Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Oral Communication Abstract – 5B.01

UNDERSTANDING STARCH METABOLISM IN PLANTS AND THE POTENTIAL TO IMPROVE STARCH CROPS

ZEEMAN S.C.

Department of Biology, ETH Zurich (Switzerland)

Starch, Arabidopsis, debranching enzymes, reversible glucan phosphorylation, amylases, protein-protein interactions

Starch is our primary source of nutrition and a key renewable resource used by industry (e.g. as a feedstock for bioethanol production). It is composed of branched and linear glucans with an architecture that allows the formation of insoluble, semi-crystalline granules. Understanding the metabolism of starch in plants gives us options for starch crop improvement by altering starch structure and properties, and by increasing yields. Much progress has been made by studying starch metabolism in model species such as *Arabidopsis thaliana*. In Arabidopsis, as in most plants, starch is a primary product of photosynthesis in leaves, where it is temporarily stored in chloroplasts for use during the night. Functional genomic studies have advanced our understanding of both starch synthesis and breakdown.

Recent data are consistent with the idea that starch synthesis requires both synthetic enzymes (starch synthases and starch branching enzymes) and degradative enzymes (debranching enzymes). Together, these enzymes create the correct glucan structure to allow crystallization. A wealth of data from different systems shows that starch structure can be manipulated in a rational way by altering the complement of these enzymes. As a result, glucans with altered properties can be obtained (e.g. in the degree of crystallinity or solubility, the ease of hydrolysis to fermentable sugars), some of which may be better suited to industrial applications than wild-type starches.

Advances have also been made in understanding starch degradation. For example, glucan phosphorylation, mediated by glucan water dikinases (GWD and PWD), is required for normal degradation to occur. Phosphorylation disrupts the starch granule surface, rendering the glucans accessible for degrading enzymes. However, dephosphorylation, mediated by the chloroplastic phosphatase SEX4 (Starch EXcess4), is also required for starch degradation. Phosphorylated intermediates of starch breakdown accumulate in *sex4* mutants. This is because the phosphate groups, while necessary to disrupt the granule surface, can also obstruct enzymes of starch degradation such as beta-amylases. Experiments with starch granules *in vitro* show that the rate of degradation is increased by simultaneous phosphorylation and dephosphorylation, corroborating the hypothesis that reversible glucan phosphorylation and glucan hydrolysis are synergistic processes. Plants have two homologues of SEX4, LSF1 and LSF2 (Like Sex Four). With collaborating labs, we recently demonstrated that the loss of LSF1 also causes a starch-excess phenotype. However, the roles of LSF1 and SEX4 differ; phospho-oligosaccharides do not accumulate in lsf1 and recombinant LSF1 protein has no phosphatase activity. These findings indicate additional complexity in the process of transient phosphorylation of the granule during starch degradation, which is the subject of ongoing research in our lab.

Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Oral Communication Abstract – 5B.02

INTEGRATION OF CHLOROPLAST STARCH METABOLISM WITH HORMONAL REGULATION OF PLANT GROWTH

PAPARELLI E.*, PARLANTI S.*, GONZALI S.*, NOVI G.*, MARIOTTI L.**,
VAN DONGEN J.T.***, PERATA P.*

*) PlantLab, Istitute of Life Science, Scuola Superiore Sant'Anna, Piazza Martiri della Libertá 33, 56127 Pisa (Italy)
**) Department of Crop Plant Biology, University of Pisa, Via Mariscoglio 34, 56124 Pisa (Italy)
***) Max Planck Institute of Molecular Plant Physiology, Energy Metabolism Research Group, Potsdam-Golm (Germany)

Starch metabolism, gibberellins response, Arabidopsis thaliana, plant growth

Mutants showing altered starch synthesis or degradation are smaller than the wild-type. This is usually explained as a direct consequence of diminished carbohydrate availability during the night phase. This work will show evidences suggesting that plant size in starch mutants results from reduced gibberellin synthesis. We show that kaurene synthesis (the precursor of gibberellins) in the chloroplast is regulated by a day/night cycle and starch mutants such as *pgm* and *sex1* loose the day peak of kaurene synthase expression. Reduced kaurene content were found at the end of the day in *pgm* and *sex1*. Exogenous gibberellin application reverts the dwarf phenotype in starch-defective mutants. These results suggest that plant growth is regulated so that it does not exceed the availability of carbohydrates by an integration of starch metabolism with hormonal-driven growth.

Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Oral Communication Abstract – 5B.03

REDOX-REGULATED BAM1 AND ITS ROLE IN DIURNAL STARCH DEGRADATION

SPARLA F.*, VALERIO C.*, MARRI L.*, COSTA A.**, ISSAKIDIS-BOURGUET E.***, PUPILLO P.*, TROST P.*

*) Department of Experimental Evolutionary Biology, University of Bologna, Via Irnerio 42, Bologna 40126 (Italy)
**) Department of Biology, University of Padova, Via U. Bassi 58/B, 35131 Padua (Italy)
***) Institut de Biotechnologie des Plantes, Unité Mixte de Recherche 8618, Centre National de la Recherche Scientifique, Université Paris-Sud, 91405 Orsay cedex (France)

Arabidopsis thaliana, starch, guard cell, redox, stomata opening

Possibly due to their sessile nature, plants show a huge metabolic plasticity, essential both for their normal growth and in response to environmental change. Post-translation modifications take part in plant metabolic plasticity, regulating the activity of metabolic enzymes. Protein phosphorylation and thiol/disulfide redox modulation are common modifications of plant enzymes and as such they are involved in the control of plant metabolism.

Starch is the major storage biopolymer synthesized by plants, and leaf starch is transiently accumulated in a daily cycle. Soluble carbohydrates resulting from starch degradation are mainly exported to sink tissues. Alternatively, starch-derived soluble sugars can also participate to osmotic adjustment under water stress or take part in the osmotic regulation of specialized cells, notably guard cells.

Here we report the characterization of BAM1 (At3g23920), a plastid-targeted beta-amylase of *Arabidopsis thaliana* specifically activated by reducing conditions. Under oxidizing conditions, the catalytic activity of BAM1 is close to zero. Among all major plastid thioredoxin isoforms, BAM1 was preferably activated by thioredoxin f1, followed by thioredoxins m1, m2, y1, y2, and m4. Alternative activation of BAM1 was also achieved by plastid-localized NADPH-thioredoxin reductase (NTRC), which allowed the recovery of about half of the BAM1 maximal activity.

In contrast with the timing of starch metabolism in mesophyll cells, redox regulation of BAM1 activity suggests that this enzyme would be mainly active in the light rather than in darkness. To elucidate this inconsistent behaviour, knockout (KO) mutants and promoter activity of BAM1 were analyzed.

Differently from KO mutants for BAM3 (At4g17090; the major chloroplast beta-amylase, insensitive to thiol/disulfide redox modulation) which show a reduced phenotype characterized by starch accumulation at night (sex phenotype), a T-DNA insertion line in which BAM1 expression was nil displays normal growth but reduced stomata opening and increased starch content in illuminated guard cells.

To study the promoter activity of BAM1, the reporter genes GUS and YFP were placed under the control of BAM1 promoter, and Arabidopsis transgenic plants were analyzed. In non-flowering plants, both YFP and GUS plants showed expression of BAM1 in leaves and roots, but expression in leaves was mainly restricted to guard cells, in agreement with the regulatory properties of the enzyme. Interestingly, BAM1 expression appeared in mesophyll cells of young plants in response to

osmotic stress. Total β-amylase activity also increased, together with its redox-sensitive fraction, in osmotically stressed wild type plants but not in KO mutants. Taken together these data suggest that thioredoxin-regulated BAM1 activates a starch degradation pathway in illuminated mesophyll cells upon osmotic stress, similar to the diurnal pathway of starch degradation in guard cells that is also dependent on thioredoxin-regulated BAM1.

Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Oral Communication Abstract – 5B.04

GENETIC MANIPULATION OF STARCH COMPOSITION TO IMPROVE NUTRITIONAL AND TECHNOLOGICAL PROPERTIES OF DURUM AND BREAD WHEAT

SESTILI F., BOTTICELLA E., LAFIANDRA D.

DAFNE, University of Tuscia, Via S. Camillo de Lellis SNC, 01100 Viterbo (Italy)

Starch, low and high amylose, genetic improvement, wheat

Reserve starch, the major component of wheat kernel, is constituted by two glucan polymers, amylose and amylopectin. The appropriate manipulation of amylose/amylopectin ratio results in the production of flours or semolina with novel functional characteristics. High amylose starch is particularly interesting because of its correlation with the amount of resistant starch in food, that plays a role similar to dietary fibre in the intestine with beneficial physiological effects for human health. On other hand many uses have been suggested for low amylose wheat, including as a source of blending flour to improve shelf-life and processing quality of baked and frozen products.

The absence of the starch synthases SSII or starch branching enzymes SBEIIa activities is associated to genotypes with an higher amylose content, whereas the knockout of the granule bound starch synthases I genes (GBSSI or waxy) produces a drastic decrease of amylose content. Three different strategies have been used to target SSII, SBEIIa and GBSSI proteins: 1) identification of natural mutants; 2) gene silencing by RNA interference; 3) TILLING (Targeting Induced Local Lesion in Genomes). Natural and EMS-induced mutants for SSII and GBSSI have been identified by SDS-PAGE analysis in bread and durum wheat. RNAi silencing of *SBEIIa* genes in durum wheat causes obvious alterations in granule morphology and starch composition, leading to high amylose wheat (>75%). Spaghetti produced with high amylose transgenic semolina showed improved quality characteristics, such as an increased firmness and decreased stickiness and water absorption. As these materials are very interesting for food industry, a non transgenic approach was chosen to silence *SBEIIa* genes in bread wheat. In particular TILLING analysis permitted to identify several allelic variants in *SBEIIa* genes, including putative *null* alleles containing non-sense or splice site mutations, responsible for the loss of functionality of the three *SBEIIa* homoeoalleles. Crossing activity is currently underway, in order to incorporate the three single null *SBEIIa* homoeoalleles in the same genotype.