Optimization of DNA Extraction from Seeds and Fresh Leaf Tissues of Soybean (*Glycine max*)

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Abstract

The effects of various components on extraction buffer such as SDS-NaCl, PVP, β-mercaptoethanol, extraction with Phenol: Chloroform:Isoamyl acetate, and incubation time on the DNA extraction from seeds and fresh leaves of Soybean (*Glycine max*) were studied. Based on results, an optimized method for DNA extraction from Soybean seeds and leaves were established. Extracting Soybean seeds with Phenol:Chloroform:Isoamyl acetate (25:24:1) two steps, incubating for 30 min and 1% SDS-2 M NaCl in extraction solution could promote the quantity and purity of DNA from seeds, respectively. The results also showed that high quantity of DNA from Soybean leaves could be extracted with Phenol:Chloroform: Isoamyl acetate (25:24:1) two steps, incubating for 30 min and adding 0.5% SDS-2% PVP-1%β-mercaptoethanol in extraction buffer. The DNA quantity could be higher than DNA quantity obtained with *Nucleospin*® Plant II method.

Keywords: *Glycine max*, DNA extraction, optimization

Introduction

Soybean (*Glycine max*) is an important commodity in Indonesian Protective Commitment in International Trading Conference[1] which is used in food and herbal supplement.[2]

Despite its important effect as herbal supplement, there were many reports that revealed its side effects on skin, gastrointestinal, and respiratory reactions and in some cases anaphylaxis.[3] Before further evaluation about its side effect, correct identification of the raw material must be performed to ensure its safety. One of the most reliable methods for identification of herbal medicine materials is by analyzing DNA.[4]

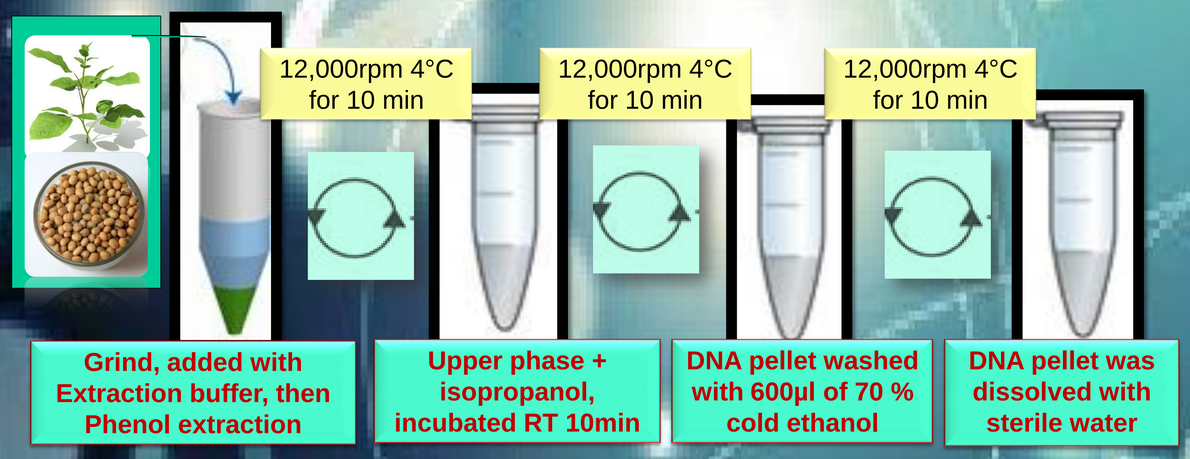
The application of genetic identification in some plant species has however been constrained by lack of efficient DNA extraction techniques, because of the presence of polyphenols and metabolites that interfere with further application of DNA, such as DNA fingerprinting.[5] Therefore this study had performed optimization of DNA extraction from seeds and fresh leaves of soybean.

Materials and Methods

Plant Material

Soybean seeds were collected from local market at Surabaya on 2011. After being cultivated several days in the soil, the leaves from the seeds were collected, washed free of dirt, mopped dry and quickly stored at -80 ºC until used.

DNA EXTRACTION METHOD (modified from [6])



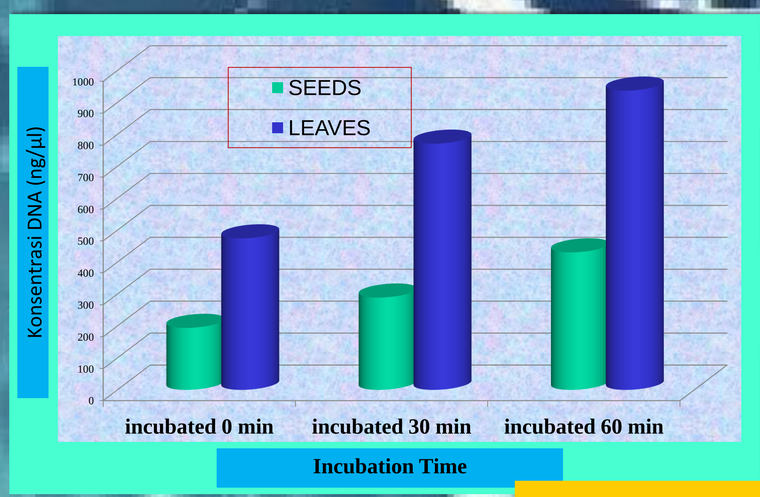
Optimization of DNA extraction

Based on extraction procedure above, following modifications on DNA extraction method were done respectively:

(1) with 2% PVP for leaves;
(2) DNA extraction with phenol, Phenol: Chloroform: Isoamyl acetate (25:24:1) one and two steps;
(3) set a different incubation time;
(4) adjust the concentration of SDS in extraction solution
(5) adjust the composition of SDS and NaCl (for seeds)
(6) adjust the concentration of *β-mercaptoethanol* for leaves.

DNA purity (A260/A280 ratio) and its concentration were measured with NanoDrop spectrophotometer

Results and Discussion



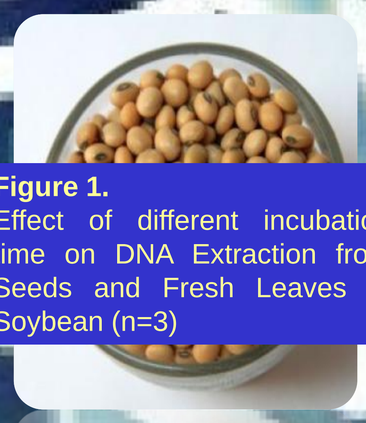


Figure 1. Effect of different incubation time on DNA Extraction from Seeds and Fresh Leaves of Soybean (n=3)

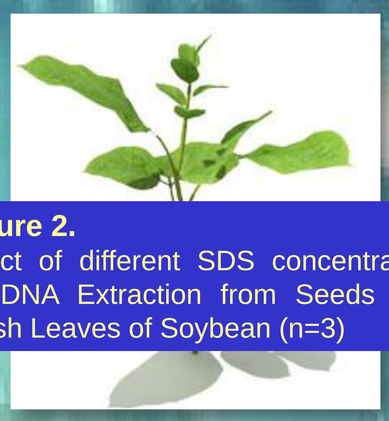


Figure 2. Effect of different SDS concentration on DNA Extraction from Seeds and Fresh Leaves of Soybean (n=3)

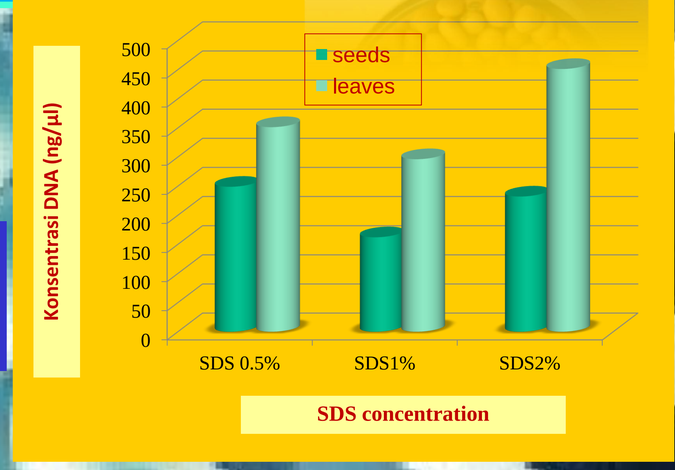


Table. The Yield of Soybean DNA from Seeds and Leaves after Optimization of DNA Extraction with Different Parameters (n=3)

Soybean Organs	Methods, Parameters, Optimal Parameters			DNA concentration (ng/µl)
Seeds 	Modification method from ICI Seeds co.[6]	Incubation time	30 min	289,07
		SDS concentration	0.5%	248.97
		SDS-NaCl ratio	SDS 1%; NaCl 2M	227.94
		Steps amount on extraction	Phenol:Chloroform: Isoamylacetate (25:24:1)-two steps	237.29
	Nucleospin® Plant II method			244.80
Leaves 	Modification method from ICI Seeds co.[6]	Incubation time	30 menit	770.87
		SDS concentration	0.5%	351.36
		PVP	2%	184.06
		Steps amount on extraction	Phenol:Chloroform: Isoamylacetate (25:24:1) -two steps	609.00
		β-mercaptoethanol	1%	770.87
	Nucleospin® Plant II method			328.29

*) A260/A280 ratio in all experiment were in the interval 1.8-2.0, which indicated that DNA is quite pure and amenable for another application

The major differences in extraction method for seeds or leaves mainly concern the ingredients of the extraction buffer. Each plant organ (seeds or leaves) may require its relevant method depending on the demand of the level of DNA purity. SDS extraction was performed to increase the efficiency of removing proteins from the extracted DNA, while reducing agents such as β-mercaptoethanol was also usually included in inhibiting oxidation process, which either directly or indirectly caused damage to DNA. Addition of high concentration of NaCl increased the solubility of polysaccharides in ethanol, effectively decreasing co-precipitation of the polysaccharides and DNA.[7]

Conclusions

Optimal condition for DNA extraction from Soybean seeds were 1%SDS-2M NaCl in the extraction buffer which incubated 30 minute and extraction by two steps of Phenol: Chloroform: Isoamyl acetate. Optimal condition for Soybean leaves were 0.5%SDS, 2%PVP, 1%β-mercaptoethanol, in the buffer mixture which incubated 30 minute and extracted by two steps of Phenol: Chloroform: Isoamyl acetate.

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Poster Presentation, International Biology Conference, Institut Teknologi 10 Nopember, Surabaya, December 6th, 2012