Synergy Visiting fellowship

Increase in oil content through redirection of carbon fluxes during seed development

ABSTRACT

Keywords : Microscopic (Gene/cell), Operation, bioenergic agrochemicals, Gene expression, Protein, Proteomic, Arabidopsis

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Project number : 1202-048
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Project type : AAP
Research units in the network :
Start date : 2013-07-01
End date : 2014-07-31
Flagship project : no

Project leader : Thomas Roscoe
Project leader's institution : CNRS
Project leader's RU : DIADE

Budget allocated : 72800 €
Total budget allocated (including co-financing) : 72800 €
Funding : Labex

GOAL

The specific objectives of the SYNERGY programme are characterisation of novel transcriptional regulators of seed oil biosynthesis and assement of their ability to influence carbon partitioning between structural carbohydrate of the seed coat, storage protein and triacylglycerol.

ACTION

Yeast-1-Hybrid screens using promoters of acyltransferases essential for triacylglycerol synthesis had isolated approximately 20 candidate regulators. Drs. Kunst and Haughn selected 7 putative DGAT1 regulators and 4 putative PDAT regulators for characterisation using a reverse genetics approach. Putative knock out mutants were obtained for the 11 candidates and were grown, genotyped and plants homozygous for the mutations obtained. The plants were regrown and the seeds were subjected to GLC analysis to determine fatty acid composition and lipid content. Three factors a NAC, ZnFinger and a TATA binding protein Associated Factor had oil contents that varied from the WT control. Three additional factors a second NAC, a bZIP and MYB factor also produced seed with varient oil contents. Subsequent characteristion of these latter three candidates in transient assays confirmed transactivation of their cogent promoters.

Populations of Arabidopsis fus3 seeds harbouring an oleosin::DAAO-napin::GFP construction were mutagenised with EMS and germinated seedlings treated with D-Serine. M3 seeds from surviving lines were phenotyped by GFP intensity and distribution of fluorescence within the embryo. Several lines were identified that may constitute seed reserve revertants. These candidate lines are being examined for seed lipid and storage protein contents.

RESULTS

During the tenure of the Agropolis Fellowships to Drs Kunst and Haughn a fus3 suppression screen was initiated which has led to the isolation of approximately 13 Arabidopsis lines that may constitute seed reserve révertants.

11 Putative regulators of genes encoding acyltansferases essential for triacylglycerol biosynthesis were examined by reverse genetics. Three putative regulators of DGAT1 were identified, a NAC factor, a Zn Finger DNA binding protein and a TBP-TAF. The seeds of Knock out mutants of each of these candidates have abnormal seed oil contents. From the 11 putative regulators initailly selected, two additional candidates, a bZIP and a MYB factor, transactivate the DGAT1 in transient assays in planta. A third factor, a NAC distinct from the factor described above transactivates both DGAT1 and PDAT1 promoters in yeast and in transient assays in planta. Thus, upto 6 transcription factors have been identified that are putative regulators of genes that control storage lipid biosynthesis in seeds.

PERSPECTIVES

The candidate fus3 révertant lines will undergo further sélection to confirm authenticity of restoration of lipid and protein reserves. Revertant lines will be mapped and génome re-sequenced to identify the suppressor mutation genes which may corresopnd to new regulators of lipid biosynthesis in seeds. The putative regulators of DGAT1 and PDAT1 will be expressed in Arabidopsis seeds to assess their capacity to enhance triacylglycerol synthesis. The validated candidates will be transferred to Camelina sativa, an emerging industrial crop plateform. Drs Kunst, Haughn and Roscoe undertake to identify homologues of validated regulatory genes in tropical species.