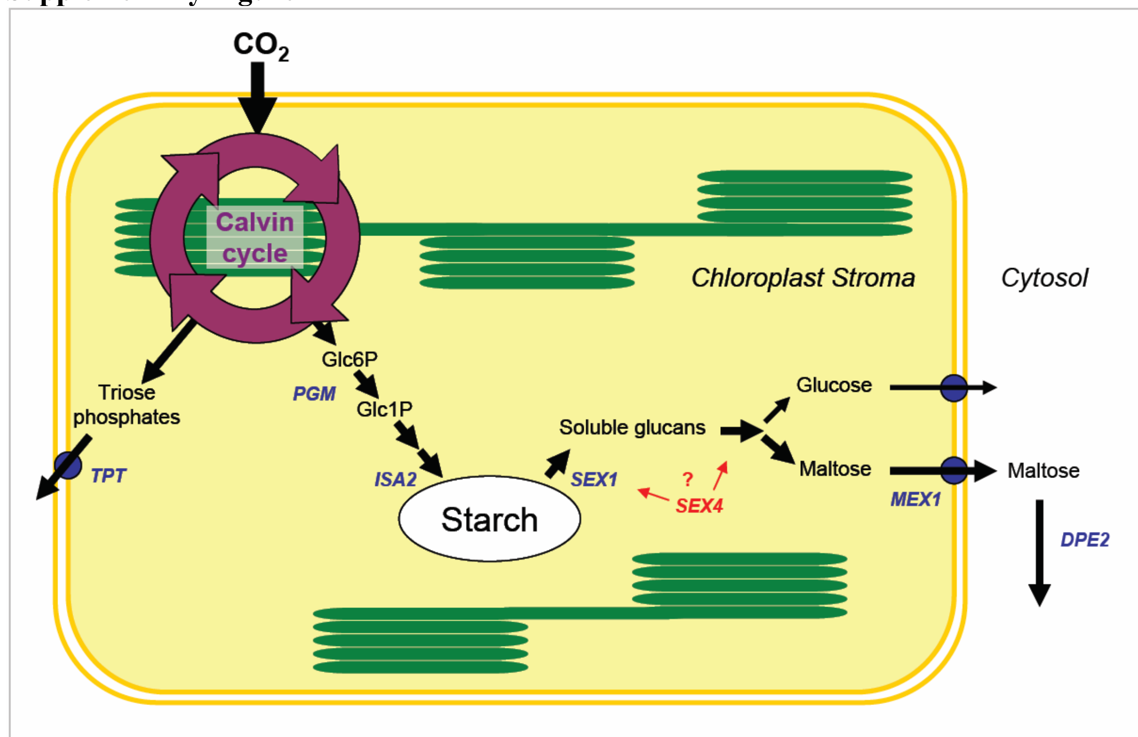
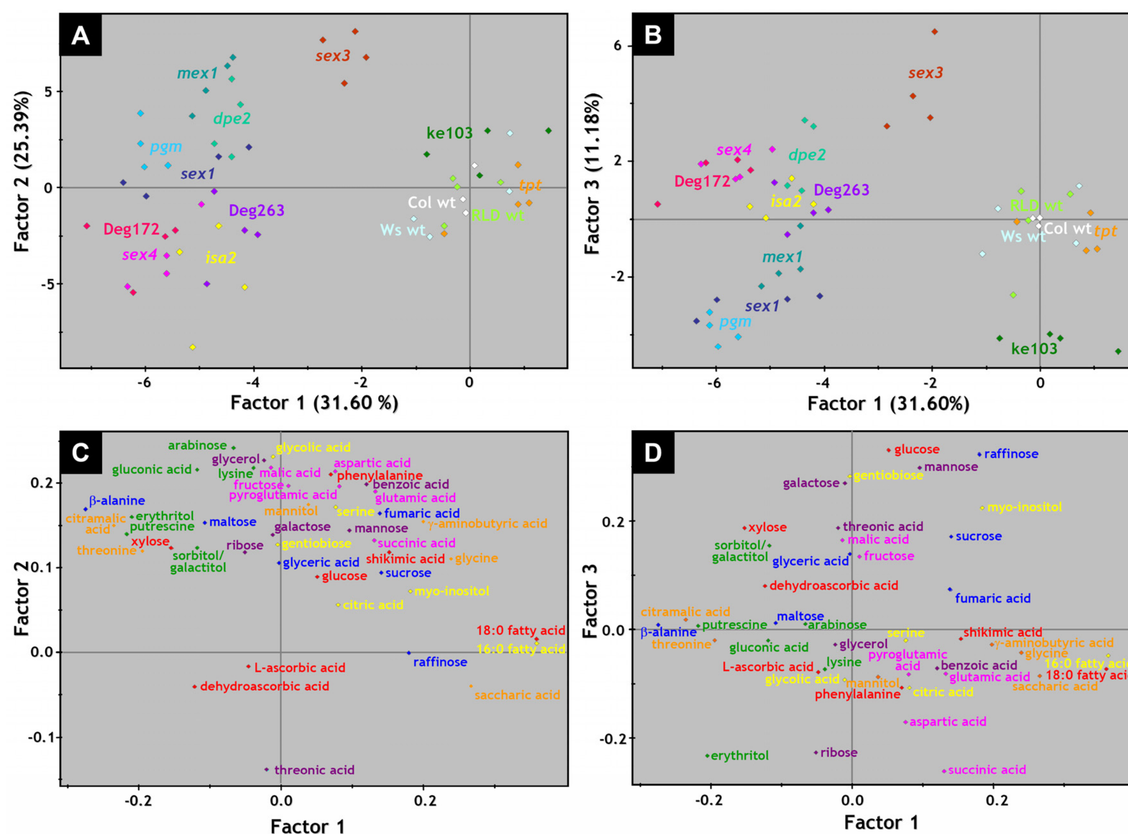
Supplementary Figure 1



Supplementary Figure 1. Scheme summarising the pathways of starch synthesis and breakdown in the chloroplasts of *Arabidopsis* leaves. Carbon dioxide assimilated via the Calvin cycle is partitioned, with part exported via the triose-phosphate/phosphate translocator (TPT) and part used for the production of glucose-6-P inside the chloroplast stroma. Mutation of the gene encoding TPT results in less export of photoassimilates and therefore a higher rate of starch synthesis in the light (Schneider et al., 2002). Consequently, *tpt* plants have a starch-excess phenotype as secondary effect of the mutation. Glucose-6-P in the chloroplast is converted into glucose-1-P via phosphoglucomutase (PGM). *pgm* plants are unable to synthesise starch (Caspar et al., 1985) and therefore have a higher level of photoassimilate export from the chloroplast during the day and accumulate sugars. However, the amount of sugar accumulated is small compared with the amount of starch made by a wild-type plant, and it is rapidly consumed at the onset of the night period. Starch is synthesised by chain elongation and branching. However, loss of a debranching enzyme comprised of ISA1 and ISA2 subunits results in reduced accumulation of glucan, which has an altered branching pattern and is mostly soluble (Zeeman et al., 1998a). Partial debranching of the branched glucan structure during synthesis probably promotes the formation of crystalline regions, resulting in an insoluble starch granule (Delatte et al., 2005; Wattebled et al., 2005). Most of

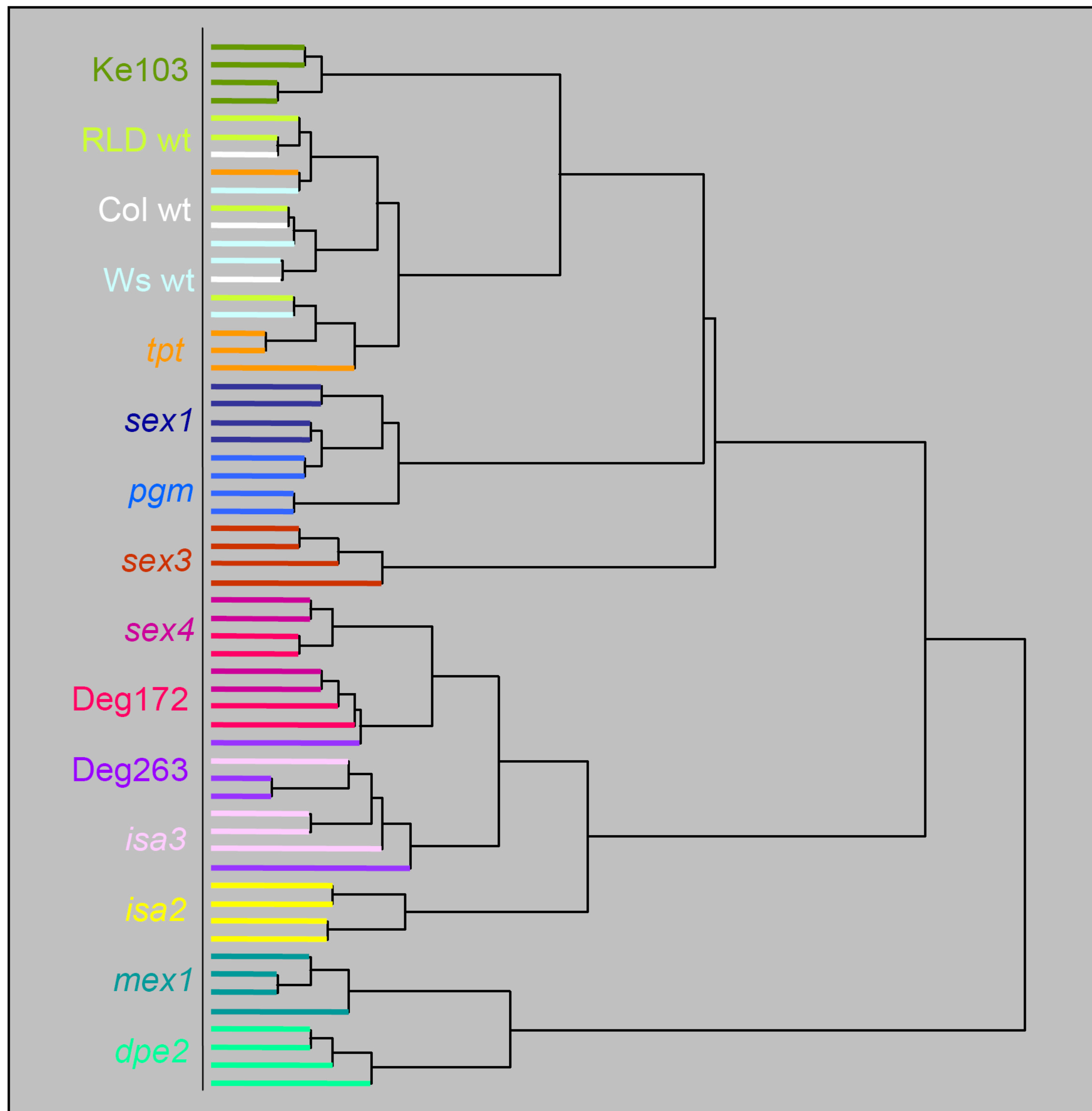
the soluble glucan in *isa1* or *isa2* mutants is degraded in the first half of the night, whereas starch in the wild type is degraded at a constant rate and lasts for the whole night. In wild-type plants, phosphorylation of glucosyl residues at the surface of the starch granule is required for normal rates of breakdown. This is mediated by glucan, water dikinase, encoded at the *SEX1* locus (Yu et al., 2001). In *sex1* mutants, phosphorylation is reduced or abolished and breakdown is inhibited, leading to the accumulation of very high levels of starch. The enzymes that catalyse starch breakdown produce soluble glucans which are metabolised to maltose and glucose for export from the chloroplast. This process is not fully understood, but recent results suggest that the pathway may be regulated by reversible protein phosphorylation. Loss of a chloroplast-targeted protein phosphatase (SEX4) leads to a reduced rate of starch breakdown, reduced night-time levels of maltose, sucrose and hexoses (glucose and fructose), and the accumulation of high levels of starch (Zeeman et al., 1998b; Niittylä et al., 2006). The phosphoprotein targets have not yet been identified. It is also possible that SEX4 is a glucan phosphatase (Worby et al., 2006), responsible for removing the phosphates added by glucan, water dikinase. This has yet to be confirmed. Maltose (the major product of starch breakdown) is exported via a chloroplast envelope transporter encoded at the *MEX1* locus (Niittylä et al., 2004). MEX1 facilitates the diffusion of maltose out of the chloroplast. Once in the cytosol, maltose is metabolised via a transglucosylation reaction catalysed by DPE2 (Chia et al., 2004; Lu and Sharkey, 2004). In both *mex1* and *dpe2* mutants, the pathway is blocked and maltose and starch accumulate to high levels.

Supplementary Figure 2



Supplementary Figure 2. Principal component analysis of metabolic profiles of wild-type plants and of mutant plants with altered starch levels. (**A**) Normalised metabolite data (Supplementary Table II) were analysed. Each point represents the complete metabolite profile of a single biological sample. Colours indicate the genotype as shown. Points are visualised in the dimensions of the first and second principal components which together explain 57 % of the variation within the entire dataset. (**B**) As in **A**, but points visualised in the dimensions of the first and third principal components which explain 43 % of the variation within the dataset. (**C**) Metabolite weightings for the first and second principal components; the distance from the origin indicates the relative importance of each metabolite in determining the separation in **A**. Colours are used simply for clarity. (**D**) Weightings for the first and third principal components indicating the relative importance of each metabolite in determining the separation in **B**.

Supplementary Figure 3



Supplementary Figure 3. Hierarchical cluster analysis of metabolic profiles of wild-type plants and mutant plants with altered starch levels and of the *isa3* mutant line GABI_KAT_280G10. Details are as in Fig. 4. Colours indicate the genotype given on the left.