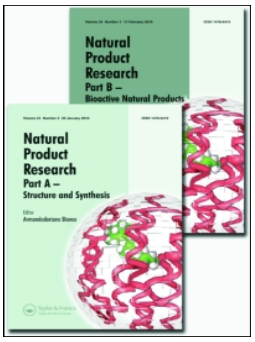


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SHORT COMMUNICATION

# Identification and determination of naturally occurring folates in grains of rice (*Oryza sativa* L.) by UPLC-MS/MS analysis

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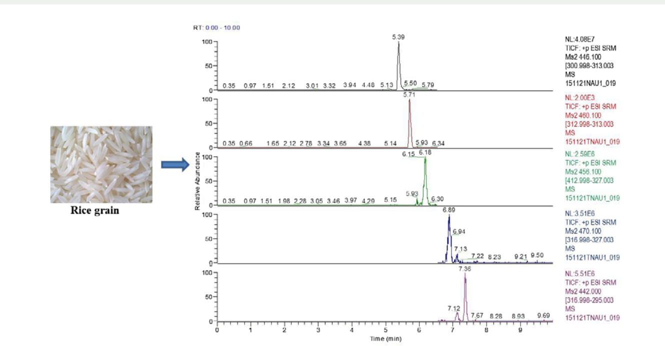
**ABSTRACT**

The genetic potential and biofortification of India-grown rice with bioavailable folate has not been studied yet. The objectives of this study were to determine the folates concentration in four cultivars of rice through UPLC–MS/MS. Total folate concentration in rice cultivars ranged from 11.0 to 51 µg/100 g with a mean of 26.0 µg/100 g. Among the four rice cultivars, the pigmented grain cultivar Nootripathu possesses two-fold rich sources of total folates than the other three non-pigmented grain cultivars. The average value of 100 g serving of rice grains could provide the amount of recommended daily allowance (% RDA) of dietary folates (6.5%) for adults, which ranged from 2.7–12.7%. Among the 5 individual forms of folates, 5-methyltetrahydrofolate was most abundant in rice cultivars followed by 10-Formylfolic acid and folic acid. The result of this study has been useful for biofortification of folates in rice.




UPLC-MS/MS folate profile of rice grain

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## 1. Introduction

Folate is the general term used to refer to different chemical forms of vitamin B, commonly known as vitamin B9 and it plays a major function in the methylation cycle and DNA biosynthesis (Scott 1992). Humans cannot synthesise folates by themselves and therefore, they must be supplied from plant-based foods as they are the main sources of folate (Rebeille et al. 2006). Currently, the recommended daily allowance (RDA) of folate is 400 µg of dietary folate equivalent for adults and 600 µg for pregnant women and lactating mothers (Rebeille et al. 2006). Folate deficiency in humans leads to a number of diseases and disorders, and it is a global health problem, which causes approximately 300,000 neural tube defects (NTDs) per year and is responsible for 10% adult deaths from heart disease (Kondo et al. 2009). Moreover, folate deficiency is the main cause of anaemia in at least 10 million pregnant women in the developing world (Rush 2000). In addition, low folate intake leads to a higher risk of cardio vascular disease (Stanger 2004) and development of a range of cancers (Choi and Friso 2005).

Based on the above comments, it is not surprising that folates have been attracting great interest. Folate analysis has been an analytical challenge for larger number of structural analogues due to their liability and low level in natural food samples. In recent years, different techniques have been employed in the analysis of folate, including microbiological (Han and Tyler 2003), HPLC (Ginting and Arcot 2004) and mass spectrometry (MS) (De Brouwer et al. 2008) detection. But, LC-MS detection has shown to have high sensitivity and selectivity. However, compared to LC-MS, LC– tandem mass spectrometry detection offers both high sensitivity and much better selectivity for the unmistakable identification and quantification of trace level of analytes in complex samples. Ultra-high performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) profiling of folates from rice grains was limited, and to our knowledge, this study was first report of naturally occurring folates in rice grains from India. Therefore, the aim of this study was to assess the five different monoglutamte folates and total folates in grains of four rice cultivars. The obtained results will be used to determine the scope for biofortification of folates in rice through conventional or molecular breeding approaches.

## 2. Results and discussion

### 2.1. Isolation of folates

Folic acid (FA), 10-formylfolic acid (10-CHOFA), tetrahydrofolate (THF), 5-methyltetrahydrofolate (5-MTHF) and 5, 10-methenyltetrahydrofolate (5, 10-$CH^{+}$THF), isolated from matured grains of rice, were determined by UPLC–MS/MS. Calibration data and MS spectra for the individual folates measured in rice grains are presented in Table 1 and Supplementary Figure S1–S4 (available online only). Total folate concentration was calculated as the sum of mean values of five individual folates.

### 2.2. Determination and comparison of folates concentration

Statistically significant differences were observed in all five individual folates and total folate concentration among the cultivars of rice (Table 2). However, through present study, we found that all five individual monoglutamate folates were present in all four rice

**Table 1.** Summary of calibration data for the individual folates measured in rice.

| Folate | Transition (eV) | LOQ(μg/100 g) | Slope (×10$^{-3}$) | Intercept (×10$^{-3}$) | Linear range (μg/100 g) | $R^2$ |
|---|---|---|---|---|---|---|
| FA | 442 → 295 (21) | 0.25 | 15.50 ± 0.10 | 4.30 ± 1.8 | 0.2–62.8 | 0.999 |
| 10-CHOFA | 470 → 295 (23) | 0.26 | 2.35 ± 0.20 | 1.40 ± 5.0 | 0.3–66.8 | 0.997 |
| THF | 446 → 299 (18) | 2.00 | 2.10 ± 0.01 | −1.10 ± 8.9 | 2.0–570.0 | 0.993 |
| 5-MTHF | 460 → 313 (16) | 4.00 | 2.91 ± 0.01 | 4.70 ± 5.8 | 4.0–1046.0 | 0.999 |
| 5,10-MTHF | 456 → 412 (29) | 0.41 | 1.01 ± 0.02 | −0.82 ± 0.5 | 0.4–107.0 | 0.998 |

Note: FA, folic acid; 10-CHOFA, 10-formyl folic acid; THF, tetrahydrofolate; 5-MTHF, 5-methyltetrahydrofolate; 5, 10-methenyltetrahydrofolate.

**Table 2.** Folate concentration and % RDA from 100 g of rice serving.

| | | Mean folate concentrations (μg/100 g)[a] | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Genotypes | Market class | FA[b] | 10-CHOFA[b] | THF[b] | 5-MTHF[b] | 5,10-CH$^+$THF [b] | Total folate [c] | % RDA from 100 g serving [d] |
| Noortipathu | Pigmented | 14.0[a] | 15.9[a] | 1.4[a] | 20.2[a] | 0.7[a] | 51.0[a] | 12.7 |
| IR20 | Non-pigmented | 6.1[b] | 6.6[b] | 0.5[b] | 11.7[b] | 0.7[a] | 23.0[a,b] | 5.7 |
| Nagina 22 | Non-pigmented | 5.5[b] | 5.9[b] | 0.6[b] | 8.6[c] | 0.8[a] | 19.1[b] | 4.8 |
| Pusa Basmati-1 | Non-pigmented | 1.9[c] | 2.7[c] | 0.7[b] | 8.3[c] | 0.3[b] | 11.0[c] | 2.7 |
| Average | – | 6.9 | 7.8 | 0.8 | 12.2 | 0.6 | 26.0 | 6.5 |
| Comprising %[e] | – | 26 | 30 | 3 | 47 | 2 | 100.0 | – |

[a]Within a column, different letters indicate significant differences at $p < 0.05$ based on DMRT.
[b]FA: Folic acid, 10-CHOFA: 10-Formylfolic acid, THF: Tetrahydrofolate, 5-MTHF: 5-Methyltetrahydrofolate, 5, 10-CH$^+$THF: 5,10-Methenyltetrahyrofolate.
[c]Total folate concentration was calculated as the sum of five individual folates.
[d]The % RDA for folates (400 μg per day for adults) was calculated on the basis of a 100 g serving of each cultivars of rice grain.
[e]Percent comprising the individual folates calculated with total folates concentrations.

cultivars. Among the four rice cultivars, the pigmented grain rice cultivar Nootripathu had highest average total folate concentration, followed by non-pigmented grain rice cultivars IR20, Nagina 22 and Pusapasmati-1. Our results showed that total folate concentration in rice ranged 11.0–51 μg/100 g and was within the range of total folates in rice 24–111 μg/100 g (Dong et al. 2011). The average total folate concentration of rice 26.0 μg/100 g was equivalent with previously reported value of 26.9 μg/100 g for pea (Jha et al. 2015).

### *2.3. Major forms of folate in rice*

Table 2 shows percentage of individual folate monoglutamates calculated with total folates concentrations in four cultivars of rice grains. Among the five monoglutamate folates, both 5-MTHF (47%) and 10-CHOFA (30%) comprised 77% of the total folate in rice. In addition, 5-MTHF only contains 47% of the total folate concentration in rice. Similar to our findings, 5-MTHF was identified as the major folate in cereals (Konings 2006), common bean and chickpea (Hefni et al. 2010).

### 2.4. Comparison of food legume folates with per cent recommended daily allowance (% RDA)

Per cent contribution to the total folate RDA varies from 2.7% (Pusa Basmati-1) to12.7% (Nootripathu) from a single serving of 100 g of rice. The results were within the range of previous reports that a single serving of 175 g of potato can supply average range of 4.5% to 12% of the RDA (Goyer and Navarre 2007). In this study, we documented single serving of 100 g of Tamil Nadu-grown rice that can supply an average of 6.5% RDA of dietary folates for adults.

### 2.5. Biofortification strategies for folate improvement in rice

The nutritional value of rice is highly important for human health in developing and developed countries. Folate biofortification strategies can be theoretically categorised into two main groups: metabolic engineering and exploiting the natural variation in folate levels (Rebeille et al. 2006). The increasing folate levels in food crops by genetic modification have been encountered with consumer resistance due to concerns for its safety (Falk et al. 2002). Therefore, enriching the nutritional contribution of staple crops through plant breeding is only option that is now widely discussed in the fields of nutrition and public health at the international level (Bouis 2002). Thus, an alternative approach of biofortification of folate in staple food crops has been adopted in many countries and is potentially a cost-effective, safe intervention and sustainable method to produce bioavailable folates for people in many parts of the world (Jha et al. 2015).

## 3. Conclusion

Significant differences were observed among four rice cultivars in total folates and relative proportions of the five folates quantified using UPLC–MS/MS. For the four rice cultivars in this study, the new understanding of the variation in folate profiles and concentration represents the first step towards biofortifying rice crop. Genetic variation detected among the four cultivars of rice is setting the stage for wider surveys to increase the folate concentration through identification of useful genetic variation. Furthermore, we hope to identify accessions with greater folate concentration after evaluation of a wider set of rice germplasm, and this trait will be introgressed into adapted cultivars using conventional and molecular breeding approaches.

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## Disclosure statement

No potential conflict of interest was reported by the authors.

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