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Genetic diversity and comparative study of genomic DNA extraction protocols in *Tamarix* L. species

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Abstract. The genus *Tamarix* consists of about 54 species that mainly grow in saline areas of deserts and semi-deserts. This genus is chemically characterized by the presence of tannins, flavonoids, anthocyanins and essential oils which interfere with the extraction of pure genomic DNA. Thus it is necessary to optimize extraction protocols to minimize the influence of these compounds to the lowest level. The present study compares the efficiency of five different approaches to extract total genomic DNA in *Tamarix* species, showing significant differences in the extracted DNA contents and quality,by using Kit (DNP ™ Kit), CTAB DNA extraction method by Murray and Thompson, Sahu et al., Nalini et al. and Bi et al., for the extraction of DNA from *Tamarix* species. Our results showed significant differences in DNA contents between these five methods. The quantity and quality of extracted genomic DNA were checked by the spectrophotometer, Nano-Drop and and agarose gel electrophoresis analysis. Finally, a PCR-based method was also applied to verify the amplification efficiency for two molecular markers (ITS and ISSR).. In the present study, the genetic diversity of 96 *Tamarix* individuals species and 8 populations were studied using 10 ISSR markerswhile for nrDNA ITS 8 species samples were used. The method of Nalini et al., provided best results (207 ng/μL) in terms of quantity and quality ofDNA. Our results proposed that this method could be effective for plants with the same polysaccharides, proteins and polyphenols components. The advantage of this method is simple and fast as it does not involve time consuming steps such as incubation at higher temperatures, and also do not requires expensive chemicals such as proteinase K, liquid nitrogen. ,. The success of this method in obtaining high-quality genomic DNA has been demonstrated in the *Tamarix* species group and the reliability of this method has been discussed.

Keywords: DNA yield, extraction protocols, *Tamarix,* ISSR, secondary metabolites.

INTRODUCTION

Tamaricaceae is relatively a small family of 4 genera and 120 species (Trease and Evans, 2002). The genus *Tamarix* L. (tamarisk, salt cedar) contain about 54 species that mainly distributed in saline areas of deserts and semi-deserts in Europe, concentrated mainly in the Mediterranean region and Eastern Europe (Gaskin, 2003). They are typically adapted to arid climate with an efficient and deep root system (Baum, 1978).

Thirty-five species of *Tamarix* occur in Iran reported by Schiman-Czeika (1964). These species have been used in plantation to prevent deforestation in Iran. The species of *Tamarix* are distributed in 21 provinces of Iran.

Some species of the genus *Tamarix* are used as ornamental plants (Baum 1967; Gaskin and Schaal, 2002). *Tamarix* species are frequently planted as windbreaks or grown for the stabilization and afforestation of sand dunes (Gaskin and Schaal 2003, Gaskin and Kazmer 2019, Mayonde *et al.,* 2019). Tamarix are also famous for medicinal purposes such as the galls and bark are used as astringent. Some species of the genus *Tamarix* are utilized, as tonic, diuretic, stimulant, and stomachic action. They are also used as diaphoretic, diuretic,hepatotonic and to treat liver disorders, relieve headache, ease prolonged or difficulty during labor. Some *Tamarix* species are melliferous and are used as a sugar substitute (Sharma and Parmar 1998; Abouzid *et al.* 2008; Orfali *et al* 2009; Bakr *et al* 2013; Orabi *et al.,* 2016). Plastid DNA (cpDNA) and Nuclear DNA (nDNA), can together be used to discourse different ecological queries. Whereas the nuclear DNA covers both unique single copy and repetitive regions (multiple copies), the chloroplast genome contains of coding segments such as ribosomal noncoding tandemly repeated units or RNA genes (Le Roux and Wieczorek, 2008). The ITS regions between the nuclear ribosomal DNA (rDNA) genes are commonly used for detecting changeability among species (Sun et al., 1994). Additionally, it is also a widely used molecular marker for rebuilding angiosperm phylogenies at different taxonomic levels as they always provide the correct level of difference at species level for well-resolved phylogenetic reconstruction (Baldwin et al., 1995). The trnS–trnG primers are used to infer phylogenetic comparisons. Moreover, chloroplast introns and intergenic spacer regions show the highest levels of intraspecific polymorphism since they are a lesser amount of inhibited through selection to preserve gene function (Hamilton, 1999).

The extraction and purification of high-quality DNA is a critical step for genomic analysis especially from the plant materials with high accumulation of interfering substances including polysaccharides, proteins, and DNA polymerase inhibitors such as tannins, alkaloids, and polyphenols. The presence of these compounds affects the quality and quantity of isolated DNA, and therefore, renders the sample non-amplifiable (Zamboni et al. 2008). Pure and rapid DNA extraction is a prerequisite for most advanced techniques such as genetic mapping, fingerprinting, marker-assisted selection, and for evaluating authenticity of exported cereal varieties.

General problems in the isolation and purification of high molecular weight DNA from medicinal and aromatic plant species include: (1) degradation of DNA due to endonucleases, consolation of highly viscous polysaccharides, and (2) inhibitor compounds like polyphenols and other secondary metabolites which directly or indirectly interfere with the enzymatic reactions (Weising et al. 1995; Jenderek et al., 1997; Zamboni et al. 2008; Sahu et al. 2012). The presence of polyphenols, as oxidizing agents present in many plant species, can reduce the production of the purified extracted DNA (Loomis 1974; Porebski et al., 1997).

Several methods to isolate DNA from plant tissues are available; however, these methods produce either small amounts or DNA of inconsistent quality. Some of the DNA extraction methods are modified versions of cetyltrimethyl ammonium bromide (CTAB) extraction and differ in time and cost (Doyle and Doyle 1990; Reichandt and Rogers, 1994). Doyle and Doyle method (1990) are applied to extract DNA in fruit trees (Jenderek *et al.,* 1997). The extraction technique of Lodhi et al. (1994) has been utilized for the grape, apple, apricot, peach, cherry and snapdragon. Sarkhosh et al. (2006) used the Bi et al. (1996) method for some Iranian pomegranate (*Punica granatum* L.) genotypes. Murray and Thompson (1980) method were used for DNA extraction in cabbage, olive, rose (Csaikl *et al.,* 1998) and sweet cherry (Khadivi-Khub et al., 2008).

Saghai-Maroof et al. (1984) method was used for DNA extraction in Mangroves and salt marsh species (Sahu *et al.* 2012). Talebi Baddaf *et al.* (2003) introduced Murray and Thompson (1980) method as the most appropriate method to achieve high-quality DNA extraction from pomegranate leaves. Because plants contain high amounts of many different substances, it is unlikely that just one nucleic acid isolation method suitable for all plants can ever exist (Loomis, 1974).

A perfect method is the one that is fast, simple, and reliable DNA extraction method, which does not require long incubations, multiple DNA precipitations, or commercial reagents, and could meet the PCR, sequencing, and next-generation library preparation requirements. Therefore, the aim of this study was to compare quality

and quantity of five different DNA extraction methods to isolate high-quality DNA from leaf tissues of different *Tamarix* species. In this study, we showed the results of tests from several DNA extraction protocols that were made to overcome the problems that mainly arise from polysaccharide contamination.

ISSR and ITS amplification was also performed to evaluate the suitability of the DNA extraction methods for PCR-based techniques. As far as, we know, this's the first report on DNA extraction from *Tamarix* leave at species level from Iran, and we expect that the suggested protocol can be an incentive to perform further studies in order to investigate the genetic diversity among the plants with same chemical components as *Tamarix* species.

MATERIALS AND METHOD

Plant samples for DNA isolation

In this study leaves of 8 *Tamarix* species were collected from different habitats in Iran (Table 1). One gram of young and mature leaf was collected and then frozen in liquid nitrogen and stored at -70 °C until extraction. For molecular studies, we used different number of plant individuals, as they were required. For example, in ISSR analysis, we used 96 individual samples of 8 species, while for nrDNA ITS 8 individual of 8 species were used for the extraction of DNA.

DNA extraction methods

One gram of the frozen leaf samples of *Tamarix* were grind into fine powder using pre-cooled mortar and pestle, and then homogenized with five different DNA extraction methods based on randomized complete block design (RCBD) with five replicates. The five extraction methods were 1) Murry and Thompson (1980); 2) Kit (DNP ™ Kit) 3) Sahu et al. (2012),4) Bi et al. (1996) 5) Nalini et al. (2003) methods. After DNA extraction and sedimentation, resulted pellet was rinsed with ethanol 75% and dissolved in 200 µL double distilled sterile water at 4 °C overnight and stored at -70 °C until next treatments.

The chemicals used for the isolation of DNA viz. Tris, EDTA were obtained from Sigma and Sodium chloride, urea, SDS, Isopropanol, sodium acetate, chloroform, Isoamlyalcohol, phenol, dNTPs, Enzyme Taq DNA Polymerase, 10X-assay buffer for Taq DNA Polymerase, Magnesium chloride and agarose.

Concentration, purity and quality of extracted DNA

The quantity (concentration and extraction efficiency) and quality (purity and intactness) of the DNA obtained at the ratio of 1:49 (20 µL of DNA stock solution + 980 µL of double distilled sterile water) were assessed using spectrophotometer at 260 and 280 nm, and the A260/A280 ratio was used to assess contamination with proteins through employing the spectrophotometry (Hitachi U-2001 UV/VIS), Nano-DropTM (Thermo Scientific) described by Brodmann (2008) and Wilmington (2008), agarose gel electrophoresis, PCR methods and molecular markers (ITS and ISSR). This spectrophotometric analysis was performed in triplicate on the samples of extracted DNA using spectrophotometer. To verify DNA integrity, 5 µL DNA from 7 sample were subjected to gel electrophoresis at 0.8% (w/v) agarose gel, stained with ethidium bromide, and a constant voltage of 120 V for 90 min. The DNA bands were visualized, and the images were acquired using Gel Doc XR+ Imaging system (Bio-Rad Laboratories Inc., Germany).

ISSR amplifications

The quality of extracted DNA was examined at 0.8% agarose gel. In total, 10 ISSR primers; $(AGC)_5GT$, (CA)

Table 1. *Tamarix* species and populations, their localities and voucher numbers.

R	Taxa	Locality	Alt (m)	Latitude	Longitude	Voucher No
1	*Tamarix arceuthoides* Bge.	Ardabil, Khalkhal-Asalem Road	1500	37°57'36"	48°61'03"	IAUH1011
2	*T. ramosissima* Ledeb	Gilan, Damash	1700	36°75'54"	49°81'07"	IAUH1012
3	*T. chinensis* Lour.	Fars, Shahr miyan	2700	30°84'40"	52°06'76"	IAUH1013
4	*T. szowitsiana* Bge.	Mazandaran,Chalus, Visar	1400	36°65'011"	51°31'051"	IAUH1014
5	*T. meyeri* Boiss.	Gilan, Damash	1700	36°75'54"	49°81'07"	IAUH1015
6	*T. androssowii* Litw.	Golestan Forest	700	37°47'50"	47°23'36.2"	IAUH1016
7	*T. mascatensis* Bge.	Mazandaran, Noshahr, Kheyrud kenar Forest	400	36°38'05"	51°29'05"	IAUH1017
8	*T. aucheriana* (Decne. ex Walp.) B.R. Baum.	Ardabil,Meshkin shahr, hatam Forest	2700	38°18'77.1"	56°41'60"	IAUH1018

Table 2. Primer sequences used in this study.

Region	Primer Sequences (5'-3')	Tm	Ref.
TAB_C	CGAAATCGGTAGACGCTACG	56	Taberlet *et al.* (1991).
TAB_F	ATTTGAACTGGTGACACGAG	56	Taberlet *et al.* (1991).
ITS4	TCCTCCGCTTATTGATATGC	57	White *et al.* (1990).
ITS5	GGA AGT AAA AGTCGT AAC AAG G	57	White *et al.* (1990).
UBS807	AGAGAGAGAGAGAGAGT	54	UBS set no. 9
UBS810	GAGAGAGAGAGAGAGAT	54	UBS set no. 9
UBC 823	TCTCTCTCTCTCTCTCC	56	UBS set no. 9
$(AGC)_5GT$	AGC AGC AGC AGC AGC GT	56	UBS set no. 9
$(CA)_7GT$	CACACACACACACAGT	56	UBS set no. 9
$(AGC)_5GG$	AGC AGC AGC AGC AGC GG	56	UBS set no. 9
$(CA)_7AT$	CACACACACACACAAT	56	UBS set no. 9
$(GA)_9C$	GAGAGAGAGAGAGAGAGAC	56	UBS set no. 9
$(GA)_9T$	GAGAGAGAGAGAGAGAGAT	55	UBS set no. 9
$(GT)_7CA$	GTGTGTGTGTGTGTCA	55	UBS set no. 9

$_7$GT, (AGC) $_5$GG, UBC 810, (CA) $_7$AT, (GA) $_9$C, UBC 807, UBC 823, (GA) $_9$T and (GT) $_7$CA commercialized by UBC (the University of British Columbia) were used (see Table 2). The final volume of 12 µL was tested in PCR reaction (2.5 µL PCR reaction buffer 10x, 0.875 µL $MgCl_2$ 50 mM, 0.5 µL dNTPs 10 mM, 1.0 µL primer 10 µM, 0.2 µL Taq DNA polymerase 5 Unit/µL, 2.0 µL template DNA (5 ng/µL). The amplification, reactions were performed in Techne thermocycler (Germany) with the following program: 5min initial denaturation step 94°C, followed by 38 cyclesfor 1 min at 95°C; 1 min at 50-55°C and 1 min at 72°C. The reaction was completed through a final extension step of 5-10 min at 72°C. The amplification products were observed at 1% agarose gel, followed by the ethidium bromide staining. The fragment size was estimated using a 100 bp molecular size ladder (Fermentas, Germany).

ITS- sequences

The ITS region was amplified using PCR with following primer pairs ITS-4 and ITS-5 (White et al. 1990). The final volume of 12 µL was tested in PCR reaction (2.5 µL PCR reaction buffer 10x, 0.875 µL $MgCl_2$ 50 mM, 0.5 µL dNTPs 10 mM, 1.0 µL primer 10 µM, 0.2 µL Taq DNA polymerase 5 Unit/µL, 2.0 µL template DNA (5 ng/µL). The amplification, reactions were performed in Techne thermocycler (Germany) with the following program: 5min initial denaturation step 94°C, followed by 38 cycles of 1 min at 94°C; 40 sec, at 55°C and 1 min at 72°C. The reaction was completed by a final extension step of 5-10 min at 72°C. The amplification products were observed at 1% agarose gel, followed by the ethidium bromide staining. The fragment size was estimated using a 100 bp molecular size ladder (Fermentas, Germany). The ITS regions were amplified using primers reported as universal primers by White et al. (1990) and Taberlet et al. (1991), respectively, for flowering plants (see Table 2).

RESULTS

Comparison of different DNA extraction methods on agarose gel electrophoresis

The quality of 8 extracted DNA sample was verified spectrophotometrically using a NanoDrop instrument and agarose gel electrophoresis. DNA purity and yield were compared between these five extracted DNA methods. Plant genomic DNA extraction of Murry and Thompson (1980); Kit (DNP ^{TM} Kit), Sahu et al. (2012), Bi et al. (1996) (Fig. 1b: 1-4), did not give best results for *Tamarix* species due to the presence of polysaccharides and proteins in the pellet and showed brown or yellow DNA precipitate that presents the gDNA gel image. The presence of phenolic compounds caused a brownish pellet (Fig. 1b).

The results confirmed that extracted DNA by Nalini et al. (2003) method from leaves showed better quality in comparison with the other extraction methods (Fig.1a). Due to the elimination of polysaccharides or protein contaminations DNA has been extracted with high quality. We believe that this method will be efficient for molecular studies of many other aro-

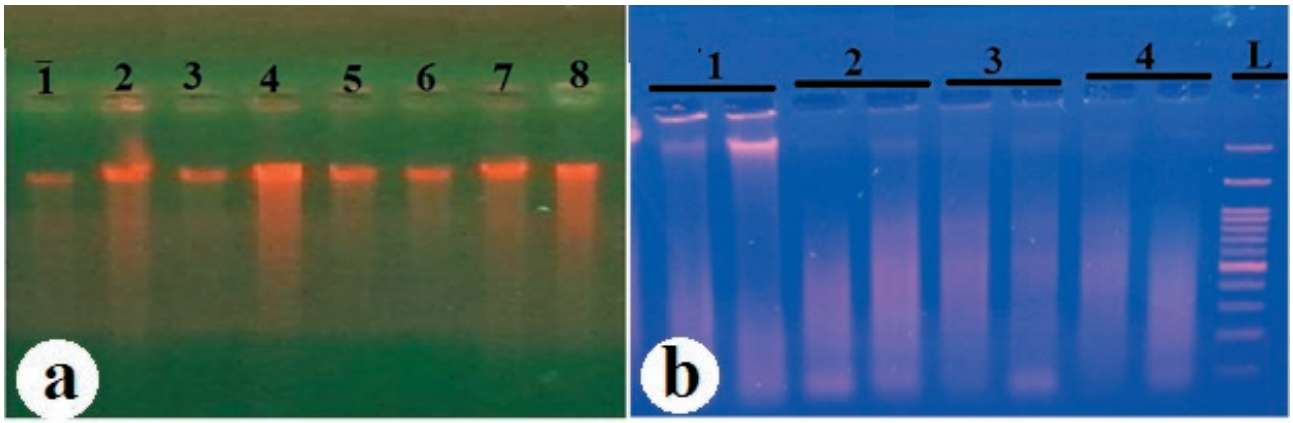

Figure 1. Electrophoretic pattern of DNA extracted by the five different methods from *Tamarix* leaves. *Note*. The electrophoresis was performed in 0.8% (w/v) agarose gel. The extraction methods were: a) Nalini et al. (2003) (1- *Tamarix arceuthoides* 2- *T. ramosissima* ,3- *T. chinensis*, 4- *T. szowitsiana*, 5- *T. meyeri*, 6- *T. androssowii*, 7- *T. mascatensis* and 8- *T. aucheriana*); b) 1- Murry and Thompson (1980); 2- Kit (DNP ™ Kit), 3- Sahu et al. (2012), 4- Bi et al. (1996); L) 100 bp DNA ladder.

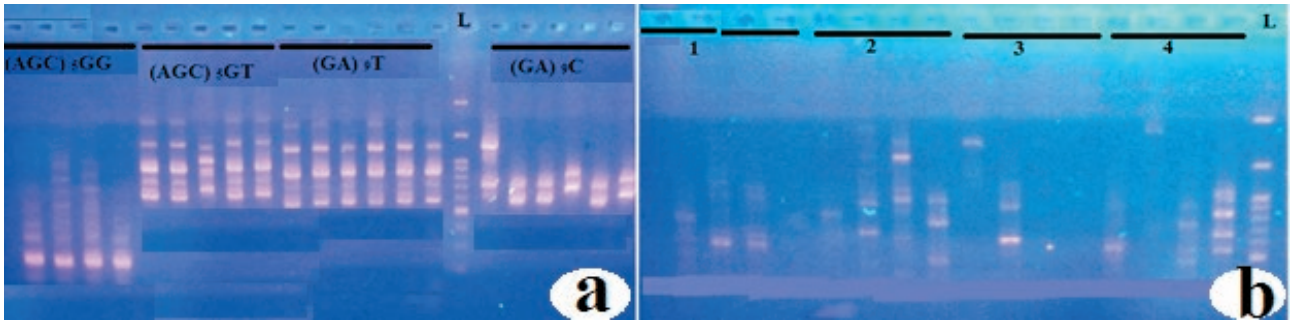

Figure 2. Amplification of DNA from *Tamarix* leaf using five different extraction methods by ISSR amplification. *Note*. Fig. 2. a) Nalini et al. (2003); Fig. 2. b) 1- Murry and Thompson (1980); 2- Kit (DNP ™ Kit), 3- Sahu et al. (2012), 4- Bi et al. (1996); L) 100 bp DNA ladder.

matic and herbal plants. In this method high level of β-mercaptoethanol successfully removed the polyphenols of the leaf tissue which may be responsible forinhibition of the DNA amplification during PCR reactions (Suman et al. 1999). It was evident that high concentration of β-mercaptoethanol resulted in the high-quality of DNA. Using of NaCl concentrations higher than 0.5 M, along with CTAB, was previously recorded to be efficient in removing polysaccharides during DNA extraction (Moreira and Oliveira 2011, Paterson et al. 1993). It was also efficient in the present study with 0.5M of NaCl concentration. Polysaccharides and secondary metabolites of *Tamarix* species were bounded by PVP and it is in concordance with previous studies (Couch and Fritz 1990, Chaudhry et al. 1999, Zhang and Stewart 2000). More replications for using chloroform: isoamyl alcohol resulted in better removing of proteins in *Tamarix* species. Sahu et al. (2012) used of sodium acetate and isopropanol only in step (xv), but we used one more time of this material in order to have the better precipitation of DNA and removing most of the secondary metabolites and polysaccharides from the DNA. The presence of higher quantities of polyphenols and polysaccharides in mature leaves are proved by Porebski et al. (1997), which makes it very difficult to isolate DNA of good quality. So, we used fresh and young leaves to overcome this problem.

Clear banding patterns were observed in the ISSR study by Nalini et al. (2003) method (Fig. 2a). It possess better quality in comparison with the other extraction methods as well as Murry and Thompson (1980); Kit (DNP ™ Kit), Sahu et al. (2012), Bi et al. (1996) (Fig.2 b, 1-4).

PCR tests findings of ITS are given in (Figs. 3. a, b) which showed that extracted DNA by the method of Nalini et al. (2003) method (Figs. 3a) from leaf samples brings an acceptable quality for PCR, and as the most appropriate method in aspect of quality of DNA extract-

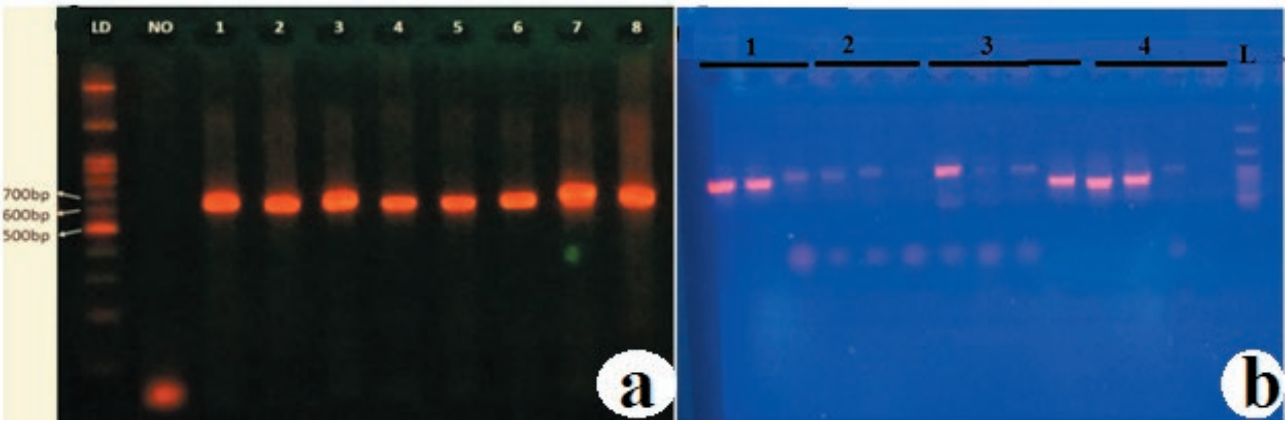

Figure 3. Agarose gel (1.5%) showing the PCR amplified ITS of the plant materials used in the present study. *Note*. Fig. a) Nalini et al. (2003) (1- *Tamarix arceuthoides*, 2- *T. ramosissima*, 3- *T. chinensis*, 4- *T. szowitsiana*, 5- *T. meyeri*, 6- *T. androssowii*, 7- *T. mascatensis* and 8- *T. aucheriana*); Fig. b) 1- Murry and Thompson (1980); 2- Kit (DNP ™ Kit), 3- Sahu et al. (2012), 4- Bi et al. (1996); L) 100 bp DNA ladder.

ed from young leaves of *Tamarix*. The PCR-amplified DNA fragments of ITS for 8 samples showed a clean single band product, when examined on an agarose gel (Fig. 3a). The PCR products were of about 600 bp.

UV spectrophotometer and NanoDrop™ 1000 spectrophotometer analysis

In spectrophotometer procedure, absorption of double-stranded DNA in wavelength of 260 nm was 50 µg/µL. In fact, the ratio of absorption amount resulted in 260 nm to 280 nm was ranged from 1.7 to 2.12. It shows the most absorption was done by nucleic acids and therefore extracted DNA was well-qualified and its purity was acceptable. If the ratio is appreciably lower in either case, it may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm. The results showed that the DNA yield and DNA purity obtained from one gram of the fresh leaf tissue in different methods using UV spectrophotometer was statistically significant ($P \leq 0.01$). A higher DNA yield was obtained with the method of Nalini et al. (2003) (333±58.1 ng/µL fresh weight), while the lowest was obtained with method of Sahu et al. (2012) (120±64.4 ng/µL fresh weight) (Table 3). Therefore, the results confirmed that extracted DNA by Nalini et al. (2003) method from leaves of *Tamarix* possess better qualitative and quantitative results as compared to other methods. DNA sample was measured with a UV spectrophotometer for the ratio of OD260/OD280 using TE buffer. The ratio of OD260/OD280 was determined to assess the purity and concentration of DNA sample. DNA concentration was calculated according to the equation of Wilmington et al. (2008). DNA concentration (ng/µL) = OD260 × a (dilution factor) × 50

Absorbance measurements made on a spectrophotometer, including any Thermo Scientific NanoDrop Spectrophotometer, will include the absorbance of all molecules in the sample that absorb at the wavelength of interest.

The ratio of absorbance at 260 nm and 280 nm was used to assess the purity of DNA and RNA. A ratio of ~1.8 was generally accepted as "pure" for DNA; a ratio of ~2.0 was generally accepted as "pure" for RNA. If the ratio appreciably lower in either case, it may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm.

Some researchers encounter a consistent 260/280 ratio change, when switching from a standard cuvette spectrophotometer to a NanoDrop Spectrophotometer. The three main explanations for this observation were listed below: Small changes in the pH of the solution will cause the 260/280 to vary*. Acidic solutions will under-represent the 260/280 ratio by 0.2-0.3, while a basic solution will over-represent the ratio through 0.2-0.3. If comparing results obtained using a NanoDrop Spectrophotometer to results obtained using other spectrophotometers, it is important to ensure that the pH of an undiluted sample measured on our instruments was at the same pH and ionic strength as the diluted sample measured on the conventional spectrophotometer.

The NanoDrop absorbance was useful for detection of contaminants such as protein, salts, and polysaccharides, which can inhibit and interfere in DNA sequencing. The NanoDrop 1000 sspectrophotometer has the capability to measure highly concentrated samples without dilution. The ratio of 260 and 280 nm absorbance

Table 3. Comparison of means for efficiency of three different DNA extraction methods in leaf samples of leaves *Tamarix* using Duncan's multiple range test ($P \leq 0.01$).

Methods	Spectrophotometer		Nano-Drop	
	DNA yield (ng/µL)	DNA purity (ng/µL)	DNA yield (ng/µL)	DNA purity (ng/µL)
Nalini et al. (2003)	333±58.1	2.12±0.15	590.4±86.5	1.94±0.15
Kit (DNP ^{TM} Kit)	178±33.8	1.8±0.18	767.5±11.8	1.80±0.09
Murray and Thompson (1980)	292±34.4	1.7±0.19	534±76.4	1.78±0.07
Sahu et al. (2012)	120±64.4	2.01±0.18	575±55.2	1.82±0.09
Bi et al. (1996)	185±44.4	2.04±0.19	655±86.4	1.74±0.09

was used to assess the purity of DNA and RNA. This ratio was between 1.7 and 1.9, and this range was generally accepted as "pure" for DNA (Table 3).

DISCUSSION

The quality and quantity of DNA required depends on the extraction method and plant group. DNA isolated from plants often contains certain compounds that inhibit PCR amplification reactions (Reichandt and Rogers, 1994). In this method Sodium chloride and β-mercaptoethanol were added in the extraction buffer to take care of the polysaccharides and the polyphenols in the leaf tissue which were the compounds that contribute to the inhibition of the DNA amplification during PCR reactions. Hence there were no additional steps needed for the removal of these compounds (Khadivi-Khub *et al.,* 2008]. The presence of the enzyme RNAse A in the DNA solution does not hamper the amplification. Hence repurification of the DNA is not needed (Csaikl *et al.,* 1998). Our results showed that the DNA isolation protocol could be successfully applied to a broad range of plant species.

Sarkhosh et al. (2006) in a study on genetic diversity of pomegranate cultivars of Iran, using Random Amplified Polymorphic DNA (RAPD) using four different genomic DNA extraction procedures; Murray and Thompson (1980), J. J. Doyle and J. L. Doyle (1990), Ziegenhagen et al. (1993) and Jenderek *et al.,* (1997) introduced Murray and Thompson's method as the most appropriate and successful method in terms of quality of DNA extraction from young leaves of pomegranate. Jenderek et al. (1997) have found the method of J. J. Doyle and J. L. Doyle as the best quality resulting method for DNA extraction form marshmallow, but its quantity was too low. Saha et al. (2016) in a study on genetic stability of *Morus alba* L. variety and Nadha et al. (2011) on genetic diversity of *Guadua angustifolia* Kunth, using RAPD and ISSR marker introduced Murray and Thompson (1980), and J. J. Doyle and J. L. Doyle (1990) methods as appropriate DNA extraction procedures, respectively. Bhatia et al. (2011) in a study on the genetic fidelity of *Gerbera jamesonii* Bolus using DNA-based markers were used Murry and Thompson (1980). PCR tests finding showed that the extracted DNA by Bi et al. (1996) method from leaf samples brings an acceptable quality forth for PCR, and the candescence of amplified DNA bands,

In this study, five DNA extraction methods were compared to isolate high-quality DNA that can be efficiently amplified using PCR techniques. Murry and Thompson (1980); Kit (DNP ^{TM} Kit), Sahu et al. (2012), Bi et al. (1996) resulted in brown or yellow DNA precipitate that could not be reliably amplified through PCR. Therefore, we used the method of Nalini et al. (2003) that produced good quality DNA., The DNA extracted by this method is successful in many land plants including; mangroves and salt marsh plants containing elevated concentrations of polysaccharide and polyphenolic compounds (Nalini et al. 2003).

Nalini et al. (2003) method are helpful to provide a pure DNA with high efficiency in *Tamarix* species. Advantages of the present method for studying medicinal plants with secondary metabolites are as follows: 1) omission of liquid nitrogen, 2) decrease of toxic effects, hazardous, expensive of some component as phenol in other methods, 3) lower amount of dried or fresh plant material, without any conservation specific condition. Although this method has many advantages but its time-consuming. The DNA extracted using this protocol can be used for whole-genome sequencing, advanced sequencing technologies, and bioinformatics tools.

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