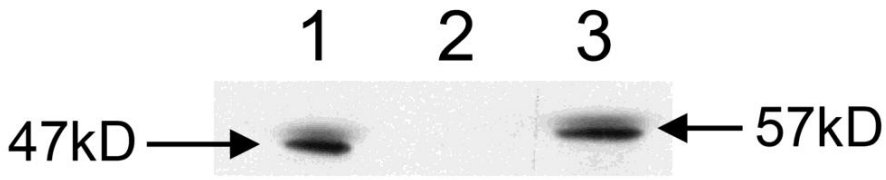
Supplemental Figure 1. CYP716A12 splicing variant



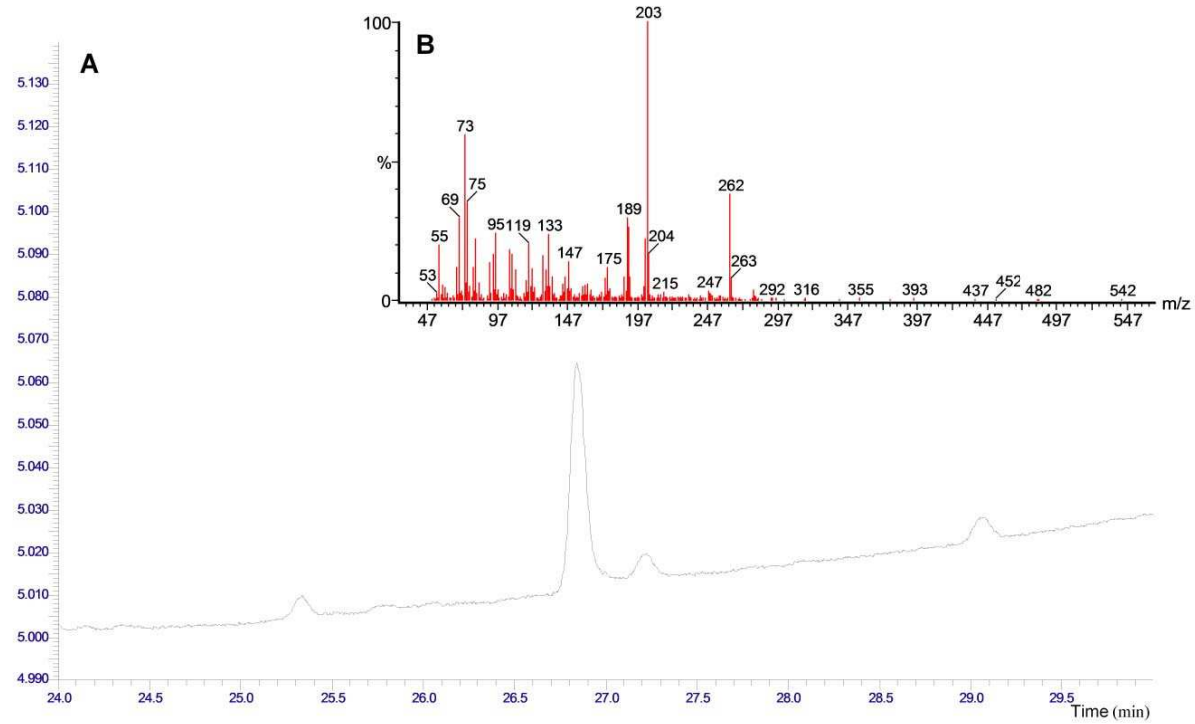
Supplemental Figure 2. Mutant alleles for CYP716A12 in TILLING collection.
(A) Alleles of CYP716A12 obtained from the TILLING collection (solid triangle): *lha-2* CYP2768 (P355→L) and *lha-3* CYP3201 (W449→ stop) lines. The position of the heme cysteine ligand is indicated (black arrow).
(B) RT-PCR analysis of transcript level of CYP716A12 gene in *lha-2* and *lha-3* mutants.



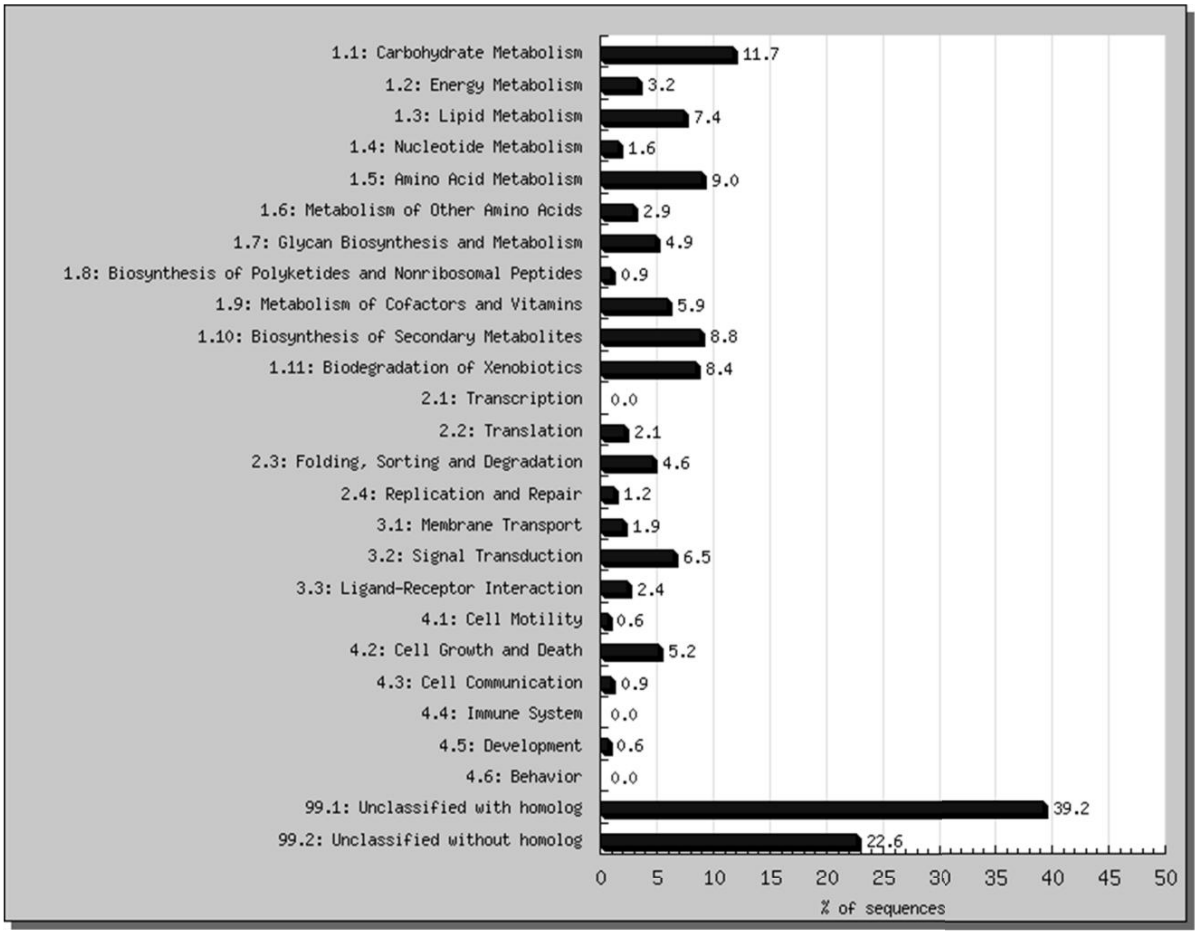
Supplemental Figure 3. GC/FID chromatograms of sapogenins of control line (E113) (red), *lha-1* mutant (green) and one transformed line derived from complementation of *lha-1* mutant (black). Identification of soyasapogenol B was achieved considering all the artefact compounds detected (soyasapogenols C, D and F).



Supplemental Figure 4. Immunoblot analysis of the CYP716A12-harbouring strains. Lane 1, FLAG-BAP™ Fusion Protein (SIGMA-Aldrich); lane 2, WAT11 expressing pESC-HIS under galactose induction; lane 3, WAT11 expressing pESC-HIS-CYP716A12:FLAG under galactose induction.



Supplemental figure 5. GC-MS analysis (A) and mass spectrum (B) of the standard oleanolic acid.



Supplemental Figure 6. Bar charts showing GO annotations of the *M. truncatula* probesets modulated in the *lha-1* mutant.

Supplemental Table 1. T_2 lines segregating for the Lha phenotype: segregation ratio and χ^2 value.

	Wild type	LHA phenotype	χ^2
E25-1	25	5	1.11
E25-2	25	5	1.11
E25-3	-	10	
E25-4	26	4	2.18
E25-5	23	7	0.04
E25-6	-	14	
E25-7	21	9	0.40
E25-8	29	-	
E25-9	29	-	
E25-10	-	30	

χ^2 value is within 95% confidence value for a 3:1 segregation ratio

Supplemental Table 2. Comparison of the *lha-1* mutant line (E25-10) and the full sib control line (E25-08). The interaction line x block was used as error term.

Characters		Control	Mutant
Germination (dd)		5.00	10.60*
Flowering (dd)		58.79	56.85*
Sum of length (cm)	main stems	393.08	201.77*
	branches	687.51	209.60**
Root DM (mg)		185.77	76.96*
Nodul Fresh	absolute	10.18	19.73†
Weight (mg)	per unit of root DM	56.62	274.47‡
Nodule number	absolute	4.33	6.47*
	per unit of root DM	0.025	0.117 ns
Pod number per unit of stem length		0.070	0.061 ns
Pod fertility (number of seeds/pod)		5.27	4.73 ns

significantly different at: *P < 0.05, **P < 0.01, †P = 0.0687, ‡P=0.0747.
ns: not significant , DM: dry matter.

Supplemental Table 3. ANOVA of sapogenin content (GC/FID) in control plants sampled in different organs at different phenological stages: test F and significance.
Only significant contrasts are reported.

Source of variation	SoyaA	SoyaB	Spg non-hem	Olea	Hed	Bayo	Med	Zan	Spg hem
Stage	1.5 ns	1.2 ns	1.4 ns	0.12 ns	2.7 ns	2.4 ns	16.1^{****}	4.7^{*}	11.9^{****}
Organ	11.8^{****}	26.1^{****}	22.9^{****}	34.7^{****}	79.4^{****}	54.2^{****}	0.7 ns	232.3^{****}	32.5^{****}
StagexOrgan	0.4 ns	0.7 ns	0.7 ns	0.12 ns	1.4 ns	2.6 ns	3.4^{*}	0.2 ns	2.0 ns
Contrast									
S1*vs*S2/ root					9.0^{**}		32.6^{****}	5.3^{*}	21.2^{****}
S1*vs*S3/ root					6.8^{*}	13.6^{***}	25.0^{****}		17.1^{****}
S2*vs*S3/ root						8.4^{**}			
L*vs*R/ S1		13.9^{***}	13.4^{***}	19.4^{****}	21.8^{****}	10.6^{***}		123.0^{****}	21.4^{****}
L*vs*S/ S1								103.1^{****}	17.7^{****}
R*vs*S/ S1		12.0^{***}	10.8^{***}	19.4^{****}	21.3^{****}	17.5^{****}			
L*vs*R/ S2	5.2^{*}	20.6^{****}	18.7^{****}	13.5^{***}	53.9^{****}	16.3^{****}	4.9^{*}	111.7^{****}	
L*vs*S/ S2								119.6^{****}	22.4^{****}
R*vs*S/ S2	10.3^{***}	21.2^{****}	18.4^{****}	13.5^{***}	51.5^{****}	21.8^{****}	6.4^{*}		9.2^{**}
L*vs*R/ S3	5.8^{*}	5.6^{*}	4.5^{*}	19.4^{****}	46.8^{****}	48.2^{****}		122.3^{****}	5.6^{*}
L*vs*S/ S3								117.6^{****}	23.6^{****}
R*vs*S/ S3	9.6^{**}	8.7^{**}	6.9^{*}	19.4^{****}	51.5^{****}	61.8^{****}			6.3^{*}

* P≤0.05; ** P≤0.01; *** P≤0.005; **** P≤0.001; ns: not significant

SoyaA: soyasapogenol A; SoyaB: soyasapogenol B; Olea: oleanolic acid; Hed: hederagenin; Bayo: bayogenin; Med: medicagenic acid; Zan: zanhic acid; Spg hem: haemolytic sapogenin; Spg non-hem: non-haemolytic sapogenin.
S1, S2, S3: stages 1, 2 and 3 respectively. L: leaves; R: roots; S: stems.
Error: within repetition (pool of 3 plants, one from each block) variation (n=3).

Supplemental Table 4. List of selected genes/TC modulated in *lha-1* mutant and cited in the text.

Probe set ID	Transcript ID	Annotation	Fold change
		2,3-oxidosqualene cyclases	
Mtr.46511.1.S1_at	Medtr8g018540		2.1 down
Mtr.46511.1.S1_x_at			
Mtr.46512.1.S1_at	Medtr8g018550		1.8 down
Mtr.46514.1.S1_x_at	Medtr8g018610	Similar to *MtbAS*1	1.8 down
		Cytochrome P450s	
Mtr.8618.1.S1_at	TC100810	CYP93E2	2.3 down
Mtr.37299.1.S1_at	TC100323	CYP72A67	2.6 up
Mtr.37298.1.S1_at	TC100322	CYP72A68	1.6 up
Mtr.10812.1.S1_at	Medtr5g102050	CYP71D64	1.7 down
Mtr.6667.1.S1_at	Medtr8g050160	Putative CYP76C2	2.4 up
Mtr.10692.1.S1_at	TC107626	CYP76E1	1.6 up
Mtr.12208.1.S1_at	TC93934	CYP76X2	1.7 up
Mtr.6322.1.S1_s_at	BQ139200	Similar to CYP72A1	1.5 down
Mtr.43267.1.S1 at	Medtr8g045080	CYP72A65	1.8 down
Mtr.4947.1.S1_at	AW127462	CYP715A2	2.0 down
Mtr.20735.1.S1_at	Medtr8g090600	Similar to CYP90C1	1.7 up
Mtr.21518.1.S1_s_at	Medtr2g104500	CYP74B, HPL3	1.6 down
Mtr.9885.1.S1_at	Medtr2g104500	CYP74B, HPL3	1.5 down
Mtr.12632.1.S1_at	TC95424	CYP81E9 isoflavone3'-hydroxylase	2.0 up
Mtr.38274.1.S1_at	TC102428	CYP90B	1.9 up
Mtr.9112.1.S1_at	TC102429	CYP90B	1.8up
Mtr.42172.1.S1_s_at	TC110889	Similar to CYP707A3	1.5 up
		Glycosyltransferases	
Mtr.12473.1.S1_a	TC94916	UGT73F3	1.5 down
Mtr.11236.1.S1_at	Medtr2g008370	UGT91H5	2.7 down
Mtr.8530.1.S1_s_at	Medtr2g008360	UGT91H6	1.5 down
Mtr.49684.1.S1_at	Medtr7g076790	Similar to *Mt*UGT73F3	1.6 down
Mtr.43628.1.S1_at	Medtr5g076110	Similar to *Mt*UGT73F3	3.5 up
Mtr.37367.1.S1_at	Medtr2g008380	Similar to *Mt*UGT91H6	1.6 down
Mtr.39289.1.S1_at	TC104594	Putative GT	1.8 up
Mtr.3013.1.S1_at	Medtr6g014410	Putative GT	1.6 down
Mtr.42552.1.S1_at	TC111804	Putative GT	1.9 up
Mtr.40639.1.S1_at	TC107730	Putative GT	1.8 up
Mtr.36069.1.S1_s_at	AJ845941	Putative GT	1.5 down
Mtr.16941.1.S1_at	Medtr4g130290	Putative GT	2.0 down
Mtr.29993.1.S1_at	Medtr3g009370	Putative GT	1.7 up
Mtr.44439.1.S1_at	Medtr6g014590	Putative GT	1.7 up
Msa.2667.1.S1_at		Putative GT	1.5down
Msa.2806.1.S1_at		Putative GT	1.6 up
Msa.1673.1.S1_at		Putative GT	2.06up
Msa.1267.1.S1_at		Putative GT	1.8up
		Pathogenesis related genes	
Mtr.47298.1.S1_at		PGIP	5.4 down
Mtr.42876.1.S1_at		Similar to *Ps*PRP4	7.5 up
Mtr.41478.1.S1_at		Similar to *Mt*PR10-1 ABR18	5.0 up

Supplemental Table 5. List of primers used in this research.

Primer name	Primer sequence (5' – 3')
Right 1	CGTTCAAGATGCCTCTACCG
Left 1	TATCTTCCACACGTGAAAATGC
Right 2	AGACGTTCCAACCACGTCTT
Left 2	TTTTTATTTCATCCGACATGG
Primer RACE 3'	TATCTCTACCAATTGATTTGCCAGG
Primer RACE 5'	CCTGGCAAATCAATTGGTAGAGATA
Primer RACE 5'nest	ATCAATTGGTAGAGATATGATTCC
ATG2fw	ATGGAGCCTAATTTCTATCTC
TAA2rw	TTAAGCTTTGTGTGGATAAAG
Msc27fw	GATGAGCTTCTGTCAGACTC
Msc27rw	GCTACCATCATCATGCATGC
CYPfw	TTTGCCACCTGGTAAAATGG
CYPrw	AGCCTTTGTTGAATGGTGTTC
InsBfw	GCCAGTGAGGTTGGCTTAGT
InsBrw	TCTCAAGAGTGAGATATACCGG
PSKI	GTAAAACGACGGCCAGTGA
P450fw	GGAAATTGCAAAATCGAAACC
P450rw	AAAGAACCGAATGAAACCAATCA
CypEcoRIfw	GAATTCATGGAGCCTAATTTCTATCTCTCCC
CypClaIrev	ATCGATTTAGCTTTGTGTGGATAAAGGC
CYP-38FW	ACTGCCATTGCACCATTGTA
CYP-220RW	CCAGGTGGCAAATTTAATGG
Msc27 269FW	CACCCAAACTAGATGCAGAGAA
Msc27 424rev	CACCATCCTTGTAGTAGGCAAA

Appendix 2.

Supplemental Dataset

Supplemental Dataset 1. Differentially expressed genes in the *lha* mutant compared to wild type R-108 (P<0.05, 1.5 fold change).

Probe Set ID	p-value	Fold Change Absolute	regulation	[Wild-type] (normalized)	[Mutant] (normalized)	Unigene (Avadis)	DFCI_MtGI_ Tentative_ Annotation	GO (Avadis)	AGI	Arabidopsis Description	Arabidopsis e-value
Mtr.23668.1.S1_s_at	8,14E-05	16,70	up	-2,1671188	1,8943874				AT4G37820	expressed protein, Kaposi's sarcoma-associated herpes-like virus ORF73gene, Kaposi's sarcoma-associated herpesvirus, U52064	0,003
Mtr.18264.1.S1_s_at	4,12E-05	16,68	up	-1,9997448	2,060476				AT3G13890	myb family transcription factor (MYB26), similar to myb-related transcription factor GI:1167486 from (Lycopersicon esculentum); contains myt	3,5
Mtr.23138.1.S1_s_at	2,07E-06	16,59	up	-1,9910122	2,060877				AT1G62970	DNAJ heat shock N-terminal domain-containing protein, low similarity to AHM1 (Triticum aestivum) GI:6691467; contains Pfam profile PF00226	1,2
Mtr.23667.1.S1_s_at	3,18E-05	16,29	up	-2,0913684	1,9347469				AT4G08200	hypothetical protein	4,00E-26
Mtr.49688.1.S1_at	0,00864159	12,03	up	-1,1271123	2,4617627	Mtr.9954		GO:0030001	AT5G05660	zinc finger (NF-X1 type) family protein, contains PF01422: NF-X1 type zinc finger	0,16
Mtr.22651.1.S1_at	1,40E-05	11,47	up	-1,768423	1,7512897				AT1G09930	oligopeptide transporter OPT family protein, similar to SP|P40900 Sexual differentiation process protein isp4 {Schizosaccharomyces pombe}; c	1,6
Mtr.22079.1.S1_x_at	6,11E-06	10,16	up	-1,6590686	1,686175				AT3G04980	DNAJ heat shock N-terminal domain-containing protein, contains Pfam profile PF00226 DnaJ domain	1,6
Mtr.6941.1.S1_at	2,59E-04	9,87	up	-1,5719957	1,7310023				AT3G46850	subtilase family protein, contains similarity to prepro-cucumisin GI:807698 from (Cucumis melo);	6,00E-24
RPTR-Mtr-U57609-2_s	0,00325886	9,66	up	-1,4482956	1,8237367				AT4G11450	expressed protein	0,61
Mtr.37338.1.S1_s_at	0,00134917	9,39	down	1,2562357	-1,9754795		homologue to (P27879) 18.1 kDa class I heat shock protein (Fragment), partial (48%)		AT2G28840	ankyrin repeat family protein, contains ankyrin repeats, Pfam:PF00023	3,00E-22
Mtr.43346.1.S1_at	0,00899027	9,26	up	-1,1911082	2,019396		similar to (Q9SUF0) Nodulin-like protein, partial (77%)		AT4G08300	nodulin MtN21 family protein, similar to MtN21 GI:2598575 (root nodule development) from (Medicago truncatula)	1,00E-31
Mtr.12531.1.S1_s_at	0,00905956	8,09	up	-1,1928989	1,8230385		weakly similar to (Q8VZ54) Putative nodulin protein, partial (33%)		AT4G08300	nodulin MtN21 family protein, similar to MtN21 GI:2598575 (root nodule development) from (Medicago truncatula)	2,00E-15
Mtr.42876.1.S1_at	0,03711187	7,57	up	-1,3507	1,5702771		homologue to (Q9M7D9) Pathogenesis-related protein 4A, partial (96%)		AT3G04720	hevein-like protein (HEL), identical to SP|P43082 Hevein-like protein precursor {Arabidopsis thaliana}; similar to SP|P09762 Wound-induced pr	2,00E-13
Mtr.24387.1.S1_at	1,35E-04	7,51	up	-1,5125376	1,3970159				AT4G31990	encodes a plastid-localized aspartate aminotransferase;aspartate aminotransferase, chloroplast / transaminase A (ASP5) (AAT1), nearly identic	14
Mtr.43717.1.S1_at	6,67E-05	6,42	down	1,3136755	-1,3695911		similar to (Q7VGA9) Hypothetical protein, partial (1%)		AT1G14290	acid phosphatase, putative, similar to acid phosphatase (Lupinus albus) GI:5360721; contains Pfam profile PF01598 sterol desaturase	16
Mtr.49642.1.S1_at	2,98E-04	6,39	up	-1,260107	1,4148445				AT3G18830	This gene encodes a plasma membrane-localized polyol/cyclitol/monosaccharide-H+-symporter. The AtPLT5 symporter is able to catalyze the e	2,00E-38
Mtr.4951.1.S1_at	0,00118457	6,07	down	1,3131529	-1,2877799				AT2G47880	glutaredoxin family protein, contains INTERPRO Domain IPR002109, Glutaredoxin (thioltransferase)	0,006
Mtr.43745.1.S1_at	0,03419145	5,91	up	-0,6736415	1,8907048		weakly similar to (Q9SUF1) Nodulin-like protein, partial (79%)		AT4G08290	nodulin MtN21 family protein, similar to MtN21 GI:2598575 (root nodule development) from (Medicago truncatula)	2,00E-36
Mtr.21428.1.S1_at	0,00171502	5,73	up	-1,2985076	1,2191538				AT5G49140	disease resistance protein (TIR-NBS-LRR class), putative, domain signature TIR-NBS-LRR exists, suggestive of a disease resistance protein.	0,037
Mtr.47298.1.S1_at	0,00152456	5,48	down	1,4739977	-0,98005706				AT5G06870	polygalacturonase inhibiting protein 2 (PGIP2), identical to polygalacturonase inhibiting protein 2 (PGIP2) (Arabidopsis thaliana) gi|7800201|gb	3,00E-27
Mtr.34636.1.S1_at	1,50E-05	5,33	up	-1,2220036	1,1911874	Mtr.18762		GO:0006810	AT2G34960	amino acid permease family protein, similar to cationic amino acid transporter 3 (Rattus norvegicus) GI:2116552; contains Pfam profile PF0032	1,00E-43
Mtr.41478.1.S1_at	0,03239612	5,08	up	-0,7067005	1,6378088		weakly similar to (Q7FNV7) Class 10 PR protein, partial (91%)		AT5G45860	Bet v I allergen family protein, low similarity to SP|P27538 Pathogenesis-related protein 2 {Petroselinum crispum}	3,00E-05
Mtr.34989.1.S1_at	0,00107014	5,08	up	-1,0339209	1,3095508				AT5G59190	subtilase family protein, contains similarity to prepro-cucumisin GI:807698 from (Cucumis melo)	2,00E-19
Mtr.33361.1.S1_at	0,00102522	4,64	up	-1,1139731	1,1007491				AT3G59370	expressed protein, hypothetical protein F12F1.4 - Arabidopsis thaliana, EMBL:AC002131	1,3
Mtr.12715.1.S1_at	0,00377859	4,53	up	-1,0126872	1,167458		weakly similar to (Q7XJE7) Putative xyloglucanase inhibitor, partial (27%)		AT1G03220	extracellular dermal glycoprotein, putative / EDGP, putative, similar to extracellular dermal glycoprotein EDGP precursor (Daucus carota) GI:28	1,00E-25
Mtr.39561.1.S1_at	1,38E-04	4,47	down	1,0639311	-1,0972596	Mtr.19156	weakly similar to F-box protein family AtFBW2 {Arabidopsis thaliana;}, partial (32%)		AT4G08980	F-box family protein (FBW2), contains similarity to N7 protein GI:3273101 from (Medicago truncatula)	2,00E-16
Mtr.48869.1.S1_at	0,01658962	4,47	up	-0,6085761	1,5517702	Mtr.6626			AT1G28330	dormancy-associated protein, putative (DRM1), identical to dormancy-associated protein (Arabidopsis thaliana) GI:2995990; similar to dorman	6,00E-24
Mtr.8498.1.S1_at	0,03537187	4,44	up	-0,48098588	1,6691083		(O24483) Asparagine synthetase, complete		AT3G59500	integral membrane HRF1 family protein, contains Pfam domain PF03878: Hrf1 family	18
Mtr.40617.1.S1_at	2,44E-05	4,39	up	-1,0449818	1,0879902		similar to (Q9FQD3) Glutathione S-transferase GST 25 (EC 2.5.1.18), partial (94%)		AT2G02380	glutathione S-transferase, putative, similar to gi:167970 gb:AAA72320 gb:AY052332;Encodes glutathione transferase belonging to the zeta cla	2,00E-31
Mtr.10331.1.S1_at	0,02733447	4,29	down	0,8401289	-1,2608255		weakly similar to (Q8L5K8) Orcinol O-methyltransferase (EC 2.1.1.6), partial (65%)		AT1G51990	O-methyltransferase family 2 protein, similar to caffeic acid O-methyltransferase GI:5031492 from (Ocimum basilicum), (SP|Q00763) (Populus	3,00E-12
Mtr.40146.1.S1_at	0,02379923	4,20	up	-0,54126865	1,5276827				AT1G78915	expressed protein	0,92
Mtr.11736.1.S1_at	6,69E-04	3,90	down	1,0178984	-0,9447427		similar to (P49173) Probable aquaporin NIP-type (Pollen-specific membrane integral protein), partial (83%)		AT5G37820	major intrinsic family protein / MIP family protein, contains Pfam profile: PF00230 major intrinsic protein (MIP)	4,00E-33
Mtr.47295.1.S1_x_at	0,01183318	3,88	down	1,0805308	-0,8742323				AT5G06860	polygalacturonase inhibiting protein 1 (PGIP1), identical to polygalacturonase inhibiting protein 1 (PGIP1) (Arabidopsis thaliana) gi|7800199|gb	4,00E-31
Mtr.6639.1.S1_s_at	0,00999668	3,81	up	-0,9878318	0,9413994	Mtr.4006			AT3G56470	F-box family protein, similar to F-box protein family, AtFBX7 (GI:20197899) (Arabidopsis thaliana)	6
Mtr.34416.1.S1_at	0,03426442	3,66	up	-0,5089194	1,363753	Mtr.8569			AT4G15530	similar to pyruvate,orthophosphate dikinase [Flaveria brownii] (GB:CAA55784.1); similar to pyruvate,orthophosphate dikinase [Mesembryanthemum crystallin	
Mtr.32795.1.S1_s_at	0,00143267	3,58	down	1,1085777	-0,7308156				AT1G06830	glutaredoxin family protein, contains INTERPRO Domain IPR002109, Glutaredoxin (thioltransferase)	5,00E-25
Mtr.43628.1.S1_at	0,01103327	3,55	up	-0,7691366	1,059792		similar to (Q7XZD0) Isoflavonoid glucosyltransferase, partial (51%)		AT4G34135	UDP-glucuronosyl/UDP-glucosyl transferase family protein, contains Pfam profile: PF00201 UDP-glucuronosyl and UDP-glucosyl transferase	6,00E-41
Mtr.492.1.S1_at	0,01289028	3,52	up	-0,86014026	0,95412415				AT5G59540	oxidoreductase, 2OG-Fe(II) oxygenase family protein, similar to desacetoxyvindoline-4-hydroxylase (Catharanthus roseus) GI:2352812; contai	6,00E-44
Mtr.35833.1.S1_s_at	0,00128153	3,39	up	-0,75954944	1,0017718		weakly similar to unknown {Arabidopsis thaliana;}, partial (24%)		AT5G40460	expressed protein	1,00E-08
Mtr.24411.1.S1_at	0,04263661	3,35	up	-0,44556308	1,299873				AT2G26570	expressed protein, contains Pfam profile PF05701: Plant protein of unknown function (DUF827); weak similarity to merozoite surface protein 3	6,00E-41
Mtr.13457.1.S1_at	0,00563344	3,23	up	-0,6835134	1,0079703		similar to unknown protein {Arabidopsis thaliana;}, partial (72%)		AT5G10580	expressed protein, contains Pfam profile PF04654: Protein of unknown function, DUF599	
Mtr.41915.1.S1_at	0,00279278	3,20	up	-0,80494565	0,87112314		similar to (O04865) Phospholipase D alpha 1 (PLD alpha 1) (Choline phosphatase 1), partial (57%)		AT1G52570	phospholipase D alpha 2 / PLD alpha 2 (PLDALPHA2) (PLD2) / choline phosphatase 2, identical to phospholipase D alpha 2 (PLD alpha 2) SP:Q9SSQ9 from (Ar	
Mtr.49438.1.S1_at	0,0142335	3,17	up	-0,54677325	1,1157755				AT1G34300	lectin protein kinase family protein, contains Pfam domains, PF01453: Lectin (probable mannose binding) and PF00069: Protein kinase domain	5,00E-18
Mtr.50082.1.S1_at	0,0045134	3,16	up	-0,6087592	1,0491009				AT3G43770	expressed protein	0,038
Mtr.5691.1.S1_at	2,45E-05	3,14	up	-0,87187237	0,78092796				AT5G25250	expressed protein	1,00E-15
Mtr.31710.1.S1_at	0,03641893	3,12	up	-0,41996303	1,2213017				AT1G60590	polygalacturonase, putative / pectinase, putative, similar to polygalacturonase PG1 (GI:5669846), PG2 (GI:5669848) from (Glycine max); contains	PF00295: (
Mtr.40558.1.S1_at	0,03263713	3,12	up	-0,6469279	0,99411964		similar to (Q9SQF9) Short-chain alcohol dehydrogenase SAD-C, partial (93%)		AT2G47140	short-chain dehydrogenase/reductase (SDR) family protein, similar to 3-beta-hydroxysteroiddehydrogenase GI:15983819 from (Digitalis lanata	1,00E-20
Mtr.12349.1.S1_at	0,02313307	3,10	up	-0,7101563	0,9913295		similar to (Q7XEJ9) Putative polyprotein, partial (55%)		AT1G60710	aldo/keto reductase family protein, contains Pfam profile PF00248: oxidoreductase, aldo/keto reductase family;auxin-induced atb2 (ATB2) mRN	8,00E-23
Msa.1138.1.S1_at	0,00364811	3,10	down	0,67657727	-0,9556405				AT5G14130	peroxidase, putative, identical to peroxidase ATP20a (Arabidopsis thaliana) gi|1546694|emb|CAA67338	
Mtr.14988.1.S1_at	1,74E-04	3,08	down	0,79133844	-0,83173496				AT1G02460	glycoside hydrolase family 28 protein / polygalacturonase (pectinase) family protein, similar to polygalacturonase PG1 GI:5669846, PG2 GI:5669848 from (Gly	
Mtr.37918.1.S1_at	0,03028111	3,07	up	-0,5052204	1,1112245	Mtr.3095	similar to (Q6KAW1) Putative esterase, partial (93%)	GO:0008152	AT2G45600	expressed protein, low similarity to PrMC3 (Pinus radiata) GI:5487873	1,00E-04
Mtr.43371.1.S1_at	0,0026493	3,06	down	0,60278386	-1,0098842		similar to (Q96509) Peroxidase 55 precursor (Atperox P55) (ATP20a) (EC 1.11.1.7 EC 1.11.1.7), partial (75%		AT4G37530	peroxidase, putative, similar to peroxidase (Arabidopsis thaliana) gi|1402906|emb|CAA66958; identical to Pfam profile PF00141: Peroxidase; i	2,00E-41
Mtr.8460.1.S1_s_at	3,35E-04	3,01	down	0,80093354	-0,7907017		similar to (Q9AXU5) Pathogen-inducible alpha-dioxygenase, partial (56%)		AT3G01420	Encodes an alpha-dioxygenase involved in protection against oxidative stress and cell death.;Induced in response to Salicylic acid and oxidative stress.	Indepen
Mtr.13171.1.S1_at	0,0131905	3,01	up	-0,55945903	1,0317003		(O82615) T9A4.6 protein (Probable wound-induced protein), partial (21%)		AT4G10270	wound-responsive family protein, similar to wound induced protein (GI:19320) (Lycopersicon esculentum)	2,00E-06
Mtr.33377.1.S1_at	0,04690795	3,00	down	0,54031867	-1,0442665	Mtr.18594			AT3G07620	exostosin family protein, contains Pfam profile: PF03016 Exostosin family	8
Mtr.11073.1.S1_at	0,04429325	2,98	up	-0,5742561	0,99872		similar to (Q6RVV4) Short-chain dehydrogenase Tic32, partial (71%)		AT4G23420	short-chain dehydrogenase/reductase (SDR) family protein, similar to WW-domain oxidoreductase (Mus musculus) GI:6934274, WW domain-containing oxidor	
Mtr.46917.1.S1_at	0,01873932	2,94	down	0,73286885	-0,8223686				AT1G09240	nicotianamine synthase, putative, similar to nicotianamine synthase (Lycopersicon esculentum)(GI:4753801), nicotianamine synthase 2 (Hordeum vulgare)(GI	
Mtr.17933.1.S1_at	0,00119009	2,93	down	0,6830206	-0,86584187				AT3G02890	PHD finger protein-related, contains low similarity to PHD-finger domain proteins	1,00E-11
Mtr.26225.1.S1_at	1,19E-04	2,91	up	-0,7832456	0,75790006				AT1G03840	zinc finger (C2H2 type) family protein, contains Zinc finger,C2H2 type,domain contains Pfam domain, PF00096: Zinc finger, C2H2 type;similar t	0,12
Mtr.38756.1.S1_at	8,42E-04	2,91	down	0,7189255	-0,8204565	Mtr.6765	(Q6TQT0) Dihydroflavonal-4-reductase 2, complete	GO:0008152	AT5G42800	dihydroflavonol 4-reductase (dihydrokaempferol 4-reductase) (DFR), nearly identical to GI:166686;dihydroflavonol reductase. Catalyzes the conversion of dihy	
Mtr.34779.1.S1_at	6,78E-04	2,90	down	0,66416407	-0,8733899	Mtr.22773			AT5G55050	GDSL-motif lipase/hydrolase family protein, similar to family II lipases EXL3 GI:15054386, EXL1 GI:15054382, EXL2 GI:15054384 from (Arabi	3,00E-41
Mtr.5963.1.S1_s_at	0,00289236	2,87	up	-0,60447294	0,91745836				AT5G17730	AAA-type ATPase family protein, contains Pfam profile: ATPase family PF00004	1,00E-13
Mtr.43199.1.S1_at	0,02735931	2,85	up	-0,63340807	0,879406		similar to unknown {Arabidopsis thaliana;}, partial (34%)		AT2G32190	expressed protein	4,00E-04
Mtr.27171.1.S1_at	1,01E-04	2,85	down	0,7785265	-0,73360157				AT4G12620	Origin Recognition Complex subunit 1b. Involved in the initiation of DNA replication. Regulated transcriptionally during cell cycle, peaking at G1	0,037
Mtr.42858.1.S1_at	0,0070914	2,84	down	0,5393941	-0,96851635		similar to (O49855) Acid phosphatase (EC 3.1.3.-), partial (84%)		AT4G25150	acid phosphatase, putative, similar to acid phosphatase-1(1); Apase-1(1) (Lycopersicon esculentum) GI:7705154, acid phosphatase (Glycine m	2,00E-38
Mtr.25983.1.S1_s_at	0,02511206	2,82	up	-0,5751204	0,9185392				AT2G45220	pectinesterase family protein, contains Pfam profile: PF01095 pectinesterase	
Mtr.35242.1.S1_at	0,00264071	2,80	up	-0,6418517	0,8432147				AT4G18960	Floral homeotic gene encoding a MADS domain transcription factor. Specifies floral meristem and carpel and stamen identity. Binds CArG box s	5,60E-45
Mtr.11236.1.S1_at	0,00348275	2,75	down	0,672311	-0,7882388		weakly similar to (Q8LKG3) UDP-glucosyltransferase, partial (26%)		AT5G65550	UDP-glucuronosyl/UDP-glucosyl transferase family protein, contains Pfam profile: PF00201 UDP-glucuronosyl and UDP-glucosyl transferase ;sim	3,00E-16
Mtr.6738.1.S1_at	0,04193889	2,75	up	-0,44945797	1,0088086				AT4G38830	protein kinase family protein, contains Pfam domain PF00069: Protein kinase domain	
Mtr.5416.1.S1_at	0,00660076	2,74	down	0,7920537	-0,66023284				AT1G12570	glucose-methanol-choline (GMC) oxidoreductase family protein, similar to mandelonitrile lyase from Prunus serotina (SP|P52706, SP|P52707);	1,00E-36
Mtr.5963.1.S1_at	0,00171849	2,72	up	-0,64436436	0,7992471				AT4G09990	expressed protein, contains Pfam profile PF04669: Protein of unknown function (DUF579)	17
Mtr.37469.1.S1_s_at	0,00663907	2,69	up	-0,51205	0,9161164		similar to (Q9SHK4) F12K11.6, partial (4%)		AT1G03400	A single copy gene that encodes a protein with sequence similarity to tomato E8 (ACC synthase, the last step in ethylene biosynthesis) involved	3,00E-19
Mtr.28727.1.S1_at	0,01671438	2,69	up	-0,54101044	0,8853505				AT3G16030	lectin protein kinase family protein, contains Pfam domains PF00069: Protein kinase domain and PF01453: Lectin (probable mannose binding)	4,5
Mtr.6637.1.S1_s_at	0,01440074	2,68	up	-0,53124094	0,89250976				AT4G37080	expressed protein, contains Pfam profile PF04784: Protein of unknown function, DUF547	30
Mtr.27194.1.S1_at	6,33E-04	2,67	up	-0,6851023	0,7310977				AT1G61690	similar to expressed protein [Arabidopsis thaliana] (TAIR:At3g28770.1); similar to FYVE zinc finger containing protein [Oryza sativa (japonica c	0,56
Mtr.16773.1.S1_x_at	2,92E-05	2,66	down	0,74248747	-0,70993954				AT4G10270	wound-responsive family protein, similar to wound induced protein (GI:19320) (Lycopersicon esculentum)	1,00E-14
Mtr.34806.1.S1_s_at	0,02088282	2,66	up	-0,48204136	0,9302518				AT1G11310	seven transmembrane MLO family protein / MLO-like protein 2 (MLO2), idenctical to membrane protein Mlo2 (Arabidopsis thaliana) gi|1409157	3,00E-16
Mtr.12607.1.S1_at	1,30E-04	2,63	up	-0,6765172	0,71981496		homologue to (Q39889) Heat shock protein (EC 3.4.21.-), partial (53%)		AT1G74310	heat shock protein 101 (HSP101), identical to heat shock protein 101 GI:6715468 GB:AAF26423 from (Arabidopsis thaliana);Heat shock protein involved in ac	
Mtr.12772.1.S1_at	0,01965666	2,63	up	-0,41959938	0,9766782		similar to H. vulgare Mlo protein homolog [imported] - Arabidopsis thaliana, partial (42%)		AT1G61560	seven transmembrane MLO family protein / MLO-like protein 6 (MLO6), idenctical to membrane protein Mlo6 (Arabidopsis thaliana) gi|1409158	7,00E-04
Msa.3125.1.S1_at	0,01062056	2,63	down	0,5427675	-0,85340947				AT4G25150	acid phosphatase, putative, similar to acid phosphatase-1(1); Apase-1(1) (Lycopersicon esculentum) GI:7705154, acid phosphatase (Glycine m	2,00E-28
Mtr.37299.1.S1_at	0,0165227	2,62	up	-0,56517905	0,8234	Mtr.3401	weakly similar to (Q9ATU2) Putative cytochrome P450, partial (51%)	GO:0055114	AT3G14640	cytochrome P450, putative, similar to GB:Q05047 from (Catharanthus roseus);putative cytochrome P450	0,001
Mtr.42983.1.S1_at	0,03221185	2,60	down	0,30409512	-1,0768676		weakly similar to At2g39220 {Arabidopsis thaliana;}, partial (46%)		AT2G39220	patatin family protein, similar to patatin-like latex allergen (Hevea brasiliensis)(PMID:10589016); contains patatin domain PF01734	6,00E-39
Mtr.43311.1.S1_at	1,24E-04	2,58	up	-0,65713626	0,7101908		similar to BURP domain-containing protein [imported] - Arabidopsis thaliana, partial (13%)		AT5G25610	dehydration-responsive protein (RD22), identical to SP|Q08298 Dehydration-responsive protein RD22 precursor {Arabidopsis thaliana};respons	8,00E-12
Mtr.37992.1.S1_s_at	0,04765051	2,54	down	0,26896253	-1,0768348		homologue to (Q6UEJ2) Mini-chromosome maintenance 7, partial (25%)		AT4G02060	prolifera protein (PRL) / DNA replication licensing factor Mcm7 (MCM7), identical to DNA replication licensing factor Mcm7 SP|P43299 PROLIFER	2,00E-33
Mtr.33453.1.S1_at	0,0328239	2,54	up	-0,36449137	0,9796397				AT5G50740	copper chaperone (CCH)-related, low similarity to copper homeostasis factor (GI:3168840)(PMID: 9701579); similar to farnesylated protein AT	2,00E-27
Mtr.26988.1.S1_at	0,04475672	2,51	up	-0,5508023	0,7745064	Mtr.18014			AT3G05120	Encodes a gibberellin (GA) receptor ortholog of the rice GA receptor gene (OsGID1). Has GA-binding activity, showing higher affinity to GA4. In	0,55
Mtr.21113.1.S1_at	0,00380393	2,50	up	-0,59542376	0,7238813				ATCG00340	Encodes the D1 subunit of photosystem I and II reaction centers.	1,2
Mtr.46426.1.S1_s_at	0,01346371	2,49	up	-0,6481096	0,66749126				AT4G24010	cellulose synthase family protein, similar to Zea mays cellulose synthase-5 (gi:9622882), -4 (gi:9622880);encodes a protein similar to cellulos	1,00E-25
Mtr.19373.1.S1_at	0,00169765	2,49	down	0,6589964	-0,6556842				AT1G18560	hAT dimerisation domain-containing protein / BED zinc finger domain-containing protein / transposase-related, weak similarity to Tam3-transpo	5,00E-09
Mtr.43585.1.S1_at	0,00347397	2,48	up	-0,742671	0,5690606	Mtr.170	(Q8L692) 1-deoxy-D-xylulose 5-phosphate synthase 2 precursor, complete	GO:0008152	AT4G15560	1-deoxy-D-xylulose 5-phosphate synthase, putative / 1-deoxyxylulose-5-phosphate synthase, putative / DXP-synthase, putative (DEF) (CLA1),	4,00E-15

Mtr.2124.1.S1_s_at	0,00423661	2,48	up	-0,57578564	0,7351702	Mtr.438					AT1G66340	ethylene receptor 1 (ETR1), identical to GB:P49333 from (Arabidopsis thaliana) (Science 262 (5133), 539-544 (1993));Similar to prokaryote se	6
Msa.2287.1.S1_at	0,04789952	2,48	down	0,35495105	-0,95418006						AT4G27010	expressed protein, ; expression supported by MPSS	0,74
Mtr.6667.1.S1_at	0,03682731	2,44	up	-0,59651166	0,6931862	Mtr.21477					AT2G45570	cytochrome P450 76C2, putative (CYP76C2) (YLS6), identical to SP|O64637 Cytochrome P450 76C2 (EC 1.14.-.-) {Arabidopsis thaliana}, cDNA	3,00E-19
Mtr.28033.1.S1_at	0,00656338	2,44	up	-0,59987086	0,6849022	Mtr.527					AT4G13345	TMS membrane family protein / tumour differentially expressed (TDE) family protein, contains Pfam domain, PF03348: TMS membrane protein,	2,3
Mtr.48866.1.S1_at	0,01425081	2,43	up	-0,6382441	0,6454037						AT1G53160	squamosa promoter-binding protein-like 4 (SPL4), nearly identical to squamosa promoter binding protein-like 4 (Arabidopsis thaliana) GI:5931	5,00E-32
Mtr.19545.1.S1_at	8,04E-05	2,43	up	-0,6037547	0,67576534						AT5G42635	glycine-rich protein	3,6
Mtr.37468.1.S1_at	0,01596205	2,42	up	-0,36045232	0,91593486		weakly similar to (Q652U1) Putative 2-oxoglutarate-dependent oxygenase, partial (36%)				AT1G03400	A single copy gene that encodes a protein with sequence similarity to tomato E8 (ACC synthase, the last step in ethylene biosynthesis) involved	2,00E-12
Mtr.39897.1.S1_at	0,01033755	2,42	down	0,48978424	-0,78503376		similar to (P93697) CPRD12 protein, partial (61%)				AT3G51680	short-chain dehydrogenase/reductase (SDR) family protein, similar to short-chain alcohol dehydrogenase GI:1877480 from (Tripsacum dactyloi	4,00E-40
Mtr.3564.1.S1_at	0,02384938	2,42	up	-0,70708424	0,56540984						AT3G25090	F-box family protein-related, contains TIGRFAM TIGR01640: F-box protein interaction domain	0,86
Mtr.21000.1.S1_at	0,03711078	2,41	up	-0,2947116	0,9736832						AT3G62550	universal stress protein (USP) family protein, similar to ER6 protein (Lycopersicon esculentum) GI:5669654; contains Pfam profile PF00582: un	2,00E-33
Mtr.5819.1.S1_at	0,02239585	2,40	up	-0,5263586	0,7390712						AT2G29110	glutamate receptor family protein (GLR2.8) (GLUR9), plant glutamate receptor family, PMID:11379626;member of Putative ligand-gated ion ch	6,00E-07
Mtr.40693.1.S1_s_at	0,00148516	2,40	up	-0,65798646	0,6060764		similar to (Q8IHD5) AT12326p (SD06669p), partial (1%)				AT1G64890	integral membrane transporter family protein, contains 11 transmembrane domains; contains Pfam PF03092: BT1 family; contains TIGRFAMS 7	1,3
Mtr.24198.1.S1_s_at	6,35E-04	2,39	down	0,72883296	-0,5305452						AT3G22400	lipoxygenase, putative, similar to lipoxygenase gi:8649004 (Prunus dulcis), gi:1495802 and gi:1495804 from (Solanum tuberosum)	2,00E-21
Mtr.34205.1.S1_at	0,00863865	2,39	down	0,42281	-0,8325308						AT2G46790	Pseudo-response regulator PRR9. Involved in clock function. PRR7 and PRR9 are partially redundant essential components of a temperature-se	0,24
Mtr.1950.1.S1_at	0,00411069	2,38	up	-0,589588	0,66224957						AT2G18470	protein kinase family protein, contains Pfam PF00069: Protein kinase domain	0,41
Mtr.20953.1.S1_at	0,00100395	2,37	up	-0,57030255	0,67424506						AT1G66920	serine/threonine protein kinase, putative, similar to receptor serine/threonine kinase PR5SK gi|1235680|gb|AAC49208; contains protein kinase	4,00E-35
Mtr.6345.1.S1_at	0,02326877	2,36	up	-0,4326965	0,8079656						AT2G17880	DNAJ heat shock protein, putative, similar to J11 protein (Arabidopsis thaliana) GI:9843641; contains Pfam profile PF00226 DnaJ domain	5,00E-10
Mtr.27324.1.S1_s_at	0,03933258	2,36	up	-0,30550337	0,93030435	Mtr.5929					AT1G32170	xyloglucan:xyloglucosyl transferase, putative / xyloglucan endotransglycosylase, putative / endo-xyloglucan transferase, putative (XTR4), ident	2,00E-12
Mtr.40782.1.S1_at	0,02641483	2,35	up	-0,3146318	0,92104083		similar to unknown protein {Arabidopsis thaliana;}, partial (66%)				AT4G22920	expressed protein	3,00E-26
Mtr.52124.1.S1_s_at	0,0298969	2,35	up	-0,51609296	0,71637726	Mtr.1969					AT5G64260	phosphate-responsive protein, putative, similar to phi-1 (phosphate-induced gene) (Nicotiana tabacum) GI:3759184; contains Pfam profile PF0	7,00E-30
Mtr.8618.1.S1_at	0,00135108	2,35	down	0,58569145	-0,6440366	Mtr.15375	similar to (Q9XHC6) Cytochrome P450 monooxygenaseCYP93D1, partial (95%)		GO:0055114	AT3G26150	cytochrome P450 71B16, putative (CYP71B16), identical to cytochrome P450 71B16 (SP:Q9LTM7) (Arabidopsis thaliana); similar to cytochrome	3,00E-27
Mtr.43937.1.S1_at	0,03683478	2,34	up	-0,35573736	0,8710038		similar to probable polygalacturonase F8A5.12 [imported] - Arabidopsis	thaliana, partial (72%)		AT1G10640	polygalacturonase, putative / pectinase, putative, similar to polygalacturonase PG1 GI:5669846, PG2 GI:5669848 from (Glycine max); contains PF00295: Glyc		
Mtr.25830.1.S1_at	1,59E-04	2,33	up	-0,54833597	0,671668						AT1G52960	hypothetical protein, very low similarity to SP|Q9UUA2 DNA repair and recombination protein pif1, mitochondrial precursor {Schizosaccharomyces pombe}	
Mtr.44991.1.S1_at	0,04829693	2,32	up	-0,3025225	0,9119441		weakly similar to (Q8S1N4) Putative subtilisin-like protease, partial (21%)				AT2G05920	subtilase family protein, contains similarity to cucumisin-like serine protease GI:3176874 from (Arabidopsis thaliana)	1,00E-37
Mtr.11773.1.S1_s_at	0,01380318	2,30	up	-0,44757652	0,75535554		similar to (Q9VTC1) CG6418-PB (LD32732p), partial (2%)				AT2G32200	expressed protein, similar to expressed protein [Arabidopsis thaliana] (TAIR:At2g32210.1)	0,085
Mtr.34717.1.S1_at	0,03138883	2,29	down	0,5005123	-0,69683695						AT1G65870	disease resistance-responsive family protein, similar to dirigent protein (Forsythia x intermedia) gi|6694693|gb|AAF25357; similar to disease re	1,00E-34
Mtr.35526.1.S1_at	0,00502801	2,26	down	0,65420276	-0,52173233		weakly similar to (Q682P3) Putative GDSL-motif lipase/hydrolase, partial (15%)				AT2G04570	GDSL-motif lipase/hydrolase family protein, similar to family II lipase EXL3 (GI:15054386), EXL1 (GI:15054382), EXL2 (GI:15054384) (Arabid	7,00E-35
Mtr.44153.1.S1_s_at	0,04776736	2,25	up	-0,22610013	0,9442127		weakly similar to (Q9FN75) AAA-type ATPase-like protein, partial (8%)				AT3G50930	AAA-type ATPase family protein, contains Pfam profile: ATPase family PF00004	7,00E-36
Mtr.9799.1.S1_at	0,00294906	2,25	down	0,59613675	-0,5732377		similar to (Q9SIM0) Expressed protein (Nucleolar protein family A member 3), partial (90%)				AT2G20490	nucleolar RNA-binding Nop10p family protein, similar to Nop10p (GI:8096260) (Homo sapiens);expressed protein, similar to hypothetical prote	2,00E-25
Mtr.37676.1.S1_at	0,03223404	2,24	up	-0,31778893	0,84848374		similar to (Q9LY87) Hypothetical protein F18O22_210 (Hypothetical protein At5g14420), partial (72%)				AT5G14420	copine-related, low similarity to SP|Q99829 Copine I {Homo sapiens}	1,00E-22
Msa.1505.1.S1_at	0,02059934	2,24	up	-0,29961237	0,866333						AT4G22920	expressed protein	
Mtr.10941.1.S1_at	0,00498833	2,24	up	-0,44171476	0,72327423	Mtr.1195	similar to (Q84TG0) At5g02580, partial (84%)				AT5G02580	expressed protein	4,00E-05
Mtr.11811.1.S1_s_at	0,01886271	2,24	up	-0,49704996	0,66744137	Mtr.22500	similar to phi-1-like protein {Arabidopsis thaliana;}, partial (47%)				AT5G64260	phosphate-responsive protein, putative, similar to phi-1 (phosphate-induced gene) (Nicotiana tabacum) GI:3759184; contains Pfam profile PF0	4,00E-34
Mtr.27822.1.S1_at	0,01698365	2,24	down	0,3555781	-0,8074729						AT1G77380	Amino acid permease which transports basic amino acids.;amino acid carrier, putative / amino acid permease, putative, strong similarity to amino acid carrier	
Mtr.12119.1.S1_at	7,34E-05	2,23	down	0,552481	-0,60591316		homologue to pseudo-response regulator 5 {Arabidopsis thaliana;}, partial (5%)				AT3G13810	zinc finger (C2H2 type) family protein, similar to finger protein pcp1 GB:S48856 from (Solanum tuberosum) contains Pfam domain, PF00096:	0,072
Mtr.41303.1.S1_at	0,00445836	2,22	up	-0,46209273	0,6902876		similar to (Q9LVB9) Emb|CAB83157.1 (Hypothetical protein At5g56550), partial (25%)				AT5G56550	expressed protein	2,00E-16
Mtr.11851.1.S1_at	0,0011399	2,22	up	-0,6245022	0,5267706		weakly similar to (Q75CR7) ACL148Cp, partial (19%)				AT1G22440	alcohol dehydrogenase, putative, similar to alcohol dehydrogenase ADH GI:7705214 from (Lycopersicon esculentum); contains Pfam zinc-bindi	3,00E-33
Mtr.37312.1.S1_at	0,00811537	2,21	up	-0,46346727	0,681756		homologue to (Q6L509) Putative hsp70, complete				AT3G12580	heat shock protein 70, putative / HSP70, putative, strong similarity to heat shock protein GI:425194 (Spinacia oleracea)	2,00E-39
Mtr.32702.1.S1_at	0,02710983	2,21	up	-0,42805734	0,7136702	Mtr.232					AT3G22550	senescence-associated protein-related, similar to senescence-associated protein SAG102 (GI:22331931) (Arabidopsis thaliana)	1,00E-17
Mtr.39945.1.S1_at	0,04893456	2,20	up	-0,5296195	0,61107635		weakly similar to unknown protein {Arabidopsis thaliana;}, partial (29%)				AT1G30755	expressed protein, contains Pfam domain PF05003: protein of unknown function (DUF668)	
Mtr.42492.1.S1_at	0,00372321	2,20	down	0,40876135	-0,72686386		similar to (Q8H5P9) Putative esterase, partial (22%)				AT5G23530	expressed protein, contains similarity to PrMC3 (Pinus radiata) GI:5487873	1,00E-42
Mtr.3858.1.S1_at	0,04804087	2,19	down	0,20410745	-0,92907935	Mtr.20724			GO:0055114	AT4G22880	leucoanthocyanidin dioxygenase, putative / anthocyanidin synthase, putative, similar to SP	P51091 (Malus domestica); contains PF03171 2OG-	2,00E-23
Mtr.11266.1.S1_at	0,00126873	2,19	up	-0,620114	0,5114444		similar to unknown protein {Arabidopsis thaliana;}, partial (14%)				AT5G56860	zinc finger (GATA type) family protein, similar to unknown protein (pir ||T04270)	0,032
Mtr.28700.1.S1_at	0,00670752	2,19	up	-0,6484351	0,48130736	Mtr.12516					AT4G30430	Member of TETRASPANIN family	0,42
Mtr.28324.1.S1_at	0,00359612	2,19	down	0,58515865	-0,54395103						AT5G52450	MATE efflux protein-related, strong similarity to unknown protein (pir||T02324); contains Pfam profile PF01554 Uncharacterized membrane pro	5,00E-43
Mtr.31456.1.S1_at	0,02253804	2,19	up	-0,49298587	0,63532925	Mtr.750					AT5G50160	Encodes a ferric chelate reductase that is expressed in shoots and flowers.	6,00E-38
Mtr.42289.1.S1_at	6,91E-04	2,18	up	-0,5716073	0,5543935		homologue to DVL4 {Arabidopsis thaliana;}, partial (85%)				AT1G13245	expressed protein	2,00E-08
Mtr.46049.1.S1_at	9,82E-05	2,18	up	-0,59157705	0,5323817						AT4G18960	Floral homeotic gene encoding a MADS domain transcription factor. Specifies floral meristem and carpel and stamen identity. Binds CArG box sequences. It is t	
Mtr.23616.1.S1_at	0,00106495	2,18	up	-0,5302186	0,5913088						AT2G40750	WRKY family transcription factor, contains Pfam profile: PF03106 WRKY DNA -binding domain;member of WRKY Transcription Factor; Group III	1,00E-18
Mtr.13004.1.S1_at	0,00624652	2,17	down	0,685709	-0,43277097						AT2G04045	Encodes a defensin-like (DEFL) family protein.	0,017
Mtr.9402.1.S1_at	0,00431932	2,16	down	0,45434475	-0,6551852	Mtr.8525	weakly similar to (Q940H4) Putative aldehyde dehydrogenase, partial (24%)		GO:0006081	AT4G34240	Aldehyde dehydrogenase induced by ABA and dehydration;aldehyde dehydrogenase (ALDH3), similar to aldehyde dehydrogenase (Arabidopsis	2,00E-34
Mtr.9820.1.S1_at	0,01864158	2,15	up	-0,3361199	0,7663803		homologue to (Q8H6F8) MADS box protein GHMADS-2, partial (95%)				AT4G09960	MADS-box protein (AGL11);a MADS box transcription factor expressed in the carpel and ovules	1,00E-36
Mtr.40942.1.S1_at	3,90E-04	2,15	down	0,57785845	-0,52446175		similar to (O04899) Caffeoyl-CoA O-methyltransferase 5 (Trans-caffeoyl-CoA 3-O-methyltransferase 5) (CCoA			AT4G34050	caffeoyl-CoA 3-O-methyltransferase, putative, nearly identical to GI:2960356 (Populus balsamifera subsp. trichocarpa), GI:684942 (Medicago s	1,00E-15
Mtr.11888.1.S1_at	8,86E-04	2,13	down	0,5561619	-0,5375894	Mtr.8525	weakly similar to (Q940H4) Putative aldehyde dehydrogenase, partial (42%)		GO:0006081	AT4G34240	Aldehyde dehydrogenase induced by ABA and dehydration;aldehyde dehydrogenase (ALDH3), similar to aldehyde dehydrogenase (Arabidopsis	7,00E-33
Mtr.16226.1.S1_at	0,02516394	2,13	down	0,43341652	-0,65945417						AT5G57630	CBL-interacting protein kinase 21, putative (CIPK21), identical to CBL-interacting protein kinase 21 (Arabidopsis thaliana) gi|14334390|gb|AAK	2,00E-26
Mtr.4724.1.S1_at	0,00509508	2,12	up	-0,39572462	0,690026						AT3G20660	similar to sugar transporter family protein [Arabidopsis thaliana] (TAIR:At1g73220.1); similar to putative organic cation transport protein [Phas	2,00E-23
Mtr.19998.1.S1_at	6,84E-04	2,11	up	-0,6234328	0,4571356						AT2G35930	U-box domain-containing protein, similar to immediate-early fungal elicitor protein CMPG1 (Petroselinum crispum) GI:14582200; contains Pfam profile PF0456	
Mtr.46511.1.S1_x_at	0,00711401	2,11	down	0,4539124	-0,62556505						AT1G78970	lupeol synthase (LUP1) / 2,3-oxidosqualene-triterpenoid cyclase, identical to lupeol synthase GI:1762150 from (Arabidopsis thaliana), 2,3-oxidosqualene-triter	
Mtr.12809.1.S1_at	0,01091986	2,11	up	-0,36435938	0,7138839		weakly similar to (Q6TFM2) Dihydroneopterin aldolase, partial (92%)				AT3G11750	dihydroneopterin aldolase, putative, similar to SP|P28823 Dihydroneopterin aldolase (EC 4.1.2.25) (DHNA) {Bacillus subtilis}; contains Pfam pr	5,00E-37
Mtr.13942.1.S1_at	0,00352039	2,11	down	0,43572283	-0,64062995		similar to (O48940) Polyphosphoinositide binding protein Ssh2p, partial (89%)				AT1G01630	SEC14 cytosolic factor, putative / phosphoglyceride transfer protein, putative, contains Pfam PF00650 : CRAL/TRIO domain and PF03765 : CRA	2,00E-23
Mtr.1946.1.S1_at	0,0144432	2,11	up	-0,2963573	0,77936524						AT4G18195	Member of a family of proteins related to PUP1, a purine transporter. May be involved in the transport of purine and purine derivatives such as	1,6
Mtr.41053.1.S1_at	0,01532822	2,10	up	-0,37948704	0,6912249		similar to (Q9LTA2) Similarity to AT-hook DNA-binding protein, partial (53%)				AT5G49700	DNA-binding protein-related, contains Pfam domain PF03479: Domain of unknown function (DUF296), found in AT-hook motifs Pfam:PF02178	7,00E-05
Mtr.35.1.S1_at	0,03061374	2,10	up	-0,32710457	0,74204284						AT2G05540	glycine-rich protein	9,4
Mtr.12632.1.S1_at	4,09E-04	2,10	up	-0,5636873	0,505192	Mtr.6713	(Q6WNQ9) Isoflavone 3'-hydroxylase (Fragment), complete		GO:0055114	AT5G67310	cytochrome P450 family protein;member of CYP81G	7,00E-11	
Mtr.23387.1.S1_x_at	0,00368816	2,09	up	-0,5612553	0,5000828						AT2G29500	17.6 kDa class I small heat shock protein (HSP17.6B-CI), contains Pfam PF00011: Hsp20/alpha crystallin family; identified in Scharf, K-D., et al, Cell Stress &		
Mtr.32795.1.S1_x_at	5,54E-04	2,09	down	0,50606894	-0,55469674						AT1G06830	glutaredoxin family protein, contains INTERPRO Domain IPR002109, Glutaredoxin (thioltransferase)	2,00E-26	
Mtr.16417.1.S1_at	6,12E-04	2,09	up	-0,54662776	0,5139775						AT3G13940	expressed protein, weak similarity to DNA-directed RNA polymerase I 49 kDa polypeptide (EC 2.7.7.6) (A49) (Swiss-Prot:O14086) (Schizosacc	2,00E-19	
Mtr.13139.1.S1_at	0,0040551	2,08	up	-0,44268194	0,6164195		similar to carbonate dehydratase-like protein {Arabidopsis thaliana;}, partial (35%)				AT4G33580	carbonic anhydrase family protein / carbonate dehydratase family protein, similar to SP|P46512 Carbonic anhydrase 1 (EC 4.2.1.1) (Carbonate	1,00E-26	
Mtr.10289.1.S1_at	0,01309419	2,08	down	0,4756743	-0,58276224		similar to (Q9LEG4) SANT/MYB domain protein (SANT/MYB protein), partial (80%)				AT2G21650	myb family transcription factor, contains PFAM profile: PF00249 myb-like DNA-binding domain	3,00E-23	
Mtr.43887.1.S1_at	0,0017805	2,08	down	0,4991986	-0,55817443		similar to At3g13310 {Arabidopsis thaliana;}, partial (30%)				AT3G13310	DNAJ heat shock N-terminal domain-containing protein, similar to J11 protein (Arabidopsis thaliana) GI:9843641; contains Pfam profile: PF002	3,00E-22	
Mtr.38909.1.S1_at	0,02294932	2,07	up	-0,31026968	0,7421877		homologue to (Q6K3R9) Putative bZIP transcription factor RF2b, partial (7%)				AT1G40133	hypothetical protein	2	
Msa.1174.1.S1_at	0,01766144	2,07	up	-0,2879038	0,7628517						AT3G55840	expressed protein	2,00E-40	
Mtr.37341.1.S1_at	0,003218	2,07	down	0,51482326	-0,5346119		similar to (Q9ZRF1) Probable mannitol dehydrogenase (NAD-dependent mannitol dehydrogenase), partial (57			AT4G37980	mannitol dehydrogenase, putative (ELI3-1), identical to GI:16267;similar to mannitol dehydrogenase, putative (ELI3-2) [Arabidopsis thaliana]	1,00E-33	
Msa.1673.1.S1_at	0,02269312	2,07	up	-0,27926302	0,7686885						AT1G32170	xyloglucan:xyloglucosyl transferase, putative / xyloglucan endotransglycosylase, putative / endo-xyloglucan transferase, putative (XTR4), identical to N-termin		
Mtr.1509.1.S1_at	0,00570967	2,07	up	-0,5557278	0,4914956						AT5G12200	telomeric DNA-binding protein 1 (TBP1), identical to telomeric DNA-binding protein 1 (Arabidopsis thaliana) gi|13641340|gb|AAK31590|Encode	1,6	
Mtr.52213.1.S1_a_at	0,03284819	2,07	up	-0,6090856	0,43805423						AT3G07960	phosphatidylinositol-4-phosphate 5-kinase family protein, similar to phosphatidylinositol-4-phosphate 5-kinase AtPIP5K1 (Arabidopsis thaliana)	1,6	
Mtr.2640.1.S1_at	1,87E-04	2,07	down	0,5015361	-0,5454071						AT5G04360	Encodes an enzyme thought to be involved in the hydrolysis of the α-1,6 linkages during starch degradation in seed endosperm. However, a knockout m		
Mtr.35741.1.S1_at	0,00609084	2,06	up	-0,4720087	0,57157993		weakly similar to unknown protein {Arabidopsis thaliana;}, partial (5%)				AT3G21330	basic helix-loop-helix (bHLH) family protein, contains Pfam profile: PF00010 helix-loop-helix DNA-binding domain	2,00E-11	
Msa.1829.1.S1_at	0,00624154	2,06	up	-0,38488913	0,6569848						AT1G60870	expressed protein	1,00E-13	
Mtr.21953.1.S1_at	0,01817881	2,05	up	-0,36543322	0,6736328						AT1G75620	glyoxal oxidase-related, contains similarity to glyoxal oxidase precursor (Phanerochaete chrysosporium) gi|1050302|gb|AAA87594		
Mtr.1427.1.S1_at	0,02093853	2,05	up	-0,39319023	0,6431448						AT4G01880	expressed protein, contains Pfam PF05206: Protein of unknown function (DUF715)	7,9	
Mtr.20884.1.S1_at	0,02571082	2,05	down	0,5006625	-0,5353133						AT4G16765	oxidoreductase, 2OG-Fe(II) oxygenase family protein, similar to SP|P40902 isp7 from Schizosaccharomyces pombe, GI:475959 flavanone-3-hydroxylase (nari		
Mtr.11118.1.S1_at	0,04291789	2,05	up	-0,3748447	0,66111976		weakly similar to probable calcium-binding protein F21C20.130 - Arabidopsis	thaliana, partial (17%		AT1G21550	calcium-binding protein, putative, contains similarity to calcium-binding protein GB:CAB63264 GI:6580549 from (Lotus japonicus)	1,00E-09	
Mtr.40178.1.S1_at	0,03164558	2,05	up	-0,43728986	0,5983381		similar to (Q9M711) Putative Hs1pro-1 homolog, partial (95%)				AT3G55840	expressed protein	2,00E-14	
Mtr.38476.1.S1_at	0,00205684	2,05	down	0,38688087	-0,64616394		homologue to (Q9T0G2) Probable cytochrome c At4g10040, complete				AT4G10040	Encodes cytochrome c. Promoter directs preferential expression in vascular tissues of cotyledons, leaves, roots, and hypocotyls, and in anthers.		
Mtr.3023.1.S1_at	5,84E-05	2,04	up	-0,49843392	0,52858084						AT1G66910	protein kinase, putative, similar to receptor serine/threonine kinase PR5K gi|1235680|gb|AAC49208		
Mtr.11846.1.S1_at	0,01474714	2,04	down	0,6947941	-0,33098158		similar to (Q40318) Coil protein, partial (74%)				AT5G14450	GDSL-motif lipase/hydrolase family protein, similar to early nodulin ENOD8 (Medicago sativa) GI:304037, elicitor-induced glycoprotein iEP4 (Da	1,00E-37	
Mtr.25722.1.S1_at	0,00568286	2,04	up	-0,42345038	0,60193187						AT2G25010	expressed protein	1,00E-07	
Msa.1305.1.S1_at	0,03343777	2,03	up	-0,3636376	0,6593569						AT1G06620	encodes a protein whose sequence is similar to a 2-oxoglutarate-dependent dioxygenase		
Mtr.49892.1.S1_at	0,00833817	2,03	up	-0,5256764	0,49557623						AT1G52570	phospholipase D alpha 2 / PLD alpha 2 (PLDALPHA2) (PLD2) / choline phosphatase 2, identical to phospholipase D alpha 2 (PLD alpha 2) SP:Q	0,011	
Mtr.20739.1.S1_s_at	0,03024604	2,03	up	-0,2894919	0,72891027						AT5G38240	serine/threonine protein kinase, putative, similar to receptor serine/threonine kinase PR5SK gi|1235680|gb|AAC49208; contains protein kinase	4,00E-23	
Mtr.4947.1.S1_at	0,02480785	2,03	down	0,31819597	-0,70009345						AT5G52400	cytochrome P450 family protein, similar to Cytochrome P450 72A1 (SP:Q05047) (cytochrome P450 lxxii hydroxylase) (ge10h) (Catharanthus r	3,00E-39	
Mtr.5980.1.S1_at	0,03265605	2,02	up	-0,4458022	0,5690225						AT5G54280	myosin heavy chain, putative, similar to myosin (Arabidopsis thaliana) gi|499045|emb|CAA84065;Type VII myosin gene	8,00E-33	
Mtr.45613.1.S1_s_at	0,04069394	2,02	down	0,80718917	-0,20678456						ATCG00700	PSII low MW protein	7,00E-15	
Mtr.2811.1.S1_at	0,00894408	2,02	up	-0,34014097	0,6718461						AT3G44300	nitrilase 2 (NIT2), identical to SP|P32962 Nitrilase 2 (EC 3.5.5.1) {Arabidopsis thaliana};encodes a nitrilase (nitrile aminohydrolase, EC 3.5.5.1	13	
Mtr.42374.1.S1_at	2,34E-04	2,01	up	-0,46897253	0,5403188		similar to unknown protein {Arabidopsis thaliana;}, partial (54%)				AT3G28760	expressed protein, contains Pfam PF01959: predicted 3-dehydroquinate synthase;similar to conserved protein [Methanothermobacter thermautotrophicus str.		

Mtr.25841.1.S1_at	0,00146026	2,01	up	-0,4757236	0,5328925				AT2G37170	plasma membrane intrinsic protein 2B (PIP2B) / aquaporin PIP2.2 (PIP2.2), identical to SP|P43287 Plasma membrane intrinsic protein 2B {Arabidopsis thaliana	
Mtr.15849.1.S1_at	0,00449497	2,01	down	0,4755977	-0,53089887				AT2G21660	encodes a glycine-rich RNA binding protein. Gene expression is induced by cold.;glycine-rich RNA-binding protein (GRP7), SP|Q03250 Glycine-r	4,6
Mtr.525.1.S1_at	7,69E-06	2,01	down	0,50661486	-0,49920782				AT2G43200	dehydration-responsive family protein, similar to early-responsive to dehydration stress ERD3 protein (Arabidopsis thaliana) GI:15320410; contains Pfam profi	
Mtr.16941.1.S1_at	0,01295574	2,01	down	0,38053575	-0,6244936				AT2G15490	UDP-glucoronosyl/UDP-glucosyl transferase family protein, contains Pfam profile: PF00201 UDP-glucoronosyl and UDP-glucosyl transferase	
Mtr.5147.1.S1_at	0,00417179	2,00	up	-0,35119882	0,65048426				AT3G46730	disease resistance protein (CC-NBS class), putative, domain signature CC-NBS exists, suggestive of a disease resistance protein.	2,00E-10
Mtr.32924.1.S1_at	0,02421994	2,00	up	-0,4808809	0,520669				AT5G52640	heat shock protein 81-1 (HSP81-1) / heat shock protein 83 (HSP83), nearly identical to SP|P27323 Heat shock protein 81-1 (HSP81-1) (Heat shock protein 83	
Mtr.16.1.S1_s_at	0,01594848	2,00	up	-0,64575034	0,35212383						
Mtr.38907.1.S1_at	0,00346975	1,99	up	-0,4053507	0,5865269	similar to (Q9LW35) Alcohol dehydrogenase-like protein, partial (36%)			AT2G38630	expressed protein	10
Mtr.26021.1.S1_x_at	0,03263372	1,99	up	-0,5753575	0,4140512				AT2G07520	hypothetical protein	
Mtr.7135.1.S1_at	5,70E-06	1,99	down	0,4790109	-0,51018333				AT5G13000	glycosyl transferase family 48 protein, contains Pfam profile: PF02364 1,3-beta-glucan synthase;encodes a gene similar to callose synthase	28
Mtr.19211.1.S1_at	0,004094	1,98	up	-0,5090697	0,47857127				AT3G48000	putative (NAD+) aldehyde dehydrogenase (At3g48000) mRNA,;aldehyde dehydrogenase (ALDH2), identical to aldehyde dehydrogenase (Arabic	9,00E-15
Mtr.17080.1.S1_s_at	0,00279905	1,98	down	0,5477087	-0,4391489				AT1G02460	glycoside hydrolase family 28 protein / polygalacturonase (pectinase) family protein, similar to polygalacturonase PG1 GI:5669846, PG2 GI:5669848 from (Gly	
Mtr.42552.1.S1_at	0,00343677	1,98	up	-0,44966903	0,53622407	weakly similar to (Q66PF2) Putative UDP-rhamnose:rhamnosyltransferase, partial (28%)			AT5G49690	UDP-glucoronosyl/UDP-glucosyl transferase family protein, contains Pfam profile: PF00201 UDP-glucoronosyl and UDP-glucosyl transferase	4,00E-34
Mtr.37525.1.S1_at	0,01378518	1,98	down	0,3110784	-0,67420244	(Q8W4T7) Multifunctional aquaporin, complete			AT4G18910	aquaglyceroporin / NOD26-like major intrinsic protein 2 (NLM2), contains Pfam profile: MIP PF00230; similar to SP:P08995 {Glycine max} Nod	0,014
Mtr.38274.1.S1_at	0,02847345	1,98	up	-0,3735547	0,61126757	similar to (O64989) Steroid 22-alpha-hydroxylase, partial (29%)			AT3G50660	steroid 22-alpha-hydroxylase (CYP90B1) (DWF4), identical to gi:2935342;Encodes a 22α hydroxylase whose reaction is a rate-limiting step in brassinost	
Mtr.35650.1.S1_at	0,00875855	1,98	down	0,3960414	-0,5880558	similar to KIC-related protein {Arabidopsis thaliana;}, partial (74%)			AT4G27280	calcium-binding EF hand family protein, similar to EF-hand Ca2+-binding protein CCD1 (Triticum aestivum) GI:9255753; contains INTERPRO:IP	1,00E-34
Mtr.11900.1.S1_at	0,00245909	1,98	up	-0,40788078	0,57457894	(Q9SC74) Hypothetical protein I1332.3, partial (6%)			AT4G10613	similar to hypothetical protein [Arabidopsis thaliana] (TAIR:At3g25720.1); similar to orf147a [Beta vulgaris subsp. vulgaris] (GB:BAD66776.1)	0,013
Mtr.36012.1.S1_at	6,59E-04	1,97	down	0,49034515	-0,4878362	Mtr.4702			AT3G12040	DNA-3-methyladenine glycosylase (MAG), identical to DNA-3-methlyadenine glycosylase (MAG) SP:Q39147 from (Arabidopsis thaliana); contai	2,00E-16
Mtr.10450.1.S1_at	0,03465901	1,97	down	0,2679901	-0,7096383	weakly similar to unknown protein {Oryza sativa (japonica cultivar-group);}, partial (70%)			AT2G19630	F-box family protein, contains F-box domain Pfam:PF00646	2,7
Mtr.15850.1.S1_at	4,63E-05	1,97	down	0,5324154	-0,4438254				AT2G21660	encodes a glycine-rich RNA binding protein. Gene expression is induced by cold.;glycine-rich RNA-binding protein (GRP7), SP|Q03250 Glycine-r	4,00E-06
Mtr.41373.1.S1_at	0,01900692	1,97	up	-0,35628986	0,61859417	Mtr.1969 similar to phi-1-like protein {Arabidopsis thaliana;}, partial (34%)			AT3G63070	PWWP domain-containing protein, putative transcription factor HUA2, Arabidopsis thaliana, EMBL:AF116556	0,42
Mtr.23387.1.S1_at	0,00367365	1,97	up	-0,4824896	0,49215984				AT5G59720	18.1 kDa class I heat shock protein (HSP18.1-CI), identical to 18.2 kDa class I heat shock protein (HSP 18.2) (SP:P19037)(Arabidopsis thaliana	9,00E-05
Mtr.15770.1.S1_at	0,00783861	1,96	down	0,40173754	-0,5727415				AT4G16146	expressed protein	2,00E-27
Mtr.22455.1.S1_at	0,00462424	1,96	down	0,4767324	-0,4955069				AT1G76520	auxin efflux carrier family protein, contains auxin efflux carrier domain, Pfam:PF03547	
Mtr.2860.1.S1_at	0,00634877	1,96	down	0,5988886	-0,36998406				AT1G24470	short-chain dehydrogenase/reductase (SDR) family protein, similar to b-keto acyl reductase GI:2586127 from (Hordeum vulgare)	1,40E-45
Mtr.42489.1.S1_at	0,01660444	1,95	up	-0,382349	0,58246756	weakly similar to unknown protein 4797-5312 [imported] - Arabidopsis thaliana {Arabidopsis thaliana;}, part	AT1G60870	expressed protein	1,00E-28
Mtr.37873.1.S1_at	0,02026118	1,95	up	-0,36262575	0,60034275				AT3G29060	similar to EXS family protein / ERD1/XPR1/SYG1 family protein [Arabidopsis thaliana] (TAIR:At2g03260.1); similar to EXS family protein / ERD	18
Mtr.27364.1.S1_at	0,00936148	1,94	down	0,4832623	-0,47577038	Mtr.19156			AT4G08980	F-box family protein (FBW2), contains similarity to N7 protein GI:3273101 from (Medicago truncatula)	2,00E-15
Mtr.8516.1.S1_at	3,39E-04	1,94	down	0,4924499	-0,4646255	similar to (Q8LLW2) Receptor-like kinase Xa21-binding protein 3, partial (71%)			AT5G12400	PHD finger transcription factor, putative, similarity to predicted proteins, Arabidopsis thaliana	1,1
Mtr.9046.1.S1_at	0,01441996	1,94	up	-0,30137697	0,6541802	similar to (Q6AUP4) Putative gibberellin-induced protein (Hypothetical protein OSJNBa0039A21.1), partial (51%	AT1G74670	gibberellin-responsive protein, putative, similar to SP|P46690 Gibberellin-regulated protein 4 precursor {Arabidopsis thaliana} GASA4; contains	1,00E-32
Mtr.47266.1.S1_at	0,02794507	1,94	up	-0,44322267	0,51132077				AT3G56400	WRKY family transcription factor, DNA-binding protein 4 WRKY4 - Nicotiana tabacum, EMBL:AF193771;member of WRKY Transcription Factor; (	7,00E-25
Mtr.27943.1.S1_at	0,00153697	1,94	down	0,41445145	-0,5382584	Mtr.16884			AT5G41970	similar to expressed protein [Arabidopsis thaliana] (TAIR:At3g49320.1); similar to LOC496075 protein [Xenopus laevis] (GB:AAH87491.1); contains InterPro d	
Mtr.17470.1.S1_at	0,01795484	1,94	up	-0,5616854	0,39091477				AT5G64770	expressed protein	7,00E-04
Mtr.13033.1.S1_at	0,00965766	1,94	up	-0,41022173	0,5421155	similar to AT5g44130/MLN1_5 {Arabidopsis thaliana;}, partial (50%)			AT2G20520	fasciclin-like arabinogalactan-protein (FLA6), identical to gi|13377780_gb_AAK20859;AF333972 Arabidopsis thaliana fasciclin-like arabinogalac	8,00E-38
Mtr.39780.1.S1_at	0,01098163	1,93	up	-0,54048115	0,4113016	similar to keratin type II cytoskeletal - mouse (fragment) {Mus musculus;}, partial (4%)			AT3G13810	zinc finger (C2H2 type) family protein, similar to finger protein pcp1 GB:S48856 from (Solanum tuberosum) contains Pfam domain, PF00096:	1,6
Mtr.32997.1.S1_x_at	0,00960979	1,93	up	-0,5683356	0,38258234				AT4G21410	protein kinase family protein, contains Pfam PF00069: Protein kinase domain	4,00E-33
Mtr.50062.1.S1_at	0,00637576	1,93	up	-0,45852223	0,48916736				AT1G01120	fatty acid elongase 3-ketoacyl-CoA synthase 1 (KCS1), nearly identical to GB:AAC99312 GI:4091810 from (Arabidopsis thaliana);Encodes a co	2,1
Mtr.9076.1.S1_at	0,00510702	1,93	up	-0,37192997	0,57375604	Mtr.150 (Q9LLM2) MTD2, complete	GO:0005515	AT5G01520	zinc finger (C3HC4-type RING finger) family protein, similar to MTD2 (Medicago truncatula) GI:9294812; contains Pfam profile PF00097: Zinc f	1,00E-28
Mtr.31464.1.S1_at	0,0208729	1,92	up	-0,52782565	0,41276646	Mtr.790			AT2G28030	aspartic protease family protein, contains Pfam domain, PF00026: eukaryotic aspartyl protease	2,7
AFFX-r2-Ec-bioB-M_at	0,02781199	1,91	down	0,6816289	-0,2546487		GO:0008152	AT2G43360	biotin synthase (BioB) (BIO2), identical to SP|P54967 Pfam profile PF04055: radical SAM domain protein;Catalyzes the conversion of dethiobiot	1,00E-30
Mtr.50426.1.S1_at	2,88E-04	1,91	down	0,42979494	-0,505894				AT1G55020	lipoxygenase (LOX1), identical to SP|Q06327;lipoxygenase, a defense gene conferring resistance Xanthomonas campestris	2,00E-40
Mtr.42587.1.S1_at	1,46E-04	1,90	down	0,49483743	-0,4337697	similar to (Q9LZR6) Heat shock transcription factor-like protein, partial (14%)			AT5G03720	member of Heat Stress Transcription Factor (Hsf) family;heat shock transcription factor family protein, contains Pfam profile: PF00447 HSF-typ	2,00E-31
Mtr.44211.1.S1_at	0,00698409	1,90	up	-0,5089081	0,41832685	similar to (Q84Z33) Hypothetical protein OSJNBa0036M16.104 (Hypothetical protein P0650C03.19), partial (5	AT4G29370	kelch repeat-containing F-box family protein, contains F-box domain Pfam:PF00646 and Kelch motif Pfam:PF01344	13
Mtr.22479.1.S1_at	0,02248909	1,90	down	0,3364277	-0,59021664				AT4G09820	similar to basic helix-loop-helix (bHLH) family protein [Arabidopsis thaliana] (TAIR:At1g63650.2); similar to basic helix-loop-helix (bHLH) famil	2,00E-31
Mtr.41625.1.S1_s_at	0,00347679	1,90	down	0,4377707	-0,48757133	homologue to (Q8H2B1) DnaJ-like protein, partial (52%)			AT1G56300	DNAJ heat shock N-terminal domain-containing protein, similar to SP|Q9QYI7 DnaJ homolog subfamily B member 8 Mus musculus; contains Pfa	1,00E-24
Mtr.12899.1.S1_at	0,00515548	1,90	up	-0,56026953	0,36316887				AT1G51480	disease resistance protein (CC-NBS-LRR class), putative, domain signature CC-NBS-LRR exists, suggestive of a disease resistance protein.	30
Mtr.17068.1.S1_at	0,0128481	1,90	down	0,2713283	-0,65145904				AT1G24130	transducin family protein / WD-40 repeat family protein, contains 7 WD-40 repeats (PF00400);similar to beta transducin-like protein HET-D2Y (GI:17225210)	
Mtr.11503.1.S1_at	0,01245578	1,89	down	0,5770559	-0,34293714	weakly similar to (Q8H0I1) Hypothetical protein B57, partial (9%)			AT5G52300	low-temperature-responsive 65 kD protein (LT165) / desiccation-responsive protein 29B (RD29B), nearly identical to SP|Q04980 Low-temperat	7,00E-12
Mtr.42021.1.S1_at	0,00219104	1,89	up	-0,5484188	0,37085852	homologue to (Q9XER4) 1-aminocyclopropane-1-carboxylate oxidase (EC 1.4.3.-), partial (36%)			AT1G12010	encodes a protein whose sequence is similar to 1-amino-cyclopropane-1-carboxylic acid oxidase	3,00E-28
Mtr.48925.1.S1_at	0,00461812	1,89	down	0,41122374	-0,5071319				AT5G40240	nodulin MtN21 family protein, similar to MtN21 (Medicago truncatula) GI:2598575; contains Pfam profile PF00892: Integral membrane protein	1,00E-35
Mtr.19820.1.S1_at	0,00345091	1,89	up	-0,47606054	0,43956184				AT5G07090	40S ribosomal protein S4 (RPS4B);similar to 40S ribosomal protein S4 (RPS4D) [Arabidopsis thaliana] (TAIR:At5g58420.1); similar to ribosomal protein S4 [Se	
Mtr.23797.1.S1_s_at	0,02191804	1,88	down	0,27976593	-0,6347658	Mtr.12650	GO:0005975	AT3G23730	xyloglucan:xyloglucosyl transferase, putative / xyloglucan endotransglycosylase, putative / endo-xyloglucan transferase, putative, similar to xy	1,00E-16
Mtr.4701.1.S1_s_at	0,01422906	1,88	up	-0,49309888	0,42142963	Mtr.1636			AT4G30250	AAA-type ATPase family protein, contains Pfam profile: ATPase family PF00004	6
Mtr.48351.1.S1_at	0,0384099	1,88	down	0,24147923	-0,67148274				AT1G17860	trypsin and protease inhibitor family protein / Kunitz family protein, similar to LeMir (miraculin homolog) GI:2654440 from (Lycopersicon esculc	2,00E-06
Mtr.43267.1.S1_at	3,16E-04	1,88	down	0,42979464	-0,4826932	Mtr.8970 weakly similar to (Q9LUC5) Cytochrome P450, partial (63%)	GO:0055114	AT3G14680	cytochrome P450, putative, similar to GB:Q05047 from (Catharanthus roseus);putative cytochrome P450	6,00E-41
Mtr.9388.1.S1_at	0,01771988	1,88	up	-0,2837474	0,6278138	similar to (Q22900) Probable WRKY transcription factor 23 (WRKY DNA-binding protein 23), partial (41%)			AT2G47260	WRKY family transcription factor, contains Pfam profile: PF03106 WRKY DNA -binding domain;member of WRKY Transcription Factor; Group I	0,002
Mtr.35539.1.S1_at	0,04785891	1,88	down	0,23874919	-0,67153263	similar to (Q6KAJ4) Putative minichromosome maintenance deficient protein 5, partial (12%)			AT3G07690	minichromosome maintenance family protein / MCM family protein, similar to SP|P55862 DNA replication licensing factor MCM5 (CDC46 homolo	4,00E-32
Mtr.10659.1.S1_s_at	0,00410443	1,88	down	0,3470583	-0,5617797	weakly similar to (Q9SS03) F12P19.3, partial (37%)			AT1G65870	disease resistance-responsive family protein, similar to dirigent protein (Forsythia x intermedia) gi|6694693|gb|AAF25357; similar to disease re	1,00E-36
Mtr.4306.1.S1_s_at	0,02854369	1,88	down	0,25733057	-0,65030986	Mtr.9079			AT1G67100	LOB domain protein 40 / lateral organ boundaries domain protein 40 (LBD40), identical to SP|Q9ZW96 LOB domain protein 40 {Arabidopsis tha	6,00E-24
Mtr.46511.1.S1_at	0,00398951	1,88	down	0,38306156	-0,5238746				AT1G78970	lupeol synthase (LUP1) / 2,3-oxidosqualene-triterpenoid cyclase, identical to lupeol synthase GI:1762150 from (Arabidopsis thaliana), 2,3-oxidosqualene-triter	
Mtr.44383.1.S1_at	0,01035802	1,87	up	-0,33296427	0,57296544	similar to (Q7XSF9) OSJNBb0051N19.4 protein, partial (57%)			AT5G38260	serine/threonine protein kinase, putative, similar to receptor serine/threonine kinase PR55K gi|1235680|gb|AAC49208; contains protein kinase domain, Pfam:I	
Mtr.20125.1.S1_at	6,47E-04	1,87	down	0,42028537	-0,48543486				AT4G17090	Transgenic BMY8 RNAi lines fail to accumulate maltose during cold shock suggesting that maltose accumulation coincides with BMY8 expression. Apart from ma	
Mtr.27314.1.S1_s_at	4,54E-04	1,87	down	0,47517666	-0,4276754				AT5G03720	member of Heat Stress Transcription Factor (Hsf) family;heat shock transcription factor family protein, contains Pfam profile: PF00447 HSF-typ	2,00E-25
Msa.985.1.S1_at	7,11E-04	1,87	down	0,44161925	-0,46064058				AT1G10760	starch excess protein (SEX1), identical to SEX1 (Arabidopsis thaliana) GI:12044358; supporting cDNA gi|12044357|gb|AF312027.1|AF312027;	3,00E-35
Mtr.37677.1.S1_s_at	0,02917275	1,87	up	-0,29603133	0,6057105	Mtr.10983 similar to unknown protein {Arabidopsis thaliana;}, partial (25%)			AT5G24890	expressed protein, weak similarity to MTD1 (Medicago truncatula) GI:9294810	5,00E-19
Mtr.20815.1.S1_at	2,37E-04	1,87	up	-0,4357965	0,46337095				AT4G23160	protein kinase family protein, contains Pfam domain PF00069: Protein kinase domain	6,00E-26
Mtr.40395.1.S1_at	0,01447492	1,86	down	0,38873133	-0,50757337	(O82701) MtN29 protein (Fragment), complete			AT5G10020	leucine-rich repeat transmembrane protein kinase, putative, receptor-like protein kinase ERECTA, Arabidopsis thaliana, EMBL:AC004484	1,2
Mtr.46512.1.S1_at	0,01144011	1,86	down	0,5196786	-0,37518406				AT1G78960	lupeol synthase, putative / 2,3-oxidosqualene-triterpenoid cyclase, putative, similar to lupeol synthase GI:1762150 from (Arabidopsis thaliana)	5,00E-14
Mtr.46462.1.S1_at	0,00795765	1,86	up	-0,5090739	0,38490406				AT2G17650	AMP-dependent synthetase and ligase family protein, similar to AMP-binding protein GI:1903034 from (Brassica napus); contains Pfam AMP-bir	8,00E-38
Mtr.26299.1.S1_at	0,0362961	1,86	down	0,24728124	-0,6452978				AT3G52820	purple acid phosphatase (PAP22), identical to purple acid phosphatase (PAP22)GI:20257494 from (Arabidopsis thaliana)	
Mtr.40354.1.S1_at	0,01601275	1,85	up	-0,4021031	0,48909998	Mtr.5203 weakly similar to (Q9FRT8) Albumin 1 precursor (A1) [Contains: Albumin 1 chain b (A1b) (Legins	GO:0009405	AT1G26960	Encodes a homeodomain leucine zipper class I (HD-Zip I) protein.	2,9
Mtr.12783.1.S1_at	0,01586	1,85	up	-0,44498983	0,44457325	homologue to (Q71EE1) Heat shock protein, complete			AT5G56010	heat shock protein, putative, strong similarity to SP|P55737 Heat shock protein 81-2 (HSP81-2) {Arabidopsis thaliana}; contains Pfam profiles	4,00E-16
Mtr.2599.1.S1_at	0,00105003	1,85	up	-0,43488193	0,4537201				AT2G19130	S-locus lectin protein kinase family protein, contains Pfam domains PF00954: S-locus glycoprotein family, PF00069: Protein kinase domain and	7,00E-07
Mtr.40639.1.S1_at	0,0279731	1,85	up	-0,31177506	0,5763604	weakly similar to (Q8S342) Putative anthocyanidine rhamnosyl-transferase, partial (33%)			AT5G65550	UDP-glucoronosyl/UDP-glucosyl transferase family protein, contains Pfam profile: PF00201 UDP-glucoronosyl and UDP-glucosyl transferase ;sim	2,00E-17
Mtr.10037.1.S1_at	0,02152522	1,85	up	-0,33169012	0,57212202	weakly similar to (Q75II0) Putative GDSL-like lipase/hydrolase, partial (23%)			AT1G29670	GDSL-motif lipase/hydrolase family protein, similar to family II lipase EXL1 GI:15054382 from (Arabidopsis thaliana); contains Pfam profile: PF	3,00E-17
Msa.1751.1.S1_at	0,02387093	1,85	down	0,29175124	-0,59388334				AT4G18910	aquaglyceroporin / NOD26-like major intrinsic protein 2 (NLM2), contains Pfam profile: MIP PF00230; similar to SP:P08995 {Glycine max} Nod	6,00E-41
Mtr.34597.1.S1_s_at	9,36E-04	1,85	down	0,5200043	-0,36477852	Mtr.8525	GO:0006081	AT2G24710	glutamate receptor family protein (GLR2.3), plant glutamate receptor family, PMID:11379626;member of Putative ligand-gated ion channel sut	4,6
Mtr.18560.1.S1_at	0,00439493	1,84	up	-0,47115836	0,40800762				AT1G08200	expressed protein;Encodes a putative UDP-D-apiose/UPD-D-xylose synthetase.	
Mtr.10700.1.S1_at	0,03933626	1,84	up	-0,35126734	0,5268464	similar to probable caltractin [imported] - Arabidopsis thaliana {Arabidopsis thaliana;}, partial (74%)			AT2G46600	calcium-binding protein, putative, similar to EF-hand Ca2+-binding protein CCD1 (Triticum aestivum) GI:9255753	1,00E-36
Msa.1267.1.S1_at	0,00101562	1,84	up	-0,37414837	0,50311804				AT2G18700	glycosyl transferase family 20 protein / trehalose-phosphatase family protein, similar to trehalose-6-phosphate synthase SL-TPS/P (Selaginella	6,00E-44
Mtr.43467.1.S1_at	0,01590125	1,83	down	0,54612154	-0,32824373	weakly similar to (Q9FRS6) F22O13.7, partial (7%)			AT3G48220	expressed protein	10
Mtr.39289.1.S1_at	0,04849969	1,83	up	-0,30143484	0,57283896	weakly similar to (Q8S342) Putative anthocyanidine rhamnosyl-transferase, partial (19%)			AT5G65550	UDP-glucoronosyl/UDP-glucosyl transferase family protein, contains Pfam profile: PF00201 UDP-glucoronosyl and UDP-glucosyl transferase ;sim	7,00E-29
Mtr.28802.1.S1_at	0,01406803	1,83	up	-0,38077512	0,4925777				AT1G12780	Encodes a UDP-glucose epimerase that catalyzes the interconversion of the sugar nucleotides UDP-glucose UDP-galactose via a UDP-4-keto-hexose intermedia	
Mtr.9112.1.S1_at	0,02279977	1,83	up	-0,3636249	0,50919753	similar to (O64989) Steroid 22-alpha-hydroxylase, partial (36%)			AT3G50660	steroid 22-alpha-hydroxylase (CYP90B1) (DWF4), identical to gi:2935342;Encodes a 22α hydroxylase whose reaction is a rate-limiting step in brassinost	
Mtr.42839.1.S1_at	0,02736536	1,83	up	-0,45013395	0,42219082	similar to (Q6NMR9) At5g45920, partial (73%)			AT5G45920	similar to GDSL-motif lipase/hydrolase family protein [Arabidopsis thaliana] (TAIR:At5g62930.1); similar to OSJNBa0038317.19 [Oryza sativa (japonica cultiva	
Mtr.38919.1.S1_at	0,00718188	1,83	down	0,3930858	-0,47685924	Mtr.16729 weakly similar to (Q7XKG1) OSJNBb0065J09.8 protein, partial (5%)	GO:0008152	AT2G36790	UDP-glucoronosyl/UDP-glucosyl transferase family protein, contains Pfam profile: PF00201 UDP-glucoronosyl and UDP-glucosyl transferase	8,00E-15
AFFX-BioB-3_at	0,04723921	1,83	down	0,6547343	-0,21432526		GO:0008152	AT2G43360	biotin synthase (BioB) (BIO2), identical to SP|P54967 Pfam profile PF04055: radical SAM domain protein;Catalyzes the conversion of dethiobiot	8,00E-16
Mtr.35826.1.S1_at	7,51E-04	1,83	down	0,39116827	-0,47706667	weakly similar to (Q680D1) Hypothetical protein At5g62200 (Fragment), partial (40%)			AT5G62200	embryo-specific protein-related, contains weak similarity to embryo-specific protein 3 (GI:3335171) (Arabidopsis thaliana)	4,00E-17
Msa.920.1.S1_at	1,61E-04	1,83	up	-0,41746235	0,4507459				AT2G29500	17.6 kDa class I small heat shock protein (HSP17.6B-CI), contains Pfam PF00011: Hsp20/alpha crystallin family; identified in Scharf, K-D., et a	2,00E-40
Mtr.39406.1.S1_at	0,01662822	1,82	up	-0,40125322	0,466468	similar to (Q8RYD3) Putative auxin response factor 32 (Putative DNA binding protein), partial (37%)			AT3G11580	DNA-binding protein, putative, similar to DNA-binding proteins from (Arabidopsis thaliana) RAV1 GI:3868857 and RAV2 GI:3868859, AP2 doma	4,20E-45
Mtr.15523.1.S1_at	0,02252973	1,82	up	-0,2692097	0,5976046				AT3G49810	U-box domain-containing protein, contains Pfam profile PF04564: U-box domain	
Msa.2535.1.S1_at	0,01054806	1,82	up	-0,44321665	0,42192206				AT2G20520	fasciclin-like arabinogalactan-protein (FLA6), identical to gi|13377780_gb_AAK20859;AF333972 Arabidopsis thaliana fasciclin-like arabinogalac	8,00E-28
Mtr.43503.1.S1_at	0,01078844	1,82	down	0,38391718	-0,4808178	weakly similar to (Q9FIJ1) Arabidopsis thaliana genomic DNA chromosome 5 P1 clone:MCA23, partial (29%)	AT5G47900	expressed protein	1,00E-37		
Mtr.46873.1.S1_at	0,00228345	1,82	down	0,3923966	-0,47192445				AT3G15810	expressed protein, contains Pfam profile PF04525: Protein of unknown function (DUF567)	7,00E-23
Msa.1384.1.S1_at	7,58E-04	1,82	down	0,39634213	-0,46760845				AT1G55020	lipoxygenase (LOX1), identical to SP|Q06327;lipoxygenase, a defense gene conferring resistance Xanthomonas campestris	8,00E-25
Mtr.3548.1.S1_at	0,00746814	1,82	up	-0,35835662	0,50290155				AT5G51910	TCP family transcription factor, putative, similar to PCF2 ((GI:2580440) Oryza sativa)	2,00E-04

Mtr.11633.1.S1_at	0,03413926	1,81	up	-0,64722365	0,2090977		similar to unknown protein {Arabidopsis thaliana;}, partial (15%)		AT1G04140	transducin family protein / WD-40 repeat family protein, contains 4 WD-40 repeats (PF00400); similar to neural cell adhesion molecule 2, large isoform precur	
Mtr.38422.1.S1_at	0,03810358	1,81	down	0,27939048	-0,576245		similar to (Q9LG09) F14J16.29, partial (24%)		AT4G38080	hydroxyproline-rich glycoprotein family protein, contains proline-rich extensin domains, INTERPRO:IPR002965; Common family member:: At2g	0,26
Mtr.6081.1.S1_at	0,02211999	1,80	down	0,32273355	-0,5278409				AT1G75890	family II extracellular lipase 2 (EXL2), EXL2 (PMID:11431566); similar to anter-specific proline-rich protein (APG) SP:P40602 (Arabidopsis thali	1,00E-22
Mtr.43230.1.S1_at	2,52E-04	1,80	up	-0,44589677	0,40462652		homologue to AT5g02020/T7H20_70 {Arabidopsis thaliana;}, partial (33%)		AT1G31260	metal transporter, putative (ZIP10), identical to putative metal transporter ZIP10 (Arabidopsis thaliana) gi|17385792|gb|AAL38436; similar to	2,4
Mtr.38209.1.S1_at	0,0055733	1,80	down	0,3238236	-0,52603406		homologue to (Q5TZ13) Novel protein similar to vertebrate protein phosphatase 1 regulatory inhibitor subunit		AT3G13225	similar to PREDICTED: similar to mKIAA1014 protein [Gallus gallus] (GB:XP_424260.1); contains InterPro domain WW/Rsp5/WWP domain (Int	2,9
Mtr.10370.1.S1_at	0,0231259	1,80	up	-0,29096714	0,5578982	Mtr.15749	similar to (Q43790) Peroxidase1B precursor (EC 1.11.1.7 EC 1.11.1.7), partial (95%)	GO:0006979	AT2G38380	peroxidase 22 (PER22) (P22) (PRXEA) / basic peroxidase E, identical to SP|P24102 Peroxidase 22 precursor (EC 1.11.1.7) (Atperox P22) (ATPE	1,00E-28
Mtr.4579.1.S1_at	0,00317852	1,80	down	0,53653	-0,31186804	Mtr.16884			AT3G49320	expressed protein, contains Pfam profile PF03690: Uncharacterised protein family (UPF0160)	1,2
Mtr.37287.1.S1_s_at	0,03724987	1,80	down	0,18726826	-0,6600456	Mtr.22282	weakly similar to (Q7XKV2) OSJNBa0022H21.5 protein, partial (20%)	GO:0005975	AT3G05790	Lon protease, putative, similar to Lon protease homolog 2 SP:P93655	6
Mtr.32156.1.S1_at	0,0337057	1,80	down	0,52449274	-0,32256866				AT3G12040	DNA-3-methyladenine glycosylase (MAG), identical to DNA-3-methlyadenine glycosylase (MAG) SP:Q39147 from (Arabidopsis thaliana); contai	1,00E-23
Mtr.47465.1.S1_at	0,03719805	1,79	up	-0,24350755	0,5991108				AT5G46050	proton-dependent oligopeptide transport (POT) family protein, contains Pfam profile: PF00854 POT family	
Mtr.45877.1.S1_at	0,00902165	1,79	up	-0,31359324	0,5289691				AT3G19130	RNA-binding protein, putative, similar to RNA Binding Protein 47 (Nicotiana plumbaginifolia) GI:9663769, DNA binding protein ACBF GB:AAC49850 from (Nicot	
Mtr.10265.1.S1_at	0,00429044	1,79	up	-0,487188	0,3551		similar to (Q9FFZ1) Arabidopsis thaliana genomic DNA chromosome 5 P1 clone:MAC12 (AT5g13800/MAC12_2	AT3G63500	expressed protein	6	
Mtr.11150.1.S1_at	0,00229092	1,79	down	0,37008762	-0,47068915		similar to (Q6ZKI2) Putative RNA recognition motif (RRM)-containing protein, partial (61%)		AT5G54580	RNA recognition motif (RRM)-containing protein, low similarity to RNA-binding protein RGP-3 (Nicotiana sylvestris) GI:1009363; contains InterF	2,00E-37
Mtr.47794.1.S1_at	0,00237726	1,79	down	0,50244904	-0,33784756				AT5G67390	expressed protein;expressed protein, similar to expressed protein [Arabidopsis thaliana] (TAIR:At5g57340.2); similar to expressed protein [Ara	1,00E-05
Mtr.12208.1.S1_at	4,24E-04	1,79	up	-0,40021515	0,43978596		pentameric polyubiquitin {Nicotiana sylvestris;}, partial (60%)		AT5G03240	polyubiquitin (UBQ3), identical to GI:928809;similar to polyubiquitin (UBQ10) (SEN3) [Arabidopsis thaliana] (TAIR:At4g05320.2); similar to polyubiquitin (UBC	
Mtr.5330.1.S1_at	0,00279919	1,79	up	-0,49282202	0,34699392				AT4G23180	receptor-like protein kinase 4, putative (RLK4), nearly identical to receptor-like protein kinase 4 (Arabidopsis thaliana) GI:13506745; contains	1,40E-45
Mtr.28497.1.S1_at	0,00947931	1,79	down	0,455592	-0,38365793				AT2G39490	F-box family protein, ; similar to SKP1 interacting partner 2 (SKIP2) TIGR_Ath1:At5g67250	0,015
Mtr.27888.1.S1_s_at	0,00708366	1,79	down	0,36600748	-0,47313404	Mtr.574			AT2G39420	esterase/lipase/thioesterase family protein, similar to monoglyceride lipase from (Homo sapiens) GI:14594904, (Mus musculus) GI:2632162; c	4,00E-23
Mtr.12900.1.S1_at	0,00505886	1,79	up	-0,46424976	0,37476778		weakly similar to (Q5XMI8) Iron reductase, partial (5%)				
Mtr.19019.1.S1_at	0,01027453	1,79	up	-0,4004186	0,43848816				AT1G69490	NAC family transcription factor genes whose expression is associated with leaf senescence.;Member of NAC gene family, expressed in floral pri	9,00E-43
Mtr.46162.1.S1_at	0,0036396	1,79	down	0,48213896	-0,35674524				AT1G67570	expressed protein	1,6
Mtr.35853.1.S1_at	0,00282808	1,79	up	-0,45354557	0,3852946		weakly similar to (Q9SZF3) Hypothetical protein F19F18.90 (Hypothetical protein AT4g37600), partial (62%)		AT2G23040	expressed protein, ; expression supported by MPSS	2,00E-04
Mtr.12358.1.S1_at	0,00381656	1,79	down	0,33872733	-0,49969658		similar to (Q9FNW7) Seed maturation protein LEA 4, partial (49%)		AT4G13140	expressed protein	5,9
Mtr.33482.1.S1_s_at	0,02080008	1,79	up	-0,35259548	0,48475012	Mtr.13241			AT4G33580	carbonic anhydrase family protein / carbonate dehydratase family protein, similar to SP|P46512 Carbonic anhydrase 1 (EC 4.2.1.1) (Carbonate	0,041
Mtr.19347.1.S1_at	0,02524466	1,79	up	-0,32160458	0,5153757						
Mtr.10681.1.S1_at	0,00905446	1,78	up	-0,4353671	0,3986044		similar to unknown protein {Arabidopsis thaliana;}, partial (64%)		AT3G58450	universal stress protein (USP) family protein, contains Pfam PF00582: universal stress protein family	0,006
Mtr.3151.1.S1_s_at	0,02849606	1,78	up	-0,41110006	0,42225137				AT3G15450	expressed protein, similar to auxin down-regulated protein ARG10 (Vigna radiata) GI:2970051, wali7 (aluminum-induced protein) (Triticum aes	6,00E-06
Mtr.40035.1.S1_at	0,02232442	1,78	down	0,3507347	-0,47933707	Mtr.22141	homologue to 21K protein precursor - alfalfa {Medicago sativa;}, partial (96%)	GO:0004857	AT4G12390	invertase/pectin methylesterase inhibitor family protein, low similarity to pectinesterase from Arabidopsis thaliana SP|Q42534, Lycopersicon es	6,00E-18
AFFX-Mtr-r2-Ec-bioB-3	0,04793272	1,78	down	0,6532844	-0,1769644				AT2G43360	biotin synthase (BioB) (BIO2), identical to SP|P54967 Pfam profile PF04055: radical SAM domain protein;Catalyzes the conversion of dethiobiot	3,00E-10
Mtr.13985.1.S1_at	0,00709869	1,78	down	0,39357123	-0,43535313		similar to (O22682) Probable cysteine synthase chloroplast precursor (O-acetylserine sulfhydrylase), partial	AT3G03630	cysteine synthase, chloroplast, putative / O-acetylserine (thiol)-lyase, putative / O-acetylserine sulfhydrylase, putative, identical to SP|O22682 Probable cystei	
Msa.686.1.S1_at	0,00166806	1,78	down	0,3448116	-0,4841021				AT3G13310	DNAJ heat shock N-terminal domain-containing protein, similar to J11 protein (Arabidopsis thaliana) GI:9843641; contains Pfam profile: PF002	2,00E-13
Mtr.10727.1.S1_at	0,03277427	1,77	up	-0,54615927	0,28021097		similar to unknown protein {Arabidopsis thaliana;}, partial (62%)		AT5G06570	expressed protein, similar to PrMC3 (Pinus radiata) GI:5487873	2,00E-34
Mtr.13536.1.S1_at	0,00718035	1,77	up	-0,35572863	0,47040161		similar to (Q8H770) Bifunctional lysine-ketoglutarate reductase/saccharopine dehydrogenase, partial (24%)	AT4G33150	lysine-ketoglutarate reductase/saccharopine dehydrogenase bifunctional enzyme, identical to lysine-ketoglutarate reductase/saccharopine dehy	7,00E-39
AFFX-r2-Ec-bioC-3_at	0,03902482	1,77	down	0,61497307	-0,21062343			GO:0008152	AT3G27785	myb family transcription factor (MYB118), contains PFAM profile: PF00249 myb-like DNA binding domain;AF334817 Arabidopsis thaliana putativ	0,54
Mtr.19208.1.S1_at	0,0132747	1,77	up	-0,26921478	0,55557853				AT1G24580	zinc finger (C3HC4-type RING finger) family protein	9,00E-26
Mtr.21028.1.S1_at	0,01419423	1,77	up	-0,45254683	0,3713951				AT2G46550	expressed protein	1,00E-05
Mtr.28722.1.S1_at	0,04285653	1,77	down	0,2046442	-0,61734295				AT5G13400	proton-dependent oligopeptide transport (POT) family protein, contains Pfam profile: PF00854 POT family	
Mtr.9984.1.S1_at	0,03753807	1,77	up	-0,2356935	0,5862624		similar to (Q84P18) Acyl-activating enzyme 17, partial (33%)		AT5G23050	acyl-activating enzyme 17 (AAE17), nearly identical to acyl-activating enzyme 17 (Arabidopsis thaliana) GI:29893266; similar to acetyl-CoA synthetase (SP|P2	
Mtr.4275.1.S1_at	0,01394309	1,76	up	-0,35454226	0,4651218				AT2G33260	tryptophan/tyrosine permease family protein, contains Pfam profile PF03222: Tryptophan/tyrosine permease family	1,00E-25
Mtr.876.1.S1_s_at	0,02061931	1,76	down	0,25289583	-0,5660329				AT3G59040	pentatricopeptide (PPR) repeat-containing protein, contains Pfam profile PF01535: PPR repeat	
Mtr.29993.1.S1_at	0,00753529	1,76	up	-0,35791603	0,46034288				AT1G22370	UDP-glucoronosyl/UDP-glucosyl transferase family protein, glycosyltransferase family	9,00E-41
Mtr.30513.1.S1_s_at	0,04365476	1,76	up	-0,21728928	0,6004775				AT5G49580	DNAJ heat shock N-terminal domain-containing protein, contains similarity to S-locus protein 5 GI:6069485 from (Brassica rapa); contains Pfar	1,6
Mtr.46690.1.S1_at	0,00741658	1,76	up	-0,44169664	0,3758184				AT3G53270	expressed protein, endopeptidase Clp ATP-binding chain C, Chlamydia pneumoniae, PIR:G72079	3,00E-19
Mtr.25317.1.S1_at	5,01E-04	1,76	down	0,39525542	-0,42164412				AT1G62660	beta-fructosidase (BFRUCT3) / beta-fructofuranosidase / invertase, vacuolar, identical to beta-fructosidase GB:CAA67560 GI:1429209 (Arabidopsis thaliana); s	
Mtr.23393.1.S1_at	0,00236865	1,76	up	-0,40799078	0,40884733				AT2G29500	17.6 kDa class I small heat shock protein (HSP17.6B-CI), contains Pfam PF00011: Hsp20/alpha crystallin family; identified in Scharf, K-D., et a	1,00E-20
Mtr.42770.1.S1_at	0,00199494	1,76	up	-0,4062244	0,40953317		similar to photosystem II 23 kDa polypeptide {Nicotiana tabacum;}, partial (80%)		AT1G06680	photosystem II oxygen-evolving complex 23 (OEC23), JBC 14:211-238 (2002); identical to 23 kDa polypeptide of oxygen-evolving comlex (OEC) GB:CAA6678	
Mtr.33440.1.S1_at	0,00197	1,76	down	0,4137856	-0,39912415				AT5G18350	disease resistance protein (TIR-NBS-LRR class), putative, domain signature TIR-NBS-LRR exists, suggestive of a disease resistance protein.	2,6
Mtr.653.1.S1_s_at	0,0022353	1,75	up	-0,40499368	0,4056603				AT3G26760	short-chain dehydrogenase/reductase (SDR) family protein, similar to sex determination protein tasselseed 2 SP:P50160 from (Zea mays)	
Mtr.25672.1.S1_a_at	0,03612009	1,75	up	-0,35483155	0,4549713				AT1G78740	similar to F-box family protein [Arabidopsis thaliana] (TAIR:At1g69630.1)	7,9
Mtr.39929.1.S1_at	4,03E-04	1,75	up	-0,38822088	0,4210612		homologue to (O24082) 17kD heat shock protein, partial (76%)		AT5G12020	17.6 kDa class II heat shock protein (HSP17.6-CII), identical to 17.6 kDa class II heat shock protein SP:P29830 from (Arabidopsis thaliana)	4,00E-35
Mtr.40978.1.S1_at	7,42E-04	1,75	down	0,3844048	-0,42468643		similar to (O82061) R1 protein precursor, partial (30%)		AT1G10760	starch excess protein (SEX1), identical to SEX1 (Arabidopsis thaliana) GI:12044358; supporting cDNA gi|12044357|gb|AF312027.1|AF312027;	1,00E-34
Mtr.18951.1.S1_at	0,02218344	1,75	up	-0,42199397	0,3866396				AT1G54560	myosin, putative, similar to myosin GI:433663 from (Arabidopsis thaliana);member of Myosin-like proteins	19
Mtr.5628.1.S1_s_at	0,00504816	1,75	down	0,48882118	-0,31938076				AT3G22400	lipoxygenase, putative, similar to lipoxygenase gi:8649004 (Prunus dulcis), gi:1495802 and gi:1495804 from (Solanum tuberosum)	2,00E-06
Mtr.6175.1.S1_at	0,00672209	1,75	up	-0,31874132	0,48795128	Mtr.18663			AT2G45680	TCP family transcription factor, putative, similar to PCF2 (GI:2580440) (Oryza sativa)	0,083
Mtr.19235.1.S1_at	0,00200302	1,75	down	0,4458548	-0,3607459				AT2G40080	expressed protein;involved in photoperiod perception and circadian regulation. ELF4 promotes clock accuracy and is required for sustained rhyt	4,00E-26
Mtr.50766.1.S1_at	0,02591602	1,75	up	-0,3890578	0,4168817				AT5G64330	non-phototropic hypocotyl 3 (NPH3), identical to non-phototropic hypocotyl 3 (Arabidopsis thaliana) gi|6224712|gb|AAF05914, PMID:10542152;Involved in bl	
Mtr.43089.1.S1_at	0,00599864	1,75	down	0,38753986	-0,416824		similar to (Q9FDY1) Seed maturation protein LEA 4, partial (44%)		AT5G06760	late embryogenesis abundant group 1 domain-containing protein / LEA group 1 domain-containing protein, low similarity to SP|P46515 11 kDa	3,00E-12
Mtr.38735.1.S1_at	0,02788328	1,75	up	-0,28835678	0,5155967		similar to (Q9M9A2) F27J15.21 (At1g49000), partial (32%)				
Mtr.17790.1.S1_at	2,78E-05	1,74	up	-0,41080984	0,39216933				AT1G07780	phosphoribosylanthranilate isomerase 1 (PAI1), identical to gi:619751;Encodes phosphoribosylanthranilate isomerase which catalyzes the third step of the tryp	
Msa.1414.1.S1_at	0,03214813	1,74	up	-0,17675225	0,62622243				AT2G40000	expressed protein	
Mtr.1796.1.S1_at	7,85E-06	1,74	down	0,41703224	-0,38542652				AT1G50110	branched-chain amino acid aminotransferase 6 / branched-chain amino acid transaminase 6 (BCAT6), contains Pfam profile: PF01063 aminotra	3
AFFX-r2-Ec-bioB-3_at	0,04564462	1,74	down	0,5968129	-0,20529413			GO:0008152	AT2G43360	biotin synthase (BioB) (BIO2), identical to SP|P54967 Pfam profile PF04055: radical SAM domain protein;Catalyzes the conversion of dethiobiot	1,00E-13
Mtr.44056.1.S1_at	0,00581321	1,74	up	-0,3667086	0,43497738		similar to hypothetical protein At2g42620 [imported] - Arabidopsis thaliana, partial (11%)		AT2G42620	F-box family protein (ORE9), E3 ubiquitin ligase SCF complex F-box subunit; identical to F-box containing protein ORE9 GI:15420162 from (Ara	1,00E-42
Mtr.20735.1.S1_at	0,01874718	1,74	up	-0,3163487	0,482799				AT4G36380	cytochrome P450 90C1 (CYP90C1) / rotundifolia3 (ROT3), identical to Cytochrome P450 90C1 (ROTUNDIFOLIA3) (SP:Q9M066) (Arabidopsis thaliana);Encode	
Mtr.35193.1.S1_s_at	0,00640262	1,74	up	-0,3574632	0,4415773	Mtr.10591			AT5G62020	member of Heat Stress Transcription Factor (Hsf) family;heat shock factor protein, putative (HSF6) / heat shock transcription factor, putative (	6,00E-39
Mtr.22597.1.S1_s_at	0,02499027	1,74	up	-0,33057466	0,46800837	Mtr.5930		GO:0019752	AT4G37010	serine decarboxylase, identical to serine decarboxylase (Arabidopsis thaliana) GI:15011302; contains Pfam profile PF00282: Pyridoxal-depende	6,00E-35
Mtr.16393.1.S1_at	8,91E-04	1,74	up	-0,41448593	0,38242593				AT4G27450	expressed protein, similar to auxin down-regulated protein ARG10 (Vigna radiata) GI:2970051, wali7 (aluminum-induced protein) (Triticum aestivum) GI:4511	
Mtr.418.1.S1_s_at	0,0020897	1,74	down	0,39522776	-0,40100622				AT3G04380	SET domain-containing protein (SUVR4), nearly identical to Su(VAR)3-9-related protein 4 (Arabidopsis thaliana) GI:17066863; contains Pfam profiles PF00856	
Mtr.22173.1.S1_at	0,00154153	1,74	up	-0,33584824	0,4597795				AT3G53690	zinc finger (C3HC4-type RING finger) family protein, contains a Zinc finger, C3HC4 type (RING finger) signature, PROSITE:PS00518 and a IBR	2,00E-07
Mtr.47478.1.S1_at	0,00169291	1,74	up	-0,3944634	0,40071854				AT3G47440	major intrinsic family protein / MIP family protein, contains Pfam profile: MIP PF00230	4,00E-07
Mtr.23575.1.S1_x_at	0,03746434	1,73	down	0,5747053	-0,21989791				AT5G06860	polygalacturonase inhibiting protein 1 (PGIP1), identical to polygalacturonase inhibiting protein 1 (PGIP1) (Arabidopsis thaliana) gi|7800199|gb	2,00E-32
Mtr.13421.1.S1_at	0,02894066	1,73	down	0,2277158	-0,5667033		similar to probable cytochrome b-561 [imported] - Arabidopsis thaliana {Arabidopsis thaliana;}, partial (77%)		AT1G26100	cytochrome B561 family protein, contains Pfam domain, PF03188: Cytochrome b561	2,00E-20
Mtr.9873.1.S1_at	6,28E-04	1,73	down	0,37661585	-0,4176086		weakly similar to (Q6VBI6) Thioredoxin H2, partial (72%)		AT1G80260	similar to tubulin family protein [Arabidopsis thaliana] (TAIR:At1g20570.1); similar to unnamed protein product [Tetraodon nigroviridis] (GB:C/	1,6
Mtr.41145.1.S1_at	0,0055654	1,73	up	-0,37915516	0,41203976		homologue to (Q9XHP4) Peroxisomal copper-containing amine oxidase, partial (33%)		AT2G42490	copper amine oxidase, putative, similar to copper methylamine oxidase precursor (MAOXI) (Arthrobacter sp.) SWISS-PROT:Q07123	2,80E-45
Mtr.40546.1.S1_at	0,01745027	1,73	down	0,34364976	-0,4475678		weakly similar to (Q9SS03) F12P19.3, partial (34%)		AT1G65870	disease resistance-responsive family protein, similar to dirigent protein (Forsythia x intermedia) gi|6694693|gb|AAF25357; similar to disease re	1,00E-25
AFFX-Mtr-r2-Ec-bioC-5	0,0397484	1,73	down	0,6307456	-0,15924168				AT5G57280	expressed protein	0,002
Mtr.46949.1.S1_at	0,02683186	1,73	down	0,22086255	-0,5666812				AT5G64700	nodulin MtN21 family protein, similar to MtN21 GI:2598575 (root nodule development) from (Medicago truncatula); contains Pfam profile PF00	4,00E-04
Mtr.8621.1.S1_s_at	0,00150197	1,72	up	-0,43166366	0,35419083	Mtr.6885	similar to (Q8GT55) Putative phosphatase, partial (34%)	GO:0008152	AT5G02230	haloacid dehalogenase-like hydrolase family protein, contains InterPro accession IPR005834: Haloacid dehalogenase-like hydrolase	4,00E-08
Mtr.38735.1.S1_s_at	0,00220969	1,72	up	-0,3604196	0,42375135		similar to (Q9M9A2) F27J15.21 (At1g49000), partial (32%)		AT1G49000	expressed protein	2,00E-22
Mtr.27697.1.S1_at	0,0111963	1,72	up	-0,33085045	0,45265865				AT1G14180	expressed protein	2,1
Mtr.41393.1.S1_at	0,00418063	1,72	down	0,30214977	-0,4810648		similar to unknown {Arabidopsis thaliana;}, partial (32%)		AT3G15110	expressed protein	3,00E-30
Mtr.19131.1.S1_at	0,04498824	1,72	up	-0,32239914	0,4599072				AT2G19810	zinc finger (CCCH-type) family protein, contains Pfam domain, PF00642: Zinc finger C-x8-C-x5-C-x3-H type (and similar)	5,00E-23
Mtr.44439.1.S1_at	0,02188708	1,72	up	-0,24331188	0,53786993		weakly similar to (Q6Z688) Putative UDP-glucose glucosyltransferase, partial (28%)		AT1G51320	F-box family protein (FBX11), contains F-box domain PF:00646	17
Mtr.27718.1.S1_at	0,01167644	1,72	up	-0,34831333	0,43198872				AT5G24820	aspartyl protease family protein, low similarity to CND41, chloroplast nucleoid DNA binding protein (Nicotiana tabacum) GI:2541876; contains I	3,5
Mtr.20438.1.S1_at	0,01077684	1,72	up	-0,260053	0,5195446	Mtr.4657		GO:0008171	AT5G54160	quercetin 3-O-methyltransferase 1 / flavonol 3-O-methyltransferase 1 / caffeic acid/5-hydroxyferulic acid O-methyltransferase (OMT1), identica	8,40E-45
Mtr.12840.1.S1_at	0,02103359	1,72	down	0,44711447	-0,33236027		weakly similar to (Q8VX12) Lipid transfer protein precursor, partial (48%)		AT2G15050	lipid transfer protein, putative, similar to SP|Q42641 nonspecific lipid-transfer protein A precursor (LTP A) (Wax-associated protein 9A) {Brassic	3,00E-17
Mtr.18646.1.S1_at	0,00667609	1,72	up	-0,43580008	0,3435656				AT4G03550	glycosyl transferase family 48 protein, contains Pfam profile: PF02364 1,3-beta-glucan synthase;Encodes a callose synthase that is required for	0,49
Mtr.31872.1.S1_at	0,00467426	1,71	up	-0,35217586	0,42589077	Mtr.8107			AT1G13580	longevity-assurance (LAG1) family protein, similar to Alternaria stem canker resistance protein (ASC1) (Lycopersicon esculentum) GI:7688742;	3,00E-23
Mtr.18035.1.S1_at	0,00324041	1,71	down	0,34763846	-0,4298655				AT2G25620	protein phosphatase 2C, putative / PP2C, putative	
Mtr.37164.1.S1_at	0,00130411	1,71	up	-0,40879202	0,36784697		similar to heat shock factor RHSF6 {Oryza sativa (japonica cultivar-group);}, partial (9%)		AT3G22830	member of Heat Stress Transcription Factor (Hsf) family;heat shock transcription factor family protein, contains Pfam profile: PF00447 HSF-typ	2,00E-10
Mtr.31257.1.S1_at	0,02594138	1,71	up	-0,2647091	0,5107941				AT5G05810	zinc finger (C3HC4-type RING finger) family protein, contains Pfam profile: PF00097 zinc finger, C3HC4 type (RING finger)	2,00E-34
Mtr.4439.1.S1_s_at	0,01253537	1,71	down	0,38347688	-0,39148712	Mtr.17708			AT5G39950	thioredoxin H-type 2 (TRX-H-2) (Gif2), identical to SP|Q38879 Thioredoxin H-type 2 (TRX-H-2) {Arabidopsis thaliana}; identical to cDNA (Gif2)	1,00E-07
Mtr.41751.1.S1_at	0,00929713	1,71	down	0,27125835	-0,5031249		similar to (Q7X699) OSJNBb0118P14.13 protein, partial (49%)		AT5G27730	expressed protein	2,00E-08
Mtr.11040.1.S1_s_at	0,01086129	1,71	down	0,40569243	-0,36843538		weakly similar to (Q9FGG3) Nodulin-like protein, partial (58%)		AT5G64700	nodulin MtN21 family protein, similar to MtN21 GI:2598575 (root nodule development) from (Medicago truncatula); contains Pfam profile PF00	1,00E-37
Mtr.22769.1.S1_at	0,02319189	1,71	up	-0,21844833	0,55512714				AT1G09530	phytochrome interacting factor 3 (PIF3), identical to phytochrome interacting factor 3 (PIF3) GI:3929585 from (Arabidopsis thaliana);Transcrip	6,00E-25
Mtr.33377.1.S1_s_at	0,01273461	1,71	down	0,42979226	-0,34375182				AT1G76720	eukaryotic translation initiation factor 2 family protein / eIF-2 family protein, similar to SP|O60841 Translation initiation factor IF-2 {Homo sapi	4,00E-18

Mtr.5478.1.S1_at	0,00950355	1,71	up	-0,35209036	0,42050967	Mtr.17741				AT3G23880	F-box family protein, contains F-box domain Pfam:PF00646	7,00E-06
Mtr.23254.1.S1_at	0,02680773	1,71	down	0,19791985	-0,5739875					AT2G28550	AP2 domain-containing transcription factor RAP2.7 (RAP2.7), nearly identical to AP2 domain transcription factor RAP2.7 (GI:2281639) (Arabido	1,00E-04
Mtr.21910.1.S1_at	0,02375088	1,71	down	0,24411853	-0,5276575					AT1G17860	trypsin and protease inhibitor family protein / Kunitz family protein, similar to LeMir (miraculin homolog) GI:2654440 from (Lycopersicon escul	2,00E-08
Mtr.43231.1.S1_s_at	0,01285029	1,71	up	-0,29606453	0,47558245		homologue to AT5g02020/T7H20_70 {Arabidopsis thaliana;}, partial (37%)			AT5G02020	expressed protein	8,00E-30
Mtr.31924.1.S1_at	0,04809944	1,71	up	-0,17612521	0,59444255					AT2G33770	Encodes a ubiquitin-conjugating E2 enzyme. UBC24 mRNA accumulation is suppressed by miR399f, miR399b and miR399c. Involved in phosph	7,00E-16
Mtr.5445.1.S1_at	0,00950975	1,71	up	-0,28470722	0,48584208					AT1G75810	expressed protein	9,00E-10
Mtr.49862.1.S1_at	0,00209813	1,71	up	-0,38272443	0,387393	Mtr.14128			GO:0008168	AT4G35150	O-methyltransferase family 2 protein, similar to caffeic acid O-methyltransferase, Pinus taeda, gb:U39301	6,00E-32
Mtr.44368.1.S1_at	0,00249541	1,70	down	0,3387092	-0,42898846		weakly similar to (Q8S9J7) AT5g65760/MPA24_11, partial (35%)			AT2G24280	serine carboxypeptidase S28 family protein, contains Pfam profile: PF05577 Serine carboxypeptidase S28	
Mtr.10812.1.S1_at	0,00618983	1,70	down	0,4146018	-0,3513042	Mtr.1422	similar to (O48923) Cytochrome P450 71D10 (EC 1.14.-.- EC 1.14.-.-), partial (70%)		GO:0055114	AT3G61040	cytochrome P450 family protein, similar to cytochrome P450 monooxygenase - Arabidopsis thaliana, EMBL:D78600;encodes a protein with cytc	4,5
Mtr.652.1.S1_at	0,00606499	1,70	up	-0,35385227	0,41193137					AT3G26744	basix helix-loop-helix (bHLH) family protein, contains Pfam profile: PF00010 helix-loop-helix DNA-binding domain;similar to basic helix-loop-helix (bHLH) famil	
Mtr.5325.1.S1_at	0,03948455	1,70	up	-0,2228508	0,5416315					AT3G51080	zinc finger (GATA type) family protein, GATA transcription factor 3 - Arabidopsis thaliana, PIR:T05288	0,11
Mtr.35779.1.S1_at	0,02703718	1,70	up	-0,22768123	0,5365928		homologue to (Q9XES7) Seed maturation protein PM27, partial (20%)			AT1G72100	late embryogenesis abundant domain-containing protein / LEA domain-containing protein, low similarity to embryogenic gene (Betula pendula)	1,00E-07
Mtr.40804.1.S1_at	4,08E-04	1,70	down	0,37571892	-0,3872199		similar to (Q94B55) Putative RING zinc finger ankyrin protein, partial (74%)			AT2G01460	phosphoribulokinase/uridine kinase family protein, contains Pfam profile PF00485: Phosphoribulokinase / Uridine kinase family	8,5
Mtr.21515.1.S1_at	0,02346492	1,70	down	0,24445565	-0,51805323					AT2G32620	cellulose synthase family protein, similar to Zea mays cellulose synthase-5 (gi:9622882), -4 (gi:9622880), -9 (gi:9622890);encodes a gene sir	2,00E-30
Mtr.35193.1.S1_at	0,03302122	1,70	up	-0,43777403	0,32439232	Mtr.10591						
Mtr.15484.1.S1_at	0,03503741	1,70	down	0,370653	-0,3912084					AT5G66390	peroxidase 72 (PER72) (P72) (PRXR8), identical to SP|Q9FJZ9 Peroxidase 72 precursor (EC 1.11.1.7) (Atperox P72) (PRXR8) (ATP6a) {Arabidopsis thaliana}	
Mtr.10681.1.S1_s_at	0,00488446	1,69	up	-0,32985625	0,43043295		similar to unknown protein {Arabidopsis thaliana;}, partial (64%)			AT3G62550	universal stress protein (USP) family protein, similar to ER6 protein (Lycopersicon esculentum) GI:5669654; contains Pfam profile PF00582: un	1,00E-35
Mtr.16639.1.S1_at	0,00493418	1,69	down	0,3176991	-0,44082466					AT5G59480	haloacid dehalogenase-like hydrolase family protein, low similarity to SP|P53078 SSM1 protein {Saccharomyces cerevisiae}; contains InterPro accession IPR0(	
Mtr.37666.1.S1_at	0,02505079	1,69	up	-0,43075624	0,32759047		weakly similar to probable cinnamoyl-CoA reductase [imported] - Arabidopsis thaliana, partial (85%			AT2G33590	cinnamoyl-CoA reductase family, similar to cinnamoyl-CoA reductase from Pinus taeda (GI:17978649), Eucalyptus gunnii (GI:2058311)	9,00E-12
Mtr.39485.1.S1_s_at	0,01632461	1,69	down	0,38919696	-0,36900273		similar to (Q8H6S8) Translation initiation factor, partial (18%)			AT1G76825	eukaryotic translation initiation factor 2 family protein / eIF-2 family protein, similar to SP|O60841 Translation initiation factor IF-2 (Homo sapi	2,00E-26
Mtr.5535.1.S1_at	0,0111427	1,69	up	-0,5157364	0,24039237					AT3G21100	RNA recognition motif (RRM)-containing protein, contains Pfam profile:PF00076 RNA recognition motif;similar to expressed protein [Arabidopsis	2,00E-09
Mtr.42610.1.S1_at	0,00416815	1,69	up	-0,4097643	0,34608746		similar to (Q84P18) Acyl-activating enzyme 17, partial (13%)			AT5G23050	acyl-activating enzyme 17 (AAE17), nearly identical to acyl-activating enzyme 17 (Arabidopsis thaliana) GI:29893266; similar to acetyl-CoA synthetase (SP|P2	
Mtr.43232.1.S1_at	0,00753588	1,69	up	-0,49974537	0,25541654		similar to (Q93XX0) Hypothetical protein At4g10430; F7L13.10 (Hypothetical protein At4g10430), partial (84%			AT3G24100	four F5 family protein / 4F5 family protein, contains Pfam PF04419: 4F5 protein family	1,00E-04
Msa.3143.1.S1_at	0,01910991	1,69	down	0,33675608	-0,41787353					AT4G15440	hydroperoxide lyase (HPL1), identical to hydroperoxide lyase GI:3822403 from (Arabidopsis thaliana);Encodes a hydroperoxide lyase. Also a member of the C	
Mtr.10890.1.S1_at	0,0024377	1,69	down	0,39571762	-0,3579251					AT3G11385	DC1 domain-containing protein, contains Pfam protein PF03107 DC1 domain	1,5
Mtr.31744.1.S1_at	0,00412109	1,69	down	0,30390677	-0,44906616	Mtr.1447				AT5G07850	transferase family protein, similar to anthranilate N-hydroxycinnamoyl/benzoyltransferase, Dianthus caryophyllus (gi:2239091); contains Pfam	0,002
Mtr.44445.1.S1_at	0,03960203	1,68	up	-0,20064354	0,55000764		similar to (Q8S8Z8) Syringolide-induced protein B13-1-9, partial (60%)			AT2G35980	harpin-induced family protein (YLS9) / HIN1 family protein / harpin-responsive family protein, similar to harpin-induced protein hin1 (GI:1619	4,00E-22
Mtr.25985.1.S1_at	0,03579802	1,68	up	-0,19964027	0,5502761					AT3G16150	L-asparaginase, putative / L-asparagine amidohydrolase, putative, similar to Swiss-Prot:P30364 L-asparaginase (EC 3.5.1.1) (L-asparagine amidohydrolase) (	
Mtr.25132.1.S1_at	0,04846412	1,68	down	0,24192905	-0,5055547					AT5G65400	expressed protein, contains Pfam profile: PF03959 domain of unknown function (DUF341) contains Pfam profile: PF03959 domain of unknown function (DUF34	
Mtr.10603.1.S1_at	0,01778287	1,68	up	-0,21952343	0,5275154		similar to (Q8GUP5) Putative cation transporter, partial (94%)			AT4G31290	ChaC-like family protein, contains Pfam profile: PF04752 ChaC-like protein	2,00E-32
Mtr.35080.1.S1_at	0,00244592	1,68	up	-0,4228622	0,32388243					AT2G18700	glycosyl transferase family 20 protein / trehalose-phosphatase family protein, similar to trehalose-6-phosphate synthase SL-TPS/P (Selaginella lepidophylla) Gl	
AFFX-Mtr-r2-Ec-bioB-M	0,02664428	1,68	down	0,51668406	-0,22904778					AT2G43360	biotin synthase (BioB) (BIO2), identical to SP|P54967 Pfam profile PF04055: radical SAM domain protein;Catalyzes the conversion of dethiobiot	1,00E-29
Mtr.37579.1.S1_at	0,02276531	1,68	up	-0,28685498	0,4578921		similar to putative branched chain alpha-keto acid dehydrogenase E2 subunit, partial (67%)			AT3G06850	branched chain alpha-keto acid dehydrogenase E2 subunit (din3), identical to branched chain alpha-keto acid dehydrogenase E2 subunit (din3) (Arabidopsis th	
Mtr.42941.1.S1_at	0,00672253	1,67	up	-0,38301167	0,36049762		similar to unknown protein {Arabidopsis thaliana;}, partial (97%)			AT4G21320	Encodes heat-stress-associated 32-kD protein. Up-regulated by heat shock. Thermotolerance in a knockout mutant was compromised following	2,00E-42
AFFX-BioB-5_at	0,04138692	1,67	down	0,5486228	-0,19488621				GO:0008152	AT2G43360	biotin synthase (BioB) (BIO2), identical to SP|P54967 Pfam profile PF04055: radical SAM domain protein;Catalyzes the conversion of dethiobiot	1,00E-26
Mtr.36016.1.S1_at	0,00289442	1,67	up	-0,332019	0,41011825	Mtr.4715				AT3G58310	receptor-like protein kinase-related, contains Pfam profile: PF01657 Domain of unknown function that is usually associated with protein kinase	4,00E-05
Mtr.38662.1.S1_at	0,00705326	1,67	up	-0,39379135	0,348056		similar to (Q6ID86) At4g34360, partial (82%)			AT4G34360	protease-related, similar to PIR|I46078 endothelin converting enzyme, Bos primigenius taurus	
Mtr.41327.1.S1_at	0,03464713	1,67	up	-0,32383503	0,41756216	Mtr.5999	(Q8GZM5) Lecithine cholesterol acyltransferase-like protein, complete		GO:0006629	AT1G75820	CLAVATA1 receptor kinase (CLV1), identical to receptor kinase (CLV1) GB:AAB58929 GI:2160756 (Arabidopsis thaliana);Putative receptor kina	2,5
Mtr.38531.1.S1_at	0,04412206	1,67	up	-0,2229555	0,51486206	Mtr.11195	similar to unknown protein {Oryza sativa (japonica cultivar-group);}, partial (12%)			AT1G52290	protein kinase family protein, contains Pfam profile PF00069: Protein kinase domain	2,1
Mtr.44674.1.S1_at	0,00829166	1,67	down	0,44679847	-0,29396057		similar to probable photomorphogenesis repressor protein [imported] - Arabidopsis thaliana, partial			AT2G46340	phytochrome A supressor spa1 (SPA1), identical to phytochrome A supressor spa1 (GI:4809171) (Arabidopsis thaliana); contains 8 WD-40 repeats (Pfam PF0(	
Mtr.15941.1.S1_at	0,04373735	1,67	up	-0,21708567	0,5227933					AT3G53380	lectin protein kinase family protein, contains Pfam domains, PF00069: Protein kinase domain, PF00138: Legume lectins alpha domain, and PF0(	2,00E-41
Mtr.43091.1.S1_s_at	0,04100012	1,67	down	0,4316144	-0,3081762		similar to (Q9FNW7) Seed maturation protein LEA 4, partial (49%)			AT5G06760	late embryogenesis abundant group 1 domain-containing protein / LEA group 1 domain-containing protein, low similarity to SP|P46515 11 kDa	6,00E-11
Mtr.34896.1.S1_at	0,00525548	1,67	up	-0,38994408	0,34938827					AT4G38830	protein kinase family protein, contains Pfam domain PF00069: Protein kinase domain	2,00E-21
AFFX-BioC-3_at	0,04730921	1,67	down	0,5777051	-0,16126347				GO:0008152	AT3G27785	myb family transcription factor (MYB118), contains PFAM profile: PF00249 myb-like DNA binding domain;AF334817 Arabidopsis thaliana putati	0,55
Mtr.40946.1.S1_at	0,00205709	1,67	up	-0,3590673	0,379577	Mtr.13728	similar to (Q9AYM5) CPRD49 protein, complete		GO:0006629	AT3G11210	GDSL-motif lipase/hydrolase family protein, contains Pfam profile PF00657: Lipase/Acylhydrolase with GDSL-like motif	6,00E-16
Mtr.9040.1.S1_at	0,00170101	1,67	up	-0,36672816	0,3712915		similar to (Q9C5U1) Histidine kinase, partial (37%)			AT2G28520	Vacuolar proton ATPase subunit VHA-a isoform 1. Localized in the trans-Golgi network.	0,86
Msa.1498.1.S1_at	0,00908994	1,67	up	-0,36662897	0,3706096					AT4G33300	disease resistance protein (CC-NBS-LRR class), putative, domain signature CC-NBS-LRR exists, suggestive of a disease resistance protein.;simi	3,00E-34
Mtr.4745.1.S1_at	0,0033047	1,67	up	-0,3915564	0,34428915	Mtr.20682				AT5G05410	encodes a member of the DREB subfamily A-2 of ERF/AP2 transcription factor family (DREB2A). The protein contains one AP2 domain. There ar	6,00E-23
Mtr.43889.1.S1_at	0,03107136	1,67	down	0,17748976	-0,5580552		similar to (Q9FLP4) Beta-1 3-glucanase-like protein, partial (52%)			AT5G55180	glycosyl hydrolase family 17 protein, similar to elicitor inducible chitinase Nt-SubE76 GI:11071974 from (Nicotiana tabacum)	
Mtr.49702.1.S1_at	0,0480397	1,66	down	0,2002651	-0,5351518					AT2G01190	octicosapeptide/Phox/Bem1p (PB1) domain-containing protein, Pfam profile PF00564: PB1 domain	1,00E-12
Mtr.34902.1.S1_at	0,04405641	1,66	up	-0,19334929	0,54168314	Mtr.2161				AT3G45850	kinesin motor protein-related, kinesin-related protein TKRP125, Nicotiana tabacum, PIR:T02017	10
Mtr.51862.1.S1_at	2,76E-05	1,66	down	0,37474155	-0,35883632	Mtr.17241				AT3G52070	expressed protein	5,00E-10
Mtr.4850.1.S1_at	0,0241663	1,66	up	-0,2512652	0,48207316	Mtr.920				AT1G67980	caffeoyl-CoA 3-O-methyltransferase, putative, similar to GI:2960356 (Populus balsamifera subsp. trichocarpa), GI:684942 (Medicago sativa su	9,00E-23
Mtr.22599.1.S1_at	0,00411508	1,66	up	-0,2731444	0,45805454	Mtr.5930			GO:0019752	AT1G43710	serine decarboxylase, identical to serine decarboxylase (Arabidopsis thaliana) GI:15011302; contains Pfam profile PF00282: Pyridoxal-dependent decarboxylas	
Mtr.24464.1.S1_at	0,02093571	1,66	up	-0,2409153	0,4901266					AT1G16670	protein kinase family protein, contains protein kinase domain, Pfam:PF00069; similar to receptor-like serine/threonine kinase GI:2465923 from	9,00E-38
Mtr.3013.1.S1_at	0,01263958	1,66	down	0,5049832	-0,2260241					AT1G22360	UDP-glucoronosyl/UDP-glucosyl transferase family protein, contains Pfam profile: PF00201 UDP-glucoronosyl and UDP-glucosyl transferase;similar to UDP-gluc	
Mtr.24746.1.S1_at	0,02422989	1,66	down	0,44559167	-0,2843816					AT3G42860	zinc knuckle (CCHC-type) family protein, contains Pfam domain, PF00098: Zinc knuckle	5,00E-04
Msa.1916.1.S1_at	0,00180181	1,66	down	0,37883916	-0,35062456					AT5G06760	late embryogenesis abundant group 1 domain-containing protein / LEA group 1 domain-containing protein, low similarity to SP|P46515 11 kDa	3,00E-18
Mtr.49684.1.S1_at	9,10E-06	1,66	down	0,36656824	-0,36250892					AT2G15490	UDP-glucoronosyl/UDP-glucosyl transferase family protein, contains Pfam profile: PF00201 UDP-glucoronosyl and UDP-glucosyl transferase	2,00E-38
Mtr.8799.1.S1_at	0,00484807	1,66	down	0,36897978	-0,3600658		similar to (Q9ZT62) Raffinose synthase (EC 2.4.1.82), partial (84%)			AT5G40390	raffinose synthase family protein, similar to galactinol-raffinose galactosyltransferase (Vigna angularis) GI:6634701, seed imbibition protein GB	4,00E-27
Mtr.37298.1.S1_at	0,03327975	1,66	up	-0,26712194	0,4618565	Mtr.7735	weakly similar to (Q9LUD0) Cytochrome P450, partial (48%)		GO:0055114	AT3G14610	cytochrome P450, putative, similar to GB:Q05047 from (Catharanthus roseus);putative cytochrome P450	2,00E-36
Mtr.8997.1.S1_at	0,02031224	1,66	down	0,22590192	-0,50279206		similar to (O04945) Enoyl-ACP reductase precursor (EC 1.3.1.9 EC 1.3.1.9), partial (89%)			AT2G05990	enoyl-(acyl-carrier protein) reductase (NADH), chloroplast, putative / NADH-dependent enoyl-ACP reductase, putative, strong similarity to enoy	6,00E-25
Mtr.38311.1.S1_at	0,04058183	1,66	down	0,19846089	-0,5298481		similar to AT4g33790/T16L1_280 {Arabidopsis thaliana;}, partial (70%)			AT4G33790	acyl CoA reductase, putative, similar to acyl CoA reductase (Simmondsia chinensis) GI:5020215; contains Pfam profile PF03015: Male sterility	6,00E-39
Mtr.29499.1.S1_at	0,01360607	1,66	up	-0,3678217	0,360072					AT2G38120	amino acid permease, putative (AUX1), identical to AUX1 GI:1531758 from (Arabidopsis thaliana);Similar to amino acid permeases. Putative transporter of am	
Mtr.3530.1.S1_at	0,00711673	1,65	down	0,33763885	-0,3882936	Mtr.21164				AT3G59890	dihydrodipicolinate reductase family protein, weak similarity to SP|Q52419 Dihydrodipicolinate reductase (EC 1.3.1.26) (DHPR) {Pseudomonas syringae}; cont	
Mtr.43865.1.S1_at	0,00687408	1,65	up	-0,32422155	0,4812772		homologue to (P51819) Heat shock protein 83, partial (77%)			AT5G52640	heat shock protein 81-1 (HSP81-1) / heat shock protein 83 (HSP83), nearly identical to SP|P27323 Heat shock protein 81-1 (HSP81-1) (Heat shock protein 83	
Mtr.20814.1.S1_at	0,00808196	1,65	up	-0,37731227	0,34739622					ATMG00710	hypothetical protein	1,00E-12
Mtr.41756.1.S1_at	0,01514752	1,65	up	-0,30418268	0,41933045		similar to (Q9T0D3) Heat shock factor protein 7 (HSF 7) (Heat shock transcription factor 7) (HSTF 7), partial (			AT5G62020	member of Heat Stress Transcription Factor (Hsf) family;heat shock factor protein, putative (HSF6) / heat shock transcription factor, putative (	2,00E-12
Mtr.18387.1.S1_at	0,01433263	1,65	up	-0,4447042	0,2788067					AT5G03150	zinc finger (C2H2 type) family protein, contains Pfam domain, PF00096: Zinc finger, C2H2 type	0,013
Mtr.50870.1.S1_at	0,04052904	1,65	up	-0,33859667	0,38410917	Mtr.22082			GO:0005515	AT1G15740	leucine-rich repeat family protein	
Mtr.23393.1.S1_x_at	0,00711123	1,65	up	-0,39821133	0,32364893					AT2G29500	17.6 kDa class I small heat shock protein (HSP17.6B-CI), contains Pfam PF00011: Hsp20/alpha crystallin family; identified in Scharf, K-D., et al, Cell Stress &	
Mtr.21098.1.S1_at	0,0392355	1,65	up	-0,34895453	0,3727243					AT5G57240	oxysterol-binding family protein, low similarity to SP|P22059 Oxysterol-binding protein 1 {Homo sapiens}; contains Pfam profile PF01237: Oxy	1,40E-45
Msa.1239.1.S1_at	0,00505952	1,65	down	0,3705152	-0,3511497					AT2G46340	phytochrome A supressor spa1 (SPA1), identical to phytochrome A supressor spa1 (GI:4809171) (Arabidopsis thaliana); contains 8 WD-40 repe	5,00E-32
Mtr.18027.1.S1_at	0,00215996	1,65	down	0,42549872	-0,29503265					AT1G10340	ankyrin repeat family protein, contains ankyrin repeat domains, Pfam:PF00023	1,00E-17
Mtr.10925.1.S1_at	0,00722337	1,65	down	0,30867279	-0,4237029		weakly similar to unknown protein {Arabidopsis thaliana;}, partial (26%)			AT1G22900	similar to disease resistance-responsive family protein [Arabidopsis thaliana] (TAIR:At5g42500.1); similar to At5g42500 [Oryza sativa (japonic	1,00E-24
Mtr.39126.1.S1_at	0,00898435	1,65	down	0,28346905	-0,43562207					AT3G59140	ABC transporter family protein, putative multi resistance protein mrp - Arabidopsis thaliana, EMBL:ATMRPPROT;member of MRP subfamily	1
Mtr.6875.1.S1_at	0,00384205	1,65	up	-0,31304947	0,40510097					AT1G07650	leucine-rich repeat transmembrane protein kinase, putative, similar to GB:AAC50043 from (Arabidopsis thaliana) (Plant Mol. Biol. 37 (4), 587-5	1,00E-39
Mtr.7199.1.S1_at	0,00470163	1,64	up	-0,27625212	0,4408822		similar to probable multiple transmembrane domain protein PFB0475c - malaria parasite, partial (5%			AT2G02450	no apical meristem (NAM) family protein, contains Pfam PF02365: No apical meristem (NAM) domain	0,26
Mtr.583.1.S1_at	0,03487857	1,64	up	-0,49769402	0,2194233					AT1G72480	expressed protein	19
Mtr.45159.1.S1_at	0,00169667	1,64	up	-0,35200667	0,36484542		similar to (Q84Z33) Hypothetical protein OSJNBa0036M16.104 (Hypothetical protein P0650C03.19), partial (5%			AT3G20340	expressed protein	1,00E-06
Mtr.28935.1.S1_at	0,00162909	1,64	down	0,35846853	-0,35617748					AT3G03050	cellulose synthase family protein (CslD3), similar to cellulose synthase catalytic subunit gi:2827143 from (Arabidopsis thaliana), cellulose synth	1,2
Mtr.23495.1.S1_s_at	0,01233567	1,64	up	-0,43113136	0,28333822					AT3G28040	leucine-rich repeat transmembrane protein kinase, putative, contains Pfam profiles: PF00560 leucine rich repeat, PF00069 eukaryotic protein ki	1,00E-25
Mtr.48768.1.S1_at	0,0034266	1,64	up	-0,2939075	0,41979885					AT5G14920	gibberellin-regulated family protein, similar to SP|P46689 Gibberellin-regulated protein 1 precursor {Arabidopsis thaliana}; contains Pfam profil	9,00E-23
Mtr.6559.1.S1_at	0,00168037	1,64	up	-0,42949256	0,28236517	Mtr.3322				AT1G06720	expressed protein, contains Pfam domain, PF04950: Protein of unknown function (DUF663)	13
Mtr.34753.1.S1_at	0,02519032	1,64	up	-0,50257903	0,2077012					AT4G36850	PQ-loop repeat family protein / transmembrane family protein, similar to SP|Q10482 Seven transmembrane protein 1 {Schizosaccharomyces p	2,00E-14
Mtr.24258.1.S1_s_at	0,01021429	1,64	up	-0,34569	0,36440015					AT5G11970	expressed protein	0,54
Mtr.39069.1.S1_at	0,00433253	1,63	up	-0,4008379	0,3084019		weakly similar to (Q8RXZ4) Hypothetical protein At1g79630, partial (35%)			AT3G02750	protein phosphatase 2C family protein / PP2C family protein, similar to protein phosphatase-2C; PP2C (GI:3643088) (Mesembryanthemum crys	1,00E-07
Mtr.37367.1.S1_at	0,00417152	1,63	down	0,3090229	-0,39890543		weakly similar to (Q8S342) Putative anthocyanidine rhamnosyl-transferase, partial (32%)			AT3G19050	PHRAGMOPLAST ORIENTING KINESIN 2 is one of the two Arabidopsis homologs isolated in yeast two-hybrid screen for interaction partners of r	10
Mtr.18798.1.S1_at	0,00390047	1,63	down	0,28657022	-0,4211413					AT1G17860	trypsin and protease inhibitor family protein / Kunitz family protein, similar to LeMir (miraculin homolog) GI:2654440 from (Lycopersicon escul	3,00E-35
Mtr.5608.1.S1_at	0,00915322	1,63	down	0,3000393	-0,407547					AT3G25570	adenosylmethionine decarboxylase family protein, contains Pfam profile: PF01536 adenosylmethionine decarboxylase	3,00E-15
Mtr.4906.1.S1_s_at	0,00114728	1,63	up	-0,3283968	0,37905788					AT1G12010	encodes a protein whose sequence is similar to 1-amino-cyclopropane-1-carboxylic acid oxidase	2,00E-05
Mtr.50058.1.S1_s_at	0,02873999	1,63	down	0,3244071	-0,38218895					AT4G33070	pyruvate decarboxylase, putative, strong similarity to SP|P51846 Pyruvate decarboxylase isozyme 2 (EC 4.1.1.1) (PDC) {Nicotiana tabacum}; contains InterPr	
Mtr.1838.1.S1_at	0,04645809	1,63	down	0,39613366	-0,31010485					AT4G35740	DNA helicase (RECQl3), identical to DNA Helicase (Arabidopsis thaliana) GI:11121447; contains Pfam profiles PF00270: DEAD/DEAH box helicase, PF00271: He	
Mtr.13505.1.S1_at	0,0286463	1,63	down	0,18955915	-0,5156021		similar to hypothetical protein similar to Arabidopsis thaliana chromosome 2 At2g03500, partial (14%)			AT1G25550	myb family transcription factor, contains Pfam domain, PF00249: Myb-like DNA-binding domain	7,00E-11
Mtr.6155.1.S1_at	0,01825193	1,63	down	0,32061943	-0,38447937					AT3G50310	protein kinase-related, contains eukaryotic protein kinase domain, INTERPRO:IPR000719;member of MEKK subfamily	0,14
Mtr.8289.1.S1_at	0,02580387	1,63	up	-0,23485756	0,4688727					AT4G20310	similar to PREDICTED: similar to Membrane-bound transcription factor site 2 protease (S2P endopeptidase) (Site-2 protease) (Sterol-regulatory	0,42

Mtr.18474.1.S1_at	0,00527603	1,63	down	0,2843902	-0,4188118					AT5G20690	leucine-rich repeat transmembrane protein kinase, putative, receptor-like protein kinase PRK1, tomato, PIR:T07865
Mtr.19295.1.S1_at	0,0210136	1,63	down	0,38789368	-0,31451735					AT4G37270	cadmium/zinc-transporting ATPase, putative (HMA1), contains InterPro accession IPR001757: ATPase, E1-E2 type; identical to Potential cadmi
Mtr.30196.1.S1_at	0,00773469	1,63	down	0,43977484	-0,26233563					AT1G19640	S-adenosyl-L-methionine:jasmonic acid carboxyl methyltransferase (JMT), nearly identical to gi:13676829;Encodes a S-adenosyl-L-methionine
Mtr.39690.1.S1_at	0,01864908	1,63	up	-0,37827602	0,32380375		(Q9CPB8) FecC, partial (3%)			AT3G06960	expressed protein
Mtr.44428.1.S1_at	0,01927726	1,63	down	0,32999453	-0,37094864		similar to (Q94C56) Putative FtsH protease (Fragment), partial (50%)			AT1G80260	similar to tubulin family protein [Arabidopsis thaliana] (TAIR:At1g20570.1); similar to unnamed protein product [Tetraodon nigroviridis] (GB:C
Mtr.33360.1.S1_at	0,03463854	1,63	up	-0,29652944	0,40413603	Mtr.22581				AT2G23690	expressed protein
Mtr.43531.1.S1_at	0,00232479	1,63	up	-0,31067243	0,38988623		homologue to (Q9SJF0) Serine/threonine protein phosphatase BSL2 (BSU1-like protein 2), partial (37%)			AT1G53930	ubiquitin family protein, contains Pfam profile: PF00240 ubiquitin family
Mtr.9658.1.S1_at	0,00746201	1,62	down	0,41813788	-0,28187022					AT3G06470	GNS1/SUR4 membrane family protein, similar to SP|P39540 Elongation of fatty acids protein 1 {Saccharomyces cerevisiae}; contains Pfam pro
Mtr.10954.1.S1_at	0,01876829	1,62	up	-0,29898134	0,39912668		similar to (Q8H2C2) Serine/threonine kinase, partial (60%)			AT5G58380	CBL-interacting protein kinase 10 (CIPK10), identical to CBL-interacting protein kinase 10 (Arabidopsis thaliana) gi|1324919|gb|AAK16685; contains Pfam pr
Mtr.35142.1.S1_at	0,01575083	1,62	down	0,25812086	-0,43956995	Mtr.77			GO:0016020	AT3G45600	Member of TETRASPANIN family
Mtr.24258.1.S1_at	0,01036113	1,62	up	-0,30492178	0,39275995					AT2G47480	expressed protein
Mtr.50519.1.S1_at	0,00518097	1,62	up	-0,40646347	0,29082775					AT1G52780	expressed protein
Mtr.38399.1.S1_at	0,01168833	1,62	down	0,26186624	-0,43524584		similar to hypothetical protein At2g46420 [imported] - Arabidopsis thaliana, partial (54%)			AT2G46420	expressed protein
Mtr.7260.1.S1_at	0,00830306	1,62	up	-0,24414594	0,45260844		weakly similar to (Q94G63) Ethylene-induced esterase, partial (24%)			AT4G37150	esterase, putative, similar to ethylene-induced esterase (Citrus sinensis) GI:14279437, polyneuridine aldehyde esterase (Rauvolfia serpentina)
Mtr.36283.1.S1_at	0,0078838	1,62	up	-0,2782739	0,4182779					AT2G26480	UDP-glucoronosyl/UDP-glucosyl transferase family protein, contains Pfam profile: PF00201 UDP-glucoronosyl and UDP-glucosyl transferase
Mtr.37477.1.S1_at	0,00594354	1,62	up	-0,23445606	0,46208224		similar to (Q9SAL0) T8K14.20 protein (At1g79380/T8K14_20), partial (81%)			AT1G79380	copine-related, low similarity to SP|Q99829 Copine I {Homo sapiens}
Mtr.43206.1.S1_at	0,02118177	1,62	up	-0,23199527	0,46402168	Mtr.2726	similar to (Q8H1G4) Putative formamidase, partial (96%)		GO:0008152	AT4G37550	formamidase, putative / formamide amidohydrolase, putative, similar to SP|Q50228 Formamidase (EC 3.5.1.49) (Formamide amidohydrolase) {Methylophilus
Mtr.40482.1.S1_at	0,03289193	1,62	down	0,17124431	-0,52467537		similar to At3g62040 {Arabidopsis thaliana;}, partial (84%)			AT3G62040	similar to haloacid dehalogenase-like hydrolase family protein [Arabidopsis thaliana] (TAIR:At5g59490.1); similar to expressed protein [Oryza s
Mtr.24534.1.S1_at	0,0455246	1,62	up	-0,5231657	0,17231798					AT3G56230	speckle-type POZ protein-related, contains Pfam PF00651 : BTB/POZ domain; similar to Speckle-type POZ protein (SP:O43791) (Homo sapiens
Mtr.36396.1.S1_at	0,04215477	1,62	down	0,22535054	-0,47009787					AT5G20040	tRNA isopentenyltransferase 9 / IPP transferase 9 (IPT9), identical to tRNA isopentenyltransferase (IPT9) (Arabidopsis thaliana) GI:14279070;A
Mtr.34165.1.S1_at	0,03304141	1,62	up	-0,45748582	0,23788102					AT2G18196	copper chaperone (CCH)-related, low similarity to copper chaperone homolog CCH (Glycine max) GI:6525011 contains Pfam profile PF00403: Heavy-metal-ass
Mtr.5040.1.S1_s_at	0,00247585	1,62	down	0,30625662	-0,3888855	Mtr.19321			GO:0005488	AT1G80130	expressed protein
Msa.1122.1.S1_at	6,01E-04	1,62	down	0,35423055	-0,34081635					AT5G40390	raffinose synthase family protein, similar to galactinol-raffinose galactosyltransferase (Vigna angularis) GI:6634701, seed imbibition protein GB
Mtr.10618.1.S1_at	0,01434932	1,62	up	-0,47143412	0,22318363		similar to (Q7XMX9) OSJNBb0006L01.10 protein (OSJNBa0001M07.1 protein), partial (45%)			AT4G34880	amidase family protein, similar to peptide amidase (Stenotrophomonas maltophilia) GI:19744118; contains Pfam profile PF01425: Amidase
Mtr.19846.1.S1_at	0,01306537	1,62	up	-0,3955307	0,2989634					AT1G44080	F-box protein-related / C-type lectin-related, contains F-box domain Pfam:PF00646, PF00059: Lectin C-type domain
Mtr.10692.1.S1_at	0,0399922	1,62	up	-0,28171936	0,41272163	Mtr.11914	weakly similar to (Q9M4G8) Putative ripening-related P-450 enzyme, partial (57%)		GO:0055114	AT1G33730	cytochrome P450, putative, Similar to cytochrome P450 76C2 (SP:O64637)(Arabidopsis thaliana); contains Pfam profile: PF00067 cytochrome
Msa.1520.1.S1_at	0,00763149	1,62	down	0,37091574	-0,32347694					AT3G15810	expressed protein, contains Pfam profile PF04525: Protein of unknown function (DUF567)
Mtr.9502.1.S1_at	0,0438093	1,62	up	-0,3613437	0,33223978		similar to (Q8S4Q4) Nodulin 6l, partial (55%)			AT3G53180	glutamine synthetase, putative, similar to glutamine synthetase (glutamate--ammonia ligase) (Bacillus subtilis) SWISS-PROT:P12425
Mtr.32638.1.S1_s_at	0,03136412	1,62	up	-0,4053278	0,2881166					AT5G63350	expressed protein
Mtr.44909.1.S1_at	0,0011591	1,61	down	0,27807173	-0,41173348		similar to (Q9FH91) Gb|AAF51525.1 (AT5g67220/K21H1_18), partial (31%)			AT5G67220	nitrogen regulation family protein, similar to unknown protein (gb|AAF51525.1) ; contains Pfam domain PF01207: Dihydrouridine synthase (Dus); similar to (S
Mtr.21518.1.S1_s_at	0,02535802	1,61	down	0,27293077	-0,41597334	Mtr.10786			GO:0004497	AT4G15440	hydroperoxide lyase (HPL1), identical to hydroperoxide lyase GI:3822403 from (Arabidopsis thaliana);Encodes a hydroperoxide lyase. Also a member of the C
AFFX-Mtr-r2-Ec-bioC-3	0,04615193	1,61	down	0,5475238	-0,14087741					AT3G27785	myb family transcription factor (MYB118), contains PFAM profile: PF00249 myb-like DNA binding domain;AF334817 Arabidopsis thaliana putati
Mtr.25206.1.S1_at	0,00210479	1,61	down	0,29726538	-0,38994074					AT2G04530	RNase Z, 97% identical to RNase Z (GI:20975607) (Arabidopsis thaliana); similar to RNase Z (GI:20975609) (Arabidopsis thaliana); identical to cDNA RNase Z
Mtr.51098.1.S1_s_at	0,00291801	1,61	down	0,34178686	-0,3451244					AT4G27590	copper-binding protein-related, low similarity to copper homeostasis factor gi:3168840 from Arabidopsis thaliana; contains Pfam profile PF0040
Mtr.4585.1.S1_s_at	0,02647629	1,61	down	0,21974818	-0,46706024					AT2G16440	DNA replication licensing factor, putative, similar to SP|P49717 DNA replication licensing factor MCM4 (CDC21 homolog) {Mus musculus}, SP|P
Mtr.6589.1.S1_at	0,0023989	1,61	up	-0,3253406	0,36073223					AT4G01100	mitochondrial substrate carrier family protein, contains Pfam profile: PF00153 mitochondrial carrier protein
Mtr.2643.1.S1_at	0,03749488	1,61	up	-0,2070837	0,47868252					AT4G11990	expressed protein, hypothetical protein F7H19.40 - Arabidopsis thaliana, PID:e1310054
Msa.2806.1.S1_at	0,04092573	1,61	up	-0,1544931	0,53113157					AT1G32170	xyloglucan:xyloglucosyl transferase, putative / xyloglucan endotransglycosylase, putative / endo-xyloglucan transferase, putative (XTR4), ident
Mtr.9739.1.S1_at	0,01085603	1,61	down	0,40125194	-0,28424263		similar to (Q9LK88) NADPH:quinone oxidoreductase, partial (94%)			AT3G27890	NADPH-dependent FMN reductase family protein, contains Pfam profile: PF03358 NADPH-dependent FMN reductase;Encodes NAD(P)H:quinone reductase which
Mtr.8207.1.S1_at	0,0035241	1,61	down	0,3406957	-0,3443891					AT2G46340	phytochrome A supressor spa1 (SPA1), identical to phytochrome A supressor spa1 (GI:4809171) (Arabidopsis thaliana); contains 8 WD-40 rep
Mtr.34167.1.S1_at	0,00843728	1,60	up	-0,34790134	0,33460012					AT4G03230	S-locus lectin protein kinase family protein, contains Pfam domins, PF00069: Protein kinase domain, PF00954: S-locus glycoprotein family and
Mtr.7064.1.S1_at	0,02447719	1,60	up	-0,29997906	0,38236442					AT2G39060	nodulin MtN3 family protein, similar to MtN3 GI:1619602 (root nodule development) from (Medicago truncatula)
Mtr.7719.1.S1_at	0,04766295	1,60	up	-0,15296555	0,5292864					AT1G09970	leucine-rich repeat transmembrane protein kinase, putative, Similar to A. thaliana receptor-like protein kinase (gb|RLK5_ARATH). ESTs gb|ATTS0475,gb|ATTS
Msa.2919.1.S1_at	0,00569575	1,60	up	-0,26976237	0,41209158					AT1G67480	kelch repeat-containing F-box family protein, similar to SKP1 interacting partner 6 (Arabidopsis thaliana) GI:10716957; contains Pfam profiles I
Mtr.4723.1.S1_at	0,02189715	1,60	down	0,23760684	-0,4441115	Mtr.1447				AT3G50280	transferase family protein, similar to anthranilate N-hydroxycinnamoyl/benzoyltransferase, Dianthus caryophyllus, PIR:T10717 (gi:2239091); c
Mtr.7238.1.S1_at	0,00122725	1,60	up	-0,30055952	0,38108492		homologue to mKIAA1365 protein {Mus musculus;}, partial (1%)			AT3G07040	disease resistance protein RPM1 (CC-NBS-LRR class), putative, domain signature CC-NBS-LRR exists, suggestive of a disease resistance protei
Mtr.41809.1.S1_s_at	0,00725869	1,60	up	-0,23985179	0,4416361		similar to (Q9FMF5) Non-phototropic hypocotyl 3, partial (27%)			AT5G64330	non-phototropic hypocotyl 3 (NPH3), identical to non-phototropic hypocotyl 3 (Arabidopsis thaliana) gi|6224712|gb|AAF05914, PMID:1054215
Mtr.46188.1.S1_x_at	9,42E-04	1,60	up	-0,3966127	0,3413067					AT5G12050	expressed protein
Mtr.4381.1.S1_at	0,02994153	1,60	up	-0,32561135	0,35477558					AT2G44270	expressed protein, contains Pfam profile PF01171: PP-loop family
Mtr.51054.1.S1_at	0,03082409	1,60	up	-0,31030655	0,36863646					AT2G39910	expressed protein
Mtr.43716.1.S1_at	0,01604806	1,60	up	-0,24709924	0,43127856	Mtr.6897	similar to (Q9M2K9) Hypothetical protein F28O9.230, partial (75%)		GO:0016740	AT2G41640	expressed protein, contains Pfam domain, PF04577: Protein of unknown function (DUF563)
Mtr.31207.1.S1_s_at	0,00346341	1,60	down	0,39552227	-0,2811462	Mtr.18701				AT2G32180	expressed protein
Mtr.10136.1.S1_at	0,00170192	1,60	down	0,33997598	-0,33657646		similar to A_IG005I10.1 {Arabidopsis thaliana;}, partial (57%)			AT1G01700	Encodes a member of KPP-like gene family, homolog of KPP (kinase partner protein) gene in tomato. Also a member of the RopGEF (guanine nucleotide excha
Mtr.41661.1.S1_s_at	0,00593349	1,60	up	-0,34011698	0,33437648		similar to (Q73NQ3) Acetyltransferase GNAT family, partial (7%)			AT1G16020	expressed protein
Mtr.38860.1.S1_at	0,01019502	1,60	up	-0,34316254	0,33113018		similar to (Q9AVI2) Putative senescence-associated protein, partial (26%)			AT3G14070	cation exchanger, putative (CAX9), similar to sodium/calcium exchanger protein (Mus musculus) gi|13925661|gb|AAK49407; Ca2+:Cation Ant
Mtr.52242.1.S1_at	2,72E-04	1,60	up	-0,30029997	0,37358633					AT3G06580	galactokinase (GAL1), identical to galactokinase (Galactose kinase) (Arabidopsis thaliana) SWISS-PROT:Q9SEE5;Encodes a galactose kinase.
Mtr.1961.1.S1_at	6,95E-04	1,59	down	0,36704636	-0,30607033					AT1G78010	RNA modification GTPase, putative, similar to tRNA modification GTPase trmE (strain PCC 7120, Anabaena sp.) SWISS-PROT:Q8YN91
Mtr.39970.1.S1_at	0,0117873	1,59	down	0,33061567	-0,34237018		similar to (Q94IC8) TRNA isopentenyltransferase, partial (16%)			AT5G20040	tRNA isopentenyltransferase 9 / IPP transferase 9 (IPT9), identical to tRNA isopentenyltransferase (IPT9) (Arabidopsis thaliana) GI:14279070;A
Mtr.20676.1.S1_at	0,02051756	1,59	up	-0,34249339	0,32996002					AT4G14070	Plastidic acyl activating enzyme involved in the elongation of exogenous medium-chain fatty acids to 16- and 18-carbon fatty acids.
Mtr.48622.1.S1_x_at	0,0352296	1,59	down	0,35394096	-0,31847072					AT1G78990	transferase family protein, low similarity to acetyl CoA: benzylalcohol acetyltransferase Clarkia breweri GI:3170250, GI:6166336, Clarkia conci
Mtr.21518.1.S1_at	0,03211204	1,59	down	0,30427408	-0,36795172	Mtr.10786			GO:0004497	AT4G15440	hydroperoxide lyase (HPL1), identical to hydroperoxide lyase GI:3822403 from (Arabidopsis thaliana);Encodes a hydroperoxide lyase. Also a m
Mtr.19547.1.S1_s_at	0,01427681	1,59	down	0,20366383	-0,46822453	Mtr.6053			GO:0016787	AT5G18280	apyrase (APY2), identical to apyrase GI:6006801, GI:7672685 from (Arabidopsis thaliana);Encodes an enzyme with ATPase and ADPase activit
Mtr.38240.1.S1_s_at	0,01836283	1,59	down	0,4355289	-0,23578723		weakly similar to (Q8LCM8) Transcription factor TINY putative, partial (44%)			AT1G01250	encodes a member of the DREB subfamily A-4 of ERF/AP2 transcription factor family. The protein contains one AP2 domain. There are 17 meml
Msa.959.1.S1_at	0,0267448	1,59	up	-0,3169807	0,35393047					AT5G61020	YT521-B-like family protein, contains Pfam profile PF04146: YT521-B-like family;
Mtr.44458.1.S1_at	0,01294251	1,59	down	0,23668401	-0,43388858		weakly similar to (Q7XXR3) Hypothetical protein, partial (53%)			AT3G09510	hypothetical protein
Mtr.49441.1.S1_at	2,20E-04	1,59	up	-0,31945166	0,35079893					AT1G12010	encodes a protein whose sequence is similar to 1-amino-cyclopropane-1-carboxylic acid oxidase
Mtr.22943.1.S1_s_at	0,01368112	1,59	up	-0,24592535	0,42356482					AT2G02650	reverse transcriptase-related, similar to reverse transcriptase (Arabidopsis thaliana) GI:976278
Mtr.4975.1.S1_s_at	0,04348854	1,59	up	-0,23095195	0,4376502	Mtr.10081				AT1G17020	oxidoreductase, 2OG-Fe(II) oxygenase family protein, similar to flavonol synthase (Petunia x hybrida)(GI:311658), leucoanthocyanidin dioxyge
Mtr.29447.1.S1_at	0,04806337	1,59	down	0,21361129	-0,4544789					AT2G30430	hypothetical protein
Mtr.43605.1.S1_at	0,00145407	1,59	up	-0,35607514	0,3114144		(Q8MQW0) SD13096p, partial (0%)			AT2G44410	expressed protein
Mtr.6672.1.S1_at	0,01710997	1,59	down	0,21045081	-0,45659527					AT5G55580	mitochondrial transcription termination factor family protein / mTERF family protein, weak similarity to mtDBP protein (Paracentrotus lividus) G
Mtr.34695.1.S1_s_at	0,03252946	1,59	down	0,22004493	-0,44682583					AT5G46890	protease inhibitor/seed storage/lipid transfer protein (LTP) family protein, similar to proline-rich 14 kDa protein {Phaseolus vulgaris} GP|14208
Mtr.11398.1.S1_at	0,03497708	1,59	down	0,16535282	-0,500711		similar to (Q9M3G9) Hypothetical protein T29H11_270, partial (34%)			AT3G48500	expressed protein
Mtr.45000.1.S1_at	0,01313456	1,58	up	-0,3579603	0,30614352		similar to probable trehalose-6-phosphate synthase [imported] - Arabidopsis thaliana, partial (20%)			AT2G18700	glycosyl transferase family 20 protein / trehalose-phosphatase family protein, similar to trehalose-6-phosphate synthase SL-TPS/P (Selaginella lepidophylla) GI
Mtr.46740.1.S1_at	0,00459946	1,58	down	0,25434935	-0,40929064					AT3G47650	bundle-sheath defective protein 2 family / bsd2 family, similar to bundle sheath defective protein 2 (Zea mays) GI:4732091
Mtr.45634.1.S1_s_at	0,04238276	1,58	down	0,45078167	-0,21287425					ATCG00360	encodes a protein required for photosystem I assembly. In Chlamydomonas reinhardtii, this protein seems to act as a PSI specific chaperone fa
Mtr.11558.1.S1_at	0,00932614	1,58	up	-0,31478038	0,34761238		similar to probable protein kinase [imported] - Arabidopsis thaliana {Arabidopsis thaliana;}, partial (48%)			AT5G13770	pentatricopeptide (PPR) repeat-containing protein, contains Pfam profile PF01535: PPR repeat
Mtr.7448.1.S1_s_at	0,03132453	1,58	up	-0,42862758	0,2335999	Mtr.22395	similar to (Q9FLI1) Arabidopsis thaliana genomic DNA chromosome 5 P1 clone:MIO24 (At5g517		GO:0045449	AT5G51780	basix helix-loop-helix (bHLH) family protein, contains Pfam profile: PF00010 helix-loop-helix DNA-binding domain
Mtr.26044.1.S1_s_at	0,00298719	1,58	up	-0,379831	0,28075442					AT2G46830	myb-related transcription factor (CCA1), identical to myb-related transcription factor (CCA1) GI:4090569 from (Arabidopsis thaliana);encodes a
Mtr.37807.1.S1_at	0,03455469	1,58	down	0,22863881	-0,4313755		similar to (Q7NQU3) Probable transporter LysE family, partial (6%)			AT2G41330	glutaredoxin family protein, contains Pfam profile PF00462: Glutaredoxin
Mtr.38212.1.S1_at	0,00817883	1,58	up	-0,29149374	0,36662102		similar to (O64645) SUPPRESSOR OF CONSTANS OVEREXPRESSION 1 protein (Agamous-like MADS box protein			AT2G45660	Controls flowering and is required for CO to promote flowering. It acts downstream of FT. Overexpression of (SOC1) AGL20 suppresses not onl
Mtr.8957.1.S1_at	0,01578377	1,58	up	-0,18579102	0,47122112		similar to Unknown protein {Arabidopsis thaliana;}, partial (26%)			AT3G21710	expressed protein
Mtr.47343.1.S1_s_at	0,00752554	1,58	up	-0,31186804	0,34375					AT3G28040	leucine-rich repeat transmembrane protein kinase, putative, contains Pfam profiles: PF00560 leucine rich repeat, PF00069 eukaryotic protein ki
Mtr.50996.1.S1_at	0,03834627	1,57	up	-0,26498517	0,39028105					AT3G10660	calcium-dependent protein kinase isoform 2 (CPK2), identical to calcium-dependent protein kinase isoform 2 (Arabidopsis thaliana) gi|9837343
Mtr.39524.1.S1_s_at	0,03754836	1,57	up	-0,35985819	0,2939755		homologue to (Q6UEI8) Circadian clock associated1, partial (7%)			AT5G01490	cation exchanger, putative (CAX4), identical to cation/proton antiporter (Arabidopsis thaliana) gi|15426028|gb|AAK97656; similar to high affini
Mtr.6631.1.S1_s_at	0,00770221	1,57	up	-0,41659212	0,23692735	Mtr.14891				AT2G32030	GCN5-related N-acetyltransferase (GNAT) family protein, contains Pfam profile PF00583: acetyltransferase, GNAT family
Msa.2667.1.S1_at	0,01030908	1,57	down	0,33449903	-0,31901613					AT2G36800	UDP-glucoronosyl/UDP-glucosyl transferase family protein, contains Pfam profile: PF00201 UDP-glucoronosyl and UDP-glucosyl transferase;Enc
Mtr.4869.1.S1_at	0,00158842	1,57	down	0,26462126	-0,3881267	Mtr.11901				AT5G67220	nitrogen regulation family protein, similar to unknown protein (gb|AAF51525.1) ; contains Pfam domain PF01207: Dihydrouridine synthase (Dus); similar to (S
Mtr.8065.1.S1_at	0,01007533	1,57	up	-0,2719009	0,38059855					AT1G53885	senescence-associated protein-related, similar to senescence-associated protein SAG102 (GI:22331931) (Arabidopsis thaliana);
Mtr.37242.1.S1_x_at	0,00414361	1,57	down	0,32887062	-0,32279888		similar to (O65781) UDP-glucose 4-epimerase GEPI48 (Galactowaldenase) (UDP-galactose 4-epimerase), part			AT4G23920	Encodes a protein with UDP-D-glucose 4-epimerase activity.
Mtr.41928.1.S1_at	0,04231558	1,57	up	-0,20736425	0,44363627		similar to (Q9ARY0) P0498A12.24 protein, partial (6%)			AT4G23330	eukaryotic translation initiation factor-related, contains weak similarity to Swiss-Prot:Q9LD55 eukaryotic translation initiation factor 3 subunit 1
Mtr.47999.1.S1_x_at	0,02692085	1,57	up	-0,23816569	0,4123219					AT2G28290	chromatin remodeling protein, putative (SYD), similar to transcriptional activator HBRM (Homo sapiens) GI:414117; contains Pfam profiles PF0
Mtr.35208.1.S1_at	0,0133009	1,57	up	-0,34261355	0,30693927	Mtr.10575				AT1G12480	C4-dicarboxylate transporter/malic acid transport family protein, contains Pfam profile PF03595: C4-dicarboxylate transporter/malic acid transp
Mtr.8954.1.S1_at	0,01554206	1,57	down	0,22496255	-0,42413378		similar to unknown {Prunus armeniaca;}, partial (37%)			AT1G06650	encodes a protein whose sequence is similar to 2-oxoglutarate-dependent dioxygenase;2-oxoglutarate-dependent dioxygenase, putative, simila
Mtr.42746.1.S1_at	0,00909462	1,57	down	0,27880144	-0,36908484	Mtr.22145	similar to (O81240) Iron superoxide dismutase 3 (Fragment) (EC 1.15.1.1), partial (72%)		GO:0006801	AT5G23310	superoxide dismutase (Fe) / iron superoxide dismutase 3 (FSD3), identical to iron superoxide dismutase 3 (Arabidopsis thaliana) gi|3273757|gb|AAC24834;Fe
Mtr.11980.1.S1_at	0,00307867	1,57	down	0,2599708	-0,3869686		similar to At1g55140 {Arabidopsis thaliana;}, partial (46%)			AT3G13740	URF 4-related, similar to URF 4 (GI:49224) (Swiss-Prot:Q05370)(Synechococcus sp.)

Mtr.39690.1.S1_x_at	0,02136382	1,57	up	-0,3860868	0,26085153		(Q9CPB8) FecC, partial (3%)				AT3G06960	expressed protein	3,5
Mtr.5468.1.S1_at	0,04336768	1,57	down	0,17512894	-0,4714168						AT2G19620	Ndr family protein, similar to SP|O23969 Pollen specific protein SF21 {Helianthus annuus}; contains Pfam profile PF03096: Ndr family	9,00E-44
Mtr.20146.1.S1_at	0,00671582	1,57	up	-0,24268882	0,4035244	Mtr.10594					AT1G15740	leucine-rich repeat family protein	3,00E-28
Mtr.5811.1.S1_at	0,00466064	1,56	up	-0,237113	0,40857124						AT1G02530	multidrug resistance P-glycoprotein, putative, similar to multidrug-resistant protein CjMDR1 GI:14715462 from (Coptis japonica)	2,00E-11
Mtr.37172.1.S1_at	0,01479999	1,56	up	-0,3586916	0,2868142		similar to (Q9UKN1) Transmembrane mucin 12 (Fragment), partial (2%)				AT3G51740	leucine-rich repeat transmembrane protein kinase, putative, brassinosteroid-insensitive protein BRI1 - Arabidopsis thaliana, PIR:T09356;encod	0,48
Mtr.50546.1.S1_at	7,89E-05	1,56	up	-0,33667406	0,3085254						AT4G31130	expressed protein	
Mtr.41622.1.S1_at	1,99E-04	1,56	up	-0,28580937	0,35929236		similar to (Q6QB11) Little protein 1, partial (95%)				AT2G26490	transducin family protein / WD-40 repeat family protein, contains 7 WD-40 repeats (PF00400); related to En/Spm transposon family of maize	0,93
Mtr.50285.1.S1_at	0,00247023	1,56	up	-0,2594277	0,38562903						AT5G55770	DC1 domain-containing protein, contains Pfam profile PF03107: DC1 domain	0,32
Mtr.29915.1.S1_at	0,00467608	1,56	up	-0,3900014	0,25457844						AT4G37190	expressed protein;similar to misato [Mus musculus] (GB:NP_659147.1)	0,77
Mtr.37931.1.S1_at	0,04427025	1,56	up	-0,16585605	0,477767	Mtr.10929	weakly similar to (Q9FKY4) Gb|AAD32907.1, partial (46%)		GO:0016021		AT1G20925	auxin efflux carrier family protein, contains auxin efflux carrier domain, Pfam:PF03547	5,00E-22
Mtr.12254.1.S1_at	0,00862087	1,56	down	0,3975576	-0,24518998	Mtr.4019	homologue to (Q70I32) Transcription factor homolog BTF3-like protein, partial (95%)				AT1G17880	nascent polypeptide-associated complex (NAC) domain-containing protein / BTF3b-like transcription factor, putative, similar to SP|P20290 Tran	8,00E-10
Mtr.12786.1.S1_at	7,98E-04	1,56	down	0,31125608	-0,3308142		similar to (Q9LR10) F10A5.10, partial (56%)				AT1G75710	zinc finger (C2H2 type) family protein, contains zinc finger, C2H2 type, domain, PROSITE:PS00028	7,00E-31
Mtr.12750.1.S1_at	0,02422561	1,56	up	-0,39628243	0,2457536		weakly similar to probable protein kinase At2g19410 [imported] - Arabidopsis thaliana, partial (8%)				AT5G57035	protein kinase family protein, contains Pfam profile: PF00069 Eukaryotic protein kinase domain	2,00E-24
Mtr.18549.1.S1_s_at	0,0457642	1,56	up	-0,37526503	0,26669487						AT1G08290	zinc finger (C2H2 type) protein (WIP3), identical to WIP3 protein (Arabidopsis thaliana) gi|18027014|gb|AAL55723; contains Pfam domain, PF(	9,00E-09
Mtr.27321.1.S1_at	0,02925085	1,56	down	0,36443186	-0,2766056	Mtr.22148			GO:0005509		AT4G32060	calcium-binding EF hand family protein, contains INTERPRO:IPR002048 calcium-binding EF-hand domain	6,00E-32
Mtr.24104.1.S1_s_at	0,0139004	1,56	up	-0,27071062	0,36982408						AT1G26510	F-box family protein, contains F-box domain Pfam:PF00646	3,6
Mtr.11985.1.S1_at	0,01150345	1,56	down	0,22891872	-0,4109807						AT5G53390	expressed protein	9,00E-25
Mtr.14004.1.S1_at	0,03991394	1,56	up	-0,4525906	0,18676312		similar to unknown protein [imported] - Arabidopsis thaliana {Arabidopsis thaliana;}, partial (15%)				AT1G42550	Encodes a plant-specific protein of unknown function that appears to be conserved among angiosperms.	9,00E-25
Mtr.22238.1.S1_at	0,01039653	1,56	up	-0,19238503	0,4466165						AT1G71250	GDSL-motif lipase/hydrolase family protein, similar to family II lipases EXL3 GI:15054386, EXL1 GI:15054382, EXL2 GI:15054384 from (Arabi	10
Mtr.50348.1.S1_s_at	0,01052057	1,55	down	0,33289036	-0,30361447						AT1G79560	encodes an FtsH protease that is localized to the chloroplast	
Mtr.10425.1.S1_at	0,02965487	1,55	up	-0,16757743	0,46852145		similar to ethylene responsive element binding factor 5 (ATERF5) {Arabidopsis thaliana;}, partial (27%)				AT5G47230	encodes a member of the ERF (ethylene response factor) subfamily B-3 of ERF/AP2 transcription factor family (ATERF-5). The protein contains	5,00E-33
Mtr.11016.1.S1_at	0,03086877	1,55	down	0,22099209	-0,41500473		weakly similar to (Q9SC76) Hypothetical protein I1332.2, partial (68%)				AT5G07220	A member of Arabidopsis BAG (Bcl-2-associated athanogene) proteins, plant homologs of mammalian regulators of apoptosis. Plant BAG protei	1,00E-17
Mtr.43691.1.S1_at	0,00441358	1,55	up	-0,27993584	0,35534286		similar to biotin holocarboxylase synthetase [imported] - Arabidopsis thaliana, partial (37%)				AT2G25710	holocarboxylase synthetase 1 (HCS1), identical to holocarboxylase synthetase 1 (hcs1) (Arabidopsis thaliana) GI:19698365;Encodes holocarboxylase synthase	
Mtr.41308.1.S1_at	0,00171771	1,55	down	0,30611148	-0,32907167						AT4G27110	phytochelatin synthetase-related, contains Pfam PF04833: Phytochelatin synthetase-like conserved region	2,3
Mtr.8530.1.S1_s_at	6,14E-04	1,55	down	0,28335032	-0,35182634		weakly similar to (Q8S342) Putative anthocyanidine rhamnosyl-transferase, partial (22%)				AT5G65550	UDP-glucoronosyl/UDP-glucosyl transferase family protein, contains Pfam profile: PF00201 UDP-glucoronosyl and UDP-glucosyl transferase ;sim	4,00E-36
Mtr.34925.1.S1_at	0,01045503	1,55	down	0,23963515	-0,3954513						AT5G40010	AAA-type ATPase family protein, contains Pfam profile: ATPase family PF00004	8,00E-34
Mtr.19970.1.S1_at	0,0111597	1,55	down	0,40854725	-0,22623198						AT1G55570	multi-copper oxidase type I family protein, nearly identical to pollen-specific BP10 protein (SP|Q00624)(Brassica napus); contains Multicopper oxidase domain	
Mtr.10243.1.S1_at	0,04290039	1,55	up	-0,17877333	0,4559938						AT3G60920	beige/BEACH domain-containing protein, contains Pfam PF02138: Beige/BEACH domain; similar to LBA isoform gamma (GI:10257405) (Mus m	1,8
Mtr.42021.1.S1_s_at	1,95E-04	1,55	up	-0,3259287	0,3086945	Mtr.4565	homologue to (Q9XER4) 1-aminocyclopropane-1-carboxylate oxidase (EC 1.4.3.-), partial (36%		GO:0016491		AT1G62380	Encodes a protein similar to 1-aminocyclopropane-1-carboxylic oxidase (ACC oxidase). Expression of the AtACO2 transcripts is affected by ethy	4,00E-32
Mtr.12862.1.S1_at	0,0062605	1,55	up	-0,2818575	0,35266113		weakly similar to (Q852L7) Putative acetyltransferase, partial (37%)				AT2G32020	GCN5-related N-acetyltransferase (GNAT) family protein, contains Pfam profile PF00583: acetyltransferase, GNAT family	3,00E-41
Mtr.50156.1.S1_at	4,63E-05	1,55	up	-0,30956778	0,32447848						AT1G09660	KH domain-containing quaking protein, putative, similar to GB:AAC67357	
Mtr.13891.1.S1_at	0,01863148	1,55	up	-0,30318975	0,32996878		weakly similar to unknown protein {Arabidopsis thaliana;}, partial (3%)				AT2G41770	expressed protein, contains Pfam domain PF03385: Protein of unknown function, DUF288	4,00E-19
Mtr.51374.1.S1_at	0,02657731	1,55	down	0,29081726	-0,34225813						AT3G13950	expressed protein	51
Mtr.42645.1.S1_at	0,03048269	1,55	down	0,25667986	-0,37610435		homologue to hypothetical protein At2g44640 [imported] - Arabidopsis thaliana, partial (2%)				AT2G44640	expressed protein	3,00E-36
Msa.2761.1.S1_at	0,01045101	1,55	up	-0,366618	0,26570478						AT1G05150	calcium-binding EF hand family protein, low similarity to O-linked GlcNAc transferase (Homo sapiens) GI:2266994; contains Pfam profiles PF0(	2,90E-44
Mtr.38441.1.S1_at	6,42E-04	1,55	down	0,34625497	-0,28586626		similar to (Q9LQ89) T1N6.8 protein, partial (20%)				AT4G00460	Encodes a member of KPP-like gene family, homolog of KPP (kinase partner protein) gene in tomato. Also a member of the RopGEF (guanine r	8,00E-30
Mtr.1688.1.S1_at	0,03888962	1,55	up	-0,17916568	0,45271268	Mtr.11417					AT4G18170	WRKY family transcription factor, similar to DNA-binding protein 2 GI:4322940 from (Nicotiana tabacum); contains Pfam profile: PF03106 WRK	17
Mtr.42172.1.S1_s_at	0,04440533	1,55	up	-0,25305495	0,3786292		similar to (Q67TP6) Putative cytochrome P450, partial (27%)				AT3G19270	cytochrome P450 family protein, similar to Cytochrome P450 85 (SP:Q43147) {Lycopersicon esculentum};member of CYP707A	
Mtr.12473.1.S1_at	0,0063225	1,55	down	0,3427008	-0,28868937		similar to (Q7XZD0) Isoflavonoid glucosyltransferase, partial (70%)				AT2G15490	UDP-glucoronosyl/UDP-glucosyl transferase family protein, contains Pfam profile: PF00201 UDP-glucoronosyl and UDP-glucosyl transferase	4,00E-10
Mtr.12087.1.S1_at	0,02278409	1,55	up	-0,3665606	0,26472044		(P26017) Polycomb protein, partial (2%)				AT1G67920	expressed protein	2,00E-09
Mtr.28079.1.S1_at	0,03496507	1,55	down	0,20799701	-0,42296967						AT2G19620	Ndr family protein, similar to SP|O23969 Pollen specific protein SF21 {Helianthus annuus}; contains Pfam profile PF03096: Ndr family	8,00E-37
Mtr.41520.1.S1_at	0,04143626	1,55	up	-0,25247636	0,3784256		weakly similar to (Q94KI8) Putative calcium channel, partial (30%)				AT4G03560	two-pore calcium channel (TPC1), identical to two-pore calcium channel (TPC1) (Arabidopsis thaliana) gi|14041819|dbj|BAB55460;Encodes a depolarization-ac	
Mtr.49481.1.S1_at	2,99E-05	1,55	up	-0,29578528	0,33506283						AT2G39705	expressed protein	1,00E-07
Mtr.38444.1.S1_at	0,0284875	1,55	down	0,27059746	-0,36020055		weakly similar to lysophospholipase homolog F12L6.8 - Arabidopsis thaliana {Arabidopsis thaliana;}, partial (8%				AT2G39420	esterase/lipase/thioesterase family protein, similar to monoglyceride lipase from (Homo sapiens) GI:14594904, (Mus musculus) GI:2632162; contains Interpro	
Mtr.10854.1.S1_at	0,00571334	1,55	up	-0,37312064	0,25697422		similar to (O49434) HyuC-like protein, partial (30%)				AT4G20070	The gene encoding Arabidopsis thaliana Allantoate Amidohydrolase (AtAAH)which catalyzes the allantoate deiminase reaction (EC 3.5.3.9)is expressed in all pa	
Mtr.45164.1.S1_at	0,03721807	1,55	up	-0,39896464	0,23107815		similar to unknown protein {Arabidopsis thaliana;}, partial (33%)				AT4G25770	expressed protein	0,12
Mtr.11362.1.S1_at	0,01404555	1,55	up	-0,27909455	0,3505071		weakly similar to (Q6R567) Ring domain containing protein, partial (40%)				AT4G03510	zinc finger (C3HC4-type RING finger) family protein (RMA1), identical to RING zinc finger protein RMA1 gi:3164222;RMA1 encodes a novel 28 k	6,00E-18
Mtr.684.1.S1_at	0,00274885	1,55	down	0,30008397	-0,3292853						AT1G61340	F-box family protein, contains Pfam PF00646: F-box domain; similar to late embryogenesis abundant protein GI:1350540 from (Picea glauca)	2,00E-29
Mtr.8562.1.S1_at	5,28E-04	1,55	down	0,34013844	-0,28919634		similar to (Q60CZ4) Hypothetical protein, partial (40%)				AT3G61700	expressed protein	
Mtr.1649.1.S1_at	0,03906512	1,55	down	0,37593666	-0,2530903						AT3G02890	PHD finger protein-related, contains low similarity to PHD-finger domain proteins	2,00E-05
Msa.1184.1.S1_at	0,00356084	1,55	down	0,32739052	-0,30114475						AT2G46420	expressed protein	
Mtr.43692.1.S1_at	0,00119145	1,55	up	-0,27750078	0,3510313		similar to (Q8RV42) Holocarboxylase synthetase 2 (Holocarboxylase synthetase hcs2.b), partial (58%)				AT1G37150	holocarboxylase synthetase 2 (HCS2.d), identical to holocarboxylase synthetase hcs2.d (Arabidopsis thaliana) GI:19698373; contains non-cons	2,00E-33
Mtr.7481.1.S1_at	0,01243793	1,54	up	-0,24529076	0,38147482	Mtr.6606	similar to unknown {Lycopersicon esculentum;}, partial (7%)				AT5G67100	DNA-directed DNA polymerase alpha catalytic subunit, putative, similar to SP|O48653 DNA polymerase alpha catalytic subunit (EC 2.7.7.7) {Or	0,58
Mtr.9514.1.S1_at	0,00271469	1,54	up	-0,32178688	0,30497393		similar to (Q7X996) Putative Serine/threonine Kinase, partial (28%)				AT5G58380	CBL-interacting protein kinase 10 (CIPK10), identical to CBL-interacting protein kinase 10 (Arabidopsis thaliana) gi|13249119|gb|AAK16685; c	1,00E-22
Mtr.26297.1.S1_at	0,00610738	1,54	down	0,2620163	-0,36449733						AT3G52820	purple acid phosphatase (PAP22), identical to purple acid phosphatase (PAP22)GI:20257494 from (Arabidopsis thaliana)	0,55
Mtr.11149.1.S1_at	0,00726315	1,54	down	0,25165114	-0,37385383		similar to (Q6EQH5) Membrane protein-like, partial (30%)				AT2G25737	expressed protein, contains Pfam profile: PF01925 domain of unknown function DUF81	8,00E-28
Mtr.37515.1.S1_at	0,00101212	1,54	down	0,2916635	-0,33305392		homologue to (Q8S2V7) Putative sodium-dependent bile acid symporter, partial (77%)				AT2G26900	bile acid:sodium symporter family protein, low similarity to SP|Q12908 Ileal sodium/bile acid cotransporter {Homo sapiens}; contains Pfam prc	1,00E-10
Mtr.2915.1.S1_at	0,03185388	1,54	up	-0,39386764	0,23082654						AT5G25510	serine/threonine protein phosphatase 2A (PP2A) regulatory subunit B', putative, similar to SWISS-PROT:Q28653 serine/threonine protein phos	4,00E-21
Mtr.16188.1.S1_at	0,01119759	1,54	down	0,2356631	-0,38844427	Mtr.9091					AT2G31040	ATP synthase protein I -related, contains weaks similarity to Swiss-Prot:P08443 ATP synthase protein I (Synechococcus sp.)	9,80E-45
Mtr.17272.1.S1_at	0,00893925	1,54	up	-0,3370749	0,28686318						AT5G12250	tubulin beta-6 chain (TUB6), nearly identical to SP|P29514 Tubulin beta-6 chain {Arabidopsis thaliana};Encodes a beta-tubulin. Expression of TUB6 has been s	
Mtr.39158.1.S1_at	0,03787829	1,54	up	-0,167857	0,455695		homologue to (Q61DX2) Hypothetical protein CBG12313, partial (3%)				AT1G10000	expressed protein	0,001
Mtr.45509.1.S1_at	0,01869208	1,54	down	0,28778362	-0,33540058		similar to (Q6IM45) HDC07503, partial (6%)				AT5G22300	nitrilase 4 (NIT4), identical to SP|P46011 Nitrilase 4 (EC 3.5.5.1) {Arabidopsis thaliana};encodes a nitrilase isomer. The purified enzyme shows	3,6
Mtr.46514.1.S1_x_at	0,00725706	1,54	down	0,37296948	-0,24966104						AT1G78960	lupeol synthase, putative / 2,3-oxidosqualene-triterpenoid cyclase, putative, similar to lupeol synthase GI:1762150 from (Arabidopsis thaliana), 2,3-oxidosqua	
Mtr.47645.1.S1_at	0,01481621	1,54	down	0,2786924	-0,34197378						AT4G17720	RNA recognition motif (RRM)-containing protein	
Mtr.46922.1.S1_at	0,00355011	1,54	up	-0,34979382	0,2707467								
Mtr.27878.1.S1_at	0,02228751	1,54	down	0,22869937	-0,39144642						AT1G75280	isoflavone reductase, putative, identical to SP:P52577 Isoflavone reductase homolog P3 (EC 1.3.1.-) {Arabidopsis thaliana}; contains Pfam pro	3,00E-41
Mtr.28579.1.S1_at	0,00337364	1,54	up	-0,33380732	0,285844						AT5G02350	DC1 domain-containing protein, contains Pfam profile PF03107: DC1 domain	2,1
Mtr.13406.1.S1_at	0,00867431	1,54	up	-0,2846632	0,33454704		weakly similar to (Q7XA84) At4g13030, partial (17%)				AT4G13030	expressed protein	2,00E-36
Mtr.36069.1.S1_s_at	0,0163818	1,54	down	0,1893533	-0,42972818	Mtr.2111					AT5G49690	UDP-glucoronosyl/UDP-glucosyl transferase family protein, contains Pfam profile: PF00201 UDP-glucoronosyl and UDP-glucosyl transferase	6,00E-05
Mtr.50462.1.S1_at	0,03066627	1,54	down	0,16077709	-0,45799002	Mtr.3682			GO:0000166		AT2G37220	29 kDa ribonucleoprotein, chloroplast, putative / RNA-binding protein cp29, putative, similar to SP|Q43349 29 kDa ribonucleoprotein, chloropla	3,00E-36
Mtr.33290.1.S1_at	0,00602226	1,54	up	-0,25417757	0,3645692						AT3G50650	scarecrow-like transcription factor 7 (SCL7)	2,7
Mtr.18215.1.S1_at	0,00139861	1,54	up	-0,27508244	0,34349313						AT3G14520	terpene synthase/cyclase family protein, similar to terpene synthase GB:CAA72074 from (Arabidopsis thaliana)	2,9
Mtr.25196.1.S1_at	0,03879243	1,54	up	-0,42354837	0,19485633						AT4G05360	zinc knuckle (CCHC-type) family protein, contains Pfam domain, PF00098: Zinc knuckle	5,8
Mtr.9323.1.S1_at	0,02154232	1,54	down	0,35933876	-0,2589744		similar to hypothetical protein At2g39670 [imported] - Arabidopsis thaliana, partial (34%)				AT2G39670	radical SAM domain-containing protein, similar to hypothetical protein PIR|S76698|S76698 contains Pfam profile PF04055: radical SAM domain protein	
Mtr.3100.1.S1_at	0,00338692	1,53	down	0,34139503	-0,2752892						AT5G17980	C2 domain-containing protein, contains INTERPRO:IPR000008 C2 domain	8,00E-19
Mtr.43797.1.S1_at	0,00206285	1,53	down	0,35067844	-0,26671663		similar to probable GDSL-motif lipase/hydrolase [imported] - Arabidopsis thaliana, partial (88%)				AT2G23540	GDSL-motif lipase/hydrolase family protein, similar to family II lipase EXL1 GI:15054382 from (Arabidopsis thaliana); contains Pfam profile PF(	4,00E-29
Mtr.42018.1.S1_at	0,04180393	1,53	up	-0,4160579	0,20005877		weakly similar to unknown protein {Arabidopsis thaliana;}, partial (61%)				AT1G20780	armadillo/beta-catenin repeat protein-related / U-box domain-containing protein, low similarity to immediate-early fungal elicitor protein CMPG1 (Petroselinum	
Mtr.33195.1.S1_at	0,03850186	1,53	up	-0,19636853	0,41912556						AT4G17080	MORN (Membrane Occupation and Recognition Nexus) repeat-containing protein /phosphatidylinositol-4-phosphate 5-kinase-related, low simila	2,4
Mtr.44795.1.S1_at	0,03728398	1,53	up	-0,41252407	0,20282681		similar to (Q76JT3) RelA-SpoT like protein PsRSH1, partial (10%)				AT3G14050	RelA/SpoT protein, putative (RSH2), nearly identical to RelA/SpoT homolog RSH2 (Arabidopsis thaliana) GI:7141306; contains Pfam profiles PF	6,00E-10
Mtr.9625.1.S1_at	0,01405715	1,53	up	-0,42990193	0,18525171		homologue to (Q39889) Heat shock protein (EC 3.4.21.-), partial (41%)				AT1G74310	heat shock protein 101 (HSP101), identical to heat shock protein 101 GI:6715468 GB:AAF26423 from (Arabidopsis thaliana);Heat shock protein involved in ac	
Mtr.27342.1.S1_at	0,02005457	1,53	down	0,18912919	-0,4257439						AT3G55400	methionyl-tRNA synthetase / methionine--tRNA ligase / MetRS (cpMetRS), identical to methionyl-tRNA synthetase MEtRS (Arabidopsis thaliana) GI:2266985	
Mtr.7333.1.S1_at	0,02967624	1,53	down	0,15024345	-0,46454954		weakly similar to (O04925) 15.5 kDa oleosin, partial (60%)				AT5G40420	glycine-rich protein / oleosin;Encodes oleosin, a protein found in oil bodies and is likely to be involved in lipid storage.	2,00E-07
Mtr.26605.1.S1_at	0,01454919	1,53	down	0,22499402	-0,3887671						AT4G02600	A member of a large family of seven-transmembrane domain proteins specific to plants, homologs of the barley mildew resistance locus o (MLO	2,00E-21
Mtr.41237.1.S1_at	0,03035518	1,53	up	-0,36463848	0,24850161		similar to (Q9XEY0) Nt-gh3 deduced protein, partial (66%)				AT2G14960	encodes a protein similar to IAA-amido synthases. Lines carrying an insertion in this gene are hypersensitive to auxin.	
Mtr.22366.1.S1_at	5,17E-04	1,53	up	-0,2916328	0,32127556						AT1G42550	Encodes a plant-specific protein of unknown function that appears to be conserved among angiosperms.	0,92
Mtr.12256.1.S1_at	0,0015855	1,53	down	0,31388697	-0,29891333		similar to (O23758) Nonspecific lipid-transfer protein precursor (LTP), complete				AT5G57480	AAA-type ATPase family protein, contains Pfam profile: PF00004 ATPase family	7,8
Mtr.23876.1.S1_at	0,01035148	1,53	down	0,2777411	-0,33430067						AT3G25420	serine carboxypeptidase S10 family protein, similar to serine carboxypeptidase I precursor (SP:P37890) from (Oryza sativa)	
Mtr.40887.1.S1_at	0,0156042	1,53	up	-0,3603975	0,25105777		similar to (Q6NKW1) At1g56230, partial (20%)				AT5G35580	protein kinase, putative, similar to auxin-regulated dual specificity cytosolic kinase (Lycopersicon esculentum) gi|14484938|gb|AAK62821	6
Mtr.10227.1.S1_at	0,03985795	1,53	down	0,31976262	-0,29104185		weakly similar to (Q7G9K0) F16N3.7 protein, partial (12%)				AT1G47620	cytochrome P450, putative, similar to cytochrome P450 GI:4688670 from (Catharanthus roseus);member of CYP96A	1,00E-22
Mtr.20822.1.S1_at	0,01317454	1,53	down	0,22394975	-0,38673463						AT2G21385	expressed protein	
Mtr.6671.1.S1_at	0,03988458	1,53	up	-0,40678072	0,20370705	Mtr.18765					AT1G25425	expressed protein	4,00E-05
Mtr.35045.1.S1_at	0,02654444	1,53	up	-0,24084814	0,36895737	Mtr.14635			GO:0043087		AT3G13330	expressed protein	68
Mtr.16025.1.S1_at	0,01126412	1,53	up	-0,34807858	0,2608118						AT1G59620	disease resistance protein (CC-NBS class), putative, domain signature CC-NBS exists, suggestive of a disease resistance protein.;CW9 mRNA fc	4,00E-12
Mtr.48263.1.S1_s_at	0,02865678	1,52	up	-0,30475044	0,3035787						AT1G33530	F-box family protein, contains F-box domain Pfam:PF00646; contains TIGRFAM TIGR01640: F-box protein interaction domain	43
Mtr.40190.1.S1_at	0,01946733	1,52	up	-0,20539124	0,40273252		similar to (Q6RIB0) Flowering promoting factor-like 1, partial (97%)				AT5G24860	expressed protein;encodes a small protein of 12.6 kDa that regulates flowering and is involved in gibberellin signalling pathway. It is expressed	6,00E-20
Mtr.4727.1.S1_a_at	0,02061885	1,52	up	-0,31451192	0,29337868						AT2G27100	C2H2 zinc-finger protein SERRATE (SE), identical to C2H2 zinc-finger protein SERRATE GI:14486602 from (Arabidopsis thaliana);Identified as a	4,7

Mtr.4281.1.S1_at	0,03837645	1,52	down	0,19740741	-0,4102335				AT5G35740	glycosyl hydrolase family protein 17, similar to elicitor inducible chitinase Nt-SubE76 GI:11071974 from (Nicotiana tabacum); C-terminal homo	1,00E-33
Mtr.6322.1.S1_s_at	0,00746852	1,52	down	0,31410536	-0,29326168				AT3G14630	cytochrome P450, putative, similar to GB:Q05047 from (Catharanthus roseus);putative cytochrome P450	
Mtr.10723.1.S1_at	0,00167243	1,52	up	-0,25952753	0,3476089	homologue to AT4g28240/F26K10_120 {Arabidopsis thaliana;}, partial (19%)			AT4G28240	wound-responsive protein-related, wound-induced protein - tomato (fragment), PIR2:S19773	5,00E-15
Mtr.17610.1.S1_at	0,01345991	1,52	down	0,24788745	-0,35857058				AT5G13930	At5g13930 encoding the CHS (chalcone synthase)is involved in the modulation of root gravitropism. Ethylene may regulate root gravitropism tl	9,00E-14
Mtr.32806.1.S1_at	0,03594928	1,52	up	-0,3326567	0,27306494	Mtr.19481			AT5G05990	mitochondrial glycoprotein family protein / MAM33 family protein, low similarity to SUAPRGA1 (Emericella nidulans) GI:6562379; contains Pfarr	4,00E-24
Mtr.14876.1.S1_s_at	2,82E-05	1,52	up	-0,30933508	0,29630026				AT2G27210	kelch repeat-containing serine/threonine phosphoesterase family protein, similar to SP|P48482 Serine/threonine protein phosphatase PP1 isozyme 2 (EC 3.1.3.	
Mtr.32532.1.S1_at	0,03253818	1,52	up	-0,3986474	0,20656435				AT1G33811	GDSL-motif lipase/hydrolase family protein, similar to family II lipases EXL3 GI:15054386, EXL1 GI:15054382, EXL2 GI:15054384 from (Arabi	3,00E-11
Mtr.20386.1.S1_s_at	0,01622187	1,52	up	-0,31745544	0,28737417				AT2G29100	glutamate receptor family protein (GLR2.9), plant glutamate receptor family, PMID:11379626;member of Putative ligand-gated ion channel sul	1,6
Mtr.33375.1.S1_at	0,02771346	1,52	up	-0,24165328	0,36236206				AT5G38460	ALG6, ALG8 glycosyltransferase family protein, similar to SP|Q9Y672 Dolichyl pyrophosphate Man9GlcNAc2 alpha-1,3-glucosyltransferase (EC 2	0,24
Mtr.12370.1.S1_s_at	0,02475346	1,52	up	-0,409557	0,19418271	Mtr.16076	(Q7RHM4) ABC transporter putative (Fragment), partial (1%)		AT1G12480	C4-dicarboxylate transporter/malic acid transport family protein, contains Pfam profile PF03595: C4-dicarboxylate transporter/malic acid transp	14
Mtr.23228.1.S1_at	0,00873616	1,52	up	-0,20096684	0,40270677				AT1G63700	protein kinase, putative, contains protein kinase domain, Pfam:PF00069: similar to MEK kinase (MAP3Ka) (Arabidopsis thaliana) gi|4204912|gb	6
Mtr.16824.1.S1_at	0,02469385	1,52	up	-0,18389082	0,41960502				AT5G44790	copper-exporting ATPase / responsive-to-antagonist 1 / copper-transporting ATPase (RAN1), identical to SP|Q9S7J8;ATP dependent copper transporter vital fo	
Mtr.9927.1.S1_at	7,62E-04	1,52	up	-0,26760387	0,33330107		similar to At5g22100 {Arabidopsis thaliana;}, partial (34%)		AT5G22120	expressed protein	7,00E-15
Mtr.5871.1.S1_at	0,00789632	1,52	up	-0,25000143	0,3503779				AT4G24740	a LAMMER-type protein kinase that co-precipitates with serine/arginine-rich (SR) proteins in vitro, interaction modulated by phosphorylation of	4,00E-34
Mtr.33735.1.S1_at	0,03604147	1,52	up	-0,2500235	0,3503262				AT1G22620	SAC domain phosphoinositide (3,5)P2 phosphatase. Colocalized with a Golgi Marker. Required for normal cell morphogenesis, cell wall synthesis, and actin org	
Mtr.8445.1.S1_s_at	0,02313204	1,52	up	-0,44269595	0,15762424		homologue to (O65760) Extensin (Fragment), partial (50%)		AT5G14230	ankyrin repeat family protein, contains ankyrin repeats, Pfam:PF00023	0,39
Mtr.28754.1.S1_at	0,04538585	1,52	down	0,3978908	-0,20193236				AT4G16810	expressed protein, similar to vernalization 2 protein (Arabidopsis thaliana) GI:16945788, embryonic flower 2 (Arabidopsis thaliana) GI:142760!	1,00E-05
Mtr.41727.1.S1_at	0,00849147	1,52	down	0,25093684	-0,3485508		similar to (Q6PW70) Soluble inorganic pyrophosphatase (EC 3.6.1.1), partial (46%)		AT1G01050	inorganic pyrophosphatase, putative (soluble) / pyrophosphate phospho-hydrolase, putative / PPase, putative, strong similarity to SP|Q43187 Soluble inorgani	
Mtr.40796.1.S1_at	0,00294241	1,52	down	0,31133357	-0,28804293		homologue to (Q7SD86) Predicted protein, partial (1%)		AT3G11800	expressed protein	7,8
Mtr.2029.1.S1_a_at	0,02577276	1,51	up	-0,2352333	0,3639404				AT5G41790	COP1-interactive protein 1 / CIP1, almost identical to CIP1 (GI:836950) (Arabidopsis thaliana);encodes a protein that physically interacts speci	1,00E-26
Mtr.44468.1.S1_at	0,00903823	1,51	down	0,29987034	-0,29890046		weakly similar to (O64600) F17O7.10, partial (21%)		AT1G23770	F-box family protein, contains Pfam PF00646: F-box domain; similar to F-box only protein 7 (SP:Q9Y3I1) {Homo sapiens} ; similar to SKP1 int	8,00E-11
Mtr.48895.1.S1_s_at	0,00495076	1,51	down	0,23340225	-0,3652309				AT3G03040	F-box family protein, contains F-box domain Pfam:PF00646	66
Mtr.21322.1.S1_s_at	0,03185323	1,51	down	0,29367113	-0,30439043	Mtr.22897			AT2G27390	proline-rich family protein, contains proline-rich extensin domains, INTERPRO:IPR002965	1,2
Mtr.35009.1.S1_at	0,03596117	1,51	up	-0,19992273	0,39801168	Mtr.18997			AT4G01870	tolB protein-related, contains weak similarity to TolB protein precursor (Swiss-Prot:P44677) (Haemophilus influenzae)	
Mtr.9885.1.S1_at	0,02317812	1,51	down	0,31842566	-0,27882972	Mtr.10786	homologue to (Q9STA2) Hydroperoxide lyase, partial (31%)	GO:0004497|GO:0005506|GO:0009055|GO:0016829|GO:0020037|GO:0046872			
Mtr.37882.1.S1_at	0,00320112	1,51	up	-0,25829253	0,3385744		similar to putative metallophosphatase {Lupinus luteus;}, partial (58%)		AT5G50400	calcineurin-like phosphoesterase family protein, contains Pfam profile: PF00149 calcineurin-like phosphoesterase	
Mtr.37155.1.S1_at	0,0060623	1,51	up	-0,24395387	0,35233608		similar to (Q65XK9) Hypothetical protein OJ1735_C10.9, partial (9%)		AT1G64380	encodes a member of the DREB subfamily A-6 of ERF/AP2 transcription factor family. The protein contains one AP2 domain. There are 8 membe	2,00E-04
Mtr.50729.1.S1_at	0,0188666	1,51	up	-0,18494892	0,41078725				AT3G18810	protein kinase family protein, contains Pfam PF00069: Protein kinase domain	0,25
Mtr.8464.1.S1_s_at	0,02005208	1,51	up	-0,2717242	0,31800905		similar to (Q8W1A0) Cysteine synthase, partial (53%)		AT3G04940	cysteine synthase, putative / O-acetylserine (thiol)-lyase, putative / O-acetylserine sulfhydrylase, putative, similar to O-acetylserine(thiol) lyas	2,00E-42
Mtr.42150.1.S1_at	0,03160301	1,51	down	0,17927487	-0,4158481		similar to (Q9ZSP3) Chaperone GrpE type 2, partial (54%)		AT4G26780	mRNA for AR192, complete cds;co-chaperone grpE family protein, similar to chaperone GrpE type 2 (Nicotiana tabacum) GI:3851640; contains Pfam profile PF	
Mtr.43614.1.S1_at	0,03642224	1,51	up	-0,17797025	0,4170672		similar to (Q9C9W6) Putative trehalose-6-phosphate synthase; 46897-44149, partial (64%)		AT1G68020	glycosyl transferase family 20 protein / trehalose-phosphatase family protein, similar to SP|Q00764 Alpha,alpha-trehalose-phosphate synthase	5,00E-32
Mtr.13750.1.S1_at	8,45E-04	1,51	down	0,35032415	-0,24441147		weakly similar to ent-kaurenoic acid hydroxylase {Arabidopsis thaliana;}, partial (17%)		AT2G32440	ent-kaurenoic acid hydroxylase, putative / cytochrome P450, putative, identical to ent-kaurenoic acid hydroxylase / cytochrome P450 CYP88A (	6,00E-36
Mtr.37902.1.S1_s_at	0,02778502	1,51	up	-0,35330948	0,24097602		weakly similar to (Q43800) Lipoxygenase (EC 1.13.11.12 EC 1.13.11.12), partial (9%)		AT3G22400	lipoxygenase, putative, similar to lipoxygenase gi:8649004 (Prunus dulcis), gi:1495802 and gi:1495804 from (Solanum tuberosum)	3,00E-24
Mtr.352.1.S1_x_at	0,00202857	1,51	down	0,33253956	-0,26161194				AT3G22800	leucine-rich repeat family protein / extensin family protein, similar to extensin-like protein (Lycsimilar to extensin-like protein (Lycopersicon esc	0,14
Sme.4601.1.S1_s_at	0,03200169	1,51	down	0,2665186	-0,32679257				AT5G53870	plastocyanin-like domain-containing protein, contains similarity to SP|Q02917 Early nodulin 55-2 precursor {Glycine max}; PF02298: Plastocya	1,3
Mtr.9893.1.S1_at	0,00542305	1,51	down	0,29939142	-0,29376522		similar to At1g14810/F10B6_6 {Arabidopsis thaliana;}, partial (38%)		AT1G14810	encodes an aspartate semialdehyde dehydrogenase, which produces the branch point intermediate for lysine and threonine/methionine biosynthesis	
Mtr.32627.1.S1_at	0,00920114	1,51	down	0,3233215	-0,26977363	Mtr.21163			AT1G07745	DNA repair family protein, contains similarity to Swiss-Prot:O75771 DNA repair protein RAD51 homolog 4 (R51H3) (TRAD) (Homo sapiens)	4,00E-12
Mtr.19850.1.S1_at	0,02043427	1,51	up	-0,27401146	0,31902328				AT5G60210	cytoplasmic linker protein-related, contains weak similarity to cytoplasmic linker protein CLIP-170 (GI:2905649) (Gallus gallus)	1,3
Mtr.47731.1.S1_x_at	0,00594239	1,51	up	-0,29116902	0,30183664				AT3G58130	N-acetylglucosaminyl-phosphatidylinositol de-N-acetylase-related, similar to SP|Q9Y2B2 N-acetylglucosaminyl-phosphatidylinositol de-N-acetyla	66
Msa.3085.1.S1_at	0,00961986	1,51	down	0,17235088	-0,420496				AT4G11650	osmotin-like protein (OSM34), nearly identical to SP|P50700|OSL3_ARATH Osmotin-like protein OSM34 precursor {Arabidopsis thaliana}; contains Pfam profile	
Mtr.34894.1.S1_at	0,02880553	1,51	down	0,36459842	-0,22812192				AT5G20040	tRNA isopentenyltransferase 9 / IPP transferase 9 (IPT9), identical to tRNA isopentenyltransferase (IPT9) (Arabidopsis thaliana) GI:14279070;A	8,00E-31
Mtr.42697.1.S1_at	0,01905522	1,51	down	0,27447525	-0,31739917		weakly similar to Unknown protein {Arabidopsis thaliana;}, partial (19%)		AT5G55580	mitochondrial transcription termination factor family protein / mTERF family protein, weak similarity to mtDBP protein (Paracentrotus lividus) G	7,00E-15
Mtr.28474.1.S1_at	0,04667997	1,51	up	-0,38257155	0,20883894				AT3G14470	disease resistance protein (NBS-LRR class), putative, domain signature NBS-LRR exists, suggestive of a disease resistance protein.	0,022
Mtr.41747.1.S1_at	0,01235972	1,51	up	-0,29276514	0,2981596		similar to (Q9XED4) Receptor-like protein kinase homolog RK20-1, partial (48%)		AT4G23180	receptor-like protein kinase 4, putative (RLK4), nearly identical to receptor-like protein kinase 4 (Arabidopsis thaliana) GI:13506745; contains Pfam domain, PI	
Mtr.10601.1.S1_at	0,02590396	1,51	up	-0,21477287	0,37609577		similar to (Q8S9J0) At2g36630/F1O11.26, partial (94%)		AT2G36630	expressed protein, contains Pfam profile: PF01925 domain of unknown function DUF81	2,00E-11
Mtr.28729.1.S1_at	0,02087031	1,51	up	-0,3351899	0,25566673				AT5G59100	subtilase family protein, contains similarity to prepro-cucumisin GI:807698 from (Cucumis melo)	9,00E-39
Mtr.13929.1.S1_at	0,0076869	1,51	down	0,29553398	-0,2951261		similar to unknown protein {Arabidopsis thaliana;}, partial (17%)		AT3G23880	F-box family protein, contains F-box domain Pfam:PF00646	2,00E-09
Msa.982.1.S1_at	0,03931211	1,51	up	-0,21333027	0,37729296				AT1G11710	pentatricopeptide (PPR) repeat-containing protein, contains Pfam profile PF01535: PPR repeat	1,1
Mtr.19716.1.S1_at	0,04825353	1,51	up	-0,35170126	0,23887222				AT1G29120	expressed protein;similar to expressed protein [Arabidopsis thaliana] (TAIR:At4g25770.1); similar to putative serine esterase [Oryza sativa (ja	5,00E-28
Mtr.37322.1.S1_at	0,00963114	1,51	up	-0,3560759	0,23441394		similar to At4g26850 {Arabidopsis thaliana;}, partial (31%)		AT4G26850	expressed protein;a gene encoding a novel protein is involved in ascorbate biosynthesis. recessive mutation has a reduced amount of vitamin C	2,00E-33
Mtr.10428.1.S1_at	0,02243914	1,50	down	0,22815435	-0,36115155		homologue to (Q7Y0W7) NADP-specific isocitrate dehydrogenase (EC 1.1.1.42), partial (39%)		AT5G14590	isocitrate dehydrogenase, putative / NADP+ isocitrate dehydrogenase, putative, strong similarity to isocitrate dehydrogenase (NADP+) (Nicotiana tabacum) GI	
Sme.1433.1.S1_at	0,01359634	1,50	down	0,26926008	-0,31891355				AT3G17160	expressed protein	0,63
Mtr.35192.1.S1_at	0,02254045	1,50	up	-0,31773916	0,26903662				AT4G33070	pyruvate decarboxylase, putative, strong similarity to SP|P51846 Pyruvate decarboxylase isozyme 2 (EC 4.1.1.1) (PDC) {Nicotiana tabacum};	6,6
Mtr.37791.1.S1_at	0,02740357	1,50	up	-0,20110433	0,38538536		weakly similar to (Q6Z6M5) Putative syntaxin-related protein(Knolle), partial (27%)		AT5G08080	syntaxin, putative (SYP132), similar to SP|Q9ZSD4 Syntaxin 121 (AtSYP121) (Syntaxin-related protein At-Syr1) {Arabidopsis thaliana};similar	4,00E-06
Mtr.12291.1.S1_s_at	0,01131317	1,50	down	0,34113598	-0,2441206		homologue to (Q6JA03) Proline dehydrogenase, partial (65%)		AT3G30775	proline oxidase, mitochondrial / osmotic stress-responsive proline dehydrogenase (POX) (PRO1) (ERD5), nearly identical to SP|P92983 Proline (	1,00E-25

Chapter 3.
Yeast expression system and microsome isolation methods

Introduction

Cytochrome P450s are membrane-bound hemeproteins characterized by their ability to catalyze oxidative attacks of molecular oxygen towards non-activated hydrocarbons (Wreck-Reichhart and Feyereisen, 2000). In plants, P450s are involved in detoxification and biosynthetic pathways of diverse molecules, such as hormones, lignins, sterols and fatty acids. Most eukaryotic P450s are not self-sufficient enzymes and their catalytic activities are strictly dependent on electron donor NADPH-cytochrome P450 reductases (CPR). CPRs and P450s are integral membrane-bound proteins, and reducing equivalents are delivered mainly through transient electrostatic interactions on the ER membranes (Ro et al., 2002).

In our laboratory, a *M. truncatula* activation-tagging collection was screened (Porceddu et al., 2008) in order to search mutants in the saponin biosynthetic pathway and one plant lacking hemolytic activity was identified (*lha* mutant). The putative gene implied in this mutation had 97% identity with the CYP716A12, a member of a cytochrome P450 family found in *M. truncatula* (Li et al., 2007). The EST of CYP716A12 was reported on GenBank (accession number DQ335781.1). Biochemical analyses and complementation experiments performed on the *lha* mutant plants revealed that CYP716A12 was involved in hemolytic sapogenin biosynthesis.

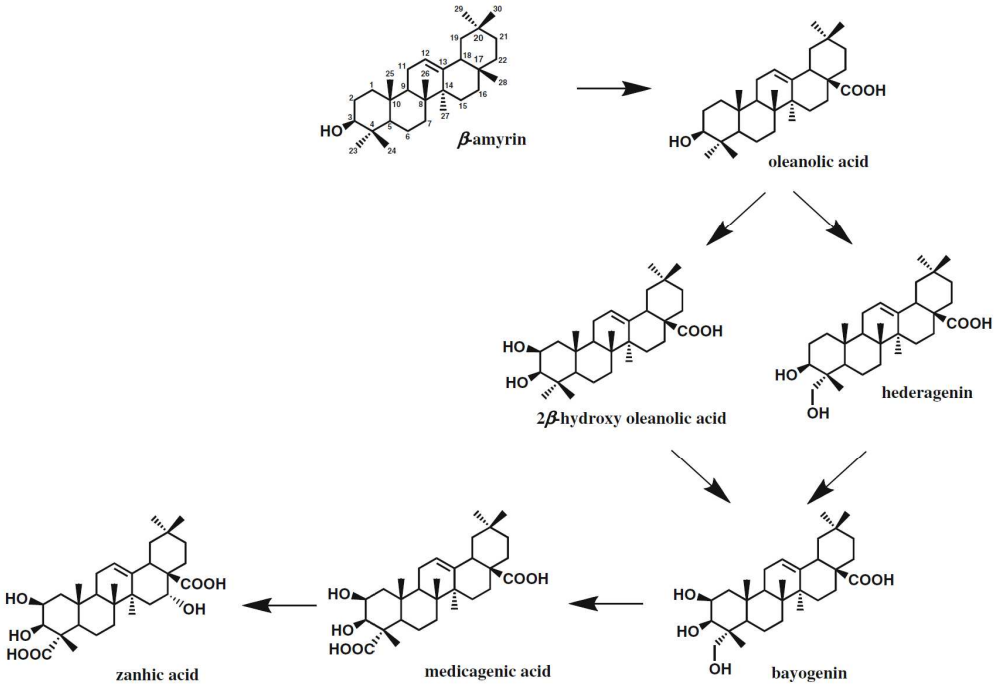


Figure 1. Proposed biosynthetic pathways for hemolytic sapogenins in *Medicago* spp.(based on figures 6 of Tava et al., 2010).

Tava et al. (2010) proposed a pathway for hemolytic sapogenins characterized by a series of step-by-step oxidations mediated by cytochrome P450s (Fig 1). As none of the hemolytic sapogenins were detected in *lha*-mutant, it was likely that the pathway was blocked at an early step. Therefore CYP716A12 was probably one of the cytochromes involved in the early steps of this pathway but the exact role of the enzyme remained unknown.

To facilitate the identification of the substrate that was oxidated and consequently the step catalyzed by CYP716A12 cytochrome, the biosynthetic step blocked in *lha* mutant plants can be investigated. In order to test which compound, among the different aglycones forming the saponin mixture, could restore the hemolytic sapogenin pathway, an enzymatic assay using plant microsomal fractions was performed. An example of biosynthetic study using plant microsomes was reported by Sibbesen et al. (1994): microsomes from sorghum seedlings catalyzed the *in vitro* conversion of L-tyrosine to p-hydroxymandelonitrile involved in the cyanogenic glucoside dhurrin biosynthesis.

Saccharomyces cerevisiae offers a low-cost and efficient way to express heterologous P450s (Pompon et al., 1996): yeast strains expressing heterologous cytochromes are supplemented with different substrates allowing characterization of P450 activities. Such a system was used to study the oxidative activity of *Glicine max* CYP93E1, involved in sapogenols biosynthesis (Shibuya et al., 2006). Similar assays were successfully adopted to analyse brassinosteroid-6-oxidase (CYP85A1) from tomato and *Arabidopsis* (Bishop et al., 1999; Shimada et al., 2001) and taxane 10β-hydroxylase from *Taxus* (Schoendorf et al., 2001). In the present PhD work, substrate specificity and catalytic properties of CYP716A12 have been investigated expressing the gene CDS in a yeast expression system.

Materials and Methods

Plant Material and Growth Conditions

M. truncatula plants (wild-type and *lha*-mutant) were grown in a greenhouse maintaining a 10°C temperature during winter and ambient temperature in other months. Leaves were collected and stored at -80°C.

Plant Microsome Preparation

Membrane preparation from *M. truncatula* leaves cells was done as previously described with minor modifications (Eren and Argüello., 2004). Briefly, cells were suspended in lysis buffer [50 mM Tris (pH 7.4), 330 mM Sucrose, 5mM EDTA, 1mM DTT, 1 mM PMSF, 1% bovine serum albumin (BSA)] at a ratio of 4:1 (v/w). Cells were ground with a mortar and pestle and the homogenate was centrifuged at 16,000g for 20 min. The supernatant was collected and centrifuged at 120,000g for 60 min. The pellet was washed using the lysis buffer and centrifuged at 120,000g for 45 min. The resulting pellet was resuspended in the KH_2PO_4

100mM buffer (pH 7.5) All procedures were performed at 0°C to 4°C. The membrane preparations were stored at -80°C. Protein amount was determined with a Bradford protein assay using BSA as standard.

Construction of *S. cerevisiae* pYES2-CYP716A12 Expression Vector

The full-length CYP716A12 ORF was amplified using primers ATG2fw (ATGGAGCCTAATTTCTATCTC) and TAA2rw (TTAAGCTTTGTGTGGATAAAG), cloned into the pGEM-Teasy vector, and sequenced. Then the CYP716A12 fragment was ligated into the restriction enzymes site (EcoRI) of pYES2-Ura vector (Invitrogen) The expression vector was transformed into yeast (*S. cerevisiae*) strains INVSc1 (Invitrogen) and WR (Pompon et al., 1996) by the lithium acetate procedure (Gietz et al., 1992). The expression of the transgene was controlled by RT-PCR. INVSc1 and WR clones transformed with pYES2-Ura empty vector were used as control.

Construction of *S. cerevisiae* pESC-CYP716A12 Expression Vector

The CYP716A12 CDS fragment was amplified using primers CypEcoRIfw (GAATTCATGGAGCCTAATTTCTATCTCTCCC) and CypClaIrev (ATCGATTTAGCTTTGTGTGGATAAAGGC), cloned into the pGEM-Teasy vector, and sequenced. Then the CYP716A12 fragment was ligated into the restriction enzymes site (EcoRI-ClaI) of the pESC-HIS vector (Agilent Technologies) to give an in-frame C-terminal fusion with the FLAG epitope. The expression vector was transformed into yeast (*S. cerevisiae*) strains WAT11 (Pompon et al., 1996) by the lithium acetate procedure (Gietz et al., 1992). The expression of the transgene was controlled by RT-PCR. WAT11 clones transformed with pESC-HIS empty vector were used as control.

In Vivo Enzymatic Activity Assay

The recombinant INVSc1 and WR strains were cultured according to to Shibuya et al. (2006). In the last induction step the substrates β-amyrin and oleanolic acid (SIGMA) were added. Cells were subjected to acid hydrolysis and sapogenin content was evaluated as described above.

Expression of CYP716A12 Gene in Yeast and Microsome Preparation

Method 1 The recombinant INVSc1 and WR strains were inoculated in 15 ml of synthetic minimal medium SC-Ura (reported on pYES2 manual, SIGMA catalog no. V825-20) containing glucose and grown overnight at 28-30°C on shaking incubator (130rpm). A 1:20 diluition in 50 ml of fresh SC-Ura, containg galactose and hemin (13 mg/mL) (SIGMA), was grown overnight at 28-30°C on shaking incubator (130rpm) until cell density reaches 1 OD. A 1:20 diluition in 1 liter of fresh SC-Ura, containg galactose and hemin (13 mg/mL) (SIGMA), was grown overnight at 28-30°C on shaking incubator (130rpm) until cell density reaches 1 OD. Cells were harvested by centrifugation at 2,500g for 10 min at 4°C and used for microsome preparation. Yeast membrane fraction was done as previously described with minor modifications (Eren and Argüello, 2004). Briefly, cells were suspended in lysis buffer [10 mM Tris (pH 7.4), 250 mM Sucrose, 10 mM ascorbic acid, 1 mM PMSF] at a ratio of 2:1 (v/w) and added of glass beads (SIGMA) at a ratio of 5:1 (w/w). Cells were disrupted mechanically by shaking for 1 min and refrigerating for 1 min for 5 times. The homogenate was centrifuged at 3,000g for 10 min. The supernatant was collected and centrifuged first at 10,000 g for 20 min and then 120,000 g for 60 min The pellet was washed using the lysis buffer and centrifuged at 120,000g for 45 min.The resulting pellet was resuspended in the KH_2PO_4 100mM buffer (pH 7.5) All procedures were

performed at 0°C to 4°C. The membrane preparations were stored at -80°C. Protein amount was determined with a Bradford protein assay using BSA as standard.

Method 2 The recombinant WAT11 and WR strains were cultured according to low density procedure (Pompon et al., 1996) with the addition of 13 mg/mL hemin (SIGMA) to the medium in the last induction step. Microsome preparation was done according to mechanical procedure (Pompon et al.,1996) except for microsome precipitation that was performed by ultracentrifugation at 100,000g for 60 min. Protein amount was determined with a Bradford protein assay using BSA as standard. The presence of CYP716A12 protein in WAT11 microsome was tested by immunoblot analysis using an anti-FLAG antibody (SIGMA). The reductase activity of the WAT11 and WR microsome was tested by Cytochrome c reductase assay kit (SIGMA catalog no. CY0100).

In Vitro Enzymatic Activity Assay

See Carelli et al. (2011) (page 3079).

Biochemical Analysis of the Sapogenin Content

See Carelli et al. (2011) (page 3077).

Biochemical Purification of the Unknown Compound

Sapogenins mixture, extracted from yeast cells by acid hydrolysis and methylated, were dissolved in hexan and submitted to a 15-40 µm silica gel 60H column (Merck). Three fractions were eluted: fraction I with hexan (3 mL), fraction II with ether (3 mL), and fraction III with ether 10% methanol (3mL). The fractions were checked by 60H silica gel TLC plates (Merck), developed with chloroform. Spots were visualized by spraying with methanol/acetic anhydride/sulfuric acid (10:1:1 v/v) followed by heating at 120°C. The three fractions were then subjected to GC-FID and GC-MS analyses as described in Tava et al. (1993) and Tava and Pecetti (1998).

Results and Discussion

Plant microsome assays

To identify which substrate could restore the saponin pathway in the *lha* mutant, an *in vitro* system employing plant microsomal fractions was used. Leaves from both wild-type and mutant plants were collected and subjected to membrane fraction isolation; Bradford assays were performed to measure protein concentration. Enzymatic characterization was performed feeding β-amyrin to microsomal fractions. Sapogenin content was evaluated by GC-MS analysis. Chromatograms showed that wild-type microsomes produced all the sapogenins (hemolytic and non-hemolytic) while in mutant microsomes only the non hemolytic soyasapogenins were found as expected. In order to verify whether a downstream substrate could overcome the pathway block, oleanolic acid, differing from β-amyrin for a carboxylic group at C-28 position, was used: the mutant microsome fraction did not restore the pathway while the wt fraction produced all the sapogenins as in the previous experiment. In contrast, when experiments were repeated using a

different microsome preparation, the results showed no substrate trasformation for either substrates either in the mutant or wild-type.

Finally, microsomes were supplemented with hederagenin, differing from oleanolic acid for carrying an hydroxylic group at C-23 position. Neither wild-type nor *lha* mutant fractions restored the saponin pathway.

These contrasting results may reflect difficulties in setting up an efficient and robust protocol for plant microsome preparation; besides, a critical step in the procedure may be the storage phase because plant microsomes seem to be subjected to rapid deterioration.

Ultimately, the plant microsome system did not yield reproducible results and was thus abandoned in favour of the yeast system, discussed in the following section.

Yeast expression system

In order to understand the role of CYP716A12 in the sapogenin pathway, a heterologous expression system was developed and optimized using first a commercial yeast strain (INVSc1) and subsequently two high efficiency yeast strains (WR and WATT11).

Commercial yeast strain

S. cerevisiae strain INVSc1 (Invitrogen) was transformed with a construct expressing CYP716A12 CDS under the control of galactose-inducibile promoter. A yeast strain transformed with an empty vector was used as negative control.

Transformed yeast cells were grown on galactose to induce cytochrome expression and used for *in vivo* assays by feeding β-amyrin or oleanolic acid as possible substrates of the cytochrome. GC-MS analysis on transformed yeast and negative control did not show any additional peak except those of the substrates. The assays were repeated with different culture volumes (500mL and 1L), in order to obtain a more detectable yield of the product, but no substrate modifications were detected. The results can be explained by a low efficiency uptake of the substrates through the yeast cell walls, also reported by some authors as a possible cause of failure in *in vivo* experiments (Shibuya et al., 2006).

To overcome these difficulties, an *in vitro* approach was used. For the enzymatic assay, yeast microsomes, obtained appling method 1, were incubated with β-amyrin or oleanolic acid. After acid hydrolysis, the sapogenin mixture was assayed by GC-MS and no substrate transformations were detected.

Results of *in vivo* and *in vitro* assays could be explained by the low efficiency of yeast CPR. In fact P450 enzymes require ancillary proteins to catalyze the transfer of electrons from NADPH to the functional heme group. Due to this fact the endogenous levels of yeast CPR may be insufficient to support the optimal activation of highly expressed recombinant P450 (Siminszky et al., 2003).

High efficiency yeast strains

To optimize electron transfer between CPR and P450, the high efficiency WR strain of *S. ceravisiae,* over-expressing endogenous yeast CPR (Pompon et al., 1996), was transformed with expression vector pYES2-CYP716A12. Increased reductase activity of transformed WR strains was verified using the cytochrome c reductase (NADPH) assay kit (SIGMA) based on the ability of CPR to transfer electrons to cytochrome b_5 which forms part of electron transport chain on the ER (Fukuchi-Mizutani et al., 1999).

For the *in vivo* assay, transformed WR cells were grown on galactose and fed β-amyrin or oleanolic acid as possible substrates. Transformed yeast cells were subjected to acid hydrolysis and then assayed by gas-chromatography to investigate sapogenin contents. GC-MS analysis did not show any additional peak except those of the substrates (data not shown).

Next, transformed WR cells were used for *in vitro* assays; microsomal fractions were prepared, using method 1, and the CYP oxygenase assay was performed using β-amyrin or oleanolic acid as substrates. The mixture was subjected to acid hydrolysis and assayed by gas-chromatography. The results indicate the presence of an additional GC peak in the transformed yeast supplied with β-amyrin (Fig. 2). The experiment was repeated twice and the result was confirmed.

To investigate the chemical structure of the compound, the total sapogenin mixture obtained by acid hydrolysis was methylated and fractionated in three groups using a silica gel column. The fractions were checked on TLC silica gel plates and finally assayed by GC/MS. Chromatograms revealed the unidentified peak was not a sapogenin but a steroidal compound probably deriving from the yeast membrane.

The negative results could be explained by low affinity between the yeast CPR and the plant cytochrome; indeed the source of the reductase can influence the efficiency of coupled reaction mediated by cytochrome P450s of plant origin (Pompon et al., 1996).

Finally, to overcome the low affinity and to maximize redox compatibility between CPR and CYP716A12, another high efficiency yeast strain was employed. *S. cerevisiae* WAT11 is engineered to over-express *Arabidopsis thaliana* CPR1 in the presence of galactose and therefore provide an optimal environment for plant P450 activity (Pompon et al., 1996).

The CYP716A12 gene was cloned into the pESC-HIS expression vector containing a C-terminal FLAG epitope tag and used for transformation of the WAT11 strain. The presence of the protein was tested by immunoblot analysis performed at CNR-IGV in Perugia. Immunoblot assay revealed a rapid deterioration of the cytocrome in microsome preparation evidenced by the presence of a minor amount of intact protein and a major proportion of degraded protein (data not shown).

The rapid deterioration observed in immunoblot assay is consistent with problems encountered when using microsomes that had been stored at -80°C before enzymatic assays

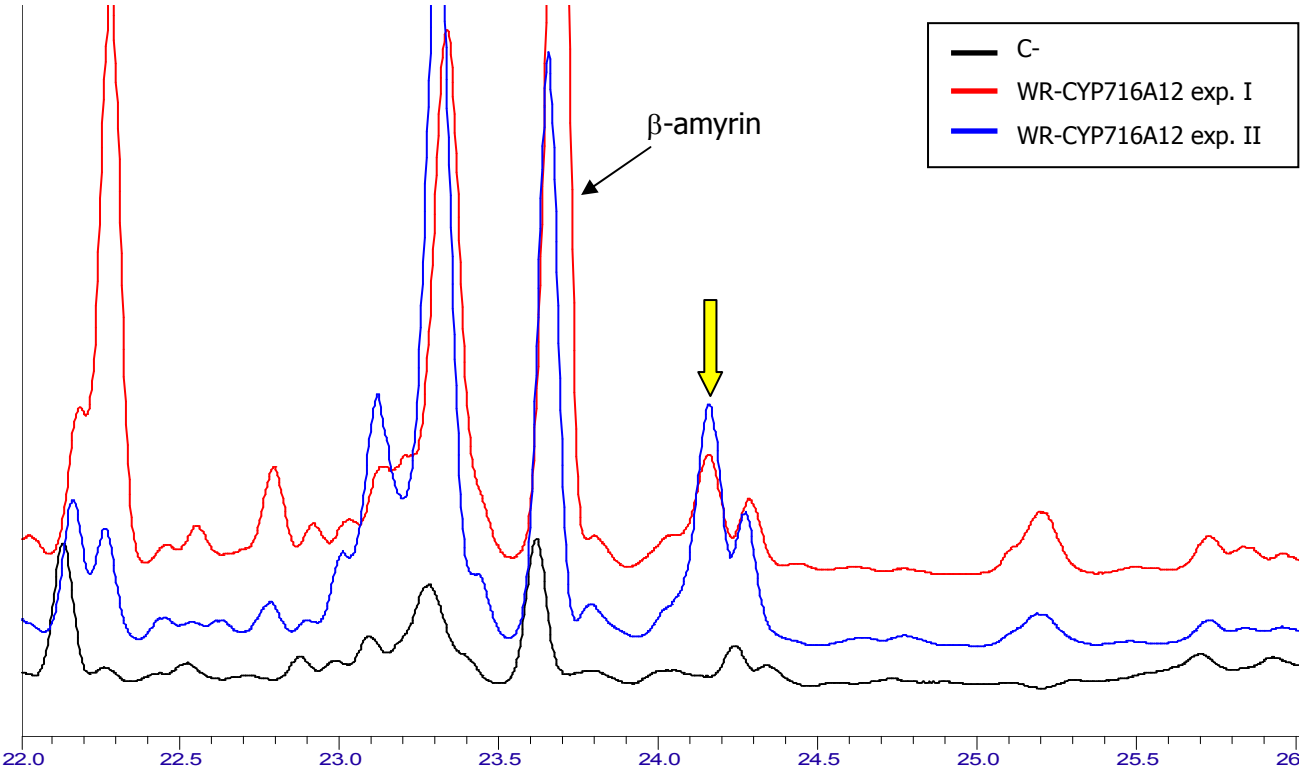


Figure 2. Chromatograms of WR transformed strain feeded of β-amyrin. The yellow arrow show the unidentified peaks.

WAT11 microsomal fractions were prepared using method 2; this microsome preparation procedure required the optimisation of yeast growth for cell collection. A cell density of 2x107 cell/ml (± 10%) turned out to be crucial for obtaining a microsome fraction effectively expressing the CYP protein; cells should be preferentially harvested and processed immediately because storage is not recommended in this step. The reductase activity of transformed WAT11 strains was verified using the cytochrome c reductase (NADPH) assay kit (SIGMA).

WAT11 membrane fractions were supplemented with β-amyrin and erythrodiol, a derivative of β-amyrin carrying a hydroxylic group at the C-28 position, and subjected to an *in vitro* oxygenase assay. GC/MS analyses revealed the presence of the same detectable GC peak in the transformed yeast supplied with β-amyrin and erythrodiol (Fig. 3). The retention time and mass spectrum of the product showed an excellent match with those of oleanolic acid. The experiments were repeated and the results were confirmed.

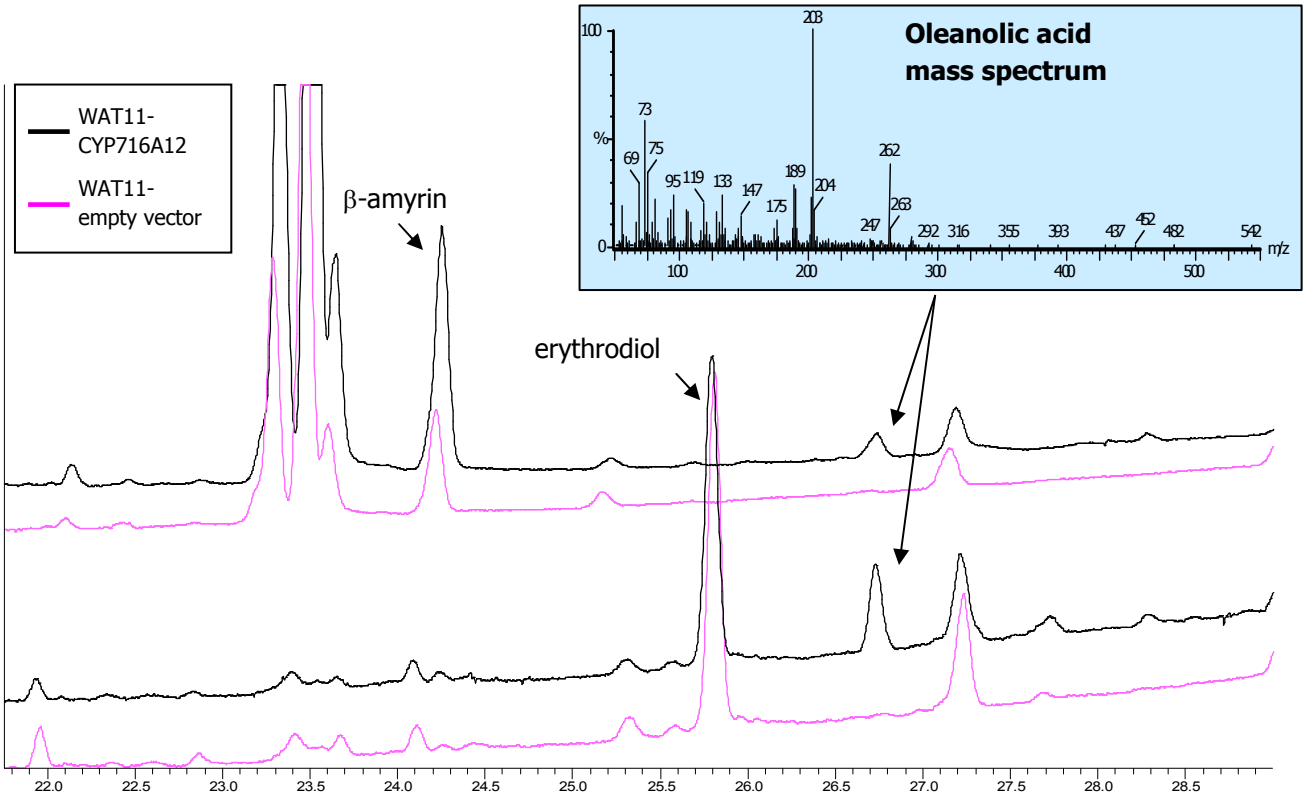


Figure 3. Chromatograms of WAT11 transformed strain feeded of β-amyrin and erythrodiol. The pale blue box showed a mass spectrum of oleanolic acid.

Following these positive results, the experiments were repeated employing WR microsomes: WR microsomes preparated with method 2 were able to transform β–amyrin and erythrodiol to yield oleanolic acid, albeit with lower efficiency compared to the WAT11 strain.

In conclusion, the results suggested that WAT11 and W(R) are both "high-quality" host strains for heterologous P450 expression systems.

Better performance of the *in vitro* assay compared to the *in vivo* assay is likely due to: i) recovery of a more purified sapogenins mixture for GC-MS analyses; ii) overcoming of substrate uptake problems.

These experiments indicate the critical phase for *in vitro* experiments is microsome preparation, method 2 being more efficient compared to method 1. In particular two steps of method 2 require high awareness: collecting the cells at the exact culture density ($2x10^7$ cell/ml) and processing the cells immediately after harvesting.

Furthermore, due to the rapid deterioration of cytochrome enzyme in membrane fractions, it is recommended to use each time fresh microsome preparations for *in vitro* assays.

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Chapter 4.
Discussion and Conclusions

1. CYP716A12 as a multifunctional oxidase

In an activation-tagging mutant collection of *M. truncatula,* one plant lacking hemolytic activity (*lha*-1 mutant) was evidenced using a microhemolytic test on leaf tissue extracts. The putative gene involved in this mutation turned out to have 97% identity with the CYP716A12 sequence, a member of a cytochrome P450 family found in *M. truncatula* (Li et al., 2007).

Biochemical analyses (TLC, HPLC and GC-MS) on *lha*-1 revealed a complete loss of the sapogenins forming the hemolytic saponins; in particular oleanolic acid, carrying a carboxylic group at C-28, was never found in mutant plants while it was present in trace amounts in roots of wild type plants.

These findings suggested that the pathway was blocked at an early and common step, likely the C-28 carboxylation of β-amyrin skeleton to give oleanolic acid. GC analyses performed on TILLING mutants (*lha*-2 and *lha*-3) gave the same results, confirming that CYP716A12 was a key gene involved in the hemolytic sapogenin pathway.

In order to validate the relation between the disruption of the gene and the *lha* phenotype, mutant plants were transformed with the complete CDS of CYP716A12. Some of the complemented lines obtained were able to restore the whole biosynthetic pathway, confirming that the knock-out of CYP716A12 gene caused the block of the hemolytic sapogenin pathway.

Further functional analysis was carried out by heterologous expression in yeast. *Saccharomyces cerevisiae* was indicated in the literature as a low-cost and efficient system to express heterologous P450s (Pompon et al., 1996). For these reasons CYP716A12 was expressed in two high efficiency yeast strains named WR and WAT11 overexpressing NADP(H) P450 reductases from *S. cerevisiae* and *Arabidopsis thaliana,* respectively. These enzymes are necessary for the electron transfer to the cytochrome P450 (Pompon et al., 1996).

Enzymatic reactions using β–amyrin and erythrodiol (a derivative of β-amyrin carrying a hydroxylic group at the C-28 position) revealed that the cytochrome uses both these substrates to yield oleanolic acid. These results demonstrated that CYP716A12 catalyzed the sequential three-step oxidation at C-28 position necessary to transform β-amyrin into oleanolic acid and that it was able to use erythrodiol too. These data were obtained using an *in vitro* system; however, as no intermediates of the three-step oxidation (erythrodiol and the corresponding aldehyde) were detected in mutant lines, it is likely that CYP716A12 catalyzes the same

reaction *in planta*. The C-28 oxidase activity was recently confirmed by an *in vitro* assay reported by Fukushima et al. (2011).

In conclusion CYP716A12 is a multifunctional oxidase that mainly catalyzes the first step of the hemolytic sapogenin pathway. To date, the order of oxidation between C-28 and C-23 in the hemolytic pathway was not clear; nevertheless, as the oxidations at C-28 position have been demonstrated to be the primary phase of the pathway, it is now evident that the oxidation at C-23 occurs later. Furthermore, CYP716A12 is a key gene because its disruption causes the block of any hemolytic sapogenin formation and the absence of C-28 glycosilated saponins.

This work demonstrates the effectiveness of coupling reverse genetics (TILLING) and yeast expression systems for functional identification of uncharacterized P450 genes involved in triterpene saponin pathway. Further candidate genes may be functionally characterized using similar approaches. A powerful technique for identifying genes putatively involved in secondary metabolism is to combine transcript and metabolite profiles with cluster analyses. In *Medicago*, these tasks are facilitated by the rapidly growing genomic resources allowing identification of potential P450 candidates. For instance, *M. truncatula* CYP93E2 displayed up-regulation by MeJA and coexpression with β-AS (Naoumkina et al., 2010) and was down-regulated in *lha*-1 mutant; the gene CDS displays 73% identity to CYP93E1 responsible for β-amyrin 24-hydroxylase activity in soybean *(Glicine max).* The oxidation at C-24 position of β-amyrin corresponds to the first step of soyasapogenol B biosynthesis (non-hemolytic sapogenin pathway) (Shibuya et al., 2006). This gene is a good candidate for heterologous expression in yeast and reverse genetic analysis using the TILLING mutant collection of *M. truncatula*. Recently*,* the 24-hydroxylase activity of *M. truncatula* CYP93E2 was demonstrated *in vivo* using yeast expression system (Fukushima et al., 2011; this work was published online while the present PhD thesis was in preparation).

Other two candidate genes found in M. truncatula are CYP72A67 and CYP72A68; these P450s have been selected as probably involved in saponin pathway because they were upregulated in *lha*-1 mutant, induced by MeJA and coexpressed with β–AS.

In our work, a high efficiency yeast strain (*S. cerevisiae* WAT11) was employed as expression system to demonstrate that cytochrome CYP716A12 catalyzes the three-step oxidase reaction from β-amyrin to oleanolic acid and also the two-step oxidation from erythrodiol to oleanolic acid. This result suggests that the cytochrome is able to employ more than one reaction intermediate. To confirm this, the next experiment may be performed using the erythrodiol corresponding aldehyde that is the last intermediate of the multi-step oxidation.

P450s typically catalyze mono-oxygenation reactions, but some have the ability to mediate sequential reactions (Sohl and Guengeric, 2010) as in the case of CYP716A12: the initial

substrate, once oxidized, may remain in the catalytic pocket of the enzyme as a substrate for a subsequent reaction (Bell-Parikh and Guengerich, 1999). On the other hand, some multi-step oxidation reactions are considered to involve exchange of the reaction intermediates with the medium (Bell-Parikh and Guengerich, 1999). In addition, the reaction may be governed by affinity of any of the ligands or by the rates of individual enzymatic steps. The kinetic mechanism of the multi-step oxidation mediated by CYP716A12 and the affinity of the substrates are yet unclear and may be the object of further investigation.

The yeast system can be also employed as a tool to validate the substrate-specificity of the catalytic site of cytochromes. In the future, we plan to use WAT11 transformed with CYP716A12, to test the ability of the enzyme to bind other sapogenins such as aglycones characterized by the absence of oxidation at C-28 and the presence of oxidation in other position that are near (C-22 or C-16) or far (C-23 or C-24) from the C-28 position on the pentacyclic skeleton.

It remains unknown whether P450 cytochromes are able to bind sapogenins and saponins indifferently and therefore which step, oxidation or glycosylation of sapogenins, occurs first. Thus CYP716A12 can be tested using aglycones with sugar moiety attached (ie saponins), such as glycosides of β–amyrin and erythrodiol, in order to verify its stereo-specificity.

2. Patterns of CYP716A12 expression and saponins accumulation

Three different tissues (leaves, roots and stems) of wild-type plants were examined at different developmental stages to investigate the expression profile of CYP716A12 gene and sapogenin content.

CYP716A12 was expressed in all plant organs and developmental stages analyzed; likewise sapogenins were detected in all tissues and stages. Roots displayed the highest and most stable expression across stages, consistent with previous observations (Li et al., 2007). In leaves, a significant increase in expression was observed in the reproductive stages, with a maximum expression at flowering: the highest and the lowest expression level were found in flowers and in pods, respectively. Thus, CYP716A12 expression appears finely regulated in different tissues and stages.

The hemolytic sapogenin content increased from vegetative to reproductive stages and varied among plant organs; non-hemolytic sapogenin (soyasapogenols A and B) content was mainly influenced by tissue. Thus, hemolytic saponin biosynthesis and accumulation appears not only organ specific (Human et al., 2005) but also developmentally regulated, in contrast to other

transcripts involved in triterpenic saponin pathway, generally restricted to the root system (Mylona et al., 2008)

Patterns of CYP716A12 expression and sapogenin accumulation and composition across plant organs and developmental stages are consistent with the idea that CYP716A12 controls a key step in the biosynthesis of the most abundant hemolytic sapogenins.

Due to their antifungal, antimicrobial and nematocidal activities, hemolytic saponins play an important role in plant defence (Sparg et al., 2004, Tava and Avato, 2006, Augustin et al., 2011); consistent with a potential involvement in plant defence responses, CYP716A12 is one of the P450 genes up-regulated by MeJA, a biotic stress signal (Naoumkina et al., 2010). Nevertheless, CYP716A12 expression and saponin production occur as part of normal growth and development and do not appear to be induced in response to biotic stress only. This indicates hemolytic saponins play additional roles in plant processes. *lha-1* and *lha-3* mutants exhibited severe root and shoot growth retardation compared with wild-type. A similar phenotype has been described for a *M. truncatula* mutant defective for UGT73F3 glycosyltransferase, affecting sugar moiety attachment at position C-28 of the aglycone. In this mutant, variation of the mixture of hemolytic saponins in roots but not in leaves was in agreement with predominant expression of UGT73F3 in roots (Naoumkina et al., 2010). Similar results were found in the oat mutant *sad4*, exhibiting an altered ratio of the triterpenic saponin avenacin A-1 and its incompletely glucosylated mutant and disruption of epidermal cell layer and hair formation in roots (Mylona et al., 2008). These findings suggest that the biosynthesis of hemolytic saponins can play a role in plant growth processes. In fact, terpenoid biosynthesis includes not only the pathways of saponins and sterols, but also pathways of important hormones such as gibberellins, abscisic acid and cytokinins.

This hypothesis is corroborated by microarray profiling of *lha* and wild-type transcriptomes showing differential expression of genes generally involved in plant development. Further investigation is required to explore the link between hemolytic saponins and plant growth.

3. Is CYP716A12 part of a gene cluster?

Operons (clusters of co-regulated genes with related functions) are common features of bacterial genomes. More recently, functional gene clustering has been reported in eukaryotes, from yeasts to filamentous fungi, plants, and animals (Osbourn and Field, 2009). The significance of gene clustering in plant secondary metabolism is unclear. Clustered genes may confer a selective advantage because they are inherited as a discrete functional unit undergoing evolutionary processes such as linkage disequilibrium or co-adaptation. Gene

clustering may also facilitate co-ordinate regulation of gene expression (Townsend et al., 2006).

In oat (*Avena* spp.), five genes encoding enzymes involved in avenacin saponin biosynthesis (P450s, acyltransferases and glycosiltransferase) are linked to the β-AS *Sad1* locus, suggesting that genes for a set of biochemically distinct enzymes are clustered within the genome (Qi et al., 2004). In *Arabidopsis thaliana,* Field and Osbourn (2008) reported an operon-like gene cluster that is required for triterpene synthesis (the thalianol pathway): the clustered genes, predicted to encode an 2,3-oxidosqualene cyclase, two CYP450s and an acyltransferase, are co-expressed. Another example of clustered genes involved in secondary metabolism is the benzoxazinoids pathway in maize including five clustered P450 genes (Papadopoulou et al., 1999).

These findings suggest that operon-like gene clusters may exist in secondary metabolite biosynthesis, in particular for triterpene saponin pathways. In *Medicago*, the possible existence of clusters is of special interest for genes involved in saponin biosynthesis.

Recently, Naoumkina et al. (2010) performed a clustering analysis on genes showing a similar expression pattern to β-AS and induced by MeJA, that were probably involved in triterpene metabolism in *M. truncatula.* The 5 genes highlighted by this analysis -a β-AS, two cytochrome P450 (CYP72A61s), an UGT and UGT73K1- were very closely linked on chromosome 4, likely the site for several genes involved in triterpene saponin biosynthesis in *Medicago* (Naoumkina et al., 2010). Although these candidate genes showed very similar expression profiles, they were not assembled into operon-like gene clusters. The CYP716A12 gene showed a similar expression profile to β-AS and was upregulated by MeJA; however, the position of CYP716A12 on the *M. truncatula* physical map is still unknown, so there is no indication as to the presence of CYP716A12 in an operon-like gene cluster. A recent study confirmed that the CYP716A12 locus is not clear in *M. truncatula* genome; a unique cluster of CYP716A genes (CYP716A21-A31) was found in the grapevine genome on chromosome 18 (Fukushima et al., 2011).

Interestingly, three genes, situated back to back on chromosome 8 displayed a similar expression pattern; phylogenetic analysis revealed that these genes belong to α/β–amyrin cluster but are not induced by MeJA (Naoumkina et al., 2010).

Future research of the genomic organization of metabolic pathways may be a complementary approach to attribute potential function to candidate genes as well as shedding further light on the evolution of chemical diversification in plants. Moreover, due to their ability to be transmitted through generations as a whole portion of genomic DNA, these "super genes" may have applications in crop breeding as a tool to confer a 'package' of adaptive advantages.

4. From model to crop legumes

Medicago truncatula has emerged during the last decade as the model system for legume biology. The availability of the complete genome sequence and integrated data obtained with various 'omics' approaches have provided a more comprehensive picture of metabolic pathways and stress tolerance mechanisms. Conserved genome structure (synteny) between model and crop plants has been proposed to facilitate the use of model species as a surrogate genome for map-based cloning of agronomically important genes in crops with complex genomes (Ané et al., 2008). Thus, genetic improvements can be expected by transfering information between the model system *M. truncatula* and legume crops, such as alfalfa (*M. sativa* L.).

In order to use the new findings in hemolytic saponin biosynthesis in crop species, the orthologous of CYP716A12 gene from *M. sativa* was amplified using the same primers utilized in *M. truncatula*. The CDS of the gene turned out to have 98% identity with the *M. truncatula* CYP716A12 CDS; the alfalfa cytochrome presents 9 aminoacid changes with respect to *M. truncatula* but the P450 typical sequences (e.g. heme cystein ligand) are thoroughly conserved. The strict correspondence between the two sequences is consistent with barrel medic and alfalfa sharing highly conserved nucleotide sequences and exhibiting nearly perfect synteny between the two genomes.

The *M. sativa* CYP716A12 obtained will be expressed using the high efficiency yeast system in order to test the ability of the alfalfa orthologous cytochrome to perform the three-step oxidation at C-28 position of β-amyrin skeleton. These future investigations might show that the orthologue of CYP716A12 has the same role also in alfalfa.

Classical breeding has successfully produced *M. sativa* varieties with low content of hemolytic saponins (such as cv. Sapko) (Pecetti et al., 2006) acting on "saponins" as a single substance and not as a mixture of compounds with specific biological properties. Novel tools enabling the dissection of the saponin pathway will result in more precise control of the individual saponin fractions in order to obtain plants with a desired saponin composition for diversified purposes. These plants could be employed as bioreactors to extract saponins for nutraceutical and pharmaceutical industries or as biological insecticide and nematocide for the use in organic farming (Tava and Avato, 2006). A possible drawback is that the modulation of saponin content and composition could have negative pleiotropic effects such as reduced dry matter production or reduced resistance to biotic stresses. This is demonstrated by mutant plants *lha*-1 and *lha*-3 where defects in saponin content are associated with dwarfism.

In our laboratory, a population derived from an interspecific cross *M. sativa* x *M. arborea* (SAC) had been developed selecting for sapogenin content and composition (Bingham and Haas,

2005). Biochemical analyses on S_1 (first selfed generation) selected plants showed wide variability both for total sapogenin content (2.05 - 14.35 mg/g DM) and composition (hemolytic/non-hemolytic ratio varying from 1 to 9). An EcoTILLING analysis will be performed on the selfed S_2 progenies in order to study the natural variation in CYP716A12 genomic sequence and its relationship with sapogenin content. This will be useful to investigate the role of CYP716A12 in alfalfa saponin biosynthesis and to identify alfalfa material with a known and stable content of specific saponin fractions.

Chapter 5.

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Summary

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Chapter 6.
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