Analysis of DNA Extraction Protocols for Transgenic Events Determination in Alfalfa

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Alfalfa (*Medicago sativa*) is one of the main forage crops in Argentina. The unclear introduction of GE glyphosate-resistant alfalfa seeds, caused contamination in different production areas, affecting the purity of the local non-GE alfalfa seeds. Also, that contamination could affect the international trade of forage to countries where such transgenic events are prohibited. The evaluation of these events could be performed by DNA evaluation. In this sense, it is essential to have methodologies that allow us to obtain a good DNA concentration and quality. Although different commercial kits (CK) are available, they are highly costly when many extractions are required. The aim of the present work was to evaluate the efficiency of two economic DNA extraction methods in alfalfa seeds, hay, and fresh leaves. The DNA extraction was performed with three methods: 1) saline precipitation with sodium dodecyl sulfate (SDS), 2) precipitation with potassium acetate (KA) and 3) with a commercial kit, as control. The DNA concentration, purity, and integrity were assessed using spectrophotometer and agarose gel electrophoresis. In addition, the activity of the *Taq polymerase* in DNA amplification by polymerase chain reaction (PCR) was evaluated. The KA method showed higher DNA concentration and purity in the tissues evaluated, followed by SDS (Table 1). Nevertheless, hay tissue could be amplified in all the samples with SDS and CK methods and seed with KA and CK. Fresh leaf tissue only amplified with CK. The results obtained suggest that hay samples should be analyzed with the SDS saline precipitation method, since it is possible to achieve extractions as pure as those of the CK, but with higher concentration. On the other hand, the seed samples could be analyzed with the KA method. Results suggest that SDS and KA methods could be an alternative protocol for hay and seed efficient as CK, cheaper, faster, and more useful for large-scale DNA extractions.

Table 1. Concentration, purity and amplification results obtained according to seeds, hay, and fresh leaves and protocol used.

	Precipitation with SDS			**Potassium Acetate (KA)**			**Commercial Kit (CK)**		
	Hay	Seed	Fresh Leaves	Hay	Seed	Fresh Leaves	Hay	Seed	Fresh Leaves
Conc. (ng/µl)	44.0	245.6	88.7	1465.80	1547.7	2411.6	16.7	33.1	35.5
Purity									
260/280	2.1	2.0	0.6	1.9	2.0	2.0	1.8	1.8	1.5
260/230	2.0	1.4	1.3	1.1	2.2	1.7	2.6	1.2	0.5
Ampl (%)	**100**	**33.33**	**33.33**	**33.33**	**100**	**0**	**100**	**100**	**100**

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