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Research Article

Existence of MeFox and Function Research in Cereal Crops

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Abstract

MeFox is the part of Prirazino-s-triazine vitamers that are oxidized from 5 methylTHF. Cereal plants (rice, maize, wheat) contain a high amount of MeFox, more than the other folate derivatives. 5-methylTHF is the most biologically active, but MeFox is not biologically active. The present study aimed to analyse the MeFox and 5-methylTHF content in rice, maize and wheat. Cereal plants rice, maize and wheat varieties are selected and determined total folate content from their germination, vegetative and mature stage of grain by liquid-chromatography tandem mass spectrometry. The MeFox levels of the cereals germination stage, vegetative stage and harvesting stage, respectively 63.85-748.02 µg/100g, 8.77- 867.44 µg/100g and 12.01-1807.35 µg/100g with an average of 396 µg/100g, 107 µg/100g and 526 µg/100g. The 5-MTHF levels of the cereals germination, vegetables and gain stages respectively 20-1.31 µg/100g, 51-1.92 µg/100g and 36.51-4.17 µg/100g with an average of 28 µg/100g, 18 µg/100g and 15 µg/100g. The highest folate content was MeFox in wheat and rice grain (> 1153.39 µg/100g). The highest 5-Methyl-tetrahydrofolate was 65.98 µg/100g in the vegetative rice stage.

Maize stems have a four-fold increase in the MeFox, and a two-fold increase in the 5-MTHF. MeFox and 5-MTHF are increasing two-fold in rice roots and stems. MeFox has a function in plant metabolism. Only one-hour MeFox solution drippinMaize and rice gave positive results of 5-MTHF and MeFox. It's clear that MeFox have a function the plant metabolism.

Keywords: MeFox; MeFox Content; MeFox Function; Cereal Crops

Introduction

Folate vitamers are vital cofactors in 1-carbon metabolism and play the in methylation reactions and DNA synthesis; suboptimal folate deficiency is associated with different diseases such as cardiovascular disease, cancer, and cognitive impairment [1].

Though, analyzing folates in serum is composite only for numerous folate derivatives in various oxidation conditions and 1-carbon substitutions. During oxidative degradation, it's shown sensitive role [2]. The initial circulating folate form of serum 5-methyltetrahydrofolate (5-methylTHF), does not severely alterable oxidation to 5-methyldihydrofolate [3]. 5-methyltetrahydrofolate (5-CH3-H4PteGlu, mentioned as L-methyl folate) is not insecure and good for depression treatment [4]. Excess corrosion can convert 5-methylTHF or 5-methyldihydrofolate to 4a-hydroxy-5-methylTHF, an internal product known as hmTHF [2]. Absence of a reducing agent, hmTHF undergoes a physical change to custom a pyrazino-s-triazine derivative [5]. MeFox is the permanent oxidative product of 5-methylTHF, and the methyl folinate oxidation product. MeFox is not physically energetic [2] and was before experimented with for a lengthy time frozen, stored serum and plasma specimens and also fresh-frozen samples [6]. The writers mentioned the compound name as hmTHF, even though they stated that it was the pyrazino-s-triazine derivative of hmTHF). Whether MeFox is made in maybe *in vitro* or *in vivo* both stages. MeFox and 5-formyltetrahydrofolate (5-formylTHF) are isobaric complexes and as such form the same mass to charge (m/z) parent to product ion pairs during ionization, making coelution and misidentification during liquid chromatography-tandem MS (LC-MS/MS) analysis likely [7].

Cereal crops contain a high level of MeFox, but no proper explanation about these elements such as function, advantage, disadvantage, etc. Moreover, why do cereal crops contain a high percentage of MeFox? Many questions are arises in this era. So, our research mainly focuses on determining the concentration of MeFox during different developmental stages of cereal plants and finding out the specific function of MeFox in cereal crops (rice, maize, and wheat).

Objectives

- To find out the concentration of MeFox during different developmental stages of cereal plants.
- To find out the specific function of MeFox in cereal crops (rice, maize and wheat).

Materials and Methods

Chemicals and reagents

Rice seeds of the ZY-10 and ZY-88 cultivars, and maize (*Zea mays* L.) seeds of inherited lines DAN3130 and GEMS31 were collected from Chinese Academy of Agricultural Science in September 2021 at Langfang, Hebei Province, China. Wheat (*Triticum aestivum* L.) grains of HN 22 and HN 79 cultivars were obtained from a 2021 field trial (at Beijing) of the Institute of Crop Science, Chinese Academy of Agricultural Sciences.

The folate standards-10-formyl-folic acid, 5,10methenyl tetrahydrofolate, 5- formyltetrahydrofolate, 5- methyl-tetrahydrofolate, dihydrofolate, folic acid, Tetrahydro folate and methotrexate (MTX) were purchased from Schircks Laboratories (Jona, Switzerland), and MeFox was obtained from Toronto Research Chemicals (Toronto, Canada).

The purity of all folate standards was>95%. Sodium phosphate monobasic (NaH_2PO_4), sodium phosphate dibasic (Na_2HPO_4), sodium ascorbate, and β-mercaptoethanol was purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultra-pure water was purified on a Heal Force ultra-pure water system (Shanghai, China). Acetonitrile and formic acid (LC-MS grade) were purchased from Fisher Scientific (Geel, Belgium). The HPLC analytical column (Kromasil 100-5 C18, 2.1 × 50 mm, 2. 5µM particle size) was bought from Akzo Nobel (Stockholm, Sweden), and an Agilent SBC18pre-column (2.1×5mm, 2.7µ Mparticle size) remained got from Agilent Technologies (California, USA). Rat serum was bought from Solarbio (Beijing, China), aliquoted in 1 mL portions into 1.5 mL tubes upon arrival and stored at -80 °C freezer before the experiment.

Folate standard solutions

The stock solution of folate was made as before mentioned [8]. The standards of folate chemical powder of folate were smelted as 0.1 mg/mL in a solution of 20 mM ammonium acetate in methanol and water (1:1, v/v) holding 1% (w/v) L-ascorbic acid and 0.5% (v/v) β-mercaptoethanol (pH6.2). The 5,10methenyl-tetrahydrofolate standard was set at a pH of 4.5 buffer. Total standard solutions were stored at -80 °C before working. The experimental solutions were mixed with folate extraction buffer for spiking and calibration. The extraction of the folate containing 50 mM phosphate buffer (pH 7.0), 0.5% (w/v) sodium ascorbate, and 0.2% β-mercaptoethanol was freshly used every day.

HPLC-MS/MS instrumentation

The quantification and separation of folate were using an Agilent 1260 HPLC system joined with an Agilent 6420 three-way-quadrupole tandem MS operated in constructive electrospray ionization (ESI) mode (Palo Alto, CA, USA). A Kromasil 100-5 C18 column (50 × 2.1 mm, 2.5 µM particle size) with an Agilent SB-C18 pre-column (2.1 × 5 mm, 2.7 µM particle size) was used. Every time the volume of injection was 15 µL. The injector and column oven temperature were maintained at four °C and 25 °C, respectively. The mobile phases were activated with 0.1% (v/v) formic acid in water (phase A) and 0.1% (v/v) formic acid in acetonitrile (phase B). The slope of the database was a total of 16.5 min. The mobile phase B was adjusted by 5% at a flow rate of 0.3 mL/min. The quantity of mobile phase B increased linearly from 5 to 9% over 2 min. In the subsequent 5.9 min, phase B increased to 9.5%, then sharply increased to 20% over 0.3 min. After holding at 20% for 3 min at a flow rate of 0.6 mL/min, the proportion of phase B decreased to 5% in 0.2 min and holding on for 3 min. Subsequently, the flow rate decreased to 0.3 mL/min in 0.1 min, followed by an equilibration time of 2 min. The mass spectrometer parameters were optimized for fragmentation of each folate standard with a gas temperature of 320 °C, drying gas flow at 11 L/min, nebulizer pressure at 35 psi, and capillary voltage at 3500 V. The precursor ion, the production and collision energy (eV) for each folate standard were m/z 470→295.1, 20 eV for 10-CHO-PteGlu; m/z 456→412, 30 eV for 5,10-CH = H4PteGlu; m/z 460.2→313, 20 eV for 5-CH3-H4PteGlu; m/z 474→327, 20 eV for 5-CHO-H4PteGlu; m/z 444→178, 20 eV for H2PteGlu; m/z 446→299, 20 eV for H4PteGlu; m/z 442→295, 20 eV for PteGlu; and m/z 455→308, 30 eV for the internal standard MTX. Mass Hunter software performed system operation, data acquisition, and data analyses.

Extraction of folates

Folate extraction was performed under subdued light to minimise light-induced degradation. The grain samples were individually making fine powder by a grinder (Geno/Grinder 2010, Boston, USA), and the leaves samples were ground by mortar and pester. Then, 50 mg of good quality powder was transferred to 1.5-mL screw-cap tubes (ST150, Axygen, and Union City, CA, USA). The extraction buffer of 1 ml was added to 50 mg of fine powder and mixed very well. The mixture of solution was boiled timely for 10 min in a 100 °C water bath, after that cooled on ice, then 50 µL of rat serum was added, and hand shaking gently few times then the mixture was incubated at 37°C for four h to deconjugate polyglutamylation tails. Then again, the sample was boiled for 10 minutes, cooled on ice for 10 minutes, and started to centrifuge at 13,000 rpm at four °C for 10 minutes. Transferred the supernatant to a 3kDa ultra-filtration tube (Millipore, Billerica, USA) for purified and centrifuged at 13,000 rpm at four °C for 20 min. As a final stage, the solution was used directly folate detection. MTX at a final concentration of 20 ng/mL was used in the beginning stage of folate extraction buffer preparation. The subsequent solution was used directly for folate analysis.

Application of the method

This method was very effective to determine calculating of folate concentration in rice, maize, wheat germination, vegetative, and harvesting stages. For each cereal, two different cultivars were selected (details mentioned in 2.1). After harvest, the total grains of maize, wheat, and wild rice seeds were stored at -80 °C until further analysis. About 20 seeds of rice, 20grains of maize and wheat were grounded into fine powder. Germination and vegetative samples are ground manually.

Determination of moisture content

Moisture content was determined in triplicate using a vacuum oven at 70°C overnight (AOAC 2007). The leaf samples were (2g) initially weighed, incubated overnight in a vacuum oven at 70°C, and weighed. The moisture content was calculated as the difference between the two weights.

Data analysis

Folate data were shown as all biological replicates' means and standard deviation (SD). The analysis of total folate was calculated as the sum of the contents of folate derivatives but focused on MeFox and 5-MTHF. All the folate contents were presented as micrograms per 100g.

Results and Discussion

Total folate contents of rice, maize, and wheat

A total of 6 cereal varieties were selected for folate analysis based on MeFox and 5-MTHF. Among them, two were rice, two were maize and two were wheat varieties. All of these samples were ready for MeFox and 5-MTHF analysis. The MeFox levels of the cereals germination stage, vegetative stage, and harvesting stage, respectively 63.85-748.02 µg/100g, 8.77- 867.44 µg/100g, and 12.01-1807.35 µg/100g with an average of 396 µg/100g, 107 µg/100g and 526 µg/100g (Table 1). The 5-MTHF levels of the cereals germination, vegetables, and gain stages respectively 20-1.31 µg/100g, 51-1.92 µg/100g, and 36.51-4.17 µg/100g with an average of 28 µg/100g, 18 µg/100g and 15 µg/100g (Table 1). The highest folate content was MeFox in wheat and rice grain (> 1153.39 µg/100g).

In the germination stage MeFox level was high in the wheat (HN-79). Variety 748.02 µg/100g, and the lowest found in the maize (3130) Variety, 63.85 µg/100g (Table 2).

The highest 5-Methyl-tetrahydrofolate was 51.37 µg/100g in the vegetative rice stage and lowest in the wheat variety (HN79) at 1.92 µg/100g (Table 3). In the harvesting stage, MeFox maximum was found in rice and wheat varieties>1094 µg/100g and lowest in maize variety 17.59 µg/100g. The maximum 5-MTHF in wheat variety (HN-79) is 36.17 µg/100g (Table 4).

MeFox was highest in the rice harvesting stage >1800 µg/100g and 5-MTHF maximum in the rice vegetative stage >50 µg/100g. The MeFox and 5-MTHF maximum data ratio was 36:1.

Crops	MeFox content (µg/100g)			
	Germination Stage	Vegetative stage	Harvesting stage	Average
Rice	482.2 ± 3.56～136.57 ± 20.2	867.44 ± 380.45～26.19 ± 21	1153.39 ± 30.52～77.2 ± 21	834
Maize	233.62 ± 16.52～63.85 ± 1.61	186.43 ± 6.31～28.57 ± 0.35	239.76 ± 8.89～12.01 ± 0.51	220
Wheat	748.02 ± 75.38～450.16 ± 75.1	265.85 ± 156.36～8.77 ± 0.90	1807.35 ± 0.61～346.39 ± 0.90	940

Crops	5-MTHF content (µg/100g)			
	Germination Stage	Vegetative stage	Harvesting stage	Average
Rice	10.51 ± 0.06～2.21 ± 0.36	51.37 ± 4.15～37.10 ± 14.85	22.25 ± 0.71～15.23 ± 6.66	28.04
Maize	19.84 ± 0.87～8.14 ± 0.80	26.49 ± 1.32～10.81 ± 3.62	7.92 ± 2.02～6.16 ± 2.90	18.08
Wheat	3.82 ± 1.29～1.31 ± 0.40	5.12 ± 0.84～1.92 ± 0.61	36.51 ± 1.5～4.17 ± 1.55	15.15

Table 1: MeFox content in rice, maize and wheat at germination, vegetative and harvesting stage.

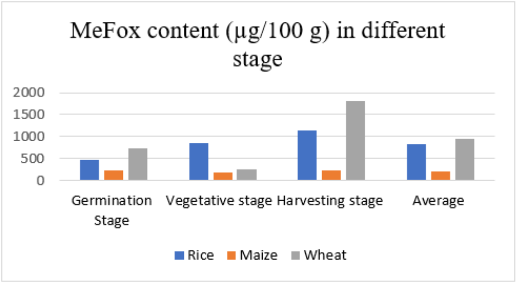

Figure 1: MEFOX CONTENT (µG/100G) IN DIFFERENT STAGES OF RICE, MAIZE AND WHEAT.

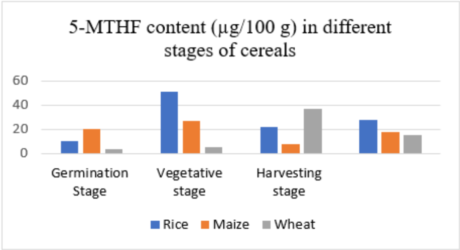

Figure 2: 5-MTHF CONTENT (µG/100G) IN DIFFERENT STAGES OF RICE, MAIZE AND WHEAT.

Variety	No. days	MeFox	10-F-FA	5,10-CH = THF	5-F-THF	5-M-THF	DHF	FA	THF	Total folates
ZY-10	3	378.15 ± 23.95	0.66 ± 0.02	0.92 ± 0.42	0.59 ± 0.07	2.21 ± 0.36	0.0 ± 0.0	0.0 ± 0.0	1.77 ± 0.07	384.32 ± 24.78
ZY-10	4	327.5 ± 25.53	0.62 ± 0.17	1.19 ± 0.16	0.70 ± 0.02	2.47 ± 0.05	0.0 ± 0.0	0.0 ± 0.0	2.82 ± 0.25	335.32 ± 25.90
ZY-10	6	482.2 ± 3.56	0.82 ± 0.06	3.07 ± 0.61	1.27 ± 0.10	5.56 ± 0.026	0.0 ± 0.0	0.0 ± 0.0	5.13 ± 0.45	498.1 ± 4.66
ZY-88	3	155.8 ± 4.87	0.93 ± 0.10	1.64 ± 0.04	0.67 ± 0.09	4.15 ± 0.81	0.0 ± 0.0	0.64 ± 0.29	3.33 ± 0.07	167.2 ± 5.27
ZY-88	4	136.57 ± 20.2	0.80 ± 0.08	1.64 ± 0.20	0.86 ± 0.15	6.29 ± 0.74	0.0 ± 0.0	0.57 ± 0.28	4.93 ± 0.45	151.69 ± 21.04
ZY-88	6	177.91 ± 27.81	3.47 ± 0.36	4.21 ± 0.44	1.89 ± 0.12	10.51 ± 0.06	0.0 ± 0.0	0.76 ± 0.34	7.41 ± 0.28	206.2 ± 29.42
DAN-3130	3	99.05 ± 1.94	0.18 ± 0.08	1.35 ± 0.045	0.79 ± 0.03	19.84 ± 0.87	0.0 ± 0.0	0.0 ± 0.0	1.90 ± 0.03	123.13 ± 2.87
DAN-3130	4	63.85 ± 1.61	0.01 ± 0.09	1.71 ± 0.49	1.17 ± 0.04	12.47 ± 0.05	0.0 ± 0.0	0.0 ± 0.0	2.49 ± 0.17	81.73 ± 2.40
DAN-3130	6	81.18 ± 0.55	0.03 ± 0.02	1.08 ± 0.14	0.99 ± 0.05	8.14 ± 0.80	0.0 ± 0.0	0.02 ± 0.01	1.38 ± 0.01	92.86 ± 1.56
GEMS-31	3	156.44 ± 0.155	0.0 ± 0.0	0.79 ± 0.31	0.90 ± 0.06	8.17 ± 0.84	0.0 ± 0.0	0.0 ± 0.0	1.47 ± 0.17	167.79 ± 1.42
GEMS-31	4	176.86 ± 8.29	0.09 ± 0.02	1.93 ± 0.07	0.69 ± 0.28	14.33 ± 1.58	0.0 ± 0.0	0.0 ± 0.0	2.84 ± 0.24	196.76 ± 10.61
GEMS-31	6	233.62 ± 16.52	0.18 ± 0.01	3.86 ± 0.22	1.76 ± 0.30	11.88 ± 0.32	0.0 ± 0.0	0.0 ± 0.0	5.67 ± 0.59	257.0 ± 17.54
HN-22	3	498.36 ± 72.3	0.22 ± 0.06	3.91 ± 0.07	1.05 ± 0.12	2.08 ± 0.89	0.0 ± 0.0	0.0 ± 0.0	2.70 ± 0.40	508.34 ± 72.76
HN-22	4	450.16 ± 75.1	0.66 ± 0.16	5.93 ± 1.16	1.69 ± 0.54	3.82 ± 1.29	0.0 ± 0.0	0.0 ± 0.0	2.58 ± 0.59	464.37 ± 78.87
HN-22	6	462.87 ± 3.13	0.46 ± 0.08	2.11 ± 0.47	0.74 ± 0.42	3.31 ± 0.05	0.0 ± 0.0	0.0 ± 0.0	1.58 ± 0.08	471.09 ± 3.68
HN-79	3	668.78 ± 17.04	0.43 ± 6.69	6.69 ± 0.25	1.93 ± 1.73	2.32 ± 0.31	0.0 ± 0.0	0.0 ± 0.0	4.05 ± 0.06	684.41 ± 18.09
HN-79	4	670.03 ± 0.03	0.10 ± 0.02	6.40 ± 0.37	1.97 ± 0.01	1.31 ± 0.40	0.0 ± 0.0	0.0 ± 0.0	3.42 ± 0.07	683.27 ± 84.07
HN-79	6	748.02 ± 75.38	0.27 ± 0.07	2.66 ± 0.27	0.98 ± 0.06	2.87 ± 0.35	0.0 ± 0.0	0.0 ± 0.0	1.99 ± 0.27	756.83 ± 75.25

Table 2: Total Folate content and composition (µg/100g) of rice, maize and wheat in germination.

Leaf samples	MeFox	10-F-FA	5,10-CH = THF	5-F-THF	5-M-THF	DHF	FA	THF	total folates
ZY-10	536.07 ± 226.19	2.82 ± 1.25	13.97 ± 5.40	4.28 ± 1.70	37.10 ± 14.85	0.15 ± 0.05	0.22 ± 0.19	9.96 ± 2.90	604.31 ± 238.7
ZY-88	44.37 ± 36.70	2.13 ± 0.43	16.69 ± 10.37	5.59 ± 3.03	51.37 ± 4.15	0.02 ± 0.01	0.37 ± 0.07	8.08 ± 2.91	128.65 ± 54.53
DAN-3130	17.42 ± 2.49	0.95 ± 0.12	1.38 ± 0.34	0.64 ± 0.28	10.81 ± 3.62	0.07 ± 0.02	0.13 ± 0.12	2.94 ± 1.02	34.37 ± 7.62
GEMS-31	178.22 ± 50.76	7.49 ± 2.85	6.37 ± 4.64	3.31 ± 1.73	26.49 ± 1.32	0.11 ± 0.07	1.04 ± 0.34	4.35 ± 2.78	324.49 ± 165.77
HN-22	33.72 ± 3.49	1.09 ± 0.12	12.54 ± 2.84	7.29 ± 1.47	5.12 ± 0.84	0.17 ± 0.03	0.14 ± 0.09	5.48 ± 0.57	65.59 ± 9.09
HN-79	114.87 ± 22.16	1.77 ± 0.77	10.34 ± 1.11	6.30 ± 1.28	1.92 ± 0.61	0.10 ± 0.04	0.36 ± 0.06	5.66 ± 0.82	141.2 ± 24.02

Table 3: Total Folate content and composition (µg/100g) of rice, maize and wheat vegetative stage.

Variety	MeFox	10-F-FA	5,10-CHTHF	5-FTHF	5-MTHF	DHF	FA	THF	Total
ZY-10	1094.47 ± 25.39	2.79 ± 2.18	1.63 ± 0.82	1.84 ± 0.79	15.23 ± 6.66	0.18 ± 0.25	0.25 ± 0.36	5.26 ± 5.80	1121.67 ± 42.29
ZY-88	247.84 ± 65.46	2.55 ± 0.13	2.49 ± 1.98	1.86 ± 0.77	22.25 ± 0.71	0.09 ± 0.06	0.18 ± 0.02	4.11 ± 1.,14	281.40 ± 70.27
DAN -3130	17.59 ± 2.41	0.98 ± 0.09	1.24 ± 0.08	0.47 ± 0.17	6.16 ± 2.90	0	0.11 ± 0.11	1.48 ± 0.20	28.05 ± 5.92
GEMS-31	239.76 ± 21.54	1.87 ± 0.28	3.58 ± 0.55	1.46 ± 0.1	7.92 ± 2.02	0	0	2.1 ± 0.36	256.76 ± 24.87
HN-22	254.08 ± 4.32	0.68 ± 0.04	14.02 ± 2.82	10.91 ± 2.33	4.51 ± 1.5	0.28 ± 0.06	0.22 ± 0.1	7.24 ± 0.64	291.98 ± 11.83
HN-79	1807.35 ± 3.30	0.72 ± 0.09	14.49 ± 3.56	7.6 ± 0.74	36.17 ± 1.55	0.099 ± 0.02	0	5.64 ± 0.54	1872.19 ± 9.75

Table 4: Folate content and composition (µg/100g) of rice, maize and wheat grain.

MeFox Dripping experiment: 10 days seedlings (rice and maize) collected from greenhouse and root zone drip in MeFox solution 1 hour and control drip in only water. MeFox solution concentration, MeFox, and water ratio was 1:20. (MeFox solution: 1µl MeFox + 20 µl water. So, 100 µl = 100*20 = 2000ml = 2L solution)

Stages	MeFox	10-F-FA	5,10-CHTHF	5-FTHF	5-MTHF	DHF	FA	THF	Total
Maize stem Control	26.41	1.73	4.41	14.74	68.04	0	0.31	3.02	92.25
Maize stem MeFox Drip	316.59	3.23	6.4	25.41	140.26	0	0.84	4	180.14
Maize root Control	5.58	0.22	4.71	11.9	53.62	0.14	0	6.77	77.36
Maize root MeFox Dripping	394.1	0.61	3.49	12.74	53.87	0	0	4.96	75.67
Rice stem Control	326.3	0.68	5.53	12.05	48.46	0	0	5.56	72.28
Rice stem MeFox drip	567.07	2.13	5.83	13.21	56.19	0	0	5.85	83.21
Rice root Control	132.31	0.76	1.39	4.47	25.41	0	0	8.54	40.57
Rice root MeFox drip	357.67	2.09	1.83	5.4	28.51	0	0	6.6	44.43

Table 5: Total Folate content and composition (µg/100g) of rice, maize and wheat plant dripping experiment.

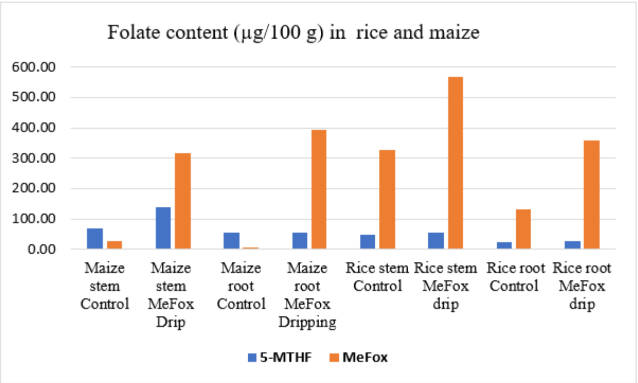

Figure 3: FOLATE CONTENT AND COMPOSITION (µG/100G) OF RICE, MAIZE AND WHEAT ROOT DRIPPING.

According to our observation, MeFox is an integral part of cereals. We observed the maize root did not have a significant difference between 5-MTHF and MeFox after dripping one hour in the MeFox solution. But maize stems have a four-fold increase in the MeFox and a two-fold increase in the 5-MTHF. Rice root has shown 5-MTHF has no significant difference but MeFox increasing two-fold. MeFox has a function in plant metabolism. Only one-hour MeFox solution dripping maize and rice gave positive results of 5-MTHF and MeFox.

Conclusion

We performed the first analysis of MeFox and 5-MTHF of cereal crops. The MeFox and 5-MTHF contents of agronomic crops were determined. The Mefox content is very high in the rice, maize and wheat grain but less in leaf analysis. On the other hand, 5-MTHF always shows expected results in grain and leaf analysis. Our results would provide helpful information about the high MeFox contain cereals variety and their day-wise folate content variation of the life cycle. MeFox is also a good part of cereal plants and has a metabolic function.

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