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Full Length Research Paper

Evaluation of Proximate Analysis, Lycopene Content And Dna Concentration Using Different Dna Extraction Protocols In Tomato Genotypes, Lycopersicon *Esculentum (Mill.)*

Adewusi, O.F

Department Of Biotechnology Federal University of Technology, Akure, Ondo–State, Nigeria.

Corresponding author. E-mail: ofadewusi@gmail.com. Tel. 08101109482

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This study was carried out on the "Evaluation of proximate analysis, lycopene content andconcentration of DNA sample usingdifferent DNA extraction protocols in Tomato Genotypes, *Lycopersicon esculentum (Mill)*".The study aimed to determine the extent of variation in the level of lycopene and proximate composition and the concentration of DNA samples in the tomato genotypes using different DNA methods among the tomato genotypes with a view of identifying and selecting promising tomato genotypes that could be utilized for further tomato breeding program. The twelvetomato genotypes used for this study were obtained from the Tomato Germplasm collection of the National Centre for Genetic Resources and Biotechnology (NACGRAB), Department of Plant Genetic Resources, Ibadan, Oyo-State, Nigeria. The experiment was conductedat the Teaching and Research Farm Obakekere, Federal University of Technology, Akure. The DNA extraction was carried out using Cetyltrimethylammonium bromide (CTAB), Sodium dodecyl sulfate (SDS), and the Zymo Kit protocol. The concentration and purity levels of the DNA samples were determined using Nano-drop (ND)-2000 spectrophotometer and 1% w/v agarose gel stain with ethidium bromide. The moisture content values were generally high for the genotypes ranging from 78.72-82.46% being the maximum in NGB00713 (82.46%). Genotypes, NGB00695, NGB00696, NGB00725 and NGB00752 recorded lower levels of moisture content. This indicates that these tomato genotypes can remain in store for an extended period compared to the other tomato genotypes. The crude protein content values ranged between 3.60% and 7.45% being the maximum in NGB00726 (7.45%). The carbohydrate content also varied between 4.77% and 10.63% being maximum in NGB00737 (10.63%). The lycopene content ranged between 2.49% and 31.20%. Genotypes, NGB00695, NGB00696, NGB00721 and NGB00735 recorded high levels of lycopene content compared to the other tomato genotypes which implies that they could be selected for improvement in future tomato breeding programs. The CTAB DNA extraction method recorded the concentration and purity levels ranging between 60.00 and 350.40 ng/µl and 1.318 and 2.153 respectively. The concentration and purity level of the Zymo kits ranged between 66.00 and 470.80 ng/µl and 1.728 and 2.210 respectively. For the SDS DNA extraction method, the concentration ranged between 57.600 – 999.600 ng/µl and 1.480 – 2.573 for the purity respectively. The results from the three DNA extraction methods imply that Zymo kits is the best for DNA extraction method in terms of DNA quality whereas SDS is the best in terms of DNA concentration that can be utilized for the extraction of DNA from tomato genotypes.

Keywords: Lycopene, Proximate, Sds, Ctab, Zymo Kits, Dnaconcentration, Tomato Genotypes

INTRODUCTION

Tomato, *Lycopersicon esculentum* (Mill.) is a member of the Solanaceae family (Motti, 2021). Tomato is a ubiquitous crop produced worldwide for a variety use ranging from high-value fresh fruit toprocessed products including ketchup, pastes, soups and stews, and canned pasta sauces (Buell *et al*., 2020). Tomato is a widely consumed fruit known for its rich content of lycopene, a potent antioxidant associated with various

health benefits, including a reduced risk of chronic diseases (Khan *et al.,* 2021). With increasing interest in functional foods and health-promoting compounds, understanding the lycopene content in different tomato genotypes is essential (Raza *et al*., 2022). Beyond lycopene, the proximate composition of tomatoes plays a significant role in determining their overall nutritional quality (Ali *et al.,* 2020). Proximate analysis is a set of standardized laboratory tests used to determine the basic chemical composition of a plant or any other material (Olaniyi *et al*., 2018). The analysis provides information on the amounts of the major components such as moisture, ash, crude protein, crude fat, crude fiber and carbohydrates present in the plant material. (Akuru *et al*., 2018). The moisture content of a plant material is important because it affects its shelf life and susceptibility to microbial spoilage (Fadiji *et al*., 2023). The ash content indicates the mineral content of the plant and is important for determining the plant's potential for use as a feed or fertilizer (Okunlola *et al*., 2019). Crude protein content is a measure of the amount of nitrogen in the plant, which is important for determining the plant's nutritional value as a food or feed source (Sanusi *et al*., 2020). Crude fat content is important for determining the energy content of the plant material (Fahey *et al*., 2019). Crude fiber content is important for determining the digestibility of the plant material (Gidenne *et al*., 2020). Isolation of high-quality genomic DNA and sufficient quantity of DNA samples in terms of the concentarationis acrucial step in modern biological techniques such as DNA fingerprinting, genome mapping, PCR, etc (Krawczak and Schmidtke, 2020). Numerous methods have been developed for genomic DNA extraction in various plant species (Aboul -Maaty and Oraby, 2019). Since high-quality genomic DNA is essential for real-time PCR knowing fully well that the presence of impurities in the genomic DNA will affect PCR amplification and reliability of real-time PCR (Arenas and Salazar, 2019). Hence, to decide on a method to be used for molecular analysis, it is necessary to ensure that the suitability of the DNA extraction and the optimization of the PCR process are key points of consideration (Kadri, 2019). Therefore, it may be necessary at times to have different methods of DNA extraction and decision taken in terms of high concentration and quality to find an ideal method suitable for isolating high-yield and high-quality DNA from tomatoes. This research was carried out to: (i) determine the proximate analysis among the tomato genotypes, (ii) determine the lycopene content in the tomato genotypes (iii) determine the DNA concentration and purity of the tomato genotypes using different extraction method.

MATERIALS AND METHODS

The experimental materials for this project consist of twelve tomato genotypes, *Lycopersicon esculentum* (Mill.). The tomato genotypes were obtained from the tomato germplasm collection of the National Center for Genetic Resources and Biotechnology (NACGRAB), Department of Plant Genetic Resources, Ibadan, Oyo – State, Nigeria. The names of thetomato, *Lycopersicon esculentum* (Mill.) genotypes are: NGB 00695, NGB 00696 , NGB 00708, NGB 00713,NGB 00721, NGB 00724, NGB 00725, NGB 00726, NGB 00729, NGB 0073, NGB 00737, NGB 00752.

PROXIMATE ANALYSIS

- **Moisture Content Determination**

The moisture content of each sample was determined as follows: A clean, oven-dried, and properly labelled petri dish was weighed (W1). About 5g of the sample was placed in the petri dish and the weight of the petri dish plus the sample was recorded (W2). The petri dish and the sample werethen transferred into the oven, maintained at 105^0C until a constant weightwas obtained (W0) and the percentage moisture content was calculated as:

$$\text{Moisture content } \% = \frac{W1-W2}{W1-W0} \times \frac{100}{1}$$

- **Fibre Content**

About 1g of the sample was weighed into the conical flask (W1) and boiled with 250 ml of 1.25% H_2SO_4 for 30mins.The mixture was filtered through muslin cloth and rinsed with distilled water. The sample was then scrapped back into the conical flask and then boiled again with 250 ml of 1.25% NaOH for 30 minutes. Thereafter it was filtered through muslin cloth and rinsed with distilled water followed by 10% HCL and finally with an organic solvent. The residue was placed in a crucible and transferred into the oven for 3 hours to remove the moisture. It was allowed to cool in the desiccator and the weight (W2) was recorded before placing in the muffle furnace at 550^0c for 3 hours, cooled, and weighed.

$$\% \text{ fibre} = \frac{W1-W2}{weight\_of\_sample} \times \frac{100}{1}$$

- **Crude Protein Determination**

Digestion

About 0.5 g of sample was weighed into 500ml kjedhal flask. Ten millilitres (10ml) of concentrated H_2SO_4 was added and a tablet of selenium catalyst was added and heated until the sample turned into clear solution. It was cooled and made up to 50ml with distilled water. The sample was stored in a sample

bottle.

Distillation

Five millilitres (5ml) of 2% boric acid (H_2BO_3) was placed in a conical flask and two drops of the mixed indicator wereadded, the conical flask was placed so that the tip of the condenser tube was in the boric acid, 5ml of the sample was pipetted into a sample holder with 10ml of 40% NaOH and this was rinsed with distilled water. The joints were tightened, and distillation was carried out till a volume of 50ml was collected in the conical flask.

Titration: The distillate was titrated with 0.1MHCl until the end point (pink colour) was reached

%Nitrogen = Molarity of acid × Titre value × 0.014 × (V1/V2)
Weight of sample

% Protein = % nitrogen × 6.25.

- **Ash ContentDetermination**

Clean crucibles were dried at 105^{0}C for about 1 hour, cooled in a desiccator and weighed (w1), 1g of sample was transferred into each of the appropriately labelled crucibles and then weighed (w2). The crucibles and their contents were transferred into the muffle furnace and ignited at 550^{0}C for about 5 hrs. After complete ashing, the crucibles were allowed to cool in a desiccator and weighed (W3). The percentage of ash was then determined as follows:

$$\% \text{ ash} = \frac{Weight\_of\_ash}{Original\_weigt\_of\_sample} \times \frac{100}{1}$$

- **Fat Content Determination**

Oven-dried filter paper was weighed (W1) and 1g of oven-dried sample was placed into the filter paper (W2). The filter paper and the content (W2) wereneatly folded and tied using thread, labelled with a pencil and arranged in the thimble. The round bottom flask (500ml) was filled with an organic solvent up to ¾ of the flask. The extractor was fitted with the reflux condenser and heated to allow the solvent to boil gently and siphon several times for 5 hours. The samples were then removed and dried in the oven for 1hr to remove any trace of the solvent, the thread was removed, and the sample was weighed back.

$$\% \text{ fat} = \frac{W1 - W0}{weight\_of\_sample} \times \frac{100}{1}$$

- **Carbohydrate Determination**

The addition of the value obtained from protein, fibre, fat, ash and moisture content minus 100 gives the value of carbohydrate in the sample (AOAC, 2005).

Determination of Lycopene Concentration in tomato genotypes.

Materials and Reagents

Test-tubes, mortar, pestle, analytical weighing balance, 5ml of Petroleum ether, 5ml of Sodium sulphate, 1cm^3 quartz cuvette, UV/Visible spectrometer.

Procedure

Mortar and pestle were used to blend the tomato samples to homogeneity. Analytical weighing balance was used to measure 2.5g of blended tomato samples and then transferred carefully into test-tubes. 5ml of petroleum ether was introduced into the samples inside the test-tubes, then 5ml of sodium sulphate was also introduced and was shaken to allow for even dissolution. The solution was allowed to stand for 5 ,minutes to allow for phase separation, thereafter the upper layer was collected into a 1cm^3 quartz cuvette, and the absorbance was taken at 543nm using UV/Visible spectrophotometer. The lycopene content of each sample was then recorded.

MOLECULAR ANALYSIS

The molecular analysis entails the extraction of the DNA from the tomato genotypes using Cetyltrimethyl ammonium bromide (CTAB), Sodium dodecyl sulfate (SDS) and Zymo Kit Protocol. After the DNA extraction, the concentration and purity will be determined using gel-electrophoresis and Nano drop spectrophotometer.

MATERIALS AND REAGENTS FOR THE EXTRACTION OF THE DNA.

Leaf samples: 15 Leaf samples each were collected on the day the experiment from the nursery in ice pack container for both the kit and two conventional methods, 70% Ethanol, Ice-cold isopropanol, Water bath, Mortar and pestle, 1.5ml Eppendorf tubes, Weighing balance, Orbital shaker, Centrifuge, Nuclease free water, Low-temperature freezer (-4°c freezer), Zymo Quick-DNA™ Plant/Seed Miniprep kit for kit method only, Lysis buffer for CTAB method only (160ml of 10x CTAB buffer and 1.3ml of B-mercaptoethanol preheated in a water bath).

SDS extraction buffer for SDS method only (20g SDS/l, 150mM NaCl, 100mM Tris/HCl, 25mM EDTA, pH 8.0), Proteinase K (10 mg/ml) for SDS method only, Phenol/chloroform/isoamyl alcohol (P: C: I, 25: 24: 1, v/v/v) (SDS method only), Chloroform/isoamyl alcohol (C: I, 24: 1, v/v) (SDS method only), Potassium acetate solution for SDS only (3M, pH 5.5), 95% glacial ice ethanol for SDS only (v/v), Glacial ice 70% ethanol, Chloroform and Isoamylalcohol (24:1 ratio).

ISOLATION OF GENOMIC DNA OF TOMATO USING CTAB METHOD

200mg (0.2g) of the leaf sample was weighed and crushed into fine paste using mortar and pestle. 800ul of the lysis or extraction buffer was added to make it a slurry. The paste was then poured into a 1.5ml Eppendorf tube. The solution was incubated at 65 °c in a water bath for 20 mins. After 20 mins, the samples were removed from the water bath and uncapped. An equal volume of SEVAGE (chloroform and isoamyl alcohol in 24:1) was added to the solution and was shaken and opened to allow gas escape. The Eppendorf tube containing the solution was transferred to the orbital shaker and was agitated at the speed of 450rpm. It was further transferred to the centrifuge and spunfor 5mins at 12000rpm. After the centrifugation, the aqueous supernatant was extracted using the micropipette and dispensed into new Eppendorf tubes. An equal volume of ice-cold Isopropanol was rocked gently and was then transferred into the -4°C freezer overnight to precipitate. After precipitation, it was centrifuged and spunat 10000rpm for 6mins. An equal volume of 70% ice-cold ethanol of the solution in the tube was added to wash the DNA pellet gently. The ethanol was decanted. This particular process was repeated three times. The pellet was allowed to air-dry until the 70% ice-cold ethanol had evaporated completely. The pellet was then suspended in 1ml of nuclease-free water to dissolve the pellet. Using a micropipette, the colorless supernatant which was the DNA extracted, and stored in a low-temperature freezer at -4°C for further analysis.

ISOLATION OF GENOMIC DNA OF TOMATO USING SDS METHOD

0.2g (200mg) of the leaf sample was measured and crushed to a fine paste using mortar and pestle. After the crushing, the paste was transferred to 1.5ml Eppendorf tube. 1ml of SDS extraction buffer (20g SDS/l, 150mM NaCl, 100mM Tris/HCl, and 25mM EDTA, pH 8.0) was preheated at 65°C, added to sample and mixed. 10µl Proteinase K (10mg/ml) was also added. The reaction tube was incubated at 65°C for 1 hour, stirring every 10 min. After incubation, the reaction tube was centrifuged for 10 mins at 12000rpm. Then the extraction of supernatant was carried out twice. The first supernatant was extracted when phenol/chloroform/isoamyl alcohol (P: C: I, 25: 24: 1, v/v/v) was added and the second supernatant extraction was carried out when chloroform/isoamyl alcohol (C: I, 24: 1, v/v) was added. Upper aqueous phase 0.1 volume potassium acetate solution (3 M, pH 5.5) and double volume of ethanol solution (95%, v/v, −20°C) was added. And this was the first precipitation. Then it was inverted gently and vortexed for 10 min at 15000rpm to precipitate the DNA. The pellet was washed with ethanol solution (70%, v/v, −20°C) twice and air dry for 5 min, and the dried pellet was dissolved with 400µl Tris/EDTA buffer (10 mM Tris, 1 mM EDTA). DNA solution. The upper layer was recovered to a new sterile tube containing 2.5 volume of ethanol (second precipitation) which helped the precipitate DNA readily. The tube was then spunat 15000rpm for 10 min and the DNA pellet was washed twice. The dried DNA was dissolved again in 200µl sterile deionized water.

ISOLATION OF GENOMIC DNA OF TOMATO USING ZYMO QUICK-DNA™ PLANT/SEED MINIPREP KIT METHOD

The Zymo Quick-DNA™ Plant/Seed Miniprep kit (Cat No: D6020) was used to extract the DNA. 200mg of finely cut tomato leaf was put inside ZR bashing Bead™ Lysis Tube (2.0 mm). 750µl Bashing Bead™ Buffer was added to the tube and cap tightly secure in a bead beater fitted with a 2ml tube holder assembly and was processed at 13,000rpm for 5 minutes. Then the ZR bashing Bead™ Lysis Tube was centrifuged in a microcentrifuge at ≥10,000 x g for 1 minute. 400µl supernatant was transferred to a Zymo-Spin™ III-F Filter in a Collection Tube and centrifuged at 8,000 x g for 1 minute. The Zymo-Spin™ III-F Filter was discarded. 1200ul of Genomic lysis buffer was added to the filtrate in the collection tube and mixed well. 800ul of the mixture above was transferred into Zymo-spin™IICR Column2 in a collection tube and centrifuged at 10000rpm for 1minute. The flow through was discarded and 200ul of DNA Pre-wash Buffer was added to the Zymo-Spin™IICR column in a new collection tube and centrifuged at 10000 rpm for 1 minute. 500ul of g-DNA Pre-Wash Buffer was added to Zymo-Spin™IICR Column and centrifuge at 10,000rpm for 1 minute. The Zymo-SpinTMIICR column was transferred to a clean 1.5ml microcentrifuge tube and 100ul of DNA Elution Buffer was added to the column matrix and centrifuged at 10,000rpm for 1 minuteto elute the DNA.

QUANTIFICATION AND PURIFICATION OF DNA

Extracted DNA samples quantification and purity were checked by measuring the absorbance at 260 and 280nm using Nano (UV-VIS) Spectrophotometer.

GEL ELECTROPHORESIS

Gel Materials

Agarose Gel, Blue-light-transilluminator, Casting tray, 0.5 × TBE buffer (2.6 g of Tris base, 5 g of Tris boric acid and 2 ml of 0.5M EDTA and adjusted to pH 8.3 with the sodium hydroxide pellet), Safeview, Combs, DNA ladder, Weighing ladder, Microwave.

Gel Procedure

Agarose powder of 0.2g was measured and dissolved

Table 1. Proximate Composition of the Tomato Genotypes

Genotypes	Moisture content (%)	Ash content (%)	Fat content (%)	Crude protein (%)	Crude Fibre (%)	Carbohydrate Content (%)
G1	79.88ab	3.10a	1.39b	5.95ab	4.27a	5.42b
G2	79.93ab	4.34a	2.54a	5.72ab	2.70ab	4.77bc
G3	80.04a	2.34ab	2.06a	3.60b	1.89b	10.09a
G5	82.46a	2.78a	1.63ab	6.80a	1.53b	4.82bc
G8	80.96a	1.71b	1.37b	6.01ab	1.20bc	8.76ab
G9	80.68a	2.05ab	1.68ab	5.94ab	1.30bc	8.36ab
G10	79.72ab	1.03c	1.38b	7.30a	0.92c	9.66a
G11	81.40a	1.98ab	1.20bc	7.45a	2.36ab	5.63b
G13	81.93a	1.85ab	1.30b	7.41a	2.08ab	5.44b
G15	81.11a	1.69b	1.35b	7.17a	2.53ab	6.15b
G16	81.69a	1.46bc	1.16bc	4.41b	0.66c	10.63a
G19	79.97ab	1.68b	1.46ab	5.51ab	1.91ab	9.48a

G1 = NGB 00695; G2 =NGB 00696 ; G3 = NGB 00708; G5 = NGB 00713; G8 = NGB 00721; G9 = NGB 00724,G10 = NGB 00725; G11 = NGB 00726; G13 = NGB 00729; G15 = NGB 00735; G16 = NGB 00737; G19 = NGB 00752.

in 12 ml of 0.5 x TBE buffer using a beaker. The solution was melted in the microwave oven for 1-2 minutes. The beaker was removed from the oven and was allowed to cool to around 55^{o}C – 65^{o}C temperature. The stain which was safeview alongside the 3µl EZ-vision (VWR Life Science) was added to enable a clear view of the result and stirred gently. The casting tray and comb were set. Following this, the gel was poured and allowed to cool for like 30 mins. After it hadcooled down, the comb was removed, and the wells were formed. The extracted DNA samples alongside the DNA ladder of size 1kb wereloaded in the wells. The tray was then connected to a direct current and was allowed to run. The expression product was visualized as bands by the Blue-light-transilluminator.

RESULTS

The estimates of the proximate composition of the tomato genotypes are presented in Table 1. The moisture content ranged from 79.72% to 82.46%. The genotype with the highest moisture content is G5 (82.46%) whereas the lowest moisture content was recorded in G10(79.72%). The ash content ranged between 1.03 and 4.34%. The highest ash content was recorded in G2 (4.34%) whereas the lowest content wererecorded in G10 (1.03%). The fat content ranged from 1.16 to 2.54%.The highest fat content was recorded in G2(2.54%) followed by G3(2.06%)whereas the lowest level of fat content was recorded in G16 (1.16%). The highest level of crude protein was recorded in G11 (7.45%) followed by G13 (7.41%) followed by G10 (7.30%)whereas the lowest protein content was recorded in G3 (3.60%). The highest fiber content was recorded in G1(4.27%) whereas the lowest level was recorded in G16 (0.66%). The highest carbohydrate content was recoded in G16 (10.63%) followed by G3 (10.09%) followed by G10 (9.66%) whereas the lowest level wererecorded in G2 (4.77%).

The estimates of the lycopene contents of the tomato genotypes are presented in Table 2. the lycopene content ranged between 2.49% and 31.20%. The highest lycopene was recorded in G15 (31.20%) followed by G8 (24.02%) followed by G2 (17.16%) whereas the lowestlycopene content was recorded in G9 (2.49%).

The estimates of the concentration and purity level of DNAsamples extracted from the tomato genotypes using the ctabdna extraction protocol arepresented in Table 3. The DNA concentration ranged from 60.00 ng/µl to 350.40 ng/µl. The highest concentration was recorded in G8(350.40 ng/µl) followed by G1(250.800ng/µl)followed by G9 (224.400 ng/µl.) whereasthelowest concentration was recorded in G15 (60.00 ng/µl). The dna purity at A260/A280 ranged between 1.852 and 2.153. 66.67% of the tomato genotypes recorded the acceptable purity range of 1.80 to 2.00. Genotypes G8, G9 and G16 exhibited DNA purity of 2.015, 2.153 and 2.055 respectively indicating that they were contaminated with RNA. Whereas the lower DNApurity was recorded in G3 (1.318) indicating contaminants from protein.

The estimates of the Concentration and Purity Level of DNA samples from the tomato genotypes using the Kit (Zymo KIT) protocol arepresented in Table 4. The DNA concentration ranged from 66.00 ng/µl to 490.80 ng/µl. The highest DNAconcentration was recorded in G9 (490.80 ng/µl) followed by G3 (475.20 ng/µl)

Table 2. Lycopene content in the tomato genotypes

GENOTYPE	LYCOPENE (mg/100g)	LYCOPENE (%)
G1	0.16	16.22a
G2	0.17	17.16a
G3	0.14	14.04ab
G5	0.13	13.73ab
G8	0.24	24.02a
G9	0.02	2.49c
G10	0.14	14.35ab
G11	0.14	14.04ab
G13	0.12	12.48ab
G15	0.31	31.20a
G16	0.14	14.97ab
G19	0.10	10.92b

G1 = NGB 00695; G2 =NGB 00696 ; G3 = NGB 00708; G5 = NGB 00713; G8 = NGB 00721; G9 = NGB 00724;G10 = NGB 00725; G11 = NGB 00726; G13 = NGB 00729; G15 = NGB 00735; G16 = NGB 00737; G19 = NGB 00752.

Table 3. Concentration and purity level of dna extracted from 12 tomato genotypes using ctab dna extraction method.

Genotypes	DNA concentration (ng/µl)	A260	A280	DNA PURITY (A260/A280)
G1	250.800	0.075	0.038	1.974a
G2	85.200	0.057	0.028	2.000a
G3	190.800	0.034	0.026	1.318b
G5	123.600	0.058	0.030	1.921a
G8	350.400	0.082	0.041	2.015a
G9	224.400	0.069	0.032	2.153a
G10	96.000	0.035	0.019	1.852ab
G11	98.400	0.042	0.022	1.904a
G13	92.400	0.086	0.045	1.925a
G15	60.000	0.075	0.038	2.000a
G16	183.600	0.087	0.042	2.055a
G19	121.200	0.080	0.043	1.860ab

G1= NGB 00695; G2=NGB 00696, G3=NGB 00708, G5=NGB 00713, G8=NGB 00721, G9=NGB 00724, G10=NGB 00725, G11=NGB 00726, G13=NGB 00729, G15=NGB 00735, G16=NGB 00737, G19=NGB 00752

followed by G5 (448.80 ng/µl) whereas the lowest concentration was recorded in G10 (66.00 ng/µl). The DNA purity ranged between 1.825 and 2.214. eight of the twelve tomato genotypes recorded dna purity within the acceptable purity range of 1.80 to 2.00. G2, G3, G11 and G15 recorded DNA purity of 2.048, 2.096, 4.043 and 2.214 respectively which is above the acceptable DNA purity of 2.00. this implies that these DNA samples were contaminated with traces of RNA. A lower DNA purity was recorded in G9 (1.728) indicating a trace of protein contaminants.

The estimates of the Concentration and Purity Level of DNA samples from the tomato genotypes using aSDS protocol arepresented in Table 5. The DNA sample concentration ranged from 57.600ng/µl to 999.600ng/µl. the DNAconcentration was maximum in G1 (999.60 ng/µl) whereas the lowest level was recorded in G3 (57.600 ng/µl), the DNA purity ranged between 1.480 and 2.573. 58% of the tomato genotypes recorded the acceptable range of DNA purity. G3 and G8 recorded DNA purity of 2.524 and 2.573 respectively. G13, G15 and G19 recorded a

Table 4. Concentration and purity level of dna extracted from 12 tomato genotypes using zymo kit dna extraction method.

Genotypes	DNA concentration (ng/µl)	A260	A280	DNA PURITY (A260/A280)
G1	271.200	0.058	0.030	1.923a
G2	157.200	0.068	0.033	2.048a
G3	475.200	0.046	0.023	2.000a
G5	448.800	0.038	0.020	1.866ab
G8	355.200	0.052	0.028	1.853ab
G9	490.800	0.038	0.022	1.728ab
G10	66.000	0.060	0.033	1.825ab
G11	369.600	0.076	0.037	2.043a
G13	104.400	0.062	0.031	1.984a
G15	361.200	0.086	0.039	2.214a
G16	309.600	0.068	0.036	1.865ab
G19	306.000	0.070	0.036	1.920a

G1= NGB 00695; G2=NGB 00696, G3=NGB 00708, G5=NGB 00713, G8=NGB 00721, G9=NGB 00724, G10=NGB 00725, G11=NGB 00726, G13=NGB 00729, G15=NGB 00735, G16=NGB 00737, G19=NGB 00752

Table 5. Concentration and purity level of dna extracted from 12 tomato genotypes using sds dna extraction method.

Genotypes	DNA concentration (ng/µl)	A260	A280	DNA PURITY (A260/A280)
G1	999.600	0.035	0.019	1.892ab
G2	649.200	0.045	0.023	1.948a
G3	57.600	0.053	0.021	2.524a
G5	248.400	0.065	0.035	1.852ab
G8	80.400	0.055	0.021	2.573a
G9	127.200	0.049	0.026	1.922a
G10	440.400	0.057	0.029	1.952a
G11	361.200	0.068	0.037	1.849ab
G13	238.800	0.040	0.023	1.732b
G15	165.600	0.045	0.030	1.480bc
G16	333.600	0.079	0.040	1.987a
G19	319.200	0.040	0.023	1.756b

G1= NGB 00695; G2=NGB 00696, G3=NGB 00708, G5=NGB 00713, G8=NGB 00721, G9=NGB 00724, G10=NGB 00725, G11=NGB 00726, G13=NGB 00729, G15=NGB 00735, G16=NGB 00737, G19=NGB 00752

lower level of DNA purity below the acceptable range of 1.732, 1.480 and 1.756 respectively which indicates contamination by protein or other impurities.

DISCUSSION

Moisture content is a key factor in determining the shelf life and texture of tomatoes. Higher moisture levels can indicate shorter shelf life due to increased susceptibility to microbial growth. The variations observed in moisture content in this study corroborates the findings of Arah *et al*., (2015). They reported that the tomato postharvest quality was affected by the high moisture content which affected the shelf life. The variations in the different nutritional composition of the tomato genotypes aresynonymous to the findings of Gupta *et al*., (2020). The variations in protein and fat content in this study are similar to the findings of Ismaeel *et al*., (2019). They also reported variability in protein and fat content across different tomato genotypes. The variations recorded in the carbohydrates in this study is similar to the

findings of Akinyele *et al*., (2020). They also reported varying levels of carbohydrate concentration in tomatoes. The DNA concentration and purity recorded in this study corroborates the findings Aboul-Maaty and Oraby (2019). They reported the effectiveness of the CTAB protocol in achievingDNA purity ratios within the ideal range for downstream molecular analyses. They also emphasized the method's suitability for overcoming contaminants such as polysaccharides and proteins. The DNApurity and concentration reported in this study using Zymo kit is synonymous withthe findings of Araujo *et al.,* **(**2021**).** They evaluated the efficiency of different plant DNA extraction kits, including Zymo methods, for obtaining high-purity DNA suitable for downstream genomic applications. They found DNA concentrations varied significantly across genotypes but generally achieved purity levels (A260/A280) around 1.8-2.0. The findings reported in this study concerning Zymo kit, also corroborates the findings of Bressan *et al.,* (2022). They compared plant DNA extraction using silica-column and Zymo kits. Their findings demonstrated that the Zymo kit consistently yielded DNA of high quality (A260/A280 ratios >1.9) and sufficient concentration required for sequencing. The variations recorded in the DNA concentration and purity recorded in this study for SDS corroborates the findings of Chanu *et al*., (2021). They reported the effectiveness of SDS protocol in yieldinghigh concentration of DNA sample in tomatoes and the optimization of SDS protocol for extracting high-quality DNA suitable for PCR amplification. Variability in purity level above or lower than 1.8 to 2.0 indicates the presence of contaminants which tend impairing and affect further downstream processes. Hence, to make the DNA sample suitable, there is the need to subject the extracted DNA samples to further purification. Overall, the Zymo Kit appears to provide generally high-quality DNA for most genotypes, with a few exceptions where the DNA purity could be optimized but all the protocols recorded the acceptable range of DNA concentration required for downstream genomic applications.

CONCLUSION

The proximate analysis revealed that NGB 00695, NGB00696, NGB00725, NGB00752 were outstanding in terms of lower moisture content implying that they will have a longer shelf life and be less prone to microbial attack. The lycopene content of the tomato genotypes revealed that NGB00695, NGB00696, NGB00721 and NGB00735 were outstanding in terms of lycopene composition.SDS provides the highest overall DNA concentration and a relatively high average DNA purity. This method can be found suitable when high DNA concentration is a priority. Zymo Kit is more consistent in its results, yielding moderately high DNA concentrations with a slightly lower average purity, making it a good choice when consistency in extraction is required.CTAB yields the lowest average DNA concentration and shows more variability in both concentration and purity, making it a less reliable option in terms of consistency, it can still provide good DNA purity in some cases. Overall, SDS and Zymo Kit are more efficient than CTAB in both concentration and purity, with SDS being the most suitable for high DNA yield, while Zymo Kit offers a more consistent and moderately pure result.

REFERENCES.

Aboul-Maaty NAF, Oraby HAS (2019). Extraction of high-quality genomic DNA from different plant orders applying a modified CTAB-based method. *Bulletin of the National Research Centre*, *43*(1), 1-10.

Araujo ES, de Almeida TG, Pio-Ribeiro G, Costa JS (2021). Evaluation of commercial plant genomic DNA extraction kits for diverse plant species and tissues. *Plant Methods, 17*(1), 117.

Bressan D, da Silva GD, Cavalcante IHL, Silva RM (2022). DNA extraction methods to obtain high DNA quality from different plant species and tissues. *Plant Methods, 18*(1), 45.

Akinyele BO, Oloruntoba OS (2020). Nutritional analysis of some selected varieties of tomatoes grown in Nigeria. Niger. J. Basic Appl. Sci., 28(1), 33-40.

Akuru UB, Amadi BA, Abbey BW (2018). Proximate. Amino Acid and Fatty Acid Composition of Eremomastaxpolysperma, Brillantaisiaowariensis Leaves and Sorghum vulgare Leaf-Sheath. Eur Exp, 8(5), 32-34.

Ali MY, Sina AAI, Khandker SS, Neesa L, Tanvir EM, Kabir A, Gan SH (2020). Nutritional composition and bioactive compounds in tomatoes and their impact on human health and disease: A review. *Foods*, *10*(1), 45.

Arah IK, Ahorbo GK, Anku EK, Kumah EK, Amaglo H (2015). "Postharvest handling practices and treatment methods for tomato handlers in developing countries: A mini review." Advances in Agriculture, 2015, 1-8.

Arenas NE, Salazar LM (2019). Steps and tools for PCR-based technique design. Biotechnology and Bioengineering, 83671.

Buell PD, Anderson EN, de Pablo Moya M, Oskenbay M (2020). Contemporary Food. In *Crossroads of Cuisine* (pp. 180-203). Brill.

Chanu N, Devi LK, Kumar KP (2021). Standardization of DNA extraction protocols in underexploited cherry tomato genotypes. *Biological Forum – An International Journal, 13*(2), 622–628. Retrieved from https://www.researchgate.net/publication/350730158_Standardization_of_DNA_Extraction_Protocols_in_Underexploited_Cherry_Tomato_Genotypes

Fadiji T, Rashvand M, Daramola MO, Iwarere SA (2023). A review on antimicrobial packaging for extending the shelf life of food. *Processes*, *11*(2),

590.
Fahey GC, Novotny L, Layton B, Mertens DR (2019). Critical factors in determining fiber content of feeds and foods and their ingredients. J. AOAC Int., *102*(1), 52-62.
Gidenne T, Carabaño R, Abad-Guamán R, García J, Blas CD (2020). Fibre digestion. In *Nutrition of the Rabbit* (pp. 69-88). Wallingford UK: CAB International.
Kadri K (2019). Polymerase chain reaction (PCR): Principle and applications. *Synthetic Biology-New Interdisciplinary Science*, 1-17.
Khan UM, Sevindik M, Zarrabi A, Nami M, Ozdemir B, Kaplan DN, Sharifi-Rad J (2021). Lycopene: Food sources, biological activities, and human health benefits. *Oxidative medicine and cellular longevity*, *2021*(1), 2713511.
Krawczak M, Schmidtke J (2020). *DNA fingerprinting*. Taylor & Francis.
Motti R (2021). The Solanaceae family: Botanical features and diversity. *The wild solanums genomes*, 1-9.
Okunlola GO, Jimoh MA, Olatunji OA, Rufai AB, Omidiran AO (2019). Proximate analysis, mineral composition, and antioxidant properties of bitter leaf and scent leaf. *International journal of vegetable science*, *25*(4), 346-354.
Olaniyi MB, Olaniyi AA, Lawal IO, (2018). Proximate, phytochemical screening and mineral analysis of Crescentia cujete L. leaves. J. Med. Plants Econ. Dev., *2*(1), 1-7.
Raza B, Hameed A, Saleem MY (2022). Fruit nutritional composition, antioxidant and biochemical profiling of diverse tomato (Solanum lycopersicum L.) genetic resource. *Frontiers in plant science*, *13*, 1035163.
Sanusi J, Habsatu S, Abubakar N, Suleiman I,Musa DD, (2020). Comparative study of proximate and minerals composition of tomato cultivars in Sokoto, Sokoto state, Nigeria. *FUDMA Journal of Sciences*, *4*(4), 409-414.