Biological Forum – An International Journal **13(2): 622-628(2021)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Standardization of DNA Extraction protocols in underexploited Cherry Tomato (*Solanum lycopersicum* L. var*. cerasiforme*) Genotypes of North East India

Naorem Bidyaleima Chanu[1], P. Sarma[1], Chandra Deo[1], B.N. Hazarika[2*], Amit Kumar Singh[3] and Md Ramjan[1]
[1]Department of Vegetable Science, College of Horticulture and Forestry,
Central Agricultural University, Pasighat-791102, (Arunachal Pradesh), India.
[2]Department of Fruit Science, College of Horticulture and Forestry,
Central Agricultural University, Pasighat-791102, (Arunachal Pradesh), India.
[3]Department of Basic Sciences and Humanities, College of Horticulture and Forestry,
Central Agricultural University, Pasighat-791102, (Arunachal Pradesh), India.

(Corresponding author: B.N. Hazarika)*
(Received 08 May 2021, Accepted 06 July, 2021)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Extraction of good quality genomic DNA is mandatory for carrying out crop improvement programme using molecular approaches. Isolation of good quality and quantity of plant genomic DNA is the key challenge due to often contamination with large amount of polysaccharides, polyphenols and secondary metabolites. In our present study, we performed DNA isolation from five native cherry tomato genotype inclusive and exclusive of liquid nitrogen using modified Doyle & Doyle method and CTAB method. Quality of the isolated DNA was determined by horizontal agarose gel electrophoresis using 1% (CTAB) and 0.8% (Doyle & Doyle) agarose in 1X TE buffer at constant voltage of 80V. Purity of extracted DNA was determined by A260/A280 ratio and observed that CTAB with liquid nitrogen (1.62: 1.7 and 472-955 ng/µl) and Doyle & Doyle method without liquid nitrogen (1.8: 1.95 and 632-1200 ng/µl) yielded PCR amenable DNA. However, Doyle & Doyle method without liquid nitrogen yield the best quality and quantity of DNA. Modified Doyle and Doyle method of plant DNA isolation gives satisfactory results in lack of liquid nitrogen whereas modified CTAB method yields good amount of DNA with provision of liquid nitrogen. Therefore, for laboratories with no facility of liquid nitrogen supply modified Doyle and Doyle method proves to be an efficient method of plant genomic DNA isolation.

Keywords: cherry tomato, CTAB, Doyle & Doyle method, DNA isolation, liquid nitrogen, standardization.

INTRODUCTION

Cherry tomato is botanically known as *Solanum lycopersicum* L. var. *cerasiforme*, a variety of the cultivated tomato (Anon, 2014). Cherry tomato is believed to be the progenitor of modern cultivated tomatoes (Kenneth, 2000). They are small round tomato and the size ranges as an intermediary between wild currant tomatoes and commercially cultivated tomatoes (Nesbitt and Tanksley, 2002). Cherry tomatoes range in size from a thumb tip up to that of like a golf ball and may vary from globular to slightly oblong shape. Although usually red, other varieties such as yellow, green, and black also exist (Anon, 2014). Oblong shape tomatoes are usually known as 'plum tomato' or 'grape tomato'. Cherry tomatoes are consumed raw, stewed, fried etc. Tomatoes also contain a wide array of beneficial nutrients and antioxidants; cherry tomato is believed to be more nutritious than regular tomato. It is known to excel in antioxidant properties that fights major human ailments such as cancer, high blood pressure and heart problems. Lycopene is the red pigment responsible for the red colour of tomatoes while β-carotene is responsible for yellow or orange colour of cherry tomatoes.

The extraction of uncontaminated, intact, and superior quality DNA is very crucial for any molecular studies (Tan and Yiap, 2009). Plant genomic DNA extraction with promising quality for molecular studies is the primary need of any plant breeder for crop improvement programme. Gene mapping, DNA fingerprinting and MAS (Marker Assisted Selection) demands pure DNA isolation for advanced studies (Abdel-Latif and Osman, 2017). The DNA isolation methods need to be standardized for every crop species and for every crop tissue as the presence of metabolites differs from crop to crop and tissue to tissue unlike in animals or microbes (Sangwan *et al*., 1998). Many factors can cause shearing of DNA during extraction. DNA deterioration by endonucleases activity causes a serious hurdle in isolating and purifying higher molecular weight genomic DNA from plant, which directly or indirectly interfere with the enzymatic reactions (Weishing *et al*., 1995). Polysaccharides may be particularly problematic when present in DNA samples, as their presence may also inhibit enzymatic activity. Inhibition of Taq polymerase activity has been shown by the presence of polysaccharides (Fang *et al*., 1992). It also inhibits restriction enzyme activity (Pandey *et al*., 1996). Formation of a highly viscous solution depicts the presence of polysaccharides in the

DNA sample (Adams and DO, 1991). The oxidized form of polyphenols covalently binds to DNA giving a brown colour and reduces maintenance time, making it useless for molecular studies (Katterman and Shattuck, 1983). While homogenizing the plant tissue polyphenols in the plant samples gets oxidized giving out brown discolouration it is also often due to protein denaturation and carbohydrates separation from nucleic acids. Such contamination can be washed out effectively by CTAB buffer. It is cationic detergent that holds the property of destroying separated proteins and carbohydrates leaving no traces of contaminants in the sample (Cheng *et al*., 2003).

North-eastern part of India harbours a good bio-diversity for cherry tomato yet due to lack of proper survey and studies mere importance is made. These genotypes are less prone to crop maladies and are often believed to carry useful resistant gene sources. Hence to exploit these valuable genotypes in modern breeding programmes the foremost and most crucial step in molecular breeding rely significantly on the isolation of good quality and quantity of plant genomic DNA. Genomic DNA isolation in plants are mostly carried out from young leaves as suggested by many researchers. There are limitations associated with the use of fresh leaves samples as it needs a careful production in protected structure throughout the study period. Additionally, sample collection, storage and preparation steps involves the requirements of liquid nitrogen in huge quantity (Ferdous *et al*., 2012). There are numerous techniques of isolating DNA in plants, like the use of CTAB, phenol and SDS in DNA extraction steps (Ángel *et al*., 2014). Doyle and Doyle method is more repeatedly used in plant DNA extraction. This method also uses CTAB and past researchers reported several advantages in plant samples (Saghai-Maroof and Soliman, 1984).

Standardizing of basic DNA isolation protocols can easily remove contaminants and obtain high purity grade plant DNA (Tiwari *et al*., 2017; Ali *et al*., 2017; Tan *et al*., 2009). Standardization can be achieved by altering basic steps established earlier. CTAB and Doyle and Doyle method being the important and simple DNA isolation method for a number of plants, it is employed for the current study by following slight modifications in the protocol established. The DNA isolation methods of each plant species needs to be studied and standardized as each plant comprises of varied chemical components unlike microbes and animals (Sangwan *et al.*, 1998). There are many methods exploited for genomic DNA extraction in plants one among which is largely used in plants is modified Doyle and Doyle method. This method is more frequently used to extract plant DNA (Varma *et al.*, 2007; Ishfaq and Qadir, 2020). Apart from conventional DNA extraction approaches, numerous commercial kits are also reported for successful extraction of sufficient quantity and quality of genomic DNA from plants (Xin and Chen 2006).

However, these rapid methods are cost incurring and less sustainable to run experiments with limited financial resources. Presently there are many established DNA isolation protocols suitable for PCR applications, yet frail in proposing simple, reliant and cheaper method that have wider applicability in laboratories worldwide. The main objective of this study was to identify simple, efficient and reliable method of DNA isolation in poorly equipped laboratories for small or large sample size on the way to provide smooth functioning of any molecular studies necessitate for crop improvement works.

MATERIALS AND METHOD

In this study, standardization of DNA isolation was executed following four different methods. The methods were focussed on isolating high quality genomic DNA amenable for PCR and enzymatic studies. Modified CTAB method (Murray and Thompson, 1980) and Doyle and Doyle (1987) method inclusive and exclusive of liquid nitrogen represented the four method of DNA isolation in the present investigation.

Plant materials: Fresh young leaves of native cherry tomatoes (Fig. 1) were collected from four weeks old plants. Seeds of these genotypes were collected from different areas of north-east India. Leaves were initially cleaned with 70 percent ethanol and allowed for surface drying (Fig. 2). Leaves were crushed using mortar and pestle to a fine powder after adding liquid nitrogen. Liquid nitrogen absorbs the excess moisture making the leaf tissues brittle and facilitates easy grinding. Further the powdered leaves are crushed with respective CTAB and Doyle and Doyle reagents used in DNA extraction. The details of the chemicals and their functions used in both the methods are presented in Table 1.

Fig. 1. Fruits of underutilized cherry tomatoes of NE India.

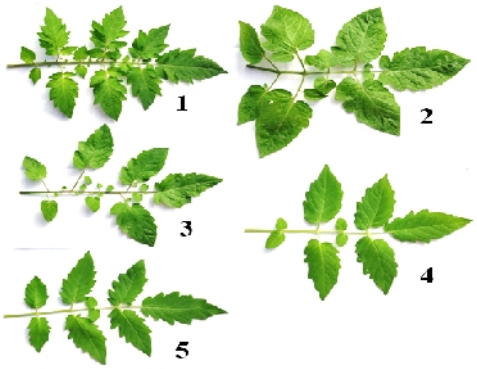

Fig. 2. Leaf samples used for DNA isolation.

Table 1: Reagents use in DNA extraction and their functions.

Reagents	Functions
CTAB (Cetyl Trimethyl Ammonium Bromide)	It acts as a strong detergent and solubilizes the plant cell wall allowing the access to inner DNA contents.
Tris HCl	It helps in maintaining pH of buffer at 8.0
NaCl (Sodium Chloride)	Maintains the ionic balance and helps in separation of different dissolved organic debris. It also facilitates the DNA precipitation process.
PVP (Polyvinylpyrrolidone)	It helps in removal of phenolic compounds by forming hydrogen bonds with them.
B-mercaptoethanol	It is a strong reducing agent that helps in removal of protein and polyphenols.
EDTA (Ethylenediaminetetraacetic acid)	Strong chelating agents which deplete Mg ions in solution, inhibit nuclease activity by that we can get nucleic acid
Chloroform	Removed proteins and help in separation of different particles in liquid and remove carbohydrates
Isoamyl alcohol	Removed proteins and avoids foaming. Also prevents phosgene from reaction to chloroform.
Iso-propanol	It prevents DNA to get dissolve in water by binding with available water molecules and thus precipitating the DNA.
Rnase	This enzyme breaks down the RNA molecule and makes the isolated DNA free of RNA contamination.
Phenol	To help in removal of histone protein and lipids from the solution.
Chloroform	It prevents the cutting of DNA during isolation and also solubilizes the lipids and proteins from the solution.
Phenol : Chloroform	To achieve removal of phenol traces.
Ammonium acetate	It helps in removal of carbohydrates and proteins and also gives stabilization to DNA
Ethanol	It dehydrates the available moisture and helps in DNA precipitation process.
TE buffer	To dissolved the DNA pellet and maintain pH 8.0
Liquid nitrogen (-196°C)	To help in removal the moisture from the leaf to facilitate easy crushing.

(a) Isolation of DNA by CTAB method

Reagents for CTAB method:

(i)CTAB buffer (200ml): 4.0g CTAB (Cetyl Trimethyl Ammonium Bromide), 20ml 1M Tris (pH 8.0), 8.0ml 0.5M EDTA (pH 8.0), 56.0ml 5M NaCl., 80.0 ml sterile distilled water and 2g PVP-40. Mix all the reagents and adjust the pH to 5.0 with HCL and make up to 200ml with distilled water.

Protocol:

(i) DNA Isolation

1. Grind 200mg of fresh plant tissue to a fine paste in approximately 500µl of CTAB buffer (W and W/ liquid nitrogen). Transfer this CTAB/plant extract mixture to a micro-centrifuge tube. Pre- chilled leaf samples along with mortar and pestle were employed for CTAB W/ method.
2. Incubate the CTAB/plant extract mixture for 15 minutes at 55°C in a circulating water bath.
3. After incubation, spin the CTAB/plant extract mixture at 12000 rpm for 5 minutes to precipitate the cell debris. Transfer the supernatant to a clean micro-centrifuge tube.
4. To each tube add 250µl of Chloroform: Isoamyl Alcohol (24:1) and mix the solution by gentle inversion. After mixing, spin the tubes at 13000 rpm for 1min.
5. Transfer the upper aqueous phase (containing DNA) only to a clean micro-centrifuge tube.
6. To each tube, add 50µl of 7.5M ammonium acetate followed by 500µl of ice-cold is opropanol. Invert the tubes gently several times to precipitate the DNA.
7. Following precipitation, precipitate can be isolated by spinning the tube at 10000rpm for 10 minutes to form a pellet. Discard the aliquot.

(ii) DNA Purification

1. Add 10µl of Rnase A (10µg/ml) and incubate at 37°C for 30 minutes in circulating water bath to remove RNA contaminants.
2. Discard the aliquot and add 500µl of 70% ice cold ethanol followed by tapping.
3. Spin the contents at 13000rpm for 1 minute and discard the supernatant.
4. Air dry the DNA pellets. The end is marked by the absence of ethanol odor from the vials.
5. Re-suspend the pellets in 200µl of sterile Dnase free water.
6. After resuspension, incubate the DNA at 65°C for 20 min to destroy any Dnases that may be present and store at -20°C.
7. Finally, the quality of DNA was confirmed by using 1 percent agarose gel electrophoresis.

(b) Isolation of DNA by Doyle and Doyle method

Reagents:

1. Extraction buffer (4x): For 1000 ml: 250 g Sorbitol, 48 g Tris (0.4 M), 7.4 g EDTA-sodium salt (20mM) and 80 ml distil water. Before use convert 4X into X by dissolving 25ml extraction buffer into 75 ml distil water.
2. Lysis buffer: For 1000 ml: 200 ml of 1M Tris pH 8.0 (maintain pH), 200ml of 250 mM EDTA (1000mM=1M), 200ml distil water, 20g CTAB and 400ml of 5M NaCl
3. T.E buffer: 10mM Tris pH 8.0, 1mM EDTA and dissolve in 100ml of distilled water. For this buffer, dissolve Tris first in 100ml of distilled water and maintain pH to 8.0 and then add EDTA.
4. Sarcosine 5%
5. Isopropanol

6. Chloroform:Isomyl alcohol mixture (24:1)
7. Ethanol 70% and 100%

Protocol:

(i) DNA Isolation

1. Grind 0.5g fresh leaf material using mortar and pestle (W and W/ liquid nitrogen) in presence of 25µl β-mercaptoethanol and a pinch of potassium metabisulphite. Add 5ml of pre-warmed (at 65ºC) 1X extraction buffer and grind well. Pre- chilled leaf samples along with mortar and pestle were employed for Doyle and Doyle W/ method.
2. Transfer the homogenate into a 50ml polypropylene centrifuge tube containing 4ml lysis buffer. Mix by inversions.
3. Add 1ml of 5% Sarcosine and mix well by inversion.
4. Incubate samples at 65°C for 8min with occasional mixing by inversion.
5. Add equal amount of Chloroform: Isoamyl alcohol and mix by inversion to emulsify. Spin at 10,000 rpm for 20 min at 4°C.
6. Take the supernatant in a clean tube and 0.6 volume of chilled isopropanol (measure the volume of supernatant in measuring cylinder and multiply it with 0.6. this multiplied volume of chilled isopropanol is added to tube containing supernatant) and mix well by quick inversion till the DNA precipitate. Keep at -20°C for an hour.
7. Centrifuge at 10,000 rpm for 20 minutes and pour off the supernatant and drain well.
8. Wash the DNA pellet in 70% ethanol followed by 100% ethanol.
9. Spin for 5 minutes at 10,000 rpm and decant ethanol.
10. Air dry the pellet, dissolve in 50µl of T.E buffer plus 500µl of molecular grade water and store at -20°C.

(ii) DNA Purification

The isolated DNA contains contaminants such as RNA and protein which can be removed by following steps:

1. About 3µl of Rnase and proteinase were added to the DNA samples and incubated at 37°C for 1 hour.
2. 400 µl of phenol was added, mixed well and centrifuged at 7500 rpm for 10 to 15 minutes and the supernatant was transferred into fresh tubes.
3. To the supernatant 400 µl of phenol: chloroform (v/v) at 1:1 volume was added to Eppendorf tube, mixed well and centrifuged at 7500 rpm for 10 minutes at 4°C and transferred the supernatant into another tube.
4. 400 µl of cold chloroform was added to the supernatant, mixed well and centrifuged at 7500 rpm for 10 minutes at 4°C.
5. Equal volume of ice-cold isopropanol was added to the supernatant and incubated overnight at 4°C.
6. The tubes were centrifuged at 8000 rpm for 10 minutes at 4°C and the supernatant was discarded.
7. The pellet was washed with 200 µl of 70 percent ethanol and centrifuged at 9000 rpm for 10 minutes at 4°C.
8. Pellet was dissolved in 100 µl to T. E buffer.
9. Finally, the quality of DNA was confirmed by using 0.8 percent agarose gel electrophoresis.

Quantification of DNA: The ratio of absorbance at 260 and 280 ŋm of spectrophotometer provides the purity assessment and DNA concentrations of the genomic DNA extracted.

RESULTS AND DISCUSSION

Following the current standardization methods, DNA was extracted from young fresh leaves of native cherry tomato. Varied concentrations were obtained from all the methods applied and are presented in Table 2 and 3. Quality of the entire genomic DNA extracted from different genotypes of cherry tomato was confirmed by performing 1% and 0.8% agarose gel electrophoresis for CTAB and Doyle and Doyle method respectively. Further, quantification of the genomic DNA was conducted spectrophotometrically by reading absorbance at 260 and 280 nm. The CTAB(W) method produced DNA purity ratio (A260/280) ranges of 1.62-1.70 while in CTAB(W/) method produces ratio ranges from 1.39- 1.56. The DNA purity ratio in in Doyle and Doyle (W and W/) methods ranges from 1.46- 1.70 and 1.80-1.95. A260/280 ratio of 1.8 depicted high purity of the extracted DNA with absence of proteins and phenols (Abdel-Latif and Osman, 2017), while ratio values more than 2.0 indicate the presence of alcohol or acetone in the DNA preparation (Kapilan, 2015, Webb and Knapp, 1990). DNA concentration of the samples following CTAB (W & W/) methods ranges from 445 – 955ng/2 µland 195 to 372.5ng/µl respectively (Table 2). While the concentration of the DNA yielded from Doyle & Doyle (W & W/) methods varies from 160-272ng/µl and 632-1200ng/µl respectively (Table 3).

Table 2: Concentration of DNA by CTAB method.

Concentration of DNA by CTAB method in presence of liquid N_2				
Sample	**A260**	**A280**	**A260/A280**	**Concentration (ng/µl)**
1	0.382	0.228	1.67	955
2	0.178	0.109	1.63	445
3	0.230	0.140	1.64	575
4	0.249	0.146	1.70	622
5	0.189	0.116	1.62	472
Concentration of DNA by CTAB method in absence of liquid N_2				
Sample	A260	A280	**A260/A280**	**Concentration (ng/µl)**
1	0.103	0.071	1.45	257.5
2	0.078	0.054	1.44	195
3	0.081	0.053	1.52	202.5
4	0.149	0.107	1.39	372.5
5	0.133	0.085	1.56	332.5

Table 3: Concentration of DNA by Doyle & Doyle method.

Concentration of DNA by Doyle & Doyle method in presence of liquid N_2				
Sample	**A260**	**A280**	**A260/A280**	**Concentration (ng/µl)**
1	0.040	0.026	1.53	160
2	0.060	0.041	1.46	240
3	0.046	0.028	1.64	184
4	0.055	0.037	1.48	220
5	0.068	0.040	1.7	272
Concentration of DNA by Doyle & Doyle method in absence of liquid N_2				
Sample	**A260**	**A280**	**A260/A280**	**Concentration (ng/µl)**
1	0.180	0.092	1.95	720
2	0.158	0.083	1.90	632
3	0.252	0.140	1.80	1008
4	0.300	0.158	1.89	1200
5	0.286	0.150	1.90	1144

Variation among extraction methods may be due to different composition of extraction buffers, different components for precipitation and purification of DNA and the time duration to complete the procedure (Weising *et al.,* 1995). Besides, variation in the quality of DNA can be due to the genetical, structural and biochemical variation among leaf samples, variation in composition of the buffers used for extraction and the differences in the chemicals, their exposure time to plant tissue and concentration of chemicals (Arumuganathan and Earle, 1991). Standardization of DNA isolation protocols needs to be formulated for each plant group since secondary metabolites existence varied in all the plant species. DNA degradation is the major limitation encountered in DNA isolation due to endonucleases activity, polyphenols and polysaccharides that reduces the purity and yield of isolated DNA (Khanuja *et al.,* 1999). Choice of leaves for isolation process should be very sceptical, recently unfurled fully green young leaves yields good quality and amount of DNA. Over matured or under matured leaves must not be considered in order to avoid contamination (Porebski, *et al.,* 1997). Moreover, in methods exclusive of liquid N_2 prechilling of mortar and pestle as well as leaf sample at −40°C/−80°C substitutes the role of liquid nitrogen effectively (Sahu *et al.,* 2012). Thorough mixing of sample and chloroform: isoamyl alcohol (24:1) must be ensured to separate the aqueous layer which otherwise will contaminate the sample with viscous contaminants in the later phase of extraction rendering high DNA contamination and degradation. It was observed that use of chilled ispopropanol instead of 70% ethanol precipitates DNA more rapidly and eliminates the additional time required in prolong precipitation at -20°C. Lesser the contamination easier is the resuspension of extracted DNA pellet in DNase free water (Abhijit and Manjushree, 2010).

Doyle & Doyle W/yields considerable amount of genomic DNA with no contamination of polyphenols, polysaccharides and other contaminats as evident by the clear bands (Fig. 3) with an A260/280 ratio ranges from 1.80-1.95 which is in accordance with the findings of Kasem *et al.,* 2008. High levels of β-mercaptoethanol use has been recommended successfully for removal of polyphenols (Suman *et al.,* 1999).

Hence, high concentration of β-mercapto ethanol was employed in the Doyle & Doyle W/method which yields white pellets without any visible discoloration that proved to be extraction of high-quality DNA. The successful cell wall disruption for easy access to cell contents is provided by the use of liquid nitrogen. Moreover, plant genomic DNA isolation procedures without the use of liquid nitrogen does not significantly affect the yield and quality DNA as evident from the purity ratios given in Table 3. Therefore, the use of liquid nitrogen for DNA isolation is a mere necessity in Doyle and Doyle method. This method provides good quality and quantity of DNA amenable for numerous PCR and enzymatic reactions with lesser cost involvement compared to the other three methods used. However, this method is slightly time consuming then CTAB method but considering the yield, quality and cost effectiveness it substantiates to be the best method among all the four standardization methods undertaken.

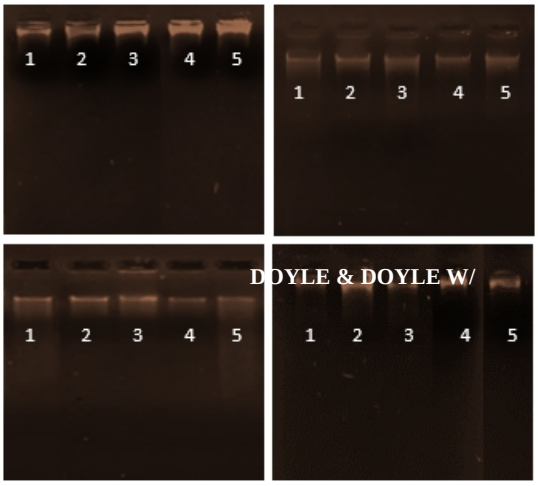

Fig. 3. Gel image of four DNA isolation methods employed.

CONCLUSION

From the present study, standardization of DNA isolation with high quality genomic DNA amenable for PCR and enzymatic studies was executed from the four different methods. Among the methods used Doyle and Doyle W/liquid nitrogen prove to be inexpensive, simple in operation, produces good amount of high

quality DNA for molecular studies. However, CTAB method supplied with liquid nitrogen produces comparable yield and quality of DNA to that of Doyle and Doyle W/liquid nitrogen method. Under critical observation of concentration and contamination by proteins or polyphenols in the isolated DNA, Doyle and Doyle W/ liquid nitrogen produces high purity genomic DNA with good yield amenable for several molecular PCR studies. Therefore, this method meets the objective of the study hence, it can be employed for isolating DNA in ill-equipped laboratories remotely present in corners of the country.

RESEARCH GAPS

High quality DNA is the prerequisite for molecular studies on MAS (Marker Assisted Selection), MABC (Marker Assisted Back Crossing), QTL (Quantitative Trait Loci) Mapping, linkage mapping and genome sequencing. Modern science offers various kinds of kits for rapid DNA isolation. They are highly efficient and reliable but often comes with a great cost. Exploitation of such kits in remote laboratories is meagre. Other conventional methods employing continuous supply of liquid nitrogen for sample preparation needs a huge investment of capital, such facilities are seldom provided in distantly located working laboratories. Moreover, simple, cost-effective yet reliable method for isolating DNA free from any possible contaminants under any working environment needs to meticulously identified. There are numerous methodologies developed in the past decades yet there is still a room for developing protocols with high reproducibility in the present day for maximizing the outputs of molecular studies.

FUTURE SCOPE

Development of simple, reliable and cost effective protocols of DNA extraction will provide some tremendous possibilities in molecular breeding of crops. Genome sequencing and identification of economic traits responsive to quantitative and qualitative traits can be easily identified from local genotypes. There may be possibilities of discovering crucial genes responsible for disease, pest, salinity, drought, alkalinity, flood and heat tolerance which can be exploited as genetic resources in breeding of crops. These genes can be used in MABC for rapid release of improved crop varieties. Such molecular crop breeding is far more advantageous than conventional breeding. Molecular breeding is playing one of the most important role in combating global hunger and nutritional security. In short molecular science is the backbone of future breeding programme.

Acknowledgements. The authors are very thankful to the Dean College of Horticulture and Forestry (CAU), Pasighat Arunachal Pradesh for encouraging and providing necessary aids.

Funding Agency: This study was supported by department of vegetable science, College of Horticulture and Forestry, CAU, Pasighat Arunachal Pradesh, India

Ethical Issues: This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of Interest. All the authors of this manuscript declared that they have no conflict of interest in publication of this research paper.

REFERENCES

Abdel-Latif, A., & Osman, G. (2017). Comparison of three genomic DNA extraction methods to obtain high DNA quality from maize. *Plant Methods*, *13*(1), 1-9.

Abhijit, S., & Manjushree, D. (2010). Standardization of DNA extraction and optimization of RAPD-PCR conditions in *Garcinia indica*. *International Journal of Botany*, *6*(3), 293-298.

Ali, N., Rampazzo, R. D. C. P., Costa, A. D. T., & Krieger, M. A. (2017). Current nucleic acid extraction methods and their *implications to point-of-care diagnostics*. *Bio. Med. Research International*, 2017.

Ángel, J. E., Hernández, E. G., Herrera, N. A., Gómez, L. Y., Castro, Á. P., Adriana Milena, S., & Ebratt, E. E. (2014). Comparison of DNA extraction methods for detection of citrus huanglongbing in Colombia. *Agronomía Colombiana*, *32*(1): 7-13.

Anonymus (2014). "BBC - Food - Cherry tomatoes recipes". Retrieved 24 October.

Anonymus (2014). *Lycopersicon esculentum* var. *cerasiforme*, nomen.at. Nomen. at. Retrieved 24 October.

Arumuganathan, K., & Earle, E. D. (1991). Nuclear DNA content of some important plant species. *Plant molecular biology reporter*, *9*(3): 208-218.

Cheng, Y. J., Guo, W. W., Yi, H. L., Pang, X. M., & Deng, X. (2003). An efficient protocol for genomic DNA extraction from Citrus species. *Plant Molecular Biology Reporter*, *21*(2): 177-178.

Adams, R. P., & Do, N. (1991). A simple technique for removing plant polysaccharides contaminants from DNA. *BioTechniques*, *10*(2), 162-164.

Doyle, J. J., & Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue (No. RESEARCH).

Fang, G., Hammar, S., & Grumet, R. (1992). A quick and inexpensive method for removing polysaccharides from plant genomic DNA. *Biotechniques*, *13*(1): 52-4.

Ferdous, J., Hanafi, M. M., Rafii, M. Y., & Muhammad, K. (2012). A quick DNA extraction protocol: Without liquid nitrogen in ambient temperature. *African Journal of Biotechnology*, *11*(27): 6956-6964.

Ishfaq, M. and Qadir M. I. (2020). *J. Biotech. Res. Biochem*. 3 1-4

Kasem, S., Rice, N., & Henry, R. J. (2008). 14 DNA Extraction from Plant Tissue. Plant genotyping II: SNP technology, 219.

Katterman, F. R. H., & Shattuck, V. I. (1983). An effective method of DNA isolation from the mature leaves of *Gossypium* species that contain large amounts of phenolic terpenoids and tannins. *Preparative biochemistry*, *13*(4) : 347-359.

Khanuja, S. P., Shasany, A. K., Darokar, M. P., & Kumar, S. (1999). Rapid isolation of DNA from dry and fresh samples of plants producing large amounts of secondary metabolites and essential oils. *Plant molecular biology Reporter*, *17*(1): 74-74.

Kiple Kenneth F (ed. 2000). The Cambridge World History of Food. *1*. 352.

Murray, M. G., & Thompson, W. F. (1980). Rapid isolation of high molecular weight plant DNA. *Nucleic acids research*, *8*(19): 4321-4326.

Nesbitt, T. C., & Tanksley, S. D. (2002). Comparative sequencing in the genus Lycopersicon: implications for the evolution of fruit size in the domestication of cultivated tomatoes. *Genetics*, *162*(1): 365-379.

Pandey, R. N., Adams, R. P., & Flournoy, L. E. (1996). Inhibition of random amplified polymorphic DNAs (RAPDs) by plant polysaccharides. *Plant Molecular Biology Reporter*, *14*(1): 17-22.

Porebski, S., Bailey, L. G., & Baum, B. R. (1997). Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant molecular biology reporter*, *15*(1): 8-15.

Kapilan, R. (2015). Comparison of the efficiency of the DNA extraction methods of some selected plant species, Sch. *Acad. J. Biosci.*, *3*(7): 611-615.

Saghai-Maroof, M. A., & Soliman, K. M., Jorgensen, R. A., Allard, R. W. (1984). Ribosomal DNA spacer-length in barley—mendelian inheritance, chromosomal location, and population-dynamics. *Proc Natl. Acad. Sci.* US A, *81*: 8014-8018.

Sahu, S. K., Thangaraj, M., & Kathiresan, K. (2012). DNA extraction protocol for plants with high levels of secondary metabolites and polysaccharides without using liquid nitrogen and phenol. *International Scholarly Research Notices*.

Sangwan, N. S., Sangwan, R. S., & Kumar, S. (1998). Isolation of genomic DNA from the antimalarial plant *Artemisia annua*. *Plant Molecular Biology Reporter*, *16*(4): 365-365.

Tan, S. C. and Yiap B. C. (2009). DNA, RNA, and protein extraction: the past and the present. *J. Biomed. Biotechnol.* Article ID 574398.

Tiwari, S., Tomar, R. S., Tripathi, M. K., & Ahuja, A. (2017). Modified protocol for plant genomic DNA isolation. *Indian Res. J. Genet. & Biotech.*, *9*(4): 478-485.

Varma, A., Padh, H., & Shrivastava, N. (2007) *Biotechnol. J. 2*: 386-392.

Webb, D. M., & Knapp, S. J. (1990). DNA extraction from a previously recalcitrant plant genus. *Plant Molecular Biology Reporter*, *8*(3): 180-185.

Weising, K., Nybom, H., Pfenninger, M., Wolff, K., & Meyer, W. (1994). DNA fingerprinting in plants and fungi. CRC press.

Xin, Z., & Chen, J. (2006). DNA sequencing II: optimizing preparation and cleanup. Extraction of Genomic DNA from Plant Tissue, *47*: 59.

How to cite this article: Chanu, N.B., Sarma, P., Deo, C., Hazarika, B.N., Singh, A.K. and Md Ramjan (2021). Standardization of DNA Extraction protocols in underexploited Cherry Tomato (*Solanum lycopersicum* L. var. *cerasiforme*) Genotypes of North East India. *Biological Forum – An International Journal*, *13*(2): 622-628.