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Science for Energy, Food and Environmental Sustainability

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IBOC 2012
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PREFACE

We would like to thank to God for blessing us until we are able to organize our first international annual event called The International Biology Conference (IBoC) 2012 at the end of this year. This conference is purposed to build a future and promising international networking between our department and other international parties which have a similar interest keeping environment balance for save the earth. Concerning to it, our first theme is "Science for Energy, Food and Environmental Sustainability".

The first outcome we would gain is having a direct and personal contact, sharing and discussion with scientists around the world. Further on we would like to build a real scientific networking doing a real work keeping the earth balance and save. We are really grateful realizing that they are from Egypt, Thailand, Bangladesh, Malaysia and Indonesia. Thank you for your participations.

We are also glad to inform that, this conference have been possible only because of the support from The Faculty of Mathematics and Natural Sciences and The Institut Teknologi Sepuluh Nopember (ITS), Surabaya-Indonesia. The Vice Rector, Prof. Darminto put several administrative and scientific advices. The big applause and thank is also going to our students from The Biology Department who has been managing the conference.

Thank you and best regards.

Surabaya, June 5th 2013
Head of Biology Department

Dr.rer.nat. Maya Shovitri

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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 above, an optimized method for DNA extraction from Soybean seeds and leaves were established. Extracting Soybean seeds twice with Phenol:Chloroform:Isoamyl acetate (25:24:1), 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 quality DNA from Soybean leaves could be extracted with Phenol:Chloroform:Isoamyl acetate (25:24:1) twice, incubating for 30 min and 0.5% SDS-2% PVP in extraction solution, although the DNA quantity was less 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 (WTO)[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. DNA markers are reliable for informative polymorphisms as the genetic composition is unique for each species and is not affected by age, physiological conditions as well as environmental factors.[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.

MATERIAL 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.

Solutions

The extraction buffer consisted of 200 mM Tris-HCl pH 8.0, 250 mM NaCl, 25 mM EDTA, and 0.5% SDS was prepared. The following solutions were also prepared and stored at 4°C : Phenol:Chloroform: Isoamylacetate (25:24:1), 70% Ethanol.

DNA extraction

Genomic DNA extraction, modified from ICI *Seeds Co.*1996[6]. Seeds (Fresh leaves) 100.0 mg were ground and 400µl of extraction buffer (200 mM Tris-HCl pH 8.0, 250 mM NaCl, 25 mM EDTA, and 0.5% SDS) were added. The mixture was added with 400µl phenol with occasional inversion and then centrifuged 12,000rpm 4°C for 10 min. Three hundreds micro littre of upper phase was mixed with 250µl isopropanol and incubated at room temperature for 10 min. After centrifugation 12,000 g at 4ºC for 10min, the DNA pellet was washed with 600µl of 70 % cold ethanol, centrifuged 12,000rpm 4°C for 10 min and DNA pellet was dissolved in 300µl sterile water (for leaves) or 100µl (for seeds) and kept at –20 ºC until used.

Optimization of DNA extraction from Soybean seeds and leaves

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) once and twice ; (3) set a different incubation time (0 min, 30 min, and 60 min); and (4) adjust the concentration of SDS in extraction solution to 0.5%, 1.0% and 2.0%, respectively; (5) adjust the composition of SDS and NaCl (0.5% SDS- 250mM NaCl; 1% SDS-2M NaCl) for seeds; (6) adjust the concentration of *β-mercaptoethanol* (1.0%, 5.0%, and 10.0%) for leaves.

Detection of quality and quantity of DNA from the seeds and leaves of Soybean

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

RESULTS and DISCUSSION

This study had used seeds and leaves of soybean for extracting the DNA. Soybean seeds were used in this study because seeds frequently being sold at the local market and they were usually consumed or developed into the another nutritive form, such as tofu, milk. The seeds were also quite stable in long term storage. The leaves were chosen for DNA extraction due to their continued availability whole year round.

Figure 1. shows DNA yield from seeds and leaves which were incubated on different incubation time. Increasing the incubation time of leaves and seeds in extraction buffer until 60 minute could increase the DNA yield until 430.47 ng/µl from seeds and 937.10 ng/µl from leaves, but the DNA qualities decreased if compared with the DNA quality from seeds and leaves which incubated 30 minute in extraction buffer. Puchooa and Venkatasamy had also used incubation time 35 minute to extract the DNA from *Trochetia boutoniana*.[7]

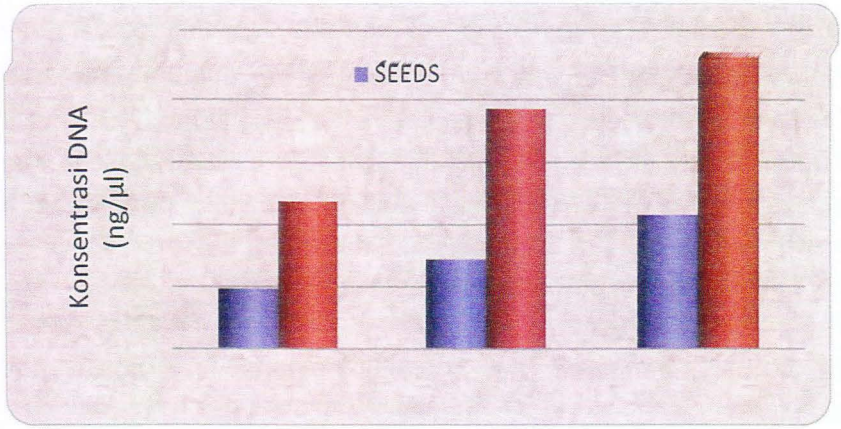

Figure 1. Effect of different incubation time on DNA yield from seeds and fresh leaves of soybean

Problems encountered in the isolation and purification of high molecular weight DNA from certain plant species include co-isolation of highly viscous polysaccharides and inhibitor compounds like polyphenols and other secondary metabolites which directly or indirectly interfere with subsequent application process, as reported by several researchers.[8,9]

Figure 2. shows DNA yield from soybean seeds and leaves which were extracted on extraction buffer containing *Sodium Dodecyl Sulphate* (SDS) at several concentrations. Extraction buffer containing 0.5% SDS could increase the DNA yield until 248.97ng/µl from seeds and 2% SDS increased the DNA yield until 451.194ng/µl from leaves. SDS extraction was usually used to increase the efficiency of removing proteins from the extracted DNA and several researchers had used 0.5%-2% SDS to extract genomic DNA.[10,11] Because of the lower purity of DNA extracted with >1%SDS from leaves, this study only used the 0.5% SDS in extraction buffer.

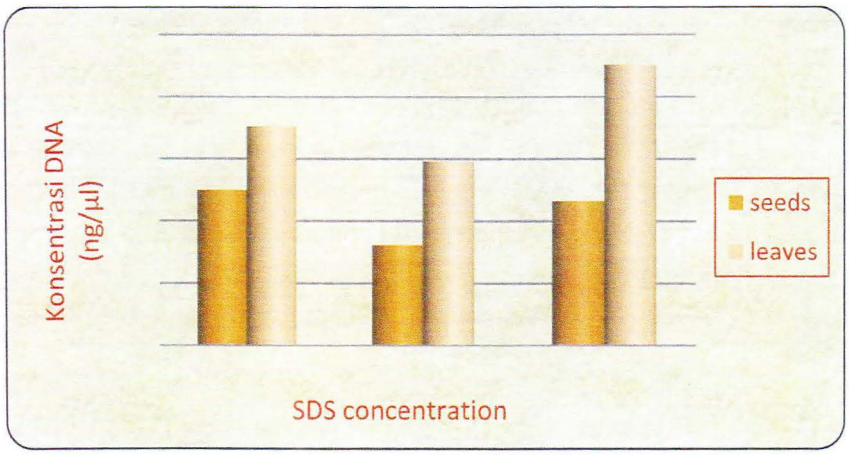

Figure 2. Effect of different incubation time on DNA yield from seeds and fresh leaves of soybean

The major differences in extraction method for seeds or leaves on table 1. mainly concern the ingredients of the extraction buffer. Each plant organ (seeds or leaves) may require its relevant protocol depending on the demand of the level of DNA purity. Reducing agents such as β-mercaptoethanol (0.5-3%) was also usually included in inhibiting oxidation process, which either directly or indirectly caused damage to DNA. PVP (2.5-3%) could also improve the colour of the DNA obtained. Addition of high concentration of NaCl (2.5-3 M) increased the solubility of polysaccharides in ethanol, effectively decreasing co-precipitation of the polysaccharides and DNA.[7,8,12]

Table 1. The Yield of Soybean DNA from Seeds and Leaves after Optimization of DNA Extraction with Different Parameters

Soybean Organs	Methods and Parameters			DNA concentration (ng/µl)
Seeds	Modified 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	Modified 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

Note:
A260/A280 ratio in all experiment were >1.8, which indicated that DNA was quite pure and amenable for another application

The yield of soybean DNA ranged from 227.94 to 289,07 ng per 100.0 mg of seeds and from 184.06 to 770.87 ng per 100.0 mg of leaves. Some experiments showed that DNA concentrations from soybean seeds and leaves with the modified ICI Seeds co. method were higher than DNA concentrations which were obtained with *Nucleospin*® Plant II method, but DNA concentration from the seeds were lower than DNA concentration from the leaves, it might be caused the abundance

protein and polysaccharides in the seeds which interfered the extraction process. A260/A280 ratio more than 1.9 of DNA extracted from the seeds could indicate polysaccharide contamination, but the DNA from the seeds should be amenable for subsequent applications such as amplification by Polymerase Chain Reaction (PCR) method because Abd-Elsalam had reported that DNA ranged from 50 to 100 ng per 100 mg of starting material, enough to conduct PCR.[11]

CONCLUSION

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%.

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