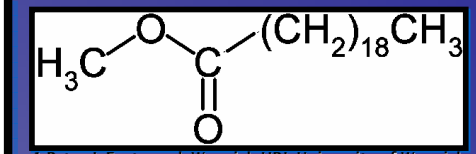
Control of storage oil mobilisation in Arabidopsis seeds by the lipase SDP1.



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Introduction:

After germination a seedling must develop as quickly as possible in order to establish photosynthetic competence and a self-sustaining root system to best ensure survival and eventual propagation. Therefore the rate of post germinative development is of utmost importance. The mobilisation of storage oil present in the seedling is critical for unlocking the energy and carbon skeletons that allow rapid growth so quickly. Mutants having altered developmental properties are therefore of interest particularly in assessing the importance of individual components, such a component is the recently identified lipase SDP1[2].

Overview:

This project used different heterozygous and homozygous mutants of *Arabidopsis thaliana* in conjunction with wild type. Seeds were plated under sterile conditions onto a medium containing essential salts and sucrose. Four or five post-germination samples of a representative number of seeds (≈20) were taken at various time points (0, 1, 2, 3 and 4 days), quenched and the fatty acids were extracted and converted to methyl esters. Then each differing fatty acid methyl ester present was separated and quantified (against an internal standard) by gas chromatography. This was applied to each individual mutant variant with particular attention paid to the fatty acid methyl ester, derived from eicosenoic acid (C20:1) as this is only present in triacylglycerol and therefore an excellent and specific marker for triacylglycerol breakdown, as well as the total fatty acid content. Subsequent metabolic control analysis[1] using the rate (of triacylglycerol breakdown) and oil body lipase activity[2] allowed for the assessment of the importance of SUGAR-DEPENDENT1 in triacylglycerol breakdown in *Arabidopsis thaliana*.

Results:

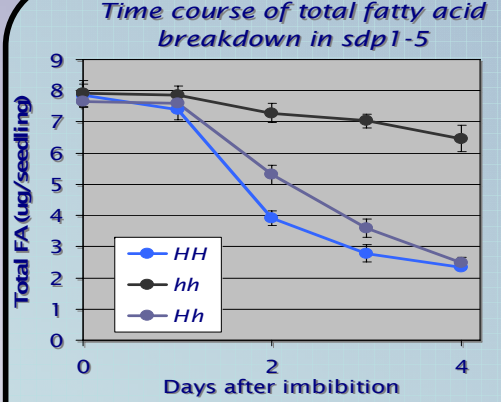

Figure 1.0: Highlighting the differences in the rate of breakdown of the total fatty acid content consequent to imbibition of the various heterozygous (Hh) and homozygous (hh) sdp1-5 mutants in comparison to wild type (HH) seedlings.

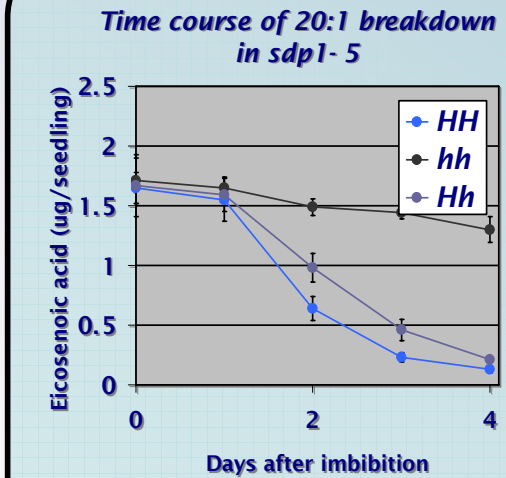

Figure 2.0: Highlighting the differences in the rate of breakdown of the key TAG indicator C20:1 subsequent to imbibition of the various heterozygous (Hh) and homozygous (hh) sdp 1-5 mutants in comparison to wild type (HH) seedlings.

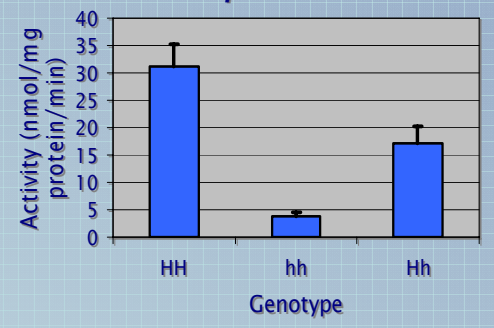

Figure 3.0: Detailing the differences in lipase activity in heterozygous (Hh) and homozygous (hh) sdp1-5 mutants in contrast with wild type (HH) lipase activity.

The data obtained and data from previous experiementation[2] presented in figures 1.0 and 2.0 show the change in the total FAME content in addition to the C20:1 FAME content obtained at each time point for wild type, homozygous and heterozygous mutants respectively. Figure 3.0 shows the activity of the lipase in each of the wild type, homozygous and heterozygous mutant seed varieties.

Metabolic Control Analysis:

Metabolic control analysis allows for the calculation of the deviation index which is numerically representative of the flux control co-efficient according to equation (1).

$$(1)\ D = \frac{(E2-E1)}{(J2-J1)} \cdot \frac{(E2)}{(J2)}$$

Where D stands for deviation index, E2 stands for mutant activity (Hh or hh), E1 stands for wild type (HH) activity, J2 stands for the rate of breakdown in mutant (Hh or hh) and J1 stands for the rate of breakdown in wild type (HH).

The numerical values obtained consequently were 0.60 (Hh) and 0.64 (hh) for C20:1 with values of 0.65 (Hh) and 0.69 (hh) for the total fatty acid content. Put into context, a value of 1 implies total control and a value of 0 implies no control. This suggests that SDP1 lipase is of important significance in the breakdown of C20:1 and indeed the total fatty acid content in post- germinative development. The remaining portion of control (approx. 0.3-0.4) must therefore be made up by other enzymes in the pathway and further research would be necessary in order quantify each individual component. In conclusion the lipase SDP1 is the most significant controlling factor in storage oil mobilisation, mutants of which possess a disadvantage phenotype as a result of being unable to utilise the energy and carbon skeleton otherwise available to wild type seedlings.

Undergraduate Research Scholarship Scheme Experience:

The URSS has entitled me to gain valuable experience and insight into how a research project is conducted especially from an academia perspective and has had unequivocal benefit in my undergraduate studies since. The practical skills that I encountered built upon and extended my practical ability and knowledge. Transferable skills, which I believe will be of importance to myself in the future in employment or in further study and has helped me understand the importance for communication, co-operation and co-ordination.

References:

1:Kacser & Small (1993). Responses of metabolic systems to large changes in enzyme activities and effectors, 1.The linear treatment of unbranched chains. *European Journal of Biochemistry*, **Vol.213** 613-624

2: Eastmond (2006). SUGAR-DEPENDANT1 encodes a patatin domain triacylglycerol lipase that initiates storage oil breakdown in germinating arabidopsis seeds. *The Plant Cell*, **Vol.18** 665-675