Preparation of buckwheat DNA extracts for further marker-assisted selection activities

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IMPROVING CROPS

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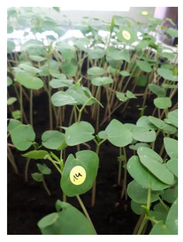
INTRODUCTION

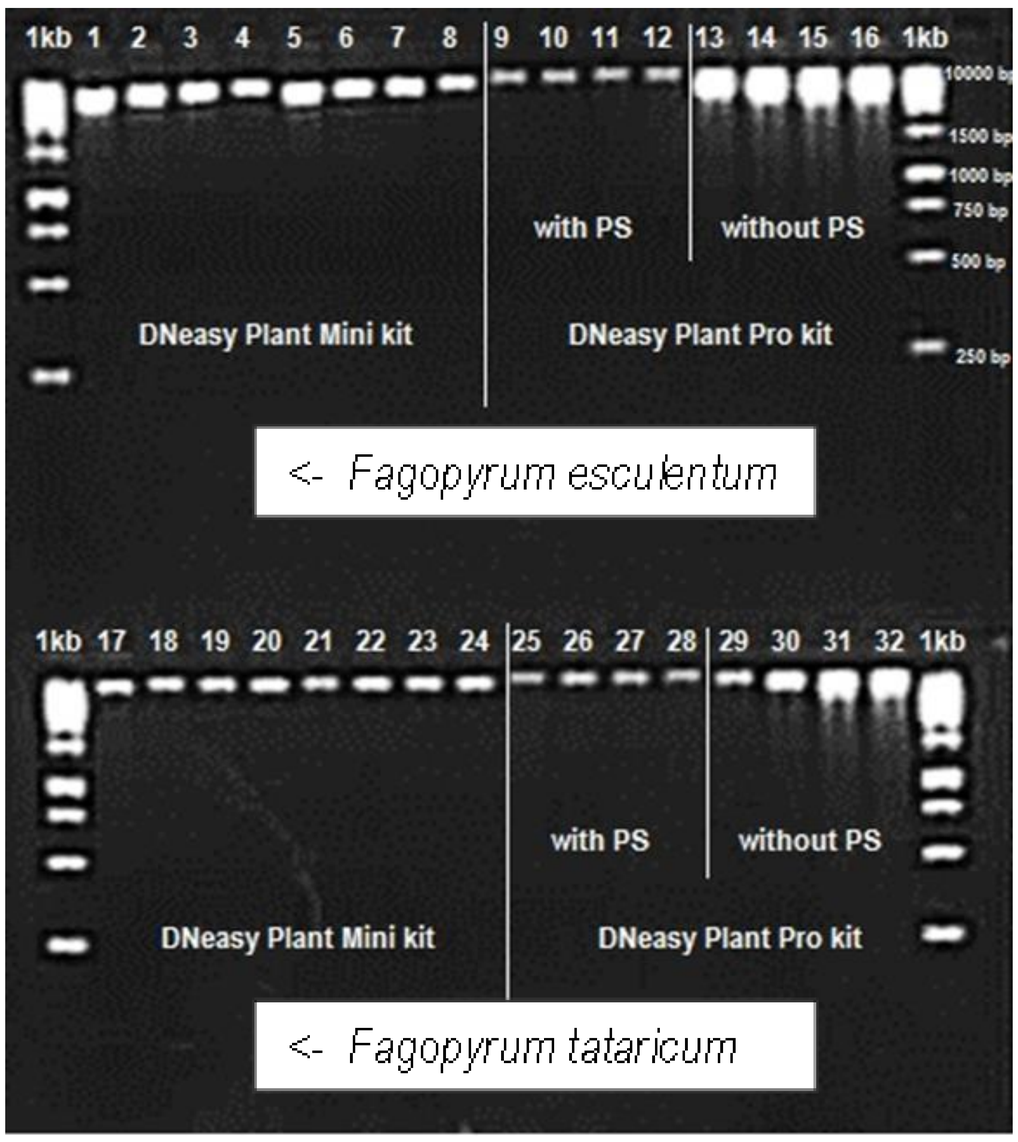
Extraction of high quality DNA is crucial for any genetic analysis. However, it is difficult to extract it from problematic plant tissues rich in secondary metabolites and other compounds whose content varies among species/varieties or other genetic resources of *Fagopyrum* sp.




METHODS and RESULTS

Three different extraction methods were used according to the manufacturer's instructions for both types of DNA from the first well-developed leaf tissue: i) magnetic extraction with the automated MagMax nucleic acid isolation robot (ThermoFicher Scientific) using the BioSprint 96 Plant Kit (Qiagen); ii) the DNeasy Plant Mini Kit (Qiagen); iii) the DNeasy Plant Pro Kit (Qiagen) with and without the addition of PS buffer (for problematic samples). DNA extraction with the MagMax kit was unsuccessful, while we did not obtain DNA from any of the buckwheat species. For the other two kits, extractions were successful for both buckwheat species based on nanodrop measurements. For common buckwheat, yields ranged from 2.4 ng/µL to 33.7 ng/µL; 260/280 ratios ranged from 1.49 to 2.46; and 260/230 ratios ranged from 0.45 to 3.3. For tatary buckwheat, DNA yields ranged from 3.7 ng/µL to 22.3 ng/µL; the 260/280 ratio ranged from 1.14 to 2.45 as the 250/230 ratio ranged from 0.68 to 2.04.

Figure 1: DNA profiles of buckwheat DNA resulted due different extraction methods



AIM OF THE STUDY

The aim of the study was to optimize the DNA extraction protocol for marker-assisted selection (MAS) of buckwheat (*Fagopyrum esculentum* L. and *Fagopyrum tataricum* L.), including molecular genotyping with functional DNA markers and/or NGS-based (Next Generation Sequencing) methods. In addition, the possibility and usefulness of extracted DNA as freeze-dried was of great importance for shipping samples worldwide.

Table 1: Nanodrop results of buckwheat DNA

Lab label	Genus, Species	Application of extraction method	Nanodrop concentration [ng/µl]	Nanodrop ratio 260/280[a]	Nanodrop ratio 260/230b	Qubit concentration [ng/ul]	Final volume [µl]	Visual scoring from the gel
1	*Fagopyrum esculentum*	DNeasy Plant Mini Kit (Qiagen)	23,9	1,76	1,91	107	200	yes
2	*Fagopyrum esculentum*		20,3	1,69	1,97	108	200	yes
3	*Fagopyrum esculentum*		13,7	1,49	1,54	62,4	200	yes
4	*Fagopyrum esculentum*		10,8	1,56	2,04	42,4	200	yes
5	*Fagopyrum esculentum*		21,2	1,69	1,62	93	200	yes
6	*Fagopyrum esculentum*		17,7	1,68	1,68	75	200	yes
7	*Fagopyrum esculentum*		9,4	1,91	3,3	48	200	yes
8	*Fagopyrum esculentum*		6,9	1,68	1,65	27,2	200	yes
9	*Fagopyrum esculentum*	DNeasy Plant Pro Kit (Qiagen) with PS	4,9	2,97	0,21	10,5	100	yes
10	*Fagopyrum esculentum*		2,4	-3,91	1,1	11,6	100	yes
11	*Fagopyrum esculentum*		5,9	2,46	0,19	10,6	100	yes
12	*Fagopyrum esculentum*		3,1	5,7	0,33	9,74	100	yes
13	*Fagopyrum esculentum*	DNeasy Plant Pro Kit (Qiagen) without PS	26,7	2	0,45	135	100	yes
14	*Fagopyrum esculentum*		40,3	1,93	1,28	187	100	yes
15	*Fagopyrum esculentum*		33,3	1,97	1,05	139	100	yes
16	*Fagopyrum esculentum*		34,7	1,98	1,16	168	100	yes
17	*Fagopyrum tataricum*	DNeasy Plant Mini Kit (Qiagen)	6,3	1,87	2,24	23	200	yes
18	*Fagopyrum tataricum*		5,7	1,41	1,18	9,02	200	yes
19	*Fagopyrum tataricum*		5,1	1,08	1,5	12,5	200	yes
20	*Fagopyrum tataricum*		5,9	1,73	1,56	17,4	200	yes
21	*Fagopyrum tataricum*		5,4	1,16	1,18	8,54	200	yes
22	*Fagopyrum tataricum*		6,1	2,25	1,54	13,8	200	yes
23	*Fagopyrum tataricum*		4	2,47	2,04	13,1	200	yes
24	*Fagopyrum tataricum*		4,5	1,85	1,65	13,8	200	yes
25	*Fagopyrum tataricum*	DNeasy Plant Pro Kit (Qiagen) with PS	3,7	1,49	1,05	6,08	100	yes
26	*Fagopyrum tataricum*		4,3	1,67	0,24	9,12	100	yes
27	*Fagopyrum tataricum*		3,7	1,34	0,37	7,88	100	yes
28	*Fagopyrum tataricum*		3,7	1,14	0,68	6,84	100	yes
29	*Fagopyrum tataricum*	DNeasy Plant Pro Kit (Qiagen) without PS	4,6	1,88	0,11	8,92	200	yes
30	*Fagopyrum tataricum*		13,2	1,63	0,26	44,2	100	yes
31	*Fagopyrum tataricum*		18,8	1,96	0,64	77,4	100	yes
32	*Fagopyrum tataricum*		22,3	1,75	1,45	86,4	100	yes

CONCLUSIONS and FUTURE PERSPECTIVES

The purity, quality and quantity of these DNA extracts would be sufficient for in-house performance of MAS. If the freeze-drying process of buckwheat DNA samples for long distance shipping is required to perform NGS-based MAS applications, these methods are less suitable. According to our results, we could suggest that the optimized CTAB-based method is more suitable before the DNA is prepared as freeze-dried powder. In addition, the time and cost of an extraction method should not be ignored, especially when a large number of samples are involved.

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