Folate Profiling in Rice

Riza Abilgos Ramos, MSc

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ABSTRACT

Folate is one of the important B vitamins needed for normal cell function, growth and development. Whilst it is naturally found in rice, the level is low in the grain and the naturally-occurring forms of the vitamin are not well studied.

Chapter 2 describes the development of LC-MS/MS based technique to profile the mono- and polyglutamated folates while Chapter 3 describes how folates in rice were initially profiled using the microbiological assay (MA). MA was used to screen 51 rice cultivars for their total folate content, and then LC-MS/MS was employed to measure naturally occurring mono- and polyglutamated forms of the vitamin in selected cultivars. This study revealed wide natural variation among rice cultivars in terms of total folate content when MA screening was made and the validated LC-MS/MS technique of simultaneous profiling of mono- and polyglutamated folates through MeOHAA/PO_4 extraction revealed that the naturally-occurring species in wild type rice are 5-CH_3-H_4PteGlu, 5/10-CHO-H_4PteGlu, 5-CH_3-H_4PteGlu$_4$, 5-CH_3-H_4PteGlu$_5$ and 5/10-CHO-PteGlu$_5$.

Chapter 4 describes the use of the LC-MS/MS techniques to characterise folate mono- and polyglutamated forms in a variety of transgenic rice lines. There was a general decrease in polyglutamated folate forms in the folate polyglutamating enzyme (FPGS Os03g02030) knockout rice line. Conversely, there was a dramatic increase in 5-CH_3-H_4PteGlu$_4$, 5/10-CHO-H_4Pteglu$_5$, 5-CH_3-H_4PteGlu$_6$, and 5/10-CHO-H_4Pteglu$_6$ levels, resulting in a 2.5 to 8.8-fold increase in the total folate pool in the unpolished grains of rice either overexpressing FPGS, or the folate binding proteins -cFBP and GNMT compared to wild type Nipponbare.

This study demonstrates that overexpression of the two rice FPGS genes (Os03g02030 and Os10g35940) or folate binding proteins (cFBP and GNMT) results in improved levels and overall folate profile which can be exploited in breeding programmes designed to enhance folate content in this important staple crop.

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ABBREVIATIONS

H_4PteGlu	tetrahydrofolate
5,10-CH^+-H_4PteGlu	5,10-methylene-tetrahydrofolate
5-CH_3-H_4PteGlu	5-methyl-tetrahydrofolate
CHO-H_4PteGlu	formyl-tetrahydrofolate
5-CH_3-H_4PteGlu_4	5-methyl-tetraglutamate
5-CH_3-H_4PteGlu_5	5-methyl-pentaglutamate
CHO-PteGlu_5	formyl-pentaglutamate
APCI	atmospheric pressure chemical ionisation
cFBP	cow’s folate binding protein
CRM	certified reference material
DHPS	dihydropteroate synthase
DMHA	dimethylhexylamine
EPI	Enhanced product ion
FPGS	folylpolyglutamate synthetase
GNMT	glycine N-methyltransferase
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LOD	limit of detection
LLOQ	lower limit of quantification
MA	microbiological assay
MRM	multiple reaction monitoring
MTX	methotrexate
pABA	*para* aminobenzoate
PteGlu	folic acid
WCV	wide compatibility variety