Bulletin UASVM Horticulture, 70(1)/2013, 265-266
Print ISSN 1843-5254; Electronic ISSN 1843-5394

# Comparison of Protocols for DNA Extraction from *Cannabis sativa* Seeds

**Salvador SOLER[1], Alicia SIFRES[1], Ernesto R. LLOSA[2], Matías LLAMAS[3], Santiago VILANOVA[1], Jaime PROHENS[1]**

[1] Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Camino de Vera 14, Valencia, Spain; jprohens@btc.upv.es
[2] Hemp Trading, S.L.U., Camino del Polio 51, Beniparell, Valencia, Spain;
[3] Maris Seed Spanish, S.L., Pl. Fernando Ferrando 1-1°, Valencia, Spain.

**Abstract.** *Cannabis sativa* is an important crop cultivated both for industrial (var. *sativa*) and medicinal and recreational (var. *indica*) purposes. Cultivation of var. *indica* plants is generally forbidden and this makes difficult genetic studies. An alternative is the extraction of DNA from seed embryos. In order to develop an efficient protocol for DNA extraction of *C. sativa* seeds we have tested six DNA extraction methods in seeds and leaves of a *C. sativa* var. *sativa* accession. We found that the best protocol is the CTAB-modified with phenol:chlorophorm:isoamyl washing, which allowed a large quantity of high quality DNA per seed. This method was tested in seeds of three C. sativa var. indica accessions and seven SSR markers tested could be amplified successfully. In summary, we have developed a highly efficient method for DNA extraction of individual seeds of C. sativa. This method will be useful for genetic studies in this species.

**Keywords**: CTAB, embryos, extraction kits, molecular markers, SSRs

**Introduction.** *Cannabis sativa* L. is cultivated both as an industrial crop cultivated for its fiber and/or achenes (var. *sativa*), and for medicinal or recreational purposes (var. *indica*). Cultivation of plants from var. *indica* is generally forbidden due to the high content of tetrahydrocannabinol (THC), which makes difficult the study of genetic diversity, crop evolution, and forensic investigation in this species (Alghanim and Almirall, 2003). An alternative is the extraction of DNA from individual seeds (Gao *et al*., 2010), which have a low THC content.

More than 50 years ago, Kirby (1956) published the first use of phenol to purify nucleic acids. Denatured proteins collect at the interface between the two phases and lipids partition efficiently into the organic layer. Whole nucleic acids highly enrich in the aqueous phase and can easily be removed from the organic phase and interphase (Sambrook and Russell, 2001).

**Aims and objectives.** This work is aimed at developing an efficient method for the extraction of high quality DNA from embryos of individual seeds of *Cannabis sativa*, and its validation using molecular markers and comparison with DNA extracted from leaves.

**Materials and methods.** DNA was extracted from individual embryos extracted from hydrated seeds from one accession of *C. sativa* var. *sativa* marketed for bird food using six extraction methods (four based on CTAB method modifications, and two commercial kits (DNeasy® Plant Mini Kit Qiagen, and NucleoSpin® Plant II Macherey-Nagel). Protocol modifications consisted of a) adding 1% PVP40 to extraction buffer, b) adding 3% PVP40 to extraction buffer, c) adding proteinase K to extraction buffer d) performing a wash with a volume of phenol: chloroform: isoamyl alcohol (25:24:1) before washing with chloroform: isoamyl alcohol (24:1).

In order to confirm the validity of the method, the selected extraction protocol was also tested on 15 seeds in each of three *C. sativa* var. *indica* varieties used for recreational

purposes (e.g., for its psychoactive properties) as well as on 15 plantlets of the *C. sativa* var. *sativa* accession. Seven genomic SSRs were tested for amplification in these materials**.**

**Results and Discussion.** The best extraction method was the CTAB-modified with phenol:chlorophorm:isoamyl alcohol washing. Using this method, a large quantity (50 µg) of high quality DNA per seed was obtained. The two commercial kits gave a low DNA yield, and the rest of CTAB methods produced low quality DNA extractions thus obtaining a white precipitate with a large absorbance at 230 nm (Figure 1). The seven SSR markers could be successfully amplified in the different *Cannabis* materials evaluated using DNA from individual embryos and leaves extracted with the selected method. This shows, that as in other species (Gao et al., 2010), extraction of large quantities of DNA from individual seeds of *C. sativa* seeds is feasible using improved protocols.

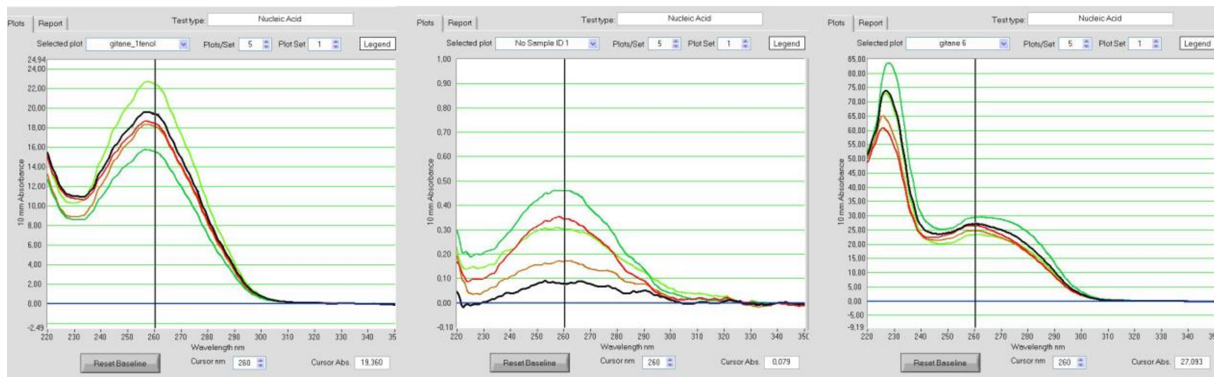


Fig. 1. NanoDrop spectrophotometric measurements for *Cannabis sativa* DNA purified with phenol (left), two extraction kits (center) and different extraction methods without phenol (right).

**Conclusion**

A highly efficient method, consisting of the CTAB-modified extraction with phenol:chlorophorm:isoamyl alcohol washing, for the DNA extraction from individual seeds of *C. sativa* has been developed. This method will be of great interest for the genetic fingerprinting, evaluation of diversity and relationships, as well as for forensic studies in *Cannabis*.

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