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Original Research Article

Genetic diversity of folate profiles in seeds of common bean, lentil, chickpea and pea

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ABSTRACT

Folates are water-soluble B vitamins and act as cofactors in many metabolic functions in the human body. Pulses have traditionally been considered as a good dietary source of folates. The objectives of this study were (1) to determine the concentration of folates in four cultivars each of common bean, lentil, chickpea and pea, and (2) to determine the effect of growing location on folate concentration. Six folate monoglutamates were quantified by ultra-performance liquid chromatography coupled with mass spectrometry (UPLC–MS/MS). Total folate concentration ranged from 351 to 589 μg/100 g in chickpea, 165 to 232 μg/100 g in common bean, 136 to 182 μg/100 g in lentil, and 23 to 30 μg/100 g in pea. The 5-methyltetrahydrofolate (5-MTHF) and 5-formyltetrahydrofolate (5-FTHF) folates were most abundant in common bean, lentil and chickpea, whereas 5-MTHF and tetrahydrofolate (THF) were the predominant forms in pea. Significant differences were detected among cultivars for all folates across the pulses, except for 5,10-methenyltetrahydrofolate (5,10-MTHF) in lentil, 5-MTHF in chickpea, and 5,10-MTHF and folic acid (FA) in pea. Significant effects for location and cultivar by location were also observed for the majority of the folates.

1. Introduction

Pulse crops play a significant role in traditional diets of people in many parts of the world as they are among the richest source of proteins and amino acids (Messina, 1999; Duranti, 2006). Globally, the consumption of pulses is increasing due to their nutritional value and many health benefits (Curran, 2012). Common bean (*Phaseolus vulgaris* L.), lentil (*Lens culinaris* Medik.), chickpea (*Cicer arietinum* L.) and pea (*Pisum sativum* L.) are among the most important pulse crops grown worldwide (Duranti, 2006). Pulses and other legumes, as well as beef liver, spinach, asparagus, lettuce, and Brussels sprouts, are rich in folates (UDA-ARS, 2012).

Tetrahydrofolate (THF) and its derivatives, collectively called folates, are involved in one carbon transfer reactions in many organisms including humans (Jabrin et al., 2003; Hayashi et al., 2007). Folates are water-soluble B vitamins and act as cofactors in many metabolic functions including the biosynthesis of nucleic acids, metabolism of amino acids, methylation of hormones, lipids, proteins, and DNA (Bailey and Gregory, 1999; Scott et al., 2000; Forges et al., 2007). In plants, folates are vital for biosynthesis of lignin, alkaloids, betaines and chlorophyll, and are indispensable in photorespiration (Hanson and Roje, 2001).

Humans cannot synthesize folates and thus depend upon plant and animal sources (Scott et al., 2000; Basset et al., 2005). The majority of people in developing countries depend on staple crops such as rice, maize, plantain and potato to fulfil their basic food requirements, however these crops are low in folates (UDA-ARS, 2012). Folate deficiency poses serious problems in both developed and developing nations and can cause serious health issues including neural tube defects (NTDs), impaired cognitive function, and cardiovascular diseases (Geisel, 2003; Ramos et al., 2005; McCully, 2007). It is also associated with numerous neurodegenerative disorders, including Alzheimer's disease (Seshadri et al., 2002), and various cancers (Choi and Friso, 2005). The Recommended Dietary Allowance (RDA) of folates is 400 μg for adults and 600 μg for pregnant women (Institute of Medicine. Food and

Abbreviations: FA, folic acid; 10-FFA, 10-formylfolic acid; 5-FTHF, 5-formyltetrahydrofolate; 5,10-MTHF, 5,10-methenyltetrahydrofolate; 5-MTHF, 5-methyltetrahydrofolate; THF, tetrahydrofolate; UPLC–MS, ultra-performance liquid chromatography–mass spectrometry..

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Nutrition Board, 1998). Folate-rich diets are recommended to pregnant women as they play an important role in various metabolic processes including nucleotide biosynthesis during cell division (Geisel, 2003). Insufficient folate consumption increases the risks of preterm delivery, low birth weight, and foetal growth retardation (Scholl and Johnson, 2000). Besides their role in megaloblastic anaemia prevention in pregnancy, folates are also essential for human reproductive health (Tamura and Picciano, 2006). Wallock et al. (2001) reported that seminal plasma folate was correlated with blood plasma folate and hence important in male reproduction.

Intake of folates can be increased by the consumption of folate-rich foods, fortification of food with folic acid, and folic acid supplements (Hefni et al., 2010). Among various approaches, biofortification, enriching the nutritional value of staple crops, is a balanced and economic way to improve the health status of low-income consumers (Bouis, 2002; Bouis et al., 2011; Blancquaert et al., 2014). In developing countries, biofortification of staple food crops including rice and pulses is a new approach to control deficiencies of folate, β-carotene, iron, and zinc (HarvestPlus, 2007).

Several methods have been used to determine the levels of folates in food samples, including legumes. Microbiological assays have been around for many years (early literature summarized in Toepfer et al. (1951)). Sample preparation in these early methods typically used a conjugase digestion, but modern methods now use a trienzyme (protease, α-amylase, and rat conjugase) treatment (Tamura et al., 1997). The trienzyme method showed significant increases in measured folates indicating that the use of conjugase alone underestimated folate levels (Tamura et al., 1997). Microbiological assays are still used to estimate total folates (Shrestha et al., 2000; Chew et al., 2012); however, more recently, methods using liquid chromatography (LC) have been employed as LC allows for the detection of specific folate forms. LC with fluorescence detection (LC-FD) (Hefni et al., 2010; Sen Gupta et al., 2013), and LC with mass spectrometry (MS) detection (Rychlik et al., 2007; De Brouwer et al., 2008, 2010; Vishnumohan et al., 2011; Camara et al., 2013) are commonly used. Tandem MS methods (MS/MS) are extremely selective as both the parent ion and a specific fragment ion are required for detection, thereby greatly reducing chemical interference. In addition, the use of isotopically labelled internal standards enables very accurate quantification, as they will account for losses in sample preparation or degradation. Recently, De Brouwer et al. (2010) used the higher resolution and faster separation capabilities of ultra-performance LC (UPLC) to develop a UPLC–MS/MS method for analysis of folates in rice. This method has a short run time that allows for a more efficient analysis of the highly labile folates before they decompose in the autosampler.

Although studies have been conducted to measure folate concentrations in various legume crops (Rychlik et al., 2007; Sen Gupta et al., 2013), knowledge of the diversity in folate profiles of pulse crop cultivars grown in different locations is not available. Biofortification of pulse crops is one of the goals of the pulse crop-breeding program at the Crop Development Centre (CDC), University of Saskatchewan. The objectives of this research were: (1) to determine the concentration of folates in four cultivars of each of common bean, lentil, chickpea and pea, and (2) to determine the effect of growing location on folate concentration. This information can be used to determine the scope for future biofortification of folates in pulse crops through conventional or molecular breeding approaches.

2. Materials and methods

2.1. Materials

Four cultivars each of common bean, lentil, chickpea and pea developed at the CDC, University of Saskatchewan, were used in the analysis (Table 1). Seeds from field trials conducted at Saskatoon (common bean, lentil and pea), Limerick (lentil, chickpea), Rosthern (common bean), Elrose (chickpea), and Meath Park (pea), Saskatchewan in 2012 were used for analyses. Trials were conducted in a randomized complete block design with three replicates per location. Saskatchewan is in the Chernozemic soil order with four soil zones. Each soil has different colour of the surface horizon based on the amount of soil organic matter (SOM) stored in the soil (www.soilsofsask.ca). Meath Park and Rosthern are located in the Black soil zone with 4.5–5.5% SOM, Saskatoon is in the Dark Brown soil zone with 3.5–4.5% SOM, Elrose and Limerick are located in the Brown soil zone with 2.5–3.5% SOM. Temperature was similar at five locations in 2012, however total precipitation differed. Meath Park experienced highest rainfall during the growing season (May 1–September 30) followed by

Table 1
Phenotypic characters of common bean, lentil, chickpea, and pea cultivars used in assessment of folates. Samples were derived from 2012 regional variety trials grown at indicated sites in Saskatchewan, Canada.

Crop	Cultivar	Species	Seed coat colour	Cotyledon colour	Grain yield (kg/ha)		Seed weight (g/100 seeds)	
					Saskatoon	Rosthern	Saskatoon	Rosthern
Common bean	CDC Blackcomb	*Phaseolus vulgaris*	Black	White	3083	1337	184	138
	CDC Pintium	*Phaseolus vulgaris*	Cream with brown flecks	White	2635	1031	370	288
	CDC Sol	*Phaseolus vulgaris*	Yellow	White	2751	1242	400	351
	CDC WM-2	*Phaseolus vulgaris*	Cream with brown flecks	White	3086	1260	377	308
					Limerick	Saskatoon	Limerick	Saskatoon
Lentil	CDC Maxim	*Lens culinaris*	Gray	Red	1847	1771	41	39
	CDC QG-1	*Lens culinaris*	Green	Green	1784	1743	47	46
	CDC SB-2	*Lens culinaris*	Gray dotted	Yellow	1964	1738	38	35
	CDC Greenstar	*Lens culinaris*	Green	Yellow	2484	1482	71	61
					Elrose	Limerick	Elrose	Limerick
Chickpea	CDC Leader	*Cicer arietinum*	Non-pigmented	Yellow	3723	3310	383	362
	CDC Consul	*Cicer arietinum*	Light tan	Yellow	3668	2849	324	311
	CDC Cory	*Cicer arietinum*	Tan	Yellow	3899	2559	279	285
	CDC Frontier	*Cicer arietinum*	Non-pigmented	Yellow	3771	2789	350	347
					Meath Park	Saskatoon	Meath Park	Saskatoon
Pea	CDC Amarillo	*Pisum sativum*	Non-pigmented	Yellow	2584	2715	NA	209
	CDC Dakota	*Pisum sativum*	Tan	Yellow	2679	2855	NA	193
	CDC Meadow	*Pisum sativum*	Non-pigmented	Yellow	2138	2099	NA	186
	CDC Striker	*Pisum sativum*	Non-pigmented	Green	2101	2377	NA	236

NA, not available.

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