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The yield and purity of DNA extracts from seeds of eight six accessions of *Treculia* species using Zymo Research mini-prep DNA extraction kit

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Abstract

There is a need for the use of efficient DNA extraction methods that will yield good quality and quantity of DNA for molecular studies. Several commercial kits are available that are used in place of conventional extraction methods due to cost, speed, and safety. Therefore, the Zymo Research mini plant/seed DNA extraction kit was used to determine the yield and purity of DNA extracts of 86 accessions of *Treculia africana* varieties. Nanodrop Thermo Scientific™ NanoDrop™ One Microvolume UV-Vis Spectrophotometer was used to determine the quality and quantity of the extracted DNA. The extracted DNA was amplified by a polymerase chain reaction using Internal Transcribed Spacer 1 and 2 (ITS 1 and ITS 2). The amplicon was run on 1% agarose gel electrophoresis. The concentration (ng/µl) at the A260/280 and A260/230 ratios of the samples were recorded. The concentration and purity values varied among the accessions. The purity at A260/280 ratio ranged from 1.5 to 2.18, obtained from accessions B22 and B43 respectively while A260/230 ratio ranged from 0.93 to 39.56, obtained from accessions C46 – Ab6 respectively. The values derived at the A260/280 ratio and A260/230 ratio were majorly within the acceptable range of 1.8 – 2.0 suggesting that the ZR kit could eliminate contaminants. Thus, further downstream applications such as PCR and sequencing could conveniently be carried out. The suitability and efficiency of using Zymo research mini prep as a DNA extraction kit were revealed on the agarose gel images. Zymo Research DNA extraction kit is appropriate for the extraction of DNA from seeds of *Treculia* species.

Keywords: Concentration, Purity, Zymo Research Kit, DNA extraction, *Treculia africana*

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INTRODUCTION

Treculia africana is an underutilized plant commonly known as African breadfruit in English and has some African names like Ukwa (Igbo), Muzinda (Luganda), Mwaya (Swahili), and Brebretim (Wolof) (Okoronkwo *et al*., 2020). It is a nutritious local edible fruit (Runsewe-Abiodun *et al*., 2018) and its seeds, leaves, timber, roots and bark are of importance. It is presently included as a threatened species of Southern Nigeria (Ojimelukwe *et al.,* 2021) and there has been a reduction in the number of trees of African breadfruit which has turned out to be an ecological concern (Nuga and Ofodile, 2010). This may be due to the massive deforestation of the trees in Nigeria as a result of an increase in population, and high demand for agriculture, livestock production and fuelwood (Ekande, 1998), leading to a problem of genetic resource erosion and extinction.

There has been an increased interest in *Treculia* species with several studies on its nutrient composition (Ezennaya and Ezeigwe, 2023), medicinal properties (Ojimelukwe *et al.,* 2021), processing (Ugwu and Iwuchukwu, 2013), physicochemical composition (Oderinde *et al*., 2020) and germination studies (Amujiri *et al*., 2020). The plant is faced with the problem of species improvement. The improvement and cultivation have not been aided by its extended gestation period of ten or more years (Nuga and Ofodile, 2010). Modern and improved techniques which would play major roles in the improvement strategy for an orphan crop (Bhattacharjee, 2009), have not been fully applied to *Treculia species.* There is a lack of molecular data on the plant on the National Center for Biotechnological Information (NCBI) gene database. Deoxyribonucleic acid (DNA) extraction is an essential step in addressing some of the species' challenges and for improvement using molecular tools. DNA isolation and purification are key in molecular techniques applied in plant research for identifying genotypes, economic traits linked to traits of interest and genetic diversity (Aboul-Maaty and Oraby, 2019).

Achieving good-quality DNA with a high yield is a major concern in plants' genetic analysis (Abdel-Latif and Osman, 2017). The appropriate DNA quantity and quality are required for further molecular activities such as PCR, construction of genomic libraries, and gene sequencing (Ibrahim, 2011). Abdullah (2016) opined that for downstream molecular analysis, extracting high-purity DNA is a limitation in genomics for plant matrices. In addition, consistent DNA quality is required for appropriate genetic analysis of several plant individuals. The purity of DNA at an absorbance ratio of 260 nm and 230 nm (A260/A230) is used to determine contaminants such as organic compounds, polysaccharides, and chaotropic salts while the absorbance ratio at 280 nm and 260 nm (A260/A280), is applied for proteins and aromatic amino acids (Japelaghi *et al*., 2011). Values within the range of 1.8 to 2.0 for the absorbance ratio of 260/280 nm are usually considered pure DNA samples (Weising *et al*., 2005; Abdel-Latif and Osman, 2017).

The DNA extraction method used determines the total DNA quality obtained which correlates with the success of the polymerase chain reaction (PCR) to analyze the sequence (Nurhasanah *et al.,* 2019). A single extraction method cannot apply to all species due to the presence of secondary metabolites including the RNA contaminants. DNA isolation and its quality are compromised by chemical defences such as tannins and phenols (Moreira and Olivera, 2011). These components interfere with DNA purification processes by forming complexes and may inhibit the restriction enzymes and Taq polymerases thereby affecting further use in molecular studies (Raimundo *et al*., 2018). Therefore, the differences in the presence and amounts of these secondary metabolites require the determination of the most appropriate method for DNA extraction for individual species (Healey *et al*., 2014). High-quality DNA extraction from plant tissues entails numerous processes however, several commercial kits are available at a high cost with a low yield of DNA (Xin and Chen, 2012). Cetyl trimethylammonium bromide (CTAB) is a widely used conventional method for plant DNA extraction (Schenk *et al*., 2023) which has been modified severally to reduce polyphenols and polysaccharides (Moller *et al*., 2014). However, the limitation lies in its failure to isolate high-quality DNA from plants with high composition of secondary metabolites or high levels of degraded DNA (Chen *et al.,* 2014). In addition, the CTAB protocol is expensive, thorough and time-consuming, does not give rapid results, and requires trained personnel (Wang *et al*., 2021).

The Zymo research mini prep extraction kit is designed to simplify, reduce cost, and reduce DNA loss while yielding high DNA quality and involves similar steps obtainable in conventional methods of DNA extraction such as CTAB (Cetyltrimethylammonium bromide). It also reduces exposure to hazardous substances such as phenol or chloroform (Zymo Research Corporation, 2022). The Zymo Research Plant/Seed Mini Prep DNA Kit isolates rapidly an inhibitor-free, high-yielding PCR-quality DNA simply and easily. The procedure has the advantage of rapid and efficient lysis through bead beating without the use of organic denaturants or proteinases. The ZymoSpin™ technology removes

polysaccharides and polyphenols/tannins from the DNA. The technology works with a Zymo-Spin™ III-HRC filter which has a removal column that removes the PCR inhibitor (Zymo Research Cooperation, Undated). The kit has been validated based on the application of the protocols for isolating DNA from *Theobroma cacao* for improved productivity and resistance to black and frost pod diseases (Navarro *et al.,* 2017) and also, in the domestication of *Theobroma cacao* (Cornejo *et al.,* 2018). Based on studies, the Zymo research protocol has not been used extensively on plants. It has been observed to give values of concentration and purity within the acceptable range in the isolation of DNA from genotypes of *Vigna unguinculata* (Ogunkanmi *et al.,* 2019) and diploid and tetraploid *Colocasia exculenta* cultivars (*Wulandari et al., 2023).* Therefore*,* this study aims to determine the yield and purity of DNA extracts from seeds of 86 accessions of *Treculia africana* using Zymo Research mini-prep plant extraction kit.

MATERIALS AND METHODS

Germplasm collection

Fruit heads of Eighty-six accessions of *Treculia africana var africana* and *Treculia africana var inversa* were collected from Abia (AB), Anambra (B), Ebonyi (C), Ogun (D), Osun (E), Oyo (F), Imo (G), Delta (H), Edo (I) and Enugu (J) States in Nigeria. The plants were identified by a plant taxonomist in the Department of Plant Science and Biotechnology. Pictures of the two varieties are shown in Plates I and II. The fruit heads were allowed to decompose and the seeds were extracted by washing from the pulp and allowed to dry at room temperature.

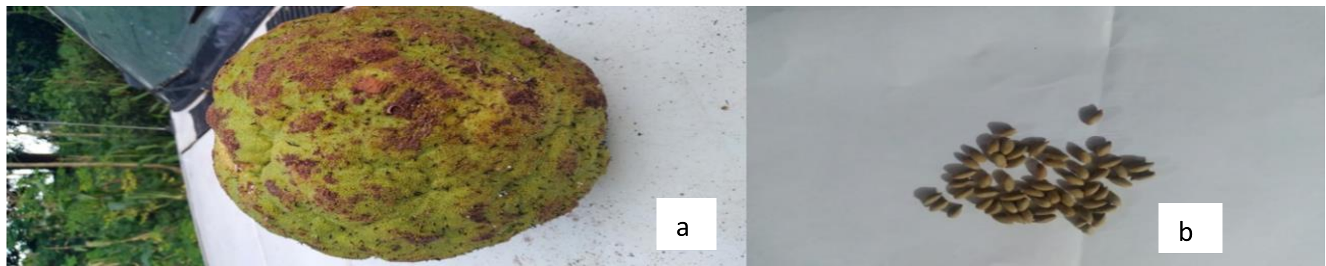

Plate I. (a) Fruit head of *Treculia africana* var *inversa* (b) Seeds of *Treculia africana var inversa*

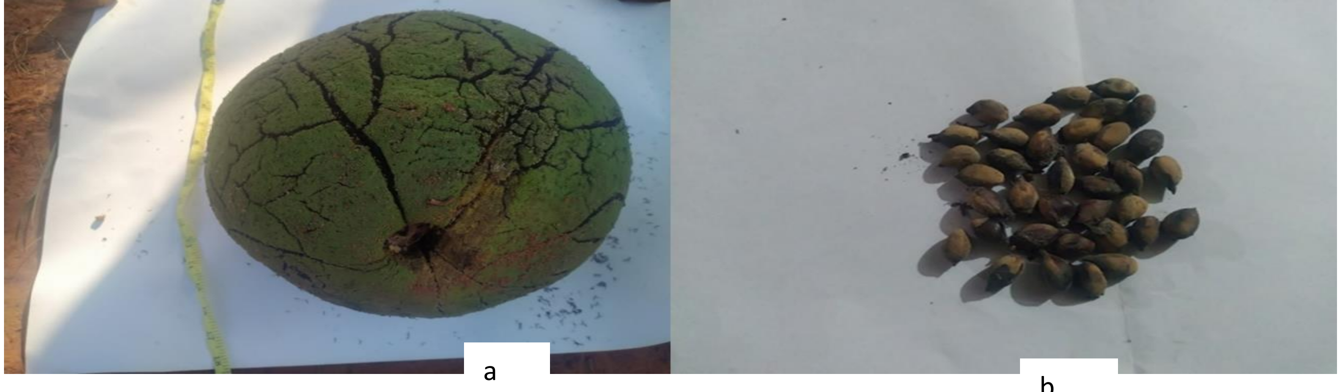

Plate II. (a) Fruit head of *Treculia africana* var *africana* (b) Seeds of *Treculia africana var africana*

DNA extraction

The molecular analysis was done at Inqaba Biotec West Africa Ltd, Ibadan. Following the instruction manual, the Zymo Research (ZR) mini-prep DNA extraction kit protocol (catalogue number D6020) was used for DNA extraction (Zymo Research Corp. Version 2.2.0). Finely ground dried seeds (150g) were used in a ZR BashingBead™ lysis tube (2.0 mm), 750 µl BashingBead™ buffer was used for lysis for 20 minutes in a bead beater (Disruptor Genie). The Eppendorf 5420 centrifuge was used for homogenizing serially using the Zymo-Spin™ III-F in different collection tubes.

DNA Quantification

A NanoDrop™ One Microvolume UV-Vis Spectrophotometer was used to assess the isolated DNA's purity and concentrations of the isolated DNA. After adding 2 µl of DNA Elution Buffer, the Nanodrop spectrophotometer was blanked. Subsequently, a sample of 2 µl of DNA was put on the pedestal the concentrations (ng/µl) were measured at A260/280 ratio, and A260/230 ratios respectively.

PCR amplification

The target region was amplified using OneTaq® Quick-Load® 2X Master Mix (NEB, Catalogue No. M0486) in a 12.5µL reaction consisting of 1 µL of template DNA, 0.25µL (10nM) of 10µM forward primer, 0.25µL (10nM) of c10µM reverse primer, 6.25µL of One Taq Quick Load 2X master mix with Standard Buffer and 4.75 µL of nuclease-free water.

PCR amplification conditions

The primers used for the reaction include ITS 1 and ITS2 F-AACAAGGTTTCCGTAGGTGA and R–TATGCTTAAAYTCAGCGGGT. Using the Eppendorf Mastercycler nexus gradient 230, the samples were then subjected to an initial denaturation at 95°C for 5mins, denaturation at 95°C for 30 seconds, annealing at 50°C for I min, extension at 68°C for I min 30 secs, final extension at 68°C for 10 mins and hold at 4°C in 35 cycles.

Gel electrophoresis

Following PCR amplification, 1% agarose gel was run on 2 µl of each PCR product, dyed with 5 µl of SafeView Red, and photographed utilizing a gel documentation system (E-BOX Vilber Lourmat, Italy). A Fast DNA ladder (NEB) molecular weight standard (50bp – 10 kb) was used. An enzymatic method (ExoSAP) was used in cleaning the PCR products. To prepare the ExoSAP master mix, 50µl of 20U/µl Exonuclease I (Catalogue No. NEB M0293L) and 200µl of 1U/µl Shrimp Alkaline Phosphatase (Catalogue No. NEB M0371, New England BioLabs (NEB) Frankfurt, Germany) were both transferred to a 0.6ml micro-centrifuge tube. The reaction mixture was made through the combination and incubating 2.5 µl of the aforementioned ExoSAP mix with 10 µl of the amplified PCR product at 37 °C for 15 minutes and 80 °C for 15 minutes.

RESULTS

DNA quantification and purity measurement

The spectrophotometer results for purity and concentrations are shown in Table I. The concentration of the DNA extracts ranged from 6 – 2652.2 ng/µl, obtained from accessions Ab6 and B24 respectively. The purity at A260/280 ratio ranged from 1.5 to 2.18 ng/µl, obtained from accessions B22 and B43 respectively while A260/230 ratio ranged from 0.93 to 39.56 ng/µl, obtained from accessions C46 – Ab6 respectively. The concentration and purity values varied among the accessions. Nineteen percent of accessions had very high concentration values (Ab9(1164.8 ng/µl), AB15(1483.3 ng/µl), G16(1687.3 ng/µl), G18(1224.9 ng/µl), Ab20(1548.4 ng/µl), Ab21(1187.5 ng/µl), B24(2089.4 ng/µl), B26(1039 ng/µl), B30 (2652.2 ng/µl), B34(1510.8 ng/µl), B35(1020.7 ng/µl), B38(1005.6 ng/µl), B42(1852.7 ng/µl), B43(2441.7 ng/µl), C45(1023.4 ng/µl), F61(1111.1 ng/µl) and J81(118.4 ng/µl)). Very high purity values above the acceptable range were observed in accessions Ab6(5.34) whereas, lower values were observed in B22(1.5 ng/µl), B31(1.52 ng/µl), and a very low value of 1.34 ng/µl in accessions J85. *Inversa* varieties had purity values slightly lower or higher than the acceptable range except accessions J73 with the value of 2.

Electrophoresis analysis of DNA extracts

DNA extraction was successful revealing clear and distinct bands without smearing. All the samples showed a good amplification process and the electropherogram to confirm the portion of ITS1 and ITS 2 regions extracted is shown in Plates III - V. The gel documentation image produced using E-BOX, Vilber Lourmat, Italy revealed that all the samples yielded bands between .300 and .500 kb of a Fast DNA ladder.

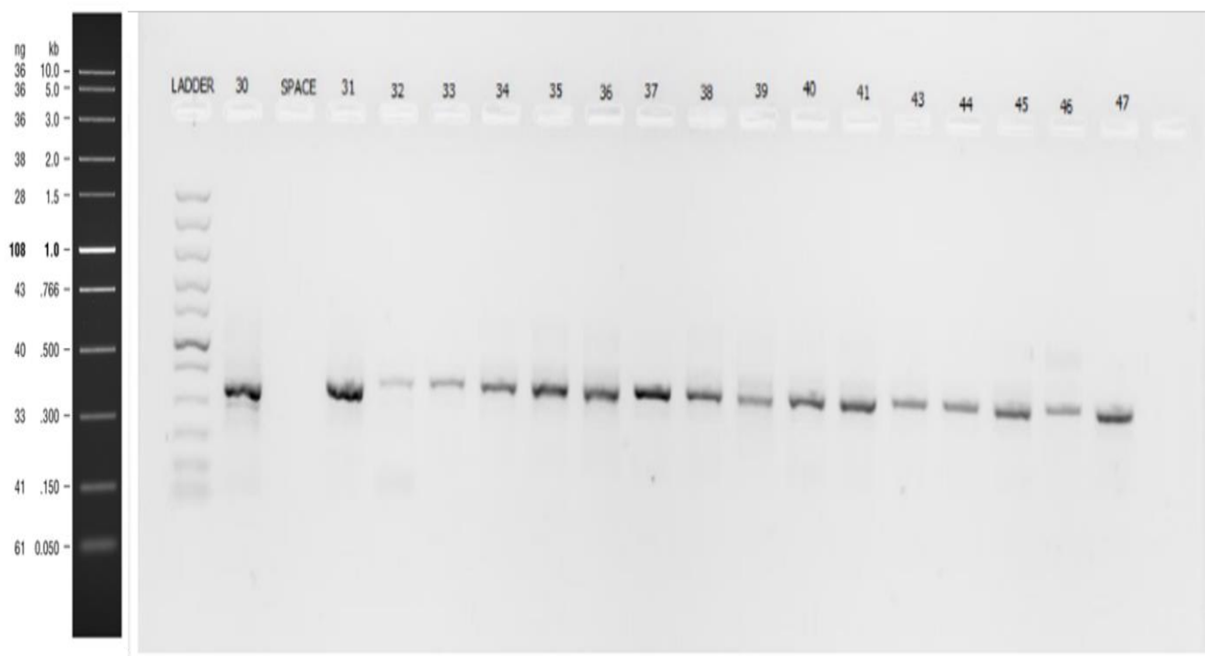

Plate III: Agarose gel electrophoresis showing amplified ITS1 and ITS 2 regions of *Treculia africana* accessions B30-C47

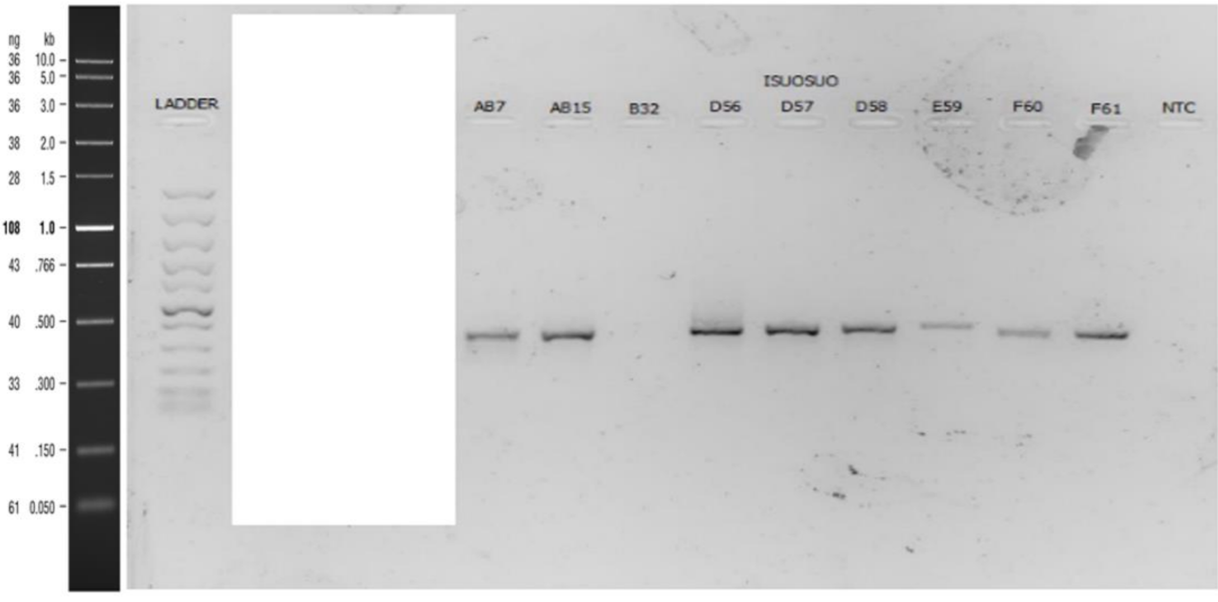

Plate IV: Agarose gel electrophoresis showing amplified ITS1 and ITS 2 regions of *Treculia africana* accessions Ab7, Ab15, B32, D56 to F61

Table I. Concentration and purity of DNA extracts of 86 accessions of *Treculia africana* varieties

Sample ID	Nucleic Acid (ng/ µl)	A260 (Abs)	A280 (Abs)	260/280	260/230
Ab 1	609.8	12.195	5.856	2.08	1.93
Ab 2	178.0	3.559	1.715	2.07	2.13
Ab 3	191.5	3.83	1.822	2.1	1.76
Ab 4	42.2	0.844	0.383	2.2	2.27
Ab 6	6.1	0.122	0.023	5.34	39.56
Ab 7	389.8	7.796	3.924	1.99	1.61
Ab 8	204.4	4.087	2.068	1.98	1.59
Ab 9	1164.8	23.296	11.182	2.08	2.02
Ab 10	587.9	11.757	6.022	1.95	1.60
Ab 11	332.0	6.64	3.145	2.11	1.87
Ab 12	616.0	12.321	5.813	2.12	2.04
Ab 13	1023.0	20.46	10.572	1.94	1.6
Ab 14	898.1	17.962	8.793	2.04	1.84
G 16	1687.3	33.746	17.029	1.98	1.69
G 17	586.1	11.722	5.537	2.12	2.11
G 18	1224.9	24.499	11.845	2.07	1.95
Ab 19	978.9	19.578	9.47	2.07	1.89
Ab 20	1548.4	30.968	15.926	1.94	1.70
Ab 21	1187.5	23.751	11.29	2.1	1.69
B 23	957.8	19.156	9.441	2.03	1.83
B 24	2089.4	41.788	19.757	2.12	2.06
B 25	397.7	7.954	5.231	1.52	0.96
B 26	1039.5	20.791	9.976	2.08	2.02
B 27	407.9	8.158	4.511	1.81	1.17
B 28	611.6	12.233	5.981	2.05	1.85
B 29	859.0	17.181	8.315	2.07	2.00
B 30	2652.2	53.044	25.621	2.07	1.94
B 32	897.6	17.952	8.611	2.08	2.02
B 33	440.6	8.813	4.321	2.04	1.74
B 34	1510.8	30.215	14.248	2.12	2.15
B 35	1020.7	20.415	9.646	2.12	2.18
B 36	360.3	7.205	3.375	2.13	2.07
B 37	342.9	6.858	3.22	2.13	1.96
B 38	1005.6	20.111	11.025	1.82	1.34
B 39	735.5	14.71	7.077	2.08	1.74
B 41	466.2	9.324	4.411	2.11	2.13
B 42	1852.7	37.053	17.321	2.14	2.17
B 43	2441.7	48.834	22.614	2.16	2.18
B 44	145.3	2.906	1.351	2.15	2.23
D 48	441.3	8.826	4.927	1.79	1.24
D 49	127.8	2.555	1.171	2.18	2.18
D 50	222.0	4.44	2.175	2.04	1.67
D 51	460.6	9.211	4.403	2.09	1.80

All samples are of variety *africana.*

Table I continued. Concentration and purity of DNA extracts of 86 accessions of *Treculia africana* varieties
Concentration Purity

Sample ID	Nucleic Acid (ng/ µl)	A260 (Abs)	A280(Abs)	260/280	260/230
D 52	155.4	3.109	1.483	2.10	1.97
D 53	377.6	7.552	3.609	2.09	1.92
D 54	155.2	3.105	1.454	2.14	2.01
D 55	358.4	7.168	3.833	1.87	1.40
D 56	467.1	9.343	4.722	1.98	1.67
E 57	338.7	6.775	3.418	1.98	1.52
E 58	269.1	5.382	2.559	2.10	1.95
F 59	211.2	4.223	2.111	2.00	1.64
F 60	326.7	6.534	3.209	2.04	1.71
F 61	1111.1	22.221	10.694	2.08	1.97
F 62	180.7	3.614	1.741	2.08	1.70
F 63	216.1	4.322	2.019	2.14	2.00
G 64	138.4	2.769	1.514	1.83	1.26
G 65	266.2	5.324	2.671	1.99	1.6
G 66	237.0	4.740	2.358	2.01	1.64
G 67	506.4	10.127	4.953	2.04	1.79
G 68	219.9	4.398	2.136	2.06	1.74
G 69	243.8	4.876	2.331	2.09	1.83
G 70	667.8	13.356	6.571	2.03	1.79
J 74	246.2	4.924	2.464	2.00	1.54
J 75	315.4	6.308	3.064	2.06	1.76
J 76	168.6	3.371	1.748	1.93	1.42
J 77	214.7	4.294	2.044	2.10	2.01
J 78	343.0	6.860	3.516	1.95	1.49
J 79	477.2	9.543	4.598	2.08	1.79
J 81	118.4	2.369	1.226	1.93	1.45
J 82	185.0	3.699	1.805	2.05	1.69
J 83	117.6	2.352	1.300	1.81	1.22
J 84	156.5	3.129	1.514	2.07	1.71
J 85	173.2	3.463	2.587	1.34	1.01
F86	589.2	11.783	5.602	2.10	1.96
Ab 5	113.5	2.270	1.096	2.07	1.80
Ab 15	1483.3	29.667	17.513	1.69	0.78
B 22	278.5	5.569	3.713	1.50	1.00
B 31	2557.0	51.140	23.833	2.15	2.17
B 40	128.7	2.573	1.204	2.14	2.09
C 45	1023.4	20.468	9.680	2.11	2.16
C 46	45.7	0.915	0.534	1.71	0.93
C 47	236.9	4.737	2.327	2.04	1.67
H 71	306.0	6.120	2.974	2.06	1.82
I 72	200.2	4.004	2.081	1.92	1.44
J 73	599.0	11.980	5.976	2.00	1.43
J 77	214.7	4.294	2.044	2.10	2.01

All samples are of variety *africana* except the last 12 samples that are of var *inversa*

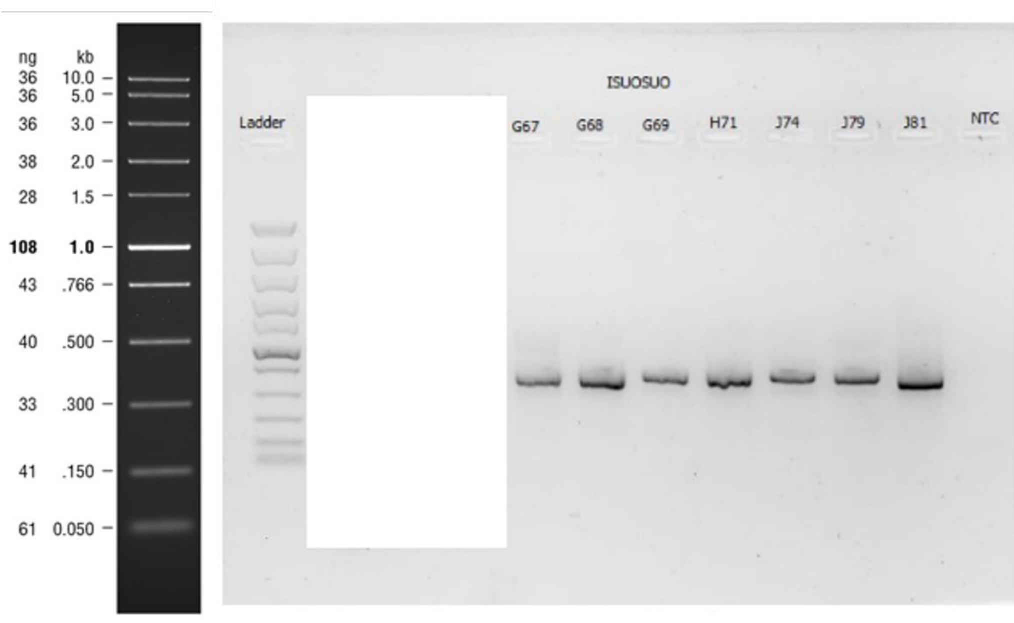

Plate V: Agarose gel electrophoresis showing amplified ITS1 and ITS 2 regions of *Treculia africana* accessions G67 - J81

DISCUSSION

Zymo Research mini prep plant DNA extraction kit was used to determine the quantity and quality of DNA isolated from T*reculia* varieties at 260/280A ratio and 260/230A ratio. There were variations in the values obtained from the DNA quantification across the accessions. The values derived at the A260/280 ratio and A260/230 ratio were majorly within the acceptable range of 1.8 – 2.0 suggesting that the ZR kit was able to eliminate contaminants. This was not in agreement with Ganiyu *et al.* (2017) in a study comparing the ZR plant extraction kit and modified methods of Dellaporta *et al.,* (1983) on five tomato cultivars. The lower range of values (1.11 – 1.45), indicating the presence of contaminants were observed with the ZR kit, unlike the modified method with better purity values of 1.73 to 2.00. A Kit (GeNei), CTAB DNA extraction methodology without using nitrogen and phenol, modified for plants with high amounts of secondary metabolites and polysaccharides, revealed similar data to this study on *Treculia* species accessions. Values ranging from 1.78 -1.84 were recorded (Sahu *et al.,* 2012).

Abdel Latif and Osman (2017) derived similar purity values with CTAB (1.2 - 2.90), DNeasy plant mini kit (1,61 – 2,00), and Qiagen (modified Mericon) (1.80 – 1.95) extraction kits in maize when compared with what was obtainable in this study. Some modified DNA extraction methods used by Huaqiang *et al.* (2013) on *Vigna unguiculata* had values ranging from 1.76 – 2.12. DNA purity values observed in this study outside the acceptable range, according to Pervaiz *et al*. (2011) may indicate insignificant levels of contamination. A very low value of 1.34 derived with accessions J85 was also observed with the Zymo kit (1.263) and the Epicentre kit (1.39) on 10 genotypes of *Vigna ungiculata* (Ogunkanmi *et al*., 2019). DNA extracts of accessions within the range of 1.8-2.0 observed are indications of high purity of samples with the absence of proteins and phenols. However, according to Latif and Osman (2017), a purity ratio greater than 1.9 and a ratio less than 1.7 in some of the samples are indications of the presence of RNA and proteins respectively. Although Abousedaa *et al*. (2015) noted that the right purity value may not account for the successful application of genes, other factors such as concentration should be considered.

The results for DNA concentrations revealed variations in values among accessions. Low, moderate to high values of DNA yield were observed. A very large value of 2089.4 observed was considered by Alfi (2021) to be a very good yield. Low values of 6.1 ng/µl, 42.2 ng/µl, and 45.7 ng/µl from accessions Ab6, Ab4, and C46 respectively were similar to values (24.2 ng/µl, 43.11 ng/µl, 25.33 ng/µl, 27.25 ng/µl, and 36.18 ng/µl) observed in five tomato cultivars extracted with ZR kit method (Ganiyu *et al.,* 2017). Easy DNA protocol produced lower values of 5.82 ng/µl, 9.38 ng/µl, 20.59 ng/µl, 21.85

ng/µl, 22.13 ng/µl, and 24.10 ng/µl extracted from ultrafine powder, seed powder, root, stem, flower, and leaf of six transgenic plants respectively (Wang *et al.*, 2021). In addition, Huaqiang *et al.* (2013) revealed similar values on *Vigna ungiculata* when comparing a commercial kit (19.48±2.51 ng/µl) and modified methods of Saghai-Maroof *et al.* (1984) (12.53±1.40 ng/µl); Doyle *et al.* (1987) (4.49±0.32 ng/µl); Dellaporta *et al.* (1983) (61.5±7.50 ng/µl); Rogers *et al.* (1985) (16.47±2.43 ng/µl and Aljanabi *et al.* (1997) (72.00±9.70 ng/µl, whereas, Ogunkanmi *et al.*, 2019 reported mean values of 103.64 ng/µl with the Zymo extraction kits.

A report by CRL-GMFF (2007) on the validation of DNA extraction methods of soybeans using modified Dellaporta methods reported an average concentration value of 457 ± 31 ng/ µL which agrees with the moderate values derived from accessions B33(440 ng/µl), B41(466.2 ng/µl), D48(441.3 ng/µl), D56(467.1 ng/µl) and J79 (477 ng/µl). The Dellaporta *et al.* (1980) method used yielded a DNA concentration value of 1430.52 ng/µl which is comparable to values derived from *Treculia* accessions 1453 ng/µl (B44), 1483 ng/µl (AB15), and 1510.4 ng/µl (B24). Higher values (1700 ng/µl) were reported using SDS-based methods on soybeans (Xia *et al.*, 2019). However, Kappa Biosystems (2014) recommended that DNA concentration values within 10-100 ng ng/µL or above could be ideal after necessary confirmations using Polymerase Chain Reaction (PCR). Alfi (2021) confirmed values as high as 755.04 ng/µlg to be good when compared with what is needed for real-time PCR due to differences in their accuracy and detection. Hence, small DNA values as low as 0.001 – 2 ng/µL could also be detected (Srisutham *et al.*, 2017). The variations in the concentrations of DNA among the seeds could be due to differences in the compositions of the accessions from different environments.

The suitability and efficiency of the use of Zymo research mini prep as a DNA extraction kit were revealed on the agarose gel images. The distinct and no significant smearing of electrophoresis bands observed according to Abdel-Latif and Osman (2017) are indications of non-degradation of samples. The agarose gel image resolution is suitable for the determination of the purity of the DNA. Good bands with high resolutions are obtained from the DNA extract provided there are no contaminants present (Abdullah *et al.*, 2016). The presence of distinct bands on the gel image are indication of good and quality DNA concentrations (Ramalah *et al.*, 2020). Very thick bands are usually associated with high DNA concentrations, Nurhaimi- Haris *et al.* (2003) which were not observed with the DNA extraction of *Treculia* species using the Zymo extraction kit. The intense bands obtained are indications of a high degree of purity and intact DNA. Smears are regarded as a sign of degradation of extracted DNA (Devi *et al.*, 2013). The quality and quantity of DNA affects the intensity of DNA band amplification (Wati *et al.*, 2014).

CONCLUSION

Zymo Research DNA extraction kit is suitable for the extraction of DNA from seeds of *Treculia* species. Its efficiency is comparable to conventional methods and other commercial kits. The use of plant parts with less amounts of secondary metabolites should be considered for DNA extraction. The use of seeds may be more favourable due to the ease of accessing samples for analysis, unlike leaf samples that require proper preservation on ice or lyophilization. Thus, the use of seeds of *Treculia* species in DNA extraction may be considered ideal due to loss of viability outside the fruit head making it difficult to use fresh leaves. The methodology of this study did not involve the comparison of the Zymo kit with other commercial kits or with conventional methods. Future studies are encouraged to compare the Zymo kit with other commercial kits in other to ascertain the ideal kits for DNA extraction of *Treculia* species.

Conflict of Interest

The authors have no conflict of interest to declare.

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Author contributions

ICC designed the experiment, wrote and edited the manuscript; AFI conceived the study, supervised, proofread, and edited the work while UUN performed the experiment and interpreted the data.

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