Metabolic Engineering of C_4 Grasses for Biofuel Applications

Submitted by

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Abstract

The amount of (1,3;1,4)-β-D-glucans or mixed-linkage-β-glucans (MLG) in biofuel crops is of interest because this is a source of fermentable hexose sugar. Recent modification of (1,3;1,4)-β-glucan in barley has opened the possibility of increasing the (1,3;1,4)-β-glucan amount in other plants, including bioethanol crops such as sorghum, a C_4 grass. As *S. viridis* is a model plant for studying C_4 biofuel crops the characteristics of MLG accumulation in *S. viridis* is of interest. The initial study determines the levels, structure and distribution of MLG in various vegetative tissues and grain in *S. viridis*, and investigates the relationship with the transcript levels of *Cellulose synthase-like* (*Csl*) genes, i.e. genes involve in MLG synthesis. Modification of MLG amount was tested by generating transformants of *S. viridis* with over-expressing *Csl F6* driven by either the oat globulin promoter (ProASGLO) or the constitutive 35S promoter (Pro35S) from barley (*HvCslF6*) and sorghum (*SbCslF6*). In addition, a previous study on the over-expression of the (1,3;1,4)-β-glucan synthase in transgenic barley showed an excessive deposition of the polysaccharide in many cell types causing vascular suffocation and sometimes plant death. To resolve this problem this study also aim to identify a sorghum promoter which may be used to regulate the over-expression of (1,3;1,4)-β-D-glucan synthase genes (such as *CslF* and *CslH*) and restrict it to certain specific cell types only. Five putative sorghum mesophyll cell-specific promoters from genes involved in C_4 photosynthesis have been fused with a *β-glucuronidase* (*uidA*) cDNA. All modifications were tested *in planta* using stable *Agrobacterium*-mediated transformation.

Statement of Authorship

I, Riksfardini Annisa Ermawar certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the join-award of this degree.

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Riksfardini Annisa Ermawar

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Dedicated with love to

My greatest inspiration, my parents

Mami Nin Kania Kowiyah and Papa Akhlan Husen

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Abbreviations

%	Percentage
°C	Degree Centigrade
µ	Micro
µL	Microlitre
µm	Micrometre
cm	Centimetre
g	Gram
h	Hours
M	Molar
mg	Milligram
min	Minutes
mL	Millilitre
mM	Millimolar
mm	Millimetre
ms	Millisecond
rpm	Revolutions Per Minute
sec	Second