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Application of InDel Markers Based on the Chloroplast Genome Sequences for Authentication and Traceability of Tartary and Common Buckwheat

*Kwang-Soo CHO, Su-Young HONG, Bong-Kyoung YUN, Hong-Sik WON, Young-Ho YOON, Ki-Beom KWON and Manjulatha MEKAPOGU**

Highland Agriculture Research Institute, National Institute of Crop Science, Rural Development Administration, Pyeongchang, Republic of Korea

**Corresponding author: manjubio7@gmail.com*

Abstract

Cho K.-S., Hong S.-Y., Yun B.-K., Won H.-S., Yoon Y.-H., Kwon K.-B., Mekapogu M. (2017): Application of InDel markers based on the chloroplast genome sequences for authentication and traceability of tartary and common buckwheat. Czech J. Food Sci., 35: 122–130.

A reliable, qualitative PCR-based detection method for the traceability and authentication of common and Tartary buckwheat was developed. Five InDel markers developed from chloroplast genome variation between the two species were applied for 96 buckwheat accessions and all accessions were easily differentiated as Tartary and common buckwheat using these markers. We also determined the sample detection limit by PCR and qPCR as 0.001 and 0.02 ng/µl, respectively. InDel markers could detect the mixture of two species flour up to 10% contamination. InDel markers were also applied to processed foods such as noodles and tea, and we found that species-specific PCR bands could be used to identify buckwheat even after processing. Hence, these InDel markers are simple with higher specificity and sensitivity and are reliable for the authentication of buckwheat processed foods.

Keywords: quantitative PCR; processed foods; insertion/deletion markers

Buckwheat is a pseudo-cereal food crop belonging to *Fagopyrum* species. It is an annual herbaceous plant with nutritional and medicinal properties and is used for dietary preparations (Jeon *et al*. 2007). *Fagopyrum* belongs to *Polygonaceae* and is classified into 20 species which largely occur in the Eurasian region and are mainly grown in highlands (Chauhan *et al*. 2010). Buckwheat was divided into two groups by Ohnishi and Matsuoka (1996) based on morphology and chloroplast genome as cymosum group, which is again classified as *F. esculentum, F. cymosum, F. tartarycum*, and *F. homotropicum*, and urophyllum group comprising *F. urophyllum.* Buckwheat is mostly cultivated in the highlands of Eurasian regions like China, Russia, Japan, South Korea, India, and Nepal (Kump & Javornik 1996; Ohsako *et al*. 2002).

Fagopyrum esculentum (common buckwheat) and *Fagopyrum tataricum* (Tartary buckwheat) are the two cultivated species of high economic importance due to their nutritional value and their usage in human consumption as both greens and grains (Li & Zhang 2001; Jeon *et al*. 2007). Buckwheat is an important functional food as it contains various polyphenols, proteins of high biological value, high contents of available minerals and relatively higher fibre content (Luthar 1992; Ikeda & Yamashita 1994; Liu *et al*. 2001; Bonafaccia *et al*. 2003). Especially, Tartary buckwheat has superior nutritional

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benefits compared to common buckwheat, due to the presence of higher levels of rutin which is a major component of flavonoids (Fabjan *et al*. 2003; Kim & Kim 2004). Rutin helps in reducing high blood pressure, reduces the risk of arteriosclerosis, decreases the permeability of the blood vessels, and has an antioxidant activity (Wojcicki *et al*. 1995; Park *et al*. 2000). It also has a crucial role in pharmaceutical research. In addition to this, Tartary buckwheat has a higher frost tolerant potential compared to common buckwheat and has higher yielding ability than common buckwheat at higher altitudes (Bisht *et al*. 2007). Hence, the cultivation of common buckwheat is declining in some areas, whereas the production of Tartary buckwheat is stable or increasing (Guo *et al*. 2010).

Buckwheat is used extensively in the food industry due to its healing effects on chronic diseases (Li *et al*. 2001). Buckwheat seeds are generally used in two kinds of foods like flour and groats dishes (Kreft 1994; Ikeda 2002). One of the most popular foods made from flour is buckwheat noodles which are largely consumed in Japan, Korea, and China. In addition, other buckwheat food products include buckwheat sprouts, beer, vinegar (Kreft 1994), Italian dishes (Barcaccia *et al*. 2016), buckwheat floral honey (Nagai *et al*. 2001; Paradkar & Irudayaraj 2002), and buckwheat tea. Buckwheat tea enables an easier absorption of nutrients by the body. Particularly, tea made from bitter buckwheat is known to help in controlling obesity, stress, high cholesterol, and blood pressure.

As Tartary buckwheat products are highly preferred for consuming due to their nutritional properties, food products made of Tartary buckwheat are expensive compared to common buckwheat products. Often, Tartary buckwheat food products are adulterated with traces of common buckwheat. In addition to food products, buckwheat seeds used for cultivation also contain a mixture of seeds from both species. Hence, a method to distinguish products from different buckwheat species is required. In this view, the study presented here shows the application of InDel (insertion/deletion) markers based on the chloroplast genome sequences of Tartary and common buckwheat to evaluate and distinguish the trace amounts of these buckwheat species in food products. The InDel markers used in this study were identified by comparing the chloroplast genome sequences of Tartary and common buckwheat in our previous study (Cho *et al*. 2015).

MATERIAL AND METHODS

Plant material. A total of 96 accessions of *F. esculentum* and *F. tataricum* were used in this study (Table 1). Among 96 accessions, 75 Tartary buckwheat accessions originated from China, Russia, Bhutan, Nepal, Japan, and Pakistan and 21 common buckwheat accessions originated from China, Russia, Japan, and Pakistan. All these genetic resources were maintained at the National Agrodiversity Centre of Rural Development Administration (http://genebank.rda.go.kr/eng/uat/uia/actionMain.do) and Highland Agriculture Research Institute, Korea. For the identification of a detection limit of two species in the mixture, flour samples were mixed at a ratio of 1 : 9, 3 : 7, 5 : 5, 7 : 3, 9 : 1 of Tartary and common buckwheat flour by weight.

Processed food product sample material. Buckwheat flour from both species, commercial noodles made from common and Tartary buckwheat, and six types of commercial buckwheat tea labelled as 100% bitter buckwheat were purchased from the local market and used in this study. Further, the applicability of the InDel marker detection method was checked in commercial food products for their authentication and to identify any mixture of trace amounts. Initially, buckwheat noodles made from common and Tartary buckwheat were purchased from the market and tested for the detectability of InDel markers. Genomic DNA was isolated from the noodles using a NucleoSpin Food kit and PCR was performed using InDel_06 primers.

In/Del markers used in the study. The InDel markers used in this study were identified by comparing the chloroplast genome sequences of Tartary and common buckwheat in our previous study (Cho *et al*. 2015). Among the total seven InDel patterns identified, five InDel markers (InDel_01, 03, 04, 05, 06) were used in this study to check their application in the authentication of buckwheat. Initially, the InDel markers identified from the comparison of chloroplast genome sequences of both Tartary and common buckwheat were validated using buckwheat germplasm.

Determination of InDel marker detection limit. The detection limit of the lowest possible trace amounts of buckwheat genomic DNA was determined with InDel markers using PCR and qRT-PCR. Around eight concentration regimes of genomic DNA ranging from the highest of 20 ng/µl to the lowest of 0.001 ng/µl from both Tartary and common buckwheat were used to perform PCR analysis with

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Table 1. List of buckwheat germplasm used for In/Del fingerprinting and their origin; No. 1–75: samples of *Fagopyrum tataricum*; No. 76–96: samples of *F. esculentum*

No.	Accession No.[z]	Origin	Remark	No.	Accession No.[z]	Origin	Remark
1	K703229	RUS	germplasm	49	K126495	NPL	germplasm
2	K035548	CHN	germplasm	50	K126496	BTN	germplasm
3	K119867	JPN	germplasm	51	K126497	BTN	germplasm
4	K126427	CHN	germplasm	52	K126498	BTN	germplasm
5	K126428	CHN	germplasm	53	K126499	BTN	germplasm
6	K126429	CHN	germplasm	54	K141188	CHN	cultivar
7	K126430	CHN	germplasm	55	K141189	CHN	cultivar
8	K126431	CHN	germplasm	56	K141190	CHN	cultivar
9	K126432	CHN	germplasm	57	K141191	CHN	cultivar
10	K126434	CHN	germplasm	58	K141192	CHN	cultivar
11	K126435	CHN	germplasm	59	K141193	CHN	cultivar
12	K126436	CHN	germplasm	60	K141194	CHN	cultivar
13	K126437	CHN	germplasm	61	K141195	CHN	cultivar
14	K126438	CHN	germplasm	62	K141196	CHN	cultivar
15	K126439	CHN	germplasm	63	K141197	CHN	cultivar
16	K126442	CHN	germplasm	64	K141198	CHN	cultivar
17	K126445	CHN	germplasm	65	K141199	CHN	cultivar
18	K126447	CHN	germplasm	66	K141200	CHN	cultivar
19	K126448	CHN	germplasm	67	K141229	CHN	cultivar
20	K126449	CHN	germplasm	68	TJ	CHN	cultivar
21	K126450	CHN	germplasm	69	M1	CHN	cultivar
22	K126452	CHN	germplasm	70	M2	CHN	cultivar
23	K126453	CHN	germplasm	71	M3	CHN	cultivar
24	K126454	CHN	germplasm	72	T8	JPN	cultivar
25	K126455	CHN	germplasm	73	T10	JPN	cultivar
26	K126457	CHN	germplasm	74	SA	CHN	cultivar
27	K126458	CHN	germplasm	75	YS	CHN	cultivar
28	K126459	CHN	germplasm	76	IT179843	RUS	cultivar
29	K126460	CHN	germplasm	77	IT179846	BLR	cultivar
30	K126461	CHN	germplasm	78	IT187869	CHN	germplasm
31	K126462	CHN	germplasm	79	IT187871	CHN	germplasm
32	K126463	CHN	germplasm	80	IT199276	uk	germplasm
33	K126464	CHN	germplasm	81	IT199286	RUS	germplasm
34	K126465	CHN	germplasm	82	IT703228	RUS	germplasm
35	K126466	CHN	germplasm	83	IT803711	CHN	germplasm
36	K126467	CHN	germplasm	84	IT910344	uk	germplasm
37	K126470	CHN	germplasm	85	IT911186	RUS	germplasm
38	K126472	CHN	germplasm	86	IT911191	RUS	germplasm
39	K126475	CHN	germplasm	87	K000694	uk	germplasm
40	K126476	CHN	germplasm	88	K000697	uk	germplasm
41	K126478	PAK	germplasm	89	K000701	uk	germplasm
42	K126479	PAK	germplasm	90	K000703	uk	germplasm
43	K126480	PAK	germplasm	91	K126516	CHN	germplasm
44	K126481	PAK	germplasm	92	K126559	JPN	germplasm
45	K126482	PAK	germplasm	93	K126561	JPN	germplasm
46	K126491	NPL	germplasm	94	K126563	JPN	germplasm
47	K126492	NPL	germplasm	95	K126569	JPN	germplasm
48	K126494	NPL	germplasm	96	K153710	PRK	germplasm

[z]Tatari buckwheat germplasm were collected and identified in Rural Development Administration Genebank and the number was assigned as K-number and IT-number tartary buckwheat germplasm from 68 to 75 was collected and maintained in Highland Agriculture Research Institute; RUS – Russia; CHN – China; JPN – Japan; PAK – Pakistan; NPL – Nepal; BTN – Bhutan; BLR – Belarus; PRK – People's Republic of Korea; uk – unknown

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the selected InDel_06 marker as a representative. For qRT-PCR InDel_01 and InDel_06 were used with various concentrations of genomic DNA (20, 2, 0.2, 0.02 ng/µl).

DNA extraction and PCR. Total genomic DNA was isolated from approximately 100 mg of fresh leaves of all buckwheat accessions using a DNeasy Plant MiniKit (Qiagen, USA) according to the manufacturer's protocol. NucleoSpin Food (Macherey-Nagel, Germany) was used to extract genomic DNA from processed food. About 200 mg of buckwheat tea and noodles was homogenised with liquid nitrogen and a subsequent procedure was performed according to the manufacturer's protocol. To amplify InDel regions, 20 ng of genomic DNA was used in a 20 µl of PCR mixture containing 2 × TOPsimple preMix-nTaq master mix (Enzynomics, Korea) consisting of 0.2 U/µl n-taq DNA polymerase, 3 mM Mg^{2+}, 0.4 mM each dNTP mixture with 10 pM of each primer. The PCR reaction was performed in a thermocycler (Veriti; Applied Biosystems, USA) using the following cycling parameters: 94°C (5 min); 35 cycles of 94°C (30 s), 55°C (30 s), 72°C (1 min); and final extension at 72°C (10 min). PCR products were analysed by 1.5% agarose gel electrophoresis and detected by DNA LoadingSTAR (DyneBio, South Korea).

Quantitative real-time PCR. Quantitative real-time PCR was performed with InDel specific primers (Table 2). Amplification and quantitative analyses were run on CFX Connect™ (Bio-Rad Pacific, Hong Kong) using Thunderbird™ SYBR® qPCR Mix (Toyobo Biotechnologies Co., Japan) according to the manufacturer's protocols. The PCR reaction mixture was prepared in 20 µl volume with 10 µl Thunderbird SYBR mix, 10 pM of each primer, and 5 µl of template DNA (20, 2, 0.2, and 0.02 ng concentration). Each PCR reaction was performed with three technical replicates and three sample replicates and the specificity of amplification products was confirmed by melting curve analysis. A serial tenfold dilution of gDNA isolated from leaves was used to make a standard curve to calculate Ct (cycle threshold) values to estimate the efficiency of InDel primers. The PCR protocol was carried out as follows: pre-incubation at 95°C for 3 min; 50 cycles of denaturation at 95°C for 10 s, annealing at 54°C for 10 s, extension at 72°C for 30 s, and data acquisition at 72°C; and melting curve analysis from 50°C to 95°C with an increment of 0.5°C.

RESULTS

Validation of InDel markers in buckwheat germplasm. PCR was carried out to amplify these InDel regions in 75 Tartary buckwheat accessions originated from China, Russia, Bhutan, Nepal, Japan, and Pakistan, and 21 common buckwheat accessions originated from China, Russia, Japan, and Pakistan. All the buckwheat accessions from both Tartary and

Table 2. List of InDel primers for the amplification of *Fagopyrum tataricum* and *F. esculentum* and their expected PCR product size

Primer name	Sequence (5' to 3')	Expected size (bp)		Remark
		F. tartaricum	*F. esculentum*	
Q_InDel_01_F Q_InDel_01_R	agttcaacggatccgagcta gggctttttcgtaaggagga	200	400	quantitative real-time PCR
Q_InDel_03_F Q_InDel_04_R	cctaccgtcttccttggaca atcaaacaaccacccccttt	150	300	quantitative real-time PCR
InDel_01_F InDel_01_R	tcaaaattagaaatacctatcgaaaaa tgtcgaacaacgaaatttaacg	538	711	semi-quantitative PCR
InDel_03_F InDel_03_R	cctaccgtcttccttggaca tctggcaaaagaggcacaat	596	710	semi-quantitative PCR
InDel_04_F InDel_04_R	cgaatcacccagtttcgttt ttcagagccgaagcctaaaa	752	644	semi-quantitative PCR
InDel_05_F InDel_05_R	tgccagttagcattggatttc tccctccctacaactcatcaa	507	682	semi-quantitative PCR
InDel_06_F InDel_06_R	acccgctgacattttgtacc gagaaagggcaggggattag	567	718	semi-quantitative PCR

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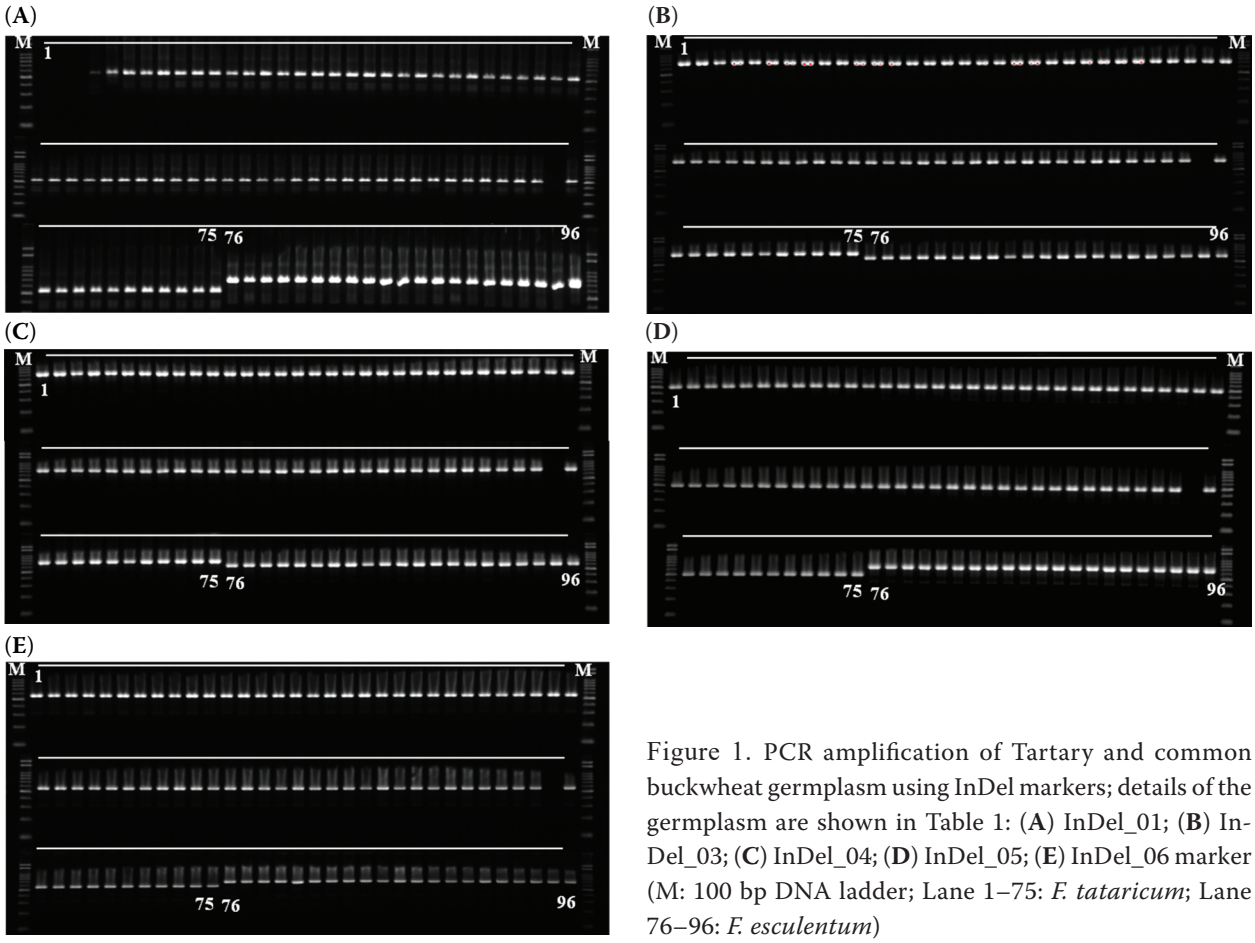

Figure 1. PCR amplification of Tartary and common buckwheat germplasm using InDel markers; details of the germplasm are shown in Table 1: (**A**) InDel_01; (**B**) InDel_03; (**C**) InDel_04; (**D**) InDel_05; (**E**) InDel_06 marker (M: 100 bp DNA ladder; Lane 1–75: *F. tataricum*; Lane 76–96: *F. esculentum*)

common buckwheat confirmed the presence of five InDel regions which can be observed by the variation in the amplicon size in both buckwheat species. Corresponding to the length of the respective InDel regions, the amplicon size variation in InDel_01, 03, 04, 05, and 06 was 173, 114, 108, 175, 151 bp, respectively (Figure 1). Hence, all the accessions in both species concurrently showed a clear variation in the InDels indicating that these InDels could be reliably used as biomarkers for the authentication of Tartary and common buckwheat traces.

Sensitivity of InDel marker amplification

InDel marker detection limit in genomic DNA by PCR analysis. The detection limit of the lowest possible trace amount of genomic DNA by InDel markers was checked by PCR analysis. Around eight concentration regimes of genomic DNA ranging from the highest of 20 ng/µl to the lowest of 0.001 ng/µl from both Tartary and common buckwheat were used to perform PCR analysis with the selected InDel_06 marker as a representative. InDel marker was successfully detected in all the eight concentrations of DNA with an expected amplicon even in the lowest concentration of 0.001 ng/µl in both Tartary and common buckwheat (Figures 2A and 2B). Hence the detection limits for these InDel markers were determined to be 0.001–20 ng/µl in genomic DNA.

Detection limit of InDel markers in genomic DNA by qRT PCR. The detection limit of InDel marker was also checked by qRT PCR using Indel_01 and InDel_03 markers. Primers for InDel_01 and 03 markers were designed for qRT PCR and were initially checked for the amplification of these InDel regions in genomic DNA from both Tartary and common buckwheat which showed the desired PCR product in both buckwheat species (Figure 3A). Further, qRT PCR was performed to check the detection limit of InDel markers with various concentrations of genomic DNA (20, 2, 0.2, 0.02 ng/µl) using InDel_01 primer in Tartary buckwheat and InDel_03 primer in common buckwheat. Amplification plots of both InDel markers showed amplification peaks at earlier cycles in samples with higher DNA concentration

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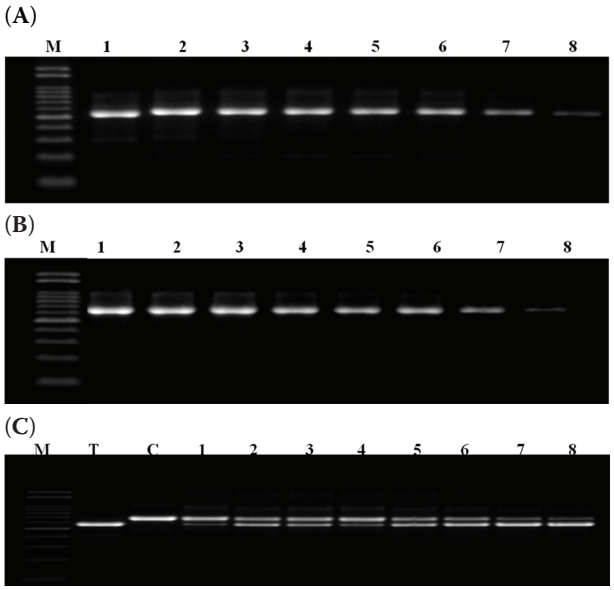

Figure 2. Analysis of the detection limit of InDel_06 marker to identify Tartary and common buckwheat genomic DNA; (**A**) *F. tataricum*; (**B**) *F. esculentum* (M: 100 bp DNA ladder; lane 1–8: genomic DNA concentration from 20, 10, 5, 1, 0.5, 0.25, 0.01 ng/µl, respectively); (**C**) in the mixed flour sample (M: 100 bp DNA ladder; T: Tartary buckwheat; C: common buckwheat; lane 1–8: mixture sample in a ratio of 1:9, 2:8, 3:7, 4:6, 6:4, 7:3, 8:2, 9:1, of Tartary and common buckwheat flour, respectively)

and samples with lower DNA concentration were amplified at later cycles (Figure 3B and 3C). Hence, it was observed that the detection limit of InDel markers by qRT PCR ranged between 0.02 and 20 ng/µl.

Application of InDel markers to identify the trace amounts in processed food. Initially, to check the sensitivity of InDel marker application in a food mixture, we tested the mixed flour samples from both buckwheat species which were mixed at a ratio of 1 : 9, 3 : 7, 5 : 5, 7 : 3, 9 : 1 of Tartary and common buckwheat flour, respectively. Genomic DNA was extracted from the mixed flour samples and PCR was performed using InDel_06 marker. It was observed that all the mixed flour samples from 1 : 9 to 9 : 1 mixtures showed an expected amplicon with a variation in the product size indicating the amplification of InDel_06 region from both Tartary and common buckwheat (Figure 4A). This suggests that the contamination of buckwheat flour as low as 10% can be easily detected and differentiated using InDel markers.

The applicability of the InDel marker detection method was checked in commercial food products for their authentication and to identify any mixture of trace amounts. This showed an amplicon of the respective product size in both common and Tartary buckwheat noodles corresponding to the amplicon obtained from the leaf genomic DNA of common and Tartary buckwheat respectively which was used as a reference (Figure 4B). This indicates that InDel markers can be reliably used in the authentication of buckwheat noodles. In addition, to determine any

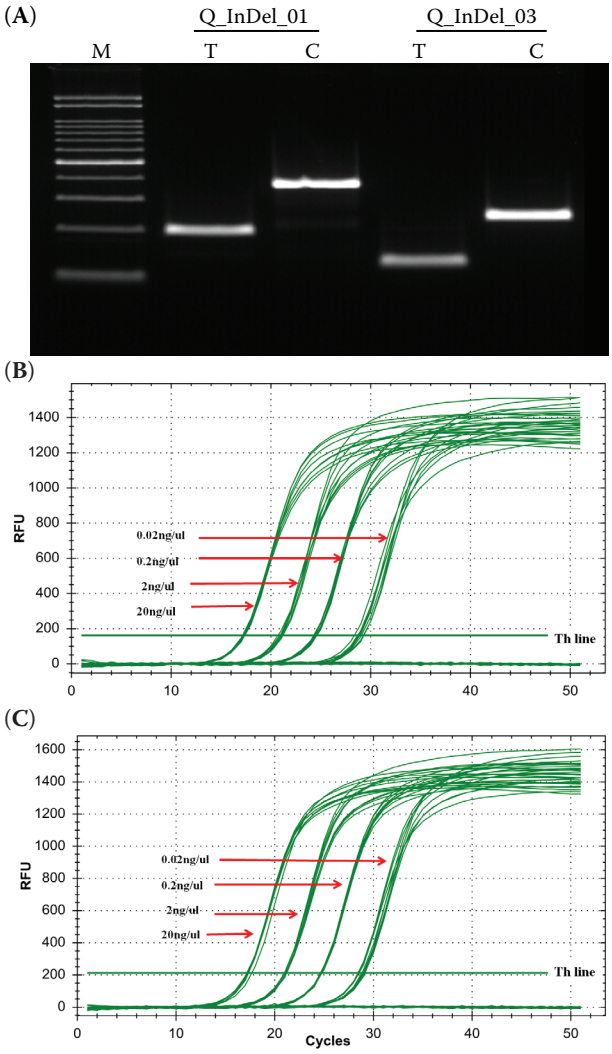

Figure 3. Analysis of the detection limit of common and Tartary buckwheat DNA by real-time PCR: (**A**) Agarose gel electrophoresis patterns with Q_InDel_01 and Q_InDel_03 markers; M – 100 bp DNA ladder; T – Tartary buckwheat; C – common buckwheat; (**B**) real-time PCR amplification plot in Tartary buckwheat using Q_InDel_01 marker; arrow indicates four concentrations of Tartary buckwheat genomic DNA (20, 2, 0.2, and 0.02 ng/µl); (**C**) real-time PCR amplification plot in common buckwheat using Q_InDel_03 marker; arrow indicates four concentrations of common buckwheat genomic DNA (20, 2, 0.2, and 0.02 ng/µl)

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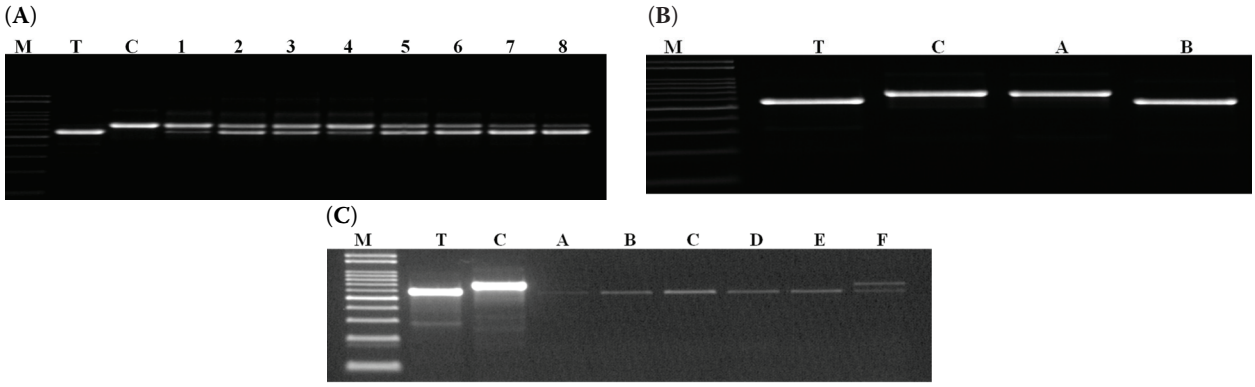

Figure 4. (**A**) Analysis of the detection limit of InDel_06 marker to identify Tartary and common buckwheat in the mixed flour sample (M: 100 bp DNA ladder; Lane 1–8: mixture sample at a ratio of 1 : 9, 2 : 8, 3 : 7, 4 : 6, 6 : 4, 7 : 3, 8 : 2, 9 : 1 of Tartary and common buckwheat flour, respectively); (**B**) Application of InDel_06 marker to identify Tartary and common buckwheat DNA from buckwheat noodles (M: 100 bp DNA ladder; T: Tartary buckwheat genomic DNA (20 ng/µl); C: common buckwheat genomic DNA (20 ng/µl); Lane A&B: DNA from buckwheat noodles made of common and Tartary buckwheat flour, respectively); (**C**) application of InDel_06 marker to identify Tartary and common buckwheat from the commercial buckwheat tea (M: 100 bp DNA ladder, T: Tartary buckwheat genomic DNA (20 ng/µl); C: common buckwheat genomic DNA (20 ng/µl); Lane A–F: different types of commercial Tartary buckwheat tea)

trace amounts in buckwheat tea, around six types of buckwheat tea which were labelled as 'made from 100% bitter buckwheat' were purchased from a local market and tested. Genomic DNA was isolated from tea by a similar method used for noodles and InDel_06 marker was detected by PCR. PCR was also performed with genomic DNA made from the leaves of common and Tartary buckwheat and used as a reference to compare the amplicon sizes. It was observed that out of the six types of bitter buckwheat tea, five showed the amplicon at a similar size of Tartary buckwheat amplicon whereas the sixth type of bitter buckwheat tea showed an amplification product similar to both common and Tartary buckwheat amplicons indicating the possibility of cross contamination with common buckwheat in 100% bitter buckwheat tea (Figure 4C).

DISCUSSION

Two major buckwheat species cultivated in various parts of the world are Tartary and common buckwheat. Due to higher rutin levels and increasing health awareness, Tartary buckwheat food products are in higher demand for consuming, which makes Tartary buckwheat expensive compared to common buckwheat (Abeywardena & Head 2001; Fabjan *et al*. 2003). More often intentional or unintentional contamination occurs in the seeds and processed food products from both species. Hence, a reliable method to identify the trace amounts and for the authentication of buckwheat food products is essential. Various detection methods are available to identify the traces of buckwheat in food products made from various other ingredients (Qin *et al*. 2011; Janes *et al*. 2012). Although most of them are quantitative methods which are laborious and time consuming, protein-based methods and PCR-based detection methods are also available (Hirao *et al*. 2005; Jeon *et al*. 2007; Yamakawa *et al*. 2008; Yoon *et al*. 2010). However, it is hard to evaluate whether these methods can detect the variation among the two buckwheat species as most of the proteins found in common and Tartary buckwheat are similar (Nair & Adachi 2002). Hence, a method to detect two cultivated buckwheat species is required for the authentication of buckwheat products. The most commonly used markers in plants are single nucleotide polymorphisms (SNPs) and InDels as they are easy to use, PCR-based, co-dominant, and relatively abundant (Pacurar *et al*. 2012). Biomarkers using DNA polymorphism can be directly analysed using tissues from individual plants or seed endosperm (Howes *et al*. 1992). PCR analysis of the InDel region can be utilised effectively as a biomarker to identify varietal contamination in a seed mixture (Yamaki *et al*. 2013). The method presented here utilises the InDel evolutionary hotspots compared between *F. tataricum* and *F. esculentum* in our previous study. Totally, six InDel regions which are identical among accessions of each species showed

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a clear polymorphism between common and Tartary buckwheat (Cho *et al*. 2015). Based on this, five InDel markers were chosen to study their application in the authentication of the two buckwheat species. This method established here is advantageous over other methods due to its sensitivity, specificity, easy detection by PCR and reliability in differentiating the two buckwheat species. The InDel evolutionary hotspot region contributes to the sequence variation and results in the amplicon size variation and hence this region is useful in developing a species-specific PCR method. PCR analyses of all the selected InDels in this study showed variations in the amplicon size which is specific for each buckwheat species and hence can be utilised in the easy discrimination of common and Tartary buckwheat. InDel markers were earlier used in rice to rapidly discriminate all genome types in the genus Oryza (Futo 2002). This method could efficiently detect the InDel marker in 0.001 ppm of buckwheat genomic DNA by PCR (Figures 2A and 2B) and successfully detected 0.02 ppm of buckwheat genomic DNA by qRT PCR (Figures 3B and 3C). In addition, it was also observed that the contamination of buckwheat flour as low as 10% can be easily detected. Hence, the sensitivity of this method is significantly higher to detect and differentiate the two buckwheat species using these InDel markers. Although earlier qualitative PCR-based detection methods could detect buckwheat genomic DNA from the mixture of wheat sample, they are not buckwheat species specific (Futo 2002; Hirao *et al*. 2005). The PCR analysis of buckwheat DNA made from common and Tartary buckwheat noodles showed amplification with different product size suggesting that the adulteration of buckwheat noodles can be easily detected by this method. Yoon *et al*. (2010) used starch granule associated proteins (SGAPs) as a biomarker to identify the botanical origin of starches used in noodle manufacture. Buckwheat DNA was detected to identify the allergens in commercial food products by PCR-based detection method (Yamakawa *et al*. 2008). Earlier reports showed the differentiation of Tartary and common buckwheat tea based on their aroma compounds (Qin *et al*. 2011). Here, six types of commercial buckwheat tea which were labelled as pure Tartary buckwheat were tested for the detection of any contamination. Among the six types, one type of tea showed the presence of common buckwheat DNA in addition to Tartary buckwheat amplicon, whereas the remaining five types showed the amplicon of Tartary buckwheat only. Although this presence of common buckwheat DNA in one tea product might be unintentional contamination during harvesting, storage or processing, these results suggest that a significantly lower amount of contamination by common or Tartary buckwheat can be efficiently detected by this method.

Hence, in conclusion, we present an efficient PCR-based InDel marker method for the specific detection of buckwheat species. This method is simple and reliable with higher sensitivity and specificity for the authentication and traceability of common and Tartary buckwheat.

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