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# Redox-regulation of starch and lipid synthesis in leaves

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This Ph.D. thesis is the account of work done between April 2002 and June 2005 in the department of Prof. Dr. Mark Stitt in the Max-Planck-Institut für Molekulare Pflanzenphysiologie, Golm, Germany. It is result of my own work and has not been submitted for any degree or Ph. D. at any other university.

Eidesstattliche Erklärung

Die Disseration ist ein Ergebnis praktischer Arbeit, welche von April 2002 bis Juni 2005 durgeführt wurde im Departament von Prof. Dr. Mark Stitt im Max-Planck-Institut für Molekulare Pflanzenphysiologie, Golm. Ich erkläre, daß ich vorliegende Arbeit selbstständig und ohne unerlaubte Hilfe angefertigt habe. Es wurden keine anderen als die angegebenen Quellen und Hilfsmittel benutzt, und die den benutzten Quellen wörtlichen und inhaltlichen Stellen sind als solche kenntlich gemacht.

Golm, Juni 2005

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# Abstract

Post-translational redox-regulation is a well-known mechanism to regulate enzymes of the Calvin cycle, oxidative pentose phosphate cycle, NADPH export and ATP synthesis in response to light. The aim of the present thesis was to investigate whether a similar mechanism is also regulating carbon storage in leaves.

Previous studies have shown that the key-regulatory enzyme of starch synthesis, ADPglucose pyrophosphorylase (AGPase) is inactivated by formation of an intermolecular disulfide bridge between the two catalytic subunits (AGPB) of the heterotetrameric holoenzyme in potato tubers, but the relevance of this mechanism to regulate starch synthesis in leaves was not investigated. The work presented in this thesis shows that AGPase is subject to post-translational redox-regulation in leaves of pea, potato and *Arabidopsis* in response to day night changes. Light was shown to trigger posttranslational redox-regulation of AGPase. AGPB was rapidly converted from a dimer to a monomer when isolated pea chloroplasts were illuminated and from a monomer to a dimer when preilluminated leaves were darkened. Conversion of AGPB from dimer to monomer was accompanied by an increase in activity due to changes in the kinetik properties of the enzyme. Studies with pea chloroplast extracts showed that AGPase redox-activation is mediated by thioredoxins *f* and *m* from spinach *in-vitro*. In a further set of experiments it was shown that sugars provide a second input leading to AGPase redox activation and increased starch synthesis and that they can act as a signal which is independent from light. External feeding of sugars such as sucrose or trehalose to Arabidopsis leaves in the dark led to conversion of AGPB from dimer to monomer and to an increase in the rate of starch synthesis, while there were no significant changes in the level of 3PGA, an allosteric activator of the enyzme, and in the NADPH/NADP+ ratio. Experiments with transgenic *Arabidopsis* plants with altered levels of trehalose 6-phosphate (T6P), the precursor of trehalose synthesis, provided genetic evidence that T6P rather than trehalose is leading to AGPase redox-activation. Compared to Wt, leaves expressing E.coli trehalose-phosphate synthase (TPS) in the cytosol showed increased

activation of AGPase and higher starch level during the day, while trehalose-phosphate phosphatase (TPP) overexpressing leaves showed the opposite. These changes occurred independently of changes in sugar and sugar-phosphate levels and NADPH/NADP$^+$ ratio. External supply of sucrose to Wt and TPS-overexpressing leaves led to monomerisation of AGPB, while this response was attenuated in TPP expressing leaves, indicating that T6P is involved in the sucrose-dependent redox-activation of AGPase. To provide biochemical evidence that T6P promotes redox-activation of AGPase independently of cytosolic elements, T6P was fed to intact isolated chloroplasts for 15 min. incubation with concentrations down to 100 µM of T6P, but not with sucrose 6-phosphate, sucrose, trehalose or Pi as controls, significantly and specifically increased AGPB monomerisation and AGPase activity within 15 minutes, implying T6P as a signal reporting the cytosolic sugar status to the chloroplast. The response to T6P did not involve changes in the NADPH/NADP$^+$ ratio consistent with T6P modulating redox-transfer to AGPase independently of changes in plastidial redox-state.

Acetyl-CoA carboxylase (ACCase) is known as key-regulatory enzyme of fatty acid and lipid synthesis in plants. At the start of the present thesis there was mainly *in vitro* evidence in the literature showing redox-regulation of ACCase by DTT, and thioredoxins f and m.  In the present thesis the in-vivo relevance of this mechanism to regulate lipid synthesis in leaves was investigated. ACCase activity measurement in leaf tissue collected at the end of the day and night in Arabidopsis leaves revealed a 3-fold higher activation state of the enzyme in the light than in the dark. Redox-activation was accompanied by change in kinetic properties of ACCase, leading to an increase affinity to its substrate acetyl-CoA . In further experiments, DTT as well as sucrose were fed to leaves, and both treatments led to a stimulation in the rate of lipid synthesis accompanied by redox-activation of ACCase and decrease in acetyl-CoA content.

In a final approach, comparison of metabolic and transcript profiling after DTT feeding and after sucrose feeding to leaves provided evidence that redox-modification is an important regulatory mechanism in central metabolic pathways such as TCA cycle and amino acid synthesis, which acts independently of transcript levels.

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# 1. Introduction

## 1.1.    Importance of Carbon Metabolism in Plants

Photosynthesis is the physico-chemical process by which plants, algae and photosynthetic bacteria use light energy to drive the synthesis of organic compounds. In plants, algae and certain types of bacteria, the photosynthetic process results in the release of molecular oxygen and the removal of carbon dioxide from the atmosphere that is used to synthesize triose-phosphates. A part of triose-phosphates can be then exported from chloroplasts and converted to end products that are required for growth and reproduction. Another part is used in biosynthetic pathways inside the chloroplast such as starch and fatty acid synthesis. Starch is the primary carbohydrate used for carbon and energy storage in plants (Dennis et al., 1997). It is a polymer of glucose, with different glycosidic linkages between glucose monomers and different degrees of branching. Alpha-amylose, one form of starch, has glucose subunits joined by alpha(1-4)-glycosidic bonds and does not have branches. These alpha-glycosidic bonds give the polymer a helical structure that aggregates, forming insoluble starch granules. Another form of starch, amylopectin, is also composed of glucose units joined largely by alpha(1-4)-glycosidic bonds, with the addition of branches at regular intervals. Advantages of starch over glucose for energy storage are that large polymers take less space, have less water associated, and have a lower osmotic pressure, making storage in the cell easier. Leaf starch represents a transient store, which is synthesised during the day and remobilized during the night to supply carbon for leaf metabolism (Geiger and Servaites, 1994). Its importance is demonstrated by the phenotype of starch-deficient mutants, which grow poorly or die in short-day conditions (Caspar et al., 1986; Schulze et al., 1991; Geiger et al., 1995; Sun et al., 2002). In plants *de novo* fatty acid synthesis occurs primarily in the plastid. In leaf tissue these fatty acids are used for the synthesis of plastidial and other cellular membranes in all cells. In certain plant tissues, mainly in seeds, they are also used for the synthesis of storage oils (TAG: triacylglycerols) (Ohlrogge et al., 1997; Stumpf, 1987; Harwood, 1988; Rawsthorne, 2002).

Both, starch and lipid synthesis are the important pathways of reduced carbon in the chloroplast. However, we do not know much about how the partitioning of carbon between these products is determined. The regulation of the pathways involved in carbon supply to fatty acid and starch synthesis in leaf chloroplasts is one of the main features of plant metabolism and is important for understanding of plant physiology. Furthermore, in seeds and other storage organs starch and lipids are economically important plant products that are common components of the human diet. They are also used industrially for many applications. The possibility of producing customized starches through manipulation of starch synthesis has contributed to the significant interest in the biochemistry of this process.

## 1.2. Photosynthesis and its regulation in leaves

All the energy consumed by biological systems ultimately comes from light energy trapped in reduced carbon skeletons by the process of photosynthesis:

$$H_2O + CO_2 => (CH_2O) + O_2$$
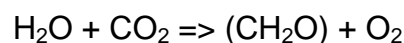
The mechanism of phtosynthesis is complex and requires the interplay of many enzymes. In green plants it takes place in chloroplasts. The light reaction is a membrane bound process. The photosynthetic membranes in plants contain two photosystems, PS I and PS II. PS I captures the energy of single photons by excitation of electrons in magnesium ions in chlorophyll. It is coupled to ferredoxin-$NADP^+$-reductase, which catalyzes the transfer of reducing equivalents from ferredoxin to thioredxin to produce reducing power in the form of $NADPH/H^+$. This reduction requires a steady supply of electrons and protons (reducing equivalents), which are provided by PS II which oxidizes water to molecular oxygen. PS II uses the energy from a second photon to reduce plastoquinone $QH_2$. The reduced quinones are deoxidized by a cytochrome *bf* complex separating electrons from protons generating a proton gradient. The proton gradient is coupled to $H^+$-ATPases synthesizing ATP from ADP + $P_i$ (Stryer, 1998, Buchanan and Balmer, 2005).

Calvin cycle uses products of light reactions (ATP and NADPH) to convert $CO_2$ into intermediates that supply carbon to various synthetic pathways inside as well as

outside the chloroplast. The $CO_2$ molecule condenses with ribulose 1.5-bisphosphate (Ru1.5bisP) to form a transient six-carbon compound, which is rapidly hydrolysed to two molecules of 3-phosphoglycerate (3PGA) in reaction catalysed by Rubisco (ribulose 1.5-bisphosphate carboxylase). 3PGA is phosphorylated by 3PGA kinase and reduced by NADP-glyceraldehyde 3-phosphate dehydrogenase to glyceraldehyde 3-phosphate. Glyceraldehyde 3-phosphate is than converted by Fru1.6bisP aldolase to fructose 1.6-bisphosphate (Fru1.6bisP). Fru1.6bisP molecule is dephosphorylated to fructose 6-phosphate (Fru6P) by FBPase (fructose 1.6-bisphosphatase). One part of the F6P is recycled to provide ribulose 5-phosphate which can be further phosphorylated by ATP hydrolysis to form ribulose 1.5-bisphosphate. The net energy balance of six rounds of the Calvin cycle to produce one mol of hexose is thus:

$$6CO_2+18ATP+12NADPH+12H_2O => C_6H_{12}O_6+18ADP+18P_i+12NADP^++6H^+$$

The Calvin cycle's hexose product is fructose 6-phosphate which can be converted to glucose 6-phosphate by the gluconeogenetic pathway. Glucose 6-phosphate can be used for starch and cellulose synthesis (Geiger and Servaites, 1994) (Fig.1).

Carbon dioxide fixation occurs during the day time and is strictly dependent on the light reaction and the formation of reductive power as NADPH. It does not occur literally in the dark (at night). The metabolic activity at night instead is mainly shifted to carbohydrate oxidation, when plants consume oxygen rather than producing it. The control of the Calvin cycle therefore means the control of regulation between light and dark reaction. The enzymes of the Calvin cycle are sensitive to the proton concentration of the chloroplast stromal compartment. The optimum pH for Rubisco activity lies around pH 8. The proton pump driven during light absorption moves protons from the stroma to the lumenal side to the thylakoid membrane increasing the stromal pH. This activates Rubisco and thus the Calvin cycle. The proton level control is coupled to magnesium interaction (chelation) with carbamates. Magnesium stabilizes carbamate formation and thus activates Rubisco. The key enzymes, fructose bisphosphatase and sedoheptulose bisphosphatase, of the Calvin cycle are also under pH control. The light-induced changes regulate synthetic pathways and opposing degradation pathways (ex. glycolysis) so that the cell can optimize available resources (Buchanan 1980, 1991).

Several Calvin cycle enzymes are known to be activated by light by a process linked to photosynthetic electron transport via the ferredoxin/thioredoxin system. Chloroplasts contain different thioredoxins showing selectivity toward different enzymes, thioredoxin *f* (named for its capability to activate fructose 1.6-bisphosphatase), *m* (named for its capability to activate NADP-malate dehydrogenase) (Jacquot et al. 1976, 1978; Buchanan et al. 1978) and two recently characterised thioredoxins: *x* and *y* (Lemaire et al., 2003; Mestres-Ortega et al., 1999). The experimental evidence suggests that thioredoxins *f* and *m* represent the major chloroplast forms, and *x* and *y*, whose functions are now being studied, are of lower abundance (Buchanan and Balmer, 2005). Thioredoxins are small proteins with a redox-active disulfide bridge present in the characteristic active site sequence -Trp-Cys-Gly-Pro-Cys-. They have a molecular mass of approximately 12 kDa and are universally distributed in animal, plant and bacterial cells. In their reduced form they constitute very efficient protein disulfide oxido-reductases and have been found to serve as electron donors in a variety of cellular redox-reactions. In chloroplasts and cyanobacterial cells, thioredoxins are reduced in response to light. Light-driven photosynthetic electron transport produces reduced ferredoxin (Fd), which serves as electron donor for ferredoxin:thioredoxin reductase (FTR). This enzyme reduces thioredoxins, which interact with target proteins involved in photosynthesis (Schürman and Jacquot, 2000). The ferredoxin/thioredoxin system has been shown to function similarly in the different types of plant photosynthesis (C3, C4, CAM) and to control, either directly or indirectly, the activity of each of the five regulatory enzymes of the Calvin cycle: fructose 1.6-bisphosphatase, sedoheptulose 1.7-bisphosphatase, phosphoribulokinase, NADP-glyceraldehyde 3-phosphate dehydrogenase and Rubisco (Breazeale et al. 1978; Buchanan et al., 1980; Schürmann and Jacquot, 2000). Regulatory enzymes of many other chloroplast processes were also found to be linked to thioredoxin: glucose 6-phosphate dehydrogenase (oxidative pentose cycle), ATP synthase (ATP synthesis), NADP-malate dehydrogenase (C4 photosynthesis, malate valve), acetyl-CoA carboxylase (fatty acid synthesis) (Buchanan 1980, 1991; Schürmann and Jacquot, 2000; Schürmann and Buchanan, 2001). Except for glucose 6-phosphate dehydrogenase and NADP-malate dehydrogenase, which are affected with thioredoxin *m*, these enzymes show a strong preference for thioredoxin *f*. Additionally, besides glucose 6-phosphate dehydrogenase, which is deactivated by thioredoxin, all these enzymes

are activated by the reduction via thioredoxin (Buchanan, 1980; Schürmann and Jacquot, 2000).

A part of triose-phosphates synthesized during Calvin cycle is exported to cytosol and converted to sucrose. Sucrose accumulates when export is decreased and this affects photosynthetic rate by repression of the expression of photosynthetic genes (Sheen et al., 1990; Krapp et al. 1991). Sugar control of metabolism, growth and development, stress, and gene expression has long been thought to be a metabolic effect. However, the control of gene expression observed with non-metabolizable or partially metabolizable hexoses or hexose and sucrose analogs clearly suggest the involvement of specific signal sensing and transduction mechanisms that do not require sugar catabolism (Stitt and Krapp, 1999; Sheen et al., 1999; Smeekens, 2000). Although hexoses are potent signals sensed in plants, sucrose-specific (Rook et al., 1998) and trehalose-mediated (Goddijn and Smeekens, 1998) signalling pathways also play important role in regulating development and gene expression. In developing seeds, it has been suggested that sucrose regulates differentiation and storage, whereas hexoses control growth and metabolism (Weber et al., 1997).

## 1.3.    The pathway of starch synthesis in leaves and its regulation

### 1.3.1.    Starch synthesis pathway

In leaves, a part of the photosynthetically fixed carbon is converted to starch within the chloroplast. The carbon precursor used for starch synthesis in leaves is provided by Calvin cycle. Inorganic $CO_2$ is converted to triose-phosphates, which are exported to cytosol and are used for sucrose synthesis. Some of the reduced carbon is retained in the chloroplast to synthesise starch as a transient carbon store, which is remobilised during the night to support non-photosynthetic leaf metabolism and sucrose export (see Fig. 1). This portion of triose-phosphates is converted via series of Calvin cycle reactions to fructose 6-phosphate (Fru6P). Fru6P can be used to

replenish the Calvin Cycle or is converted to glucose 6-phosphate (Glc6P) by the enzyme phosphoglucomutase (PGM) and subsequently via phosphoglucoisomerase (PGI) to glucose 1-phosphate (Glc1P), which is used for starch synthesis. Glc1P and ATP are converted by ADP-glucose pyrophosphorylase (AGPase) to ADP-glucose (ADPGlc) and inorganic pyrophosphate (PPi). PPi is further hydrolysed by pyrophosphatase to ortophosphates (Pi). Different isoforms of starch synthases, soluble and granule-bound starch synthases (GBSS), catalyse the addition of ADPGlc units to the non-reducing ends of linear glucan chains via $\alpha$-1.4 linkages. Branches are produced by starch branching enzymes (SBE), which hydrolytically cleave a linear glucan chain and transfer it to $\alpha$-1.6 position. Additional enzymes, such as debranching enzymes also participate in amylopectin synthesis (Smith, 2001). A simplified pathway of starch metabolism in *Arabidopsis* leaf is shown in Figure 1.
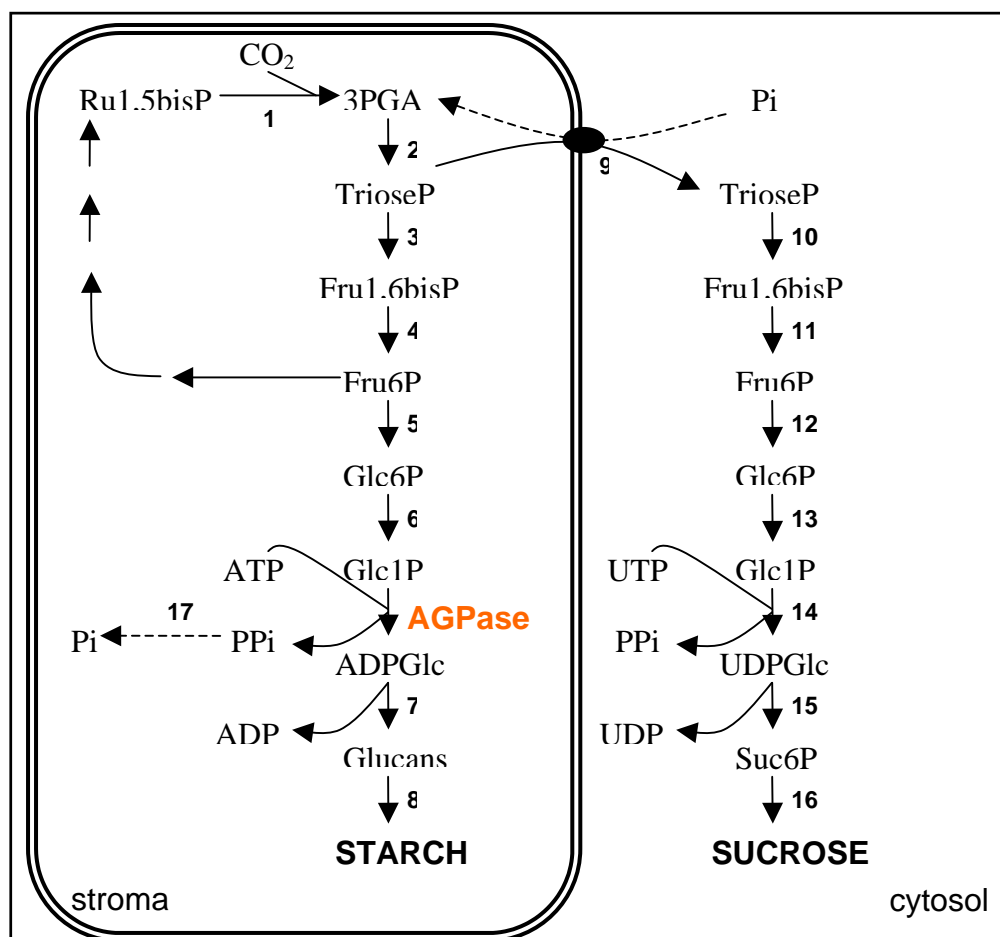


**Figure 1.** Starch and sucrose synthesis pathway. 1 - Rubisco, 2 - 3PGA kinase and NADP-GAPDH, 3 and 10 - Fru1.6bisP aldolase, 4 and 11 - FBPase, 5 and 12 - PGI, 6 and 13 - PGM, 7 - GBSS, 8 - SBE, debranching enzymes, 9 - TPT, 14 - UGPase, 15 - SPS, 16 - SPPase, 17 - pyrophosphatase.

## 1.3.2.     Regulation of starch synthesis

Previous studies showed a relationship between the activity of ADP-glucose pyrophosphorylase (AGPase, EC 2.7.7.27) and starch accumulation in several plant species and tissues. Isolated mutants of maize endosperm deficient in AGPase activity were shown to be deficient in starch content (Tsai and Nelson, 1966; Dickinson and Preiss, 1969). In confirmation, Müller-Röber et al. (1992) showed that expression of a chimeric gene encoding antisense RNA for AGPase in potato plants resulted in a reduction in enzymatic activity to 2-5% of the wild type levels and a commensurate reduction in starch content in growing tubers. Furthermore, expression of a mutated *E.coli*-AGPase in potato plants led to an increase in the starch content of the tubers by 30-60% (Stark et al. 1992). More recently, flux control analysis of the pathway of starch synthesis in *Arabidopsis* leaves (Neuhaus and Stitt 1990), potato tubers (Geigenberger, Müller-Röber and Stitt 1999; Geigenberger et al. 2004) and seeds of oil-seed rape (Vigeolas et al. 2004) showed that AGPase exerts a high degree of control on the pathway of starch synthesis, indicating that the enzyme is catalysing a near rate-limiting reaction.

AGPase from higher plants is a heterotetramer consisting of two bigger regulatory subunits (AGPS) and two smaller catalytical subunits (AGPB) (Morell et al., 1987; Okita et al., 1990). Studies of subunits expressed in *E. coli* indicate that small subunits alone can form catalytically active enzyme, and that large subunits are responsible for modulation of the sensitivity of the enzyme to effectors (Preiss and Sivak, 1996; Ballicora et al., 2000). The size of the two AGPB subunits has been found to range from 50 to 56 kDa and the size of two AGPS subunits varies between 51 and 60 kDa, depending on the plant species under investigation (Morell et al., 1987). cDNA clones encoding small and large AGPase subunits from a variety of monocot and dicot plant species have been isolated in recent years (Vailland et al., 1992; Ainsworth et al., 1993). Comparison of the primary structures of 11 plant AGPase proteins (Smith-White and Preiss 1992) revealed existence of at least three types of AGPase polypeptides in higher plants: the small subunit expressed in both photosynthetic and nonphotosynthetic tissues, a large subunit present in nonphotosynthetic tissues and a large subunit found in photosynthetic cells. The precise functions of the various AGPase polypeptides are not known, however there

are several possible explanations for the presence of these isoforms. 1) All four AGPase polypeptides contribute to the holoenzyme which is believed to function as a tetramer. Iglesias et al. (1993) showed that only when both subunits from potato were expressed simultaneously in *E.coli* high AGPase activity was detected. Expression of either subunit alone led to only minor AGPase activities. The precise composition of the enzyme could vary with the developmental stage and with changes in metabolism of the plant, allowing a finetuning of starch biosynthesis (La Cognata et al., 1995). 2) The various AGPase polypeptides could have different intracellular locations. Although the majority of the enzyme is located within chloroplasts and amyloplasts, it can not be excluded that at least some of the enzyme is also located in the cytoplasm (La Cognata et al., 1995). 3) The various AGPase polypeptides could contribute differently to cell-specific isozymes of the AGPase. In the transgenic potato and tobacco plants, GUS activity in leaves was restricted to stomatal guard cells and to the starch sheath, which is adjacent to the vascular tissues. No GUS activity was detected in mesophyll cells upon histochemical analysis (Müller-Röber et al., 1994; La Cognata et al., 1995).

Transcript levels of AGPS are increased by light and sucrose (Sokolov et al., 1998) and decreased by nitrates and phosphates (Scheible et al., 1997; Nielsen et al., 1998). This mechanism allows AGPase to react on changes of the carbon level and nutritional status on the long-term scale (Stitt and Krapp, 1999), but the changes in enzyme activity require up to days to develop (Geigenberger et al., 1994). In case of *Arabidopsis* leaves AGPase activity changes much slower than transcript levels during photoperiod (Sokolov et al., 1998). In antisense potato transformants large changes in AGPB transcripts were required to produce a significant decrease in AGPase activity (Müller-Röber et al., 1992; Zrenner et al., 1995). This indicates that transcriptional regulation on its own is not an efficient method to alter enzyme activities and fluxes.

AGPase is exquisitively sensitive to allosteric regulation, being activated by 3-phosphoglyceric acid (3PGA) and inhibited by inorganic orthophosphate (Pi) (Preiss, 1984, 1988; Sowokinos and Preiss, 1982). Studies with isolated chloroplasts led to the concept that starch synthesis is stimulated when low Pi restricts carbon export from the plastid (Heldt et al., 1977). In these conditions ATP falls, leading to an inhibition of 3PGA reduction. Rising 3PGA/Pi ratio provides a sensitive signal that

carbon fixation is exceeding the rate of export and activates AGPase. A similar situation occurs in leaves when the rate of the end products synthesis falls below the rate of photosynthesis. For example, feedback regulation of sucrose synthesis will lead to the accumulation of phosphorylated intermediates, depletion of Pi, and activation of AGPase by the rising 3PGA/Pi ratio, resulting in a stimulation of starch synthesis (Herold, 1980; Stitt et al., 1987).

For very long time, this biochemical model has provided the framework to explain how the photosynthate allocation between sucrose and starch is regulated. However, several situations have been reported in which changes in the rate of starch synthesis could not be explained by allosteric regulation or changes in expression of AGPase, for example treatments that modify partitioning by altering sugar levels in the leaf. Starch synthesis was stimulated in the absence of an increase of 3PGA when sugars were supplied to detached spinach leaves (Krapp et al., 1991), when spinach leaves were cold-grigled to decrease export (Krapp and Stitt, 1995), and when phloem transport was inhibited by phloem-specific expression of *E. coli* pyrophosphorylase in tobacco (*Nicotiana tabacum*, Geigenberger et al, 1996). In at least some conditions, decreased expression of SPS in *Arabidopsis* leads to decreased rather than inceased starch synthesis (Strand et al., 2000). Moreover, transgenic potato (*Solanum tuberosum*) plants with increased levels of 3PGA due to antisense inhibition of cytosolic phosphoglycerate mutase did not show any increase of starch in their leaves (Westram et al., 2002). These results indicate that there are gaps in our understanding of the regulation of photosynthate partitioning.

Recent studies provided *in vitro* evidence for post-translational redox-regulation of AGPase. When potato tuber *AGPB* and *AGPS* were heterologously overexpressed in *E. coli* an intermolecular bridge formed between the Cys[12] residues of the two AGPB subunits (Fu et al. 1998). To obtain active enzyme, it was necessary to incubate the complex with dithiothreitol (DTT) or reduced plastidal thioredoxins to break this link (Fu et al., 1998; Ballicora et al., 2000). The kinetic properties of the thioredoxin-reduced enzyme differed from the oxidized form by an increase in the affinity towards the activator 3-PGA. Thioredoxin *f* was shown to be more efficient than thioredoxin *m* in the reduction and activation process. Fifty percent activation was at 4.5 and 8.7 µM for reduced thioredoxin *f* and *m* respectively. The activation was reversed by oxidized thioredoxin (Ballicora et al., 2000). Regulation of starch synthesis in leaves may

occur in a similar way as the light-dependent regulation of Calvin cycle enzymes, where reduced thioredoxins act as activators (Scheibe, 1991; Schürmann and Jacquot, 2000). One of the major questions asked in the present thesis was whether reduction mediated by the ferredoxin-thioredoxin system could play a role in the fine regulation of the AGPase in leaves.

## 1.4. The pathway of sucrose synthesis in leaves and its regulation

Sucrose is synthesized in leaves as one of the primary end products of leaf photosynthesis. During the day, the substrate for sucrose synthesis is triose phosphate, released from the chloroplast on the triose phosphate translocator in exchange for inorganic phosphate (Huber et al., 1993; Winter and Huber, 2000). Trisose phosphate is converted by fructose 1.6-bisphosphate aldolase (Fru1.6bisP aldolase) to Fru1.6bisP and than by FBPase to Fru6P. PGI converts Fru6P to Glc6P and Glc6P is further converted to Glc1P by PGM. UDPglucose pyrophosphorylase (UGPase) by incorporating UTP and releasing inorganic pyrophosphate (PPi) produces UDPglucose, which is further converted to sucrose 6-phosphate (Suc6P) and sucrose using sucrose phosphate synthase (SPS) and sucrose phosphate synthase (SPPase), respectively. At night, starch mobilization provides the substrate for sucrose biosynthesis released on the hexose transporter (Schleucher et al., 1998). Several control mechanisms are involved in regulating sucrose synthesis. When sucrose accumulates in leaves SPS activity decreases because of an increase in the phosphorylation state of the enzyme (Foyer, 1990; Siegl and Stitt, 1990). SPS has been shown to be a substrate for SNF-1 related protein kinases (SnRKs; Sugden et al, 1999) which may be important in modulating SPS activity when sucrose accumulates. Control of sucrose synthesis includes also metabolite regulation of SPS by inorganic phosphate (inhibitor) and glucose 6-phosphate (activator) (Doehlert and Huber, 1983). Thus, sucrose synthesis is only promoted when metabolites are aboundant. Decreased hexose phosphate utilization by sucrose synthesis stimulates fructose 2.6-bisphosphate (F2.6BP) synthesis. In some plants this results in decreased cytosolic FBPase activity (Neuhaus et al., 1990; Stitt, 1990). As a result of the decreased Pi liberation imposed by low cytosolic FBPase activity more carbon is

retained in chloroplasts for starch synthesis. The importance of F2.6BP in controlling sucrose synthesis has been emphasized in transgenic tobacco where elevated F2.6BP concentrations , produced by the activity of a modified mammalian gene encoding 6-phosphofructo-2-kinase, led to decreased fluxes of carbon to soluble sugars, organic acids and amino acids while enhancing starch accumulation (Scott et al., 1995).

# 1.5. Pathway and Regulation of Fatty Acid Synthesis in Leaves

## *1.5.1. The pathway of fatty acid synthesis*

Fatty acids are an essential component of the cellular membranes of all living organisms excepting the *Archebacteria* (Davis et al., 2000). In plants *de novo* synthesis of fatty acids takes place in chloroplasts (Ohlrogge and Browse, 1995). It starts with the carboxylation of acetyl-CoA and its convertion to malonyl-CoA (Fig. 2). This reaction is the first commited step of the pathway and is catalysed by acetyl-CoA carboxylase (ACCase). Since acetyl-CoA is not imported by plastids it must be generated by metabolism within the plastid (Ke et al. 2000). In chloroplasts, photosynthesis provides an endogenous source of fixed carbon. Whether this fixed carbon can be utilised for the synthesis of acetyl-CoA depends upon the enzyme complement within the plastid in different species and during different developmental stages. Plants appear to have multiple pathways for generating acetyl-CoA.

Four possible routes for the acetyl-CoA synthesis in plastids have been proposed. In the first, free acetate deriving from hydrolysis of mitochondrial acetyl-CoA is taken up by diffusion into the plastid where it is converted to acetyl-CoA by plastidial ACS (acetyl-CoA synthetase) in an ATP-dependent reaction (Kuhn et al., 1981). Suggested alternative acetate sources include the combined actions of pyruvate decarboxylase and acetaldehyde dehydrogenase (Cui et al., 1996) or the action of O-acetyl-Ser thiol-lyase in forming Cys from O-acetyl-Ser (Leustek and Saito, 1999). In

the second, pyruvate is converted to acetyl-CoA by the PDC (pyruvate dehydrogenase complex) in a reaction that generates NADH. This pathway requires the conversion of 3PGA to acetyl-CoA via 2PGA, PEP and then pyruvate and is sometimes called C3-C2 pathway. A modification of this pathway occurs, if not all the glycolytic steps occur in the plastid, but instead some reactions are located in the cytosol and PEP or pyruvate are re-imported into the chloroplast (Fischer et al., 1997). During photosynthesis, a further source of pyruvate can be Rubisco, which at a low frequency catalyses a side reaction in which pyruvate is generated. A third possible route for intraplastidial synthesis of acetyl-CoA is through a plastidial ACC (carnitine acetyltransferase) reaction in which acetate is transferred from acetyl-carnitine to CoA. It has been proposed that this activity represents part of a carnitine-dependent acetyl/acyl transfer mechanism in the plant cell (Thomas et al., 1993). However, this proposed mechanism is controversial since it was not possible to demonstrate the incorporation of acetate from acetyl-carnitine into fatty acids by chloroplasts from spinach and pea leaves (Roughan et al., 1993). The fourth route is through the ACL (ATP-citrate lyase) reaction. The proposed role of this enzyme in *de novo* fatty acid synthesis is also controversial. Overexpression of the ACL from a rat liver in the plastid was reported to increase the fatty acid content of tobacco leaves by 16% compared to control plants. Whether this overexpression actually increases the rate of fatty acid synthesis has not been shown, nor has the source of plastidial citrate been identified (reviewed by Rawsthorne, 2002).

After acetyl-CoA is synthesised, its conversion to malonyl-CoA is catalysed by ACCase in an ATP-dependent reaction. After this step, elongation cycle starts. In subsequent reactions the condensation of malonyl-CoA and another acetyl-CoA to form acetoacetyl-ACP, the reduction, the dehydration and another reduction leading to production of butyryl-ACP occur. The reactions are catalysed by β-ketoacyl-ACP synthase III (KAS III), β-ketoacyl-ACP reductase, β-hydroxyacyl-ACP dehydratase and enoyl-ACP reductase. In the next rounds of synthesis C2 elements are successively added, but there are some differences in enzymes involved in this pathway. First, the condensation is catalysed by β-ketoacyl-ACP synthase I (KAS I), leading to production of palmitoyl-ACP (C-16:0-ACP). Second, the elongation to stearoyl-ACP (C18:0-ACP) and desaturation to oleoyl-ACP (C18:1-ACP) requires β-ketoacyl-ACP synthase II (KAS II) and stearoyl-ACP desaturase, respectively.

Some of the 16:0, 18:0 and 18:1 acyl groups are transferred from ACP to Gly3P by plastidic Gly3P:acyltransferases, thereby entering the so-called 'procaryotic' pathway leading to characteristic set of phospholipids (mono- and digalactosyldiacylglycerol, phosphatidylglycerol, sulphoquinovosyldiacylglycerol) (Ohlrogge and Browse, 1995). Alternatively, these groups can be released via acyl-ACP-thioesterases and move to cytosol, where they are reconverted to acyl-CoA and enter the 'eucaryotic' pathway for lipid synthesis, leading to another set of phospholipids ( phosphatidyl-choline, - ethanoloamine, -glycerol, - inositol) and TAG (Ohlrogge and Browse, 1995). Paralell reactions in the endoplasmic reticulum and plasmid convert acyl groups to phosphatidic acid (PA) and diacylglycerol (DAG) via combined action of sn2-Gly3P:acyltransferases and lysophosphatidic acid:acyltransferases, followed by dephosphorylation of PA by phosphatidic acid phosphatase. DAG can be also substrate for diacylglycerol:acyltransferase, which synthesises triacylglycerol (TAG) (Ohlrogge and Browse, 1995).
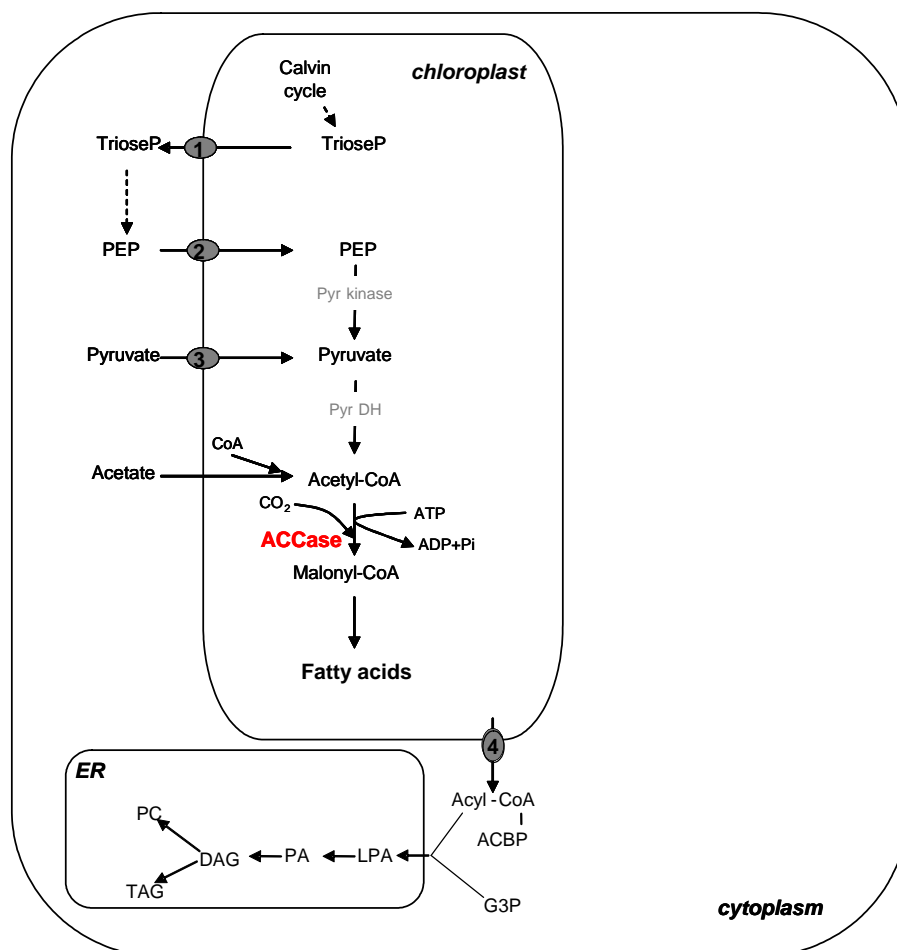


**Figure 2.** Lipid synthesis in chloroplast. 1- TrioseP/Pi transporter (TPT), 2- PEP/Pi transporter, 3-Pyr/Pi transporter, 4-Fatty acids transporter.

### 1.5.2.    Regulation of fatty acid synthesis

Acetyl-CoA carboxylase (ACCase, EC 2.4.1.13) regulates the rate of fatty acid synthesis in yeasts, animals, bacteria and plants (Sasaki et al., 1997; Ohlrogge and Jaworski, 1997; Rawsthorne, 2002). There are several lines of evidence suggesting that ACCase does represent a regulatory step in plants. Intermediates of fatty acid synthesis change during the transition to darkness in leaves and chloroplasts in a manner consistent with control at the level of ACCase (Ohlrogge and Jaworski, 1997). This observation is entirely consistent with the more recent reports of light-dependent regulation of chloroplast ACCase by the redox status of the plastid whereby the enzyme is more active under the reducing conditions found in the light (Kozaki and Sasaki, 1999). In leaves, the light-dependent regulation of fatty acid synthesis through ACCase activity is logical if the synthesis from malonyl-CoA is in turn dependent upon the supply of reducing power and ATP from the light reactions of photosynthesis. Other aspects of the control of carbon flux through fatty acid synthesis by ACCase have been addressed in a number of ways. By using specific enzyme inhibitors, Page et al. (1994) have reported that ACCase exerts strong control over flux into fatty acid synthesis in isolated barley and maize leaf chloroplasts. Indirect evidence for control by ACCase can be drawn from experiments in which fatty acids were supplied to tobacco suspention cell cultures (Shintani and Ohlrogge, 1995). This resulted in inhibition of fatty acid synthesis, which was explained by feedback inhibition of ACCase by an undefined mechanism.

Two forms of ACCase are known to occur in plants: type I ACCase, which is a multifunctional, homodimeric polypeptide analogous to that found in mammals and yeasts, or type II, which is a heteromeric, multi-subunit form analogous to that found in prokaryotes (Ohlrogge and Jaworski, 1997). In most of higher plants ACCase II is active in plastids, with the exception of *Gramineae* species such as maize and wheat where the plastidic ACCase has a homodimeric (type I) structure. (Alban et al., 1994; Ohlrogge and Jaworski,1997). All higher plants possess an extraplastidial type I enzyme which is proposed to be cytosolic (Ohlrogge and Jaworski, 1997). In contrast to the plastidial isoform, the cytosolic ACCase does not catalyse the *de novo* fatty acid biosynthesis, but it is required for the fatty acids elongation (over 18-C). In

addition to this it is involved in the synthesis of variety of phytochemicals such as epicutical waxes, suberin, flavonoids, stilbenoids, a variety of malonylated chemicals and free malonic acid (Nikolau et al., 1984; Caffrey et al., 1998).

Heteromeric ACCase from plastids is composed of a dissociable complex of biotin carboxylase (BC), catalysing the carboxylation of biotin on the biotin carboxyl carrier protein (BCCP) subunit, and the carboxyltransferase $\alpha$- and $\beta$-subunits ($\alpha$-CT and $\beta$-CT), which catalyse the transfer of $CO_2$ from carboxybiotin to acetyl-CoA to form malonyl-CoA (Sasaki et al, 1995; Ohlrogge and Browse, 1995). The $\beta$-subunit is encoded by the chloroplast genome, while the other three sunbunits are encoded by the nuclear genome (Sasaki et al., 1995).

Plastidial ACCase has pH optimum near 8.0 to 8.5. It is activated by ATP, CoA, malonyl-CoA, propionyl-CoA and butyryl-CoA (Hunter and Ohlrogge, 1998). Competitive inhibitors of ACCase are ADP and to lesser extent AMP (Nikolau et al., 1984). Studies in isolated pea chloroplasts showed that the plastidial ACCase in plants is subject to phosphorylation. Dephosphorylation of pea chloroplast extracts by an alkaline phosphatase reduced *in vitro* plastidial ACCase activity by 67%. Evidence was presented pointing to the carboxyltransferase $\beta$-subunit as a candidate for regulation by protein phosphorylation/dephosphorylation (Savage and Ohlrogge, 1999).

Plastid ACCase has also been shown to be redox-regulated via reduction of a disulfide bridge between the nuclear encoded $\alpha$ and plastidial encoded $\beta$ subunits of carboxyltransferase. Reducing agents led to an increase in ACCase activity in spinach chloroplasts and preparations of partially purified pea ACCase (Sauer and Heise, 1984; Sasaki et al., 1997). Reduced thioredoxin activated the enzyme more efficiently than DTT alone, and thioredoxin *f* was more effective than thioredoxin *m* or *E. coli* thioredoxin. Western blot analysis of partially purified ACCase from pea chloroplasts showed that in the absence of reducing agents Cys-267 and Cys-442 between $\alpha$- and $\beta$-CT subunits form a disulfide bridge leading to the dimerisation and inactivation of the enzyme. In the presence of 2-mercaptoethanol CT subunits were present in monomerised active form (Kozaki et al., 2001). Comparison of the redox-state of ACCase measured in chloroplasts isolated from dark- or light-adapted pea

seedlings showed that in the light CT subunits are partially monomerised, whereas in the dark all the enzyme is in the dimerised non-active form (Kozaki et al., 2001).

However, direct *in-planta* evidence for the regulation of lipid synthesis by redox regulation is lacking. Fatty acid synthesis in pea chloroplasts is increased in the light and decreased in the dark. It was demonstrated (Sauer and Heise, 1983) that isolated chloroplasts are able to incorporate acetate into malonyl-CoA within minutes when exposed to light and the incorporation decreases after the exposure ends. These changes were at least partly explained by changes in ACCase activity via light-dependent changes of pH, $Mg^{2+}$ and adenine nucleotide levels in the stroma. During illumination, increases in pH from 7 to 8 and in $Mg^{2+}$ from 1 to 3 mM have been measured for chloroplast stroma. The activity of ACCase at pH 8 and 2.5 mM $Mg^{2+}$ was 90-fold that at 7 and 1 mM $Mg^{2+}$, suggesting that stromal ACCase could be activated very strongly by these changes in the light (Kozaki and Sasaki,1999). In the dark, ATP and ADP concentrations have been found to be in the range of 0.3-0.8 mM and 0.6-1.0 mM, respectively, in chloroplast stroma. Upon illumination, photophosphorylation causes a rise in the ATP concentration to 0.8-1.4 mM while ADP decreases to 0.3-0.6 mM. Such changes have been shown to lead to a 2-fold increase in the stimulated ACCase activity *in-vitro*. (Nikolau and Hawke, 1984).

Further, in addition to the unclarified role of light for the redox-activation of ACCase in leaves, the involvement of sugars as a second input in analogy to the situation for AGPase in potato tubers (see above) has not been considered. One of the aims of the present thesis is to investigate whether lipid synthesis is redox-regulated *in planta* and what signals are leading to reductive activation of ACCase.

## 1.6.    Aims and objectives of the present work

Post-translational redox-regulation has been found many years ago to regulate enzymes of the Calvin cycle, oxidative pentose phosphate cycle, NADPH export and ATP synthesis in response to light via the ferredoxin/thioredoxin system (Buchanan 1980, 1991; Schürmann and Jacquot, 2000; Schürmann and Buchanan, 2001). In the present thesis it should be investigated whether this concept can be extended to the

regulation of carbon storage in leaves. It has been shown by other research groups that heterologously overexpressed AGPase is activated by DTT and thioredoxins *f* and *m in vitro*, which involves reduction of a disulfide-bond between $Cys^{12}$ of regulatory subunits (Fu et al., 1998; Ballicora et al., 2000). In the first part of the thesis, the *in vivo* importance of this mechanism in the regulation of starch synthesis in leaves in response to light should be investigated. In the second part it should be investigated whether sugars provide a second input that leads to redox-regulation of AGPase and starch synthesis, and possible factors that are involved in this response should be identified. In the third part it should be investigated whether lipid synthesis is regulated by a similar mechanism which involves reductive activation of acetyl-CoA carboxylase *in vivo*. Up to the start of the thesis, redox-regulation of ACCase has mainly been investigated *in vitro* (Sasaki et al., 1997).

The following questions should be investigated in this thesis:

1. Is it possible to measure the redox-activation state of AGPase in leaves or is it necessary to adjust the current protocols?

2. Are there changes in AGPase redox-activation during day-night alterations in leaves?

3. Is there a direct light effect leading to reductive activation of AGPase in leaves?

4. Are there sugars involved in reductive activation of AGPase and do they provide a second input that is different from light? Is an increase in AGPase redox-activation accompanied by an increase in the rate of starch synthesis?

5. What could be the link between sugars and redox activation of AGPase? Do sugars lead to an increase in the overall redox-state or to changes in signal metabolites that report the cytosolic sugar status to the plastid?

6. Is it possible to measure the redox-activation state of ACCase in leaves or is it necessary to adjust the current protocols?

7. Is there a decrease in ACCase redox-activation during the night?

8. Is it possible to re-activate ACCase and to stimulate lipid synthesis by DTT feeding?

9. Does sugar feeding lead to redox-activation of ACCase and stimulation of lipid synthesis similar to its effect on AGPase?

10. Is post-translational redox-regulation of general importance for the regulation of metabolic pathways in leaves and what is the role of transcriptional regulation in this context?

The work was mainly focused on *Arabidopsis* as a model system, where the complete genome sequence is known and mutants are available. Some experiments were also performed with leaves from potato and pea to show the general importance of the results. Pea was also used as a convenient system to perform experiments with isolated chloroplasts. Overall, leaves provide an excellent system to investigate the effect of light on starch and lipid synthesis. It also represents a convenient system to manipulate sugar levels.

# 2. Materials and methods

All experiments were reproduced at least once with independent biological material. Data points are at least the average of duplicate measurements of the same biological sample. When error bars are shown, they represent the standard deviation (SD) of the average of the measurements on at least two biological samples of the same experiment or standard error (SE) when indicated.

Some of the specific conditions and experimental procedures have changed during the course of the thesis. In these cases the figure legends explain the detailed conditions that were used for that particular set of experiments.

## 2.1.    Enzymes and chemicals

Biochemical enzymes and substrates were purchased from Roche (Mannheim) and Sigma (München), chemicals were obtained from Roche (Mannheim), Merck (Darmstadt), Sigma (München) and Fluka. Reagents for SDS-PAGE were used from BioRad (München). Radiochemicals were from Amersham-Pharmacia (Braunschweig). X-ray films were from Kodak. Most enzymes were grade II and chemicals were of analytical purity.

## 2.2.    Plant material and growth

Pea (*Pisum sativum* cv Marcia) was grown either in a greenhouse with a 16-h day of 180 µE, 21℃/19℃ (day/night),  and 50% humidity or in a high-light phytotron with a 14-h day, 20℃/16℃, and 60%/75% humidity. The pea cv Kelvedo n Wonder was grown in a short-day phytotron (8-h day of 180 µE, 20℃/16℃, and 60%/75% humidity day/night). *Arabidopsis thaliana* var Col-0 (wild type) and transgenic lines overexpressing otsA encoding for *E.coli* TPS (line A19.3) or otsB encoding for an *E.coli* TPP (line B12.1) (Schluepmann et al., 2003) were grown in the same short-day phytotron. Potato (*Solanum tuberosum* cv Desiree) and tobacco (*Nicotiana tabacum*) Wt and transgenic plants overexpressing otsA encoding for *E.coli* TPS or otsB

encoding for an *E.coli* TPP (Pellny et al., 2004) were grown in a greenhouse at 400 µE, 14-h day, 20°C/16°C day/night, and 50% humidity throughout.

## 2.3. Harvesting procedure, sample storage

Leaves were harvested while leaving the plants in place. Only source leaves that were not shaded by other leaves were collected. Leaves were put directly into liquid nitrogen, and stored at −80°C until use.

## 2.4. Homogenisation of frozen leaf tissue

A swingmill (MM 200, Retch) was used to homogenise the frozen leaf material to a fine powder. The metal ball (2 cm) and containers (stainless steal of 20 ml volume) were precooled in liquid nitrogen and the frozen tissue was homogenised at 30 Hz for at least 1 minute. The frozen powder was then transferred to precooled eppis or falcons for the different extractions. Work was done quickly to not allow the frozen powder to melt and water to condensate on it. The powder was kept free of liquid nitrogen to allow an accurate mass determination and avoid explosion of safelock eppis after storage at -80$^{o}$C.

## 2.5. Incubation of Leaves with Sugars in the Darkness

At the end of the dark period (or at the end of day period in case of over night feeding), plants were taken from the phytotron. Non-shaded source leaves were cut, and their petioles were recut under buffer solution. The recut petioles were inserted into the feeding solution, containing 2 mM MES, pH 6.5, and varying concentrations of sorbitol, sucrose, trehalose or glucose. The leaves were put in the growth cabinet (where the conditions were the same as in phytotron, but the light was switched off) and incubated there (times of incubations are indicated in figure legends). After incubation, leaves were frozen immediately in liquid nitrogen, after excising that part of the petiole, which had been immersed in the feeding solution.

## 2.6. Incubation of leaf discs with DTT

Incubation with DTT (dithiotreitol) was performed similar to the sugar incubations. The difference was that leaf discs not whole leaves were incubated in the medium

containing 2 mM Mes, pH 6.5 (control) or 2 mM Mes, pH 6.5 with different concentrations of DTT. The discs were put in the growth cabinet (where the conditions were the same as in phytotron, but the light was switched off and incubated there for 1 hour. After incubation, discs were frozen immediately in liquid nitrogen.

## 2.7.     Trichloroacetic acid (TCA) extraction

The frozen plant tissue (0.1 g FW) was resuspended in 0.8 ml of 16% (w/v) TCA dissolved in diethyl ether and incubated shortly on ice. Then 300 µl of 16% (w/v) TCA in water including 5 mM EGTA was added, mixed well and incubated for 2h in thermomixer at $4^{o}C$. The samples were centrifuged at 10000 g for 10 min at $4^{o}C$. The water soluble phase was transferred to a new tube and washed 3 times with diethyl ether to remove TCA. Samples were neutralised (pH 6-7) with 5 M KOH including 1 M triethanoloamine and centrifuged 5 min 10000 g at $4^{o}C$. The supernatant was frozen in liquid nitrogen and stored at $-80^{o}C$ for metabolite analysis.

## 2.8.     Extraction of AGPase for western blot and procedures for gels

Frozen leaf material was homogenized using a liquid nitrogen cooled ball-mill, and 50 mg of leaf material was extracted in cold 16% (w/v) TCA in diethyl ether, mixed, and stored at $-20°C$ for at least 2 h. The pellet was collected by centrifugation at 13,000 rpm for 5 min at $4°C$. The pellet was washed three times with ice-cold acetone, dried briefly under vacuum, and resuspended in 1x Laemmli sample buffer containing no reductant (Laemmli, 1970). After heating the sample for 3 min at $95°C$, the insoluble material was settled by a 1-min spin, and the supernatant was used for gel electrophoresis on 10% or 7.5%(w/v) acrylamide gels in presence of SDS. Proteins coming from 0.5 or 1 mg of fresh weight were loaded per small or broad lane, respectively and separated at constant voltage (140 V) for 2 hours (Laemmli, 1970). The gels were blotted onto polyvinylidene difluoride (PVDF) membrane according to standard procedures. After transfer of the proteins to membrane, it was incubated for at least 1 hour with blocking solution consisting of 1xTBS (20 mM Tris-HCl pH 7.5, 500 mM NaCl) and 5% (w/v) fat-free milk (Bio-Rad). AGPase antigen was detected for 1 h at RT, using a primary rabbit antibody raised against the His-tagged AGPB of

potato, diluted 1:10000 in blocking buffer (Tiessen et al., 2002*). Thereafter one short washing step with 1xTBST (1xTBS containing 0.1% v/v Tween) and two washing steps with 1xTBS were performed. Incubation with a second antibody (peroxidase-conjugated secondary goat anti-rabbit antibody, Bio-Rad Laboratories, Hercules, CA) was done for 1 h at RT and the same washing steps were repeated.. The peroxidase was detected on film using the ECL kit of Amersham Biosciences (Uppsala). To quantify the amount of AGPase present as monomer, the films were scanned with standardized settings, saved as tif files, and analyzed with Scion image software (Raytest Isotopenmessgeräte GmbH, Straubenhardt, Germany). Gel samples from chloroplasts were prepared by mixing 1 volume of chloroplasts with 1 volume of 2x Laemmli sample buffer without reductant. The samples were heated at 95°C for 3 min and stored at room temperature until use. Proteins coming from 6.25 µg of chlorophyll were loaded per lane. Electrophoresis, blotting, and immunolabeling procedures were as described above. For detection of the peroxidase, the ECL or ECL advance kit was used (Amersham).

## 2.9.    Extraction and assay of AGPase

Activity measurements were performed essentially as described (Tiessen et al., 2002): Fifty milligrams of material was extracted with 0.5 ml of extraction buffer (50 mM K-HEPES, pH 7.5, 5 mM MgCl$_2$, 1 mM EGTA, 1 mM EDTA, 1 mM benzamidine, and 1 mM ε-aminocaproic acid). The sample was centrifuged for 30s at 4°C. The supernatant was used directly at 1/10 or 1/20 of the volume in the activity assay containing 50 mM K-HEPES, pH 7.5, 5 mM MgCl$_2$, 1.5 mM G1P, and varying amounts of ATP and 3PGA. After 15 min at 30°C, the reaction was stopped by boiling for 3 min. After a 5-min centrifugation, the supernatant was stored at 4°C or –80°C until the ADP-Glucose content was determined by HPLC. Chloroplasts suspensions or desalted protein extracts used for activity measurements were first incubated for 15 minutes with different concentrations of T6P, DTT or reduced thioredoxins and than assayed as described above.

## 2.10.   HPLC (measurement of ADP-glucose)

According to Geigenberger et al. (1996, 1997).

ADPglucose was measured by high performance liquid chromatography using a system from Kontron Instruments (München). The AGPase assays were kept at 4°C and 20 µl were injected by the auto sampler to the HPLC column. Buffer A consisted of 10 mM $NH_4H_2PO_4$ pH 2.8 (adjusted with H3PO4). Buffer B consisted of 750 mM $NH_4H_2PO_4$ pH 3.7. Both buffers were first filtered (Cellulose acetate filter, 3 µm mesh, Sarstedt) and then degassed by ultrasonification. The gradient program used to separate the nucleotides was as follows: flow 1 ml/min, 0 min 7.5% B, 0.5 min inject, 12 min 7.5 B, 15 min 50% B, 19.5 min 90% B, 34 min 100% B, 38 min Acq. Off, 39 min end. Detection was performed with UV light at 254 nm and at 230 nm. A partisil-10-SAX ion exchange column (5 mm x 250 mm, Whatman, UK) and a precolumn (5 mm x 20 mm) were used for the separation of the nucleotides. The nucleotides were identified by comparison of the elution time of standards and the 254 nm/ 230 nm ratio of absorbance. Standards consisted of 20 µM, 50 mM and 100 µM of the mono-, di- and tri-nucleotides, respectively. The amount of metabolite was calculate via software (Kroma System 3000, Kontron) from the peak area by using a calibration factor obtained from standard runs.

## 2.11. Extraction and assay of ACCAse

50 mg of leaf material was extracted with 0.5 ml of extraction buffer (50 mM K-HEPES, pH 7.6, 5 mM $MgCl_2$, 1 mM EGTA, 1 mM EDTA, 1 mM benzamidine, and 1 mM ε-aminocaproic acid). The sample was centrifuged for 30s at 4°C and supernatant preincubated 15 min without or with 5 mM DTT in RT. 5 µl of supernatant was added to 20 µl of reaction mixture (50 mM Tricine/KOH pH 9, 1 mM ATP, 2.5 mM $MgCl_2$, 50 mM KCl, 200 µM Acetyl-CoA, 3.4 mM $NaH^{14}CO_3$ [14.8 kBq], +/- 5 mM DTT), incubated for 20 min in 30°C, stopped with 5 µl of 6 M HCl and transferred on Whatman paper (3 mm). Control samples were prepared by adding HCl before incubation with the reaction mixture. The paper was dried and the radioactivity determined (modified from Sasaki et al., 1997).

## 2.12. Chloroplast preparation

Ten- to 16-day-old pea seedlings were subjected to an extended night by 5 h to deplete the internal starch pools and subsequently were transferred to light for about 30 min to induce photosynthesis. Chloroplasts were then prepared essentially as

described by Lunn et al. (1990), but with some adaptations. 500 µl of extraction buffer (0.33 M sorbitol, 0.01 M Mes/KOH pH 6.5, 0.005 M MgCl2, 0.002 M ascorbate, 0.5% BSA) and 80 g of pea leaves were blended 2x 10 sec, filtered through miracloth and nylonmesh to discard leaf tissue, poured into plastic tubes, centrifuged 1 min 1700g at 4$^o$C. Pellet was dissolved in 1 ml of resuspension buffer (0.33 M sorbitol, 0.05 M Hepes/KOH pH 7.5, 0.002 M EDTA, 0.001 M MgCl$_2$), filtered through 40% (v/v) percoll mixed with resuspension buffer by centrifugation for 1 min, 1700g, 4$^o$C. Pellet was dissolved in 250 µl resuspension buffer, mixed and kept in darkness on ice until use. The chlorophyll content of the final preparation was determined in MeOH extracts (Porra et al., 1989) and calculated according to the equation:

Chlorophyll a+b = 22.12*($A^{652}$-$A^{750}$)+2.71*($A^{665}$-$A^{750}$).

Intactness of chloroplasts was determined routinely by microscopy or determined on oxygen electrode. Intact chloroplasts measurement mixture: 500 µl 2x concentrated degassed resuspention buffer (containing 0.004 M NaHCO$_3$ additionally), 20 µl chloroplasts extract, 500 µl degassed water. Mixture was darkened for 5 min, next 50 µl of 0.2 M glyceraldehyde and 5 µl of 1M ferricyanate were added, darkened for 2 min and 20 µl of 0.25 M ammonium chloride added. Destroyed chloroplasts measurement mixure contained the same solutions, but water was added first, than chloroplasts and buffer (Lilley et al., 1975).

## 2.13. Incubations of chloroplasts and photosynthesis measurement

Oxygen evolution was measured in an oxygen electrode at 25℃ on the chloroplasts suspension of 50 µg chlorophyll ml$^{-1}$ in resuspension buffer containing 4 mM NaHCO$_3$ and additives as indicated in the figure legends. The cuvette was darkened for 5 min before the dark sample was taken. After restabilisation of the evolution trace, the sample was illuminated using the beam of a slide projector.

## 2.14. Desalting of proteins from isolated chloroplasts

Freshly isolated intact chloroplasts were destroyed by 5-fold dilution with water (osmotic shock) and cleaned on desalting Sephadex G-50 column.  Sephadex G-50

resin was prepared according to Amersham Biosciences procedure and 10 ml of the resin was used for column preparation. 10 mM $Na_2HPO_4$ buffer, pH 6.8, was used for the equilibration of the column and elution of the sample. After desalting samples were stored on ice until use and used within one day.

## 2.15. Thioredoxin reduction

Plastidial thioredoxins f and m from spinach leaves (provided by Prof. Renate Scheibe (Osnabrück). were reduced as described (Sasaki et al., 1997). The mixture containing 50 mM Hepes pH 7.8, 0.1 mM EDTA, 5 mM DTT, 1 mM thioredoxin was incubated for 10 minutes in $37^o$C, cooled on ice and used for AGPase reduction.

## 2.16. Extraction and assay of FBPase and NADP malate dehydrogenase

FBPase (fructose bisphosphatase) and NADP-MDH (NADP-malate dehydrogenase) activities in chloroplasts were measured by mixing 20 μl of the chloroplasts solution from the oxygen electrode with 180 μl of reaction mixture containing 50 mM K-Tricine, pH 8.0, 5 mM $MgCl_2$, and 0.1% (v/v) Triton X-100. For FBPase, the mixture additionally contained 0.1 mM $NADP^+$, 40 μM Fru-1,6-bisphosphate, and 1.75 mM EDTA. The reaction was stopped by the addition of 20 μl of 1 M NaOH either directly or after a 3- or 10-min incubation at room temperature. The reaction mix for NADP-malate dehydrogenase assays instead contained 0.1 mM NADPH, and 0 or 2 mM oxaloacetate additionally. It was stopped after 10 min by addition of 20 μl 1 M HCl/0.1 M Tricine, pH 9. In both cases, the difference in NADP(H) content of the two samples was taken as a measure for enzyme activity. The samples were stored at $4°C$ until further processing. Heating of the samples for 5 min at $95°C$ ensured the complete disrupture of all unused nucleotide-adenine substrate (NADP or NADPH). Five or 10 μl of the reaction was brought to pH 9 by the addition of 25 mM HCl/50 mM Tricine, pH 9, for FBPase or 0.1 M NaOH for MDH. The NADP(H) content was determined directly after the pH adjustment by an enzymatic cycling assay (like for glucose 6-phosphate, see 2.13.1). Both assays were shown to be linear with time for over 10 min.

## 2.17.   Ethanolic extraction

Frozen plant material was extracted in two subsequent steps with 80% (v/v) ethanol (250 µl per 10 mg FW and 150 µl per 10 mg FW), mixed for 20 min at $80^{o}C$ and centrifuged at max speed for 5 min. The pellet was re-extracted in with 50% (v/v) ethanol (250 µl per 10mg FW), the supernatants were combined and used for immediate assay or stored at $-20^{o}C$ until use. The pellet of the ethanol extraction was used for starch analysis.

## 2.18.   Assay of sucrose, reducing sugars, starch

Sucrose, glucose, fructose and starch were determined enzymatically in ethanol extracts (Stitt et al., 1989) using a microplate spectrophotometer. The assay contained a final volume of 220 µl (179 µl of 100 mM Hepes/KOH with 3 mM $MgCl_2$ (pH 7), 10 µl of 12 mg/ml NADP, 10 µl of 20 mg/ml ATP, 0.5 U glucose 6-phophate dehydrogenase from yeast and 20 µl of ethanol extract. The reactions were started by successive addition of 1U hexokinase (from yeast), 1 U phosphoglucoisomerase (from yeast) and 20 U  invertase (from yeast).

For starch determination, the pellets of the ethanol extraction were solubilized by heating them to $95°C$ in 0.1 M NaOH for 30 min. After acidification  to pH 4.9 with an HCl/sodium-acetate, pH 4.9, mixture, part of the suspension was digested overnight with amyloglucosidase and α-amylase. The glucose content of the supernatant was then used to assess the starch content of the sample.

## 2.19.   Assay of hexose-phosphates

Hexose-phosphates were determined as described in Gibon et al. (2002).

### 2.19.1.    Glucose 6-phosphate

Aliquots of 5 µl of ethanol extracts or hexose-P standards (0-50 pmol) were disposed into 1.5 ml microtubes, containing 20 µl of 50 mM Tricine/KOH buffer pH 9 containing 2.5 mM $MgCl_2$, 0.02 U G6PDH and 6.5 nmol NADP added, incubated for 20 min at

RT, 20 µl 0.5 M NaOH added, heated at 95°C for 5 min, cooled on ice, centrifuged 30 sec and contents transferred to a 96-well microplate containing 20 µl of 0.5 M HCl in buffer. After mixing, 50 µl buffer containing 1.2 U G6PDH, 0.3 µmol G6P, 0.5 µmol EDTA, 0.04 µmol PMS and 0.1 µmol MTT was added and absorbance read at 570 nm for 10 min.

### 2.19.2.     Glucose 1-phosphate

Aliquots of 10 µl of ethanol extracts or hexose-P standards (0-20 pmol) were disposed into 1.5 ml microtubes, containing 20 µl of 50 mM Tricine/KOH buffer pH 9 containing 2.5 mM $MgCl_2$, 0.02 U G6PDH and 6.5 nmol NADP added, incubated for 20 min at RT, 10 µl of 0.25 M HCl was added and incubated 10 min at RT. This incubation removed G6P and destroyed the resulting NADPH. After neutralising with 10 µl of 0.25 M a in buffer, 20 µl of 50 mM buffer containing 0.2 U G6PDH, 0.04 U PGM and 0.5 nmol G1,6-bisP were added, incubated 20 min at RT, 20 µl 0.5 M NaOH added, heated at 95°C for 5 min, cooled on ice, centrifuged 30 sec and contents transferred to a 96-well microplate containing 20 µl of 0.5 M HCl in buffer. After mixing, 50 µl buffer containing 1.2 U G6PDH, 0.3 µmol G6P, 0.5 µmol EDTA, 0.04 µmol PMS and 0.1 µmol MTT was added and absorbance read at 570 nm for 10 min.

### 2.19.3.     Fructose 6-phosphate

Aliquots of 10 µl of ethanol extracts or hexose-P standards (0-50 pmol) were disposed into 1.5 ml microtubes containing 20 µl of 50 mM Tricine/KOH buffer pH 9 containing 2.5 mM $MgCl_2$, 0.02 U G6PDH and 6.5 nmol NADP added, incubated for 20 min at RT, 10 µl of 0.25 M HCl was added and incubated 10 min at RT. This incubation removed G6P and destroyed the resulting NADPH. After neutralising with 10 µl of 0.25 M a in buffer, 20 µl of 50 mM buffer containing 0.2 U G6PDH, 0.04 U PGI and 0.5 nmol G1,6-bisP were added, incubated 20 min at RT, 20 µl 0.5 M NaOH added, heated at 95°C for 5 min, cooled on ice, centrifuged 30 sec and contents transferred to a 96-well microplate containing 20 µl of 0.5 M HCl in buffer. After mixing, 50 µl buffer containing 1.2 U G6PDH, 0.3 µmol G6P, 0.5 µmol EDTA, 0.04 µmol PMS and 0.1 µmol MTT was added and absorbance read at 570 nm for 10 min.

## 2.20. Pyruvate and phosphoenolpyruvate

The TCA extracts were used to measure pyruvate and phosphoenolpyruvate enzymatically (Stitt et al., 1989) in a dual-wavelengh spectrophotometer (Sigma). The assay (600 µl) contained 50 mM Hepes/KOH pH 7, 5 mM $MgCl_2$, 50 µM NADH, 1 mM ADP and 40 µl extract. The reactions were started by successive addition of 1 U 1-lactate-dehydrogenase and 2 U pyruvate kinase.

## 2.21. 3PGA

The TCA extracts were used to measure 3-phosphoglyceric acid (3PGA) enzymatically (Stitt et al., 1989) in a dual-wavelengh spectrophotometer (Sigma). The assay (600 µl) contained contained 50 mM Hepes/KOH pH 7, 5 mM $MgCl_2$, 50 µM NADH, 1.5 mM ATP and 20 µl extract. The reaction was started by simultaneous addition of 5 U glycerine-aldehyd-3-phosphate-dehydrogenase (rabbit muscle) and 5 U 3-phosphoglycerate-kinase (yeast).

## 2.22. Glycerol-3-phosphate

Gly3P was determined as described in Gibon et al. (2002).

Aliquots of extract (10 µl) or Gly3P standard (0-20 pmol) were added to 40 µl 200 mM buffer, heated for 20 min, $95^{o}C$ to destroy DAP, cooled on ice, centrifuged 30 sec, the supernatants transferred to a 96-well microplate, mixed with 40 µl 50 mM buffer containing 2 U Gly3POX, 130 U catalase, 0.4 U Gly3PDH and 0.12 µmol NADH, and absorbance by 340 nm was read for 20 min.

## 2.23. Acetyl-coenzyme A

Acetyl-Coa was determined as described in Gibon et al. (2002).

Aliquots of 20 µl extract or Acetyl-CoA standards (0.5-10 pmol) were disposed into a microplate and 0 or 1 µmol of N-ethylmaleimide was added (20 µl) in order to remove CoASH. After 10 min incubation, 100 µl of 200 mM Tris/HCl ph 7.4 containig 0 or 1

µmol DTT and 20 µmol malate were added and the plate was incubated for 15 min at RT. Finally 50 µl of a mixture containing 0.5 µmol DTT, 0.92 µmol acetyl phosphate, 0.28 µmol NAD, 2.8 U malate dehydrogenase in 100 mM Tris/HCl were added and absorbance was read at 340 nm for 10 min.

## 2.24.   NADPH/NADP$^+$ ratio measurement

NADPH/NADP$^+$ ratio was determined in ethanol extracts as described in Lowry et al. (1961). 500µl of ethanol extract was pipeted to 50µl of 0.5M NaOH (for NADPH measurement) or to 50µl of 0.5M HCl, heated at 90$^o$C for 2 minutes, cooled on ice for 1 minute and centrifuged in 4$^o$C on maximum speed. 20µl of supernatant was used for cycling assay (as for glucose 6-phosphate, see 2.13.1) immediately.

## 2.25.   Labeling experiments and label separation

Labeling experiments were carried out with whole *Arabidopsis* leaves cut directly from the plant, with ends of petioles re-cut under water. Leaves were incubated in the dark for 13 h at 20℃ (humidity of 60%) in medium containing 2 mM MES-KOH (pH 6.5) and 0.66 mM or 0.33 mM [U-$^{14}$C]-glucose (specific activity, 111 kBq/mmol; Amersham-Buchler, Braunschweig, Germany) together with various concentrations of sucrose (see legends to figures for details). Incubations were done in petri dishes (5-ml volume). Wet ends of petioles of incubated leaves were cut and discarded, and leaves were frozen immediately in liquid nitrogen. After ethanol extraction, 1 ml of chloroform and 1 ml water were added to the combined supernatants and the extracts were vigorously shaken. The upper phase (ethanol/water) were transferred into a small tube and dried under an air stream at 45℃, taken up in 1 ml H$_2$O (soluble fraction), and separated into neutral, anionic and cationic fractions by ion-exchange chromatography (Geigenberger et al., 1997). The chloroform fraction was dried and counted for total lipids or freeze-dired, taken up in 75 µl of water and further separated by thin-layer chromatography (Geigenberger et al., 1997), developing 8 times with ethyl acetate/pyridine /water (100:35:25, by vol.), and quantifying using a linear analyser (Tracemaster 20, Berthold, Bad Wildbad, Germany). Peaks were identified using radiolabelled standards. The insoluble material (starch, proteins and cell wall) left after ethanol extraction was analysed to determine the amount of label in starch, protein and cell walls as in Merlo et al.

(1993). Label in the hexose phosphate pool was analyzed as by Geigenberger et al. (1997), and total carbon in the hexose phosphate pool was determined in ethanol extracts as described above using non-radioactive replicates incubated in parallel. Total fatty acids were extracted according to the method of Bligh and Dyer (1959) and the lipid content was counted for the radioactivity.

## 2.26. Extraction and derivatisation for GC/MS (gas chromatography/mass spectrometry)

The polar metabolite fraction was obtained from wild type (ecotype Columbia-0) or transgenic *Arabidopsis thaliana* plants overexpressing *E.coli* genes for trehalose metabolism. Samples were harvested at the end of day and night or incubated with different sugars or DTT (see Materials and Methods and description of experiments in Results chapter). After freezing in liquid nitrogen and homogenization the tissue was extracted in 1400µl 100% methanol containing 60µl quantitative internal standard (2mg ribitol in 1ml water) for 15 min at 70$^o$C and centrifuged. The supernatant was then transferred to glass vials and vigorously mixed with 750µl of chloroform and 1500µl water and subsequently centrifuged at 4000rpm. Aliquots of the methanol/water supernatant (400 and 800µl) were dried in speed vac for 6-16 hours.

The dried residue was redissolved and derivatised in 40µl of 20mg/ml methoxyamine hydrochloride in pyridine for 2h at 37$^o$C followed by treatment with 70ml MSTFA for 30min at 37$^o$C. Retention time standard mixture (10µl) was added to trimethylsilylation. The retention time standard mixture contained heptanoic, nonanoic , undecanoic, tridecanoic and pentadecanoic acid – 3.7% (w/v) each, tricosanoic and nonadecanoic acid – 7.4% (w/v) each, heptacosanoic acid – 22.2% (w/v) and hentriacontanoic acid – 55.5% (w/v) in tetrahydrofuran at 10mg/ml total concentration.

## 2.27. GC/MS analysis

All GC/MS analyses were carried out using a GC/MS system consisted of an AS 2000 autosampler, a GC 8000 gas chromatograph and a Voyager quadrupole mass

spectrometer (ThermoQuest, Manchester, GB). The mass spectrometer was tuned according to the manufacturer's recommendations using tris-(perfluorobutyl)-amine (CF43). Gas chromatography was performed on a 30m SPB-50 comlumn with 0.25mm inner diameter and 0.25µm film thickness (Supelco, Belfonte, CA). Injection temperature was 230$^o$C, the interface set to 250$^o$C and the ion source adjusted to 200$^o$C. The carrier gas used was helium set at a constant flow rate of 1ml/min. The temperature program was 5min isothermal heating at 70$^o$C, followed by a 5$^o$C/min oven temperature ramp to 310$^o$C and a final 1min heating at 310$^o$C. The system was then temperature equilibrated for 6min at 70$^o$C prior to injection of the next sample. Mass spectra were recorded at 2scan/s with m/z 50-600 scanning range.

## 2.28.    Determination of relative metabolite levels

The chromatograms and mass spectra were evaluated using the Masslab program (ThermoQuest, Manchester, GB). A retention time and mass spectral library for automatic peak quantification of metabolite derivatives was implemented within the Masslab method format. For evaluation of relative values within each chromatogram the peak areas derived from specific ion traces indicative of each analysed compound were normalized by the peak area derived from an internal standard present within the same chromatogram.

At least 5 replicates were measured and standard error (SE) was determined. Significance was determined using t-test analysis. Significant data are marked on red colour on the list of measured metabolites (Appendix).

## 2.29.    RNA isolation

Total RNA was isolated according to Trizol RNA extraction protocol (adapted by O. Bläsing). 5ml Trizol was added to 0.5 g of frozen leaf sample, vortexed for 30 sec and incubated at RT for 5 min. Next it was centrifuged 20 min in 4$^o$C, 4000 rpm, supernatant was transferred to a new tube and 3ml pure chlorophorm was added, the miture was vortexed and incubated at RT for 5 min. After centrifugation (20 min, 4000 rpm, 4$^o$C), RNA containing aqueous phase was transferred to a new tube. 1/10 volume 3M sodium acetate and 0.6 volume of isopropyl alcohol was added, mixed and incubated at 4$^o$C for 15 min. Next samples were centrifuged 10 min, 4000 rpm at

$4^{\circ}$C to pellet RNA. Supernatnat was removed, pellet was washed twice with 1ml 70% Ethanol, spinned 5 min, 4000 rpm at $4^{\circ}$C. Finally pellet was air dryed for 10 min at RT, resuspended in 50µl DEPC treated water and stored in freezer until use.

## 2.30. Transcript levels measurement and analysis

Chip hybridization was performed as in Thimm et al. (2004) MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. The Plant Journal 37: 914-939

Data analysis was done using the Bioconductor package in the R software (Robert C Gentleman and Vincent J. Carey and Douglas M. Bates and Ben Bolstad and Marcel Dettling and Sandrine Dudoit and Byron Ellis and Laurent Gautier and Yongchao Ge and Jeff Gentry and Kurt Hornik and Torsten Hothorn and Wolfgang Huber and Stefano Iacus and Rafael Irizarry and Friedrich Leisch Cheng Li and Martin Maechler and Anthony J. Rossini and Gunther Sawitzki and Colin Smith and Gordon Smyth and Luke Tierney and Jean Y. H. Yang and Jianhua Zhang, 2004, Bioconductor: Open software development for computational biology and bioinformatics. Genome Biology, 5: R80).

Normalisation of the raw data and estimation of signal intensities was done using RMA methodology (Bolstad, B.M., Irizarry, R. A., Astrand, M. and Speed, T.P. (2003), A comparison of normalization methods for high density oligonucleotide array data based on bias and variance.Bioinformatics, 19, 185–193) combining all experiments with the same experimental treatment.

Cluster analysis was performed using self-made selection macros written in EXCEL, as well as the TIGRE MeV software package (Saeed, A.I., Sharov, V., White, J., Li, J., Liang, W., Bhagabati, N., Braisted, J., Klapa, M., Currier, T., Thiagarajan, M., Strun, A., Snuffin, M., Rezantsev, A., Popov, D., Ryltsov, A., Kostukovich, E., Borisovsky, I., Liu, Z., Vinsavich, A., Trush, V. and Quackenbush, J. (2003) TM4: a free, open-source system for microarray data management and analysis. Biotechniques, 34, 374–378.)

# 3. Results

## 3.1. AGPase is subject to post-translational redox-activation in leaves in response to light signals

AGPase is a key-regulatory step in the pathway of starch synthesis. Previous studies provided *in-vitro* evidence for post-translational redox-regulation of AGPase. (Fu et al., 1998; Ballicora et al., 2000). However, the *in-vivo* importance of this mechanism for the regulation of photosynthetic starch synthesis and its relation to the well-establisehd light-dependent regulation of the activities of Calvin cycle and other enzymes invovled in photosynthesis by thioredoxin-mediated redox activation remained unclear.

In the following chapter it is investigated whether AGPase is subject to redox-regulation in response to light-dark changes in leaves. To do this, a protocol was established to measure *in-vivo* AGPB-dimerisation state and activation in leaves. Then AGPB dimerisation state and activation was measured in leaves at the end of the day and night and in response to more rapid light-dark transitions. AGPB dimerisation was also measured in isolated chloroplasts to investigate whether there is direct response to light.

### 3.1.1. Establishing of a new protocol to measure changes in the reduction state of AGPase in leaves

To investigate whether leaf AGPB undergoes reversible dimerisation *in vivo*, extracts were initially prepared rapidly in degassed SDS solutions and subjected to non-reducing SDS-PAGE and AGPB protein was detected using a rabbit-antibody raised against AGPB from potato. When extracts from growing potato tubers were prepared

this way, they contained a mixture of monomeric and dimeric AGPB (Tiessen et al., 2002). In case of leaves, all the AGPB protein ran with an apparent molecular mass of about 100 kDa, irrespective of whether extracts were prepared from illuminated or darkened plants. When extracts from leaves were mixed 1:1 with extracts from growing potato tubers, the immunosignal was also obtained at only 100 kDa (data not shown). These results indicated that leaf extracts contain unknown compounds that rapidly oxidize AGPB to a dimer. To prevent this, leaves were extracted in a trichloroacetic acid (TCA)-diethyl ether mixure at 4°C to rapidly denature AGPase and physically separate AGPB subunits that were present as monomers, and analysed in non-reducing SDS-PAGE electrophoresis. Rapid oxidation of AGPB in leaf extracts probably explains why posttranslational redox-regulation was overlooked in earlier studies.

### 3.1.2. Changes in AGPB dimerisation and activity in response to day/night changes in leaves

In leaves, starch is accumulating during the day and decreasing during the night. Pea (*Pisum sativum*), potato (*Solanum tuberosum*), and *Arabidopsis thaliana* leaves were harvested during the night, and in the second half of the light period to investigate whether leaf AGPB is subject to reversible dimerisation *in vivo* using the new extraction procedure. Results show that pea leaf AGPB subunit is completely dimerised in the night, and partly converted to a monomer during the day (Fig. 3). When the extracts were separated in reducing conditions (samples contained DTT), immunosignal was found only at 50 kDa, showing that the intermolecular link involves a disulfide bridge. Similar results were obtained for potato and *Arabidopsis* leaves. The proportion converted to a monomer was lower in *Arabidopsis* compared to pea and potato, possibly reflecting the lower growth light intensity.
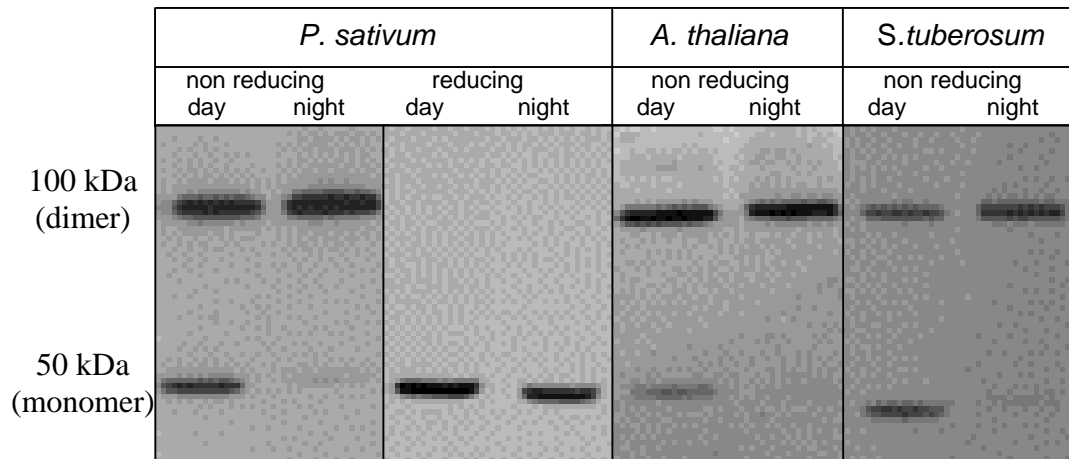
**Figure 3.** Dimerisation of AGPB varies between the day and night in potato, pea, and *Arabidopsis* leaves. Western blot of pea (cv Marcia), *Arabidopsis* and potato leaf tissue harvested during the second half of the day (day) and at the end of the night (night). Samples were prepared with TCA ether and run directly (non-reducing) or after adding 4 mM DTT to part of the sample (reducing).

DTT feeding to leaf discs was performed to check whether external supply of reductants can lead to increased AGPase redox state in leaf discs during the night. Leaf discs were taken from *Arabidopsis* plants at the end of the night and incubated in a medium without reducing agent or with 5 or 20 mM DTT for one hour in the darkness (Fig. 4). In leaf discs incubated without DTT AGPB was almost completely dimerised, while discs incubated with increasing DTT concentrations showed progressive increase in AGPase monomerisation. This shows that the decrease in reductive activation of AGPase that occurs during the night (see above) can be partially reverted by short-term feeding of DTT to the tissue.

**A**
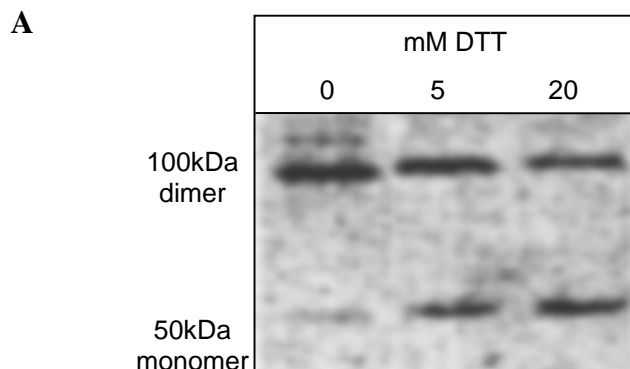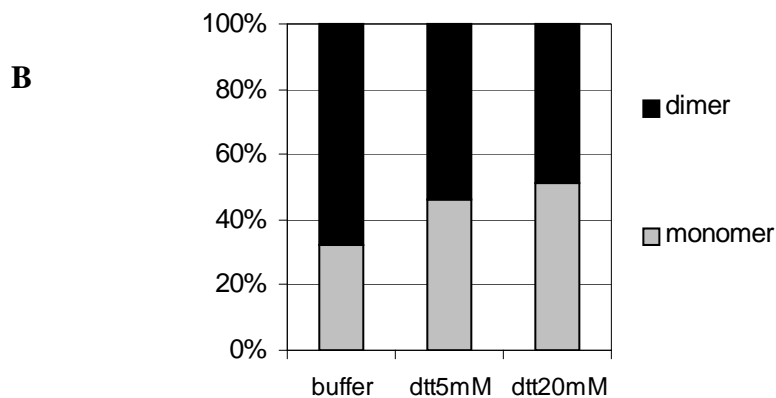
**Figure 4.** DTT feeding to *Arabidopsis* leaf discs. Leaf discs were collected in the darkness at the end of night and incubated for 1h in media containing 2mM Mes buffer, pH 6.5 (control) or 2mM Mes buffer with 5 or 20mM DTT. **A**, western blot; **B**, percentage of protein bands density calculated with Scion Image software.

It was shown by Fu et al. (1998) that dimerisation of AGPB leads to inactivation of AGPase by changing the kinetic properties of the enzyme leading to an increase of the $K_m$(ATP) and decrease of the sensitivity to activation by 3PGA. One of the questions asked in the present work was whether dimerisation of the leaf AGPase is also accompanied by changes in AGPase activity. Fully maturated *Arabidopsis* leaves were harvested at the end of the night (in the dark) and at the end of the day (in the light) and the activity in forward direction was measured using HPLC. Appearance of the monomer in *Arabidopsis* leaves during the day was accompanied by an increase in AGPase activity when assayed in absence of 3PGA. In the presence of saturating amounts of 3PGA the activity in both, light and dark kept leaves, was increased. The 3PGA effect overcomes redox-activation and leads to a change in the kinetic properties such rather than a change of the Vmax activity (Fig. 5 A). Illumination led to a marked increase of the affinity of AGPase for ATP in the absence of 3PGA, which could be overcome at high 3PGA concentrations (Fig. 5 B– C). The sensitivity to activation by 3PGA was also changed. Whereas AGPase from illuminated leaves achieved significant activities in the absence of 3PGA and was stimulated 4- to 10-fold by 3PGA depending on the ATP level, activity of AGPase from leaves collected at the end of the night was very low in the absence of 3PGA

and was stimulated 10-fold by 3PGA in the presence of high ATP and up to 25-fold in the presence of low ATP levels (Fig. 5 D).
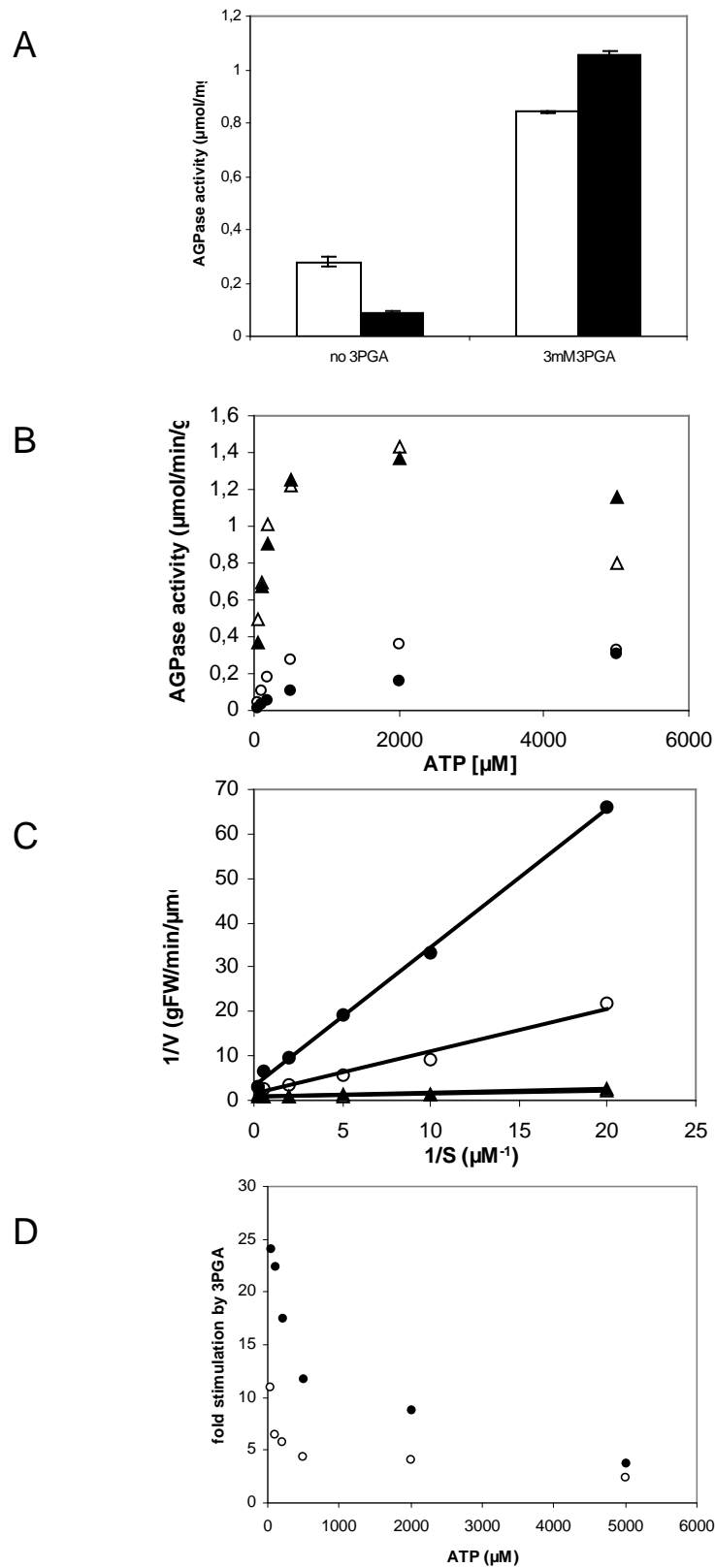


**Figure 5.** Increased monomerization of AGPB in the light leads to a change in the kinetic properties of AGPase. **A**, AGPase activity in presence of 0 or 3 mM 3PGA in presence of 1.5

mM ATP and 1.5 mM G1P in *Arabidopsis* leaves harvested during the end of the night (■) or the second half of the day (□). **B**, ATP substrate saturation curves of AGPase from *Arabidopsis* leaves harvested at the end of the day (▽, ○) and end of the night (▼, ●) assayed in presence of no (○, ●) or 3 mM 3PGA (▽, ▼) and 1.5 mM G1P. **C**, Lineweaver-Burk presentation of the data in B. **D**, The activation factor by 3 mM 3PGA compared with 0 mM 3PGA for the day (□) and the night sample (■). Leaves were taken from 8-week-old plants.

### 3.1.3.   Rapid Changes in AGPB Dimerisation as a Response to Light/Dark Transitions

There are two possible explanations for the observed changes: the appearance of AGPB monomer during the day in leaves could be due to illumination or it could be an indirect effect of, for example, increased sugars levels in leaves in the light, in analogy to the situation in potato tubers (Tiessen et al., 2002). To check if there are rapid light-dependent changes in AGPB monomerisation, we darkened pre-illuminated *Arabidopsis* plants. This treatment was chosen because it leads to an abrupt change in photosynthesis, whereas illumination leads only to slow changes due to the need to induce photosynthesis and increase stomatal conductance.

*Arabidopsis* plants were illuminated for 6.5 h, samples were taken in the light, the remaining plants were darkened, and samples taken after 6, 15, and 60 minutes. In the light, a small proportion of AGPB was monomerised (Fig. 6 A; see also Fig. 3). After darkening, the monomer decreased within 6 min and almost totally vanished within 15 minutes. Sugar levels were measured in the same leaf material (Fig. 6 B). There were no significant changes in sucrose, glucose and fructose levels in the first 6 min and only small changes after 60 minutes of darkening.

**B**
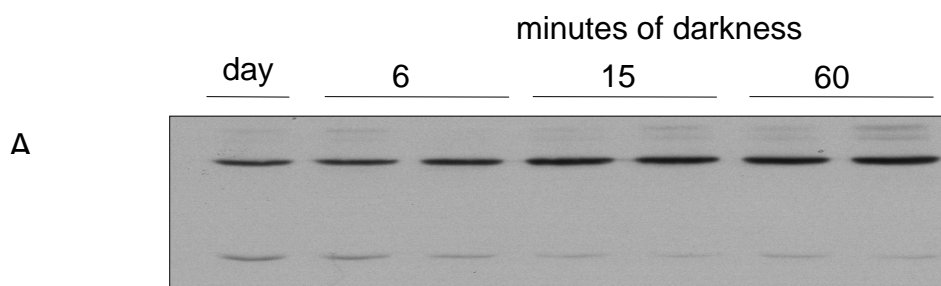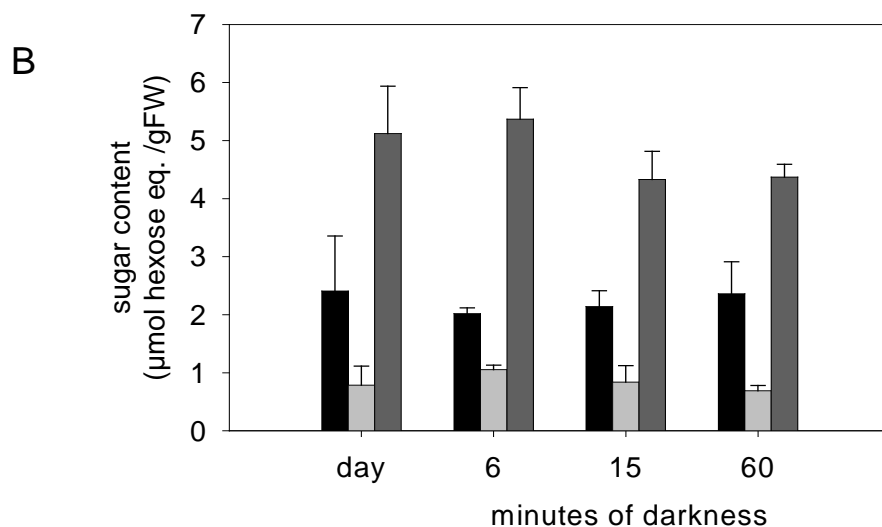
**Figure 6.** Darkening rapidly reverses the light-dependent monomerization of AGPB. **A**, Non-reducing western blot of leaf samples of 6.5-week-old *Arabidopsis* harvested 6.5 h into the day, and after darkening the plants for 6, 15, and 60 min. **B**, Sugar content in these leaves.

### 3.1.4. Light leads to monomerisation of AGPB in isolated pea chloroplasts

In order to confirm the results obtained in the darkening experiment made on *Arabidopsis* leaves, we investigated the light responses in isolated pea chloroplasts. It is known that chloroplasts do not contain or synthesize sucrose or other sugars, so the influence of them could be excluded. These experiments were carried out with chloroplasts from young pea plants. Pea chloroplasts have the advantage that it is possible to manipulate the adenylate content. Addition of inorganic pyrophosphate (PPi) leads to the loss of adenylates from the chloroplast, which can be than reversed by adding ATP or ADP (Lunn and Douce, 1993).

AGPB was almost completely dimerised when chloroplasts were incubated in the dark with PPi, ATP and 3PGA (Fig. 7 A). After 3 minutes of illumination about 50% of the protein was monomerised, after 6 minutes the conversion proceeded further and almost all the protein was in monomerised form. This paralleled the increase of

plastidial FBPase activity, a representative of the enzyme that are already known to be subject to thioredoxin-mediated light activation in chloroplasts (Fig. 7 B).
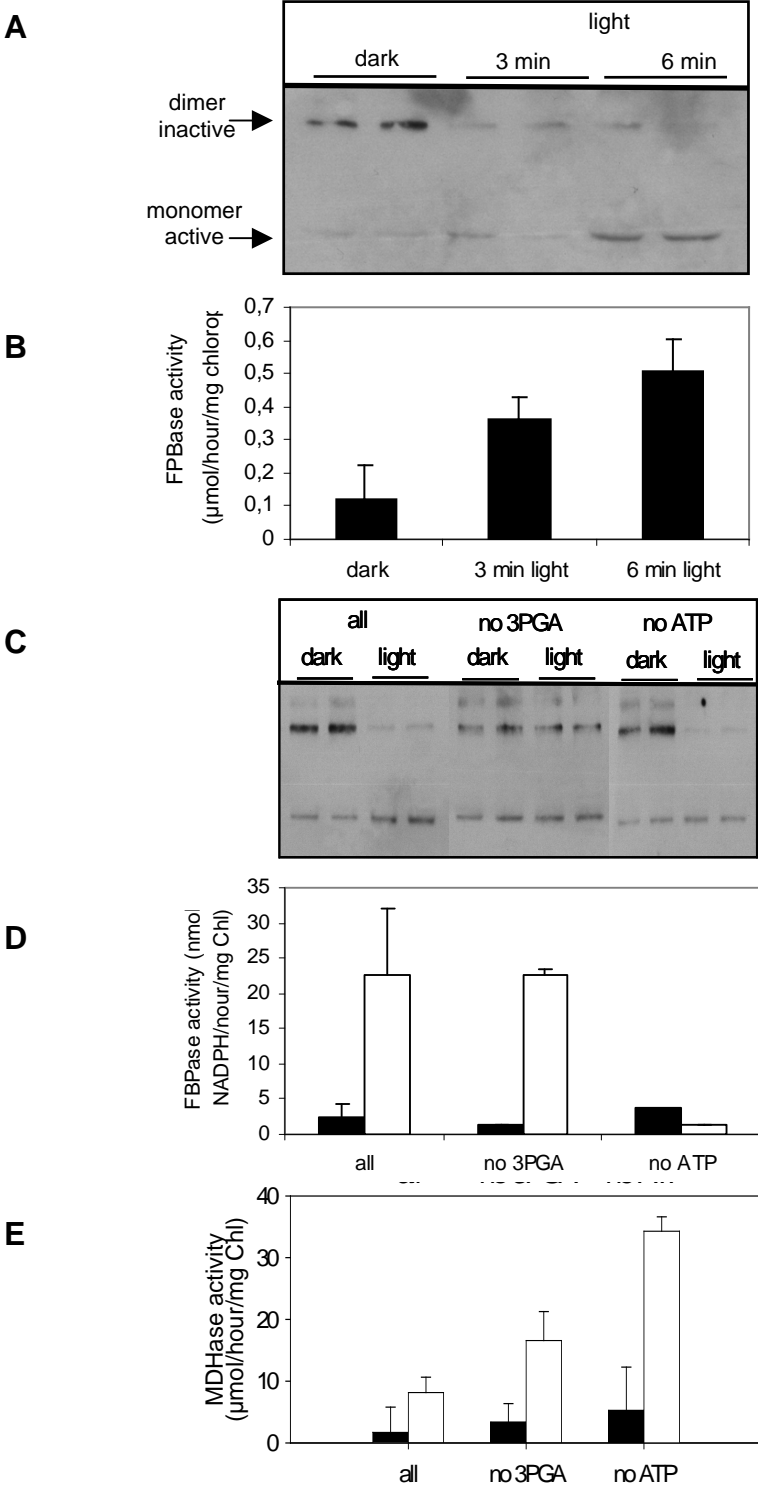


**Figure 7.** Changes in dimerization of AGPB also occur in isolated pea chloroplasts. A and B, Non-reducing western-blot (**A**) and FBPase activity (**B**) of chloroplasts incubated in the

dark, and 3 and 6 min after turning the lamp on in presence of 0.67 mM NaPPi, 1 mM 3PGA, and 1 mM ATP. C through E, Non-reducing western-blot (**C**), FBPase (**D**), and NADP-malate dehydrogenase activity (**E**) in chloroplasts in the dark (■) and after 6 min in the light (□) in the presence of 0.67 mM PPi, 1 mM 3PGA, and 1 mM ATP (all), or when either 3PGA or ATP was left out of the incubation medium.

Experiments made with heterologously expressed potato tuber AGPase have indicated that monomerisation can be mediated by plastidial thioredoxins (Ballicora et al., 2000). In many cases, substrate levels can modulate the activation of enzymes regulated by thioredoxins (Scheibe, 1991; Stitt, 1996; Schürmann and Jacquot, 2000). For example NADP-MDH (NADP-malate dehydrogenase) can be activated by a high NADPH/NADP ratio. Chloroplasts were incubated in full medium and in the absence of 3PGA or ATP. Appearance of monomer in the illuminated samples was suppressed when 3PGA was omitted, but did not change when ATP was omitted (Fig. 7 C). The activity of NADP-MDH as well as plastidial FBPase (fructose-bisphosphatase) was measured and compared with the monomerisation state of AGPase in the same chloroplast samples (Fig. 7 C, D, E). FBPase activity was high in conditions without 3PGA and comparable to the conditions when all substrates were included, but decreased strongly in samples without ATP (Fig. 7 D). This may be because in these conditions photosynthesis by isolated pea chloroplasts is inhibited (data not shown). Activation of NADP-MDH was relatively low in full medium, increased when 3PGA was omitted and also increased further when ATP was omitted (Fig. 7 E). Both of these conditions will decrease photosynthesis and lead to a higher NADPH/NADP$^+$ ratio which (see references above) activates NADP-MDH.

### 3.1.5.    AGPase activation in chloroplast extracts incubated with thioredoxins

It was shown for potato tuber AGPase (Ballicora et al., 2000) that reduced thioredoxin *m* and *f* from spinach leaves caused monomerisation of AGPB *in vitro*, leading to activation of the enzyme at low concentrations of 3PGA. The activation

was reversed by oxidized thioredoxin. Since it is not clear whether thioredoxins are present in amyloplasts of tubers it remained unclear if this mechanism is active in tubers *in vivo.* In photosynthetic tissues light-dependent reduction of Calvin cycle enzymes is known to be mediated by ferredoxin-thioredoxin system (Scheibe et al., 1991) and there is the possibility that AGPase could be activated in a similar way. Therefore, leaves were used as an experimental system to investigate thioredoxin involvement in AGPase redox-regulation.

Chloroplasts were isolated from 15-days old pea seedlings, lysed by osmotic shock and desalted on Sepharose G-50 columns, before incubation with reduced thioredoxins for 15 minutes (8 A-B). In these extracts the concentrations of AGPase and thioredoxins will be much lower than in the intact stroma. Thioredoxins purified from spinach leaves were provided by Prof. Renate Scheibe (Osnabrück). They were reduced by incubation with DTT in concentrations that were 5 fold higher than the thioredoxin concentration according to Sasaki et al. (1997). Control incubations containing DTT but no thioredoxin are shown for comparison. Stromal proteins were subsequently extracted in non-reducing sample buffer, separated using non-reducing SDS-gels and AGPB detected by immunoblotting as described in Materials and methods section. Thioredoxin *m* (Fig. 8 A) and *f* (Fig. 8 B) led to a partial conversion of AGPB from dimer (100 kDa) to monomer (50 kDa). There was no significant increase in monomerisation in control incubations containing 100μM DTT but no added thioredoxin. Higher levels of DTT did lead to monomerisation of AGPase, presumably acting via endogenous thioredoxins in the stromal extract.

To investigate whether changes in monomerisation were causally related to changes in activity, the stromal protein fraction incubated with 20 μM of thioredoxin *f* was used to measure AGPase activity in the ADPGlc synthesis direction using an enzymatic assay. Thioredoxin *f* led to an increase of up to 3-fold in AGPase activity, due to a marked increase in the sensitivity of the enzyme to activation by 3PGA (Fig. 8 C).
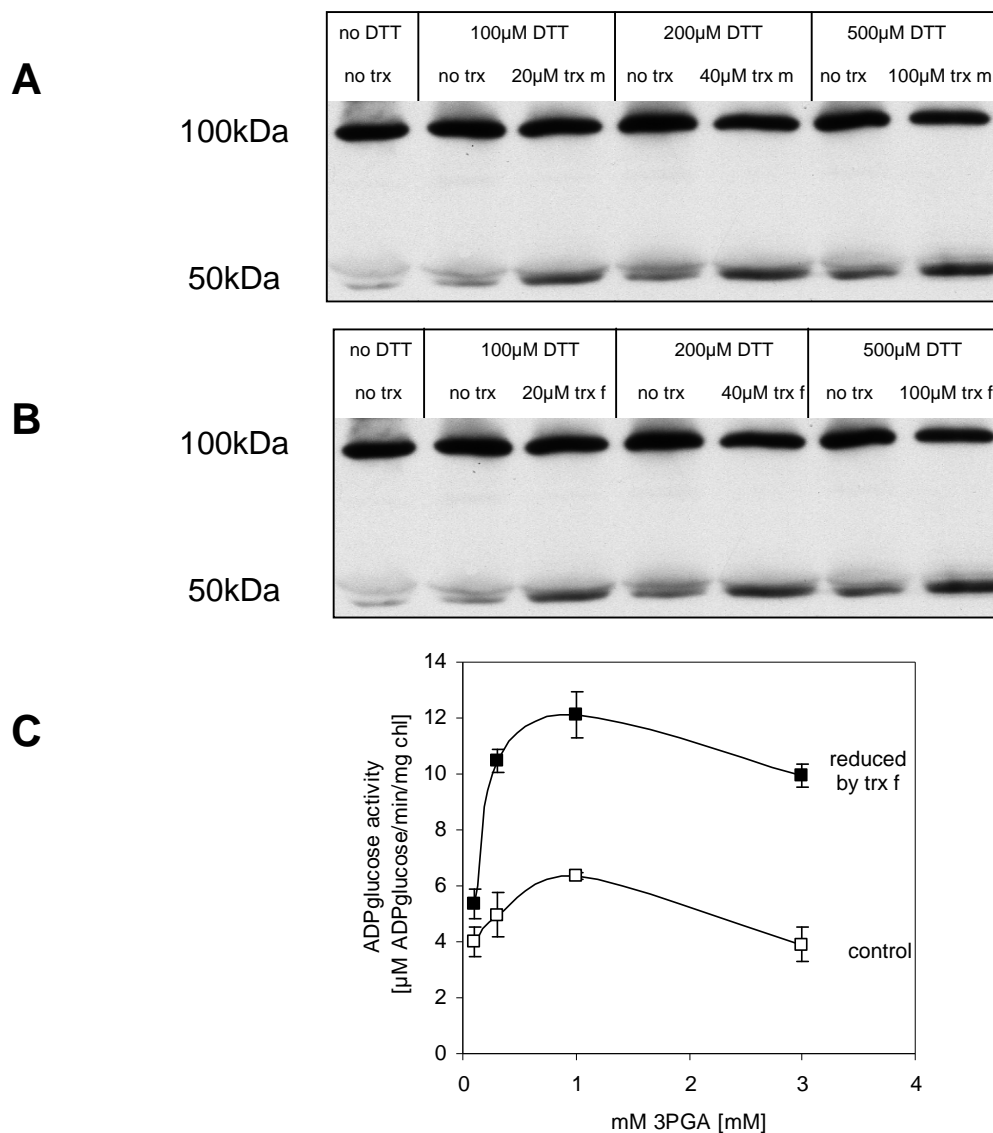
**Figure 8.** ADP-glucose pyrophosphorylase (AGPase) from pea-leaf chloroplasts is redox-activated by thioredoxins. Chloroplasts were prepared from pea leaves as in Hendriks et al. (2003), lysed by osmotic shock and subsequently desalted on Sepharose-50 columns, before incubation with reduced thioredoxins for 15 min. (**A-B**) Stromal proteins were subsequently extracted in non-reducing sample buffer, separated using non-reducing SDS-gels and AGPB detected by immunoblotting according to Hendriks et al. (2003). Thioredoxin *f* (**A**) and *m* (**B**) led to a partial conversion of AGPB from dimer (100 kD) to monomer (50 KD). (**C**) To investigate whether changes in monomerisation were causally related to changes in activity, the stromal protein fraction incubated with 20 µM of thioredoxin *f* was used to measure AGPase activity in the ADPGlc synthesis direction using an enzymatic test according to Hendriks et al. (2003). Thioredoxin *f* led to an increase of up to 3-fold in AGPase activity, due to a marked increase in the sensitivity of the enzyme to activation by 3PGA (**C**). Thioredoxin *f* and *m* purified from spinach leaves were kindly provided by Prof. Renate Scheibe (Osnabrück). They were reduced by incubation with DTT in concentrations

that were 5 fold higher than the thioredoxin concentration according to Sasaki et al. (1997). Control incubations containing DTT but no thioredoxin are shown for comparison.

### 3.1.6.    Conclusions

The results provide evidence that AGPase is subject to posttranslational redox regulation in leaves *in vivo*. This involves reversible interconversion between a less active form in which AGPB is present as a dimer and an active form in which AGPB exists as monomers. The shift from dimer to a monomer is mediated by thioredoxins and accompanied by an increase in AGPase activity. The increase of AGPase activity involved a change in the kinetic properties, including increased affinity for ATP and altered sensitivity to regulation by 3PGA. AGPase is redox-activated in response to a light-dependent signal. Evidence for this is provided by two independent experiments: First, AGPB monomerisation decreases rapidly after darkening of wild type plants even though sugar levels do not change and second AGPB monomerisation increases rapidly after illumination of isolated chloroplasts.

# 3.2.    AGPase is activated by posttranslational redox-modification in response to sugars in leaves

While the experiments presented above show that light leads to monomerisation and activation of leaf AGPase, in the next set of experiments it was asked whether sugars can also increase AGPB monomerisation and whether they can act as an independent signal. To manipulate sugar levels in the leaf tissue, short and long term incubations of *Arabidopsis* leaves in different sugars solutions in the dark were performed. To investigate candidate components that may link reductive activation of AGPase to sugars, leaves of transgenic *Arabidopsis* plants with increased and decreased T6P contents were analysed. It was shown by Schluepmann et al. (2003) that the metabolic precursor of trehalose, T6P (trehalose 6-phosphate), is essential for the regulation of sugar utilization in *Arabidopsis* but the sites of action remained unresolved. To provide further evidence for the involvement of T6P in AGPase redox-

modification, chloroplasts from pea leaves were isolated and incubated with T6P and a variety of other metabolites.

### 3.2.1. Supplying sugars to leaves during the night leads to conversion of AGPB from a dimer to a monomer and to increased rates of starch synthesis

Leaves from 8-week-old *Arabidopsis* plants were fed via their petioles with buffer and varying concentrations of sucrose in the dark during their natural night (0, 50, 100, and 200 mM). For comparison, leaves harvested from intact plants at the start (end of day [ED]) and at the end of the experiment (end of night [EN]) are also presented (Fig. 9).

Western blot in non-reducing conditions shows that AGPB is converted into the monomeric form by supplying sugars to leaves in the dark. The proportion of AGPB converted to monomer in response to sucrose feeding in the dark was similar to that seen in the light under normal growth conditions. In the leaf extracts from the end of the night and in leaves incubated in buffer without sucrose, AGPB was almost completely dimerised (Fig. 9 A).

Sucrose feeding led to a progressive increase in sugars levels (Fig. 9 B), but 3PGA remained unaltered (Fig. 9 E). There was also an increase of starch levels (Fig. 9 C). This might be due to stimulation of starch synthesis or to slower breakdown of starch during the 13-h dark treatment. To measure the rate of starch synthesis, the unlabeled sucrose was spiked with high specific activity [$^{14}$C]-glucose. The rate of starch synthesis was calculated by dividing the label incorporated into starch by the specific activity of the hexose phosphate pool (for a detailed discussion of this approach, see Geigenberger et al., 1997; and Materials and methods section). Sucrose feeding led to a concentration-dependent stimulation of starch synthesis in the dark (Fig. 9 D).
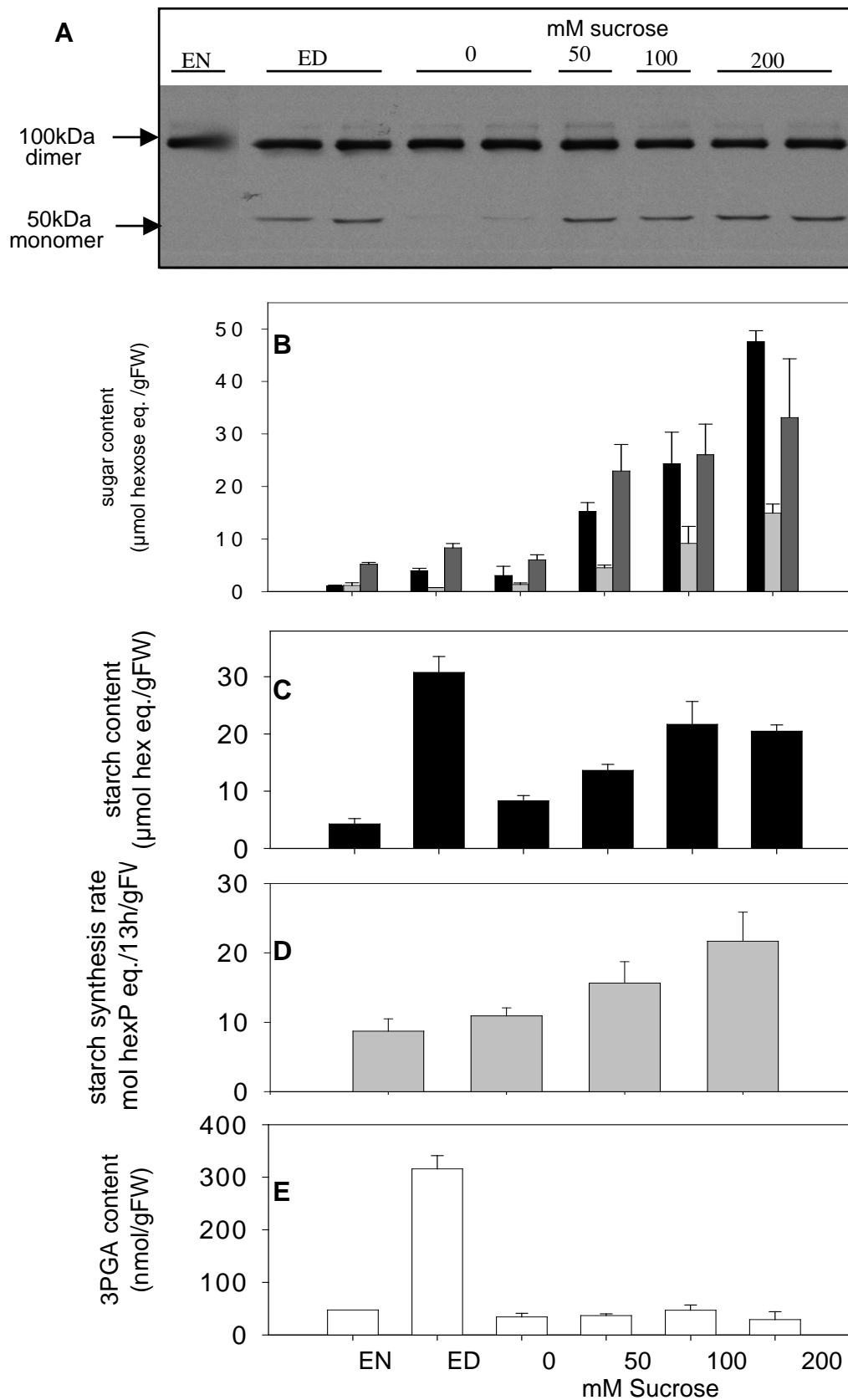
**Figure 9.** AGPB is converted into the monomeric form by supplying sugars to leaves in the dark. Leaves from 8-week-old *Arabidopsis* plants were fed via their petioles with buffer and varying concentrations of sucrose in the dark during their natural night (0, 50, 100, and 200

mM). For comparison, leaves harvested from intact plants at the start (end of day [**ED**]) and at the end of the experiment (end of night [**EN**]) are also shown. **A**, Non-reducing western blot of AGPB; **B**, sugar content; **C**, starch content; **D**, In parallel incubations, high specific [U-$^{14}$C]-glucose was supplied together with the various concentrations of unlabeled sucrose, to investigate the rate of starch synthesis. **E**, 3PGA content.

Further evidence showing that sugars increase monomerisation of AGPB was provided by Janneke Hendriks (Hendriks et al., 2003), who carried out a set of experiments comparing diurnal changes in wild-type Col 0 and the *pgm* mutant (Caspar et al., 1986). Obtained results are shown in Appendix (Fig. 36, 37).

### 3.2.2. Short-term feeding of sucrose or trehalose to leaf discs leads to increased AGPB monomerisation without affecting hexose-P levels and NADPH/NADP$^+$ ratio

A time-course of the effect of sucrose feeding on AGPB monomerisation using leaf discs in the dark was investigated. Discs were also incubated with sorbitol or trehalose as further controls, since both of these sugars are not metabolised to starch. .

*Arabidopsis* leaves were harvested at the end of the night and discs incubated in media containing 100mM sorbitol, 100 mM sucrose or 100 mM trehalose for 15, 30 minutes and 3 hours (Fig. 10). In discs from leaves sampled at the end of the night and incubated with sorbitol as osmotic control, AGPB was completely dimerised (Fig. 10 A) and sucrose and starch were relatively low (Fig. 10 B). Sucrose feeding led to partial monomerisation of AGPB (Fig. 10 I) and increased starch content after 3 hours (Fig. 10 J) without changing the glucose 6-phosphate level (Fig. 10 K). Surprisingly, trehalose incubation resulted in similar but even more rapid changes in AGPB monomerisation and starch accumulation as observed after sucrose feeding. AGPB monomers were visible already after 15 minutes (Fig. 10 A) and increased further during the course of the experiment. Trehalose led to a significant increase in starch levels within 30 min which increased further in the next 2.5 h. Crucially, this

was not accompanied by any increase in sucrose (Fig. 10 F), other soluble sugars (figure legend) or phosphorylated intermediates (Fig. 10 G).
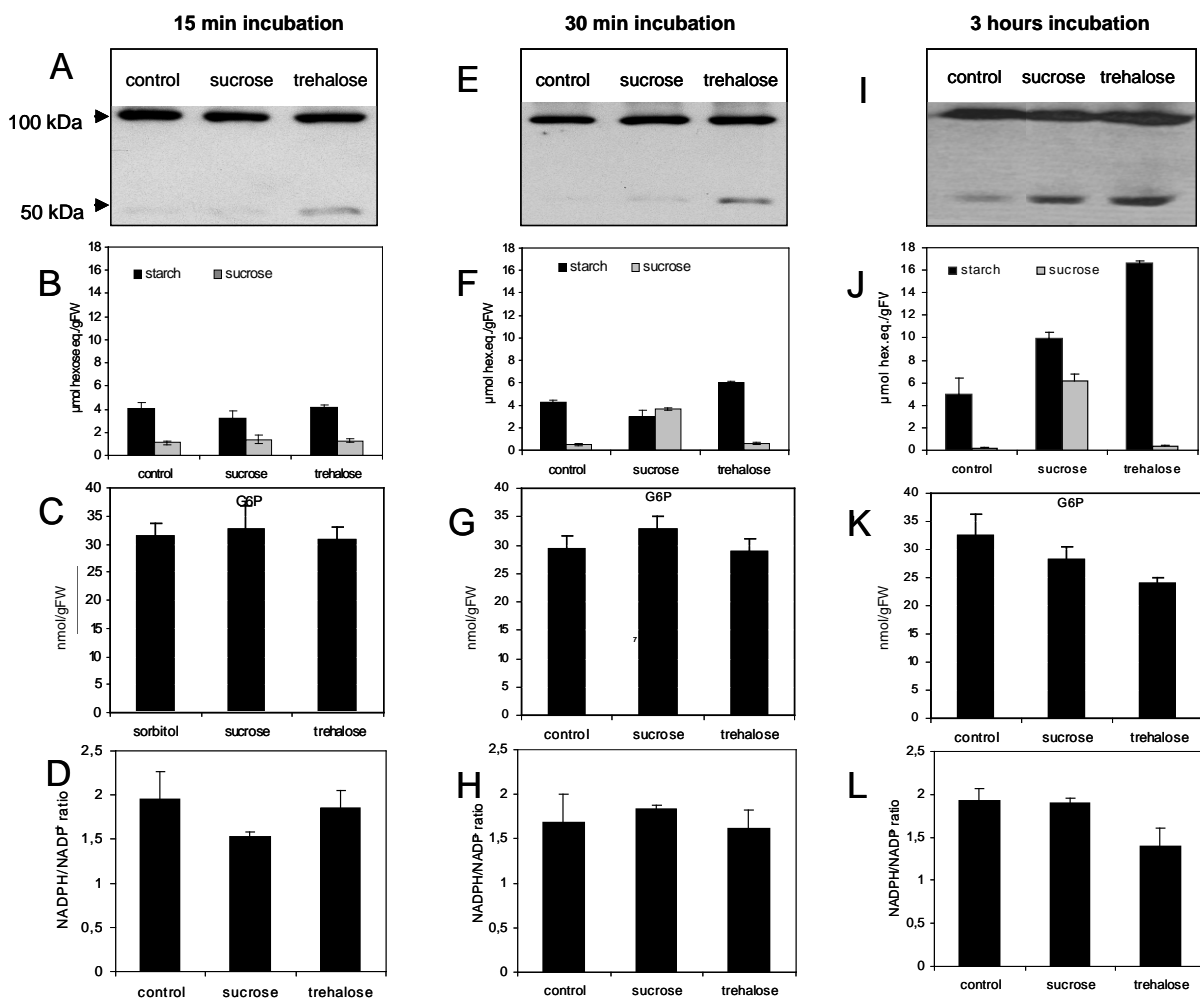


**Figure 10.** Short-term feeding of trehalose to leaf discs of *Arabidopsis* plants during the end of the night leads to redox-activation of AGPase and increased starch levels without changing the NADPH/NADP$^+$ ratio. Leaf discs were incubated for 15 min (**A, B, C, D**), 30 min (**E, F, G, H**) and 3h (**I, J, K, L**) in 100 mM sorbitol, sucrose or trehalose, before samples were frozen to analyse (**A, E, I**) AGPase redox-activation by measuring AGPB monomerisation using non-reducing SDS gels, (**B, F, J**) starch and sucrose levels, (**C, G, K**) hexose-phosphate levels and (**D, H, L**) NADPH/NADP$^+$ ratios. Results are means $\pm$ SE ($n$ = 3-4). For comparison, the NADPH/NADP$^+$ ratios measured in intact leaves at the end of the night were 1.44 +/- 0.35 (mean +/- SE, n = 3), while leaves that were sampled upon illumination had a 2-fold higher NADPH/NADP$^+$ ratio (3.3 +/- 0.2, mean +/- SE, n = 3).

The reduction state of the NADPH/NADP$^+$ system is indicative for the redox-potential of the plastid. To investigate, whether sugars lead to redox-activation of AGPase via an increase in the reduction state of the NADPH/NADP$^+$ system, NADPH and NADP+ levels were measured in sugars-incubated leaves and leaf tissue sampled in parallel. The NADPH/NADP$^+$ ratio in control incubations using sorbitol ranged between 1.7-1.9 and did not change significantly with incubation time (Fig. 10 D, H, L). It was also similar to the value measured in intact leaves at the end of the night at the start of the incubation experiment (1.44+/- 0.35, mean +/- SE, n= 3), while leaves that were sampled upon illumination had a 2-fold higher NADPH/NADP+ ratio (3.29 +/- 0.21, mean +/- SE, n= 3). Crucially, neither feeding of trehalose nor sucrose led to significant changes in the NADPH/NADP$^+$ ratio, compared to osmotic control. This shows that the effect of feeding trehalose or sucrose on AGPase redox-activation was not attributable to an overall increase in plastidial redox-status.

### 3.2.3. Manipulation of T6P metabolism in transgenic Arabidopsis plants leads to AGPase redox-modification and changes in starch content

Resuts above show that trehalose feeding leads to an increase in AGPase redox-activation which was more rapid than in response to sucrose feeding. Interestingly, recent studies of Schluepmann et al. (2004) provided evidence that trehalose and sucrose feeding lead to increased trehalose 6-phosphate (T6P) levels in *Arabidopsis*. The time-course of the increase in T6P after adding sucrose or trehalose resembled activation of AGPase in experiments presented in Fig. 10 above. This prompted the hypothesis that T6P might be involved in the activation of AGPase. T6P is an intermediate in trehalose metabolism pathway and has been proposed to be a regulator of sugar utilisation in yeast and plants (Eastmond and Graham, 2003; Gacendo and Flores, 2004). It is synthesised by T6P-synthase (TPS) from UDPglucose and glucose-6-phosphate. T6P is subsequently dephosphorylated by T6P-phosphorylase (TPP) to trehalose. Trehalose can be hydrolyzed into two glucoses by trehalase (TRE) (Fig. 11).
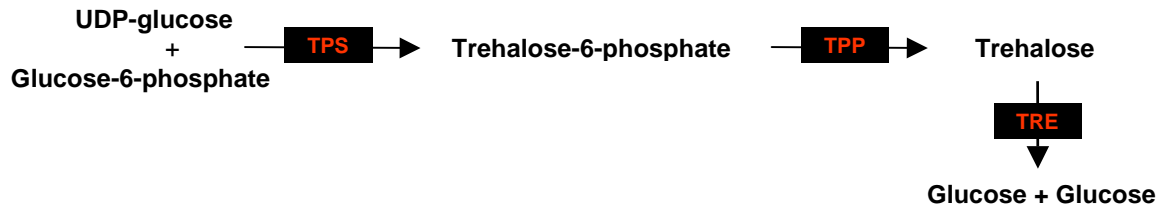
**Figure 11.** Trehalose metabolism pathway. **TPS** – trehalose-6-phosohate synthase, **TPP** – trehalose-6-phosphate phosphatase, **TRE** - trehalase.

To provide genetic evidence that the stimulation of starch synthesis after trehalose feeding was linked to T6P, leaves of transgenic *Arabidopsis* plants with T6P levels increased by expression of *E.coli* TPS or decreased by expression of *E.coli* TPP in the cytosol, but with no changes in trehalose content (Schluepmann et al. 2003), were analysed. Leaves of wild-type (Wt) and of transgenic *Arabidopsis* plants were sampled in the middle of the day and in the middle of the night to analyse AGPase redox-state and carbohydrate levels. Compared to wild-type, TPS-expressing plants showed increased redox-activation of AGPase reflected by increased monomerisation of the AGPB subunits (Fig. 12 A) and increased starch accumulation in leaves during the day (Fig. 12 B), whereas TPP plants showed the opposite. This indicates that increase in the level of cytosolic T6P is involved in activation of AGPase. Changes in redox-activation of AGPase occurred independently of changes in the levels of sugars (Fig. 12 C-E). This indicates that changes in T6P override changes in metabolite levels, which is consistent with T6P acting downstream of sucrose (see below for more data).
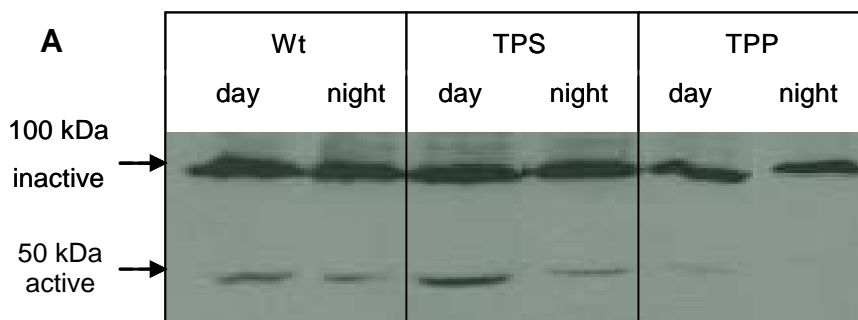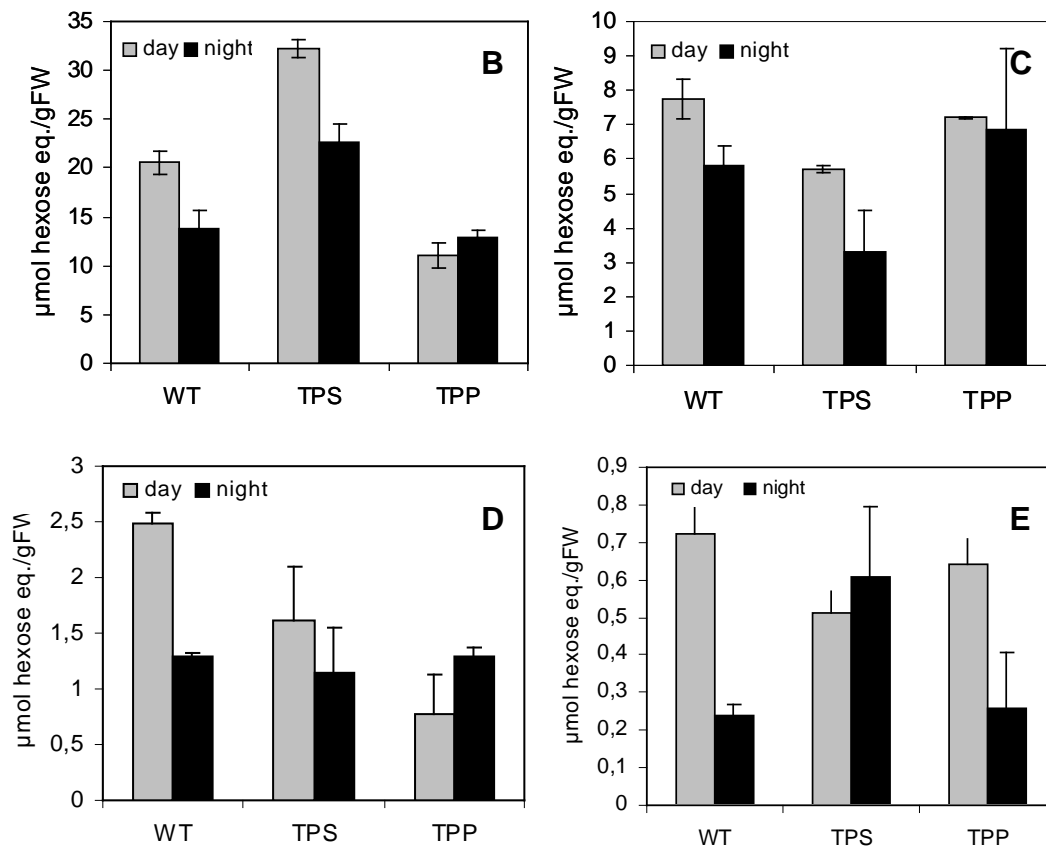
**Figure 12.** Transgenic *Arabidopsis* plants show a crucial role of trehalose-6-phosphate (T6P) in regulating starch synthesis via post-translational redox-activation of AGPase. Leaves of wild-type (Wt) and of transgenic *Arabidopsis* plants overexpressing T6P synthase (TPS) or T6P phosphatase (TPP) in their cytosol were sampled in the middle of the day and in the middle of the night to analyse (**A**) redox-activation of AGPase by measuring AGPB monomerisation using non-reductive SDS gels, (**B**) starch levels, (**C**) sucrose levels, (**D**) glucose levels, (**E**) fructose levels. Results are means $\pm$ SE ($n = 4$).

To provide additional evidence that T6P is involved in AGPase redox-regulation, leaves of transgenic tobacco (*Nicotiana tabacum*) plants with T6P levels increased by expression of *E.coli* TPS or decreased by expression of *E.coli* TPP in the cytosol, but with no changes in trehalose content (Pellny et al., 2004), were analysed. Tobacco leaves of Wt and transformants were sampled and analysed in the same way as *Arabidopsis* leaves. Similarly to *Arabidopsis*, TPS-expressing tobacco plants showed increased redox-monomerisation of AGPB subunits (Fig. 13 A) and increased starch accumulation during the day (Fig. 13 B), whereas TPP-expressing plants showed

decreased activation of AGPase, and a higher sucrose level (Fig. 13 C), compared to Wt. This indicates that T6P is also involved in AGPase activation in tobacco plants.
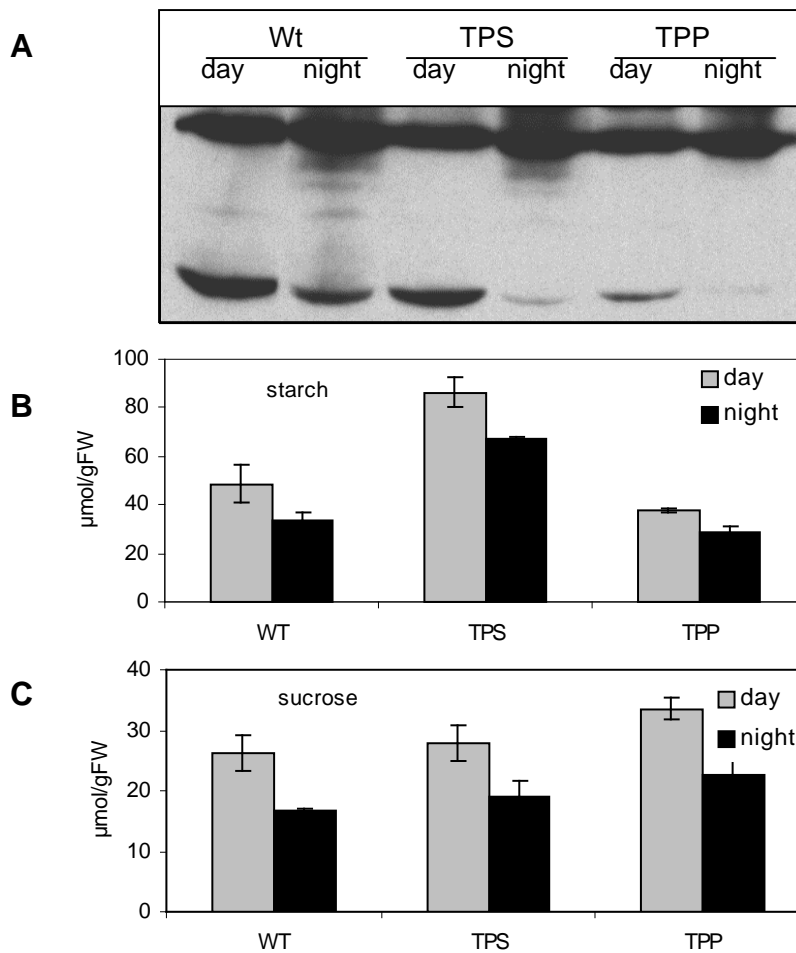


**Figure 13.** Transgenic tobacco (*Nicotiana tobaccum*) plants show that T6P plays a crucial role in regulating starch synthesis via post-translational redox-activation of AGPase. Leaves of wild-type (Wt) and of transgenic tobacco plants overexpressing T6P synthase (TPS) or T6P phosphatase (TPP) in their cytosol were sampled in the middle of the day and in the middle of the night to analyse (**A**) redox-activation of AGPase by measuring AGPB monomerisation using non-reductive SDS gels, (**B**) starch levels, (**C**) sucrose levels. Results are means ± SE (*n* = 4).

### 3.2.4. Sucrose and trehalose dependent redox-activation of AGPase is attenuated in transgenic plants expressing TPP in the cytosol

To further investigate whether T6P is a component of the sucrose-dependent AGPase redox-activation in leaves, mature leaves of *Arabidopsis* wild type and the TPS and TPP overexpressing transformants were fed with solutions containing 100mM sorbitol (osmotic control) or 100 mM sucrose over-night via the transpiration stream (13 hours), and then harvested to measure AGPB dimerisation state and the content of sugars, sugar phosphates and 3PGA.
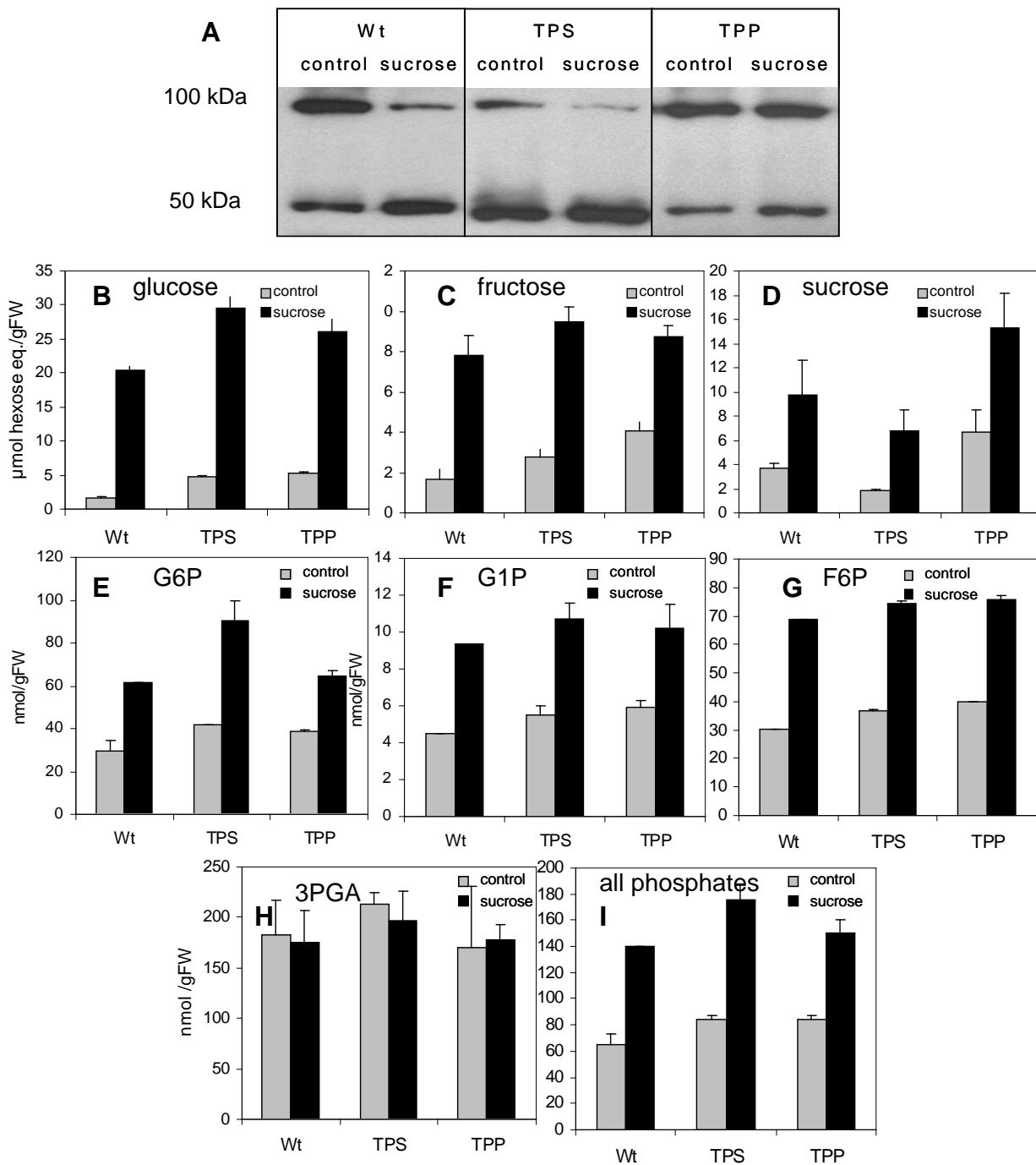


**Figure 14.** Over night sucrose feeding to Wt, TPS and TPP leaves leads to redox-activation of AGPase and increased starch levels. **A**, western blot in non-reducing conditions; **B**

glucose; **C** fructose; **D** sucrose; **E** G6P; **F** G1P; **G** F6P; **H** 3PGA; **I**, sume of hexose phosphates. Results are means $\pm$ SE ($n = 4$).

While AGPB monomerisation was clearly increased by feeding sucrose to leaves of wild-type and TPS expressing plants, this response was attenuated in TPP-expressing plants (Fig. 14 A). In inspecting these Figures, it is helpful to look at the behaviour or the dimer at 100 kDa in combination with that of the monomer at 50 kDa. Crucially, redox-activation of AGPase remained low in leaves of TPP plants despite a strong increase in internal sucrose level, which was larger than in wild-type or TPS plants (Fig. 14 D). This indicates that T6P is a crucial component in the sucrose-dependent redox-activation of AGPase in leaves.

Levels of hexose phosphates and 3PGA (Fig. 14 E - I) were also measured. Hexose phosphates showed increase in samples fed with sucrose, but 3PGA remained unchanged.

To check whether also short-tem feeding of sugars lead to a similar response, sucrose and trehalose were supplied to leaves of Wt and plants with decreased T6P content. Fully maturate leaves of *Arabidopsis* wild type and TPP transformants were harvested at the end of the night, and discs were taken and incubated with solutions containing 100mM sorbitol (control), trehalose or sucrose for 1 hour in the dark. Western blot in non-reducing conditions was performed to compare the influence of different sugars on AGPB monomerisation. In leaf discs incubated with sorbitol, AGPB was almost completely dimerised. Incubation with trehalose or sucrose of Wt leaf discs led to increased monomerisation of AGPase. AGPB dimerisation from TPP leaf discs was not affected by sucrose or trehalose feeding (Fig.15 A). Soluble sugars (glucose, fructose and sucrose) were measured in the same leaf discs (Fig. 15 B, C, D). In Wt there were observed strong increases in sugars levels in sucrose incubated samples, but not in sorbitol and trehalose fed discs. In TPP transformant soluble sugars content rose more strongly than in wild-type in sucrose and in trehalose fed discs.
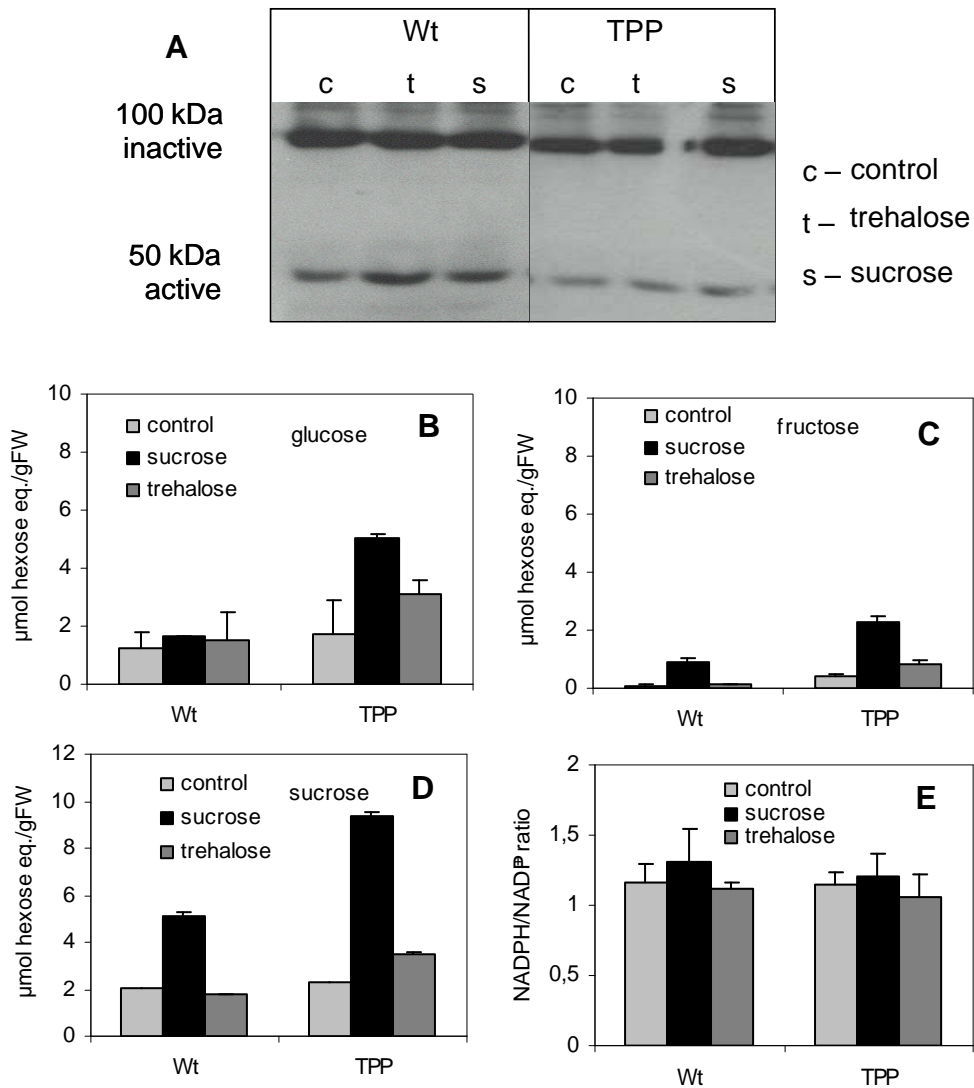
**Figure 15.** Short-term feeding of trehalose and sucrose to leaf discs of *Arabidopsis* plants during the end of the night leads to redox-activation of AGPase without changing the NADPH/NADP$^+$ ratio. Leaf discs were incubated for 1h in 100 mM sorbitol, sucrose or trehalose, before samples were frozen to analyse (**A**) AGPase redox-activation by measuring AGPB monomerisation using non-reducing SDS gels, (**B**) glucose, (**C**) fructose, (**D**) sucrose and (**E**) NADPH/NADP$^+$ ratios. Results are means $\pm$ SE (*n* = 3-4).

NADPH/NADP$^+$ ratio measurement showed that the redox state of the cell was not changed by supply of sucrose or trehalose, either in the Wt or the transformant (Fig. 15 E). This shows that neither the effect of feeding trehalose or sucrose nor the effect

of TPP expression on AGPase redox-activation was due to increase in plastidial redox-status.

### 3.2.5. Short-term feeding of trehalose-6-phosphate to intact purified chloroplasts leads to a specific increase in redox-activation of AGPase without changing the plastidial redox-state

To provide biochemical evidence that T6P promotes redox-activation of AGPase independently of other cytosolic factors, it was investigated whether addition of T6P to isolated chloroplasts leads to post-translational activation of AGPase. Chloroplasts were prepared from pea leaves and purified by centrifugation in Percoll. The intactness of the chloroplasts was verified by microscopy and by comparing the rate of ferricyanide-dependent uncoupled electron transport in chloroplast preparations before and after osmotic shock (Lilley et al, 1975). This assay is based upon the inability of the ferricyanide to cross the chloroplast envelope and to react with the electron transport system in the thylakoid membranes. Ferricyanide reduction occurs only when ruptured chloroplasts are present in the preparation. The degree of integrity of the chloroplast preparation is assessed by comparing the rate of ferricyanide reduction upon illumination before and after osmotic shock of the chloroplasts. Intactness of isolated pea chloroplasts was above 90%.

The chloroplasts were incubated in the dark in the presence of low level of the reductant DTT (0.3mM), to allow partial reduction of the stromal ferredoxin/NADPH and ferredoxin/thioredoxin systems. In these conditions, there was insignificant monoerisation of AGPB.  Addition of 100 μM T6P in the medium significantly increased AGPB monomerisation (Fig. 16 A) and AGPase activity (Fig. 16 B). This occurred within 15 min. No significant changes were observed after feeding trehalose, sucrose, sucrose 6-phosphate or trehalose together with Pi to chloroplasts as controls (Fig. 17 A).  In a further control experiment, T6P was added to chloroplasets that had been disrupted by osmotic shock. While adding T6P to intact chloroplasts resulted in an increase in AGPase activity assayed in the presence of 3PGA, disruption of chloroplasts prevented T6P activation (Fig. 16 D).
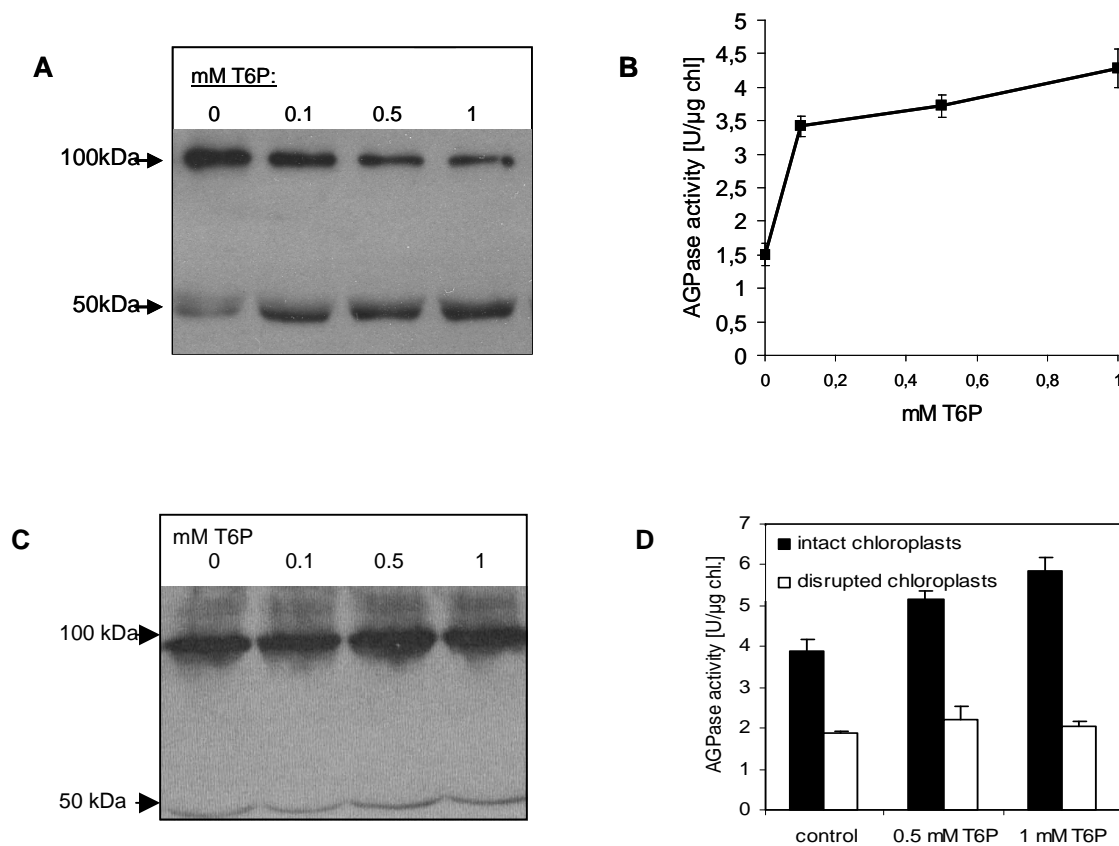
**Figure 16.** Changes in AGPB monomerisation (**A, C**) and AGPase activity (**B, D**) after feeding different concentrations of T6P to intact (**B**) isolated chloroplasts or to disrupted chloroplasts (**D**) for 15 min. After isolation chloroplasts were diluted 1:5 with resuspention buffer or disrupted by incubation in hypo-osmotic medium and incubated for 15 minutes with different T6P concentrations and in the presence of 3PGA and low DTT concentration (0,3 mM) (**B**) or without DTT (**C**) and used for gel electrophoresis (1 mM 3PGA) or AGPase assay (3 mM 3PGA). AGPase activity is shown as mean ± SE (n= 2).
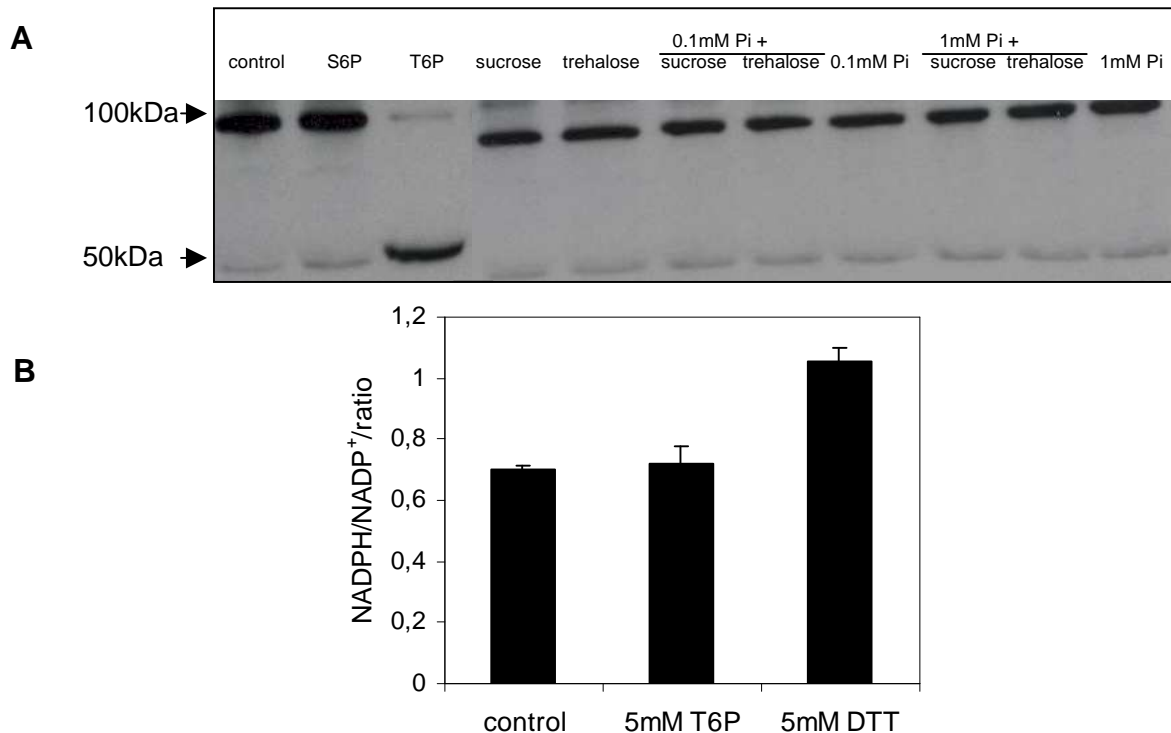
**Figure 17. A,** Changes in AGPB monomerisation after incubation of intact isolated chloroplsts in the presence of 1 mM 3-phosphoglycerate (3PGA) and 0.3 mM DTT for 15 minutes. Control – chloroplasts in resuspention buffer; concentrations used for incubations – 5 mM S6P (sucrose-6-phosphate), 5 mM T6P, 10 mM sucrose, 10 mM trehalose, 0.1 or 1 mM Pi alone or in mixture with 10 mM sucrose or trehalose; **B,** NADPH/NADP$^+$ ratio measured in intact pea chloroplasts. Chloroplasts after isolation were diluted 5 times with resuspention buffer (see Materials and Methods, Chloroplasts Isolation), incubated for 15 minutes in the presence of 1 mM 3PGA with 5 mM T6P or 5 mM DTT or only in buffer (control) and used for assay as described.

The above experiments were carried out with a low level of DTT in the medium to provide a source of reducing equivalents for the plastid. When experiments were performed using chloroplasts in the absence of any external source of reductants, T6P did not affect redox-activation of AGPase (Fig. 16 C). Feeding DTT to chloroplasts led to an increase in the NADPH/NADP$^+$ ratio, which however, on its own, did not lead to a significant increase in monomerisation of AGPB (see above). The response to T6P did not involve further changes in the reduction state of the chloroplast NADP-system (Fig. 17 B). This is consistent with T6P acting by modulating redox-transfer from ferredoxin or NADPH via thioredoxins to AGPase, rather than increasing the plastidial redox-state.

### 3.2.6. Conclusions

The results show that AGPase is subject to posttranslational redox regulation in leaves *in vivo* in response to sugars, which can act as an independent signal. Evidence is provided by experiments showing that AGPase monomerisation is increased by supplying exogenous sugars to wild type leaves in the dark. Over night sucrose feeding leads to conversion of AGPB from a dimer to a monomer and to increased rates of starch synthesis without any significant changes in the level of allosterical activator 3PGA. The effect of sucrose feeding on AGPB redox-state and starch synthesis is visible after 3 hours.

The results also implicate T6P in the signal transduction chain. After trehalose feeding changes in AGPB redox state were much quicker; AGPB was partially monomerised already after 15 minutes and starch increased within 30 minutes without any increase in the levels of other sugars or glycolytic intermediates. Trehalose feeding is known to lead to an increase of T6P. NADPH/NADP$^+$ ratio in the tissue was not affected by supply of any tested sugars showing that the effect of sugars feeding on AGPB redox-state was not dependent on changes in plastidial redox-status in leaves. Transgenic *Arabidopsis* and tobacco plants with increased and decreased T6P levels in cytosol provide evidence that T6P rather than trehalose leads to redox-activation of AGPase. TPS-overexpressing plants showed increased redox-activation of AGPase and higher starch levels during the day, while TPP-overexpressing plants showed the opposite, when compared to Wt. Changes in AGPase redox-activation occurred independently of changes in the levels of sugars, sugar-phosphates and NADPH/NADP$^+$ ratio. This indicates that T6P overrides light-dependent changes in metabolite levels and redox-status. While AGPase was activated and starch levels increased by feeding sucrose to leaves of wild-type and TPS-overexpressing plants, this response was attenuated in TPP-overexpressing plants, providing evidence that T6P links AGPase redox-activation to sucrose. Incubation with T6P clearly increased AGPB monomerisation and AGPase activity within 15 minutes, while no significant changes were observed after sucrose or trehalose feeding to the chloroplasts. This shows that the effects of sucrose and trehalose on AGPase redox-activation in leaf tissue require T6P synthesis in the cytosol.

### *3.3.* Redox-regulation of ACCase is involved in regulating lipid synthesis in response to day/night changes and sugars in *Arabidopsis* leaves

The results presented so far show that starch synthesis is redox-regulated in response to light and sugars in *Arabidopsis* leaves. In the following it was investigated, whether lipid synthesis is regulated in a similar way. It has been proposed for chloroplasts of photosynthesising leaves that fatty acid synthesis is subject to redox-regulation in response to light. The evidence for this is two-fold: First, it has been measured that lipid synthesis shows diurnal changes that were suggested to be due to light-regulation of ACCase (Sauer and Heise, 1983). Second, Kozaki et al. (2001) showed that ACCase activity can be activated via reduction of a disulfide bridge between $\alpha$ and $\beta$ subunits of carboxyltransferase in response to DTT and thioredoxin. By investigating partially purified ACCase from isolated pea chloroplasts it was shown that the shifted bands of about 230 kDa were observed in both the $\alpha$ and $\beta$ polypeptides in the absence of 2-mercaptoethanol, suggesting that both the $\alpha$ and $\beta$ polypeptides formed an S-S bond. The disulfide form of about 230 kDa was more abundant in chloroplasts isolated from dark-adapted plants than from light-adapted plants. The reverse was found for thiol forms of about 98 kDa for $\alpha$ polypeptide and 90 kDa for $\beta$ polypeptide, suggesting that a light-dependent reduction of the disulfide bond occurred.

In the following experiments it was investigated whether similar mechanism occur *in-planta*. To do this, a protocol was established to measure changes in *in-vivo* ACCase redox-activation state in leaves during day and night. Further, the reductant DTT was fed to leaf discs in the night to investigate whether ACCase activation state, the rate of lipid synthesis and acetyl-CoA content can be altered *in situ*. In another set of experiments sucrose was supplied to detached leaves in the dark in order to check if sugars can act on reductive activation of ACCase and lipid synthesis independently of light.

### 3.3.1. Establishing of a protocol to measure diurnal changes in reductive activation of ACCase in Arabidopsis Leaves

In the present work enzyme assays to detect changes in the kinetic properties of ACCase in response to DTT incubation were developed. ACCase was quickly extracted from leaves at the end of the night and activity was measured at various acetyl-CoA substrate concentrations in the absence or presence of 5mM DTT.

Redox-activation led to a marked increase of affinity for acetyl-CoA. The $K_m$ (acetyl-CoA) without reductant was 250µM, and when DTT was added it decreased 3-fold (Fig. 18 A). From this results, two assay conditions were selected showing activity under selective conditions (Vsel) using 200 µM acetyl-CoA without adding DTT and under reduced conditions (Vred) using 200 µM acetyl-CoA and 5 mM DTT. The ratio between Vsel and Vred was used as an estimate of the redox-activation state of the enzyme. Some attempts to show ACCase dimerisation state on westerns were made, but they were not successful since the ACC antibodies that were used by Kozaki et al. (2001) did not work specifically or strongly with our material (data not shown).

Fatty acid synthesis in pea chloroplasts is increased in the light and decreased in the dark (Sauer and Heise, 1983). To investigate whether reductive activation of ACCase is involved in this response, leaves collected at the end of the day (in light) and at the end of the night (in darkness) were frozen in liquid nitrogen, rapidly extracted and activity measured using the two different assay conditions (see above). The redox-activation state of the enzyme was calculated as the ratio between Vsel and Vred.

In leaves collected at the end of the night and assayed without addition of DTT (Vsel) ACCase activation was about 3-fold lower than in leaves that were harvested in the light period. When the ACCase activity was assayed with DTT included (Vred), activity was similar in samples taken from leaves in day or night. (Fig. 18 B, C). This shows that ACCase is redox-activated during dark-light transitions in *Arabidopsis* leaves. The ratio of Vsel/Vred increased almost 3-fold between the night and the day.
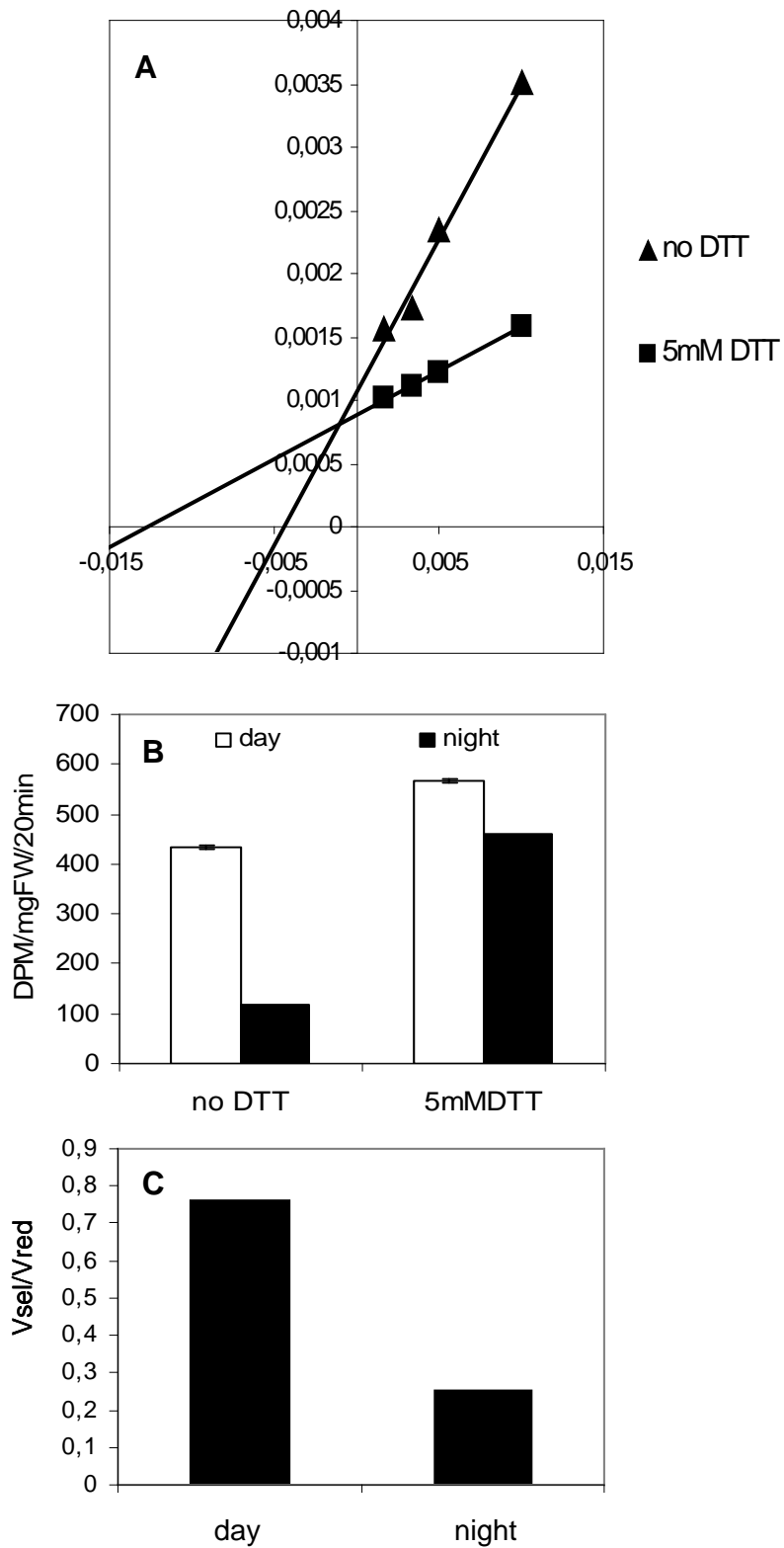
**Figure 18.** Increase of ACCase activity in the light leads to a change in its kinetic properties.
Lineveaver-Burk presentation of acetyl-CoA substrate saturation curves of ACCase in leaves
harvested at the end of the night and assayed in absence or presence of 5mM DTT(**A**),

ACCase activity in presence of 0 or 5mM DTT(**B**), redox-activation during the light-period in *Arabidopsis* leaves (**C**).

### 3.3.2. *Feeding of the reductant DTT to leaves leads to increased rates of lipid synthesis by increasing redox-activation of ACCase*

#### *3.3.2.1. Increase in carbon flux to lipids in response to DTT feeding to leaves in the dark*

To investigate the rate of lipid synthesis, high specific activity [U-$^{14}$C]-glucose was fed to *Arabidopsis* leaf discs. Leaf discs were taken at the end of the night and incubated in the medium containing $^{14}$C-glucose without reducing agent or with different concentrations of DTT (5–50 mM) for one hour in the dark. The DTT was used to obtain an activation of ACCase *in vivo*. Fig. 19 shows the uptake of $^{14}$C-glucose into the discs and the percentage that was metabolised to other components. DTT feeding resulted in a slight inhibition of glucose uptake, which might be attributable to inhibition of transport proteins by DTT. In response to 5 mM DTT feeding, the percentage of carbon that was allocated to starch, lipids, amino acids, proteins and cell wall increased, whereas the percent incorporated into sucrose decreased. The changes were especially marked between 0 and 20mM DTT.

| | BUFFER | | | 5mM DTT | | | 20mM DTT | | | 30mM DTT | | | 50mM DTT | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Uptake [µmol hex.*gFW-1*h-1] | 1762 | | | 1370 | | | 1523 | | | 1340 | | | 1312 | | |
| | % of total uptake | | SE | % of total uptake | | SE | % of total uptake | | SE | % of total uptake | | SE | % of total uptake | | SE |
| LIPIDS | 23,30 | ± | 1,05 | 29,63 | ± | 1,24 | 45,41 | ± | 4,16 | 45,01 | ± | 1,84 | 44,97 | ± | 1,55 |
| STARCH | 12,97 | ± | 0,66 | 14,33 | ± | 1,47 | 9,24 | ± | 1,64 | 6,27 | ± | 0,47 | 3,12 | ± | 0,42 |
| PROTEINS | 4,07 | ± | 1,09 | 7,64 | ± | 1,80 | 2,24 | ± | 0,55 | 5,73 | ± | 1,51 | 3,69 | ± | 0,65 |
| AMINO ACIDS | 25,19 | ± | 1,26 | 36,67 | ± | 1,95 | 30,40 | ± | 2,88 | 33,88 | ± | 2,54 | 37,40 | ± | 1,11 |
| ORGANIC ACIDS | 5,75 | ± | 0,58 | 5,77 | ± | 0,71 | 6,67 | ± | 2,58 | 3,02 | ± | 0,57 | 2,93 | ± | 0,22 |
| CELL WALL | 0,14 | ± | 0,02 | 0,56 | ± | 0,13 | 1,72 | ± | 0,49 | 1,30 | ± | 0,37 | 1,22 | ± | 0,27 |
| SUCROSE | 28,58 | ± | 2,08 | 5,40 | ± | 0,51 | 4,32 | ± | 0,53 | 4,79 | ± | 0,22 | 6,67 | ± | 0,46 |

**Figure 19.** % of label converted to lipids, starch, proteins, amino acids, organic acids, cell wall and sucrose calculated as % of total uptake after DTT feeding to *Arabidopsis* leaf discs in the dark. Specific activity of hexose phosphate pool: control – 326.23 +/- 64.82, 5mM DTT

– 123.79 +/- 26.34, 20mM DTT – 207.17 +/- 48.62, 30mM DTT – 121.62 +/- 30.98, 50mM DTT – 604.81 +/- 92.69 nmol hexose-units/gFW. SE calculated for n=4.

The decreased rate of ^{14}C-glucose uptake could have led to isotopic dilution of internal glucose pools. Therefore, carbon flux into lipids and other cellular components (starch, proteins, amino acids, cell wall, sucrose) was calculated by dividing the incorporated label by the specific activity of the hexose-phosphate pool (as described in Geigenberger et al., 1997) and results presented in Fig. 20.
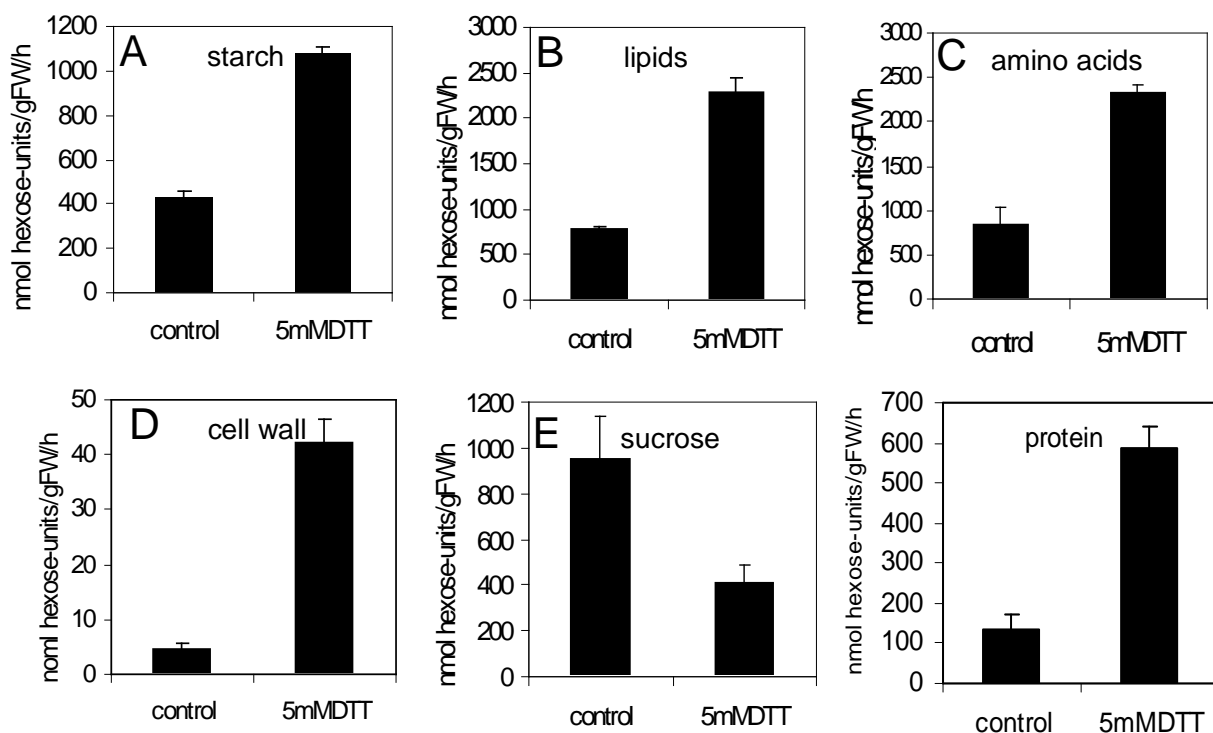


**Figure 20.** DTT feeding to leaf discs stimulates metabolism of ^{14}C-glucose tracer to starch(**A**), lipids (**B**), amino acids (**C**), cell wall (**D)**, protein (**F**) whereas metabolism to sucrose (**E**) is decreased. Specific activity of hexose phosphate pool: control – 326.23 +/- 64.82 nmol hexose-units/gFW, 5mM DTT – 123.79 +/- 26.34 nmol hexose-units/gFW. Fluxes were calculated by dividing the radioactivity incorporated in different fractions by the specific activity of hexose phosphate pool. SE calculated for n=4.

In response to 5 mM DTT, carbon fluxes into lipids, starch, proteins, amino acids and cell wall increased by 2-, 3-, 2- and 10-fold, whereas flux to sucrose decreased.

Separation of label incorporated in lipid fraction by TLC was used to investigate which class of lipids was affected by different concentrations of DTT. Lipid extract was separated into six fractions: phospholipids, pigments, diacylglycerol (DAG), triacylglycerol (TAG), fatty acids and hydrosolubles. The experiment showed that mainly polar lipids were increased (Fig. 21).
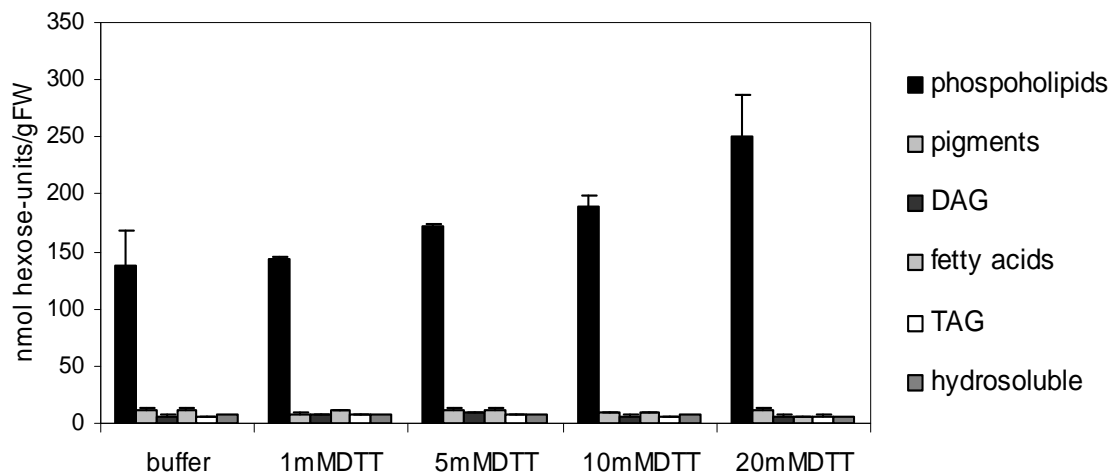


**Figure 21.** Increase in phospholipids fraction after DTT feeding. **DAG** – diacylglycerol, **TAG** – triacylglycerol. Carbon flux into lipids was calculated by dividing the incorporated label by the specific activity of hexose-phosphate pool (Geigenberger et al., 1997) as presented on Fig. 20. Lipid extract was separated into fractions using TLC (Thin Layer Chromatography).

### 3.3.2.2. DTT feeding leads to an increase in ACCase activation state in leaves in the dark

To investigate whether stimulation of lipid synthesis in response to DTT feeding to leaves in the dark was due to redox-activation of ACCase, extracts were prepared and ACCase activity measured in two different assay conditions: with Vred including 5mM DTT and Vsel omitting DTT (Fig. 22 A, B). The redox-activation state was calculated as the ratio between Vsel and Vred (Fig. 22 C). DTT feeding to leaves led to a 2-fold increase in redox-activation state of the ACCase, corresponding to the increased rate of lipid synthesis.
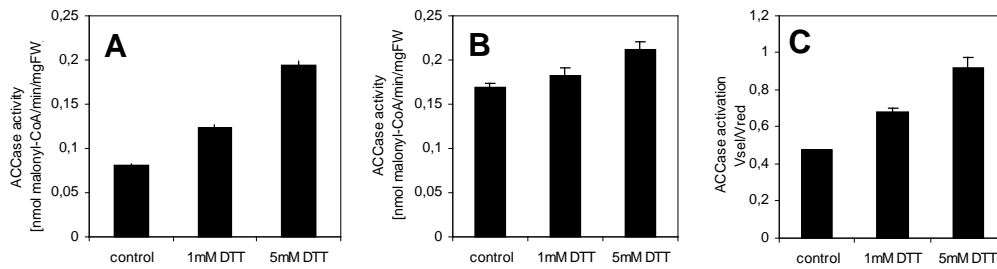
**Figure 22.** DTT feeding to leaf discs leads to an increase in the redox-activation state of ACCase in-situ. ACCase activity in two different assay conditions with Vsel at 200 mM acetyl-CoA and omitting reductant (**A)** and Vred at 200 mM acetyl-CoA including 5mM DTT (**B**). The redox-activation state is calculated as the ratio between Vsel and Vred (**C**).

### 3.3.2.3. Changes in metabolite levels after DTT feeding to leaves

To investigate whether increase in the ACCase redox-activation and lipid synthesis rate were accompanied by any changes substrates, levels of phosphoenolpyruvate (PEP), pyruvate, acetyl-CoA, hexose-phosphates and 3PGA were measured spectrophotometrically after extraction of leaves that had been incubated with 5mM DTT. There was a 30% decrease in the level of acetyl-CoA, which is the immediate substrate of ACCase. There was a small and non-significant decrease in the levels of pyruvate and PEP, which are the two preceeding intermediates in glycolysis, but not change of hexose-phosphates, 3PGA and glycerol-3-phosphate contents (Fig. 23).
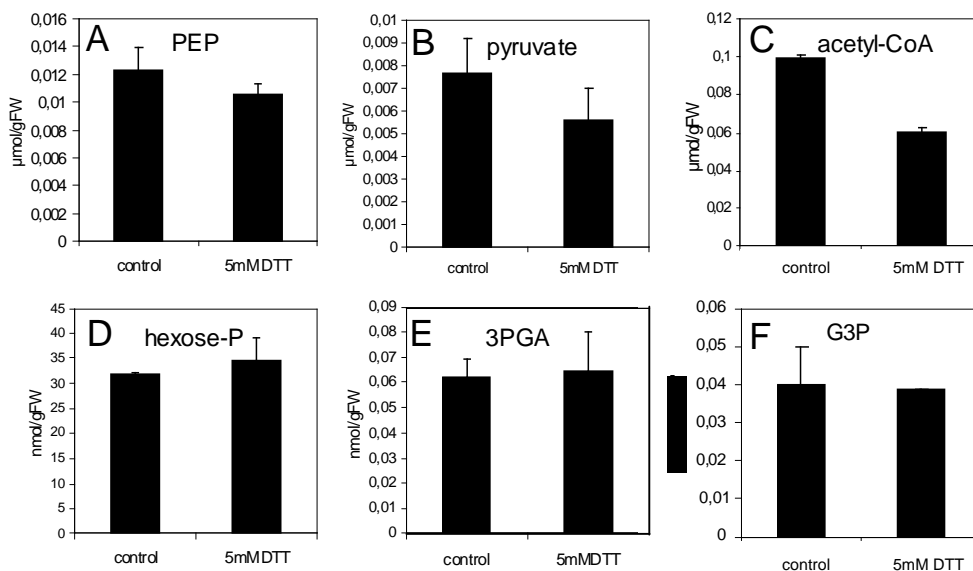


**Figure 23.** Metabolite levels measured enzymatically in samples after DTT incubation. The levels of phosphoenolpyruvate (**A**), pyruvate (**B**) do not change significantly. The level of

acetyl-CoA (the immediate substrate of ACCase) (**C**) decreases. The levels of hexose phosphates (**D**) 3PGA (**E**) and G3P (**F**) do not change.

### 3.3.3. Sugar feeding to leaves in the dark lead to an increase in ACCase activation state and the rate of lipid synthesis which is accompanied by a decrease of acetyl-CoA content

#### 3.3.3.1. Increase in flux to lipids by sucrose feeding

Sucrose feeding leads to redox-activation of AGPase and increased starch synthesis in leaves (see chapter 3.2 of this thesis). The following experiments investigated whether sucrose also leads to an increase in the rate of lipid synthesis and whether this is accompanied by an increase in the redox-activation of ACCase.

*Arabidopsis* leaves were collected at the end of the day and supplied via the transpiration stream with zero, 50 or 100 mM sucrose in the medium over night for 13h. High specific activity [U-$^{14}$C]-glucose (concentration < 1 mM) was included in all three treatments to allow flux determination. Carbon flux into lipids and other cellular components (starch, proteins, amino acids, organic acids, cell wall) was calculated by dividing the incorporated label by the specific activity of hexose-phosphate pool (Geigenberger et al., 1997). The results are presented in fig. 24. There was a simulation of carbon flux into lipids (5-8 fold), starch (3-4 fold), proteins (3-fold), amino acids (2 fold), organic acids and cell wall (3-5 fold) in leaf discs incubated with 50 and 100 mM sucrose. The pattern of changes was similar to that obtained for DTT feeding, which also shown significant increase (from about 3 to 8 fold) of fluxes to lipids, starch, amino acids, proteins and cell wall.
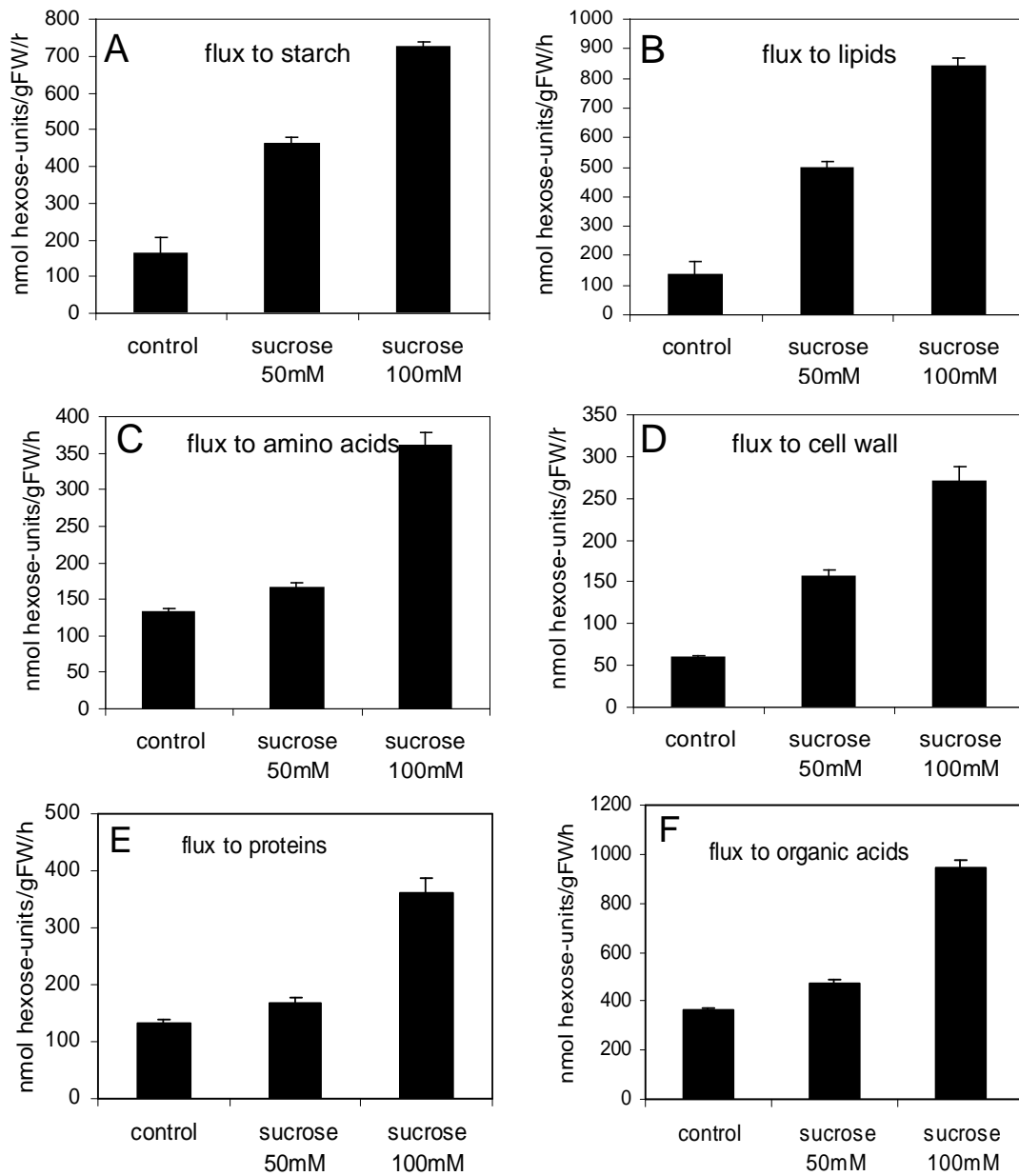
**Figure 24.** Metabolism of ^{14}C-glucose tracer to starch (**A**), lipids (**B**), amino acids (**C**), cell wall (**D**), proteins (**E**) and organic acids (**F**) was measured in parallel in samples after sucrose feeding, the data show absolute fluxes corrected by the specific activity of the hexose-P pool. Specific activity for hexose-P: control – 2.22+/-0.01, 50mM sucrose – 1.33+/-0.05, 100mM sucrose – 0.8+/-0.02 nmol hexose-units/gFW/h. SE calculated for n=4.

### 3.3.3.2. Increase in ACCase activation state after sucrose feeding

To investigate whether stimulation of lipid synthesis in response to sucrose was due to redox-activation of ACCase, leaves were harvested, extracted and ACCase activity was measured in two different assay conditions: with Vred including 5mM DTT and Vsel omitting DTT (Fig. 25 A, B). The redox-activation state was calculated as the ratio between Vsel and Vred (Fig. 25 C). Overnight sucrose feeding to leaves led to a progressive increase in redox-activation state of the ACCase with increasing sucrose concentration, corresponding to the increased rate of lipid synthesis.
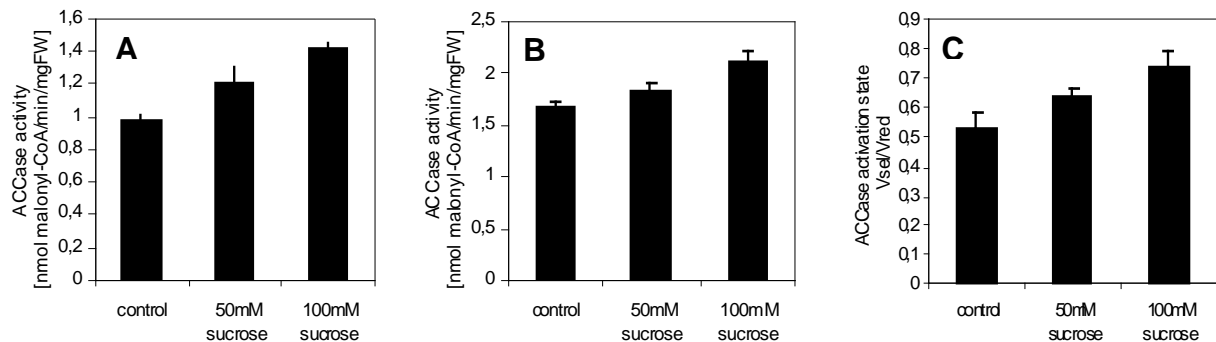


**Figure 25.** Sucrose feeding to leaf discs leads to an increase in the redox-activation state of ACCase in-situ. ACCase activity in two different assay[I] conditions with Vsel omitting reductant (**A)** and Vred including 5mM DTT (**B**). The redox-activation state is calculated as the ratio between Vsel and Vred (**C**).

### 3.3.3.3. Changes in metabolite levels after sucrose feeding

To investigate whether the increase in ACCase redox-activation  and lipid synthesis rate after sugar supply were accompanied by any changes in substrates, levels of PEP, pyruvate, acetyl-CoA, hexose-phosphates and 3PGA were measured spectrophotometrically using *Arabidopsis* leaves. There was a decrease in acetyl-CoA level, indicating more rapid use of ACCase substrate after external sucrose supply (Fig. 26 A). There was also a decrease in PEP and pyruvate (Fig. 26 B, C), whereas 3PGA remained stable (Fig. 26 D). The results indicate that there is a stimulation of synthetic pathways after sucrose

feeding. Interestingly, the level of glycerol 3-phosphate, which together with fatty acids is a component of lipids, increased progressively with the increase of sucrose concentration (Fig. 26 E).
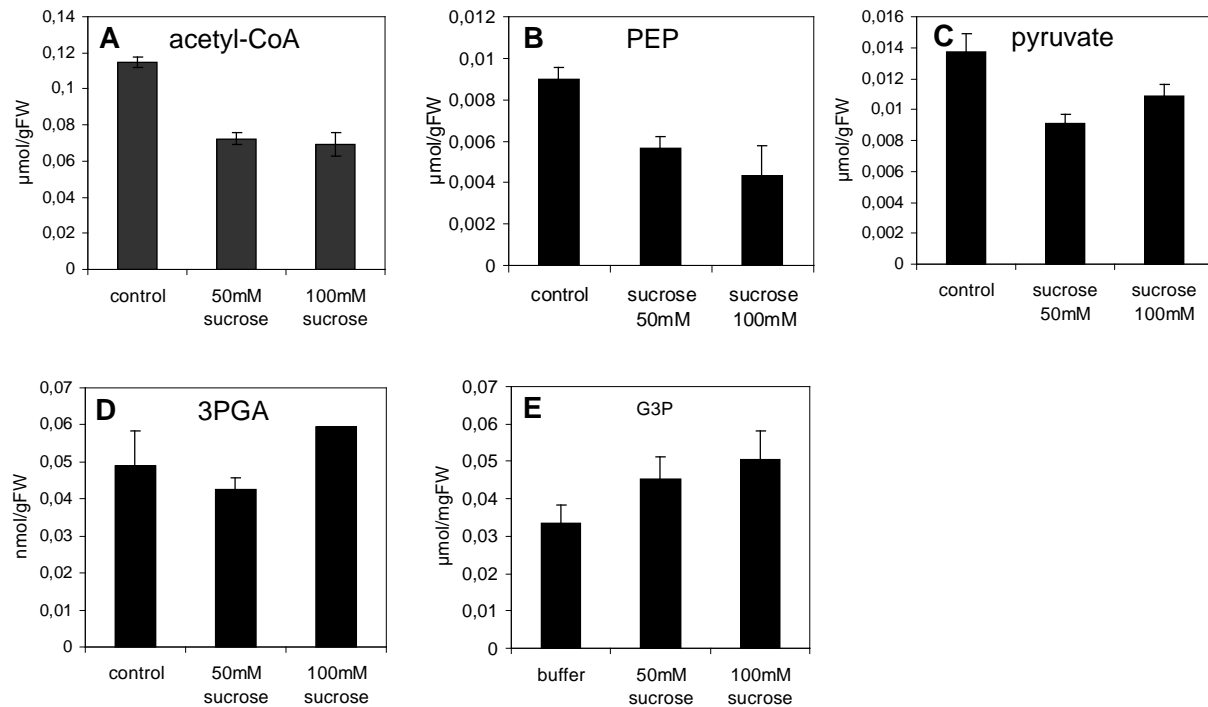


**Figure 26.** Sucrose feeding via the transpiration stream to leaves of Arabidopsis plants leads to decrease of the acetyl-CoA (**A**), phosphoenolpyruvate (**B**), pyruvate (**C**) level, but 3PGA (**D**) does not change and G3P (**E**) increases. SE calculated for n=4.

## 3.3.4. Conclusions

The results provide *in-planta* evidence that lipid synthesis is redox-regulated in response to light and sugars, involving redox-modification of ACCase in leaves. The measurement of ACCase activity in leaf tissue collected at the end of the day and night revealed a 3-fold higher redox-activation state in the day than in the night , which reflected a change in the kinetic properties of the enzyme. Feeding of the reductant DTT to leaf discs in the night led to a 2-8-fold stimulation of flux into starch, lipid, amino acid, proteins and cell wall synthesis and a decrease in sucrose synthesis. Separation of label in lipid showed that mainly polar lipids were increased.

DTT feeding led to an increase in ACCase redox-activation state, corresponding to an increased rate of lipid synthesis and a decrease of acetyl-CoA content. ACCase was also redox-activated in response to sucrose feeding during the night. The increase in ACCase redox- activation state was accompanied by an increase of lipid synthesis and a decrease of acetyl-CoA level. These results show that redox regulation of ACCase provides a mechanism to increase lipid synthesis in reponse to light, and in response to the sugar supply.

## 3.4. Changes in metabolite and transcript profiles in Arabidospis leaves in response to manipulation of the redox-state by feeding DTT

The results presented above show that DTT can be used to alter the NADPH/NADP$^+$ ratio of isolated chloroplast and to induce changes in the activation state of redox-regulated enzymes like AGPase and ACCase when supplied to intact leaf tissue. This indicates that DTT can be used as a tool to manipulate the redox-state of plant tissues. In the following the effect of DTT feeding on metabolite and transcript profiles was investigated in leaf discs incubated in the dark. This systemic approach might allow identification of further redox-regulated sites in leaf metabolism.

### 3.4.1. Metabolic Profiling of Arabidopsis Leaves after DTT Feeding

Leaf discs were taken from *Arabidopsis* plants at the end of the night and incubated in the medium without reducing agent or with 5 mM DTT for one hour in the darkness. After harvest and extraction, metabolite contents were measured and calculated as ratio between DTT and buffer incubated samples. Fig. 27 presents a schematic overview of metabolites placed in metabolic pathways to show which metabolites increase or decrease in response to DTT feeding using a color code. The list of all measured metabolites is presented in Table 1, Appendix, where the extent and significance of the changes is also documented.
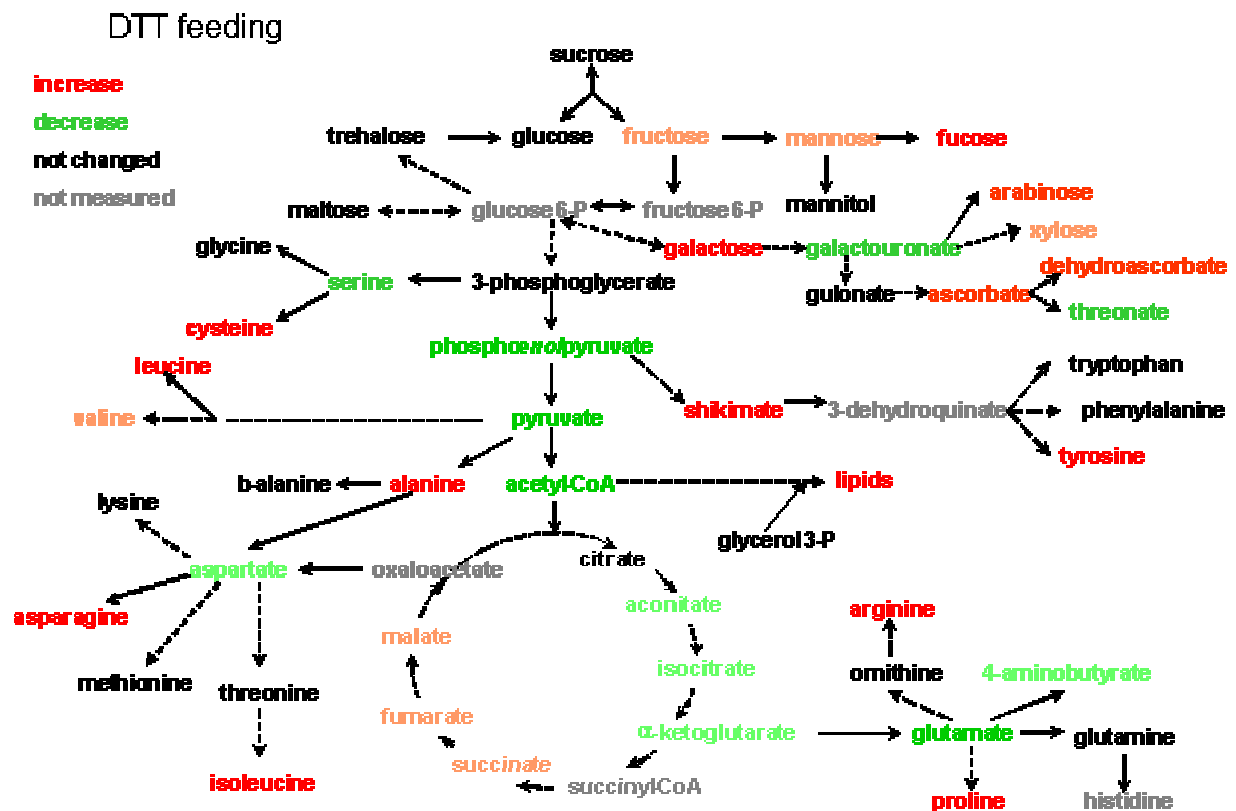
**Figure 27.** Metabolic profiling after incubation of *Arabidopsis* leaf discs in 5 mM DTT. Changes in metabolite levels calculated as ratio between DTT and buffer incubated samples. Not all the data presented on graphs are significant, the increase or decrease over 20% indicated with red or green letters respectively, the increase or decrease over 10% indicated with light red or light green respectively. The list of all data (showing also the significance) is presented in Appendix, Table 1. Measurement is mean of n=5 +/- SE.

DTT led to major changes in the levels of metabolites of central metabolic pathways. There was a decrease in the levels of glycolytic intermediates such asphosphoenolpyruvate and pyruvate, a decrease in the metabolites of the first half of TCA cycle such as aconitate, isocitrate and α-ketoglutarate, while succinate, fumarate and malate slightly increased. This was accompanied by major changes in amino acid levels, such as a decrease in glutamate, aminobutyrate, aspartate and serine, while many other amino acids such as arginine, proline, asparagine, isoleucine, alanine, leucine and cysteines increased. There was also increase in shikimate and the aromatic amino acid tyrosine. Interestingly, DTT feeding did not lead to significant changes in the levels of the major sugar pools such as sucrose or glucose. There were marked changes in the levels of minor sugars that are involved

in ascorbate synthesis and cell wall metabolism. Galactouronate and threonate decreased, but the levels of galactose, arabinose, fucose, ascorbate and dehydroascorbate increased. The changes in metabolite levels were consistent with labelling studies using high-specific activity of $^{14}$C-glucose (see chapter 3.3) that were performed in parallel, revealing an increase in the flux to cell wall, amino acids, proteins and decrease in the flux to sucrose. These results presented in fig. 27 show that a short-term increase in the redox-state also leads to an increased flux of carbon into cell wall synthesis, major respiratory pathways and amino acids.

### 3.4.2. Changes in transcript profile in response to DTT feeding

To investigate the changes in transcriptome of *Arabidopsis* leaves in response to manipulation of redox-status by feeding DTT, Affymetrix ATH1 GeneChip analysis was used. DTT feeding led to a relatively small number (206 of 22817 genes investigated) of changes in transcript levels (Fig. 28, 29 and Table 2 in Appendix). To the genes with altered transcript profiles belong components of the photosynthetic electron transport (several PSII components), mitochondrial electron transport (such as NADH dehydrogenases, ubiquinol-cytochrome-c reductase and uncoupling proteine) uptake of sugars (SUT1), high affinity sulfate and nitrate transporters, amino acid transport and metabolism (amino acid permease, proline transporter, proline oxidase, alanine acetyltransferase), protein folding (calreticulin and calnexin) and disulfide-bond formation (thioredoxin, protein-disulfide-isomerases, disulfide-bond forming proteins), cell-wall metabolism (glucuronosyl transferase, chitinase, endochitinase, pectinesterases, cinnamyl-alcohol dehydrogenase, cinamoyl-CoA reductase, arabinogalactan, beta-glucosidases, beta-1,3-glucanase, glucosyltransferase), disease resistance and oxidative burst (quinon-oxidoreductase) and signaling components (protein kinase APK1, receptor-like kinases, protein phosphatase 2C, Ca2+ transporting ATPase, calmodulin, MYB transcription factor, phosphatidylinositol kinase, ethylene responsive element binding factor).
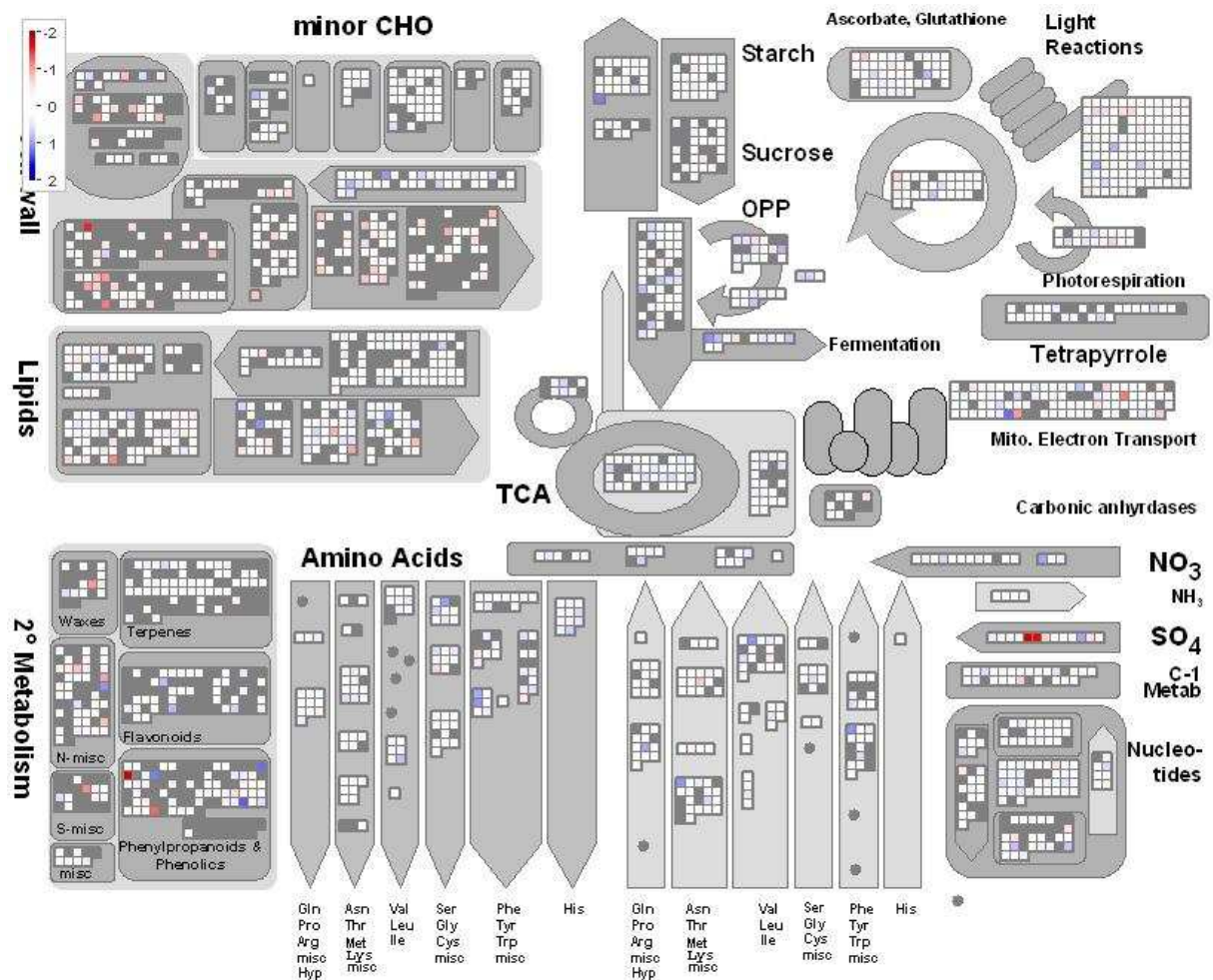
**Figure 28.** Transcript profiling after DTT feeding to leaf discs – metabolism overview. Figure shows graphical presentation of the ratio of DTT incubated sample in comparison to control in log2 scale. Red colour – decrease, blue colour – increase. Data were normalized using RMA software, only detection values over 100 were treated as present and ratios were calculated. Expression data were filtered to select only genes showing a coinciding change-call in the two biological replicates for each experimental condition.

There were no significant changes observed in transcript levels of genes encoding for enzymes involved in central metabolic pathways (Table 2 in Appendix and Fig. 28, 29) such as glycolysis, TCA cycle, starch metabolism, Calvin cycle, pentose phosphate pathway and most genes of amino acid metabolism. This indicates that the changes in fluxes and metabolite levels in these pathways obtained after short-term DTT feeding were due to post-translational changes rather than to changes in gene expression.

| metabolic pathway | number of genes involved | percentage of genes involved in different pathways showing decrease (–2 to –0.22), no changes (–0.22 to 0.22) or increase (0.22 to 3) in transcription levels or being absent: | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | transcription level (in log2 scale): | | | | | | | | | | |
| | | (-2.00, -1.55) | (-1.55, -1.11) | (-1.11, -0.66) | (-0.66, -0.22) | (-0.22, 0.22) | (0.22, 0.66) | (0.66, 1.11) | (1.11, 1.55) | (1.55, 2.00) | (2.00, 3.00) | absent (%) |
| light reactions | 127 | 0,0 | 0,0 | 0,0 | 14,2 | 68,5 | 11,8 | 2,4 | 0,0 | 0,0 | 0,0 | 3,1 |
| photorespiration | 20 | 0,0 | 0,0 | 5,0 | 5,0 | 40,0 | 35,0 | 0,0 | 0,0 | 0,0 | 0,0 | 15,0 |
| Calvin cycle | 32 | 0,0 | 0,0 | 3,1 | 3,1 | 68,8 | 12,5 | 3,1 | 0,0 | 0,0 | 0,0 | 9,4 |
| starch/sucrose | 103 | 0,0 | 0,0 | 0,0 | 4,9 | 53,4 | 17,5 | 1,0 | 0,0 | 0,0 | 0,0 | 23,3 |
| glycolysis | 62 | 0,0 | 0,0 | 0,0 | 6,5 | 37,1 | 25,8 | 4,8 | 0,0 | 0,0 | 0,0 | 25,8 |
| TCA | 78 | 0,0 | 0,0 | 0,0 | 1,3 | 41,0 | 34,6 | 0,0 | 0,0 | 0,0 | 0,0 | 23,1 |
| fermentation | 12 | 0,0 | 0,0 | 0,0 | 0,0 | 25,0 | 25,0 | 25,0 | 0,0 | 0,0 | 0,0 | 25,0 |
| gluconeogenesis | 10 | 0,0 | 0,0 | 0,0 | 0,0 | 50,0 | 50,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 |
| OPP | 31 | 0,0 | 0,0 | 0,0 | 9,7 | 35,5 | 32,3 | 6,5 | 0,0 | 0,0 | 0,0 | 16,1 |
| mitoch. e transport | 99 | 0,0 | 1,0 | 2,0 | 11,1 | 47,5 | 19,2 | 1,0 | 1,0 | 0,0 | 0,0 | 17,2 |
| minor CHO | 104 | 0,0 | 0,0 | 0,0 | 4,8 | 51,0 | 13,5 | 1,0 | 0,0 | 0,0 | 0,0 | 29,8 |
| cell wall | 485 | 0,0 | 0,4 | 4,3 | 14,2 | 21,4 | 6,0 | 0,4 | 0,0 | 0,0 | 0,0 | 53,2 |
| lipids | 384 | 0,0 | 0,0 | 2,1 | 14,1 | 37,5 | 13,8 | 0,8 | 0,0 | 0,0 | 0,0 | 31,8 |
| amino acids | 318 | 0,0 | 0,0 | 0,6 | 5,3 | 49,4 | 23,0 | 3,5 | 0,0 | 0,0 | 0,0 | 18,2 |
| redox | 176 | 0,0 | 0,0 | 0,6 | 8,0 | 48,9 | 23,3 | 2,8 | 0,6 | 0,6 | 1,1 | 14,2 |
| tetrapyrrole | 32 | 0,0 | 0,0 | 0,0 | 0,0 | 53,1 | 21,9 | 0,0 | 0,0 | 0,0 | 0,0 | 25,0 |
| nitrate metabolism | 23 | 0,0 | 0,0 | 0,0 | 0,0 | 65,2 | 21,7 | 8,7 | 0,0 | 0,0 | 0,0 | 4,3 |
| sulfur metabolism | 13 | 15,4 | 0,0 | 0,0 | 15,4 | 38,5 | 23,1 | 7,7 | 0,0 | 0,0 | 0,0 | 0,0 |
| nucleotides | 139 | 0,0 | 0,0 | 0,0 | 9,4 | 41,0 | 24,5 | 2,2 | 0,0 | 0,0 | 0,0 | 23,0 |
| 2nd metabolism | 406 | 0,0 | 0,0 | 1,2 | 7,4 | 26,8 | 11,1 | 0,7 | 0,7 | 0,0 | 0,0 | 52,0 |

**Figure 29.** Transcript profiling after DTT feeding to leaf discs – metabolism overview. Data from **Fig. 28** recalculated and shown as percentage of total amount of genes involved in different metabolic pathways.

## 3.5. Changes in metabolite and transcript profiles in *Arabidospis* leaves in response sucrose feeding

The results in chapters 3.1 – 3.3 of this thesis showed that sucrose and DTT feeding both lead to a similar increase in redox-activation of enzymes such as AGPase and ACCase and related metabolic fluxes. However, in contrast to DTT, sucrose led to these changes without increasing the overall redox-state of the tissue (see above). It was therefore investigated, whether sucrose also led to a different response concerning the changes in metabolite and transcript profiles.

### 3.5.1. *Metabolic profiling of Arabidopsis leaves after sucrose feeding*

To investigate the effect of sucrose feeding on metabolite profiles, leaves were taken from *Arabidopsis* plants at the end of the day and incubated in the medium without or with 50 and 100 mM sucrose over-night (13 hours). After freezing in liquid nitrogen and extraction, metabolite contents were measured on GC/MS and changes in metabolite levels were calculated as ratio between 50 or 100 mM sucrose and buffer incubated (control) leaves. Similarly to DTT feeding, metabolites were placed in metabolic pathways and presented on the scheme in Fig. 30 to show which of the metabolite increase or decrease in response to redox using a color-code. All the metabolites measured are listed in Table 3, Appendix, where the significance of the changes is documented.
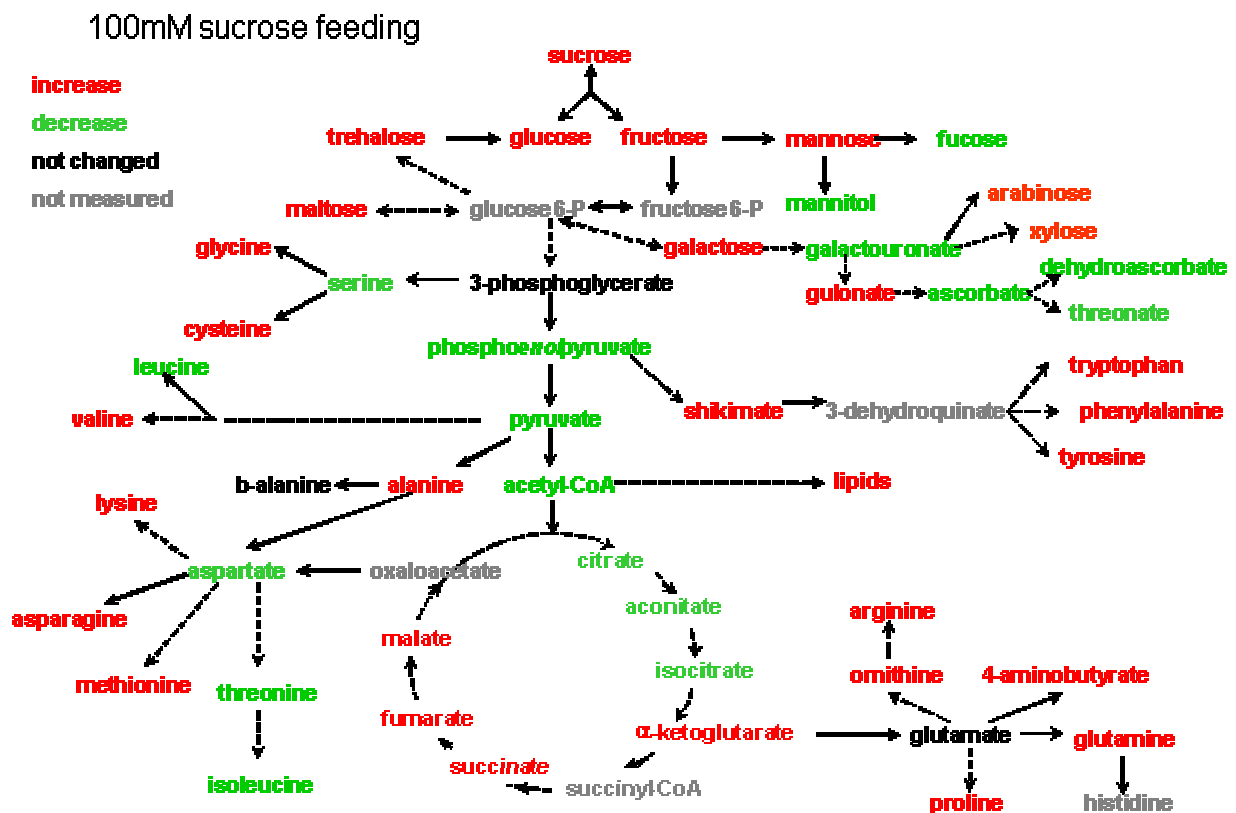


**Figure 30.** Metabolic profiling after feeding of *Arabidopsis* leaves with 100mM sucrose. Changes in metabolite levels calculated as ratio between sucrose and sorbitol incubated samples. Not all the data presented on graphs are significant and are presented to show a tendency to increase or decrease. The list of all data (showing also the significance) is presented in Appendix, Table 6. Measurement is mean of n=5 +/- SE.

100 mM sucrose led to a strong and significant increase in the levels of the major sugar pools such as sucrose, glucose and fructose and also most of other sugars (trehalose, maltose, galactose), and sugar-alcohols (Fig. 30 and Table 3, Appendix). Galactouronate, threonate, ascorbate and dehydroascorbate decreased. There was also a decrease in the levels of glycolytic intermediates such as phosphoenolpyruvate and pyruvate, a decrease in the metabolites of the first half of TCA cycle such as citrate, aconitate, isocitrate, while α-ketoglutarate, succinate, fumarate and malate slightly increased. This was accompanied by major changes in amino acid levels, such as a decrease in aspartate, threonine, isoleucine, leucine and histidine, while most other amino acids such as arginine, ornithine, proline, glutamine, alanine, lysine, asparagines, methionine, glycine and cysteine increased. There was also increase in shikimate and the aromatic amino acids (tryptophan, phenylalanine, tyrosine).

### 3.5.2.  Transcript profiling of Arabidopsis leaves after sucrose feeding

To investigate the changes in transcriptome of *Arabidopsis* leaves in response to sucrose feeding in the dark, Affymetrix ATH1 GeneChip analysis was used. External sucrose supply led to changes in transcript levels of 2896 out of 22817 genes investigated (Fig. 31, 32 and Table 4 in Appendix). To the genes with altered transcript profiles belong many genes involved in central metabolite pathways as for example: glycolysis (UDP-glucose pyrophosphorylase, phosphoglucomutase, hexokinase, glyceraldehydes 3-phosphate dehydrogenase), gluconeogenesis (fructose 6-phosphate dehydrogenase, ATP citrate lyase, malate dehydrogenase), TCA cycle (citrate synthase, aconitase, isocitrate dehydrogenase), sucrose and starch metabolism (large subunit of AGPase, b-amylase, isoamylase, sucrose phosphate synthase, invertase), amino acid metabolism (prephenate dehydrogenase, aminotransferase, serin acetyltransferase) and lipid metabolism (desaturase, phospholipase, acetyl-CoA synthase, acyl carrier protein).  There are also a lot of genes with altered transcript profiles in different other metabolic pathways such as trehalose metabolism (TPS and TPP), nucleotide metabolism (adenine phosphoribosyltransferase, thymidine kinase), secondary metabolism

(anthocyanidin synthase, flavonoid 3-hydroxylase, flavonol synthase), N metabolism (ferredoxin-nitrite reductase, glutamate dehydrogenase), hormone metabolism (auxin-induced protein, gibberelin-regulated protein, squalene epoxidase), stress response components (disease resistant protein), redox regulation (glutaredoxin, thioredoxins, peroxiredoxins, protein-disulfide-isomerases), protein folding (calreticulin and calnexin) and many other genes.
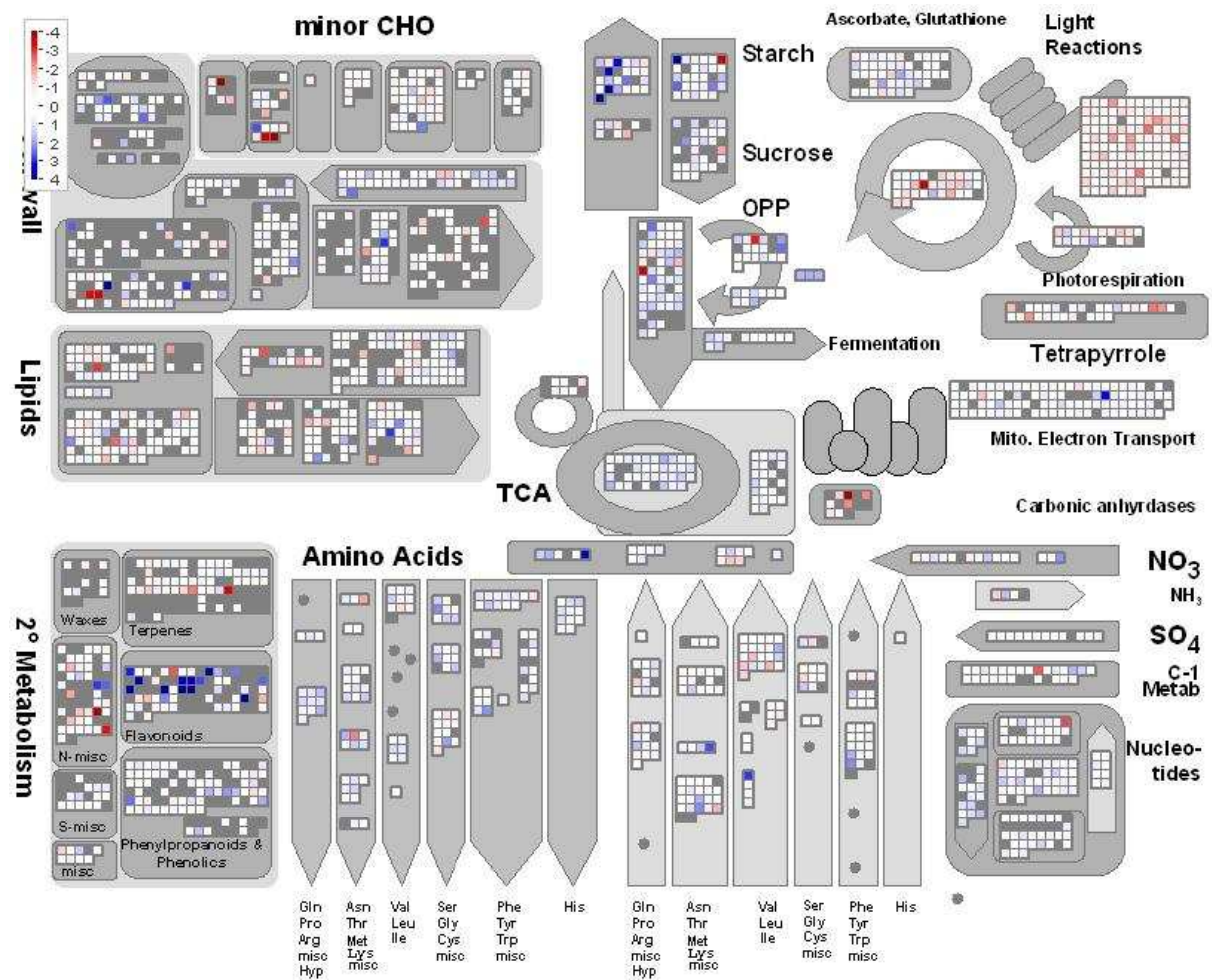


**Figure 31.** Transcript profiling after sucrose feeding to leaves – metabolism overview. Figure shows graphical presentation of the ratio of DTT incubated sample in comparison to control in log2 scale. Red colour – decrease, blue colour – increase. Data were normalized using RMA software, only detection values over 100 were treated as present and ratios were calculated. Expression data were filtered to select only genes showing a coinciding change-call in the two biological replicates for each experimental condition.

| metabolic pathway | number of genes involved | percentage of genes involved in different pathways showing decrease (−5 to −0.44), no changes (−0.44 to 0.44) or increase (0.44 to 5) in transcription levels or being absent: | | | | | | | | | | | |
| | | transcription level (in log2 scale): | | | | | | | | | | | |
| | | (-5.00, -4.00) | (-4.00, -3.11) | (-3.11, -2.22) | (-2.22, -1.33) | (-1.33, 0.44) | (-0.44, 0.44) | (0.44, 1.33) | (1.33, 2.22) | (2.22, 3.11) | (3.11, 4.00) | (4.00, 5.00) | absent |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| light reactions | 127 | 0,0 | 0,0 | 2,4 | 10,2 | 53,5 | 30,7 | 1,6 | 0,0 | 0,0 | 0,0 | 0,0 | 1,6 |
| photorespiration | 20 | 0,0 | 0,0 | 5,0 | 15,0 | 25,0 | 25,0 | 15,0 | 10,0 | 0,0 | 0,0 | 0,0 | 5,0 |
| Calvin cycle | 32 | 0,0 | 3,1 | 0 | 15,6 | 34,4 | 31,3 | 6,3 | 3,1 | 0,0 | 0,0 | 0,0 | 6,3 |
| starch/sucrose | 103 | 0,0 | 1,0 | 0 | 1,9 | 8,7 | 30,1 | 24,3 | 5,8 | 2,9 | 1,9 | 2,9 | 20,4 |
| glycolysis | 62 | 0,0 | 1,6 | 0,0 | 1,6 | 4,8 | 17,7 | 41,9 | 11,3 | 3,2 | 0,0 | 0,0 | 17,7 |
| TCA | 78 | 1,3 | 0,0 | 0,0 | 3,8 | 3,8 | 23,1 | 43,6 | 5,1 | 0,0 | 0,0 | 0,0 | 19,2 |
| fermentation | 12 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 41,7 | 41,7 | 8,3 | 0,0 | 0,0 | 0,0 | 8,3 |
| gluconeogenesis | 10 | 0,0 | 0,0 | 0,0 | 0,0 | 10,0 | 40,0 | 10,0 | 0,0 | 0,0 | 0,0 | 0,0 | 40,0 |
| OPP | 31 | 0,0 | 3,2 | 0,0 | 0,0 | 12,9 | 32,3 | 19,4 | 19,4 | 3,2 | 0,0 | 0,0 | 9,7 |
| mitoch. e transport | 99 | 0,0 | 0,0 | 0,0 | 1,0 | 5,1 | 42,4 | 35,4 | 2,0 | 1,0 | 0,0 | 0,0 | 13,1 |
| minor CHO | 104 | 1,9 | 1,0 | 0,0 | 3,8 | 17,3 | 37,5 | 12,5 | 1,0 | 1,9 | 0,0 | 0,0 | 23,1 |
| cell wall | 485 | 0,0 | 0,4 | 0,0 | 1,2 | 8,5 | 25,4 | 13,6 | 2,7 | 1,2 | 0,0 | 0,0 | 47,0 |
| lipids | 384 | 0,0 | 0,0 | 0,8 | 3,9 | 12,0 | 36,2 | 17,2 | 3,1 | 0,3 | 0,3 | 0,0 | 26,3 |
| amino acids | 318 | 0,0 | 0,0 | 0,3 | 2,2 | 10,7 | 34,9 | 30,8 | 9,7 | 0,6 | 0,3 | 0,0 | 10,4 |
| redox | 176 | 0,6 | 0,0 | 1,1 | 4,0 | 10,8 | 38,6 | 22,2 | 4,5 | 1,7 | 0,6 | 0,6 | 15,3 |
| tetrapyrrole | 32 | 0,0 | 0,0 | 3,1 | 9,4 | 12,5 | 43,8 | 12,5 | 3,1 | 0,0 | 0,0 | 0,0 | 15,6 |
| nitrate metabolism | 23 | 0,0 | 0,0 | 0,0 | 4,3 | 13,0 | 43,5 | 17,4 | 13,0 | 0,0 | 0,0 | 0,0 | 8,7 |
| sulfur metabolism | 13 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 69,2 | 23,1 | 0,0 | 0,0 | 0,0 | 0,0 | 7,7 |
| nucleotides | 139 | 0,0 | 0,0 | 0,7 | 0,7 | 7,9 | 36,7 | 33,1 | 5,0 | 0,0 | 0,0 | 0,0 | 15,8 |
| 2nd metabolism | 406 | 0,0 | 0,0 | 1,2 | 7,4 | 26,8 | 11,1 | 0,7 | 0,7 | 0,0 | 0,0 | 0,0 | 52,0 |

**Figure 32.** Transcript profiling after sucrose feeding to leaves– metabolism overview. Data from **Fig. 31** recalculated and shown as percentage of total amount of genes involved in different metabolic pathways.

### 3.5.3.    Conclusions

The changes in metabolite profile in response to sucrose resembled in many aspects that observed after feeding DTT (see Fig. 33) in leading to an increase in amino acid and cell-wall synthesis that was accompanied by a decrease in the levels of glycolytic intermediates and of metabolites in the first half of the TCA cycle and of sugars such as arabinose, xylose, galactose. Sucrose differed from DTT feeding in leading to distinct changes in amino acid pattern, a decrease in ascorbate and dehydroascorbate rather that an increase, and a decrease of fucose.

There were hardly any similarities between DTT and sucrose feeding concerning changes in transcripts. Sucrose led to a multitude of changes in transcript levels (about 12 %), including many changes in the transcripts of genes involved in the pathways of central metabolism such as glycolysis, TCA, sucrose, starch and lipid metabolism. However, only a small part of the changes in transcripts that were observed after DTT feeding were also obtained after sucrose feeding (Fig. 34), and it was conspicuous that the genes invovled central metabolic pathways whose expression is changed by sucrose are not affected by DTT.
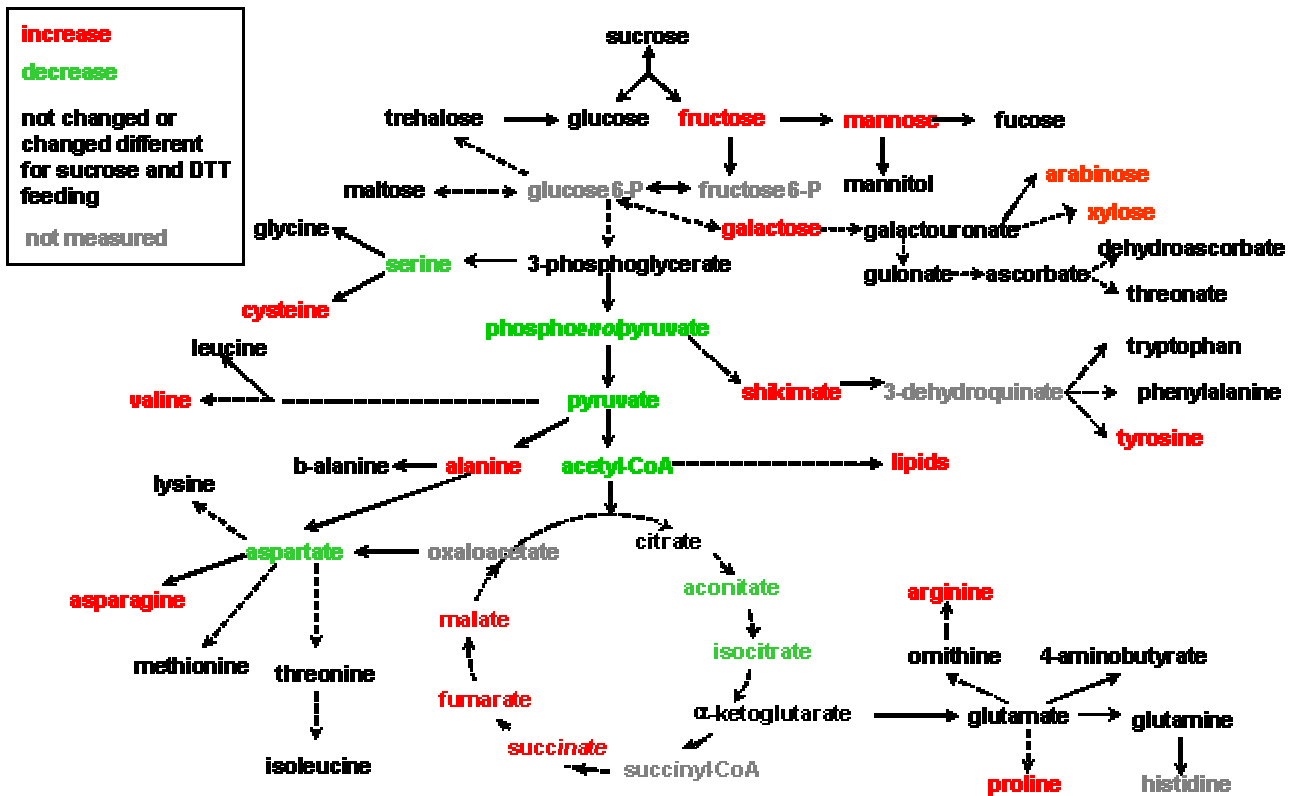
**Figure 33.** Changes in metabolite levels after DTT feeding compared with changes after sucrose feeding. Similarities between DTT and sucrose feeding marked on red (increase in both cases) or green (decrease in both cases). See also **Fig. 27** and **30** and **Tables 1** and **3** in Appendix.

**Figure 34.** **A**) Comparison of changes in transcript levels after DTT and sucrose feeding. Data are calculated as ratio between DTT incubated sample and control or sucrose incubated sample and control and are shown as log2. Measurements are mean of n=2 +/- SE. **B**) Data from **Fig. 34 A** shown as Venn diagram.

# 4. Discussion

Posttranslational redox-regulation has been intensively researched during the last 25 years. This mechanism was found to regulate enzymes of the Calvin cycle, oxidative pentose phosphate cycle, NADPH export and ATP synthesis in response to light (Buchanan, 1980, 1991; Schürmann and Jacquot, 2000; Schürmann and Buchanan, 2001). The aim of this work was to investigate whether this concept can be extended to the regulation of starch and lipid synthesis in leaves *in vivo*. In addition, prompted by work showing that sugars regulate starch synthesis in potato tubers by post-translational redox modulation of AGPase, a second aim was to investigate if sugars regulate starch and lipid synthesis by an analogous mechanism in leaves. If so, this would establish a immediate link between light regulation, sugar regulation and redox regulation.

The results in the present thesis showed, that redox-modulation of AGPase provides a powerful mechanism to regulate starch synthesis in leaves. It was overlooked until now, because redox modification of AGPase is rapidly reversed under the standard methods used to extract the protein and analyse its activity. Applying the new TCA-diethyl ether extraction method followed by SDS-PAGE electrophoresis in non-reducing conditions allowed the identification of changes in AGPase redox-activation during day-night alterations. Light (chapter 3.1) and sugars (chapter 3.2) were shown to provide two independent inputs leading to reductive activation of AGPase accompanied by an increase in the rate of starch synthesis. It was also demonstrated, that sucrose acts via trehalose 6-phosphate, which reports the cytosolic sugar status to the plastid, without changing the redox-state of the cell.

At the start of this thesis there was *in vitro* evidence showing ACCase to be redox-regulated in response to light. The development of a new protocol for ACCase activity measurement enabled finding of changes in ACCase redox-state *in-vivo*. In combination wioth flux analyses, this allowed the demonstration that fatty acid synthesis is redox-regulated in leaves in a similar manner to starch synthesis (chapter 3.3). ACCase activation decreased during the night and increased during

the day. Feeding of the reducing agent DTT to leaf discs led to re-activation of the enzyme and stimulation of lipid synthesis *in situ*. Supply of external sugars to leaves in the dark also led to post-translational redox-activation of ACCase and stimulation of lipid synthesis.

Metabolic and transcript profiling (chapter 3.4) provided evidence that post-translational redox-modification is an important regulatory mechanism in many biosynthetic pathways, which acts independently of changes in transcript levels.

In the following section the importance of posttranslational redox-regulation in starch, lipid and variety of other metabolic pathways occurring in leaf tissue will be discussed.

## 4.1. Starch synthesis is regulated via redox-modification of AGPase in response to light and sugar signals in leaves

The new extraction method presented in this work revealed that AGPase is subject to posttranslational redox-regulation in leaves of *Arabidopsis*, potato and pea (Fig. 3). When leaves were extracted under either dark or light conditions, using trichloroacetic acid to quickly denature AGPase before separation on non-reducing SDS gels, AGPB was found to be completely dimerised in the dark and partly converted to monomer in the light. Fu et al. (1998) found that an intermolecular bridge forms between the Cys-82 residues of the two AGPB subunits of the potato AGPase, when heterologously overexpressed in *E. coli*. To obtain an active enzyme it was necessary to incubate the complex with 1-5 mM DTT to break this link. DTT led to monomerisation of AGPB also in potato tuber extracts (Tiessen et al. 2002).

The shift from a dimer to monomer was accompanied by an increase in AGPase activity (Fig. 5). This involved the change in kinetic properties, including an increased affinity for ATP and increased sensitivity to activation by 3PGA. The increase in the activity is less marked than Tiessen et al. (2002) reported for potato tubers. This may

be due to technical difficulties in retaining AGPB in the *in planta* status in leaf extracts. Alternatively, it may reflect a real difference in sensitivity between AGPase in different plants and organs, due, for example, to association with a different AGPS isoform.

Light-dependent signal modulates posttranslational redox-activation of AGPase. Evidence for a light-dependent input is provided by two experiments: AGPB monomerisation decreases rapidly after darkening wild-type leaves even though sugar levels do not change (Fig. 6) and increases rapidly after illumination of isolated chloroplasts (Fig. 7). This is analogous to the way that several Calvin cycle enzymes and other proteins involved in photosynthesis are regulated (Scheibe, 1991; Schürmann and Jacquot, 2000).

The light-dependent redox-activation of AGPase can be envisaged to be a direct result of increased reduction by thioredoxin. To show this a desalted extract of stromal proteins prepared from chloroplasts of dark-adapted pea leaves was used for incubation with reduced thioredoxins (Fig. 8). Obtained data indicate that AGPB can interact with thioredoxin *f* or *m*. Results presented in this thesis are also supported by the studies Ballicora et al. (2000), who was able to show that reduced forms of both thioredoxins *f* and *m* from spinach leaves were able to activate the recombinant AGPase from potato tubers by up to 4-fold. Fifty percent activation was obtained at 4.5 and 8.7 µM for reduced thioredoxin *f* and *m*, respectively. Oxidised thioredoxins reversed the activation.

However, in both studies the AGPB dimer was not completely converted to monomers, even when higher concentrations of thioredoxins up to 100 µM were supplied. One explanation for this is that other thioredoxin isoforms might be more effective to activate AGPase than thioredoxin *f* and *m*. The degree of monomerisation that was obtained after incubation with these thioredoxins is, however, similar to that usually observed in illuminated leaves.

A second explanation is that interaction of thioredoxins with AGPase is modulated by additional factors allowing a further degree of fine control. It has been found for photosynthetic enzymes that activation by thioredoxin is modified by pH, $Mg^{2+}$ and

the levels of substrates and products, all of which show marked changes upon light-dark transitions (Scheibe, 1991). Interestingly, *in vitro* activation of potato AGPase by DTT requires the presence of substrates: These are also needed to attain the active conformation after reduction (Fu et al., 1998).

Monomerisation of AGPase in isolated chloroplasts was promoted by 3PGA but not by ATP (Fig. 7). This indicates that the reductive activation of AGPase may be promoted by high 3PGA in leaves. This could provide a mechanism to prevent depletion of phosphorylated intermediates due to excessive posttranslational activation of AGPase. The light-dependent activation of photosynthetic enzymes by thioredoxin was also shown to be modulated by metabolites which modify the mid-redox potential of the cysteine in the target protein (Sheibe, 1991; Schürmann and Jacquot, 2000). This provides a mechanism to fine-tune the activity of enzymes at different sites around the Calvin cycle and poise ATP and NADPH levels production (Stitt, 1996). More detailed studies are necessary using purified AGPB enzyme to investigate the affinities of leaf AGPase for different thioredoxin isoforms, and to investigate whether interaction of AGPase with thioredoxins is modified by low molecular weight factors.

Sugar-related signal is a second input which modulates AGPase redox-activation in leaves. It acts independently of light, evidence is provided by experiments showing that AGPase monomerisation and starch synthesis rate were increased by supplying exogenous sugars to wild-type leaves in the dark, but 3PGA level was not involved in the regulation (Fig. 9). When sugars and the light regime are both varied they can interact in a additive or super-additive manner, as shown in the studies with the *pgm* mutant (Appendix, Fig. 36 and Hendriks et al., 2003).

There are two ways how sugars can be linked to redox-regulation: First, via an increase in the platidial redox-status, second via a signal metabolite that is produced in the cytosol and transferred to the plastid. After illumination, the $NADPH/NADP^+$ ratio increases, which will favour reductive activation of AGPase. After feeding sucrose or trehalose, AGPase redox-activation increases even though the $NADPH/NADP^+$ ratio remained unaltered (Fig. 10). This resembles the situation when sucrose leads to post-translational redox-activation of AGPase in non-photosynthetic potato tubers (Tiessen et al. 2002). Studies in growing potato tubers showed that

sucrose and glucose lead to a redox-activation of AGPase via two different signalling pathways involving an SNF 1-related protein kinase (SnRK1) and an endogenous hexokinase, respectively (Tiessen et al., 2003). Glucose signalling, acting via hexokinase, led to an increase in NADPH/NADP$^+$ ratio, whereas sucrose did not affect it (Tiessen et al., 2003). Both hexokinase and SnRK1 are part of regulatory network that is proposed to control the expression and phosphorylation of cytosolic enzymes in response to sugars (Smeekens et al., 2000).

The increased redox-state in response to light reflects the generation of NADPH by photosynthetic electron transport. The increase after feeding glucose may be indicative for a stimulation of the oxidative pentose phosphate cycle or other processes leading to increased reduction of NADP$^+$ to NADPH. An elevated NADPH/NADP$^+$ ratio will lead to an increase in the reduction state of thioredoxins via the NADP/thioredoxin reductase or the ferredoxin/NADP reductase systems, and activate AGPase.

The increase in the NADPH/NADP$^+$ ratio after feeding glucose in the dark probably involves a plastidic isoform of glucose-6-P dehydrogenase (P2-G6PDH) identified recently in potato (Wendt et al., 2000) and tobacco (Debnam et al., 2004) that is much less susceptible to inactivation by thioredoxin and to end-product inhibition by NADPH (Wendt et al., 2000). It has been proposed that P2-G6PDH could play a role in the provision of reductant in heterotrophic plastids, where photochemical processes are absent or contribute to the capacity of chloroplasts to generate NADPH. Interestingly, overexpression of P2-G6PDH in transgenic tobacco plants led to an increase in the starch to soluble sugar ratio in leaves (Debnam et al, 2004). More studies are needed to identify the thioredoxin isoforms in heterotrophic plastids and the way they are reduced by the activity of the oxidative pentose phosphate pathway.

Sucrose does not affect the redox-state, which indicates that the sucrose-signal activates AGPase by modifying the redox-transfer form NADPH via thioredoxins to AGPase by an unknown factor. In analogy to the well-characterised systems like the NADP-MDH, these may act by lowering the mid redox point potential of the cysteines involved in the reversiblke cystein bridge, allowing them to be reduced even though

the redox state of the NADP(H) and thioredoxin pools does not change. Alternatively, if AGPase is reduced by a specific thioredoxin *in situ*, these unknown factors might modulate transfer of reducing equivalents from NADPH or ferredoxin to this specific thioredoxin.

It was proposed in this work that this additional factor is, or is closely linked to, trehalose-6-phosphate. In previous studies, T6P has been identified as a cytosolic signal metabolite that is indispensable for sugar utilisation in plants and other organisms, but the site of action were largely unresolved (Schluepmann et al., 2003).

## 4.2. Trehalose 6-phosphate provides a link between changes in sucrose status of the cytosol and redox-regulation of AGPase in the plastid

Trehalose ($\alpha$-D-glucopyranosyl-[1,1]-$\alpha$-D-glucopyranoside) is a non-reducing sugar that is made up of two glucose units joined by an $\alpha,\alpha$-1,1 linkage and is common in the nature. It functions as a storage carbohydrate and can protect against cellular damage caused by different stresses in invertebrates, microorganisms and fungi (Goddijn and Dun, 1999). Recent studies show that the precursor of trehalose, T6P is an indispensable regulator of sugar utilisation in eucariotic organisms as different as yeasts and plants (Gacendo and Flores, 2004; Schluepmann et al., 2003). However, apart from regulating glycolysis by inhibiting hexokinase in some yeast species, other sites of T6P action are not known (Gacendo and Flores, 2004). No target of T6P has been found in plants, where hexokinases are insensitive to T6P (Eastmond and Graham, 2003). In this thesis independent lines of genetic and biochemical evidence are provided that T6P acts on AGPase by promoting post-translational redox-activation of the enzyme in response to sucrose thereby controlling starch synthesis independently of light signals.

In *Arabidopsis* leaves short-term feeding of trehalose leads to increased T6P levels within 30 minutes (Schluepmann et al., 2004). In the present work it was shown that there is an increase in reductive activation of AGPase and starch synthesis following a similar time frame after supplying trehalose (Fig. 10). This was not paralleled by an

increase in the levels of other sugars or glycolytic intermediates, making it unlikely that the effect of trehalose was due to its metabolism to glucose, sucrose or glycolysis. This is consistent with previous studies suggesting that trehalose affects T6P levels via feedback inhibition of TPP (Schluepmann et al., 2004). Moreover, the time-course of reductive activation of AGPase in response to sucrose feeding was different to that of trehalose feeding, with 30 min of sucrose feeding having no effect on redox-activation of AGPase. Sucrose feeding led to redox-activation of AGPase and increased starch levels after 3 h, following a similar kinetic as the increase of T6P under these conditions (Schluepmann et al., 2004).

Transgenic *Arabidopsis* and tobacco plants with alterations in T6P metabolism provide genetic evidence that T6P rather than trehalose leads to redox-activation of AGPase (Fig. 12 and 13). Compared to wild-type, *Arabidopsis* and tobacco leaves with T6P levels increased by expression of *E.coli* TPS in their cytosol (Schluepmann et al., 2003) showed increased redox-activation of AGPase and increased starch accumulation during the day, while leaves with T6P levels decreased by overexpression of TPP showed the opposite. These changes in redox-activation of AGPase occurred independently of changes in sugars, sugar-phophates or NADPH/NADP$^+$ ratio, indicating that T6P overrides light-dependent changes in redox-status and metabolite levels. While AGPase was activated and starch levels increased by feeding sucrose to leaves of wild-type and TPS-expressing plants, this response was strongly attenuated in TPP-expressing plants, providing evidence that T6P links redox-activation of AGPase to sucrose (Fig. 14, 15).

Feeding experiments with isolated chloroplasts provide direct biochemical evidence that T6P promotes redox-activation of AGPase independently of cytosolic elements (Fig. 16). Incubation with T6P significantly and specifically increased AGPB monomerisation and AGPase activity within 15 minutes, while no significant changes were observed after feeding trehalose or sucrose to the chloroplasts (Fig. 17 A).

Taken together these different approaches provide cumulative evidence that T6P promotes post-translational redox-activation of AGPase in leaves as a part of a rapid sugar-signalling response that stimulates carbon storage in response to sugar availability, reporting on metabolite status between cytosol and chloroplast. The

response most likely involves synthesis of T6P in the cytosol and its subsequent perception at the chloroplast membrane or, after transport, within the chloroplast. First, expression of TPS and TPP in the transgenic plants was targeted to the cytosol which will perturb cytosolic levels of T6P. Second, feeding experiments show that T6P in concentrations down to 100 µM can act on isolated chloroplasts leading to redox-activation of AGPase. The T6P content in plants has been reported to be 3-12 nmol/gFW (Schluepmann et al., 2003; 2004). Assuming T6P is in the cytosol and plastids, representing ca. 10% of the cell volume, 100 µM is within the physiological range of cytosolic concentrations in plants. For comparison, internal T6P concentrations in yeast cells have been determined to be in a similar range between 150 and 200 µM, and to transiently rise to 1.5 mM in response to sugars (Blazquez et al., 1994; van Vaeck et al., 2001; Gacendo and Flores, 2004). T6P had no effect when fed to chloroplasts that have been disrupted by hypo-osmotic shock showing that intact chloroplast structures are needed for T6P action. This might be attributable to specific transport characteristics leading to an accumulation of T6P in intact chloroplasts, to the importance of T6P interacting factors being present concentrations similar to those fund in intact chloroplast envelope, or to the importance of interacting proteins such as thioredoxins being present in concentrations usually found in intact chloroplast stroma. More experiments are needed to define also the sensing and/or transport mechanisms of T6P to intact chloroplasts.

Interestingly, changes in the chloroplast redox-state were not involved in the reductive activation of AGPase in response to T6P. Neither sugar feeding to leaves (Fig. 10) nor T6P supply to chloroplasts (Fig. 17 B) led to significant changes in the platidial redox status. This indicates that T6P promotes redox-transfer from NADPH or ferredoxin to AGPase rather than leading to increased reduction state of these redox-systems.

Redox-activation of AGPase in response to sucrose feeding was strongly attenuated in TPP-expressing plants, showing T6P to be a crucial element in the response of AGPase to sucrose (Fig. 14, 15). There is evidence documenting that the sensing of the sugar occurs in the cytosol rather than in the plastid. First, the response of AGPase to sucrose was dependent on SnRK1 expression in the cytosol (Tiessen et

al., 2003). Second, in the present thesis it was shown that the effect of sucrose on AGPase was absent in isolated chloroplasts (Fig. 17 A).

The link between sucrose and T6P has not been fully resolved yet, but may involve regulation of enzymes of T6P metabolism at the transcriptional or post-transcriptional levels. *Arabidopsis* contains a family of genes encoding TPS and/or TPP and recent studies using transcript arrays indicate large diurnal fluctuations in the expression of these genes (Gibon et al. 2004) which are probably due to changes in the carbon status of the leaves (Schluepmann et al., 2004). Microarrays also show that increased T6P levels in transgenic *Arabidopsis* lines and wild-type plants exogenously supplied with sugars correlate with increased expression of SnRK1 (Schluepmann et al., 2004; and own data). Whether SnRK1 is acting upstream or downstream of TPS can not be fully resolved at the moment. The presence of SnRK1 phosphorylation motives in TPS sequences (see Halford et al. 2003) and the finding that T6P acts on isolated chloroplasts without requiring further cytosolic elements would be consistent with SnRK1 being an upstream component in this signaling pathway.

## 4.3. Importance of redox-regulation of AGPase in other plant tissues

Almost all dicotyl plant AGPB sequences (with the exception of *Citrus unshui*) contain a conserved SQTCLDPDAS motif at the N terminus, which includes the regulatory cystein identified by Fu et al. (1998) in potato. The importance of redox-regulation of AGPase for the synthesis of starch in other plant tissues still has to be investigated. Starch is the major carbon store in cereal seeds, such as maize, rise and wheat. Monocots contain two types of AGPB transcript: one encodes proteins that contain this motif, and the other encodes proteins that lack it. The pathway of starch synthesis in the cereal endosperm is different to other plant species and tissues, such as leaves and tubers, and involves a cytosolic AGPase in addition to the plastidic isoform (Teltow et al., 2004). The regulatory cystein is missing in these cytosolic AGPB isoforms, indicating that the cytosolic AGPase of cereal endosperm

is redox-insensitive (Fig. 35). However, more studies are needed to confirm this interpretation.

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Z.mais_Q941P2       RPFLFSPKGVSDSRSSQTCLDPDASTSVLGIIILGGGAGTRLYPLTKKRAKPAVPLGANYR 140
O.sativa_Q9ARH9     RPFVFTPRAVSDSRSSQTCLDPDASTSVLGIIILGGGAGTRLYPLTKKRAKPAVPLGANYR 140
Z.mais_Q947B9       RPFVFSPRAVSDSKSSQTCLDPDASTSVLGIIILGGGAGTRLYPLTKKRAKPAVPLGANYR 140
H.vulgare_P55238    RPFFFSPRAVSDSKSSQTCLDPDASTSVLGIIILGGGAGTRLYPLTKKRAKPAVPLGANYR 140
T.eastivum_Q8H6H5   RPFFFSPRAVSDSKSSQTCLDPDASTSVLGIIILGGGAGTRLYPLTKKRAKPAVPLGANYR 140
T.eastivum_P30523   ------------SSSKHADLNPHVDDSVLGIIILGGGAGTRLYPLTKKRAKPAVPLGANYR 128
H.vulgare_Z48562    ------------SSSKHADLNPHAIDSVLGIIILGGGAGTRLYPLTKKRAKPAVPLGANYR 128
Z.mais_Q947C0       ----------------TYLNPQAHDSVLGIIILGGGAGTRLYPLTKKRAKPAVPLGANYR 123
O.sativa_P15280     -----------NSSKNKNLDRSVDESVLGIIILGGGAGTRLYPLTKKRAKPAVPLGANYR 128
P.frutescens_Q9M4W7 -PVIVSPKAVSDSQNSQTCLDPDASRSVLGIIILGSGAGTKLYPLTKKRAKPAVPFGANYR 139
S.oleracae_Q43152   ---------vsdskNSQTCLDPEASRSVLGIIILGGGAGTRLYPLTKKRAKPAVPLGANYR 126
P.sativum_Q43815    -PFIVSPKAVSDSKNSQTCLDPDASRSVLGIIILGGGAGTRLYPLTKKRAKPAVPLGANYR 139
V.faba_P524172      -PFIVSPKAVSDSKNSQTCLDPDASRSVLGIIILGGGAGTRLYPLTKKRAKPAVPLGANYR 139
C.arietinum_Q9AT06  -PIIVSPKAVSDSKNAQTCLDPDASRSVLGIIILGGGAGTRLYPLTKKRAKPAVPLGANYR 139
C.lanatus_O22657    -PSIVSPKAVSDSKNTQTCLDPDASRSVLGIIILGGGAGTRLYPLTKKRAKPAVPLGANYR 139
C.melo_O22629       -PSIVSPKAVSDSKNSQTCLDPDASRSVLGIIILGGGAGTRLYPLTKKRAKAAVPLGANYR 139
C.unshiu_Q9SP43     --SLCLLQAVSDSKNSP--LDPEASRRVLGIIILGGGAGTRLYPLTKKRAKPAVPLGANYR 136
V.faba_P52416       -HVFLTPKAVSDSQNSQTCLDPDASRSVLGIIILGGGAGTRLYPLTKKRAKPAVPLGANYR 139
P.sativum_Q43816    -HVIVTPKAVSDSQNSQTCLDPDASRSVLGIIILGGGAGTRLYPLTKKRAKPAVPLGANYR 139
C.arietinum_Q9AT05  -HVIVTPKAVSDSQNSQTCLDPDASRSVLGIIILGGGAGTRLYPLTKKRAKPAVPLGANYR 139
A.thaliana          -PIIVSPKAVSDSQNSQTCLDPDASSSVLGIIILGGGAGTRLYPLTKKRAKPAVPLGANYR 139
S.tuberosum_P23509  -PMIVSPKAVSDSQNSQTCLDPDASRSVLGIIILGGGAGTRLYPLTKKRAKPAVPLGANYR 139
L.esculentum_Q42882 SPLIVSPKAVSDSQNSQTCLDPDASRSVLGIIILGGGAGTRLYPLTKKRAKPAVPLGANYR 140
B.napus_Q9M462      -PSIVSPKAVSDSQNSQTCLDPDASRSVLGIIILGGGAGTRLYPLTKKRAKPAVPLGANYR 139
B.rapa_Q43152       -NAIDSPKAVSDSQNSQTCLDPDASRSVLGIIILGGGAGTRLYPLTKKRANRAVPLGANYR 139
I.batata_P93477     -PIIVSPKAVSDSQNSQTCLDPDASRSVLGIIILGGGAGTRLYPLTKKRAKPAVPLGANYR 139
I.batata_P93476     -PFIVSPKAVSDSQNSQTCLDPDASRSVLGIIILGGGAGTRLYPLTKKRAKPAVPLGANYR 139
B.vulgaris_P55232   -PIVVSPKAVSDSKNSQTCLDPEASRSVLGIIILGGGAGTRLYPLTKKRAKPAVPLGANYR 139
M.sagu_Q8LLJ5       -PVLVSPKAVSDSKSSQTCLDPDASRSVLGIIILGGGAGTRLYPLTKKRAKPAVPLGANYR 139
P.frutescens_Q9M4W6 SARVVSPKAVSDTSSSLTCLEPDANFSELAFFL-EWSGTRLYLLRKKRAKPAVPIGANYR 139
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**Figure 35.** Alignment of all complete AGPB sequences of plants. The incomplete spinach sequence was included, after completion of its N-terminus to the mature N-terminus with the peptide sequence data (non-capitals). The predicted start of the mature proteins is indicated by an arrow. Residues that are conserved within the plant sequences are highlighted; residues only conserved within plant sequences are highlighted in light grey, for the part of the alignment where homology to bacterial genes exist in dark grey. The conserved N-terminal cysteine is highlighted in black. Residue numbers are indicted on the right. The alignment was made using ClustalX (Thompson et al., 1997). Figure was kindly provided by Janneke Hendriks (Hendriks et al., 2003).

## 4.4.    Light and sugars are regulating lipid synthesis via redox-activation of ACCase

The use of new protocol for measuring ACCase activity revealed that the activation of ACCase is subject to redox-modulation in response to changes in DTT concentration.

In the present work two different assay conditions were used showing activity under selective conditions (Vsel) using 200 µM acetyl-CoA without adding DTT and under reduced conditions (Vred) using 200 µM acetyl-CoA and 5 mM DTT (Fig. 18). Vsel represents the *in vivo* activity of the tissue, which can change depending on different conditions (ex. day/night), whereas Vred shows the maximum activity of the enzyme. The ratio between Vsel and Vred shows redox-activation of the enzyme. The activity of ACCase varied between the day and night being 3-times lower in the dark than in the light. The light-activation was mimicked by reducing agent DTT fed to leaf discs in the dark indicating the involvement of redox-regulation of ACCase during light/dark transitions in leaves. Redox-activation led also to changes in kinetic properties of ACCase, showing increase of affinity for acetyl-CoA. In the present thesis it was shown for the first time that redox-activation of ACCase caused by DTT feeding to leaf discs in the dark was also accompanied by an increase in fatty acid synthesis rate and a decrease in the level of acetyl-CoA providing evidence that the key enzyme of lipid synthesis pathway is regulated by redox-modification *in vivo* (Fig. 19, 20, 23).

This is consistent with the *in vitro* evidence provided by Kozaki et al. (2001) showing that DTT causes reduction and activation of partially purified plastidial ACCase and demonstrating the redox-regulation of carboxyltransferase (CT) composed of the nuclear-encoded α and the chloroplast-encoded β subunits and identified the cysteine residues involved in this regulation. They expressed the recombinant CT in *E.coli* and found that the partly deleted CT was, like the full-length CT, sensitive to redox state. Site-directed mutagenesis of the deleted CT showed that replacement by alanine of the cysteine residue 267 in the α polypeptide or 442 in the β polypeptide resulted in the redox-insensitive CT and broke the intermolecular disulfide bond between the α and β polypeptides. Similar results were also confirmed in the full-length CT, indicating that the two cysteines in recombinant CT are involved in redox regulation by intermolecular disulfide/dithiol exchange between the α and β subunits. This was supported by the immunoblots from plastidial proteins isolated from dark- or light-adapted plants showing that the acetyl-CoA carboxylase activity can be modulated via redox-changes occurring in cysteines between α and β subunits of carboxyltransferase in response to dark/light transitions (Kozaki et al., 2001). Interestingly, the activity of biotin carboxylase was not influenced by DTT (Kozaki and

Sasaki, 1999). Sasaki et al. (1997) provided *in vitro* evidence that reduced thioredoxin, which is transducer of redox potential during illumination, activated ACCase more efficiently that DTT alone. Similarly to AGPase it was affected by both, thioredoxin *f* and *m*, but thioredoxin *f* was more effective than *m*. The ACCase was also activated by thioredoxin reduced enzymaticaly with NADPH and NADP-thioredoxin reductase. These findings suggest that the reduction of ACCase is needed for the activation of the enzyme, and a redox potential generated by photosynthesis is involved in its activation through thioredoxin as for the enzymes of the reductive pentose phosphate cycle (Sasaki et al., 1997). A redox cascade is likely to link between light and fatty acid synthesis, resulting in coordination of fatty acid synthesis with photosynthesis.

In this work novel *in-vivo* evidence was provided, showing that analogous to AGPase, the second input modulating posttranslational redox-regulation of leaf ACCase independently of light is sugar-related signal. Evidence is provided by long-term sucrose feeding to leaves in the dark (Fig. 24). ACCase was redox-activated what was accompanied by an increase in the rate of lipid synthesis and a decrease in acetyl-CoA level (Fig. 24, 25, 26). Importance of redox-regulation of fatty acid synthesis in response to sugars has not been studied before although fatty acid synthesis is also required in the absence of light. This strongly suggests that additional factors linked to the metabolism of sugars will be involved in regulating ACCase. The decrease in metabolite levels when ACCase is activated and lipid synthesis is stimulated indicates that sucrose provides a signal for the enzyme regulation without being metabolized. Further studies are needed to identify components of the signalling pathway leading to ACCase redox-activation in response to sugars and to answer the question whether similarly to AGPase regulation, trehalose feeding is also affecting lipid synthesis and ACCase activation in leaves and whether T6P supply to isolated chloroplasts has a direct effect on ACCase redox-state.

Little is known about the importance of redox-regulation of ACCase in lipid storing seeds. These seeds are green and photosynthetically active, and lipid synthesis has been shown to be stimulated in response to light in developing rape seeds (Ruuska et al., 2004).The major factors involved in this regulatory process still have to be resolved in seeds, but it may involve redox-activation of ACCase. Recent studies

made in our lab (P. Waldeck, H. Vigeolas, P. Geigenberger, unpublished) show, that, both, redox-activation of ACCase and flux to storage lipids were increased in a similar manner when rape seeds were subjected to light or to external feeding of DTT or sucrose *in planta*.

## 4.5.    Post-translational redox-regulation may control different pathways in leaves

DTT feeding was used as a systemic strategy to identify redox-regulated sites of metabolic pathways. In order to check if redox-modulation is of general consequence in leaf tissue, DTT feeding experiments were performed and metabolic and transcript profiles and carbon fluxes to variety of metabolites were analyzed. Obtained results showed that a short-term increase in the redox-state led to an increased flux of carbon into lipid, starch, cell wall, major respiratory pathways and amino acid synthesis without involving significant changes in the levels of major sugars like sucrose or glucose in leaves *in vivo* (Fig. 27). This provides the evidence that redox-modification can be involved in variety of metabolic pathways and control the key enzymes regulating these pathways in response to physiological and environmental inputs. This is consistent with the recent proteomic studies, which allowed the identification of a large number of redox-regulated targets that are involved in different metabolic pathways inside and outside the plastid (Balmer et al., 2003; Lee et al., 2004).

DTT feeding led to relatively small number of changes in the transcript profile, that mainly involved transport of C, N and S-compuonds, mitochondrial electron transport, some genes involved in amino acid transport and metabolism, protein folding and disulfide-bond formation and some genes coding for proteins involved in cell wall assembly. DTT also affected some of the genes coding for signalling components that may be involved in mediating changes in redox-state to key regulatory sites of metabolism (Fig. 28, 29 and Table 2, Appendix). Martinez and Chrispeels (2003) showed that DTT is responsible for UPR (unfolded protein resoponse) in *Arabidopsis* plants. When stress causes that protein folding in the endoplasmic reticulum slowes down, the temporary presence of an aboundance of unfolded proteins triggers the

UPR. The UPR results in enhanced expression of those genes known to encode proteins that create the optimal-folding environment, such as protein disulfide isomerase, calreticulin, clanexin and binding protein.

Interestingly, no significant changes were observed for genes coding for enzymes involved in central metabolic pathways (Fig. 28, 29 and Table 2 in Appendix) such as glycolysis, TCA cycle, starch metabolism, Calvin cycle, penthose phosphate pathway, most genes of amino acid metabolism. This indicates that the changes in metabolite levels and flux in these pathways obtained after DTT feeding were due to posttranslational redox-regulation rather than to changes in gene expression. For example, there was a decrease in α-ketoglutarate and an increase in succinate, which is consistent with α-ketoglutarate dehydrogenase being a redox-regulated step in the TCA cycle in plants as it is known from animal mitochondria (Bunik, 2003). Indeed, it is kown that α-ketoglutarate dehydrogenase is redox regulated by mitochondrial thioredoxins in animals, where thioredoxin efficiently protects the complexes from self-inactivation during catalysis at low $NAD^+$ (Bunik, 2003). α-ketoglutarate is also an important precursor for ammonia assimilation via GS/GOGAT (glutamine synthetase/ glutamate:oxoglutarate amino transferase) cycle. There are studies providing evidence that the two enzymes, Fd-GOGAT and GS2, are subject to thioredoxin-dependent redox-regulation *in vitro* (Choi et al., 1999; Motohashi et al., 2001; Balmer et al., 2003). The decrease of α-ketoglutarate while the levels of most of amino acids increased after DTT feeding to leaf discs may be indicative for reductive activation of the GS/GOGAT cycle *in situ*. It was also shown, that isocitrate level decreased after DTT feeding to leaf tissue. Another candidate enzyme from TCA cycle, which might be redox-regulated could therefore be isocitrate dehydrogenase. Proteomic studies using resin-bound *E.coli* thioredoxins to capture target proteins have shown that this enzyme is subject to redox-modification in bacteria (Kumar et al., 2004). Balmer et al. (2003) confirmed also some of already identified chloroplast thioredoxins targets in spinach, like Calvin cycle enzymes (sedoheptulose bisphosphatase, phosphoribulokinase, GAP dehydrogenase, Rubisco activase, Rubisco small subunit), nitrogen metabolism enzymes (glutamine synthetase), C4 cycle/malate valve (malate dehydrogenase) or fatty acid synthesis (ACCase) by proteomic analysis.

Similar to DTT feeding, sucrose has been shown to stimulate starch and fatty acid synthesis by promoting post-translational redox-regulation of key enzymes of these pathways. In contrast to DTT, sucrose led to these changes without increasing the overall redox-state of the tissue.

Long-term sucrose feeding increased sugars pool in the leaf tissue leading to stimulation of cell wall, amino acids and lipid synthesis and metabolites of second part of TCA cycle ($\alpha$-ketoglutarate, succinate, fumarate and malate increased). This was accompanied by a decrease in the levels of glycolytic metabolites and of metabolites from the the first half of TCA cycle (citrate, aconitate, isocitrate) (Fig. 30). The changes in metabolite levels were consistent with labelling studies using high-specific activity of $^{14}$C-glucose (see chapter 3.3) that were performed in parallel, revealing an increase in the flux to lipids, starch, cell wall, amino acids, organic acids and proteins.

Sucrose resembled DTT feeding in leading to a decrease in the levels of glycolytic intermediates and of metabolites of the first half of the TCA cycle and an increase of metabolites of the second half of TCA cycle. Some of sugars, which are cell wall components (arabinose, xylose, mannose, galactose) showed a tendency to increase similar to DTT feeding. Sucrose led to changes in the amino acids pattern, similar to DTT. For example, decrease in serine level and increase in cysteine content, decrease in aspartate amount and increase in arginine (Fig. 27, 30, 33).

Sucrose feeding led to massive changes of about 12% transcript levels being involved in most of metabolic pathways such as central metabolism, hormone metabolism, nucleotides metabolism, trehalose metabolism, N metabolism, secondary metabolism, stress response, redox regulation, protein folding (Fig. 31, 32 and Table 4, Appenidx). For plants in particular, carbohydrate-regulated genes represent an especially valuable mechamism for adjusting to environmental change. Plants are extremely sensitive and responsive to their surroundings because immobility leaves them few options for survival other than acclimation. Sugar concentrations vary over a wide range in plant tissues. This range typically exceeds that found in more homeostatic systems (such as mammalian blood stream) and provides plants with both a broader range of signals and a greater challenge to adjustment. Sugar-mediated changes in gene expression are also unique in plants

because changes in carbohydrate allocation can ultimately modulate form through processes affecting import/export balance (photosynthesis vs utilization) (Koch, 1996). However, there is an evidence suggesting that transcriptional regulation on its own is not an efficient method of altering pathway enzyme activities and fluxes. Geigenberger and Stitt (2000) shown that diurnal changes in AGPase and SuSy expression were not accompanied by changes in the maximal activities of the encoded enzymes. Both activities also remained high for several days after detaching tubers, even though transcripts fell to low levels within 24 h (Geigenberger et al., 1994). This indicates that transcriptional regulation in response to sucrose allows only gradual changes in enzyme activity, which require up to days to develop. Further, large changes in *AGPB* and *SUS1* transcripts were required to produce a significant decrease in AGPase or SuSy activity in antisense potato transformants (Müller-Röber et al., 1992; Zrenner et al., 1995). Studies of Smith et al. (2004) made on *Arabidopsis* leaves supported the results obtained for potato tubers showing the lack of corresponce between RNA and protein for some enzymes of starch metabolism (AGPase, $\alpha$-amylase, water dikinase and disproportionating enzyme-like protein). This indicates the importance of posttranslational control in regulating amounts of enzymes of starch metabolism and the fluxes throughout the key pathways (Gibon et al., 2004).

There were hardly any similarities concerning changes in transcripts between sucrose and DTT feeding. Sucrose led to a multitude of alterations in transcript levels, including many changes in the transcripts of genes involved in the pathways of central metabolism such as glycolysis, TCA, sucrose, starch and lipid metabolism. However, only a small part of the changes in transcripts that were observed after sucrose feeding were also obtained after DTT feeding (Fig. 34). This provides an evidence that posttranslational redox-modulation is of general importance for variety pathways of primary metabolism without the requirement of changes in transcript levels. In addition to direct regulation by changing redox-status of the cell, post-translational redox-regulation provides a mechanism that allows changes in activity of variety enzymes when sucrose accumulates in the leaf due to a decreased export to the growing sinks.

## 4.6.    Final conclusions

The results of this PhD thesis demonstrated that (I) leaf starch synthesis is regulated via post-translational  redox-modification of AGPase in response to light and sugars without involving changes in metabolite levels, (II) trehalose 6-phosphate provides a link between changes in sucrose status of the cytosol and redox-regulation of AGPase in plastid, (III) light and sugars also regulate lipid synthesis via redox-activation of ACCase, (IV) posttranslational redox-regulation rather than transcriptional regulation may also be involved in the redox-dependent short-term control of other important pathways of primary metabolism in leaves.

# Abbreviations

| | |
|---|---|
| **[U]-14C-glucose** | unilabelled 14-C glucose |
| **2PGA** | 2-phosphoglycerate |
| **3PGA** | 3-phosphoglycerate |
| **ACBP** | ACP-binding protein |
| **ACC** | carnitine acetyltransferase |
| **ACCase** | acetyl-coenzyme A carboxylase |
| **acetyl-CoA** | acetyl-coenzyme A |
| **ACL** | ATP-citrate lyase |
| **ACP** | pantathein-containing acyl carrier protein |
| **ACS** | acetyl-coenzyme A synthetase |
| **ADP** | adenosine 5'-diphosphate |
| **ADP-glucose** | adenosine 5'-diphospho-lucose |
| **AGPase** | ADP-glucose pyrophosphorylase |
| **AGPB** | catalycal subunit of AGPase |
| **AGPS** | regulatory subunit of AGPase |
| **AMP** | adenosine 5'-monophosphate |
| **ATP** | adenosin 5'-triphosphate |
| **BC** | biotin carboxylase |
| **BCCP** | biotin carboxyl carrier protein |
| **Bq** | bequerell (decays per second) |
| **BSA** | bovine serum albumine |
| **CAM** | crassulacean acid metabolism |
| **CF43** | tris-(perfluorobutyl)-amine |
| **Chl** | chlorophyll |
| **CoASH** | coenzyme A |
| **Col-0** | Columbia-0 |
| **CT** | carboxyltransferase |
| **DAG** | diacylglycerol |
| **DHAP** | dihydroxyacetone phosphate |
| **DPM** | disintegrations per minute |
| **DTT** | dithiotreitol |
| **E.coli** | Escherichia coli |
| **EDTA** | ethylenediamine tetraacetic acid |
| **EGTA** | ethylene glycol-bis(b-aminoethyl ether)-N,N,N,N-tetraacetic acid |
| **FBPase** | fructose 1.6-bisphosphatase |
| **Fd** | ferredoxin |
| **Fru1.6bisP** | fructose 1.6-bisphosphate |
| **Fru6P** | fructose 6-phosphate |
| **FTR** | ferredoxin:thioredoxin reductase |
| **FW** | fresh wieght |
| **G3P** | glycerol 3-phosphate |
| **G6PDH** | glucose 6-phosphate dehydrogenase |
| **GAP** | glyceraldehyde 3-phosphate |
| **GAPDH** | glyceraldehyde 3-phosphate dehydrogenase |
| **GBSS** | granule-bound starch synthase |
| **GC/MS** | gas chromatography/mass spectrometry |
| **Glc** | glucose |
| **Glc1.6bisP** | glucose 1.6-bisphosphate |
| **Glc1P** | glucose 1-phosphate |
| **Glc6P** | glucose 6-phosphate |
| **Gly3PDH** | gylcerol 3-phosphate dehydrogenase |
| **Gly3POX** | glycerol 3-phosphate oxidase |
| **h** | hour |
| **Hepes** | 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid |
| **Hexose-P** | hexose phosphates |
| **HK** | hexokinase |
| **HPLC** | high performance liquid chromatography |
| **KAS I** | $\beta$-ketoacyl-ACP reductase |

| | |
|---|---|
| **KAS II** | β-ketoacyl-ACP synthetase II |
| **KAS III** | β-ketoacyl-ACP synthetase III |
| **kDa** | kilodalton |
| **Km** | Michaelis constant |
| **LPA** | lysophosphatidic acid |
| **M** | molar concentration (mol/litre) |
| **MDH** | malate dehydrogenase |
| **Mes** | 4-morpholine-ethanesulphonic acid |
| **MSTFA** | N-methyl-N-(trimetylsilyl)trifluoroacetamide |
| **MTT** | 3-[4.5-dimethylthiazol-2-yl]-2.5-diphenyl tetrazolium bromide |
| **n** | number of measurements used to calculate the mean |
| **NAD+** | nicotinamide adenine dinucleotide (oxidized) |
| **NADH** | nicotinamide adenine dinucleotide (reduced) |
| **NADP+** | nicotinamide adenine dinucleotide phosphate (oxidized) |
| **NADPH** | nicotinamide adenine dinucleotide phosphate (reduced) |
| **PAGE** | polyacryloamide gel electrophoresis |
| **PC** | phosphatydylcholine |
| **PDC** | pyruvate dehydrogenase complex |
| **PEP** | phosphoenolpyruvate |
| **PGI** | phosphoglucoisomerase |
| **PGM** | phosphoglucomutase |
| **pH** | negative decaic logarithm of the proton concentration in mol per litre |
| **Pi** | inorganic orthophosphate |
| **PK** | pyruvate kinase |
| **PMS** | phenazine methosulphate |
| **Ppi** | inorganic orthophosphate |
| **Pyr DH** | pyruvate dehydrogenase |
| **Pyr kinase** | pyruvate kinase |
| **RNA** | ribonucleic acid |
| **RT** | room temperature |
| **Rubisco** | ribulose 1.5-bisphosphate carboxylase |
| **SBE** | starch branching enzyme |
| **SD** | standard deviation |
| **SDS** | sodium dodecylsulfate |
| **SE** | standard error |
| **SNF1** | sucrose non-fermeting protein kinase |
| **SnRK1** | SNF-like protein kinase |
| **SPPase** | sucrose-phosphate phosphatase |
| **SPS** | sucrose-phosphate synthase |
| **Suc** | sucrose |
| **T6P** | trehalose 6-phosphate |
| **TAG** | triacylglycerol |
| **TCA** | trichloroacetic acid |
| **TEMED** | N,N,N,N-tetramethyl ethylenediamine |
| **TLC** | thin layer chromatography |
| **TPP** | T6P phosphatase |
| **TPS** | T6P synthase |
| **TPT** | triose-phosphate transporter |
| **TPT** | triose-phosphate transporter |
| **TRE** | trehalase |
| **Tricine** | N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine |
| **Tris** | 2-amino-2-hydroxymethylpropane-1.3-diol |
| **U** | unit of enzyme activity (1μmol substrate per minute) |
| **UDP** | uridin diphosphate |
| **UGPase** | UDP-glucose pyrophosphorylase |
| **w/v** | weight per volume (g in 100 ml) |
| **w/w** | weight per weight |
| **Wt** | wild type |

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# Appendix

## GC/MS measurement of Arabidopsis leaves - DTT feeding

SUGAR ALCOHOLS

| AND SUGARS | CONTROL | | SE | 5mMDTT | | SE |
|---|---|---|---|---|---|---|
| arabinose | 1,000 | ± | 0,0111 | 1,2110 | ± | 0,0117 |
| erythritol | 1,000 | ± | 0,0094 | 1,2378 | ± | 0,1176 |
| fructose | 1,000 | ± | 0,0226 | 1,1311 | ± | 0,0211 |
| fucose | 1,000 | ± | 0,1684 | 1,3568 | ± | 0,0205 |
| galactose | 1,000 | ± | 0,0102 | 1,4900 | ± | 0,0095 |
| glucose | 1,000 | ± | 0,0336 | 1,0826 | ± | 0,0296 |
| glycerol | 1,000 | ± | 0,0345 | 1,1798 | ± | 0,0580 |
| isomaltose | 1,000 | ± | 0,0495 | 1,0867 | ± | 0,0625 |
| maltitol | 1,000 | ± | 0,0620 | 1,0569 | ± | 0,0634 |
| maltose | 1,000 | ± | 0,0481 | 1,0802 | ± | 0,0966 |
| mannose | 1,000 | ± | 0,0380 | 1,1323 | ± | 0,0266 |
| mannitol | 1,000 | ± | 0,0357 | 0,9491 | ± | 0,0533 |
| myo-inositol | 1,000 | ± | 0,0088 | 1,0064 | ± | 0,0040 |
| ononitol | 1,000 | ± | 0,0332 | 1,0313 | ± | 0,0544 |
| raffinose | 1,000 | ± | 0,5163 | 1,0001 | ± | 0,5427 |
| rhamnose | 1,000 | ± | 0,0331 | 0,9882 | ± | 0,0282 |
| ribose | 1,000 | ± | 0,0155 | 1,9542 | ± | 0,0239 |
| sorbitol/galactitol | 1,000 | ± | 0,0520 | 0,8623 | ± | 0,1360 |
| sucrose | 1,000 | ± | 0,0451 | 1,0587 | ± | 0,1147 |
| trehalose | 1,000 | ± | 0,0248 | 0,9796 | ± | 0,0241 |
| xylose | 1,000 | ± | 0,0175 | 1,1510 | ± | 0,0144 |

ORGANIC ACIDS

| | CONTROL | | SE | 5mMDTT | | SE |
|---|---|---|---|---|---|---|
| aconitate | 1,000 | ± | 0,1092 | 0,8412 | ± | 0,1921 |
| a-kt-glutarate | 1,000 | ± | 0,1687 | 0,8344 | ± | 0,1809 |
| a-kt-gulonate | 1,000 | ± | 0,1175 | 0,9455 | ± | 0,0879 |
| benzoate | 1,000 | ± | 0,0224 | 1,0922 | ± | 0,0255 |
| c-caffeate | 1,000 | ± | 0,1225 | 1,1511 | ± | 0,2115 |
| chlorogenate | 1,000 | ± | 0,1146 | 0,7503 | ± | 0,1911 |
| citramalate | 1,000 | ± | 0,0252 | 1,0684 | ± | 0,0173 |
| citrate | 1,000 | ± | 0,1160 | 0,9900 | ± | 0,1096 |
| c-sinapate | 1,000 | ± | 0,0897 | 0,8283 | ± | 0,1001 |
| dehydroascorbate | 1,000 | ± | 0,0685 | 1,2359 | ± | 0,0520 |
| D-isoascorbate | 1,000 | ± | 0,1901 | 1,7155 | ± | 0,1259 |
| fumarate | 1,000 | ± | 0,0200 | 1,0867 | ± | 0,0211 |
| galactonate | 1,000 | ± | 0,0413 | 0,9931 | ± | 0,0945 |
| galacturonate | 1,000 | ± | 0,0911 | 0,8998 | ± | 0,1189 |
| g-aminobutyrate | 1,000 | ± | 0,1810 | 0,8629 | ± | 0,1629 |
| gluconate | 1,000 | ± | 0,0185 | 0,8244 | ± | 0,0859 |
| glycerate | 1,000 | ± | 0,0117 | 1,0216 | ± | 0,0077 |
| gulonate | 1,000 | ± | 0,1748 | 0,9152 | ± | 0,1687 |
| isocitrate | 1,000 | ± | 0,1463 | 0,8881 | ± | 0,1313 |
| L-ascorbate | 1,000 | ± | 0,1128 | 1,8999 | ± | 0,0937 |
| maleate | 1,000 | ± | 0,0689 | 1,0269 | ± | 0,0742 |
| malate | 1,000 | ± | 0,0351 | 1,1257 | ± | 0,0492 |
| nicotinate | 1,000 | ± | 0,2579 | 1,1269 | ± | 0,3635 |
| phospate | 1,000 | ± | 0,1282 | 0,8578 | ± | 0,1540 |
| pyroglutamate | 1,000 | ± | 0,0570 | 0,8662 | ± | 0,0311 |
| quinate | 1,000 | ± | 0,2397 | 0,9786 | ± | 0,1908 |
| shikimate | 1,000 | ± | 0,0244 | 1,1968 | ± | 0,0433 |
| succinate | 1,000 | ± | 0,0112 | 1,0927 | ± | 0,0068 |
| threonate | 1,000 | ± | 0,0309 | 0,7965 | ± | 0,0400 |
| t-sinapate | 1,000 | ± | 0,1864 | 0,6911 | ± | 0,2059 |

| AMINO ACIDS | | | | | | |
|---|---|---|---|---|---|---|
| alanine | 1,000 | ± | 0,1522 | 2,0599 | ± | 0,0759 |
| arginine | 1,000 | ± | 0,0666 | 2,2987 | ± | 0,1302 |
| asparagine | 1,000 | ± | 0,1256 | 1,4950 | ± | 0,0849 |
| aspartate | 1,000 | ± | 0,2874 | 0,8530 | ± | 0,3684 |
| b-alanine | 1,000 | ± | 0,1372 | 0,9381 | ± | 0,1299 |
| cysteine | 1,000 | ± | 0,1044 | 1,3180 | ± | 0,1531 |
| glutamate | 1,000 | ± | 0,5199 | 0,5200 | ± | 0,7232 |
| glutamine | 1,000 | ± | 0,0917 | 1,0009 | ± | 0,0607 |
| glycine | 1,000 | ± | 0,0672 | 0,9924 | ± | 0,2592 |
| homocysteine | 1,000 | ± | 0,0731 | 1,3524 | ± | 0,0777 |
| homoserine | 1,000 | ± | 0,0445 | 1,2558 | ± | 0,0550 |
| isoleucine | 1,000 | ± | 0,0422 | 1,1922 | ± | 0,0509 |
| leucine | 1,000 | ± | 0,0228 | 1,2414 | ± | 0,0302 |
| lysine | 1,000 | ± | 0,1844 | 0,9822 | ± | 0,0906 |
| methionine | 1,000 | ± | 0,2189 | 1,0456 | ± | 0,1994 |
| N-acet-serine | 1,000 | ± | 0,0829 | 0,3587 | ± | 0,2912 |
| noradrenalin | 1,000 | ± | 0,3188 | 0,6726 | ± | 0,1381 |
| O-acet-serine | 1,000 | ± | 0,2816 | 0,3111 | ± | 0,1349 |
| ornithine | 1,000 | ± | 0,0528 | 1,0381 | ± | 0,0331 |
| phenylalanine | 1,000 | ± | 0,3964 | 0,9922 | ± | 0,4534 |
| proline | 1,000 | ± | 0,0565 | 1,1988 | ± | 0,0516 |
| putrescine | 1,000 | ± | 0,0719 | 0,8679 | ± | 0,0568 |
| serine | 1,000 | ± | 0,0351 | 0,7000 | ± | 0,0812 |
| spermidine | 1,000 | ± | 0,1504 | 0,8204 | ± | 0,0583 |
| HO-proline | 1,000 | ± | 0,0460 | 0,8686 | ± | 0,0179 |
| threonine | 1,000 | ± | 0,0465 | 1,0207 | ± | 0,0403 |
| tryptophan | 1,000 | ± | 0,1325 | 0,9917 | ± | 0,1498 |
| tyramine | 1,000 | ± | 0,0826 | 1,0656 | ± | 0,0715 |
| tyrosine | 1,000 | ± | 0,4752 | 1,4619 | ± | 0,2834 |
| uracil | 1,000 | ± | 0,1723 | 1,0755 | ± | 0,2215 |
| valine | 1,000 | ± | 0,0188 | 1,0942 | ± | 0,0212 |

**Table 1.** List of all known metabolites measured on GC/MS in *Arabidopsis* leaf extracts after incubation with buffer or 5mM DTT. Changes in metabolite levels calculated as the ratio between DTT and buffer incubated sample. Significant data (according to Student's t-test) are marked on red. SE calculated for n=5, p< or = 0.05.

| # | gene name | log2 ratio | | # | gene name | log2 ratio | | # | gene name | log2 ratio |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | At2g02990 | -2,84 | | 77 | At3g15820 | -0,84 | | 153 | At5g47220 | 0,82 |
| 2 | At1g04770 | -2,32 | | 78 | At3g54140 | -0,84 | | 154 | At4g14430 | 0,83 |
| 3 | At2g32860 | -2,28 | | 79 | At1g51790 | -0,84 | | 155 | At1g19025 | 0,84 |
| 4 | At2g47550 | -1,83 | | 80 | At2g38540 | -0,83 | | 156 | At3g49530 | 0,85 |
| 5 | At4g15610 | -1,67 | | 81 | At4g03390 | -0,83 | | 157 | At4g23810 | 0,87 |
| 6 | At5g24660 | -1,59 | | 82 | At5g46590 | -0,82 | | 158 | At5g47420 | 0,88 |
| 7 | At1g68290 | -1,50 | | 83 | At4g28350 | -0,82 | | 159 | At3g15760 | 0,90 |
| 8 | At4g05110 | -1,48 | | 84 | At5g55050 | -0,82 | | 160 | At1g29060 | 0,90 |
| 9 | At5g25450 | -1,46 | | 85 | At2g22470 | -0,81 | | 161 | At4g14420 | 0,91 |
| 10 | At3g16370 | -1,45 | | 86 | At4g37450 | -0,81 | | 162 | At4g14450 | 0,92 |
| 11 | At5g10820 | -1,43 | | 87 | At5g01380 | -0,80 | | 163 | At1g66090 | 0,93 |
| 12 | At2g47260 | -1,40 | | 88 | At3g07390 | -0,80 | | 164 | At5g50460 | 0,93 |
| 13 | At5g47330 | -1,39 | | 89 | At5g04250 | -0,80 | | 165 | At1g29310 | 0,94 |
| 14 | At4g27730 | -1,38 | | 90 | At1g79410 | -0,79 | | 166 | At5g14700 | 0,95 |
| 15 | At4g04610 | -1,36 | | 91 | At1g70160 | -0,79 | | 167 | At1g66500 | 0,98 |
| 16 | At5g48850 | -1,35 | | 92 | At2g01610 | -0,79 | | 168 | At5g54860 | 1,00 |
| 17 | At5g59580 | -1,34 | | 93 | At2g34510 | -0,78 | | 169 | At4g27657 | 1,06 |
| 18 | At1g51620 | -1,33 | | 94 | At1g78000 | -0,78 | | 170 | At5g67080 | 1,07 |
| 19 | At3g47380 | -1,29 | | 95 | At1g23800 | -0,78 | | 171 | At1g42980 | 1,10 |
| 20 | At4g21990 | -1,28 | | 96 | At3g51970 | -0,78 | | 172 | At5g52750 | 1,10 |
| 21 | At2g38010 | -1,23 | | 97 | At2g15080 | -0,77 | | 173 | At1g56340 | 1,12 |
| 22 | At2g22170 | -1,22 | | 98 | At4g21410 | -0,77 | | 174 | At4g16660 | 1,13 |
| 23 | At1g14780 | -1,22 | | 99 | At5g55090 | -0,76 | | 175 | At3g53670 | 1,13 |
| 24 | At4g21960 | -1,19 | | 100 | At3g52470 | -0,76 | | 176 | At1g09210 | 1,13 |
| 25 | At5g16970 | -1,18 | | 101 | At2g12290 | -0,76 | | 177 | At1g27330 | 1,14 |
| 26 | At3g54420 | -1,17 | | 102 | At1g21790 | -0,76 | | 178 | At2g25110 | 1,17 |
| 27 | At4g37990 | -1,15 | | 103 | At1g52400 | -0,76 | | 179 | At1g21750 | 1,17 |
| 28 | At1g74010 | -1,13 | | 104 | At2g26680 | -0,76 | | 180 | At2g32030 | 1,17 |
| 29 | At4g25260 | -1,12 | | 105 | At3g30775 | -0,75 | | 181 | At1g56580 | 1,17 |
| 30 | At1g22830 | -1,11 | | 106 | At2g40180 | -0,75 | | 182 | At4g29330 | 1,17 |
| 31 | At1g14890 | -1,11 | | 107 | At2g46800 | -0,74 | | 183 | At1g21120 | 1,21 |
| 32 | At2g43620 | -1,10 | | 108 | At3g61150 | -0,74 | | 184 | At1g64460 | 1,22 |
| 33 | At5g11410 | -1,10 | | 109 | At1g30820 | -0,74 | | 185 | At1g65040 | 1,24 |
| 34 | At5g12420 | -1,09 | | 110 | At4g03400 | -0,73 | | 186 | At5g60890 | 1,31 |
| 35 | At2g38250 | -1,09 | | 111 | At5g54300 | -0,73 | | 187 | At4g24190 | 1,32 |
| 36 | At1g26560 | -1,08 | | 112 | At5g13400 | -0,72 | | 188 | At1g21130 | 1,32 |
| 37 | At2g16630 | -1,08 | | 113 | At3g49580 | -0,72 | | 189 | At2g44660 | 1,33 |
| 38 | At3g04070 | -1,08 | | 114 | At5g12010 | -0,70 | | 190 | At5g07340 | 1,49 |
| 39 | At3g20570 | -1,07 | | 115 | At3g28210 | -0,67 | | 191 | At1g04980 | 1,56 |
| 40 | At3g47780 | -1,07 | | 116 | At2g42360 | -0,66 | | 192 | At3g53660 | 1,56 |
| 41 | At1g32860 | -1,06 | | 117 | At5g55180 | -0,65 | | 193 | At5g64905 | 1,57 |
| 42 | At4g34990 | -1,06 | | 118 | At2g18660 | -0,61 | | 194 | At3g62600 | 1,58 |
| 43 | At5g41800 | -1,06 | | 119 | At4g34230 | -0,60 | | 195 | At5g22290 | 1,61 |
| 44 | At1g70130 | -1,05 | | 120 | At5g08200 | -0,60 | | 196 | At1g77510 | 1,67 |
| 45 | At2g43510 | -1,04 | | 121 | At2g40230 | -0,59 | | 197 | At3g08970 | 1,87 |
| 46 | At2g39200 | -1,04 | | 122 | At3g14210 | -0,59 | | 198 | At3g24090 | 1,90 |
| 47 | At3g16670 | -1,04 | | 123 | At4g18170 | -0,58 | | 199 | At5g03160 | 1,96 |
| 48 | At5g55930 | -1,04 | | 124 | At3g60450 | -0,55 | | 200 | At2g02810 | 2,11 |
| 49 | At3g28740 | -1,03 | | 125 | At5g06570 | -0,54 | | 201 | At5g28540 | 2,17 |
| 50 | At2g48050 | -1,01 | | 126 | At1g71880 | -0,52 | | 202 | At5g61790 | 2,30 |
| 51 | At3g25180 | -1,01 | | 127 | At5g62000 | -0,40 | | 203 | At4g29520 | 2,57 |
| 52 | At1g35230 | -1,01 | | 128 | At4g17260 | 0,55 | | 204 | At1g14360 | 2,59 |
| 53 | At1g62510 | -1,00 | | 129 | At2g38210 | 0,56 | | 205 | At5g64510 | 2,71 |
| 54 | At3g54400 | -1,00 | | 130 | At1g26380 | 0,56 | | 206 | At4g34630 | 3,06 |
| 55 | At3g55740 | -1,00 | | 131 | At1g72280 | 0,59 | | | | |
| 56 | At1g51800 | -0,99 | | 132 | At1g30370 | 0,59 | | | | |
| 57 | At4g12730 | -0,96 | | 133 | At1g25370 | 0,62 | | | | |
| 58 | At3g63380 | -0,96 | | 134 | At1g54090 | 0,62 | | | | |
| 59 | At1g43160 | -0,94 | | 135 | At4g39950 | 0,62 | | | | |
| 60 | At1g62790 | -0,91 | | 136 | At5g03240 | 0,62 | | | | |
| 61 | At3g45060 | -0,91 | | 137 | At3g50770 | 0,64 | | | | |
| 62 | At3g10410 | -0,90 | | 138 | At5g35080 | 0,65 | | | | |
| 63 | At1g60960 | -0,90 | | 139 | At2g32920 | 0,67 | | | | |
| 64 | At3g10740 | -0,90 | | 140 | At4g24570 | 0,67 | | | | |
| 65 | At2g38530 | -0,90 | | 141 | At3g23170 | 0,67 | | | | |
| 66 | At3g48460 | -0,88 | | 142 | At2g36690 | 0,70 | | | | |
| 67 | At1g58270 | -0,88 | | 143 | At3g50920 | 0,72 | | | | |
| 68 | At5g50200 | -0,88 | | 144 | At2g38470 | 0,74 | | | | |
| 69 | At2g03470 | -0,87 | | 145 | At5g22250 | 0,76 | | | | |
| 70 | At1g69580 | -0,87 | | 146 | At2g17040 | 0,77 | | | | |
| 71 | At3g20510 | -0,87 | | 147 | At5g55620 | 0,77 | | | | |
| 72 | At1g10370 | -0,86 | | 148 | At1g18570 | 0,78 | | | | |
| 73 | At5g15740 | -0,86 | | 149 | At2g45790 | 0,79 | | | | |
| 74 | At1g21980 | -0,85 | | 150 | At2g47470 | 0,81 | | | | |
| 75 | At5g25100 | -0,85 | | 151 | At4g29960 | 0,81 | | | | |
| 76 | At2g43520 | -0,85 | | 152 | At5g48530 | 0,82 | | | | |

**Table 2.** Transcript profiling after DTT feeding. Data were normalized using RMA software, only detection values over 100 were treated as present and ratios were calculated. Presented values show ratio of DTT incubated sample in comparison to control in log2 scale. Expression data were filtered to select only genes showing a coinciding change-call in the two biological replicates samples for each experimental condition.

# GC/MS measurement of Arabidopsis leaves - sucrose feeding

| SUGARS AND SUGAR ALCOHOLS | control | | SE | 50mM sucrose | | SE | 100mM sucrose | | SE |
|---|---|---|---|---|---|---|---|---|---|
| arabinose | 1,000 | ± | 0,041 | 1,170 | ± | 0,019 | 1,275 | ± | 0,014 |
| erythritol | 1,000 | ± | 0,028 | 1,178 | ± | 0,028 | 1,715 | ± | 0,020 |
| fructose | 1,000 | ± | 0,037 | 11,993 | ± | 0,183 | 16,450 | ± | 0,010 |
| fucose | 1,000 | ± | 0,038 | 0,936 | ± | 0,010 | 0,832 | ± | 0,028 |
| galactose | 1,000 | ± | 0,081 | 1,703 | ± | 0,031 | 1,782 | ± | 0,091 |
| glucose | 1,000 | ± | 0,039 | 15,956 | ± | 0,027 | 17,367 | ± | 0,015 |
| glycerol | 1,000 | ± | 0,086 | 35,185 | ± | 0,199 | 1,165 | ± | 0,018 |
| isomaltose | 1,000 | ± | 0,433 | 8,645 | ± | 0,038 | 3,626 | ± | 0,030 |
| maltitol | 1,000 | ± | 0,109 | 1,874 | ± | 0,047 | 1,794 | ± | 0,049 |
| maltose | 1,000 | ± | 0,058 | 35,951 | ± | 0,017 | 64,488 | ± | 0,019 |
| mannose | 1,000 | ± | 0,130 | 1,918 | ± | 0,031 | 1,905 | ± | 0,031 |
| myo-inositol | 1,000 | ± | 0,031 | 1,028 | ± | 0,017 | 0,926 | ± | 0,013 |
| mannitol | 1,000 | ± | 0,059 | 0,501 | ± | 0,091 | 0,716 | ± | 0,128 |
| ononitol | 1,000 | ± | 0,084 | 1,063 | ± | 0,045 | 0,636 | ± | 0,081 |
| raffinose | 1,000 | ± | 0,135 | 0,500 | ± | 0,338 | 0,680 | ± | 0,229 |
| rhamnose | 1,000 | ± | 0,080 | 1,699 | ± | 0,026 | 1,498 | ± | 0,052 |
| ribose | 1,000 | ± | 0,049 | 1,268 | ± | 0,009 | 2,054 | ± | 0,018 |
| sorbitol/galactitol | 1,000 | ± | 0,178 | 5,135 | ± | 0,034 | 13,179 | ± | 0,016 |
| sucrose | 1,000 | ± | 0,036 | 5,757 | ± | 0,030 | 9,254 | ± | 0,026 |
| trehalose | 1,000 | ± | 0,036 | 1,988 | ± | 0,034 | 1,749 | ± | 0,037 |
| xylose | 1,000 | ± | 0,046 | 1,592 | ± | 0,016 | 1,761 | ± | 0,022 |
| | | | | | | | | | |
| **ORGANIC ACIDS** | | | | | | | | | |
| aconitate | 1,000 | ± | 0,058 | 0,764 | ± | 0,046 | 0,818 | ± | 0,026 |
| a-kt-glutarate | 1,000 | ± | 0,096 | 2,167 | ± | 0,040 | 3,148 | ± | 0,062 |
| a-kt-gulonate | 1,000 | ± | 0,096 | 0,730 | ± | 0,200 | 0,119 | ± | 0,322 |
| benzoate | 1,000 | ± | 0,019 | 0,943 | ± | 0,034 | 0,936 | ± | 0,018 |
| c-caffeate | 1,000 | ± | 0,344 | 2,244 | ± | 0,074 | 2,304 | ± | 0,059 |
| chlorogenate | 1,000 | ± | 0,811 | 0,086 | ± | 0,187 | 0,111 | ± | 0,209 |
| citramalate | 1,000 | ± | 0,033 | 1,139 | ± | 0,015 | 1,475 | ± | 0,017 |
| citrate | 1,000 | ± | 0,067 | 0,762 | ± | 0,034 | 0,848 | ± | 0,044 |
| c-sinapate | 1,000 | ± | 0,037 | 0,780 | ± | 0,030 | 0,881 | ± | 0,037 |
| dehydroascorbate | 1,000 | ± | 0,061 | 1,157 | ± | 0,034 | 0,522 | ± | 0,059 |
| D-isoascorbate | 1,000 | ± | 0,230 | 2,367 | ± | 0,211 | 3,000 | ± | 0,360 |
| fumarate | 1,000 | ± | 0,016 | 1,181 | ± | 0,030 | 1,117 | ± | 0,018 |
| galactonate | 1,000 | ± | 0,023 | 17,154 | ± | 0,022 | 23,563 | ± | 0,017 |
| galacturonate | 1,000 | ± | 0,062 | 1,055 | ± | 0,060 | 0,830 | ± | 0,057 |
| g-aminobutyrate | 1,000 | ± | 0,126 | 8,195 | ± | 0,064 | 19,753 | ± | 0,056 |
| gluconate | 1,000 | ± | 0,044 | 2,109 | ± | 0,018 | 4,602 | ± | 0,023 |
| glutarate | 1,000 | ± | 0,129 | 0,858 | ± | 0,050 | 0,815 | ± | 0,047 |
| glycerate | 1,000 | ± | 0,051 | 1,008 | ± | 0,019 | 1,469 | ± | 0,014 |
| gulonate | 1,000 | ± | 0,163 | 2,072 | ± | 0,187 | 4,291 | ± | 0,306 |
| isocitrate | 1,000 | ± | 0,024 | 0,662 | ± | 0,119 | 0,776 | ± | 0,044 |
| L-ascorbate | 1,000 | ± | 0,100 | 1,561 | ± | 0,018 | 0,122 | ± | 0,050 |
| maleate | 1,000 | ± | 0,058 | 1,055 | ± | 0,062 | 1,091 | ± | 0,056 |
| malate | 1,000 | ± | 0,030 | 1,414 | ± | 0,010 | 1,761 | ± | 0,015 |
| nicotinate | 1,000 | ± | 0,391 | 0,662 | ± | 0,228 | 0,854 | ± | 0,360 |
| phosphorate | 1,000 | ± | 0,115 | 0,789 | ± | 0,041 | 0,932 | ± | 0,058 |
| pyroglutamate | 1,000 | ± | 0,035 | 1,323 | ± | 0,027 | 1,212 | ± | 0,016 |
| quinate | 1,000 | ± | 0,466 | 0,449 | ± | 0,087 | 0,650 | ± | 0,298 |
| shikimate | 1,000 | ± | 0,055 | 1,314 | ± | 0,014 | 1,238 | ± | 0,024 |
| succinate | 1,000 | ± | 0,018 | 1,412 | ± | 0,030 | 1,389 | ± | 0,015 |
| t-caffeate | 1,000 | ± | 0,511 | 0,564 | ± | 0,182 | 0,873 | ± | 0,347 |
| t-ferulate | 1,000 | ± | 0,330 | 0,725 | ± | 0,173 | 0,856 | ± | 0,129 |
| threonate | 1,000 | ± | 0,030 | 0,681 | ± | 0,015 | 0,817 | ± | 0,018 |
| t-sinapate | 1,000 | ± | 0,079 | 0,757 | ± | 0,022 | 0,867 | ± | 0,042 |

AMINO ACIDS

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| alanine | 1,000 | ± | 0,093 | | 1,839 | ± | 0,041 | | 4,167 | ± | 0,033 |
| arginine | 1,000 | ± | 0,171 | | 1,666 | ± | 0,098 | | 2,530 | ± | 0,082 |
| asparagine | 1,000 | ± | 0,215 | | 1,413 | ± | 0,305 | | 1,410 | ± | 0,353 |
| aspartate | 1,000 | ± | 0,240 | | 0,531 | ± | 0,155 | | 0,415 | ± | 0,205 |
| b-alanine | 1,000 | ± | 0,142 | | 0,585 | ± | 0,066 | | 0,958 | ± | 0,068 |
| cysteine | 1,000 | ± | 0,290 | | 1,488 | ± | 0,404 | | 1,806 | ± | 0,411 |
| glutamate | 1,000 | ± | 0,308 | | 1,039 | ± | 0,263 | | 0,943 | ± | 0,300 |
| glutamine | 1,000 | ± | 0,307 | | 3,300 | ± | 0,235 | | 4,249 | ± | 0,191 |
| glycine | 1,000 | ± | 0,160 | | 2,596 | ± | 0,054 | | 2,981 | ± | 0,040 |
| homocysteine | 1,000 | ± | 0,134 | | 0,700 | ± | 0,299 | | 1,005 | ± | 0,134 |
| homoserine | 1,000 | ± | 0,057 | | 3,503 | ± | 0,046 | | 1,054 | ± | 0,048 |
| isoleucine | 1,000 | ± | 0,049 | | 0,564 | ± | 0,027 | | 0,786 | ± | 0,083 |
| leucine | 1,000 | ± | 0,049 | | 0,581 | ± | 0,020 | | 0,679 | ± | 0,046 |
| lysine | 1,000 | ± | 0,432 | | 0,697 | ± | 0,238 | | 1,150 | ± | 0,250 |
| methionine | 1,000 | ± | 0,146 | | 1,442 | ± | 0,136 | | 1,679 | ± | 0,149 |
| N-acet-serine | 1,000 | ± | 0,117 | | 0,261 | ± | 0,319 | | 0,404 | ± | 0,399 |
| noradrenalin | 1,000 | ± | 0,139 | | 1,453 | ± | 0,135 | | 5,148 | ± | 0,079 |
| O-acet-serine | 1,000 | ± | 0,130 | | 1,158 | ± | 0,178 | | 1,237 | ± | 0,142 |
| ornithine 3 | 1,000 | ± | 0,367 | | 1,462 | ± | 0,201 | | 2,359 | ± | 0,319 |
| ornithine 4 | 1,000 | ± | 0,056 | | 0,928 | ± | 0,027 | | 1,358 | ± | 0,120 |
| phenylalanine | 1,000 | ± | 0,297 | | 0,897 | ± | 0,147 | | 1,218 | ± | 0,266 |
| proline | 1,000 | ± | 0,046 | | 2,675 | ± | 0,061 | | 3,564 | ± | 0,059 |
| putrescine | 1,000 | ± | 0,064 | | 5,602 | ± | 0,161 | | 1,859 | ± | 0,538 |
| serine | 1,000 | ± | 0,057 | | 0,614 | ± | 0,016 | | 0,630 | ± | 0,036 |
| spermidine | 1,000 | ± | 0,213 | | 2,872 | ± | 0,586 | | 13,764 | ± | 0,735 |
| HO-proline | 1,000 | ± | 0,041 | | 1,515 | ± | 0,015 | | 1,355 | ± | 0,018 |
| threonine | 1,000 | ± | 0,058 | | 0,961 | ± | 0,031 | | 0,665 | ± | 0,048 |
| tryptophan | 1,000 | ± | 0,172 | | 3,760 | ± | 0,131 | | 1,249 | ± | 0,207 |
| tyramine | 1,000 | ± | 0,051 | | 0,551 | ± | 0,061 | | 0,883 | ± | 0,057 |
| tyrosine | 1,000 | ± | 0,485 | | 0,714 | ± | 0,267 | | 1,747 | ± | 0,318 |
| uracil | 1,000 | ± | 0,154 | | 0,663 | ± | 0,066 | | 1,008 | ± | 0,096 |
| valine | 1,000 | ± | 0,052 | | 0,854 | ± | 0,023 | | 1,139 | ± | 0,025 |

**Table 3.** List of all known metabolites measured on GC/MS in *Arabidopsis* leaf extracts after incubation in sorbitol or sucrose solutions. Changes in metabolite levels calculated as the ratio between sucrose and sorbitol incubated sample. Significant data (according to Student's t-test) are marked on red. SE calculated for n=5, p< or = 0.05.

| No. | gene name | log2 ratio | No. | gene name | log2 ratio | No. | gene name | log2 ratio |
|---|---|---|---|---|---|---|---|---|
| 1 | At5g18600 | -4,70 | 77 | At4g17245 | -2,35 | 153 | At1g43670 | -2,00 |
| 2 | At5g49730 | -4,53 | 78 | At3g02170 | -2,34 | 154 | At5g54130 | -1,98 |
| 3 | At4g35770 | -4,18 | 79 | At4g16410 | -2,34 | 155 | At3g47160 | -1,98 |
| 4 | At3g62410 | -4,05 | 80 | At2g05310 | -2,34 | 156 | At4g08920 | -1,98 |
| 5 | At1g71030 | -4,05 | 81 | At1g69530 | -2,33 | 157 | At3g22420 | -1,98 |
| 6 | At3g22210 | -4,04 | 82 | At3g16770 | -2,32 | 158 | At5g50335 | -1,98 |
| 7 | At5g49360 | -4,04 | 83 | At4g25080 | -2,31 | 159 | At1g09340 | -1,98 |
| 8 | At3g02380 | -3,99 | 84 | At5g45680 | -2,31 | 160 | At1g44000 | -1,98 |
| 9 | At4g33666 | -3,96 | 85 | At1g75100 | -2,30 | 161 | At4g38690 | -1,98 |
| 10 | At5g02160 | -3,76 | 86 | At1g15980 | -2,29 | 162 | At5g57655 | -1,97 |
| 11 | At1g53160 | -3,75 | 87 | At2g04795 | -2,29 | 163 | At4g09010 | -1,97 |
| 12 | At4g38840 | -3,69 | 88 | At2g40670 | -2,28 | 164 | At1g16260 | -1,96 |
| 13 | At5g14740 | -3,65 | 89 | At3g01500 | -2,28 | 165 | At4g09350 | -1,95 |
| 14 | At5g17300 | -3,64 | 90 | At2g15960 | -2,28 | 166 | At3g07310 | -1,95 |
| 15 | At5g35790 | -3,63 | 91 | At5g57340 | -2,28 | 167 | At5g16400 | -1,95 |
| 16 | At3g15450 | -3,58 | 92 | At1g26800 | -2,26 | 168 | At5g17170 | -1,94 |
| 17 | At5g24490 | -3,54 | 93 | At1g64720 | -2,26 | 169 | At3g45860 | -1,94 |
| 18 | At5g21170 | -3,52 | 94 | At4g18740 | -2,25 | 170 | At1g44446 | -1,94 |
| 19 | At1g08550 | -3,42 | 95 | At1g58290 | -2,25 | 171 | At5g59750 | -1,94 |
| 20 | At1g14150 | -3,25 | 96 | At3g55630 | -2,25 | 172 | At1g73870 | -1,94 |
| 21 | At2g41250 | -3,22 | 97 | At3g17040 | -2,24 | 173 | At3g10740 | -1,93 |
| 22 | At1g66100 | -3,20 | 98 | At5g57345 | -2,24 | 174 | At3g11930 | -1,93 |
| 23 | At4g39710 | -3,06 | 99 | At5g35490 | -2,23 | 175 | At3g53920 | -1,93 |
| 24 | At3g62550 | -3,03 | 100 | At1g18620 | -2,20 | 176 | At1g55000 | -1,93 |
| 25 | At1g13080 | -3,01 | 101 | At5g27290 | -2,20 | 177 | At4g25910 | -1,92 |
| 26 | At5g66490 | -2,99 | 102 | At1g55960 | -2,20 | 178 | At3g61060 | -1,92 |
| 27 | At3g16250 | -2,99 | 103 | At1g11260 | -2,20 | 179 | At1g10150 | -1,92 |
| 28 | At3g15850 | -2,92 | 104 | At4g28080 | -2,20 | 180 | At1g74840 | -1,91 |
| 29 | At2g25200 | -2,91 | 105 | At3g47560 | -2,19 | 181 | At5g58650 | -1,91 |
| 30 | At5g61590 | -2,88 | 106 | At2g39570 | -2,18 | 182 | At1g64500 | -1,89 |
| 31 | At4g36540 | -2,85 | 107 | At5g13770 | -2,18 | 183 | At5g57170 | -1,89 |
| 32 | At5g06870 | -2,85 | 108 | At4g13560 | -2,16 | 184 | At3g46780 | -1,89 |
| 33 | At2g39470 | -2,85 | 109 | At1g12250 | -2,16 | 185 | At3g56060 | -1,89 |
| 34 | At3g19800 | -2,84 | 110 | At5g60680 | -2,16 | 186 | At2g21560 | -1,89 |
| 35 | At1g30260 | -2,83 | 111 | At3g54050 | -2,16 | 187 | At1g50290 | -1,88 |
| 36 | At4g22570 | -2,82 | 112 | At5g52960 | -2,15 | 188 | At1g51400 | -1,88 |
| 37 | At1g53870 | -2,79 | 113 | At2g05540 | -2,14 | 189 | At1g76100 | -1,88 |
| 38 | At3g26510 | -2,77 | 114 | At4g30690 | -2,13 | 190 | At3g61080 | -1,88 |
| 39 | At1g65230 | -2,73 | 115 | At1g50730 | -2,13 | 191 | At5g15850 | -1,87 |
| 40 | At2g38210 | -2,70 | 116 | At3g01440 | -2,12 | 192 | At1g23390 | -1,85 |
| 41 | At5g44530 | -2,66 | 117 | At3g52070 | -2,12 | 193 | At1g73750 | -1,85 |
| 42 | At2g24550 | -2,64 | 118 | At2g15080 | -2,12 | 194 | At2g46830 | -1,85 |
| 43 | At1g02340 | -2,63 | 119 | At3g51420 | -2,11 | 195 | At4g27710 | -1,85 |
| 44 | At1g75690 | -2,61 | 120 | At3g13750 | -2,11 | 196 | At5g36700 | -1,84 |
| 45 | At5g64570 | -2,59 | 121 | At1g64360 | -2,11 | 197 | At5g38520 | -1,84 |
| 46 | At2g01590 | -2,59 | 122 | At3g55330 | -2,10 | 198 | At4g31820 | -1,84 |
| 47 | At1g80440 | -2,57 | 123 | At1g68520 | -2,10 | 199 | At3g26890 | -1,83 |
| 48 | At1g08980 | -2,57 | 124 | At2g30510 | -2,10 | 200 | At5g18630 | -1,83 |
| 49 | At2g03750 | -2,56 | 125 | At5g48790 | -2,09 | 201 | At2g24540 | -1,83 |
| 50 | At2g46220 | -2,56 | 126 | At5g45490 | -2,08 | 202 | At1g06460 | -1,83 |
| 51 | At5g17670 | -2,55 | 127 | At5g64040 | -2,08 | 203 | At3g17510 | -1,83 |
| 52 | At3g27690 | -2,55 | 128 | At4g05070 | -2,08 | 204 | At1g17200 | -1,82 |
| 53 | At2g18700 | -2,55 | 129 | At3g07350 | -2,07 | 205 | At5g64410 | -1,82 |
| 54 | At1g21500 | -2,53 | 130 | At1g03130 | -2,07 | 206 | At1g20020 | -1,82 |
| 55 | At5g40890 | -2,53 | 131 | At4g35090 | -2,07 | 207 | At4g00780 | -1,82 |
| 56 | At5g04810 | -2,53 | 132 | At1g32060 | -2,07 | 208 | At2g22190 | -1,82 |
| 57 | At2g44920 | -2,52 | 133 | At1g65490 | -2,05 | 209 | At1g60260 | -1,82 |
| 58 | At1g19150 | -2,52 | 134 | At1g72820 | -2,05 | 210 | At2g38820 | -1,81 |
| 59 | At5g20250 | -2,49 | 135 | At3g47430 | -2,04 | 211 | At1g66130 | -1,81 |
| 60 | At4g24700 | -2,49 | 136 | At4g19170 | -2,04 | 212 | At1g21920 | -1,81 |
| 61 | At5g58260 | -2,47 | 137 | At5g53490 | -2,03 | 213 | At4g10120 | -1,81 |
| 62 | At3g23700 | -2,45 | 138 | At4g27440 | -2,03 | 214 | At1g21680 | -1,80 |
| 63 | At3g47070 | -2,44 | 139 | At1g64680 | -2,03 | 215 | At5g14120 | -1,80 |
| 64 | At5g53160 | -2,44 | 140 | At1g35420 | -2,02 | 216 | At1g10740 | -1,80 |
| 65 | At2g34620 | -2,44 | 141 | At1g29440 | -2,02 | 217 | At3g51510 | -1,80 |
| 66 | At3g23080 | -2,43 | 142 | At1g80920 | -2,02 | 218 | At1g71695 | -1,79 |
| 67 | At1g10360 | -2,42 | 143 | At5g63800 | -2,02 | 219 | At3g56290 | -1,79 |
| 68 | At1g70760 | -2,41 | 144 | At1g12240 | -2,01 | 220 | At1g72150 | -1,79 |
| 69 | At3g13062 | -2,40 | 145 | At4g25050 | -2,01 | 221 | At5g11070 | -1,78 |
| 70 | At4g16690 | -2,40 | 146 | At2g42220 | -2,01 | 222 | At1g26665 | -1,78 |
| 71 | At5g02840 | -2,40 | 147 | At5g02120 | -2,00 | 223 | At1g71500 | -1,78 |
| 72 | At1g09415 | -2,39 | 148 | At1g16720 | -2,00 | 224 | At4g15630 | -1,78 |
| 73 | At1g75460 | -2,39 | 149 | At1g22740 | -2,00 | 225 | At3g26230 | -1,77 |
| 74 | At2g23590 | -2,37 | 150 | At3g21260 | -2,00 | 226 | At1g56220 | -1,77 |
| 75 | At5g02020 | -2,35 | 151 | At4g13830 | -2,00 | 227 | At5g40450 | -1,77 |
| 76 | At3g48200 | -2,35 | 152 | At4g02075 | -2,00 | 228 | At3g15760 | -1,77 |

| | gene name | log2 ratio | | | gene name | log2 ratio | | | gene name | log2 ratio | |
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| 229 | At4g24800 | -1,77 | | 305 | At4g38470 | -1,61 | | 381 | At1g07320 | -1,49 | |
| 230 | At5g44680 | -1,77 | | 306 | At5g53170 | -1,61 | | 382 | At3g26650 | -1,49 | |
| 231 | At1g03600 | -1,76 | | 307 | At5g18140 | -1,61 | | 383 | At5g02760 | -1,49 | |
| 232 | At1g22710 | -1,76 | | 308 | At4g14020 | -1,61 | | 384 | At1g21050 | -1,48 | |
| 233 | At1g32470 | -1,76 | | 309 | At3g10720 | -1,61 | | 385 | At1g05385 | -1,48 | |
| 234 | At2g40960 | -1,75 | | 310 | At5g42070 | -1,61 | | 386 | At2g07180 | -1,48 | |
| 235 | At4g27700 | -1,75 | | 311 | At4g39970 | -1,60 | | 387 | At5g38510 | -1,48 | |
| 236 | At2g39850 | -1,75 | | 312 | At2g33810 | -1,60 | | 388 | At2g36430 | -1,48 | |
| 237 | At4g22540 | -1,75 | | 313 | At4g34090 | -1,60 | | 389 | At1g05840 | -1,48 | |
| 238 | At3g55800 | -1,75 | | 314 | At1g73655 | -1,60 | | 390 | At4g10060 | -1,48 | |
| 239 | At2g28200 | -1,74 | | 315 | At3g25690 | -1,60 | | 391 | At1g33240 | -1,47 | |
| 240 | At5g24800 | -1,74 | | 316 | At1g52550 | -1,60 | | 392 | At3g16370 | -1,47 | |
| 241 | At1g49500 | -1,74 | | 317 | At1g68010 | -1,60 | | 393 | At3g61260 | -1,47 | |
| 242 | At5g42900 | -1,74 | | 318 | At4g19500 | -1,60 | | 394 | At5g59430 | -1,47 | |
| 243 | At3g59940 | -1,73 | | 319 | At4g23300 | -1,60 | | 395 | At1g27290 | -1,47 | |
| 244 | At5g62430 | -1,73 | | 320 | At1g80180 | -1,60 | | 396 | At1g69523 | -1,47 | |
| 245 | At5g52900 | -1,72 | | 321 | At5g55620 | -1,60 | | 397 | At5g54120 | -1,47 | |
| 246 | At2g21185 | -1,72 | | 322 | At5g25630 | -1,59 | | 398 | At1g03610 | -1,47 | |
| 247 | At4g33790 | -1,72 | | 323 | At1g01070 | -1,59 | | 399 | At5g56100 | -1,46 | |
| 248 | At5g44190 | -1,72 | | 324 | At4g31040 | -1,58 | | 400 | At5g53580 | -1,46 | |
| 249 | At4g14890 | -1,72 | | 325 | At4g34920 | -1,58 | | 401 | At5g02710 | -1,46 | |
| 250 | At1g07440 | -1,72 | | 326 | At3g08010 | -1,57 | | 402 | At1g58032 | -1,46 | |
| 251 | At1g69490 | -1,72 | | 327 | At2g39730 | -1,57 | | 403 | At5g15230 | -1,46 | |
| 252 | At3g02690 | -1,71 | | 328 | At5g63850 | -1,57 | | 404 | At4g23890 | -1,46 | |
| 253 | At5g01920 | -1,71 | | 329 | At2g15580 | -1,57 | | 405 | At2g05620 | -1,46 | |
| 254 | At5g09660 | -1,71 | | 330 | At1g42970 | -1,57 | | 406 | At1g29720 | -1,45 | |
| 255 | At5g18650 | -1,71 | | 331 | At4g00050 | -1,57 | | 407 | At1g49010 | -1,45 | |
| 256 | At3g13450 | -1,71 | | 332 | At3g47610 | -1,57 | | 408 | At3g01770 | -1,45 | |
| 257 | At1g75750 | -1,70 | | 333 | At1g76080 | -1,56 | | 409 | At1g01240 | -1,45 | |
| 258 | At2g03710 | -1,70 | | 334 | At1g52190 | -1,56 | | 410 | At1g13640 | -1,45 | |
| 259 | At2g15830 | -1,70 | | 335 | At5g24165 | -1,56 | | 411 | At5g05690 | -1,45 | |
| 260 | At2g42690 | -1,69 | | 336 | At4g03210 | -1,56 | | 412 | At4g23880 | -1,45 | |
| 261 | At5g66440 | -1,69 | | 337 | At1g73110 | -1,56 | | 413 | At3g06483 | -1,45 | |
| 262 | At5g64840 | -1,69 | | 338 | At5g12050 | -1,56 | | 414 | At3g01480 | -1,45 | |
| 263 | At1g74880 | -1,69 | | 339 | At2g48020 | -1,56 | | 415 | At1g54500 | -1,44 | |
| 264 | At2g47180 | -1,69 | | 340 | At5g24160 | -1,56 | | 416 | At3g48420 | -1,44 | |
| 265 | At5g19220 | -1,68 | | 341 | At3g20270 | -1,56 | | 417 | At5g02150 | -1,44 | |
| 266 | At1g78460 | -1,68 | | 342 | At1g17990 | -1,56 | | 418 | At4g03510 | -1,44 | |
| 267 | At5g47110 | -1,68 | | 343 | At1g14290 | -1,55 | | 419 | At5g48485 | -1,43 | |
| 268 | At4g37760 | -1,68 | | 344 | At3g27050 | -1,55 | | 420 | At2g44130 | -1,43 | |
| 269 | At3g11170 | -1,68 | | 345 | At1g51805 | -1,55 | | 421 | At1g64860 | -1,43 | |
| 270 | At3g21600 | -1,67 | | 346 | At5g64770 | -1,55 | | 422 | At2g39400 | -1,43 | |
| 271 | At5g26820 | -1,67 | | 347 | At5g64380 | -1,54 | | 423 | At1g19720 | -1,43 | |
| 272 | At5g24120 | -1,67 | | 348 | At5g59030 | -1,54 | | 424 | At4g19100 | -1,42 | |
| 273 | At5g39610 | -1,67 | | 349 | At3g26740 | -1,54 | | 425 | At4g17730 | -1,42 | |
| 274 | At4g28260 | -1,67 | | 350 | At4g33660 | -1,54 | | 426 | At4g14870 | -1,42 | |
| 275 | At5g35170 | -1,67 | | 351 | At5g58800 | -1,54 | | 427 | At1g05690 | -1,42 | |
| 276 | At3g21055 | -1,67 | | 352 | At5g16650 | -1,53 | | 428 | At2g28305 | -1,42 | |
| 277 | At2g37540 | -1,66 | | 353 | At3g50820 | -1,53 | | 429 | At1g66330 | -1,42 | |
| 278 | At2g18050 | -1,66 | | 354 | At2g25590 | -1,53 | | 430 | At3g25560 | -1,42 | |
| 279 | At1g18060 | -1,66 | | 355 | At4g01026 | -1,53 | | 431 | At2g36990 | -1,41 | |
| 280 | At1g62480 | -1,66 | | 356 | At1g52870 | -1,53 | | 432 | At3g14595 | -1,41 | |
| 281 | At3g48740 | -1,66 | | 357 | At3g49140 | -1,53 | | 433 | At1g64355 | -1,41 | |
| 282 | At1g12900 | -1,66 | | 358 | At2g45180 | -1,53 | | 434 | At1g72430 | -1,41 | |
| 283 | At5g66590 | -1,66 | | 359 | At2g45170 | -1,53 | | 435 | At1g28100 | -1,41 | |
| 284 | At2g26690 | -1,65 | | 360 | At2g29180 | -1,53 | | 436 | At4g23260 | -1,40 | |
| 285 | At3g22120 | -1,65 | | 361 | At3g29240 | -1,52 | | 437 | At1g01170 | -1,40 | |
| 286 | At1g78170 | -1,65 | | 362 | At4g35780 | -1,52 | | 438 | At5g39660 | -1,40 | |
| 287 | At3g47295 | -1,65 | | 363 | At5g38850 | -1,52 | | 439 | At5g58120 | -1,40 | |
| 288 | At1g07180 | -1,65 | | 364 | At2g19650 | -1,51 | | 440 | At4g18370 | -1,40 | |
| 289 | At4g25170 | -1,65 | | 365 | At1g16880 | -1,51 | | 441 | At2g13360 | -1,40 | |
| 290 | At2g47940 | -1,65 | | 366 | At1g29070 | -1,51 | | 442 | At4g09650 | -1,40 | |
| 291 | At3g23760 | -1,65 | | 367 | At2g02710 | -1,51 | | 443 | At3g19850 | -1,40 | |
| 292 | At2g29310 | -1,65 | | 368 | At2g21960 | -1,51 | | 444 | At1g32200 | -1,40 | |
| 293 | At3g18890 | -1,64 | | 369 | At3g19480 | -1,51 | | 445 | At5g23340 | -1,39 | |
| 294 | At3g54500 | -1,64 | | 370 | At5g63190 | -1,51 | | 446 | At2g29340 | -1,39 | |
| 295 | At4g37560 | -1,63 | | 371 | At5g37300 | -1,51 | | 447 | At2g32560 | -1,39 | |
| 296 | At4g24930 | -1,63 | | 372 | At5g09820 | -1,50 | | 448 | At3g17650 | -1,39 | |
| 297 | At5g16030 | -1,63 | | 373 | At3g15770 | -1,50 | | 449 | At4g20070 | -1,39 | |
| 298 | At2g01870 | -1,63 | | 374 | At1g54820 | -1,50 | | 450 | At4g17340 | -1,38 | |
| 299 | At1g78290 | -1,63 | | 375 | At3g19860 | -1,50 | | 451 | At3g49470 | -1,38 | |
| 300 | At1g32080 | -1,63 | | 376 | At2g40970 | -1,50 | | 452 | At1g60600 | -1,38 | |
| 301 | At5g25190 | -1,62 | | 377 | At1g72030 | -1,50 | | 453 | At5g52780 | -1,38 | |
| 302 | At1g51115 | -1,62 | | 378 | At4g27435 | -1,50 | | 454 | At1g15550 | -1,38 | |
| 303 | At2g41120 | -1,62 | | 379 | At4g14270 | -1,50 | | 455 | At4g19160 | -1,38 | |
| 304 | At1g14345 | -1,61 | | 380 | At5g48900 | -1,49 | | 456 | At1g71480 | -1,38 | |

| | gene name | log2 ratio | | | gene name | log2 ratio | | | gene name | log2 ratio | |
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| 457 | At3g27160 | -1,38 | | 533 | At3g01660 | -1,28 | | 609 | At4g02770 | -1,21 | |
| 458 | At3g16670 | -1,38 | | 534 | At4g01800 | -1,28 | | 610 | At2g36320 | -1,21 | |
| 459 | At2g17640 | -1,37 | | 535 | At4g33110 | -1,28 | | 611 | At3g01690 | -1,20 | |
| 460 | At1g56505 | -1,37 | | 536 | At5g48590 | -1,27 | | 612 | At5g49100 | -1,20 | |
| 461 | At2g18710 | -1,37 | | 537 | At1g17140 | -1,27 | | 613 | At3g04350 | -1,20 | |
| 462 | At3g29180 | -1,37 | | 538 | At2g03550 | -1,27 | | 614 | At1g67480 | -1,20 | |
| 463 | At2g40540 | -1,37 | | 539 | At5g44520 | -1,27 | | 615 | At1g76890 | -1,20 | |
| 464 | At2g29360 | -1,37 | | 540 | At3g15900 | -1,27 | | 616 | At1g74910 | -1,20 | |
| 465 | At5g19140 | -1,37 | | 541 | At2g35260 | -1,27 | | 617 | At5g10180 | -1,20 | |
| 466 | At5g10380 | -1,36 | | 542 | At3g08030 | -1,27 | | 618 | At1g64510 | -1,20 | |
| 467 | At4g23820 | -1,36 | | 543 | At5g13630 | -1,27 | | 619 | At1g26560 | -1,20 | |
| 468 | At4g01310 | -1,36 | | 544 | At1g67700 | -1,27 | | 620 | At2g06850 | -1,20 | |
| 469 | At1g48840 | -1,36 | | 545 | At1g09010 | -1,27 | | 621 | At2g33450 | -1,20 | |
| 470 | At5g41050 | -1,36 | | 546 | At3g07870 | -1,27 | | 622 | At3g56910 | -1,20 | |
| 471 | At3g16000 | -1,36 | | 547 | At3g60130 | -1,27 | | 623 | At3g46530 | -1,20 | |
| 472 | At3g56940 | -1,36 | | 548 | At4g17870 | -1,27 | | 624 | At4g26700 | -1,20 | |
| 473 | At1g07110 | -1,36 | | 549 | At1g13570 | -1,27 | | 625 | At1g51110 | -1,20 | |
| 474 | At4g38160 | -1,36 | | 550 | At2g37130 | -1,27 | | 626 | At3g62010 | -1,19 | |
| 475 | At1g79510 | -1,35 | | 551 | At5g02830 | -1,26 | | 627 | At3g01060 | -1,19 | |
| 476 | At4g22890 | -1,35 | | 552 | At1g31820 | -1,26 | | 628 | At3g57190 | -1,19 | |
| 477 | At5g25460 | -1,35 | | 553 | At5g28020 | -1,26 | | 629 | At1g66820 | -1,19 | |
| 478 | At5g10930 | -1,35 | | 554 | At1g08520 | -1,26 | | 630 | At4g19830 | -1,19 | |
| 479 | At5g19940 | -1,35 | | 555 | At3g62750 | -1,26 | | 631 | At4g32190 | -1,19 | |
| 480 | At1g20010 | -1,35 | | 556 | At1g58180 | -1,26 | | 632 | At2g36080 | -1,19 | |
| 481 | At3g60300 | -1,35 | | 557 | At5g14910 | -1,26 | | 633 | At2g38140 | -1,19 | |
| 482 | At4g34620 | -1,35 | | 558 | At1g04040 | -1,26 | | 634 | At1g04530 | -1,19 | |
| 483 | At5g42680 | -1,35 | | 559 | At5g27400 | -1,26 | | 635 | At4g29060 | -1,19 | |
| 484 | At3g23050 | -1,35 | | 560 | At4g37550 | -1,26 | | 636 | At5g14320 | -1,19 | |
| 485 | At1g53560 | -1,35 | | 561 | At3g44610 | -1,26 | | 637 | At3g47500 | -1,19 | |
| 486 | At5g04140 | -1,35 | | 562 | At2g42620 | -1,25 | | 638 | At1g33050 | -1,19 | |
| 487 | At1g30250 | -1,35 | | 563 | At5g57030 | -1,25 | | 639 | At5g17290 | -1,19 | |
| 488 | At1g74940 | -1,34 | | 564 | At3g05900 | -1,25 | | 640 | At1g63180 | -1,18 | |
| 489 | At2g22550 | -1,34 | | 565 | At2g21330 | -1,25 | | 641 | At4g28025 | -1,18 | |
| 490 | At3g54890 | -1,34 | | 566 | At5g64240 | -1,25 | | 642 | At5g23920 | -1,18 | |
| 491 | At3g28270 | -1,34 | | 567 | At4g11360 | -1,25 | | 643 | At5g47370 | -1,18 | |
| 492 | At3g18780 | -1,34 | | 568 | At2g33480 | -1,25 | | 644 | At5g47650 | -1,18 | |
| 493 | At4g27800 | -1,34 | | 569 | At2g03530 | -1,25 | | 645 | At5g18170 | -1,18 | |
| 494 | At4g39090 | -1,34 | | 570 | At5g24890 | -1,25 | | 646 | At3g48530 | -1,18 | |
| 495 | At1g54780 | -1,34 | | 571 | At4g24810 | -1,24 | | 647 | At3g29185 | -1,18 | |
| 496 | At5g16110 | -1,34 | | 572 | At5g51110 | -1,24 | | 648 | At3g12730 | -1,18 | |
| 497 | At4g27130 | -1,34 | | 573 | At2g27050 | -1,24 | | 649 | At5g23120 | -1,18 | |
| 498 | At1g12780 | -1,34 | | 574 | At5g16540 | -1,24 | | 650 | At1g18460 | -1,18 | |
| 499 | At4g04040 | -1,33 | | 575 | At3g26060 | -1,24 | | 651 | At5g49660 | -1,18 | |
| 500 | At1g42550 | -1,33 | | 576 | At5g14920 | -1,24 | | 652 | At5g52570 | -1,17 | |
| 501 | At4g02440 | -1,33 | | 577 | At3g26710 | -1,24 | | 653 | At1g32520 | -1,17 | |
| 502 | At1g10470 | -1,33 | | 578 | At3g53960 | -1,24 | | 654 | At1g02860 | -1,17 | |
| 503 | At4g00560 | -1,33 | | 579 | At4g38860 | -1,24 | | 655 | At5g08050 | -1,17 | |
| 504 | At1g75180 | -1,33 | | 580 | At2g20500 | -1,24 | | 656 | At2g04039 | -1,17 | |
| 505 | At3g56680 | -1,33 | | 581 | At1g47530 | -1,24 | | 657 | At1g19680 | -1,17 | |
| 506 | At4g01330 | -1,32 | | 582 | At3g20820 | -1,24 | | 658 | At2g46900 | -1,17 | |
| 507 | At5g20220 | -1,31 | | 583 | At3g16520 | -1,24 | | 659 | At3g59400 | -1,17 | |
| 508 | At5g14410 | -1,31 | | 584 | At1g19450 | -1,24 | | 660 | At1g05140 | -1,17 | |
| 509 | At1g47270 | -1,31 | | 585 | At5g16120 | -1,23 | | 661 | At4g15560 | -1,17 | |
| 510 | At1g74470 | -1,30 | | 586 | At1g01140 | -1,23 | | 662 | At2g29980 | -1,17 | |
| 511 | At2g41560 | -1,30 | | 587 | At4g04955 | -1,23 | | 663 | At5g54980 | -1,17 | |
| 512 | At1g18730 | -1,30 | | 588 | At4g31850 | -1,23 | | 664 | At4g00880 | -1,17 | |
| 513 | At3g48720 | -1,30 | | 589 | At3g16220 | -1,23 | | 665 | At5g38140 | -1,17 | |
| 514 | At1g33110 | -1,30 | | 590 | At4g30950 | -1,23 | | 666 | At2g45850 | -1,16 | |
| 515 | At5g25840 | -1,30 | | 591 | At4g33670 | -1,23 | | 667 | At1g01770 | -1,16 | |
| 516 | At1g78820 | -1,30 | | 592 | At5g14080 | -1,23 | | 668 | At5g14940 | -1,16 | |
| 517 | At5g67420 | -1,29 | | 593 | At3g20060 | -1,23 | | 669 | At2g18280 | -1,16 | |
| 518 | At2g26500 | -1,29 | | 594 | At1g59840 | -1,23 | | 670 | At5g17870 | -1,16 | |
| 519 | At5g44420 | -1,29 | | 595 | At2g17550 | -1,23 | | 671 | At1g63800 | -1,16 | |
| 520 | At5g62350 | -1,29 | | 596 | At1g62750 | -1,23 | | 672 | At5g10470 | -1,16 | |
| 521 | At5g36170 | -1,29 | | 597 | At5g58480 | -1,22 | | 673 | At2g16350 | -1,16 | |
| 522 | At1g47960 | -1,29 | | 598 | At5g13140 | -1,22 | | 674 | At5g57960 | -1,16 | |
| 523 | At3g10020 | -1,28 | | 599 | At1g32450 | -1,22 | | 675 | At1g76560 | -1,16 | |
| 524 | At3g30720 | -1,28 | | 600 | At1g02280 | -1,22 | | 676 | At5g47390 | -1,16 | |
| 525 | At2g46710 | -1,28 | | 601 | At1g78630 | -1,22 | | 677 | At4g22200 | -1,15 | |
| 526 | At4g21810 | -1,28 | | 602 | At4g19530 | -1,21 | | 678 | At1g08570 | -1,15 | |
| 527 | At5g06980 | -1,28 | | 603 | At1g18010 | -1,21 | | 679 | At1g01620 | -1,15 | |
| 528 | At4g24770 | -1,28 | | 604 | At2g21210 | -1,21 | | 680 | At4g09830 | -1,15 | |
| 529 | At3g55130 | -1,28 | | 605 | At1g26230 | -1,21 | | 681 | At4g14385 | -1,15 | |
| 530 | At3g62190 | -1,28 | | 606 | At5g46840 | -1,21 | | 682 | At2g42890 | -1,15 | |
| 531 | At2g35370 | -1,28 | | 607 | At1g26920 | -1,21 | | 683 | At2g23840 | -1,15 | |
| 532 | At2g43010 | -1,28 | | 608 | At1g01360 | -1,21 | | 684 | At2g40460 | -1,15 | |

| | gene name | log2 ratio | | | gene name | log2 ratio | | | gene name | log2 ratio | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 685 | At4g30310 | -1,15 | | 761 | At5g62140 | -1,09 | | 837 | At1g19670 | -1,03 | |
| 686 | At1g60010 | -1,15 | | 762 | At1g35680 | -1,09 | | 838 | At3g14415 | -1,03 | |
| 687 | At3g04760 | -1,15 | | 763 | At5g24520 | -1,09 | | 839 | At4g02510 | -1,03 | |
| 688 | At3g47650 | -1,15 | | 764 | At4g14930 | -1,08 | | 840 | At2g26340 | -1,03 | |
| 689 | At1g28150 | -1,15 | | 765 | At5g22500 | -1,08 | | 841 | At3g13120 | -1,03 | |
| 690 | At3g22460 | -1,15 | | 766 | At3g59060 | -1,08 | | 842 | At3g56010 | -1,03 | |
| 691 | At5g24610 | -1,15 | | 767 | At2g45820 | -1,08 | | 843 | At5g40950 | -1,03 | |
| 692 | At3g15840 | -1,14 | | 768 | At3g18390 | -1,08 | | 844 | At5g23050 | -1,03 | |
| 693 | At1g44920 | -1,14 | | 769 | At2g29400 | -1,08 | | 845 | At1g55850 | -1,03 | |
| 694 | At5g13650 | -1,14 | | 770 | At1g26580 | -1,08 | | 846 | At4g26860 | -1,02 | |
| 695 | At5g07010 | -1,14 | | 771 | At1g12280 | -1,08 | | 847 | At3g21760 | -1,02 | |
| 696 | At4g36730 | -1,14 | | 772 | At2g30170 | -1,08 | | 848 | At4g25300 | -1,02 | |
| 697 | At2g33250 | -1,14 | | 773 | At3g52230 | -1,08 | | 849 | At4g31720 | -1,02 | |
| 698 | At2g16380 | -1,14 | | 774 | At1g74970 | -1,07 | | 850 | At4g22710 | -1,02 | |
| 699 | At3g27770 | -1,14 | | 775 | At5g64880 | -1,07 | | 851 | At4g18230 | -1,02 | |
| 700 | At5g67260 | -1,14 | | 776 | At2g26430 | -1,07 | | 852 | At1g66890 | -1,02 | |
| 701 | At5g19530 | -1,14 | | 777 | At2g40490 | -1,07 | | 853 | At5g04790 | -1,02 | |
| 702 | At1g01790 | -1,14 | | 778 | At2g20920 | -1,07 | | 854 | At5g59250 | -1,02 | |
| 703 | At4g39320 | -1,14 | | 779 | At1g52510 | -1,07 | | 855 | At1g53510 | -1,02 | |
| 704 | At1g51440 | -1,14 | | 780 | At5g06710 | -1,07 | | 856 | At1g68590 | -1,02 | |
| 705 | At5g61410 | -1,13 | | 781 | At1g22750 | -1,07 | | 857 | At2g03730 | -1,02 | |
| 706 | At5g62900 | -1,13 | | 782 | At1g45474 | -1,07 | | 858 | At2g41290 | -1,02 | |
| 707 | At2g45740 | -1,13 | | 783 | At2g37460 | -1,07 | | 859 | At1g29670 | -1,02 | |
| 708 | At4g21280 | -1,13 | | 784 | At3g04730 | -1,07 | | 860 | At5g23660 | -1,01 | |
| 709 | At1g52220 | -1,13 | | 785 | At1g16080 | -1,07 | | 861 | At1g73660 | -1,01 | |
| 710 | At1g67740 | -1,13 | | 786 | At1g34000 | -1,06 | | 862 | At2g36835 | -1,01 | |
| 711 | At1g60160 | -1,13 | | 787 | At1g52740 | -1,06 | | 863 | At3g15780 | -1,01 | |
| 712 | At1g02205 | -1,12 | | 788 | At5g36250 | -1,06 | | 864 | At1g18400 | -1,01 | |
| 713 | At3g26900 | -1,12 | | 789 | At4g17810 | -1,06 | | 865 | At3g51370 | -1,01 | |
| 714 | At3g51840 | -1,12 | | 790 | At2g01420 | -1,06 | | 866 | At4g17600 | -1,01 | |
| 715 | At1g49470 | -1,12 | | 791 | At5g40500 | -1,06 | | 867 | At4g36530 | -1,01 | |
| 716 | At2g40110 | -1,12 | | 792 | At4g34350 | -1,06 | | 868 | At1g20340 | -1,01 | |
| 717 | At5g20380 | -1,12 | | 793 | At1g05890 | -1,06 | | 869 | At1g61180 | -1,01 | |
| 718 | At3g08940 | -1,12 | | 794 | At2g35800 | -1,06 | | 870 | At2g03390 | -1,01 | |
| 719 | At1g63880 | -1,12 | | 795 | At2g43680 | -1,06 | | 871 | At5g27360 | -1,01 | |
| 720 | At5g43750 | -1,12 | | 796 | At4g16515 | -1,05 | | 872 | At5g64460 | -1,01 | |
| 721 | At3g60910 | -1,12 | | 797 | At3g17770 | -1,05 | | 873 | At5g50915 | -1,01 | |
| 722 | At5g35480 | -1,12 | | 798 | At2g26020 | -1,05 | | 874 | At1g04620 | -1,01 | |
| 723 | At1g70580 | -1,12 | | 799 | At1g19660 | -1,05 | | 875 | At1g01490 | -1,01 | |
| 724 | At3g10230 | -1,12 | | 800 | At3g16857 | -1,05 | | 876 | At1g10660 | -1,01 | |
| 725 | At3g26170 | -1,11 | | 801 | At4g28030 | -1,05 | | 877 | At5g05740 | -1,01 | |
| 726 | At2g43920 | -1,11 | | 802 | At5g51540 | -1,05 | | 878 | At5g54630 | -1,01 | |
| 727 | At4g01050 | -1,11 | | 803 | At5g45950 | -1,05 | | 879 | At3g63490 | -1,01 | |
| 728 | At2g02100 | -1,11 | | 804 | At5g17400 | -1,05 | | 880 | At4g18240 | -1,01 | |
| 729 | At5g27950 | -1,11 | | 805 | At4g17560 | -1,05 | | 881 | At1g31330 | -1,00 | |
| 730 | At1g11350 | -1,11 | | 806 | At3g15520 | -1,05 | | 882 | At2g17710 | -1,00 | |
| 731 | At5g46330 | -1,11 | | 807 | At2g38800 | -1,05 | | 883 | At4g17880 | -1,00 | |
| 732 | At3g26520 | -1,11 | | 808 | At3g54210 | -1,05 | | 884 | At3g04910 | -1,00 | |
| 733 | At2g18440 | -1,11 | | 809 | At3g46600 | -1,04 | | 885 | At2g38330 | -1,00 | |
| 734 | At5g08430 | -1,10 | | 810 | At5g18640 | -1,04 | | 886 | At4g37470 | -1,00 | |
| 735 | At5g63180 | -1,10 | | 811 | At5g61670 | -1,04 | | 887 | At1g09280 | -1,00 | |
| 736 | At4g33470 | -1,10 | | 812 | At1g20070 | -1,04 | | 888 | At5g08650 | -1,00 | |
| 737 | At5g46110 | -1,10 | | 813 | At5g19500 | -1,04 | | 889 | At1g67860 | -1,00 | |
| 738 | At3g62650 | -1,10 | | 814 | At3g58670 | -1,04 | | 890 | At1g80380 | -1,00 | |
| 739 | At1g24340 | -1,10 | | 815 | At1g03520 | -1,04 | | 891 | At4g33500 | -1,00 | |
| 740 | At4g27600 | -1,10 | | 816 | At5g24150 | -1,04 | | 892 | At2g43560 | -1,00 | |
| 741 | At1g76450 | -1,10 | | 817 | At4g24750 | -1,04 | | 893 | At5g54600 | -1,00 | |
| 742 | At1g50900 | -1,10 | | 818 | At1g17220 | -1,04 | | 894 | At2g22240 | -1,00 | |
| 743 | At5g23210 | -1,10 | | 819 | At5g04040 | -1,04 | | 895 | At2g32150 | -1,00 | |
| 744 | At5g62470 | -1,10 | | 820 | At3g25920 | -1,04 | | 896 | At3g09600 | -0,99 | |
| 745 | At3g54920 | -1,10 | | 821 | At4g19410 | -1,04 | | 897 | At4g09890 | -0,99 | |
| 746 | At1g75450 | -1,10 | | 822 | At3g06380 | -1,04 | | 898 | At1g53885 | -0,99 | |
| 747 | At5g03470 | -1,10 | | 823 | At1g05805 | -1,04 | | 899 | At3g62980 | -0,99 | |
| 748 | At3g57320 | -1,09 | | 824 | At2g39450 | -1,04 | | 900 | At4g32060 | -0,99 | |
| 749 | At3g03940 | -1,09 | | 825 | At5g51010 | -1,04 | | 901 | At5g49630 | -0,99 | |
| 750 | At5g35970 | -1,09 | | 826 | At1g35670 | -1,04 | | 902 | At3g25070 | -0,99 | |
| 751 | At4g15780 | -1,09 | | 827 | At1g21640 | -1,04 | | 903 | At2g21530 | -0,99 | |
| 752 | At1g19330 | -1,09 | | 828 | At4g26400 | -1,03 | | 904 | At5g66190 | -0,99 | |
| 753 | At1g70410 | -1,09 | | 829 | At4g39330 | -1,03 | | 905 | At4g08330 | -0,98 | |
| 754 | At4g15545 | -1,09 | | 830 | At3g12320 | -1,03 | | 906 | At1g63690 | -0,98 | |
| 755 | At5g47330 | -1,09 | | 831 | At1g70610 | -1,03 | | 907 | At3g21670 | -0,98 | |
| 756 | At4g31390 | -1,09 | | 832 | At1g04350 | -1,03 | | 908 | At3g21390 | -0,98 | |
| 757 | At2g04690 | -1,09 | | 833 | At1g32990 | -1,03 | | 909 | At3g24430 | -0,98 | |
| 758 | At4g25570 | -1,09 | | 834 | At1g15740 | -1,03 | | 910 | At1g04640 | -0,98 | |
| 759 | At1g19000 | -1,09 | | 835 | At3g06750 | -1,03 | | 911 | At1g28960 | -0,98 | |
| 760 | At3g63140 | -1,09 | | 836 | | | | 912 | At1g55480 | -0,98 | |

| | gene name | log2 ratio | | | gene name | log2 ratio | | | gene name | log2 ratio | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 913 | At1g67050 | -0,98 | | 989 | At3g05890 | -0,92 | | 1065 | At2g33800 | -0,87 | |
| 914 | At5g35330 | -0,98 | | 990 | At1g07700 | -0,92 | | 1066 | At2g38780 | -0,87 | |
| 915 | At5g08410 | -0,98 | | 991 | At2g01620 | -0,92 | | 1067 | At1g49380 | -0,86 | |
| 916 | At2g38460 | -0,97 | | 992 | At3g10770 | -0,92 | | 1068 | At1g14270 | -0,86 | |
| 917 | At5g64920 | -0,97 | | 993 | At5g22875 | -0,92 | | 1069 | At5g06290 | -0,86 | |
| 918 | At2g05380 | -0,97 | | 994 | At4g32980 | -0,92 | | 1070 | At1g12520 | -0,86 | |
| 919 | At4g35250 | -0,97 | | 995 | At1g12800 | -0,92 | | 1071 | At3g14660 | -0,86 | |
| 920 | At2g03420 | -0,97 | | 996 | At4g33010 | -0,92 | | 1072 | At1g78150 | -0,86 | |
| 921 | At1g07010 | -0,97 | | 997 | At5g41700 | -0,92 | | 1073 | At3g20230 | -0,86 | |
| 922 | At2g23390 | -0,97 | | 998 | At2g25080 | -0,92 | | 1074 | At1g75080 | -0,86 | |
| 923 | At3g60070 | -0,97 | | 999 | At2g17040 | -0,92 | | 1075 | At4g14480 | -0,86 | |
| 924 | At2g30390 | -0,97 | | 1000 | At3g20550 | -0,92 | | 1076 | At1g24440 | -0,86 | |
| 925 | At4g19510 | -0,97 | | 1001 | At3g55080 | -0,92 | | 1077 | At1g77490 | -0,86 | |
| 926 | At1g60000 | -0,97 | | 1002 | At1g23310 | -0,92 | | 1078 | At2g39420 | -0,86 | |
| 927 | At4g23400 | -0,96 | | 1003 | At5g58770 | -0,91 | | 1079 | At1g51500 | -0,85 | |
| 928 | At1g70660 | -0,96 | | 1004 | At5g23870 | -0,91 | | 1080 | At2g27290 | -0,85 | |
| 929 | At5g66030 | -0,96 | | 1005 | At1g32700 | -0,91 | | 1081 | At5g55300 | -0,85 | |
| 930 | At2g15695 | -0,96 | | 1006 | At5g47190 | -0,91 | | 1082 | At3g23640 | -0,85 | |
| 931 | At1g78180 | -0,96 | | 1007 | At1g75220 | -0,91 | | 1083 | At2g36900 | -0,85 | |
| 932 | At2g28800 | -0,96 | | 1008 | At4g14210 | -0,91 | | 1084 | At2g37850 | -0,85 | |
| 933 | At3g26590 | -0,96 | | 1009 | At5g13740 | -0,91 | | 1085 | At1g80960 | -0,85 | |
| 934 | At5g42310 | -0,96 | | 1010 | At1g15730 | -0,91 | | 1086 | At4g31310 | -0,85 | |
| 935 | At3g09210 | -0,96 | | 1011 | At2g06520 | -0,91 | | 1087 | At2g31750 | -0,85 | |
| 936 | At2g46910 | -0,96 | | 1012 | At5g05080 | -0,91 | | 1088 | At4g29380 | -0,85 | |
| 937 | At5g58570 | -0,96 | | 1013 | At1g28410 | -0,91 | | 1089 | At4g18910 | -0,84 | |
| 938 | At1g65190 | -0,96 | | 1014 | At1g14280 | -0,91 | | 1090 | At5g07580 | -0,84 | |
| 939 | At1g75410 | -0,96 | | 1015 | At5g05800 | -0,91 | | 1091 | At5g37260 | -0,84 | |
| 940 | At5g19430 | -0,96 | | 1016 | At2g31810 | -0,91 | | 1092 | At1g66150 | -0,84 | |
| 941 | At1g72450 | -0,96 | | 1017 | At2g32500 | -0,90 | | 1093 | At2g31040 | -0,84 | |
| 942 | At1g50250 | -0,96 | | 1018 | At5g44410 | -0,90 | | 1094 | At1g01060 | -0,84 | |
| 943 | At1g60950 | -0,96 | | 1019 | At1g56280 | -0,90 | | 1095 | At1g64150 | -0,84 | |
| 944 | At5g39030 | -0,96 | | 1020 | At4g16330 | -0,90 | | 1096 | At1g34640 | -0,84 | |
| 945 | At2g22310 | -0,95 | | 1021 | At5g20935 | -0,90 | | 1097 | At4g19985 | -0,84 | |
| 946 | At1g67330 | -0,95 | | 1022 | At2g20180 | -0,90 | | 1098 | At2g42580 | -0,84 | |
| 947 | At4g35020 | -0,95 | | 1023 | At3g61490 | -0,90 | | 1099 | At3g52380 | -0,84 | |
| 948 | At1g48350 | -0,95 | | 1024 | At2g32440 | -0,90 | | 1100 | At1g73500 | -0,83 | |
| 949 | At1g70530 | -0,95 | | 1025 | At1g17160 | -0,90 | | 1101 | At5g58250 | -0,83 | |
| 950 | At1g10960 | -0,95 | | 1026 | At4g37790 | -0,90 | | 1102 | At1g70100 | -0,83 | |
| 951 | At4g04830 | -0,95 | | 1027 | At1g48100 | -0,90 | | 1103 | At5g37055 | -0,83 | |
| 952 | At1g56200 | -0,95 | | 1028 | At3g50685 | -0,90 | | 1104 | At1g54210 | -0,83 | |
| 953 | At1g77480 | -0,95 | | 1029 | At3g52150 | -0,90 | | 1105 | At3g12080 | -0,83 | |
| 954 | At4g31560 | -0,95 | | 1030 | At1g77990 | -0,90 | | 1106 | At5g14390 | -0,83 | |
| 955 | At4g24340 | -0,95 | | 1031 | At1g15170 | -0,90 | | 1107 | At5g03430 | -0,83 | |
| 956 | At2g30600 | -0,94 | | 1032 | At1g75350 | -0,90 | | 1108 | At1g14030 | -0,82 | |
| 957 | At3g05640 | -0,94 | | 1033 | At3g55770 | -0,90 | | 1109 | At1g17650 | -0,82 | |
| 958 | At5g58575 | -0,94 | | 1034 | At3g43800 | -0,90 | | 1110 | At1g32160 | -0,82 | |
| 959 | At4g18160 | -0,94 | | 1035 | At2g40100 | -0,89 | | 1111 | At3g43540 | -0,82 | |
| 960 | At4g37300 | -0,94 | | 1036 | At2g24060 | -0,89 | | 1112 | At5g57910 | -0,82 | |
| 961 | At1g74730 | -0,94 | | 1037 | At3g16140 | -0,89 | | 1113 | At5g55910 | -0,82 | |
| 962 | At4g28085 | -0,94 | | 1038 | At4g03110 | -0,89 | | 1114 | At1g71970 | -0,82 | |
| 963 | At2g46820 | -0,94 | | 1039 | At5g24420 | -0,89 | | 1115 | At1g14210 | -0,82 | |
| 964 | At1g03400 | -0,94 | | 1040 | At3g52290 | -0,89 | | 1116 | At2g38530 | -0,82 | |
| 965 | At1g09770 | -0,94 | | 1041 | At3g10060 | -0,89 | | 1117 | At3g50530 | -0,82 | |
| 966 | At4g30993 | -0,94 | | 1042 | At4g35750 | -0,89 | | 1118 | At4g04640 | -0,82 | |
| 967 | At1g11860 | -0,94 | | 1043 | At1g62250 | -0,89 | | 1119 | At2g33830 | -0,82 | |
| 968 | At3g03990 | -0,94 | | 1044 | At5g65730 | -0,89 | | 1120 | At2g45960 | -0,82 | |
| 969 | At5g65220 | -0,94 | | 1045 | At1g36980 | -0,89 | | 1121 | At3g13360 | -0,81 | |
| 970 | At5g54080 | -0,94 | | 1046 | At3g44630 | -0,89 | | 1122 | At1g70250 | -0,81 | |
| 971 | At2g26080 | -0,93 | | 1047 | At4g01120 | -0,89 | | 1123 | At3g06510 | -0,81 | |
| 972 | At1g75440 | -0,93 | | 1048 | At4g32590 | -0,89 | | 1124 | At3g59490 | -0,81 | |
| 973 | At5g25120 | -0,93 | | 1049 | At2g01320 | -0,89 | | 1125 | At3g60340 | -0,81 | |
| 974 | At5g55640 | -0,93 | | 1050 | At5g64940 | -0,88 | | 1126 | At3g45290 | -0,81 | |
| 975 | At1g03630 | -0,93 | | 1051 | At3g62910 | -0,88 | | 1127 | At4g04350 | -0,81 | |
| 976 | At2g21240 | -0,93 | | 1052 | At3g16240 | -0,88 | | 1128 | At5g53020 | -0,81 | |
| 977 | At1g01120 | -0,93 | | 1053 | At1g20160 | -0,88 | | 1129 | At4g13670 | -0,81 | |
| 978 | At4g28240 | -0,93 | | 1054 | At5g47840 | -0,88 | | 1130 | At4g29220 | -0,81 | |
| 979 | At2g21320 | -0,93 | | 1055 | At3g18980 | -0,88 | | 1131 | At5g44250 | -0,80 | |
| 980 | At5g67030 | -0,93 | | 1056 | At1g33290 | -0,88 | | 1132 | At4g25960 | -0,80 | |
| 981 | At2g46340 | -0,93 | | 1057 | At4g03280 | -0,88 | | 1133 | At3g12610 | -0,80 | |
| 982 | At3g49590 | -0,93 | | 1058 | At2g35410 | -0,87 | | 1134 | At4g26710 | -0,80 | |
| 983 | At1g63980 | -0,93 | | 1059 | At1g05160 | -0,87 | | 1135 | At2g45530 | -0,80 | |
| 984 | At2g42750 | -0,93 | | 1060 | At5g23330 | -0,87 | | 1136 | At5g52440 | -0,80 | |
| 985 | At1g03675 | -0,93 | | 1061 | At3g14560 | -0,87 | | 1137 | At3g53130 | -0,80 | |
| 986 | At1g28260 | -0,93 | | 1062 | At1g04400 | -0,87 | | 1138 | At5g13180 | -0,80 | |
| 987 | At5g58330 | -0,93 | | 1063 | At1g34310 | -0,87 | | 1139 | At3g55310 | -0,80 | |
| 988 | At2g20260 | -0,92 | | 1064 | At3g26070 | -0,87 | | 1140 | At5g16710 | -0,80 | |

| | gene name | log2 ratio | | | gene name | log2 ratio | | | gene name | log2 ratio | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1141 | At1g52230 | -0,79 | | 1217 | At4g33220 | -0,74 | | 1293 | At1g18880 | -0,63 | |
| 1142 | At2g21385 | -0,79 | | 1218 | At2g38310 | -0,73 | | 1294 | At5g56860 | -0,63 | |
| 1143 | At4g02210 | -0,79 | | 1219 | At1g26180 | -0,73 | | 1295 | At4g25700 | -0,63 | |
| 1144 | At1g66940 | -0,79 | | 1220 | At3g17590 | -0,73 | | 1296 | At5g57100 | -0,63 | |
| 1145 | At3g46630 | -0,79 | | 1221 | At5g62790 | -0,73 | | 1297 | At3g14650 | -0,63 | |
| 1146 | At1g62030 | -0,79 | | 1222 | At2g42590 | -0,73 | | 1298 | At1g01725 | -0,63 | |
| 1147 | At5g13240 | -0,79 | | 1223 | At1g57790 | -0,73 | | 1299 | At5g16140 | -0,63 | |
| 1148 | At2g20890 | -0,79 | | 1224 | At1g08110 | -0,73 | | 1300 | At1g32070 | -0,62 | |
| 1149 | At5g66570 | -0,79 | | 1225 | At4g11100 | -0,73 | | 1301 | At2g45990 | -0,62 | |
| 1150 | At5g46270 | -0,79 | | 1226 | At2g40020 | -0,73 | | 1302 | At4g34630 | -0,62 | |
| 1151 | At5g59780 | -0,79 | | 1227 | At1g51270 | -0,72 | | 1303 | At3g06590 | -0,62 | |
| 1152 | At4g31030 | -0,79 | | 1228 | At2g41870 | -0,72 | | 1304 | At2g24280 | -0,62 | |
| 1153 | At1g21065 | -0,79 | | 1229 | At1g73170 | -0,72 | | 1305 | At5g22620 | -0,61 | |
| 1154 | At4g39620 | -0,79 | | 1230 | At2g28950 | -0,72 | | 1306 | At3g59780 | -0,61 | |
| 1155 | At2g38400 | -0,79 | | 1231 | At1g10900 | -0,72 | | 1307 | At3g27170 | -0,61 | |
| 1156 | At3g50790 | -0,79 | | 1232 | At2g29720 | -0,72 | | 1308 | At4g37200 | -0,61 | |
| 1157 | At2g35810 | -0,78 | | 1233 | At1g23400 | -0,72 | | 1309 | At4g23180 | -0,60 | |
| 1158 | At5g04490 | -0,78 | | 1234 | At1g58200 | -0,72 | | 1310 | At1g44575 | -0,60 | |
| 1159 | At3g04940 | -0,78 | | 1235 | At5g47435 | -0,72 | | 1311 | At1g06500 | -0,60 | |
| 1160 | At5g29771 | -0,78 | | 1236 | At5g57930 | -0,72 | | 1312 | At5g54730 | -0,60 | |
| 1161 | At2g35760 | -0,78 | | 1237 | At1g19700 | -0,71 | | 1313 | At4g20380 | -0,60 | |
| 1162 | At5g58870 | -0,78 | | 1238 | At1g55670 | -0,71 | | 1314 | At1g04550 | -0,59 | |
| 1163 | At4g28290 | -0,78 | | 1239 | At1g20650 | -0,71 | | 1315 | At3g26580 | -0,59 | |
| 1164 | At1g55260 | -0,78 | | 1240 | At1g66980 | -0,71 | | 1316 | At2g31450 | -0,58 | |
| 1165 | At1g18650 | -0,78 | | 1241 | At5g38990 | -0,71 | | 1317 | At2g30350 | -0,58 | |
| 1166 | At2g34860 | -0,78 | | 1242 | At5g25770 | -0,71 | | 1318 | At5g46420 | -0,58 | |
| 1167 | At1g29700 | -0,78 | | 1243 | At1g21130 | -0,71 | | 1319 | At5g10460 | -0,57 | |
| 1168 | At3g26280 | -0,78 | | 1244 | At5g51140 | -0,71 | | 1320 | At2g35920 | -0,57 | |
| 1169 | At1g01540 | -0,78 | | 1245 | At1g18170 | -0,71 | | 1321 | At3g53410 | -0,57 | |
| 1170 | At1g79670 | -0,78 | | 1246 | At1g28440 | -0,70 | | 1322 | At2g30520 | -0,54 | |
| 1171 | At3g51820 | -0,78 | | 1247 | At1g16810 | -0,70 | | 1323 | At1g73060 | -0,53 | |
| 1172 | At3g21510 | -0,78 | | 1248 | At1g45976 | -0,70 | | 1324 | At5g66460 | -0,53 | |
| 1173 | At5g13090 | -0,78 | | 1249 | At3g45780 | -0,70 | | 1325 | At2g33310 | -0,53 | |
| 1174 | At1g08380 | -0,77 | | 1250 | At5g27350 | -0,70 | | 1326 | At2g33255 | -0,53 | |
| 1175 | At5g13410 | -0,77 | | 1251 | At3g51895 | -0,70 | | 1327 | At2g40000 | -0,52 | |
| 1176 | At4g10300 | -0,77 | | 1252 | At2g35960 | -0,70 | | 1328 | At1g15670 | -0,17 | |
| 1177 | At2g37220 | -0,77 | | 1253 | At3g54900 | -0,70 | | 1329 | At1g07590 | -0,16 | |
| 1178 | At5g18590 | -0,77 | | 1254 | At4g25620 | -0,70 | | 1330 | At1g05560 | -0,09 | |
| 1179 | At1g05810 | -0,77 | | 1255 | At1g78895 | -0,69 | | 1331 | At3g23550 | -0,02 | |
| 1180 | At4g15110 | -0,77 | | 1256 | At1g56500 | -0,69 | | 1332 | At2g38240 | 0,15 | |
| 1181 | At3g04260 | -0,77 | | 1257 | At1g29660 | -0,69 | | 1333 | At4g31290 | 0,18 | |
| 1182 | At1g66920 | -0,77 | | 1258 | At4g12010 | -0,69 | | 1334 | At4g27410 | 0,25 | |
| 1183 | At5g55220 | -0,77 | | 1259 | At5g64330 | -0,69 | | 1335 | At3g21230 | 0,28 | |
| 1184 | At2g24090 | -0,77 | | 1260 | At2g47910 | -0,69 | | 1336 | At2g37970 | 0,52 | |
| 1185 | At5g42240 | -0,77 | | 1261 | At1g08540 | -0,69 | | 1337 | At4g30650 | 0,53 | |
| 1186 | At3g16010 | -0,76 | | 1262 | At1g05900 | -0,69 | | 1338 | At1g66250 | 0,53 | |
| 1187 | At1g78020 | -0,76 | | 1263 | At5g28750 | -0,68 | | 1339 | At1g13210 | 0,53 | |
| 1188 | At3g63190 | -0,76 | | 1264 | At2g23610 | -0,68 | | 1340 | At5g14790 | 0,54 | |
| 1189 | At5g06265 | -0,76 | | 1265 | At5g13690 | -0,68 | | 1341 | At4g33040 | 0,54 | |
| 1190 | At4g10770 | -0,76 | | 1266 | At1g30360 | -0,68 | | 1342 | At1g01250 | 0,54 | |
| 1191 | At4g38430 | -0,76 | | 1267 | At4g11175 | -0,68 | | 1343 | At5g55640 | 0,55 | |
| 1192 | At3g06770 | -0,76 | | 1268 | At1g01080 | -0,68 | | 1344 | At5g46910 | 0,55 | |
| 1193 | At3g52060 | -0,76 | | 1269 | At5g27560 | -0,67 | | 1345 | At4g02350 | 0,56 | |
| 1194 | At1g78510 | -0,76 | | 1270 | At4g22753 | -0,67 | | 1346 | At1g22650 | 0,56 | |
| 1195 | At5g58350 | -0,76 | | 1271 | At5g42765 | -0,67 | | 1347 | At5g18440 | 0,56 | |
| 1196 | At4g18210 | -0,76 | | 1272 | At1g60140 | -0,67 | | 1348 | At1g33940 | 0,56 | |
| 1197 | At4g15430 | -0,76 | | 1273 | At1g13090 | -0,66 | | 1349 | At3g05220 | 0,56 | |
| 1198 | At1g03820 | -0,76 | | 1274 | At1g22050 | -0,66 | | 1350 | At5g35980 | 0,57 | |
| 1199 | At5g07020 | -0,75 | | 1275 | At1g63850 | -0,66 | | 1351 | At1g08970 | 0,57 | |
| 1200 | At4g31530 | -0,75 | | 1276 | At1g33590 | -0,66 | | 1352 | At1g79560 | 0,57 | |
| 1201 | At3g19580 | -0,75 | | 1277 | At4g24350 | -0,66 | | 1353 | At3g09310 | 0,58 | |
| 1202 | At3g03710 | -0,75 | | 1278 | At5g06060 | -0,66 | | 1354 | At4g02280 | 0,58 | |
| 1203 | At4g30790 | -0,75 | | 1279 | At4g02530 | -0,65 | | 1355 | At1g76510 | 0,59 | |
| 1204 | At5g52550 | -0,75 | | 1280 | At3g25805 | -0,65 | | 1356 | At4g16990 | 0,59 | |
| 1205 | At3g52155 | -0,75 | | 1281 | At4g05090 | -0,65 | | 1357 | At5g01010 | 0,59 | |
| 1206 | At5g22270 | -0,75 | | 1282 | At3g11090 | -0,65 | | 1358 | At2g16480 | 0,59 | |
| 1207 | At1g20840 | -0,75 | | 1283 | At5g12260 | -0,65 | | 1359 | At4g27380 | 0,59 | |
| 1208 | At3g50440 | -0,74 | | 1284 | At1g11410 | -0,64 | | 1360 | At1g06380 | 0,59 | |
| 1209 | At5g42030 | -0,74 | | 1285 | At2g34930 | -0,64 | | 1361 | At1g59960 | 0,60 | |
| 1210 | At5g05480 | -0,74 | | 1286 | At2g21280 | -0,64 | | 1362 | At4g34555 | 0,60 | |
| 1211 | At3g61870 | -0,74 | | 1287 | At4g17740 | -0,64 | | 1363 | At2g32990 | 0,60 | |
| 1212 | At2g21540 | -0,74 | | 1288 | At2g33050 | -0,64 | | 1364 | At1g67940 | 0,60 | |
| 1213 | At5g16520 | -0,74 | | 1289 | At3g02450 | -0,64 | | 1365 | At1g09940 | 0,60 | |
| 1214 | At2g24820 | -0,74 | | 1290 | At5g07690 | -0,63 | | 1366 | At2g30140 | 0,61 | |
| 1215 | At3g07360 | -0,74 | | 1291 | At5g03230 | -0,63 | | 1367 | At5g42080 | 0,61 | |
| 1216 | At4g38530 | -0,74 | | 1292 | At3g46900 | -0,63 | | 1368 | At3g47450 | 0,61 | |

| | gene name | log2 ratio | | | gene name | log2 ratio | | | gene name | log2 ratio | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1369 | At1g80410 | 0,62 | | 1445 | At5g53140 | 0,69 | | 1521 | At3g48250 | 0,75 | |
| 1370 | At2g37860 | 0,62 | | 1446 | At1g74810 | 0,69 | | 1522 | At5g66680 | 0,75 | |
| 1371 | At5g16040 | 0,62 | | 1447 | At4g39390 | 0,69 | | 1523 | At1g16900 | 0,75 | |
| 1372 | At5g22540 | 0,62 | | 1448 | At1g59910 | 0,69 | | 1524 | At4g33490 | 0,75 | |
| 1373 | At1g49400 | 0,62 | | 1449 | At3g23210 | 0,69 | | 1525 | At2g41180 | 0,75 | |
| 1374 | At3g49810 | 0,62 | | 1450 | At5g61310 | 0,69 | | 1526 | At5g45350 | 0,75 | |
| 1375 | At1g56120 | 0,62 | | 1451 | At2g45070 | 0,70 | | 1527 | At3g07230 | 0,75 | |
| 1376 | At4g30440 | 0,62 | | 1452 | At3g50480 | 0,70 | | 1528 | At4g25320 | 0,75 | |
| 1377 | At3g10520 | 0,62 | | 1453 | At5g40580 | 0,70 | | 1529 | At5g57090 | 0,75 | |
| 1378 | At3g49100 | 0,62 | | 1454 | At4g34135 | 0,70 | | 1530 | At3g54090 | 0,75 | |
| 1379 | At5g56610 | 0,63 | | 1455 | At1g30120 | 0,70 | | 1531 | At4g31985 | 0,75 | |
| 1380 | At2g33845 | 0,63 | | 1456 | At1g64600 | 0,70 | | 1532 | At1g63000 | 0,75 | |
| 1381 | At1g02500 | 0,63 | | 1457 | At1g22510 | 0,70 | | 1533 | At5g65880 | 0,75 | |
| 1382 | At2g17870 | 0,63 | | 1458 | At2g27580 | 0,70 | | 1534 | At1g16350 | 0,76 | |
| 1383 | At2g19860 | 0,63 | | 1459 | At2g32240 | 0,70 | | 1535 | At1g41830 | 0,76 | |
| 1384 | At3g10800 | 0,63 | | 1460 | At1g11700 | 0,71 | | 1536 | At4g35260 | 0,76 | |
| 1385 | At5g46350 | 0,64 | | 1461 | At1g68370 | 0,71 | | 1537 | At1g07430 | 0,76 | |
| 1386 | At1g13950 | 0,64 | | 1462 | At3g52580 | 0,71 | | 1538 | At3g53430 | 0,76 | |
| 1387 | At5g67600 | 0,64 | | 1463 | At3g14790 | 0,71 | | 1539 | At4g23600 | 0,76 | |
| 1388 | At1g73880 | 0,64 | | 1464 | At5g13460 | 0,71 | | 1540 | At4g00700 | 0,76 | |
| 1389 | At4g16250 | 0,64 | | 1465 | At2g18740 | 0,71 | | 1541 | At2g23930 | 0,76 | |
| 1390 | At4g01300 | 0,64 | | 1466 | At5g45410 | 0,71 | | 1542 | At1g08130 | 0,76 | |
| 1391 | At5g16060 | 0,64 | | 1467 | At5g49910 | 0,71 | | 1543 | At2g19120 | 0,76 | |
| 1392 | At5g51070 | 0,64 | | 1468 | At1g75390 | 0,71 | | 1544 | At3g22980 | 0,76 | |
| 1393 | At2g35605 | 0,64 | | 1469 | At3g08720 | 0,71 | | 1545 | At2g29550 | 0,76 | |
| 1394 | At3g53980 | 0,64 | | 1470 | At4g28640 | 0,71 | | 1546 | At1g27330 | 0,76 | |
| 1395 | At5g04170 | 0,65 | | 1471 | At2g39660 | 0,71 | | 1547 | At3g07630 | 0,76 | |
| 1396 | At5g39320 | 0,65 | | 1472 | At1g57660 | 0,71 | | 1548 | At4g23270 | 0,76 | |
| 1397 | At2g43500 | 0,65 | | 1473 | At3g05230 | 0,71 | | 1549 | At3g52800 | 0,76 | |
| 1398 | At1g04710 | 0,65 | | 1474 | At2g20900 | 0,71 | | 1550 | At1g19690 | 0,76 | |
| 1399 | At2g29120 | 0,65 | | 1475 | At1g15470 | 0,71 | | 1551 | At5g04540 | 0,76 | |
| 1400 | At3g12740 | 0,65 | | 1476 | At5g66070 | 0,72 | | 1552 | At2g46420 | 0,76 | |
| 1401 | At2g07687 | 0,65 | | 1477 | At3g02470 | 0,72 | | 1553 | At3g58700 | 0,77 | |
| 1402 | At2g43105 | 0,65 | | 1478 | At1g19180 | 0,72 | | 1554 | At3g25940 | 0,77 | |
| 1403 | At4g12720 | 0,65 | | 1479 | At2g39080 | 0,72 | | 1555 | At5g64860 | 0,77 | |
| 1404 | At2g46640 | 0,65 | | 1480 | At2g05990 | 0,72 | | 1556 | At4g24970 | 0,77 | |
| 1405 | At4g24270 | 0,65 | | 1481 | At1g64950 | 0,72 | | 1557 | At3g18820 | 0,77 | |
| 1406 | At2g37710 | 0,66 | | 1482 | At4g38360 | 0,72 | | 1558 | At4g12250 | 0,77 | |
| 1407 | At1g19025 | 0,66 | | 1483 | At5g65860 | 0,72 | | 1559 | At5g38530 | 0,77 | |
| 1408 | At5g41100 | 0,66 | | 1484 | At1g27680 | 0,72 | | 1560 | At1g07280 | 0,77 | |
| 1409 | At3g15090 | 0,66 | | 1485 | At3g03610 | 0,72 | | 1561 | At1g22270 | 0,77 | |
| 1410 | At4g28390 | 0,66 | | 1486 | At3g55450 | 0,72 | | 1562 | At4g21830 | 0,77 | |
| 1411 | At5g64300 | 0,66 | | 1487 | At1g30270 | 0,72 | | 1563 | At3g09840 | 0,77 | |
| 1412 | At2g34570 | 0,66 | | 1488 | At5g13610 | 0,73 | | 1564 | At4g27070 | 0,77 | |
| 1413 | At4g02130 | 0,66 | | 1489 | At3g28900 | 0,73 | | 1565 | At5g62530 | 0,77 | |
| 1414 | At1g72900 | 0,66 | | 1490 | At5g11790 | 0,73 | | 1566 | At3g26970 | 0,77 | |
| 1415 | At5g61220 | 0,66 | | 1491 | At4g34910 | 0,73 | | 1567 | At5g65660 | 0,77 | |
| 1416 | At5g59350 | 0,66 | | 1492 | At4g27490 | 0,73 | | 1568 | At1g24793 | 0,77 | |
| 1417 | At5g07340 | 0,66 | | 1493 | At3g18560 | 0,73 | | 1569 | At2g21160 | 0,78 | |
| 1418 | At3g57550 | 0,66 | | 1494 | At5g04800 | 0,73 | | 1570 | At2g24050 | 0,78 | |
| 1419 | At3g01160 | 0,66 | | 1495 | At5g51300 | 0,73 | | 1571 | At2g41000 | 0,78 | |
| 1420 | At5g38720 | 0,66 | | 1496 | At5g04930 | 0,73 | | 1572 | At2g39700 | 0,78 | |
| 1421 | At5g23310 | 0,66 | | 1497 | At5g66410 | 0,73 | | 1573 | At4g30270 | 0,78 | |
| 1422 | At3g11010 | 0,67 | | 1498 | At4g28540 | 0,73 | | 1574 | At1g57560 | 0,78 | |
| 1423 | At2g33700 | 0,67 | | 1499 | At1g48370 | 0,73 | | 1575 | At5g43980 | 0,78 | |
| 1424 | At5g11670 | 0,67 | | 1500 | At5g23690 | 0,73 | | 1576 | At3g02630 | 0,78 | |
| 1425 | At3g05200 | 0,67 | | 1501 | At2g04305 | 0,73 | | 1577 | At1g15430 | 0,78 | |
| 1426 | At3g01780 | 0,67 | | 1502 | At1g47550 | 0,73 | | 1578 | At1g73940 | 0,78 | |
| 1427 | At1g78280 | 0,67 | | 1503 | At5g64140 | 0,73 | | 1579 | At5g15870 | 0,78 | |
| 1428 | At5g56270 | 0,67 | | 1504 | At1g17620 | 0,73 | | 1580 | At3g10490 | 0,78 | |
| 1429 | At5g19820 | 0,67 | | 1505 | At2g27775 | 0,73 | | 1581 | At2g18220 | 0,78 | |
| 1430 | At5g42890 | 0,68 | | 1506 | At4g14220 | 0,74 | | 1582 | At1g72930 | 0,78 | |
| 1431 | At5g08415 | 0,68 | | 1507 | At3g19520 | 0,74 | | 1583 | At2g45860 | 0,78 | |
| 1432 | At3g07330 | 0,68 | | 1508 | At1g18390 | 0,74 | | 1584 | At4g38600 | 0,79 | |
| 1433 | At1g20270 | 0,68 | | 1509 | At3g13920 | 0,74 | | 1585 | At1g15140 | 0,79 | |
| 1434 | At4g14430 | 0,68 | | 1510 | At2g29990 | 0,74 | | 1586 | At5g23020 | 0,79 | |
| 1435 | At5g20540 | 0,68 | | 1511 | At3g18420 | 0,74 | | 1587 | At2g38860 | 0,79 | |
| 1436 | At5g46760 | 0,68 | | 1512 | At2g25110 | 0,74 | | 1588 | At1g26770 | 0,79 | |
| 1437 | At3g19950 | 0,68 | | 1513 | At5g12080 | 0,74 | | 1589 | At3g02190 | 0,79 | |
| 1438 | At4g08685 | 0,68 | | 1514 | At1g13250 | 0,74 | | 1590 | At3g48990 | 0,79 | |
| 1439 | At4g29490 | 0,68 | | 1515 | At1g08640 | 0,74 | | 1591 | At5g50200 | 0,79 | |
| 1440 | At5g43745 | 0,69 | | 1516 | At3g45620 | 0,74 | | 1592 | At4g15620 | 0,79 | |
| 1441 | At3g27080 | 0,69 | | 1517 | At1g70140 | 0,74 | | 1593 | At5g19750 | 0,79 | |
| 1442 | At3g15210 | 0,69 | | 1518 | At3g50210 | 0,74 | | 1594 | At2g35190 | 0,79 | |
| 1443 | At2g01710 | 0,69 | | 1519 | At3g55280 | 0,74 | | 1595 | At3g03270 | 0,80 | |
| 1444 | At2g20850 | 0,69 | | 1520 | At2g41410 | 0,74 | | 1596 | At3g57000 | 0,80 | |

| | gene name | log2 ratio | | | gene name | log2 ratio | | | gene name | log2 ratio | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1597 | At2g30990 | 0,80 | | 1673 | At1g66260 | 0,85 | | 1749 | At1g07135 | 0,89 | |
| 1598 | At5g08380 | 0,80 | | 1674 | At1g48570 | 0,85 | | 1750 | At1g23490 | 0,89 | |
| 1599 | At1g54090 | 0,80 | | 1675 | At5g17520 | 0,85 | | 1751 | At1g16670 | 0,89 | |
| 1600 | At2g30050 | 0,80 | | 1676 | At3g27230 | 0,85 | | 1752 | At4g30340 | 0,89 | |
| 1601 | At5g03290 | 0,80 | | 1677 | At1g28680 | 0,85 | | 1753 | At3g27300 | 0,89 | |
| 1602 | At1g19850 | 0,80 | | 1678 | At5g57870 | 0,85 | | 1754 | At1g66430 | 0,89 | |
| 1603 | At5g02610 | 0,81 | | 1679 | At4g37990 | 0,85 | | 1755 | At5g17190 | 0,89 | |
| 1604 | At5g08630 | 0,81 | | 1680 | At3g27380 | 0,85 | | 1756 | At4g17520 | 0,89 | |
| 1605 | At1g24360 | 0,81 | | 1681 | At5g48970 | 0,85 | | 1757 | At5g45750 | 0,89 | |
| 1606 | At2g39220 | 0,81 | | 1682 | At5g26360 | 0,85 | | 1758 | At1g18540 | 0,89 | |
| 1607 | At1g09210 | 0,81 | | 1683 | At4g22530 | 0,85 | | 1759 | At3g24954 | 0,89 | |
| 1608 | At3g25860 | 0,81 | | 1684 | At2g17220 | 0,85 | | 1760 | At4g02400 | 0,89 | |
| 1609 | At4g36940 | 0,81 | | 1685 | At1g55690 | 0,85 | | 1761 | At1g10270 | 0,89 | |
| 1610 | At4g25640 | 0,81 | | 1686 | At4g25030 | 0,85 | | 1762 | At4g00790 | 0,89 | |
| 1611 | At1g67300 | 0,81 | | 1687 | At3g20050 | 0,85 | | 1763 | At5g13880 | 0,90 | |
| 1612 | At1g16780 | 0,81 | | 1688 | At2g25970 | 0,85 | | 1764 | At2g19730 | 0,90 | |
| 1613 | At3g51660 | 0,81 | | 1689 | At5g52540 | 0,85 | | 1765 | At1g70890 | 0,90 | |
| 1614 | At1g70190 | 0,81 | | 1690 | At5g35700 | 0,85 | | 1766 | At4g35490 | 0,90 | |
| 1615 | At1g73080 | 0,81 | | 1691 | At2g30360 | 0,85 | | 1767 | At2g05710 | 0,90 | |
| 1616 | At2g43320 | 0,81 | | 1692 | At4g36710 | 0,85 | | 1768 | At1g18850 | 0,90 | |
| 1617 | At3g27240 | 0,81 | | 1693 | At5g57050 | 0,86 | | 1769 | At4g18800 | 0,90 | |
| 1618 | At2g27260 | 0,81 | | 1694 | At3g46540 | 0,86 | | 1770 | At3g62810 | 0,90 | |
| 1619 | At4g29670 | 0,81 | | 1695 | At1g17510 | 0,86 | | 1771 | At5g57550 | 0,90 | |
| 1620 | At3g33530 | 0,81 | | 1696 | At3g22845 | 0,86 | | 1772 | At4g34230 | 0,90 | |
| 1621 | At1g47400 | 0,81 | | 1697 | At3g15350 | 0,86 | | 1773 | At3g28700 | 0,90 | |
| 1622 | At5g18790 | 0,81 | | 1698 | At4g24920 | 0,86 | | 1774 | At4g23490 | 0,90 | |
| 1623 | At2g46610 | 0,81 | | 1699 | At1g76160 | 0,86 | | 1775 | At3g58570 | 0,90 | |
| 1624 | At4g31480 | 0,82 | | 1700 | At1g55265 | 0,86 | | 1776 | At4g03550 | 0,90 | |
| 1625 | At2g28600 | 0,82 | | 1701 | At5g38890 | 0,86 | | 1777 | At3g59020 | 0,90 | |
| 1626 | At1g74560 | 0,82 | | 1702 | At2g37190 | 0,86 | | 1778 | At5g58710 | 0,90 | |
| 1627 | At3g04840 | 0,82 | | 1703 | At3g05560 | 0,86 | | 1779 | At3g15080 | 0,90 | |
| 1628 | At1g58080 | 0,82 | | 1704 | At1g76300 | 0,86 | | 1780 | At4g24820 | 0,90 | |
| 1629 | At3g03770 | 0,82 | | 1705 | At5g04660 | 0,86 | | 1781 | At4g39510 | 0,90 | |
| 1630 | At2g25210 | 0,82 | | 1706 | At5g14590 | 0,86 | | 1782 | At2g29690 | 0,91 | |
| 1631 | At1g22780 | 0,82 | | 1707 | At1g16740 | 0,87 | | 1783 | At4g39840 | 0,91 | |
| 1632 | At1g16060 | 0,82 | | 1708 | At1g34260 | 0,87 | | 1784 | At1g27400 | 0,91 | |
| 1633 | At3g60540 | 0,82 | | 1709 | At5g55400 | 0,87 | | 1785 | At2g04030 | 0,91 | |
| 1634 | At1g09970 | 0,82 | | 1710 | At2g11520 | 0,87 | | 1786 | At4g23010 | 0,91 | |
| 1635 | At5g39790 | 0,83 | | 1711 | At5g59690 | 0,87 | | 1787 | At1g05630 | 0,91 | |
| 1636 | At3g11670 | 0,83 | | 1712 | At5g50210 | 0,87 | | 1788 | At3g57560 | 0,91 | |
| 1637 | At2g21620 | 0,83 | | 1713 | At5g64960 | 0,87 | | 1789 | At2g39830 | 0,91 | |
| 1638 | At4g21990 | 0,83 | | 1714 | At5g09810 | 0,87 | | 1790 | At5g01270 | 0,91 | |
| 1639 | At4g30390 | 0,83 | | 1715 | At3g21110 | 0,87 | | 1791 | At4g38420 | 0,91 | |
| 1640 | At3g46620 | 0,83 | | 1716 | At5g65470 | 0,87 | | 1792 | At1g06180 | 0,91 | |
| 1641 | At3g05180 | 0,83 | | 1717 | At2g17840 | 0,87 | | 1793 | At4g26310 | 0,91 | |
| 1642 | At5g06460 | 0,83 | | 1718 | At1g75670 | 0,87 | | 1794 | At1g53760 | 0,91 | |
| 1643 | At1g71170 | 0,83 | | 1719 | At5g15750 | 0,87 | | 1795 | At5g58420 | 0,91 | |
| 1644 | At4g38580 | 0,83 | | 1720 | At3g22422 | 0,87 | | 1796 | At1g61250 | 0,91 | |
| 1645 | At3g49430 | 0,83 | | 1721 | At5g56710 | 0,87 | | 1797 | At1g55890 | 0,91 | |
| 1646 | At1g78380 | 0,83 | | 1722 | At1g77120 | 0,87 | | 1798 | At2g27730 | 0,91 | |
| 1647 | At2g11890 | 0,83 | | 1723 | At4g37090 | 0,87 | | 1799 | At3g27360 | 0,91 | |
| 1648 | At4g08180 | 0,83 | | 1724 | At5g20080 | 0,87 | | 1800 | At4g18900 | 0,91 | |
| 1649 | At1g55330 | 0,83 | | 1725 | At3g24080 | 0,87 | | 1801 | At5g40980 | 0,92 | |
| 1650 | At3g60420 | 0,83 | | 1726 | At4g24130 | 0,88 | | 1802 | At3g10500 | 0,92 | |
| 1651 | At4g30910 | 0,83 | | 1727 | At3g24770 | 0,88 | | 1803 | At2g44520 | 0,92 | |
| 1652 | At5g46280 | 0,83 | | 1728 | At5g08570 | 0,88 | | 1804 | At5g46160 | 0,92 | |
| 1653 | At1g33265 | 0,83 | | 1729 | At5g11520 | 0,88 | | 1805 | At2g37760 | 0,92 | |
| 1654 | At1g28670 | 0,83 | | 1730 | At2g41190 | 0,88 | | 1806 | At1g15710 | 0,92 | |
| 1655 | At3g56370 | 0,83 | | 1731 | At5g52650 | 0,88 | | 1807 | At5g65720 | 0,92 | |
| 1656 | At3g43810 | 0,83 | | 1732 | At5g54930 | 0,88 | | 1808 | At1g10410 | 0,92 | |
| 1657 | At2g16430 | 0,83 | | 1733 | At5g57990 | 0,88 | | 1809 | At5g37600 | 0,92 | |
| 1658 | At2g01970 | 0,84 | | 1734 | At5g18900 | 0,88 | | 1810 | At1g64610 | 0,92 | |
| 1659 | At4g20010 | 0,84 | | 1735 | At1g54630 | 0,88 | | 1811 | At3g09805 | 0,92 | |
| 1660 | At1g26690 | 0,84 | | 1736 | At5g61640 | 0,88 | | 1812 | At1g48850 | 0,92 | |
| 1661 | At3g26980 | 0,84 | | 1737 | At4g30220 | 0,89 | | 1813 | At2g25625 | 0,92 | |
| 1662 | At5g22440 | 0,84 | | 1738 | At5g08610 | 0,89 | | 1814 | At3g06930 | 0,92 | |
| 1663 | At1g18800 | 0,84 | | 1739 | At2g27020 | 0,89 | | 1815 | At4g12300 | 0,92 | |
| 1664 | At5g19320 | 0,84 | | 1740 | At3g53630 | 0,89 | | 1816 | At2g39390 | 0,92 | |
| 1665 | At1g54270 | 0,84 | | 1741 | At2g26730 | 0,89 | | 1817 | At1g67970 | 0,93 | |
| 1666 | At1g72970 | 0,84 | | 1742 | At2g46170 | 0,89 | | 1818 | At5g54650 | 0,93 | |
| 1667 | At5g06760 | 0,84 | | 1743 | At3g16080 | 0,89 | | 1819 | At1g80615 | 0,93 | |
| 1668 | At3g51860 | 0,84 | | 1744 | At4g24160 | 0,89 | | 1820 | At2g19385 | 0,93 | |
| 1669 | At1g56340 | 0,84 | | 1745 | At1g68470 | 0,89 | | 1821 | At5g09510 | 0,93 | |
| 1670 | At1g09830 | 0,84 | | 1746 | At3g52960 | 0,89 | | 1822 | At2g45030 | 0,93 | |
| 1671 | At1g29150 | 0,84 | | 1747 | At4g01220 | 0,89 | | 1823 | At1g66580 | 0,93 | |
| 1672 | At3g06540 | 0,84 | | 1748 | At2g20450 | 0,89 | | 1824 | At4g29210 | 0,93 | |

| | gene name | log2 ratio | | | gene name | log2 ratio | | | gene name | log2 ratio | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1825 | At4g34480 | 0,93 | | 1901 | At1g56450 | 0,97 | | 1977 | At5g43010 | 1,01 | |
| 1826 | At1g15500 | 0,93 | | 1902 | At1g66070 | 0,97 | | 1978 | At4g22580 | 1,01 | |
| 1827 | At1g18590 | 0,93 | | 1903 | At1g17290 | 0,97 | | 1979 | At3g62590 | 1,01 | |
| 1828 | At3g43510 | 0,93 | | 1904 | At4g19390 | 0,97 | | 1980 | At1g42440 | 1,01 | |
| 1829 | At4g02450 | 0,93 | | 1905 | At5g53820 | 0,97 | | 1981 | At4g37980 | 1,01 | |
| 1830 | At1g53500 | 0,93 | | 1906 | At3g05490 | 0,97 | | 1982 | At1g67430 | 1,01 | |
| 1831 | At5g41010 | 0,93 | | 1907 | At1g24180 | 0,97 | | 1983 | At3g14067 | 1,01 | |
| 1832 | At4g29680 | 0,93 | | 1908 | At2g33120 | 0,97 | | 1984 | At4g35300 | 1,01 | |
| 1833 | At2g30200 | 0,93 | | 1909 | At5g39410 | 0,97 | | 1985 | At4g31580 | 1,02 | |
| 1834 | At1g31230 | 0,93 | | 1910 | At4g05440 | 0,97 | | 1986 | At2g20585 | 1,02 | |
| 1835 | At5g63890 | 0,93 | | 1911 | At1g76260 | 0,97 | | 1987 | At3g57280 | 1,02 | |
| 1836 | At5g48580 | 0,93 | | 1912 | At3g46560 | 0,97 | | 1988 | At2g15690 | 1,02 | |
| 1837 | At5g52760 | 0,93 | | 1913 | At2g40360 | 0,97 | | 1989 | At3g54960 | 1,02 | |
| 1838 | At4g15000 | 0,93 | | 1914 | At5g61170 | 0,97 | | 1990 | At3g53890 | 1,02 | |
| 1839 | At2g28000 | 0,93 | | 1915 | At1g14810 | 0,98 | | 1991 | At1g63830 | 1,02 | |
| 1840 | At3g57780 | 0,93 | | 1916 | At5g50810 | 0,98 | | 1992 | At2g47630 | 1,02 | |
| 1841 | At3g07480 | 0,94 | | 1917 | At3g51330 | 0,98 | | 1993 | At5g43140 | 1,02 | |
| 1842 | At3g13670 | 0,94 | | 1918 | At3g09390 | 0,98 | | 1994 | At2g36970 | 1,02 | |
| 1843 | At4g22670 | 0,94 | | 1919 | At1g28210 | 0,98 | | 1995 | At5g66200 | 1,02 | |
| 1844 | At1g63840 | 0,94 | | 1920 | At1g70090 | 0,98 | | 1996 | At3g46450 | 1,02 | |
| 1845 | At5g22770 | 0,94 | | 1921 | At4g16660 | 0,98 | | 1997 | At3g52500 | 1,02 | |
| 1846 | At3g21690 | 0,94 | | 1922 | At1g77750 | 0,98 | | 1998 | At4g10390 | 1,02 | |
| 1847 | At4g23420 | 0,94 | | 1923 | At1g29310 | 0,98 | | 1999 | At2g37110 | 1,02 | |
| 1848 | At1g62740 | 0,94 | | 1924 | At3g13230 | 0,98 | | 2000 | At2g20370 | 1,02 | |
| 1849 | At4g11890 | 0,94 | | 1925 | At5g16750 | 0,98 | | 2001 | At4g01660 | 1,02 | |
| 1850 | At1g79150 | 0,94 | | 1926 | At4g09510 | 0,98 | | 2002 | At1g33260 | 1,02 | |
| 1851 | At1g09140 | 0,94 | | 1927 | At3g02880 | 0,98 | | 2003 | At3g58990 | 1,02 | |
| 1852 | At2g37600 | 0,94 | | 1928 | At1g74230 | 0,98 | | 2004 | At3g13930 | 1,02 | |
| 1853 | At5g53590 | 0,94 | | 1929 | At3g05660 | 0,99 | | 2005 | At1g03870 | 1,03 | |
| 1854 | At5g16300 | 0,95 | | 1930 | At1g16520 | 0,99 | | 2006 | At4g18050 | 1,03 | |
| 1855 | At5g42650 | 0,95 | | 1931 | At1g07210 | 0,99 | | 2007 | At3g11964 | 1,03 | |
| 1856 | At1g77540 | 0,95 | | 1932 | At2g23350 | 0,99 | | 2008 | At3g62360 | 1,03 | |
| 1857 | At4g22380 | 0,95 | | 1933 | At1g29250 | 0,99 | | 2009 | At3g29810 | 1,03 | |
| 1858 | At4g01480 | 0,95 | | 1934 | At5g03030 | 0,99 | | 2010 | At1g20950 | 1,03 | |
| 1859 | At3g63010 | 0,95 | | 1935 | At2g39795 | 0,99 | | 2011 | At3g03560 | 1,03 | |
| 1860 | At3g13860 | 0,95 | | 1936 | At5g47050 | 0,99 | | 2012 | At5g37580 | 1,03 | |
| 1861 | At4g28200 | 0,95 | | 1937 | At4g34150 | 0,99 | | 2013 | At1g15440 | 1,03 | |
| 1862 | At5g58900 | 0,95 | | 1938 | At3g05590 | 0,99 | | 2014 | At2g27710 | 1,03 | |
| 1863 | At2g17500 | 0,95 | | 1939 | At1g53750 | 0,99 | | 2015 | At3g14450 | 1,03 | |
| 1864 | At1g42990 | 0,95 | | 1940 | At2g03480 | 0,99 | | 2016 | At2g20020 | 1,03 | |
| 1865 | At2g31610 | 0,95 | | 1941 | At1g74380 | 0,99 | | 2017 | At1g07140 | 1,03 | |
| 1866 | At5g59310 | 0,95 | | 1942 | At4g13170 | 0,99 | | 2018 | At4g26940 | 1,03 | |
| 1867 | At2g22480 | 0,95 | | 1943 | At1g09690 | 0,99 | | 2019 | At3g07430 | 1,03 | |
| 1868 | At3g60450 | 0,95 | | 1944 | At3g60360 | 0,99 | | 2020 | At3g49390 | 1,03 | |
| 1869 | At5g63680 | 0,95 | | 1945 | At1g67950 | 0,99 | | 2021 | At2g20060 | 1,03 | |
| 1870 | At2g18660 | 0,95 | | 1946 | At5g42060 | 0,99 | | 2022 | At2g01540 | 1,03 | |
| 1871 | At3g49780 | 0,95 | | 1947 | At2g35040 | 1,00 | | 2023 | At4g02360 | 1,03 | |
| 1872 | At1g31130 | 0,95 | | 1948 | At1g31860 | 1,00 | | 2024 | At4g36030 | 1,03 | |
| 1873 | At1g54020 | 0,95 | | 1949 | At5g61780 | 1,00 | | 2025 | At5g40770 | 1,04 | |
| 1874 | At5g52510 | 0,95 | | 1950 | At5g03160 | 1,00 | | 2026 | At1g41880 | 1,04 | |
| 1875 | At3g15460 | 0,95 | | 1951 | At2g29140 | 1,00 | | 2027 | At3g13330 | 1,04 | |
| 1876 | At2g47650 | 0,95 | | 1952 | At4g12130 | 1,00 | | 2028 | At1g76810 | 1,04 | |
| 1877 | At3g16870 | 0,95 | | 1953 | At3g46970 | 1,00 | | 2029 | At2g45710 | 1,04 | |
| 1878 | At1g64140 | 0,95 | | 1954 | At3g25520 | 1,00 | | 2030 | At1g26740 | 1,04 | |
| 1879 | At3g25220 | 0,95 | | 1955 | At5g61570 | 1,00 | | 2031 | At3g04240 | 1,04 | |
| 1880 | At1g72260 | 0,95 | | 1956 | At5g07090 | 1,00 | | 2032 | At3g23390 | 1,04 | |
| 1881 | At3g04090 | 0,95 | | 1957 | At1g62560 | 1,00 | | 2033 | At4g05400 | 1,04 | |
| 1882 | At3g47370 | 0,96 | | 1958 | At1g23190 | 1,00 | | 2034 | At3g13520 | 1,04 | |
| 1883 | At5g43970 | 0,96 | | 1959 | At4g00570 | 1,00 | | 2035 | At1g57590 | 1,04 | |
| 1884 | At4g26950 | 0,96 | | 1960 | At5g17010 | 1,00 | | 2036 | At5g65270 | 1,04 | |
| 1885 | At3g09200 | 0,96 | | 1961 | At1g43310 | 1,00 | | 2037 | At4g24190 | 1,04 | |
| 1886 | At1g72440 | 0,96 | | 1962 | At3g27210 | 1,00 | | 2038 | At1g26880 | 1,04 | |
| 1887 | At4g38630 | 0,96 | | 1963 | At3g09630 | 1,00 | | 2039 | At5g42050 | 1,04 | |
| 1888 | At3g05050 | 0,96 | | 1964 | At5g14520 | 1,00 | | 2040 | At1g72370 | 1,04 | |
| 1889 | At5g40830 | 0,96 | | 1965 | At2g31360 | 1,00 | | 2041 | At5g14700 | 1,04 | |
| 1890 | At2g04530 | 0,96 | | 1966 | At5g59290 | 1,01 | | 2042 | At2g36620 | 1,04 | |
| 1891 | At4g26900 | 0,96 | | 1967 | At4g38710 | 1,01 | | 2043 | At2g37500 | 1,04 | |
| 1892 | At1g50010 | 0,96 | | 1968 | At5g28370 | 1,01 | | 2044 | At1g13810 | 1,04 | |
| 1893 | At5g25780 | 0,96 | | 1969 | At1g74020 | 1,01 | | 2045 | At4g17550 | 1,04 | |
| 1894 | At4g17720 | 0,97 | | 1970 | At4g35310 | 1,01 | | 2046 | At1g28600 | 1,04 | |
| 1895 | At1g14830 | 0,97 | | 1971 | At3g01280 | 1,01 | | 2047 | At2g35720 | 1,04 | |
| 1896 | At2g44860 | 0,97 | | 1972 | At5g14640 | 1,01 | | 2048 | At4g25210 | 1,05 | |
| 1897 | At4g31800 | 0,97 | | 1973 | At1g05010 | 1,01 | | 2049 | At4g29410 | 1,05 | |
| 1898 | At5g61520 | 0,97 | | 1974 | At1g70770 | 1,01 | | 2050 | At1g28395 | 1,05 | |
| 1899 | At1g31780 | 0,97 | | 1975 | At3g11820 | 1,01 | | 2051 | At4g23200 | 1,05 | |
| 1900 | At5g47120 | 0,97 | | 1976 | At4g18100 | 1,01 | | 2052 | At3g11710 | 1,05 | |

| | gene name | log2 ratio | | | gene name | log2 ratio | | | gene name | log2 ratio | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2053 | At3g61100 | 1,05 | | 2129 | At5g17380 | 1,10 | | 2205 | At4g37660 | 1,14 | |
| 2054 | At5g67280 | 1,05 | | 2130 | At1g52890 | 1,10 | | 2206 | At2g17630 | 1,14 | |
| 2055 | At2g27530 | 1,05 | | 2131 | At1g67120 | 1,10 | | 2207 | At1g20330 | 1,14 | |
| 2056 | At4g25890 | 1,05 | | 2132 | At2g39480 | 1,10 | | 2208 | At5g60670 | 1,14 | |
| 2057 | At1g75040 | 1,05 | | 2133 | At1g15810 | 1,10 | | 2209 | At5g40480 | 1,15 | |
| 2058 | At5g09440 | 1,05 | | 2134 | At5g19550 | 1,10 | | 2210 | At3g44590 | 1,15 | |
| 2059 | At5g64680 | 1,05 | | 2135 | At2g07698 | 1,10 | | 2211 | At3g04230 | 1,15 | |
| 2060 | At1g16470 | 1,05 | | 2136 | At5g27760 | 1,10 | | 2212 | At1g67250 | 1,15 | |
| 2061 | At3g22300 | 1,05 | | 2137 | At3g17240 | 1,10 | | 2213 | At4g24380 | 1,15 | |
| 2062 | At2g29540 | 1,05 | | 2138 | At3g15430 | 1,10 | | 2214 | At5g48240 | 1,15 | |
| 2063 | At2g46650 | 1,05 | | 2139 | At1g19440 | 1,10 | | 2215 | At5g41460 | 1,15 | |
| 2064 | At3g55270 | 1,06 | | 2140 | At5g46290 | 1,10 | | 2216 | At3g62720 | 1,15 | |
| 2065 | At2g32920 | 1,06 | | 2141 | At3g54560 | 1,10 | | 2217 | At1g12840 | 1,15 | |
| 2066 | At1g66400 | 1,06 | | 2142 | At1g60770 | 1,11 | | 2218 | At3g52400 | 1,15 | |
| 2067 | At1g09100 | 1,06 | | 2143 | At5g54860 | 1,11 | | 2219 | At5g55280 | 1,15 | |
| 2068 | At2g05920 | 1,06 | | 2144 | At4g28140 | 1,11 | | 2220 | At1g16090 | 1,15 | |
| 2069 | At5g11110 | 1,06 | | 2145 | At5g54900 | 1,11 | | 2221 | At5g60390 | 1,15 | |
| 2070 | At1g69250 | 1,06 | | 2146 | At3g54950 | 1,11 | | 2222 | At3g45930 | 1,15 | |
| 2071 | At5g01740 | 1,06 | | 2147 | At1g27760 | 1,11 | | 2223 | At4g30350 | 1,15 | |
| 2072 | At4g18040 | 1,06 | | 2148 | At5g62450 | 1,11 | | 2224 | At4g25340 | 1,15 | |
| 2073 | At4g11120 | 1,06 | | 2149 | At3g05060 | 1,11 | | 2225 | At3g23110 | 1,15 | |
| 2074 | At3g11400 | 1,06 | | 2150 | At3g49210 | 1,11 | | 2226 | At2g43020 | 1,15 | |
| 2075 | At3g57630 | 1,06 | | 2151 | At4g36020 | 1,11 | | 2227 | At1g67680 | 1,15 | |
| 2076 | At5g67500 | 1,06 | | 2152 | At1g70780 | 1,11 | | 2228 | At1g09560 | 1,16 | |
| 2077 | At5g67480 | 1,06 | | 2153 | At3g10610 | 1,11 | | 2229 | At1g63720 | 1,16 | |
| 2078 | At3g60860 | 1,06 | | 2154 | At2g35480 | 1,11 | | 2230 | At1g65540 | 1,16 | |
| 2079 | At1g61730 | 1,06 | | 2155 | At5g54960 | 1,11 | | 2231 | At5g59850 | 1,16 | |
| 2080 | At1g64330 | 1,06 | | 2156 | At3g49580 | 1,11 | | 2232 | At4g31300 | 1,16 | |
| 2081 | At1g11840 | 1,07 | | 2157 | At1g24510 | 1,11 | | 2233 | At4g15640 | 1,16 | |
| 2082 | At3g50970 | 1,07 | | 2158 | At5g40690 | 1,12 | | 2234 | At2g17720 | 1,16 | |
| 2083 | At1g79390 | 1,07 | | 2159 | At2g47950 | 1,12 | | 2235 | At1g07000 | 1,16 | |
| 2084 | At5g10840 | 1,07 | | 2160 | At2g02860 | 1,12 | | 2236 | At1g76040 | 1,16 | |
| 2085 | At4g12600 | 1,07 | | 2161 | At5g27520 | 1,12 | | 2237 | At5g02870 | 1,16 | |
| 2086 | At5g03190 | 1,07 | | 2162 | At1g62300 | 1,12 | | 2238 | At5g66400 | 1,16 | |
| 2087 | At5g10730 | 1,07 | | 2163 | At5g20160 | 1,12 | | 2239 | At3g11250 | 1,16 | |
| 2088 | At2g40590 | 1,07 | | 2164 | At3g59080 | 1,12 | | 2240 | At5g08180 | 1,16 | |
| 2089 | At1g29940 | 1,07 | | 2165 | At3g49530 | 1,12 | | 2241 | At4g24570 | 1,16 | |
| 2090 | At3g06700 | 1,07 | | 2166 | At2g34350 | 1,12 | | 2242 | At3g01790 | 1,17 | |
| 2091 | At2g19540 | 1,07 | | 2167 | At5g17650 | 1,12 | | 2243 | At1g69830 | 1,17 | |
| 2092 | At4g39300 | 1,07 | | 2168 | At5g13750 | 1,12 | | 2244 | At2g02930 | 1,17 | |
| 2093 | At2g31410 | 1,07 | | 2169 | At3g56950 | 1,12 | | 2245 | At4g39190 | 1,17 | |
| 2094 | At5g06550 | 1,07 | | 2170 | At5g59870 | 1,12 | | 2246 | At2g30490 | 1,17 | |
| 2095 | At5g38830 | 1,07 | | 2171 | At5g50460 | 1,13 | | 2247 | At1g80750 | 1,17 | |
| 2096 | At1g04820 | 1,08 | | 2172 | At5g27140 | 1,13 | | 2248 | At5g62620 | 1,17 | |
| 2097 | At4g29510 | 1,08 | | 2173 | At3g22850 | 1,13 | | 2249 | At3g04710 | 1,17 | |
| 2098 | At2g29530 | 1,08 | | 2174 | At3g54640 | 1,13 | | 2250 | At3g03600 | 1,17 | |
| 2099 | At5g48300 | 1,08 | | 2175 | At3g07750 | 1,13 | | 2251 | At2g23810 | 1,17 | |
| 2100 | At3g10530 | 1,08 | | 2176 | At5g53460 | 1,13 | | 2252 | At1g09780 | 1,17 | |
| 2101 | At3g26670 | 1,08 | | 2177 | At3g10050 | 1,13 | | 2253 | At5g67360 | 1,17 | |
| 2102 | At1g70740 | 1,08 | | 2178 | At1g54990 | 1,13 | | 2254 | At5g03040 | 1,17 | |
| 2103 | At5g65360 | 1,08 | | 2179 | At1g69295 | 1,13 | | 2255 | At5g01500 | 1,17 | |
| 2104 | At4g39980 | 1,08 | | 2180 | At1g76790 | 1,13 | | 2256 | At5g26780 | 1,17 | |
| 2105 | At4g15770 | 1,08 | | 2181 | At1g75170 | 1,13 | | 2257 | At2g21580 | 1,17 | |
| 2106 | At3g60980 | 1,08 | | 2182 | At1g69070 | 1,13 | | 2258 | At3g03920 | 1,17 | |
| 2107 | At1g76550 | 1,08 | | 2183 | At5g16200 | 1,13 | | 2259 | At5g23250 | 1,18 | |
| 2108 | At5g62050 | 1,08 | | 2184 | At4g00600 | 1,13 | | 2260 | At3g07910 | 1,18 | |
| 2109 | At1g35720 | 1,08 | | 2185 | At2g31390 | 1,14 | | 2261 | At3g20000 | 1,18 | |
| 2110 | At5g67350 | 1,09 | | 2186 | At1g80840 | 1,14 | | 2262 | At1g60890 | 1,18 | |
| 2111 | At4g11010 | 1,09 | | 2187 | At5g61770 | 1,14 | | 2263 | At3g02570 | 1,18 | |
| 2112 | At4g01070 | 1,09 | | 2188 | At1g10650 | 1,14 | | 2264 | At3g57150 | 1,18 | |
| 2113 | At5g24380 | 1,09 | | 2189 | At1g17745 | 1,14 | | 2265 | At1g23290 | 1,18 | |
| 2114 | At5g04280 | 1,09 | | 2190 | At3g08630 | 1,14 | | 2266 | At5g47060 | 1,18 | |
| 2115 | At2g29570 | 1,09 | | 2191 | At2g39650 | 1,14 | | 2267 | At1g29900 | 1,18 | |
| 2116 | At5g19400 | 1,09 | | 2192 | At4g18010 | 1,14 | | 2268 | At5g54500 | 1,18 | |
| 2117 | At1g03310 | 1,09 | | 2193 | At3g63640 | 1,14 | | 2269 | At1g63090 | 1,18 | |
| 2118 | At2g04400 | 1,09 | | 2194 | At1g15410 | 1,14 | | 2270 | At3g56150 | 1,18 | |
| 2119 | At5g23070 | 1,09 | | 2195 | At5g22880 | 1,14 | | 2271 | At1g03860 | 1,18 | |
| 2120 | At1g71840 | 1,09 | | 2196 | At2g43520 | 1,14 | | 2272 | At2g46520 | 1,18 | |
| 2121 | At5g47070 | 1,09 | | 2197 | At2g32060 | 1,14 | | 2273 | At5g14050 | 1,18 | |
| 2122 | At1g16870 | 1,09 | | 2198 | At3g07590 | 1,14 | | 2274 | At5g10550 | 1,19 | |
| 2123 | At5g50360 | 1,09 | | 2199 | At1g52690 | 1,14 | | 2275 | At4g04940 | 1,19 | |
| 2124 | At3g02910 | 1,09 | | 2200 | At3g17130 | 1,14 | | 2276 | At1g62840 | 1,19 | |
| 2125 | At5g19440 | 1,09 | | 2201 | At5g51770 | 1,14 | | 2277 | At1g62540 | 1,19 | |
| 2126 | At5g47210 | 1,09 | | 2202 | At2g40700 | 1,14 | | 2278 | At3g13100 | 1,19 | |
| 2127 | At1g63780 | 1,09 | | 2203 | At2g41220 | 1,14 | | 2279 | At1g49410 | 1,19 | |
| 2128 | At5g48410 | 1,10 | | 2204 | At2g41010 | 1,14 | | 2280 | At1g17430 | 1,19 | |

| | gene name | log2 ratio | | | gene name | log2 ratio | | | gene name | log2 ratio | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2281 | At1g31817 | 1,19 | | 2357 | At3g12270 | 1,25 | | 2433 | At1g59990 | 1,31 | |
| 2282 | At1g77760 | 1,19 | | 2358 | At3g16780 | 1,25 | | 2434 | At3g03780 | 1,31 | |
| 2283 | At5g67510 | 1,19 | | 2359 | At5g22860 | 1,25 | | 2435 | At3g44190 | 1,31 | |
| 2284 | At3g55410 | 1,19 | | 2360 | At3g11130 | 1,25 | | 2436 | At1g18080 | 1,31 | |
| 2285 | At3g47520 | 1,19 | | 2361 | At2g03140 | 1,25 | | 2437 | At2g01140 | 1,32 | |
| 2286 | At1g24150 | 1,19 | | 2362 | At3g17420 | 1,25 | | 2438 | At3g62460 | 1,32 | |
| 2287 | At2g30870 | 1,19 | | 2363 | At1g50630 | 1,25 | | 2439 | At5g65630 | 1,32 | |
| 2288 | At5g24810 | 1,19 | | 2364 | At2g38670 | 1,25 | | 2440 | At1g55680 | 1,32 | |
| 2289 | At4g39880 | 1,20 | | 2365 | At3g55510 | 1,25 | | 2441 | At2g25530 | 1,33 | |
| 2290 | At5g11240 | 1,20 | | 2366 | At3g18760 | 1,25 | | 2442 | At5g18150 | 1,33 | |
| 2291 | At1g08580 | 1,20 | | 2367 | At2g41110 | 1,25 | | 2443 | At1g30530 | 1,33 | |
| 2292 | At3g49680 | 1,20 | | 2368 | At4g16143 | 1,25 | | 2444 | At5g49280 | 1,33 | |
| 2293 | At1g78050 | 1,20 | | 2369 | At1g02400 | 1,25 | | 2445 | At2g15000 | 1,33 | |
| 2294 | At3g20630 | 1,20 | | 2370 | At5g53420 | 1,26 | | 2446 | At3g23810 | 1,33 | |
| 2295 | At3g49080 | 1,20 | | 2371 | At4g04910 | 1,26 | | 2447 | At4g33550 | 1,34 | |
| 2296 | At1g29690 | 1,20 | | 2372 | At1g29980 | 1,26 | | 2448 | At1g28480 | 1,34 | |
| 2297 | At2g42560 | 1,20 | | 2373 | At5g56500 | 1,26 | | 2449 | At2g16660 | 1,34 | |
| 2298 | At2g22870 | 1,20 | | 2374 | At1g78000 | 1,26 | | 2450 | At2g39030 | 1,34 | |
| 2299 | At1g75270 | 1,20 | | 2375 | At3g50760 | 1,26 | | 2451 | At4g20980 | 1,34 | |
| 2300 | At1g77590 | 1,20 | | 2376 | At1g44800 | 1,26 | | 2452 | At1g24140 | 1,34 | |
| 2301 | At3g23940 | 1,20 | | 2377 | At1g18740 | 1,26 | | 2453 | At5g64080 | 1,34 | |
| 2302 | At1g22180 | 1,20 | | 2378 | At5g14040 | 1,26 | | 2454 | At5g61790 | 1,34 | |
| 2303 | At3g57260 | 1,21 | | 2379 | At2g22880 | 1,26 | | 2455 | At5g63120 | 1,34 | |
| 2304 | At5g66420 | 1,21 | | 2380 | At1g32900 | 1,27 | | 2456 | At3g54810 | 1,34 | |
| 2305 | At2g38550 | 1,21 | | 2381 | At1g14620 | 1,27 | | 2457 | At1g28190 | 1,34 | |
| 2306 | At5g16990 | 1,21 | | 2382 | At1g07890 | 1,27 | | 2458 | At1g10585 | 1,34 | |
| 2307 | At5g10390 | 1,21 | | 2383 | At5g52640 | 1,27 | | 2459 | At3g26910 | 1,35 | |
| 2308 | At4g22590 | 1,21 | | 2384 | At3g06680 | 1,27 | | 2460 | At3g09410 | 1,35 | |
| 2309 | At5g20280 | 1,21 | | 2385 | At1g65900 | 1,27 | | 2461 | At5g24030 | 1,35 | |
| 2310 | At2g34160 | 1,21 | | 2386 | At4g39730 | 1,27 | | 2462 | At4g44720 | 1,35 | |
| 2311 | At1g07750 | 1,21 | | 2387 | At2g45290 | 1,28 | | 2463 | At5g43380 | 1,35 | |
| 2312 | At2g18950 | 1,21 | | 2388 | At3g53560 | 1,28 | | 2464 | At1g02850 | 1,35 | |
| 2313 | At3g51160 | 1,21 | | 2389 | At4g26970 | 1,28 | | 2465 | At5g15640 | 1,35 | |
| 2314 | At2g43360 | 1,21 | | 2390 | At3g55470 | 1,28 | | 2466 | At2g19750 | 1,36 | |
| 2315 | At5g64120 | 1,21 | | 2391 | At3g20860 | 1,28 | | 2467 | At5g47700 | 1,36 | |
| 2316 | At2g19450 | 1,21 | | 2392 | At1g74450 | 1,28 | | 2468 | At5g62720 | 1,36 | |
| 2317 | At3g57520 | 1,21 | | 2393 | At2g20940 | 1,28 | | 2469 | At4g10040 | 1,36 | |
| 2318 | At4g36420 | 1,22 | | 2394 | At2g34260 | 1,28 | | 2470 | At4g00900 | 1,36 | |
| 2319 | At3g20330 | 1,22 | | 2395 | At2g24590 | 1,28 | | 2471 | At1g02930 | 1,37 | |
| 2320 | At1g68410 | 1,22 | | 2396 | At5g33290 | 1,28 | | 2472 | At1g77840 | 1,37 | |
| 2321 | At4g34390 | 1,22 | | 2397 | At2g37585 | 1,28 | | 2473 | At3g15020 | 1,37 | |
| 2322 | At4g35850 | 1,22 | | 2398 | At1g23800 | 1,28 | | 2474 | At3g13160 | 1,37 | |
| 2323 | At5g22250 | 1,22 | | 2399 | At5g48470 | 1,28 | | 2475 | At2g39050 | 1,37 | |
| 2324 | At5g59550 | 1,22 | | 2400 | At4g14365 | 1,28 | | 2476 | At5g15450 | 1,38 | |
| 2325 | At1g55900 | 1,22 | | 2401 | At2g36460 | 1,28 | | 2477 | At3g21500 | 1,38 | |
| 2326 | At1g51060 | 1,22 | | 2402 | At4g04020 | 1,28 | | 2478 | At3g17820 | 1,38 | |
| 2327 | At3g01970 | 1,22 | | 2403 | At1g16850 | 1,28 | | 2479 | At4g02940 | 1,38 | |
| 2328 | At2g20530 | 1,22 | | 2404 | At2g20690 | 1,29 | | 2480 | At5g59220 | 1,38 | |
| 2329 | At3g17390 | 1,22 | | 2405 | At2g44350 | 1,29 | | 2481 | At2g37940 | 1,38 | |
| 2330 | At5g57280 | 1,22 | | 2406 | At5g05190 | 1,29 | | 2482 | At4g25230 | 1,38 | |
| 2331 | At5g37770 | 1,22 | | 2407 | At2g24200 | 1,29 | | 2483 | At1g11140 | 1,38 | |
| 2332 | At4g36990 | 1,23 | | 2408 | At3g60260 | 1,29 | | 2484 | At3g29360 | 1,39 | |
| 2333 | At2g36870 | 1,23 | | 2409 | At5g55070 | 1,29 | | 2485 | At4g22470 | 1,39 | |
| 2334 | At3g23620 | 1,23 | | 2410 | At2g39020 | 1,29 | | 2486 | At4g13940 | 1,39 | |
| 2335 | At3g28930 | 1,23 | | 2411 | At5g56090 | 1,30 | | 2487 | At4g27280 | 1,39 | |
| 2336 | At4g10480 | 1,23 | | 2412 | At1g08940 | 1,30 | | 2488 | At2g43090 | 1,39 | |
| 2337 | At1g65840 | 1,23 | | 2413 | At1g74030 | 1,30 | | 2489 | At2g31060 | 1,39 | |
| 2338 | At2g21790 | 1,23 | | 2414 | At2g30110 | 1,30 | | 2490 | At1g02470 | 1,40 | |
| 2339 | At4g01560 | 1,23 | | 2415 | At1g17500 | 1,30 | | 2491 | At2g18510 | 1,40 | |
| 2340 | At3g19970 | 1,23 | | 2416 | At1g55500 | 1,30 | | 2492 | At1g71140 | 1,40 | |
| 2341 | At1g51090 | 1,23 | | 2417 | At4g31790 | 1,30 | | 2493 | At4g18880 | 1,40 | |
| 2342 | At3g16860 | 1,23 | | 2418 | At2g40140 | 1,30 | | 2494 | At1g51660 | 1,40 | |
| 2343 | At5g53400 | 1,23 | | 2419 | At5g04250 | 1,30 | | 2495 | At4g40010 | 1,40 | |
| 2344 | At1g24600 | 1,24 | | 2420 | At1g14980 | 1,30 | | 2496 | At5g05990 | 1,41 | |
| 2345 | At4g20170 | 1,24 | | 2421 | At3g62090 | 1,30 | | 2497 | At3g27880 | 1,41 | |
| 2346 | At4g21140 | 1,24 | | 2422 | At3g04120 | 1,30 | | 2498 | At2g20560 | 1,41 | |
| 2347 | At4g31700 | 1,24 | | 2423 | At4g26910 | 1,30 | | 2499 | At3g15000 | 1,41 | |
| 2348 | At5g22460 | 1,24 | | 2424 | At1g31660 | 1,31 | | 2500 | At5g47730 | 1,41 | |
| 2349 | At2g32070 | 1,24 | | 2425 | At3g53880 | 1,31 | | 2501 | At1g76600 | 1,41 | |
| 2350 | At3g25290 | 1,24 | | 2426 | At3g14390 | 1,31 | | 2502 | At2g21380 | 1,41 | |
| 2351 | At5g52410 | 1,24 | | 2427 | At4g26270 | 1,31 | | 2503 | At2g24850 | 1,42 | |
| 2352 | At1g53310 | 1,24 | | 2428 | At1g04990 | 1,31 | | 2504 | At2g43290 | 1,42 | |
| 2353 | At5g13190 | 1,24 | | 2429 | At2g43620 | 1,31 | | 2505 | At1g12000 | 1,42 | |
| 2354 | At1g52930 | 1,24 | | 2430 | At1g07390 | 1,31 | | 2506 | At2g47470 | 1,42 | |
| 2355 | At3g56430 | 1,24 | | 2431 | At1g80130 | 1,31 | | 2507 | At1g24020 | 1,42 | |
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| | gene name | log2 ratio | | | gene name | log2 ratio | | | gene name | log2 ratio | |
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| 2509 | At5g10750 | 1,43 | | 2585 | At3g56070 | 1,55 | | 2661 | At1g33960 | 1,73 | |
| 2510 | At3g14050 | 1,43 | | 2586 | At1g53645 | 1,55 | | 2662 | At5g60790 | 1,73 | |
| 2511 | At1g17420 | 1,43 | | 2587 | At3g47780 | 1,56 | | 2663 | At4g21380 | 1,73 | |
| 2512 | At3g52370 | 1,43 | | 2588 | At1g57990 | 1,56 | | 2664 | At5g17020 | 1,73 | |
| 2513 | At5g55915 | 1,43 | | 2589 | At4g08950 | 1,56 | | 2665 | At3g60440 | 1,73 | |
| 2514 | At4g20020 | 1,43 | | 2590 | At3g15650 | 1,56 | | 2666 | At5g02580 | 1,73 | |
| 2515 | At1g06720 | 1,44 | | 2591 | At2g37870 | 1,56 | | 2667 | At5g27390 | 1,74 | |
| 2516 | At3g25230 | 1,44 | | 2592 | At5g52300 | 1,57 | | 2668 | At5g55450 | 1,74 | |
| 2517 | At3g54150 | 1,44 | | 2593 | At5g42150 | 1,57 | | 2669 | At1g11740 | 1,74 | |
| 2518 | At1g25370 | 1,44 | | 2594 | At3g55605 | 1,58 | | 2670 | At5g46230 | 1,74 | |
| 2519 | At5g52810 | 1,44 | | 2595 | At3g18600 | 1,58 | | 2671 | At5g01340 | 1,74 | |
| 2520 | At3g14720 | 1,45 | | 2596 | At2g36880 | 1,58 | | 2672 | At4g29780 | 1,75 | |
| 2521 | At5g06320 | 1,45 | | 2597 | At1g56300 | 1,58 | | 2673 | At3g22550 | 1,75 | |
| 2522 | At1g79920 | 1,45 | | 2598 | At4g12580 | 1,58 | | 2674 | At2g37040 | 1,75 | |
| 2523 | At1g79550 | 1,45 | | 2599 | At5g49480 | 1,59 | | 2675 | At5g09590 | 1,75 | |
| 2524 | At4g28450 | 1,46 | | 2600 | At3g13470 | 1,59 | | 2676 | At5g64870 | 1,75 | |
| 2525 | At5g60960 | 1,46 | | 2601 | At1g22410 | 1,59 | | 2677 | At3g60160 | 1,75 | |
| 2526 | At1g61370 | 1,46 | | 2602 | At5g02050 | 1,59 | | 2678 | At5g55200 | 1,76 | |
| 2527 | At4g02380 | 1,46 | | 2603 | At1g61570 | 1,59 | | 2679 | At4g08390 | 1,76 | |
| 2528 | At5g15550 | 1,46 | | 2604 | At1g11330 | 1,60 | | 2680 | At1g64880 | 1,76 | |
| 2529 | At5g39740 | 1,46 | | 2605 | At2g38470 | 1,60 | | 2681 | At2g18690 | 1,76 | |
| 2530 | At3g52040 | 1,46 | | 2606 | At3g04640 | 1,60 | | 2682 | At2g36580 | 1,77 | |
| 2531 | At1g64110 | 1,46 | | 2607 | At5g15490 | 1,60 | | 2683 | At5g64550 | 1,77 | |
| 2532 | At4g32870 | 1,46 | | 2608 | At1g33030 | 1,60 | | 2684 | At5g03610 | 1,77 | |
| 2533 | At3g20510 | 1,47 | | 2609 | At1g64900 | 1,60 | | 2685 | At5g13170 | 1,77 | |
| 2534 | At2g45440 | 1,47 | | 2610 | At2g32030 | 1,60 | | 2686 | At5g40850 | 1,77 | |
| 2535 | At2g18330 | 1,47 | | 2611 | At1g06550 | 1,60 | | 2687 | At4g13890 | 1,78 | |
| 2536 | At5g57290 | 1,47 | | 2612 | At4g39030 | 1,61 | | 2688 | At2g27820 | 1,78 | |
| 2537 | At3g01800 | 1,47 | | 2613 | At1g17020 | 1,61 | | 2689 | At3g15352 | 1,78 | |
| 2538 | At3g01650 | 1,47 | | 2614 | At3g08640 | 1,61 | | 2690 | At2g30020 | 1,78 | |
| 2539 | At5g08300 | 1,47 | | 2615 | At5g26340 | 1,62 | | 2691 | At2g36530 | 1,79 | |
| 2540 | At5g13420 | 1,48 | | 2616 | At3g57490 | 1,62 | | 2692 | At3g23830 | 1,79 | |
| 2541 | At4g29520 | 1,48 | | 2617 | At5g54970 | 1,62 | | 2693 | At1g61870 | 1,79 | |
| 2542 | At3g13940 | 1,48 | | 2618 | At4g33920 | 1,62 | | 2694 | At3g59820 | 1,79 | |
| 2543 | At4g35630 | 1,48 | | 2619 | At1g71330 | 1,63 | | 2695 | At2g37770 | 1,79 | |
| 2544 | At5g67470 | 1,48 | | 2620 | At5g02590 | 1,63 | | 2696 | At2g40840 | 1,79 | |
| 2545 | At1g61580 | 1,48 | | 2621 | At4g30800 | 1,63 | | 2697 | At5g18400 | 1,79 | |
| 2546 | At5g19470 | 1,49 | | 2622 | At4g36360 | 1,63 | | 2698 | At2g25140 | 1,80 | |
| 2547 | At3g23990 | 1,49 | | 2623 | At3g18130 | 1,63 | | 2699 | At3g16530 | 1,80 | |
| 2548 | At1g67920 | 1,49 | | 2624 | At3g02650 | 1,64 | | 2700 | At1g02270 | 1,80 | |
| 2549 | At3g46660 | 1,49 | | 2625 | At5g56350 | 1,64 | | 2701 | At3g08590 | 1,80 | |
| 2550 | At3g09350 | 1,50 | | 2626 | At5g22650 | 1,64 | | 2702 | At5g66760 | 1,80 | |
| 2551 | At5g56000 | 1,50 | | 2627 | At3g22660 | 1,64 | | 2703 | At1g73600 | 1,80 | |
| 2552 | At5g52310 | 1,50 | | 2628 | At4g03450 | 1,65 | | 2704 | At5g47880 | 1,80 | |
| 2553 | At3g48020 | 1,50 | | 2629 | At5g09420 | 1,65 | | 2705 | At1g56110 | 1,82 | |
| 2554 | At1g51890 | 1,50 | | 2630 | At2g26560 | 1,65 | | 2706 | At5g56030 | 1,82 | |
| 2555 | At2g43780 | 1,50 | | 2631 | At3g07770 | 1,65 | | 2707 | At5g03650 | 1,82 | |
| 2556 | At5g48180 | 1,50 | | 2632 | At1g19270 | 1,65 | | 2708 | At5g03630 | 1,83 | |
| 2557 | At2g27840 | 1,51 | | 2633 | At5g62440 | 1,65 | | 2709 | At5g13030 | 1,83 | |
| 2558 | At4g10450 | 1,51 | | 2634 | At1g55210 | 1,65 | | 2710 | At4g18950 | 1,84 | |
| 2559 | At4g05020 | 1,51 | | 2635 | At3g58660 | 1,66 | | 2711 | At4g12120 | 1,85 | |
| 2560 | At3g27570 | 1,51 | | 2636 | At3g17520 | 1,66 | | 2712 | At1g78410 | 1,85 | |
| 2561 | At5g50800 | 1,52 | | 2637 | At1g53070 | 1,66 | | 2713 | At1g32870 | 1,85 | |
| 2562 | At1g73260 | 1,52 | | 2638 | At1g09070 | 1,66 | | 2714 | At3g22231 | 1,85 | |
| 2563 | At4g21910 | 1,52 | | 2639 | At1g72750 | 1,66 | | 2715 | At3g29320 | 1,85 | |
| 2564 | At4g37430 | 1,52 | | 2640 | At4g36220 | 1,66 | | 2716 | At5g20960 | 1,86 | |
| 2565 | At3g22600 | 1,52 | | 2641 | At1g77810 | 1,67 | | 2717 | At5g62150 | 1,86 | |
| 2566 | At5g02270 | 1,52 | | 2642 | At3g13110 | 1,67 | | 2718 | At1g80270 | 1,87 | |
| 2567 | At1g69790 | 1,52 | | 2643 | At2g14560 | 1,67 | | 2719 | At1g27730 | 1,87 | |
| 2568 | At4g23885 | 1,52 | | 2644 | At1g04980 | 1,67 | | 2720 | At4g16146 | 1,87 | |
| 2569 | At1g62790 | 1,52 | | 2645 | At4g36680 | 1,68 | | 2721 | At1g24280 | 1,88 | |
| 2570 | At5g46240 | 1,53 | | 2646 | At3g53260 | 1,68 | | 2722 | At4g26780 | 1,88 | |
| 2571 | At5g02490 | 1,53 | | 2647 | At4g00630 | 1,69 | | 2723 | At5g10695 | 1,88 | |
| 2572 | At1g22070 | 1,53 | | 2648 | At5g56630 | 1,70 | | 2724 | At3g02230 | 1,88 | |
| 2573 | At3g22240 | 1,53 | | 2649 | At5g67300 | 1,70 | | 2725 | At5g03350 | 1,89 | |
| 2574 | At4g24830 | 1,53 | | 2650 | At1g02200 | 1,70 | | 2726 | At5g53870 | 1,90 | |
| 2575 | At3g09010 | 1,53 | | 2651 | At4g39890 | 1,71 | | 2727 | At1g21750 | 1,91 | |
| 2576 | At1g66500 | 1,53 | | 2652 | At3g50910 | 1,71 | | 2728 | At1g48920 | 1,91 | |
| 2577 | At1g24070 | 1,53 | | 2653 | At1g10580 | 1,71 | | 2729 | At1g22400 | 1,92 | |
| 2578 | At1g18320 | 1,54 | | 2654 | At3g51800 | 1,71 | | 2730 | At1g32920 | 1,92 | |
| 2579 | At2g46630 | 1,54 | | 2655 | At1g48630 | 1,72 | | 2731 | At1g56650 | 1,93 | |
| 2580 | At1g78610 | 1,54 | | 2656 | At1g61640 | 1,72 | | 2732 | At2g43570 | 1,93 | |
| 2581 | At1g61120 | 1,54 | | 2657 | At1g10370 | 1,72 | | 2733 | At5g13110 | 1,93 | |
| 2582 | At4g27520 | 1,55 | | 2658 | At5g17450 | 1,72 | | 2734 | At2g25620 | 1,93 | |
| 2583 | At1g63940 | 1,55 | | 2659 | At5g51830 | 1,72 | | 2735 | At3g02480 | 1,93 | |
| 2584 | At1g09200 | 1,55 | | 2660 | At3g55620 | 1,72 | | 2736 | At2g46400 | 1,94 | |

| | gene name | log2 ratio | | | gene name | log2 ratio | | | gene name | log2 ratio | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2737 | At1g54570 | 1,94 | | 2813 | At1g57630 | 2,32 | | 2889 | At3g44990 | 3,93 | |
| 2738 | At5g25930 | 1,97 | | 2814 | At3g55430 | 2,32 | | 2890 | At2g32210 | 4,01 | |
| 2739 | At3g53230 | 1,97 | | 2815 | At2g22500 | 2,34 | | 2891 | At3g22840 | 4,02 | |
| 2740 | At1g30510 | 1,97 | | 2816 | At4g34590 | 2,35 | | 2892 | At2g41730 | 4,10 | |
| 2741 | At5g28540 | 1,97 | | 2817 | At5g27420 | 2,36 | | 2893 | At1g05680 | 4,17 | |
| 2742 | At2g17230 | 1,97 | | 2818 | At3g05500 | 2,37 | | 2894 | At4g22870 | 4,31 | |
| 2743 | At2g33210 | 1,97 | | 2819 | At5g15740 | 2,37 | | 2895 | At1g15520 | 4,61 | |
| 2744 | At4g17615 | 1,97 | | 2820 | At1g69870 | 2,37 | | 2896 | At2g29470 | 4,62 | |
| 2745 | At3g49620 | 1,98 | | 2821 | At2g45570 | 2,38 | | | | | |
| 2746 | At1g05000 | 1,98 | | 2822 | At5g20230 | 2,41 | | | | | |
| 2747 | At1g06830 | 1,98 | | 2823 | At3g57450 | 2,42 | | | | | |
| 2748 | At1g78570 | 1,98 | | 2824 | At4g05390 | 2,43 | | | | | |
| 2749 | At4g02930 | 1,98 | | 2825 | At2g21640 | 2,43 | | | | | |
| 2750 | At3g52930 | 1,99 | | 2826 | At4g34200 | 2,44 | | | | | |
| 2751 | At4g13850 | 1,99 | | 2827 | At5g39520 | 2,46 | | | | | |
| 2752 | At5g15090 | 1,99 | | 2828 | At4g37910 | 2,48 | | | | | |
| 2753 | At4g25630 | 1,99 | | 2829 | At4g34710 | 2,48 | | | | | |
| 2754 | At5g07440 | 2,00 | | 2830 | At2g41380 | 2,53 | | | | | |
| 2755 | At5g67340 | 2,00 | | 2831 | At1g69880 | 2,55 | | | | | |
| 2756 | At3g48520 | 2,01 | | 2832 | At4g17770 | 2,55 | | | | | |
| 2757 | At5g05410 | 2,01 | | 2833 | At2g36770 | 2,56 | | | | | |
| 2758 | At3g25780 | 2,01 | | 2834 | At2g03760 | 2,56 | | | | | |
| 2759 | At1g17830 | 2,02 | | 2835 | At5g57560 | 2,60 | | | | | |
| 2760 | At3g22310 | 2,03 | | 2836 | At5g41670 | 2,60 | | | | | |
| 2761 | At1g11210 | 2,03 | | 2837 | At3g61190 | 2,62 | | | | | |
| 2762 | At2g29670 | 2,03 | | 2838 | At4g01870 | 2,63 | | | | | |
| 2763 | At5g05220 | 2,04 | | 2839 | At5g41740 | 2,67 | | | | | |
| 2764 | At5g17760 | 2,05 | | 2840 | At1g01560 | 2,68 | | | | | |
| 2765 | At1g60730 | 2,05 | | 2841 | At2g26400 | 2,69 | | | | | |
| 2766 | At1g77510 | 2,06 | | 2842 | At1g76650 | 2,72 | | | | | |
| 2767 | At5g65300 | 2,06 | | 2843 | At4g39210 | 2,73 | | | | | |
| 2768 | At4g20830 | 2,07 | | 2844 | At2g27550 | 2,75 | | | | | |
| 2769 | At3g28340 | 2,08 | | 2845 | At3g56710 | 2,77 | | | | | |
| 2770 | At4g15910 | 2,08 | | 2846 | At3g50930 | 2,78 | | | | | |
| 2771 | At1g76960 | 2,09 | | 2847 | At4g36010 | 2,80 | | | | | |
| 2772 | At2g15620 | 2,10 | | 2848 | At2g14620 | 2,83 | | | | | |
| 2773 | At1g30620 | 2,10 | | 2849 | At2g28900 | 2,85 | | | | | |
| 2774 | At3g49120 | 2,11 | | 2850 | At5g64310 | 2,85 | | | | | |
| 2775 | At5g13200 | 2,11 | | 2851 | At5g38900 | 2,86 | | | | | |
| 2776 | At4g34860 | 2,11 | | 2852 | At5g62480 | 2,87 | | | | | |
| 2777 | At3g44750 | 2,11 | | 2853 | At1g66090 | 2,87 | | | | | |
| 2778 | At2g27510 | 2,12 | | 2854 | At1g19020 | 2,88 | | | | | |
| 2779 | At3g22370 | 2,12 | | 2855 | At4g34950 | 2,88 | | | | | |
| 2780 | At3g09440 | 2,13 | | 2856 | At2g02010 | 2,93 | | | | | |
| 2781 | At1g72520 | 2,13 | | 2857 | At1g56060 | 2,94 | | | | | |
| 2782 | At5g17050 | 2,13 | | 2858 | At2g14610 | 2,95 | | | | | |
| 2783 | At5g13490 | 2,14 | | 2859 | At4g15210 | 3,00 | | | | | |
| 2784 | At4g27570 | 2,14 | | 2860 | At1g32960 | 3,02 | | | | | |
| 2785 | At3g25610 | 2,16 | | 2861 | At1g59860 | 3,03 | | | | | |
| 2786 | At1g12845 | 2,16 | | 2862 | At2g30770 | 3,05 | | | | | |
| 2787 | At5g25260 | 2,16 | | 2863 | At3g01830 | 3,07 | | | | | |
| 2788 | At2g16900 | 2,16 | | 2864 | At3g26830 | 3,09 | | | | | |
| 2789 | At1g64660 | 2,16 | | 2865 | At5g59820 | 3,12 | | | | | |
| 2790 | At2g26440 | 2,17 | | 2866 | At3g28210 | 3,16 | | | | | |
| 2791 | At5g63450 | 2,18 | | 2867 | At1g13340 | 3,16 | | | | | |
| 2792 | At3g55120 | 2,18 | | 2868 | At5g17220 | 3,19 | | | | | |
| 2793 | At3g12580 | 2,18 | | 2869 | At4g33905 | 3,20 | | | | | |
| 2794 | At2g15480 | 2,19 | | 2870 | At2g22470 | 3,20 | | | | | |
| 2795 | At1g70810 | 2,19 | | 2871 | At5g35735 | 3,20 | | | | | |
| 2796 | At2g22170 | 2,20 | | 2872 | At4g37370 | 3,22 | | | | | |
| 2797 | At1g32940 | 2,22 | | 2873 | At1g59730 | 3,27 | | | | | |
| 2798 | At2g29350 | 2,22 | | 2874 | At1g43910 | 3,28 | | | | | |
| 2799 | At2g21660 | 2,22 | | 2875 | At3g51240 | 3,35 | | | | | |
| 2800 | At4g39950 | 2,23 | | 2876 | At5g13930 | 3,38 | | | | | |
| 2801 | At1g62710 | 2,25 | | 2877 | At3g21560 | 3,41 | | | | | |
| 2802 | At2g29460 | 2,25 | | 2878 | At3g48650 | 3,43 | | | | | |
| 2803 | At1g68620 | 2,26 | | 2879 | At4g25810 | 3,46 | | | | | |
| 2804 | At3g03250 | 2,26 | | 2880 | At1g05340 | 3,47 | | | | | |
| 2805 | At5g48880 | 2,28 | | 2881 | At4g39670 | 3,49 | | | | | |
| 2806 | At4g01080 | 2,28 | | 2882 | At5g54100 | 3,51 | | | | | |
| 2807 | At4g37295 | 2,30 | | 2883 | At1g17170 | 3,52 | | | | | |
| 2808 | At1g67360 | 2,30 | | 2884 | At5g42800 | 3,52 | | | | | |
| 2809 | At4g09600 | 2,30 | | 2885 | At4g09020 | 3,62 | | | | | |
| 2810 | At4g17260 | 2,31 | | 2886 | At5g07990 | 3,70 | | | | | |
| 2811 | At5g34940 | 2,32 | | 2887 | At2g32190 | 3,70 | | | | | |
| 2812 | At3g52180 | 2,32 | | 2888 | At1g03495 | 3,88 | | | | | |

**Table 4.** Transcript profiling after over night sucrose feeding. Data were normalized using RMA software, only detection values over 100 were treated as present and ratios were calculated. Presented values show ratio of sucrose incubated sample in comparison to control in log2 scale. Expression data were filtered to select only genes showing a coinciding change-call in the two biological replicates samples for each experimental condition.
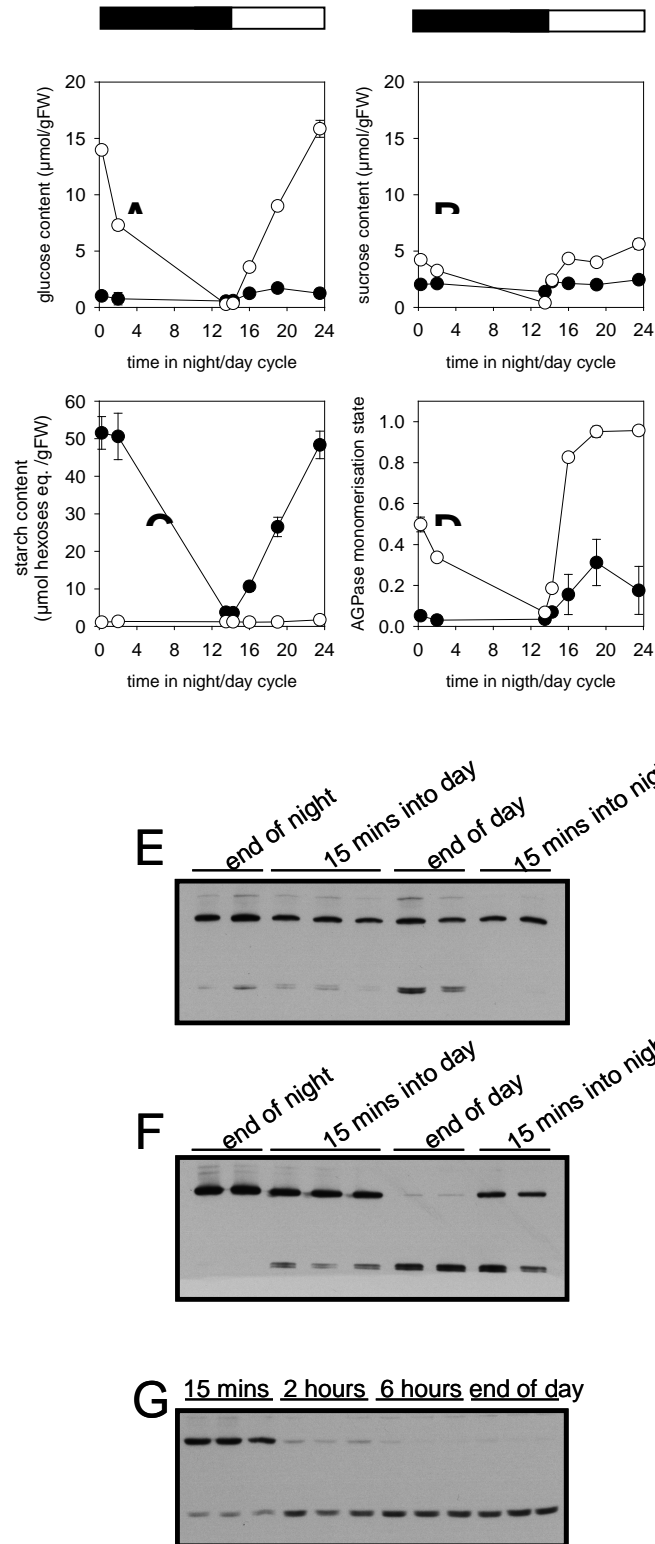
**Figure 36.** Monomerisation of AGPB is increased in the starch-deficient *pgm* mutant. Glucose (**A**), sucrose (**B**), and starch content (**C**) in *Arabidopsis* leaves of 6-weeks-old Col 0 ( ○ ) and 11-week-old *pgm* ( ● ) during a night/day cycle (indicated by black and white bar above the figures). The reduction state of the AGPase antigen in the same

samples (**D**). Non-reducing western of leaves of Col 0 (**E**) and pgm (**F**) at the change of light. Non-reducing western of *pgm* samples harvested at the indicated times after the start of the illumination (**G**). Figures were kindly provided by Janneke Hendriks (Hendriks et al., 2003).
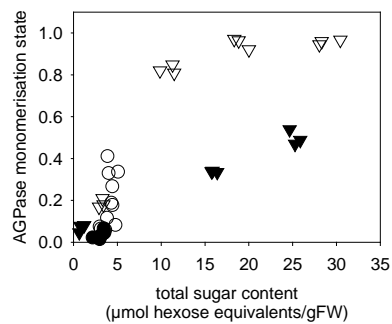


**Figure 37.** Relation between AGPB dimerisation and the leaf sugar content in the light and dark. The data from **Fig. 36** were replotted showing the relation between the AGPB dimerisation state and the total sugar content (sum of glucose, fructose and sucrose) for Col 0 (•,o ) and *pgm* (▼, ∇) during the night (•,▼) and the day (o,∇). When extracts from darkened leaves are compared, there is a correlation between leaf sugar levels and the appearance of AGPB monomer. Illumiation leads to increased monomerisation at a given sugar content in the dark. Figure was kindly provided by Janneke Hendriks (Hendriks et al., 2003).

# Zusammenfassung

Es ist bereits seit längerem bekannt, dass viele Enzyme des Calvinzyklus, des oxidativen Pentosephosphatwegs, des NAD(P)H-Exports und der ATP-Synthese durch post-translationale Redox-Modifikation in Antwort auf Licht reguliert werden. In der vorliegenden Arbeit sollte untersucht werden, ob ein ähnlicher Mechanismus auch die Kohlenstoffspeicherung in Blättern reguliert.

Vorangegangene Studien mit Kartoffelknollen zeigten, dass das Schlüsselenzym der Stärkesynthese ADP-Glukose-Pyrophosphorylase (AGPase) durch die Bildung einer Disulfidbrücke zwischen den zwei kleinen Untereinheiten (AGPB) des tetrameren Proteins inaktiviert wird, die Bedeutung dieses Mechanismus für die Stärkesynthese in Blättern blieb jedoch bislang ungeklärt. Die vorliegenden Arbeiten zeigen, das AGPase in Erbsen-, Kartoffel- und Arabidopsis-Blättern über post-translationale Redox-Modifikation in Antwort auf Tag-Nacht Änderungen reguliert wird. Dies erfolgt über ein Licht-abhängiges Signal, da, erstens, AGPB in isolierten Chloroplasten durch Belichtung sehr schnell von Dimer zu Monomer umgewandelt wird und, zweitens, ein Abdunkeln der Blätter zu einer schnellen Umwandlung von AGPB von Monomer zu Dimer führt. Die Monomerisierung von AGPB ging mit Änderungen in den kinetischen Eigenschaften des Enzyms einher, die zu einer Aktivierung führten. Studien mit Extrakten aus Erbsenchloroplasten zeigten, dass die AGPase-Redoxaktivierung *in-vitro* durch die Thioredoxine *f* und *m* aus Spinat vermittelt wird. In einem weiteren experimentellen Ansatz konnte gezeigt werden, dass auch Zucker zu Redox-Aktivierung der AGPase und erhöhter Stärkesynthese in Blättern führen, und dass diese unabhängig von Licht wirken. Externe Zugabe von Zuckern wie Saccharose oder Trehalose an Arabidopsis-Blätter im Dunkeln führten zu Monomerisierung von AGPB und einer Erhöhung der Stärkesyntheserate , während die Spiegel des allosterischen Aktivators 3PGA unverändert blieben und keine Änderungen im NADPH/NADP+-Verhältnis auftraten. Experimente mit transgenen Arabidopsis-Pflanzen mit veränderten Spiegeln des Vorläufers der Trehalosesynthese, Trehalose-6-phosphat (T6P), zeigten, dass T6P und nicht Trehalose zu Redox-Aktivierung von AGPase führt. Expression einer E. coli T6P synthase (TPS) im Zytosol führte zu erhöhter Redox-Aktivierung von AGPase und erhöhter Stäreksynthese in Blättern, während die Expression einer T6P-Phosphatase

(TPP) gegenteilige Änderungen bewirkte. Diese Auswirkungen erfolgten unabhängig von Änderungen in den Spiegeln von Zuckern und Zuckerphosphaten oder im NADPH/NADP+-Verhältnis. Externe Zugabe von Saccharose führte zu Monomerisierung von AGPB in Wildtyp und TPS exprimierenden Blättern, während diese Antwort in TPP exprimierenden Blättern stark abgeschwächt war. Dies zeigt, dass T6P eine wesentliche Komponente darstellt, die die Redox-Aktivierung der AGPase in Antwort auf Saccharose vermittelt. T6P wurde auch für 15 min direkt an intakte, isolierte Erbsenchloroplasten gefüttert. T6P Konzentrationen im Bereich von 100 µM bis 10 mM führten zu einem signifikanten und spezifischen Anstieg der AGPB-Monomersierung und der AGPase Aktivität. Dies zeigt, dass T6P auch ohne zytosolische Elemente die Redox-Aktivierung der AGPase stimuliert und somit ein Signal zwischen Zytosol und Plastid darstellt. Diese Antwort erfolgte ohne Änderungen im NADPH/NADP+-Verhältnis, was zeigt, dass T6P eher den Redox-Transfer zu AGPase als den Redoxzustand des Chloroplasten moduliert.

Acetyl-CoA-Carboxylase (ACCase) ist als Schlüsselenzym der Fettsäure- und Lipidsynthese in Pflanzen bekannt. Zu Beginn der vorliegenden Arbeit lagen hauptsächlich in-vitro Befunde vor, die zeigten, dass ACCase durch DTT und thioredoxine *f* und *m* über Redox-Modulation reguliert wird. In der Arbeit sollte daher die in-vivo Relevanz dieses Mechanismus für die Regulation der Lipidsynthese in Blättern untersucht werden. ACCase zeigte einen höheren Redox-Aktivierungszustand in Arabidopsis-Blätter, die während des Tages im Vergleich zur Nacht geerntet wurden. Die Redox-Aktivierung der ACCase wurde von Änderungen in den kinetischen Eigenschaften begleitet und führte zu einer erhöhten Affinität des Enzymes gegenüber Acetyl-CoA als Substrat. In weiteren Versuchen wurde sowohl DTT als auch Saccharose an Blätter gefüttert, und beide Behandlungen führten zu Redox-Aktivierung von ACCase, was mit erhöhten Lipidsynthesraten und einem Rückgang des Acetyl-CoA-Spiegels einherging.

In einem abschließenden Ansatz, wurden Metabolit- und Transkriptprofile verglichen, die nach DTT oder Saccharosefütterung an Blättern erhalten wurden. Sie zeigen, dass auch andere zentrale Stoffwechselwegen in Pflanzen (wie TCA-Zyklus und Aminosäuresynthese) über post-translationale Redoxregulation und unabhängig von Änderungen in Transkriptspiegeln reguliert werden.

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# Curriculum vitae

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