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Full Paper

# Evaluation of USM Biotech DNA extraction method for selected agricultural crops in comparison with existing methods

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## Abstract

Different DNA extraction protocols have been used in plant DNA extraction. However, no comparative analysis has been done to determine their efficiency, cost effectiveness and time requirement for the extraction. Three (3) established protocols and the USM Biotech modified protocols were used in this study. It aimed to evaluate the efficiency of the four DNA extraction protocols in terms of DNA yield, purity and processing time; to determine and compare the cost of sample analysis per protocol and to assess which protocol is universally applicable in extracting DNA of selected agricultural crops (banana, cacao, durian, mango and rubber). The experiment was carried out in 4X5 factorial arranged in Complete Randomized Design (CRD), Factor A as protocols and Factor B as crops used. Results of the study showed that among four (4) protocols, the protocol developed by Ferdous et al. (2012) was the most cost effective. It was the least expensive and fastest method of extracting DNA resulting to high genomic DNA yield. Likewise, University of Southern Mindanao (USM) Biotech modified protocol was found to be another efficient, economical and effective method of extracting DNA with sufficient amount of DNA yields. The protocol developed by Ray et al. (2016) produced the highest DNA yield; however, it was the most time-consuming method among the four (4) protocols. The Diversity Array Technology (DArT) protocol on the other hand, was the most expensive method among the four protocols because it required the use of expensive reagents and liquid nitrogen.

**Keywords** - agricultural crops, cost-effectiveness, DNA extraction protocols, DNA purity, DNA yield

## Introduction

Extraction of DNA has been the foundation of many important molecular studies, such as: marker-assisted selection, diversity assessment, germplasm identification, quantitative trait loci analysis and transformants screening (Post et al., 2003). Due to the importance of DNA extraction, numerous DNA extraction protocols have been developed by several authors (Dellaporta et al., 1983; Doyle & Doyle 1990; Sharma et al., 2002; Haque et al., 2004 & Kumari et al., 2012). Along with this many DNA extraction kits such as DNeasy, Plant Mini kits and others are also available. However, the problem with these commercially available kits is their exorbitant cost (Ahmed et al., 2009). In addition, some kits are found to be not cost-effective (Varma et al., 2007) and are not readily available or accessible (Bashalkhanov & Rajora, 2008; Nui et al., 2008).

Generally, although numerous DNA extraction protocols are available they are rarely compared comprehensively (Zimmermann et al., 1998). There is a need to compare the protocols in order to determine the cost effectiveness, simplicity and reliability of the different methods. Aside from lack of comparative studies on the different DNA extraction protocols, a universal DNA extraction protocol needs to be established to develop the most efficient method and highly applicable protocol in a wide range of specimens.

In order to address the aforementioned concerns, this study was conducted to evaluate the efficiency of four (4) protocols developed by Ray et al. (2016), Ferdous et al. (2012), Diversity Array Technology (DArT) 2001 and University of Southern Mindanao (USM) Biotech modified protocol in terms of their nucleic acid yield, purity and processing time. It also aimed to determine

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and compare the cost of sample analysis per protocol and assess which extraction protocol could be universally applied in various plant species such as: banana, cacao, durian, mango and rubber.

The study used these four protocols because they had been successfully applied in extracting DNA of different agricultural crops. The protocol developed by Ray et al. (2016) was applied in extracting DNA of cold tolerant rice; protocol developed by Ferdous et al. (2012) was successfully applied in rice, banana and oil palm while the protocol developed by DArT (2001) was used in extracting DNA of sugarcane and routinely used by the laboratory. They are known for high DNA yield and found to be effective. However, they all require the use of liquid nitrogen which is not readily available in some laboratories. In addition to the three protocols, the USM Biotech modified protocol which was routinely used in the USM Biotech laboratory was also evaluated in comparison to the three mentioned protocols. This study was conducted to evaluate the efficiency of four (4) protocols developed by Ray et al. (2016), Ferdous et al. (2012), DArT (2001) and USM Biotech modified protocol in terms of their nucleic acid yield, purity and processing time; to determine and compare the cost of sample analysis per protocol and to determine which extraction protocol could be universally applied in various plant species such as *Musa sp*. (banana), *Theobroma* cacao (cacao), *Durio zibethenos* (durian), *Hevea brasilensis* (rubber) and *Mangifera indica* (mango).

## Methodology

### Plant Materials

Young leaves of banana, cacao, durian, mango and rubber were used in the study. The collected leaves were put on ice.

### Experimental Design and Treatments

The experiment was carried out using the 4x5 factorial laid out in Complete Randomized Design with DNA extraction method as Factor A while crop species as the Factor B. All extraction protocols were replicated three times (3x).

### Evaluation of the Four DNA Extraction Protocols

The four protocols were evaluated in five crops species. In each protocol 50 mg of leaf samples were ground with mortar and pestle with addition of extraction buffer or liquid nitrogen. Samples were placed in labeled tubes. The samples were then incubated in a water bath before the ratio of 24:1 chloroform-isoamyl alcohol was added. The tubes were centrifuged and the supernatant or aqueous phase was transferred in new tubes. The DNA was precipitated with an ethanol or an Isopropanol, and the pellet was washed with an alcohol or ethanol then air-dried. Tris Edta (TE) buffer was used for the re-suspension of DNAs. Meanwhile, RNAase was added to the tubes to purify the DNAs. The tubes were then stored in -20ºC. On the other hand, some of the protocols required additional steps, such as: re-precipitation of DNAs with the use of sodium acetate, centrifugation of the tubes, followed by air drying of pellets and re-suspension of DNAs.

The different reagents and chemicals used in every protocol are presented in Table 1. On the other hand, the differences among the steps involved in each protocol are shown in Table 2.

### DNA yeld and Purity Measurement

The yield and quality of extracted DNA was measured with the use of spectrophotometer. The purity of DNAs was assessed based on the A260/280 nm absorbance ratio.

### Statistical Data Analysis

The data gathered were analyzed using ANOVA. The treatment means were separated using Tukey's HSD Test.

### Cost Analysis

The cost of analysis per sample was based on the cost of the reagents and solutions used in each protocol as well as the man days dedicated to the analysis.

## Results and Discussion

### Comparative Evaluation of the Genomic DNA Yields of Five (5) Test Crops from the Four (4) Protocols Tested

Figure 1 showed the interaction effect of protocols on test crops. Results revealed that the protocol developed by Ray et al. (2016) gave the highest (1,815.45 ng/µL) genomic DNA yield in banana. In contrast, the protocol developed by Ferdous et al. (2012) generated the lowest (45.53 ng/µL) genomic DNA yield in banana. Moreover, the three remaining protocols

generated low DNA yields that were comparable to one another.

For cacao, results showed that the four protocols did not give significant difference among the genomic DNA yields. This implied that any of the four protocols can be alternatively used in extracting DNA of cacao.

In the case of durian, the protocol developed by Ray et al. (2016) generated the highest genomic DNA yield with an average of 358.19 ng/µL. Statistical analysis revealed no significant difference in the DNA yield generated from the protocols developed by Ray et al. (2016), Ferdous et al. (2012) and USM Biotech modified protocol. The DNA yield generated from the protocol developed by DArT (2001) was the lowest and was significantly different from the other three protocols. This proved that the protocol developed by DArT (2001) was not efficient for durian.

Table 1. Different reagents and chemicals used per protocol evaluated.

| Chemicals and Reagents | Ray et al. (2016) | DArT Extraction Protocol (2001) | Ferdous et al. (2012) | USM Biotech Modified Protocol |
|---|---|---|---|---|
| 1.Chloroform: Isoamyl alcohol (24:1) | 900µL | 1000µL | 400µL (5% phenol) | 800µL |
| 2. CTAB | 10%X CTAB solution | 2% (Extraction buffer) | 2X CTAB solution | 2% (Extraction buffer) |
| 3. EDTA | 0.5M | 5mM | 0.5M | 5mM |
| 4. Liquid Nitrogen | N/A | No exact amount | N/A | N/A |
| 5. PVP | N/A | 2% | 1% | 2% |
| 6. Sodium Chloride | 5M | 2M | 3.5M | 2M |
| 7. Sodium metabisufite | N/A | 0.5% | N/A | 0.5% |
| 8. Sorbitol | N/A | 0.35M | N/A | 0.35M |
| 9. Tris HCl | 1M | 0.2M | 1M | 0.2M |
| 10. Sodium dodecyl sulfate | 20% | N/A | 0.2% | N/A |

Table 2. Different steps involved in the four protocols evaluated.

| DNA Extraction Protocol (Steps) | Ray et al. (2016) | DArT Extraction Protocol (2001) | Ferdous et al. (2012) | USM Biotech Modified Protocol |
|---|---|---|---|---|
| Water Bath Duration and Temperature | 1st -10 min at 65$^0$C<br>2nd - 10 min at 65$^0$C (after addition of CTAB) | 1-1.5 hours at 65$^0$C -invert every 20 min | N/A | 1-2 hrs at 65$^0$C -invert every 10 min |
| Chloroform:Isoamyl Application | 900µL | 1,000µL | 400µL | 800µL |
| Centrifuge Duration | 5 spin (35.40min) at maximum capacity (1st and 2nd spin) , 12, 000 rpm (3rd, 4th and 5th spin) | 1st-20 mins X 3,000 rpm<br>2nd- 30mins X 10,000 rpm | 1st- 10 mins X 8,4000 rpm<br>2nd- 5 mins X 8,4000 rpm | 1st- 8 mins X 13,000 rpm<br>2nd-5mins X 13,000 rpm |
| Drying Duration | 12 hrs | N/A | N/A | 1-2 hrs |
| Grinding Method | Mortar and pestle with 670µL of extraction buffer | Mortar and pestle under liquid nitrogen to fine powder | Mortar and pestle with 600µL of extraction buffer | Mortar and pestle without exact amount of extraction buffer |
| TE Buffer Re-suspension | 30µL | 200-250µL | 50µL | 200µL |

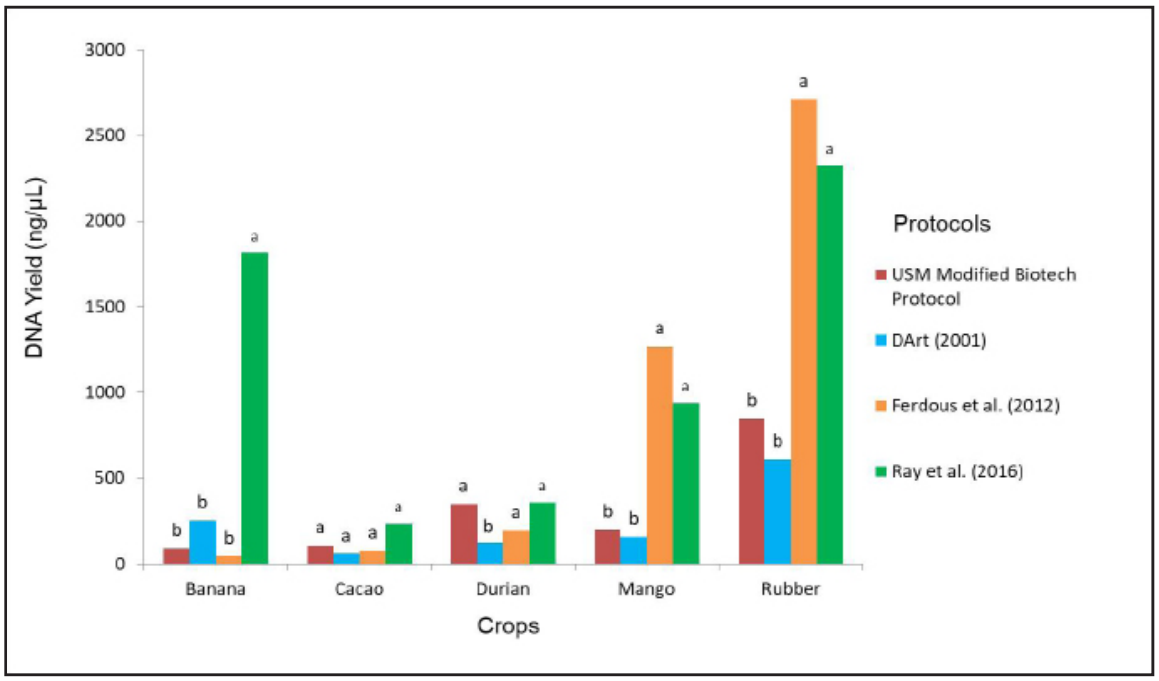


Figure 1. Comparison of the DNA yield generated from the five crops tested using the four protocols. Within a crop, means with common letter are not significantly different from each other (Tukey's HSD test).

For mango and rubber, the protocol developed by Ferdous et al. (2012) generated the highest genomic DNA yield with an average of 1,267.09 ng/µL, while the protocol developed by Ray et al. (2016) produced 2,709.53 ng/µL. Comparatively, the results were not significantly different from each other. However, results obtained from the DArT and USM Biotech modified protocol showed significant difference from that of the protocol developed by Ferdous et al. (2012) and Ray et al. (2016). This therefore implies that if given the choice, the protocol developed by Ferdous et al. (2012) and Ray et al. (2016) can be recommended as DNA extraction protocols for mango and rubber.

Overall results showed that the protocols developed by Ray et al. (2016) and Ferdous et al. (2012) were more efficient in generating genomic DNA across all crops tested than that of the protocol developed by DArT (2001) and USM Biotech modified protocol. This might be due to the use of higher concentration of sodium chloride for the said protocols. It should be noted that the protocols developed by Ray et al. (2016) and Ferdous et al. (2012) required higher concentration of NaCl than that of the protocols developed by DArT (2001) and USM Biotech modified protocol. For Ray et al. (2016) 5M NaCl (2012) was used, while for Ferdous et al. (2012) 3.5M NaCl was added to the buffer. On the other hand, for the protocol developed by DArT (2001) and the USM Biotech modified protocol, a lower concentration of NaCl (2M) was required.

This result supported the findings of Paterson et al. (1993) where they reported that the addition of more than 0.5M NaCl with CTAB could easily remove high polysaccharides. Furthermore, higher genomic DNA from the two protocols could also be attributed to the use of higher concentration of Tris HCL and EDTA. A 1M concentration of Tris HCl was used in the protocols developed by Ferdous et al. (2012) and Ray et al. (2016), while 0.2M Tris HCl was used in both DArT (2001) and USM Biotech modified protocols. Another reason might be due to the addition of 10X CTAB (Ray et al., 2016) and 2X CTAB (Ferdous et al., 2012) in the whole extraction process could have improved the efficiency of the two protocols. This result appeared to be congruent with the findings of several authors (Murray and Thompson 1980; Paterson et al., 1993; Suman et al., 1999), where they observed that higher concentration of CTAB could increase genomic DNA yield. This could be further attributed to the successful removal of most polysaccharides.

Another unique feature of the two protocols is the use of sodium dodecyl sulfate (SDS) solution. Phenol:chloroform:isoamyl was also

used in the protocol developed by Ferdous et al. (2012), which supported the findings of Dipankar et al. (2006) in chickpea (*Cicer arietinum* L.). They observed that the use of phenol removed CTAB polysaccharides complex formed earlier in the reaction.

With the foregoing results, it was further observed that the efficiency of the protocols was dependent on the plant species used. This result was congruent with the findings of Weishing et al. (1995) which revealed that the DNA quantity and quality often varied among representatives of different genera, and sometimes even among different species of a genus or among different plant tissues that were closely related plants might require different DNA isolation protocols.

Although there were differences among the generated genomic DNA of the four (4) protocols used, it could be pointed that any of the four (4) protocols can be alternately used in any genotyping studies since genomic DNA yields of the four (4) protocols were all higher than 50ng/µL, the minimum amount required in performing complex genotyping studies (http://genepoolbio.ed.ac.uk/illumina/samples.html). Moreover, previous studies obtained good amplification product below 50ng/µL (Dacumos et al., 2011; Sales et al., 2011 & Sales et al., 2017). Hence, all of these protocols tested can be used and could give an acceptable DNA yield for banana, cacao, durian, mango, and rubber.

## Comparative evaluation of the DNA Purity of Five (5) Crops from the Four (4) Protocols Evaluated

Figure 2 presents the comparative evaluation of the purity index of genomic DNA generated from the five (5) crops using the four (4) protocols tested. In terms of the purity of DNA obtained from banana using the different protocols, data showed that the protocol developed by Ray et al. (2016) gave a purer DNA with a purity index of 2.01 than the protocol developed by DArT (2001), showing a purity index of 1.94. Although the purity index of banana using DArT (2001) protocol was numerically lower than that of the protocol developed by Ray et al. (2016), statistical analysis showed no significant difference between the two. Moreover, the protocol developed by Ferdous et al. (2012) generated the lowest purity index of 1.78. However, this was not significantly different from that of the modified protocol developed by USM Biotech. This finding further showed that the protocol developed by Ray et al. (2016) and DArT (2001) could produce purer DNA than that of the protocol developed by Ferdous et al. (2012) and USM Biotech modified protocol.

For cacao, the protocol developed by DArT (2001) generated the highest purity index of

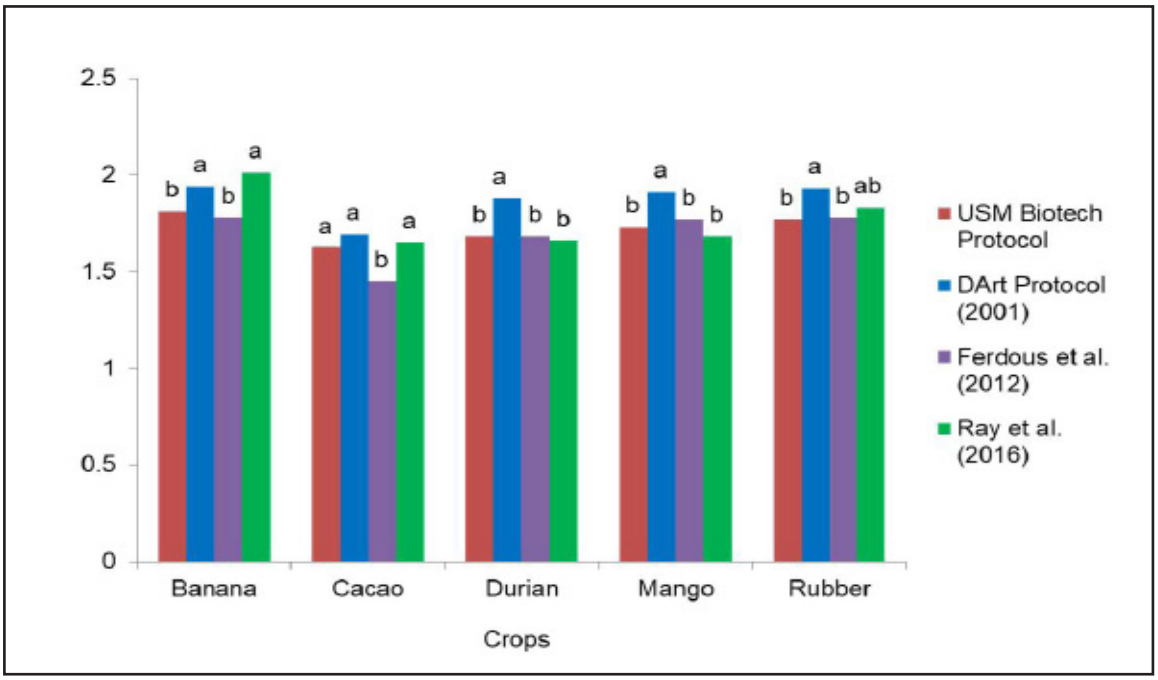


Figure 2. Comparison of the DNA yield generated from the five crops tested using the four protocols. Within a crop, means having a common letter are not significantly different from each other (Tukey's HSD test).

1.69, followed by the protocol of Ray et al. 2016 (1.65) and USM Biotech modified protocol (1.63). Notably, however statistical analysis showed no significant differences among the three. On the other hand, these three protocols were significantly different from that of the protocol developed by Ferdous et al. (2012), which produced DNA with the lowest purity index of 1.45. This indicated that the protocols developed by DArT (2001), Ray et al. 2016 and USM Biotech could produce purer DNA of cacao than the protocol developed by Ferdous et al. (2012).

On the other hand, for the DNA obtained from durian and mango, data revealed that the protocol developed by DArT (2001) generated DNA with higher purity index than the other three protocols. Statistical analysis showed significant difference between the protocol developed by DArT (2001) and the three protocols used. However, among the three protocols used, data showed no significant difference. This suggests that for durian and mango, the protocol developed by DArT (2001) could generate purer or cleaner DNA than that of the protocols developed by Ferdous et al. (2012), Ray et al. (2016) and USM Biotech.

For rubber, DArT protocol yielded DNA with the highest purity index of 1.93. Data indicated a significant difference between DArT (2001) protocol and the other three protocols. Data further showed that there was no significant difference among the protocols developed by Ferdous et al. (2012), Ray et al. (2016) and the USM Biotech modified protocol. This suggested that the protocol developed by DArT (2001) could produce cleaner DNA than that of the protocols developed by Ferdous et al. (2012), Ray et al. (2016) and the USM Biotech for rubber as indicated by the purity index obtained.

Moreover, it was observed that among the crop species used DNA extracted from cacao were always less pure than that of the other four crop species used in whatever extraction protocol applied. This might be attributed to the higher phenolic compounds present in cacao. Dai & Mumper (2010) reported that phenolics are present in plant foods (chocolate) and beverages (tea and coffee). Furthermore, Briz (2015) reported that phenols, such as flavonoids, epicatechin quercetin and catechin are present in cacao. These compounds might have hindered the production of good quality DNA from cacao. It should be noted that the DNA extraction in cacao was more difficult because pellets produced from cacao were highly viscous, sticky and difficult to handle. Aside from this, browning of the pellets from cacao was also observed. Loodhi et al. (1994) reported that browning of the pellets indicated the presence of secondary metabolites and polysaccharides.

## Evaluation of Cost and Time Efficiency of the Four (4) DNA Extraction Protocols Evaluated

To further evaluate the efficiency and utility of the four protocols, time and cost spent were taken into consideration (Table 3). The time and cost associated with DNA extraction and purification methods are factors that can greatly influence molecular diversity analysis, fingerprinting and genome mapping (Weishing et al.,1995).

Furthermore, yield and purity of genomic DNA play a vital role in the analysis of molecular diversity and optimization of different parameters for PCR. Data in Table 3 show the cost incurred

Table 3. Comparative data showing the cost and time required among the four (4) different DNA extraction methods with genomic DNA yield and purity of their product.

| Protocols | Yield (ng/µL) | Purity (A260/A280) | Cost (Philippines Peso) | Cost US Dollar* | Time (hrs) |
|---|---|---|---|---|---|
| USM Biotech Modified Protocol | 318.92 | 1.72 | 351.8 | 6.76 | 8.16 |
| DArT Protocol (2001) | 240.97 | 1.87 | 1541.25 | 29.6 | 7.15 |
| Ferdous et al. (2012) | 858.14 | 1.69 | 174 | 3.34 | 6.10 |
| Ray et al. (2016) | 1134.80 | 1.77 | 174.54 | 3.34 | 19.11 |

and time dedicated to every extraction protocol in the terms of genomic DNA yield and purity. The results obtained show that USM Biotech modified protocol produced 318.92 ng/µL DNA yield with a purity index of 1.72 at a cost of Php 351.80 (US$6.77) and 8.16 hours time spent. The DArT protocol (2001) produced lesser DNA yield 240.97 ng/µL compared to USM Biotech modified method but has higher purity index (1.87) with 7.15 required hours and incurred the highest cost of Php 1,541.25 (US$29.64). Ferdous et al. (2012) produced higher DNA yield (858.14 ng/µL) with 1.69 purity index with a lowest cost of Php 174.00 (US$3.36) in 6.10 hours. Ray et al. (2016) protocol has the highest DNA yield (1,134.80 ng/µL) with 1.77 purity at a cost of Php174.54 (US$3.36) in 19.11 hours.

Comparing the four (4) protocols evaluated, the protocol developed by Ray et al. (2016) was more labor intensive as it requires 19.11 hours to complete the extraction process. The protocol developed by Ferdous et al. (2012) although considered the "fastest" but it is the least efficient in cleaning the DNA among the three (3) protocols. It requires the least time generated but have the lowest purity index. The protocol of Ferdous et al. (2012) shows to be the most cost effective among the four protocols. In contrast, the protocol developed by DArT (2001) was the most expensive among the four protocols, which could be probably because, it requires more expensive chemicals and reagents, like, liquid nitrogen. On the other hand, the modified protocol developed by USM Biotech could be used across all crops although it ranked third in terms of cost, purity, yield and time efficiency.

## Conclusion

The protocols developed by Ray et al. (2016) and Ferdous et al. (2012) could produce higher genomic DNA yield than the DArT (2001) and USM Biotech modified protocol. Although the protocols developed by DArT (2001) and USM Biotech produced lower DNA yield compared to the two protocols, their DNA yields, however, were still sufficient to carry out further genomic studies, since genomic DNA obtained was more than the minimum DNA required in performing complex genotyping studies. This implies that the four protocols are all efficient in generating sufficient genomic DNA yield to carry out further analysis. It showed that all protocols applied produced good DNA quality. More so, among the four (4) protocols Ferdous et al. (2012) showed to be the most cost effective. In addition, it was the fastest method for extracting DNA with high genomic DNA yield. USM Biotech modified protocol, on the other hand, could be recommended as an alternate method as it ranked third in terms of cost and yield.Based on the results and findings of the study, a follow up study on the efficiency of each protocol in the genetic analysis using some molecular markers be carried out to confirm their efficiency in generating excellent molecular data.

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