

# Molecular characterization of some landraces and varieties of Date palm (*Phoenix dactylifera* L.) from Afar region of Ethiopia using ISSR markers

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## Research article

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# Abstract

Background Date palm (*Phoenix dactylifera* L.) is one of the oldest fruit trees in hot arid region of the world including North Africa. In some areas of Afar region of Ethiopia, date palm grow as landraces, are in danger due to introduction of improved cultivars. Present study was carried out to fill knowledge gap about molecular diversity of this crop in Afar region. Molecular studies of 5 landraces and 3 introduced cultivars of date palm from Afar region of Ethiopia were tested using 21 randomly selected ISSR primers for amplification and polymorphism detection using genomic DNA. ISSR markers across 8 date palm varieties were scored for their presence (1) or absence (0). Shannon's Information index (I) and polymorphic information content (PIC) were analyzed by popGENE 32 and online PIC calculator respectively. Results 17 out of 21 ISSR markers used for this study produced a total of 557 scorable DNA fragments with average of 33.52 per marker and 61.68, 43.93 and 68.22% polymorphism were obtained within local landraces, introduced varieties and among all samples respectively. The genetic distance among all samples ranged from 0.1402 to 0.5953; and the dendrogram separated date palm varieties into seven clusters. ISSR markers used for this study have high discrimination power and the average values of Shannon's information index and PIC were 0.318 and 0.76 respectively. Conclusion Genetic diversity was observed among all date palms studied in this investigation. To have better understanding on genetic diversity of date palm in the Afar region, further research should be done using SNP markers and landraces should be registered.

# Background

*Phoenix dactylifera* L. belongs to family Arecaceae which comprises 200 genera and more than 2500 species [1, 2] and believed to be the most ancient cultivated tree in North Africa [3, 4] Zaid and Wet, 2002; Zohary and Hopf, 2000]. Date palm was introduced in areas around the Awsa delta and Afambo regions of Afar region in Ethiopia a very long time ago and plays an important socioeconomic role [5, 6]. Afar region is located in Danakil depression and experiences the harshest climate reaches up to 50oC and is home to virgin land that is suitable for date plantation [6, 7]. According to FAO 2019 [8], socio-economic and ecological importance of nutritional rich date palm as a cash crop and its cultivation native to their regions around the world. There are more than 5000 date palm species popular all over the globe [9] and the landraces of Afar region was not drawn global attention. Date palm's slow growth, dioecy, and adult characteristics could be known only after maturation severely restrict its improvement [10]. Introduction of elite varieties results date palm plants vulnerability to genetic erosion and evaluation of genetic diversity is critical step in germplasm conservation of date palm landraces [11]. Introduction of predominant varieties Medjool, Khalas, Barhe with good yield and fruit quality and lack of improved local landraces are important constraints of date palm production in Afar region [12]. Developing suitable method to study date palm diversity will help to initiate date improvement program [13] in Afar region. Molecular markers are important tools in identifying genetic relationships of different varieties of date palm, estimating genetic diversity for plant breeding, germplasm management, utilization [14], monitoring genetic erosion, and removing duplicates from germplasm collections [15]. Only a small part of the total

date palm genetic diversity has been characterized, evaluated, and used for crop breeding and improvement purposes [16]. Several studies used ISSR markers [17-20] for genetic diversity studies for date palm [21-23] and to identify date palm cultivars [17, 24, 25]. ISSR studies require small amount of DNA, simple, fast, reliable [26], high stringency at annealing temperature [27] and used in various plant species [28-32]. Several studies using ISSR, in combination with other markers showed polymorphism between 73% to 85.45 percentage of polymorphism [33-36]. There were no documented reports on molecular studies, description and variability of date palm landraces of Afar region from Ethiopia [12, 37]. It was therefore important to aim at the evaluation of the genetic diversity of the Afar date palm germplasm. So, the present study was carried out to understand the genetic diversity of five local landraces and three introduced varieties of Afar region of Ethiopia using ISSR markers.

## Results

### ISSR analysis for Per cent polymorphism

In the present study, leaf samples from the five different local landraces (Gewane, ABRD, ABRW, ABW and Harisa and three introduced varieties of date palm (Medjhool, Barhe and Kahlas) were assessed the genetic variation present in between date palm trees collected from different part of Afar region. The ISSR pattern of genomic DNA of collected samples were analyzed with respect to the fragments, informativeness of the markers and polymorphism for the assessment of genetic diversity present. Reproducible and clear banding patterns were obtained (Figure 1).

The primer wise amplification detail of the genomic DNA of all samples and per cent polymorphism across the 17 ISSR primers were presented in the Table 6. A total of 557 scorable DNA fragments were produced and among them 266 DNA fragments were found to be polymorphic. The minimum number (0) of polymorphic fragments produced by primer ID-438228 and 438233, while the maximum number of polymorphic fragments was found to be 33 by primer ID-438229. The average per cent polymorphism across the 17 primers within five local landraces, within three introduced varieties and among all collected date palm found to be 61.68, 43.93 and 68.22% respectively. The lowest polymorphism percentage (0 %) was shown by the primer ID-438228 and 438233 whereas highest polymorphism percentage (100 %) was observed in the primer ID-438229, 438238 and 438239. The primer ID 438228 and 438233 produced monomorphic banding pattern. The product size ranged from 250 bp to 550 bp by primer ID-438245 and 438243 respectively.

Figure 1: PCR amplification products obtained with ISSR primers.

## Genetic distance within local landraces

The overall range of genetic distance with in local landraces was 0.1842 to 0.5620. The minimum distance was observed between ABRD and ABW (0.1842), while the highest distance was observed between Harisa and ABW (0.5620). The dendrogram separated local date palm landraces into four

clusters, cluster I contains ABRD and ABW, cluster II contains cluster I and ABRW, cluster III contains cluster II and Gewane and cluster IV contains cluster III and Harisa.

Table 1. Nei's genetic identity (above diagonal) and genetic distance (below diagonal) within local landraces.

Figure 2. Dendrogram depicting Genetic distance within local landraces: Method = UPGMA

## Genetic distance within introduced varieties

The genetic distance was computed considering all samples from introduced date palm varieties and the dendrogram was constructed, the overall range of the dissimilarity with in introduced varieties was 0.1955 to 0.4832. The minimum distance was observed between Kahlas and Madjule (0.1955), while the highest distance was observed between Barhe and Madjule (0.4832). The dendrogram separated introduced date palm varieties into two clusters, Cluster I contains Madjule and Barhe, cluster 2 contains cluster I and Kahlas.

Table 2. Nei's genetic identity (above diagonal) and genetic distance (below diagonal) within introduced varieties.

Figure 3. Dendrogram depicting genetic distance within introduced varieties: Method = UPGMA

## Genetic distance among all date palm grown in Afar region

The genetic distance among all date palms from both introduced varieties and local landraces ranged from 0.1402 to 0.5953. The minimum distance was observed between Kahlas and ABRD (0.1402), while the highest distance was observed between Harisa and Kahlas (0.5953). The dendrogram separated all local and introduced date palms into seven clusters. Cluster I contains Kahlas and ABRD, cluster II contains Barhe and ABRW, cluster III contains cluster I and ABW, cluster IV contains cluster III and Madjule, cluster V contains cluster IV and Gewane, cluster VI contains cluster V and cluster II, cluster VII contains cluster VI and Harisa.

Table 3. Nei's genetic identity (above diagonal) and genetic distance (below diagonal) among all collected date palms.

Figure 4. Dendrogram depicting genetic distance among all collected date palms: Method = UPGMA

## Shannon's Information index (I)

Shannon's Information index for each primer ranged from (0) to (0.5454) with an average of (0.3181). The highest value obtained by primer ID 438229 and the minimum value observed by primer ID 438228 and 438233.

Figure 5. a and b: a) for the study of Shannon's information index and b) PIC value of primers used for the study

## Polymorphic Information Content (PIC)

To measure informativeness of ISSR primers PIC was calculated and the maximum (0.864) and minimum (0.565) value was obtained by primer ID 438226 and ID 438246 respectively. The average polymorphic information content of all primers was 0.760.

## Discussion

Date palm (*Phoenix dactylifera* L.) is fruit crop which is successful in arid and semiarid tropical and subtropical habitats. Understanding the amount and distribution of the genetic variation present in the genetic pool determine the progress of any genetic preservation [38, 39]. Assessing the genetic diversity and population structure of date palm germplasm is important not only for developing strategies for date palm conservation but also for broadening the genetic base for date palm breeding. Number of molecular techniques is available for characterization of the variation at the DNA level, e.g., random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSRs). Besides, they can reveal a virtually unlimited number of markers. Both ISSR [40-42] and RAPD [43, 44] used to study genetic polymorphism among date palm cultivars. Simple sequence repeat (SSR) technique also used to study genetic diversity of date palm [33, 45]. In the present investigation, three introduced varieties and five local landraces of *Phoenix dactylifera* L. were analyzed for polymorphism based on ISSR analysis. This technique has been already exploited to detect polymorphic differences between different date palm varieties [34-36, 46-52]. For the present investigation sample was taken from five local landraces and three introduced varieties to characterize and assess the genetic variation by using 21 ISSR primers. Among 21 ISSR primers, 17 of them produce a total of 570 scorable DNA fragments. The average per cent polymorphism across the 17 primers within local landraces was higher than the polymorphism within introduced varieties, however the average polymorphism obtained among all samples of collected date palm was higher than the result for both introduced varieties and local landraces. The average polymorphism obtained among all samples of collected date palm was also higher than a similar report on date palm diversity using 29 ISSR primers reported that, 54.5% genetic diversity among 18 female date palm [53]. Another study using 8 RAPD observed 36% of polymorphism and 32.09 % of polymorphism observed using 8 ISSR primers [54] and 28 AFLP primers in combinations to examine the phylogenetic relationships among five Upper Egypt date palm cultivars and observed 41.59% polymorphism across cultivars [18]. However the polymorphism percentage obtained among all samples of collected date palm was less when compared to a date palm diversity study [46] which reported 95% genetic diversity using 45 genotypes of date palm, with 37 RAPD

primers and 90% polymorphism with 53 ISSR primers [2] from five AFLP primer combinations to analyze 3 date palm cultivars and found an average polymorphism of 72%. The band size ranged from 250 to 550 bp with primer ID 438243 and 438245 respectively and it was less than those found by two groups, [50, 55] and who amplified the bands sizes ranged from 200 to 2000-bp and 100 to 1400 bp respectively. The product size obtained in our study is somewhat similar with a study [56], obtained 50-700 bp.

For vulnerability reduction and crop improvement in plants, genetic diversity is the valuable tool which can be used in several breeding programs to enhance the genetic variation through crossing the cultivars at high genetic distance or introgression of the exotic germplasm in base population. In our study the overall range of the genetic distance among all study samples of date palm was found to be 0.1402 to 0.5953, which is very low compared with a result (0 to 4.04) reported [54]. As the dendrogram and genetic distance value indicate the polymorphism/genetic divergence within local date palms grown in Afar region is high. These variations were expected due to the dioecious nature of date palm trees. Furthermore, the dendrogram revealing the relationships between date palm tree which is local landrace ABRD and introduced variety Kahlas was relatively close than between two landraces ABRD and Harissa. PIC is a measure that indicates allelic richness and discriminatory power of the markers [57], in this study the polymorphic information content found to be from 0.566 to 0.85, which is higher than those obtained [36, 53, 58] which ranges 0.22 to 0.3, 0.12 to 0.38 and 0.31 to 0.37 respectively. In the present study, the mean for Shannon's Information index was 0.32 which is in agreement with the result reported [30] which was 0.32, and was less than the result obtained by another group [58] and was 0.57.

## Conclusion And Recommendation

Diversity analysis in date palm has been done by morphological traits, biochemical and molecular markers. Morphological and biochemical markers have shortages such as long juvenile phase, high cost, long-term of field evaluation, environment factors and limited number of available phenotypic markers. However, since molecular markers are detectable at all stages of development and can cover the entire genome, they, can detect variation at DNA level, which overcome most limitations of morphological and biochemical markers. Keeping these in view, molecular markers were employed for the characterization of date palm landraces and varieties grown in Afar region and as the dendrogram and genetic distance value indicate the polymorphism/genetic divergence within local date palms grown in Afar region is high. These variations were expected due to the dioecious nature of date palm trees. Furthermore, the dendrogram revealing the relationships between date palm tree which are local landrace ABRD and introduced variety Kahlas were relatively close than between two landraces ABRD and Harisa. The result obtained for polymorphic information content during present investigation shows that the ISSR markers used for this study have high discriminating power.

Even though results are obtained from present investigation on the genetic diversity of date palm found in the Afar region using ISSR markers, to have better understanding on genetic diversity of date palm in the Afar region further research should be done using morphological parameters and other molecular markers with better area coverage to include date palm trees that are not studied in this investigation. Up

to date all the landraces of date palm in Afar region are not registered properly, consequently there will be genetic erosion due to expansion of improved varieties thus proper registration should be done for local landraces.

## Methods

### Ethics statement

No specific permissions were required for the activities conducted in this study. The study did not involve endangered or protected species. The research work was carried out in molecular laboratory of Institute of Biotechnology, University of Gondar during the academic year 2017-2018.

### Study area description

The samples were collected from three woredas of Afar National regional state which is located in the northeast part of Ethiopia. It is geographically located between 39.34' and 42.28' East Longitude and 8.49' and 14.30' North Latitude. The region shares boundary with four national regional states in the northwest Tigray region, in south west Amhara region, in south Oromiya and in southeast Somalia region and has two International boundaries, in east Djibouti and northeast Eritrea. Gewanē woreda is found at 10.17° North latitude, 40.65° East longitude and 618 meters elevation above the sea level and it is part of Zone One of Afar Regional State. The annual mean temperature and rainfall of the woreda is 29.5oC and 500 ml respectively. The soil of the woreda is 60% loam, 20% sandy and 20% clay. Afambo is one the woredas under Zone one of Afar regional state and situated at 11o, 14' North latitude, 41o, 39' East longitude. The altitude of the woreda is 300 meters above sea level. Its annual rainfall is 200–250 ml. The average annual temperature of this woreda is 35oC. Awash River is the main source of water for this woreda. The soil of the woreda is composed of 98% of loam and 2% of it is sandy. Aysaita woreda is also part of Zone one of Afar regional state and situated at 11°, 34' North latitude, 41°, 26' East longitude and the altitude of the woreda is 348 meters above sea level. The annual mean temperature and rain fall is 32.55oC and 10.4 ml respectively. The soil of the woreda is composed of 85% of loam and 15% of it is sandy [59, 60].

### Plant material, DNA extraction and quantification

#### Plant material

For the present experimental study, fresh tender disease free leaves from 8 to 10 year old date palm (three introduced tissue cultured varieties of England and five local landraces) (*Phoenix dactylifera* L.) were collected from agro- pastoral's field and APARI research sites in Gewane, Aysaita and Afambo woredas of afar region. These areas represent more than 85% of the total date palm Ethiopia. The collected samples are transported from Afar region to University of Gondar and stored at -20o C and used for extraction of genomic DNA and further analysis. The entire laboratory work was done in the molecular biology laboratory of Institute of Biotechnology, University of Gondar, Gondar, Ethiopia during the

academic year 2017-2018. Analyses were performed on individual trees belonging to the 8 cultivars (Table 1), with 2 replications for each cultivar.

Figure 6. The sites of collected date palm samples from Afar region of Ethiopia [61]

Table 5: Details of Ethiopian date palm varieties collected used in this study.

### **Extraction of genomic DNA and quantification**

Deep freeze stored tender leaves were lyophilized using freeze drying chamber (model BK-FD10S, Biobase, Shandong Co. Ltd.). Freeze dried leaves were grounded by kitchen blender (Mixer grinder Preeti, India). Total DNA of 8 date palm cultivars were isolated as per Doyle and Doyle (1987) [62] with slight modifications in buffer composition and incubation period (Unpublished data). 500 mg of leaf tissue was taken and 800 µl extraction buffer was added and leaf tissue macerated gently for few seconds and kept at 65°C for 2 hours 30 minutes. Then sample was cooled to room temperature and centrifuged at 10,000 rpm for 10 minutes. Aqueous layer was transferred to fresh eppendorf tube and equal volume of chloroform:isoamyl alcohol (24:1) was added and mixed by gentle inversion for 5-6 times. The contents were then centrifuged at 12,000 rpm for 10 minutes. This step is repeated twice. Supernatant was transferred to fresh eppendorf tube and mixed with double volume of chilled Isopropanol and incubated at -20°C for overnight. On the next day, the solution was centrifuged at 10,000 rpm for 25 minutes and pellet was collected. Pellet was washed twice with 300 µl of 70% ethanol followed by centrifugation at 10,000 rpm for 10 minutes. Pellet was dried and re-suspended in 100 µl of 1X TE buffer. Purified total DNA was quantified and its quality was checked by Nano drop reader (model: Optizen nano Q) and stored at -20° C till further use.

### **Reagents**

PCR buffer, Taq DNA polymerase, magnesium chloride, dNTPs were purchased from Solis Biodyne and provided by Institute of Biotechnology, University of Gondar.

### **ISSR genotyping**

DNA polymorphism was detected by polymerase chain reaction (PCR) using ISSR primers. We used 21 ISSR primers in this study (Table 6). A set of 21 primers composed wholly of defined, short tandem repeat sequences with anchor, and representing different microsatellites (di and tri-repeats) have been used as generic primers in PCR amplification of inter simple sequence repeat regions (Bioserve, Hyderabad, India). Template DNA: Crude extracts of DNA from date palm leaf samples, dNTPs mixture of 100 mM, thermal cycler (TECHNE-TC-412) was used for DNA amplification.

Table 6: List of different ISSR markers, primer sequences, %GC, annealing temperature, number of fragments amplified, polymorphic bands primers (Bioserve Technologies (India) Pvt. Ltd, a CGI company) used for ISSR analysis of 8 date palm cultivars of Afar region of Ethiopia. \*Y = C/T, R = A/G

## Preparation of master mixture

PCR master mix was standardized by changing the quantity of each component and optimum concentration of each component in master mix which gave better amplification was chosen for amplification purpose (Unpublished data). Initially the thermo profile of PCR was standardized by changing the temperature of each step in PCR cycle i.e. initial denaturation, denaturation, annealing, initial primer extension and final extension.

Thermal cycling: Sterile eppendorf tubes were numbered from 1 to 8 for each primer and 2µl of template DNA from each plant cultivar was taken and 23 µl of master mix was added to all the tubes and gently mixed with the of magnetic spinner and DNA was amplified for 40 cycles and samples were kept at 4°C until the samples loaded for agarose gel electrophoresis.

## Agarose gel electrophoresis

Amplified DNA products of ISSR reactions were separated on 3.0% agarose gel electrophoresis containing Ethidium Bromide (1 µg/ml) in 1x TAE Buffer (pH 8.0) by applying constant voltage of 80 volts for 1 hour. 100 bp DNA ladder was used to calculate the DNA bands (Sambrook *et al.*, 1989). Gels were visualized using a gel documentation system (Bio-Rad, U.S.A) and photographed using UV viewing cabinet model WO-9403F. The size of the amplicons were estimated by comparing with 1kb DNA ladder (Fermentas, Germany). Experiment was repeated for twice and only reproducible patterns were used for data analysis.

## Statistical analysis

ISSR markers across 8 date palm varieties were scored for their presence (1) or absence (0) of bands for each primer. Based on presence/absence data, genetic dissimilarity was calculated to estimate all pairwise difference in the amplification products for all genotypes. Based on the collected data, cluster analysis was done to estimate relationship among genotypes. Genetic identity and genetic distance was done within introduced varieties, with in local landraces and among all samples which are collected from both introduced varieties and local landraces of date palm according to Nei's Original measures of genetic identity and genetic distance, using pop GENE 32 population genetics software. All dendograms were constructed based on Unweighted Pair Group Method of Arithmetic Means (UPGMA) [63] using pop GENE 32 population genetics software. Shannon's Information index and Polymorphic information content among all samples which are collected from both introduced and local landraces were analyzed by popGENE 32 and online PIC calculator respectively.

## Declarations

### Ethics approval and consent to participate

Not applicable

### Consent for publication

Not applicable

### **Availability of data and material**

Not applicable

### **Competing interests**

The authors declare that they have no competing interests

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DT sponsored by his host organization (APRI, Afar region, Ethiopia), is a research staff gets salary and contingency (to spend on his travel, and so) during his master's program in Biotechnology, at loB, University of Gondar, Ethiopia and no fund was received by the any of the researcher to carry out the present research and facilities and chemicals were provided by the loB, UoG.

### **Authors' contributions**

**MI, MT, DT** contributed in conceiving the research design

**DT and MT** performed the experiments

**MI and MT** overall supervision and management

**DT, MI and MT** the acquisition, analysis, interpretation of data

**DT and MI** have drafted the work

**MI, DT and MT** substantively revised the draft

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## **References**

1. El-Hadrami A, Al-Khayri JM. Socioeconomic and traditional importance of date palm. Emir J Food Agric. 2012; 24 (5): 371-385.
2. Diaz S, Pire C, Ferrer J, Bonete M. Identification of Phoenix dactylifera L. varieties based on amplified fragment length polymorphism (AFLP) markers. Cellular and Molecular Biology Letters. 2003; 8:891-899. PMID: 14668912.

3. Zaid A, Wet PFD. Origin, geographical distribution and nutritional values of date palm. Date Production Support Programme. FAO. 2002; 25-40.
4. Zohary D, Hopf M. Domestication of plants in the old world: The origin and spread of cultivated plants in West Asia, Europe, and the Nile Valley. Oxford University Press, Oxon, UK, 2000.
5. Demeli M. Date palm research in Wererar Agricultural Research Center (WARC), Ethiopian Institute of Agricultural Research, Addis Ababa: Ethiopia, 2013.
6. Hussen AH. Potential of Date Palm Plantation in Afar Region, Ethiopia and Its Market. *Acta Horticulturae*. 2010;882:pp.43–48..<http://www.nal.usda.gov/>. DOI: 10.17660/ActaHortic.2010.882.3.
7. Salah MB. Date Palm Status and Perspective in Sub-Saharan African Countries: Burkina Faso, Chad, Ethiopia, Mali, Senegal, and Somalia,” *Date Palm Genetic Resources and Utilization*, pp. 369–386, 2015.
8. FAO (Food and Agriculture Organization). 2019.  
<http://www.fao.org/news/story/en/item/1184643/icode/> <http://faostat.fao.org>.
9. Bashah M. Date variety in the Kingdom Of Saudi Arabia, In: King Abdulaziz Univ. guidance booklet palms and dates. King Abdul Aziz Univ. Press Riyadh Saudi Arabia. 1996; p 1225-1319.
10. Al Khateeb AA, Ali-Dinar HM. Date palm in Kingdom of Saudi Arabia: cultivation, production and processing. Translation, Authorship and Publishing Center, King Faisal University, Kingdom of Saudi Arabia. 2002; p 188.
11. Krueger RR. Date palm germplasm. In: Jain SM, Al-Khayri JM, Johnson DV (eds) *Date palm biotechnology*. Springer, Dordrecht. 313–336, 2011.
12. Aregawi L, Melkamu A, Mossa E. Date Palm Production Practices and Constraints in the Value Chain in Afar Regional State, Ethiopia. *Hindawi Advances in Agriculture*. 2018; 1-10.  
[doi.org/10.1155/2018/6469104](https://doi.org/10.1155/2018/6469104).
13. Monire M, Ali Ashraf M. Molecular identification and genetic diversity of Iranian date palm (*Phoenix dactylifera* L.) cultivars using ISSR and RAPD markers. *Aust J Crop Sci*. 2013; 7(8):1160-1166.
14. Maria D, Angela P, Chialexei L. Characteristics of RAPD markers inbreeding of *Cucumis sativus* L. *Roum Biotech Lett*. 2008; 13:3843–3850.
15. Khadari B, Breton C, Moutier N, Roger JP, et al. The use of molecular markers for germplasm management in a French olive collection. *Theor Appl Genet*. 2003; 106:521–529.
16. Dhanapal AP. Genomics of crop plant genetic resources. *Adv Biosci Biotechnol*. 2012; 3:378– 385.
17. Ahmed T. Genetic and environmental variation among Qatari date palm cultivars assessed by DNA markers. Paper presented at symposium on the Foundation Annual Research Forum Proceedings of

Energy and Environment, Qatar University, Doha, December 2010.

18. Adawy SS, Hussein EHA, El-Khishin D, Saker MM, et al. Genotyping Egyptian date palm cultivars using RAPD, ISSR, AFLP markers and estimation of genetic stability among tissue culture derived plants. Arab J Biotech. 2004; 8(1): 99-114.
19. Al-Hadidi SH, Al-Agha HN, Ahmed T. Genetic diversity of date palm in Qatar. Paper presented at symposium on the Foundation Annual Research Forum Proceedings of Energy and Environment, Qatar University, Doha, December 2010.
20. Zehdi S, Trifi M, Ould Mohamed Salem A, et al. Survey of inter simple sequence repeat polymorphisms in Tunisian date palms (*Phoenix dactylifera* L.). J Genet Breed. 2002; 56:77/83.
21. Hussein EH, Adawy SS, El-Khishin D, Moharam H, El-Itriby H. Genetic variability studies and molecular fingerprinting of some Egyptian date palm (*Phoenix dactylifera* L.) cultivars. I. A preliminary study using RAPD markers. Arab J Biotechnol. 2002; 5:217–224.
22. Khierallah HS, Bader SM, Baum M, Hamwiah A. Assessment of genetic diversity for some Iraqi date palms (*Phoenix dactylifera* L.) using AFLP markers. J Amer Soc Hort Sci. 2011; 136(4):282–287. 2011.
23. Billotte N, Marseillac N, Brottier P, Noyer JL, et al. Nuclear microsatellite markers for the date palm (*Phoenix dactylifera* L.): Characterization and utility across the genus *Phoenix* and in other palm genera. Mol. Ecol. Notes. 2004; 4:256– 258.
24. Adawy SS, Hussein Ebtissam HA, ElKhishin D, Moharam H, ElItriby HA. Genetic variability studies and molecular fingerprinting of some Egyptian date palm (*Phoenix dactylifera* L.) cultivars: II. RAPD and ISSR profiling. Arab J. Biotech. 2002; 5(2): 225-236.
25. Ben Saleh M, El-Helaly R. Using ISSR to investigate the similarities and dissimilarities between some Tunisian date palm (*Phoenix dactylifera* L.) cultivars. International Conference on Date Palm, Kingdom of Saudia Arabia, Ministry of Higher Education, King Saud University, Qaseem branch, College of Agriculture and Veterinary Medicine (16-19 September 2003).
26. Reddy MP, Sarla N, Siddiq EA. Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. Euphytica. 2002; 128: 9-17.
27. Gürcan K, Mehlenbacher SA, Cristofori V. Inter-simple sequence repeat (ISSR) markers in hazelnut. Acta Hort. (ISHS). Vol. 845: pp. 159-162, 2009.
28. Venkatachalam L, Sreedha RV, Bhagyalakshmi N. Micropropagation in banana using high levels of cytokinins does not involve any genetic changes as revealed by RAPD and ISSR markers. Plant Growth Reg. 2007; 51:193-205.

29. Joshi P, Dhavan V. Assessment of genetic fidelity of micropropagated *Swertia chirayita* plantlets by ISSR marker assay. *Biol Plant*. 2007; 51: 22-26.
30. Santos MDM, Buso GCS, Torres AC. Evaluation of genetic variability in micropropagated propagules of ornamental pineapple [*Ananas comosus* var. *bracteatus* (Lindley) Coppens and Leal] using RAPD markers. *Genet Mol Res*. 2008; 7: 1097-1105.
31. Ahmed O, Chokri B, Noureddine D, Mohamed M, Mokhtar T. Regeneration and molecular analysis of date palm (*Phoenix dactylifera* L.) plantlets using RAPD markers. *African J. Biotech*. 2009; 8: 813-820.
32. Huang WJ, Ning GG, Liu GF, Bao MZ. Determination of genetic stability of long-term micropropagated plantlets of *Platanus acerifolia* using ISSR markers. *Biol Plant*. 2009; 53: 159-163.
33. Moghaieb REA, Abdel-Hadi AA, Ahmed MRA, Hassan AGM. Genetic diversity and sex determination in date palms (*Phoenix dactylifera* L.) based on DNA markers. *Arab J Biotech*. 2010; 13(2): 143-156.
34. Purayil FT, Robert GA, Gothandam KM, Kurup SS, et al. Genetic variability in selected date palm (*Phoenix dactylifera* L.) cultivars of United Arab Emirates using ISSR and DAMD markers. *3 Biotech*. 2018;109:1-8.
35. Guettouchi A, Elshibli S, Haider N, Nabulsi I, Ykhlef N. Molecular diversity in date palm (*Phoenix dactylifera* L.) cultivars from Algeria indicated by RAPD and ISSR polymorphism. *Plant Cell Biotech and Mol Bio*. 2017; 18(1&2): 76-89.
36. Marsafari M, Mehrabi AA, Tahmasebi Z. The identification of RAPD and ISSR informative markers with some quality traits of fruit in some of Iranian date palm. *Inter J of Plant Anim and Env Sci*. 2014; 4(2): 714-722.
37. Chao CT and Krueger RR. The Date Palm (*Phoenix dactylifera* L.): Overview of Biology, Uses, and Cultivation. *HortScience*. 2007; 42 (5): 1077–1082. <https://doi.org/10.21273/HORTSCI.42.5.1077>.
38. Saker MM, Moursy HA. Molecular characterization of Egyptian date palm: II – RAPD fingerprints. *Arab J Biotech*. 1999; 2: 71–78.
39. Jubrael JMS, Udupa SM, Baum M. Assessment of AFLP-based genetic relationships among date palm (*Phoenix dactylifera* L.) varieties of Iraq. *J Am Soc Hortic Sci*. 2005; 130: 442–447.
40. Ouarda HEF, Walker JW, Khouja ML. Phenotypic and nuclear DNA variation in Tunisian cultivars of date palm (*Phoenix dactylifera* L.) *Afr J Biotechnol*. 2012; 11: 6034–6042.
41. Zehdi S, Sakka H, Rhouma A, Ould MSA, Marrakchi M, Trifi M. Analysis of Tunisian date palm germplasm using simple sequence repeat primers. *Afr J Biotechnol*. 2004; 3:215– 219.

42. Soliman KA, Rizk RM, El-Sharabasy SF. Genetic polymorphism of semi-dry date palm (*Phoenix dactylifera* L.) cultivars in Egypt. *Egypt J Biotechnol.* 2006; 22, 261–273.
43. Soliman SS, Ali BA, Ahmed MMM. Genetic comparisons of Egyptian date palm cultivars (*Phoenix dactylifera* L.) by RAPDPCR. *Afr J Biotechnol.* 2003; 2:86–87.
44. Emoghene BO, Omorefe A, Idu M, Eke CR. Molecular characterization of some date palms in Nigeria using RAPD markers. *J Appl Biol Biotech.* 2015; 3: 026–030.
45. Zhao YL, Williams R, Prakash CS, He GH. Identification and characterization of gene-based SSR markers in date palm (*Phoenix dactylifera* L.). *BMC Plant Biol.* 2013; 12: 237.
46. Mitra C, Kharb P, Uppal S, Jain S. Genetic diversity analysis in date palm (*Phoenix dactylifera* L.): a comparative assessment using ISSR and RAPD marker assays. *The J of Hort Sci and Biotech.* 2011; 86 (4): 398-402.
47. Mirbahar AA, Markhand GS, Khan S, Kauser N, Saeed R. DNA Fingerprinting of some Pakistani Date palm (*Phoenix dactylifera* L.) cultivars using ISSR. *Pak J Bot.* 2016; 48(5): 2005-2010.
48. Karim KB, Choki S, Amel H, Wafa H. Richid. Genetic diversity of Tunisian date palm germplasm using ISSR markers. *Int J Bot.* 2010; 6: 182-186.
49. Sabir JSM, Abo-Aba S, Bafeel S, et al. Characterization of ten date palm (*Phoenix dactylifera* L.) cultivars from Saudi Arabia using AFLP and ISSR markers. *Comptes Rendus Biol.* 2014; 337: 6-18.
50. Haider N, Nabulsi I, Ali MN. Phylogenetic relationships among date palm (*Phoenix dactylifera* L.) cultivars in Syria using RAPD and ISSR markers. *J Plant Biol Res.* 2012; 1: 12-24.
51. Srivashtav VS, Kapadia CV, Mahatma MK, Jha SK, et al. Genetic diversity analysis of date palm (*Phoenix dactylifera* L.) in the Kutch region of India using RAPD and ISSR markers. *J Food Agric.* 2012; 25:907-915.
52. Munshi A, Osman G. Investigation on molecular phylogeny of some date palm (*Phoenix dactylifera* L.) cultivars by protein, RAPD and ISSR markers in Saudi Arabia. *Aust J Crop Sci.* 2010; 4: 23-28.
53. Elmeer K, Alghanem M, Al-Latifi L, Alhemairi H. Efficiency of RAPD and ISSR markers for the detection of polymorphisms and genetic relationships in date palm. *Biotechnol.* 2017; 16: 19-26.
54. Aladadi W, Moustafa MF, Alruman SA. Genetic variability among seven cultivars of date palm (*Phoenix dactylifera* L.) based on embryonic DNA of old fruit, *Kuwait J of Sci.* 2018; 45 (1): 108-114.
55. Elsheikh MH, El-Motty EZA, Elsabagh AS, Yassin R. Fruit properties and molecular characterization using ISSR markers of six Libyan date palm cultivars. *Res J of Agri and Biol Sci.* 2014; 10(1): 47-52.

56. Cao BR, Chao CT. Identification of date cultivars in California using AFLP markers. *Hort Science*. 2002; 37:966-968.
57. Boopathi NM. Genetic Mapping and Marker Assisted Selection Basics, Practice and Benefits Plant Molecular Biology & Bioinformatics Tamil Nadu Agricultural University Coimbatore , TN, India. 2013; 303.
58. Ghariani S, Elazreg H, Ghorbel NC, Chakroun M, Farah NF. Genetic diversity analysis in Tunisian perennial ryegrass germplasm as estimated by RAPD, ISSR, and morpho- agronomical markers. *Genetics and Molecular Research*. 2015; 14 (4): 18523-18533.
59. Pastoral Agriculture and Rural Development Bureau (PARDB). Basic agriculture report of year 2000.
60. Afar National Regional State Finance and Economic Development Buearu (ANRSFEDB). 2009; Regional Atlas of Afar Region.
61. Abdulatif M. Condition and chemical composition of major feed resources in chifra district of Afar regional state, Ethiopia, 2009.
62. Doyle JJ, Doyle JL. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*. 1987; 19:11-15.
63. Nei M. Molecular evolutionary genetics. Columbia University Press, New York.1987.

## Tables

[Note: Tables 1 - 3 could not be included due to technical limitations and can be found in the supplemental file section. No Table 4 was included in the submission]

Table 5: Details of Ethiopian date palm varieties collected used in this study.

Number	Date palm type	Name of the date palm	Collection location and date	Code assigned
1	Landrace	Awssa bollo red dry	Afambo, Nov 16, 2017	ABRD
2	Landrace	Awssa bollo red wet	Afambo, Nov 16, 2017	ABRW
3	Landrace	Awssa bollo white	Afambo, Nov 16, 2017	ABW
4	Landrace	Gewene	Gewene, Nov 15, 2017	G
5	Landrace	Harissa	Afambo, Nov 16, 2017	H
6	Introduced variety	Brhee	Aysaita, Nov 17, 2017	B
7	Introduced variety	Khalas	Aysaita, Nov 17, 2017	K
8	Introduced variety	Medjool	Aysaita, Nov 17, 2017	M

Table 6: List of different ISSR markers, primer sequences, %GC, annealing temperature, number of fragments amplified, polymorphic bands primers (Bioserve Technologies (India) Pvt. Ltd, a CGI company) used for ISSR analysis of 8 date palm cultivars of Afar region of Ethiopia. \*Y = C/T, R = A/G

Sr. No.	Primer Id.	*Primer sequence (5'-3')	Tm	%GC content	Total number of replicated alleles	Number of polymorphic alleles
1	438226	AGAGAGAGAGAGAGAGAGG	51	52.632	8	8
2	438227	AGAGAGAGAGAGAGAGAT	50	45	6	6
3	438228	AGAGAGAGAGAGAGAGAA	50	45	4	0
4	438229	ACACACACACACACACAT	46	44.444	8	8
5	438230	ATATATATATATATATTYC	30	5.556	0	0
6	438231	ATATATATATATATATTYG	30	5.556	0	0
7	438232	AGAGAGAGAGAGAGAGYC	48	50	8	8
8	438233	AGAGAGAGAGAGAGAGYG	48	50	4	0
9	438234	TATATATATATATATARG	30	5.556	0	0
10	438235	GAGAGAGAGAGAGAGAYT	46	44.444	8	8
11	438236	GAGAGAGAGAGAGAGAYC	48	50	8	8
12	438237	CTCTCTCTCTCTCTRG	48	50	0	0
13	438238	GTGTGTGTGTGTGTGYA	46	44.444	6	6
14	438239	ACACACACACACACACYT	46	44.444	7	7
15	438240	ACACACACACACACACYA	46	44.444	7	7
16	438241	AGAGAGAGAGAGAGAGC	47	52.941	6	6
17	438242	CAGCAGCAGCAGCAGT	49	62.5	7	7
18	438243	CAGCAGCAGCAGCAGG	51	68.75	7	7
19	438244	GCACACACACACACACA	47	52.941	5	5
20	438245	CCAGGTGTGTGTGTGTGT	50	55.558	4	4
21	438246	CTCCTCCTCCTCCTC	47	66.667	4	4

## Figures

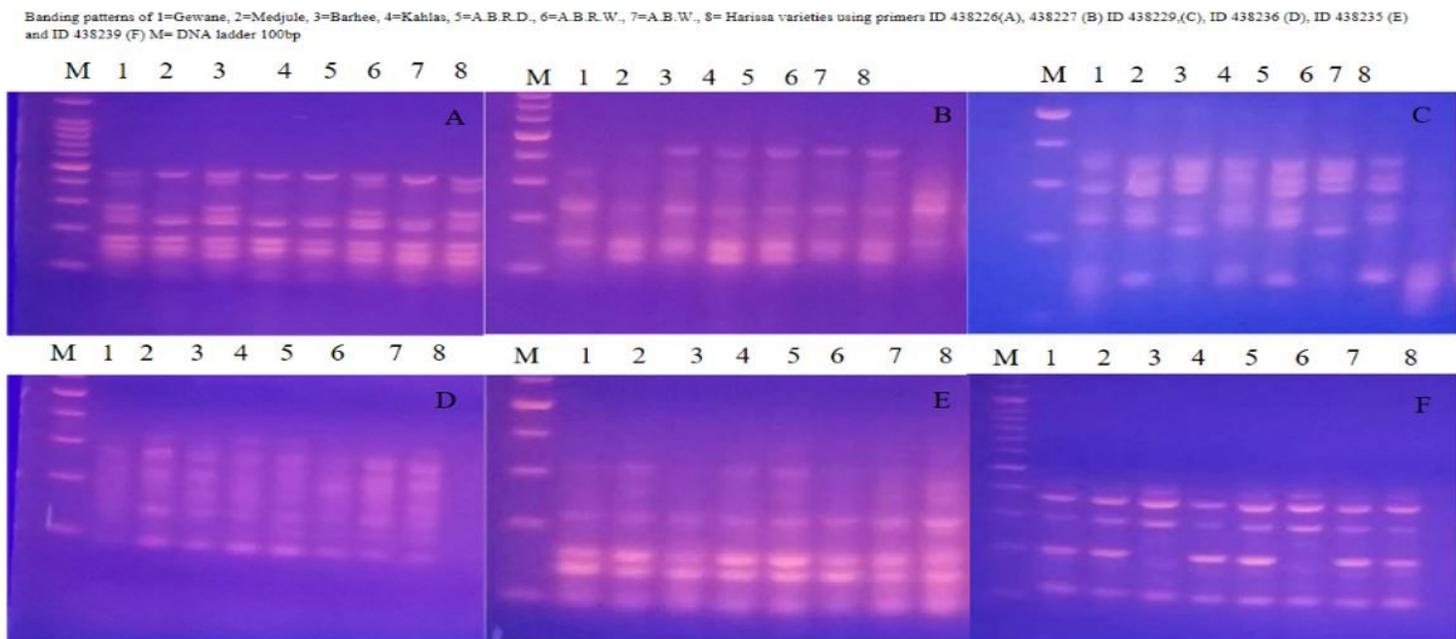
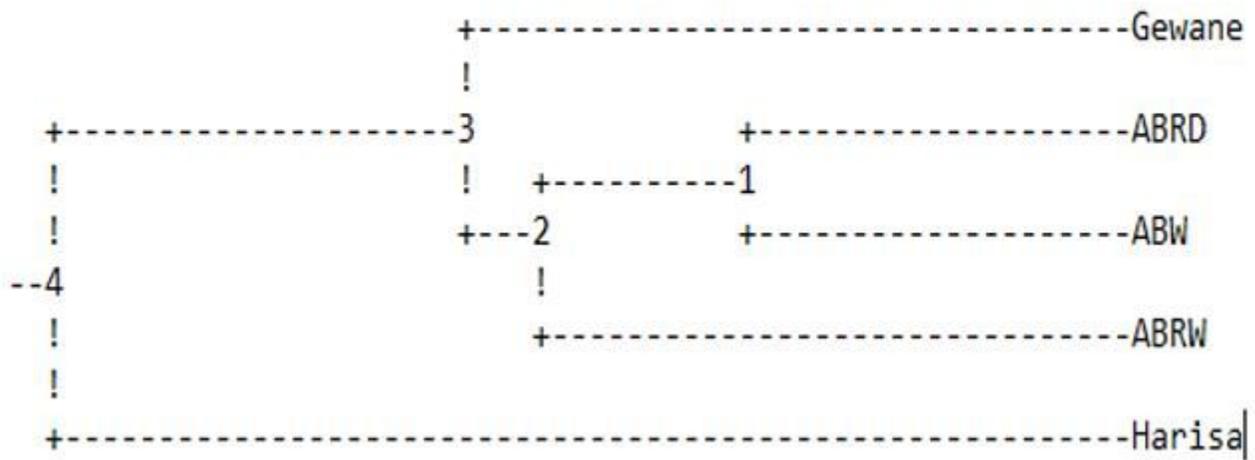


Figure 1

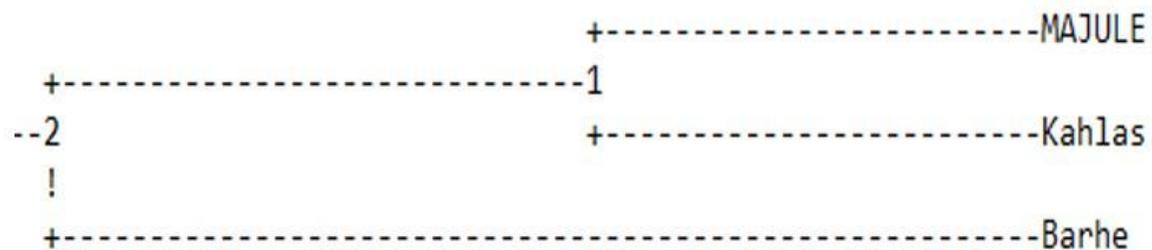
PCR amplification products obtained with ISSR primers



Between	And	Length
4	3	9.70632
3	pop4	15.83067
3	2	1.81625
2	1	4.80480
1	pop5	9.20962
1	pop7	9.20962
2	pop6	14.01443
4	pop8	25.53699

Figure 2

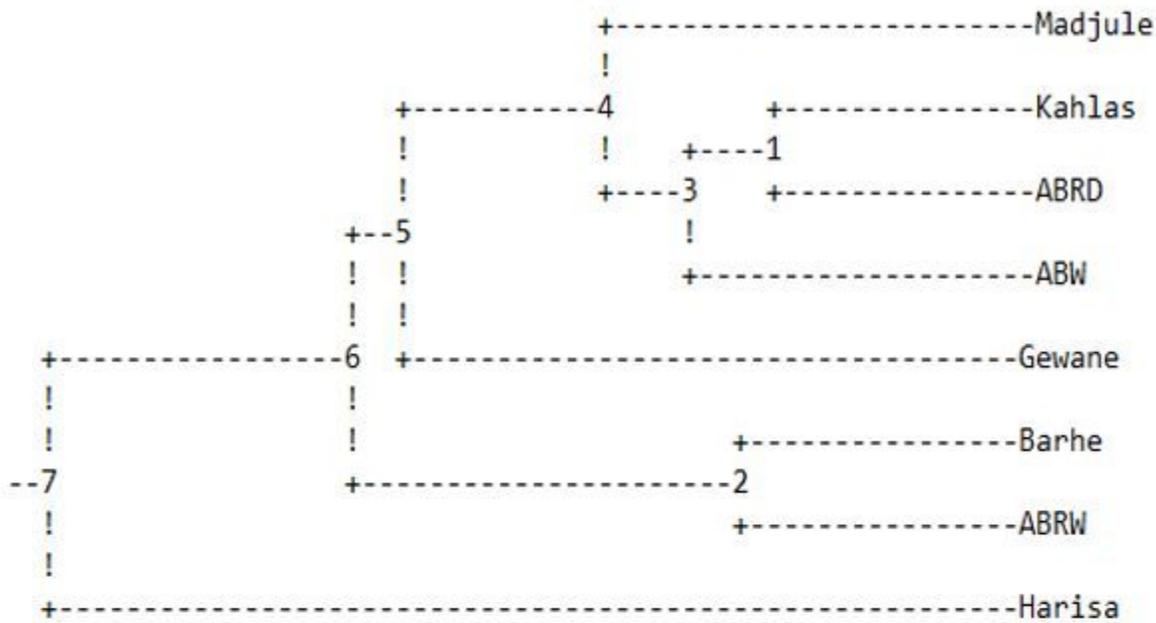
Dendrogram depicting Genetic distance within local landraces: Method = UPGMA



Between	And	Length
2	1	11.86399
1	pop1	9.77460
1	pop3	9.77460
2	pop2	21.63859

**Figure 3**

Dendrogram depicting genetic distance within introduced varieties: Method = UPGMA



Between	And	Length
7	6	7.92058
6	5	1.08572
5	4	5.30270
4	pop1	11.36310
4	3	2.15348
3	1	2.19816
1	pop3	7.01147
1	pop5	7.01147
3	pop7	9.20962
5	pop4	16.66580
6	2	10.19951
2	pop2	7.55201
2	pop6	7.55201
7	pop8	25.67210

Figure 4

Dendrogram depicting genetic distance among all collected date palms: Method = UPGMA

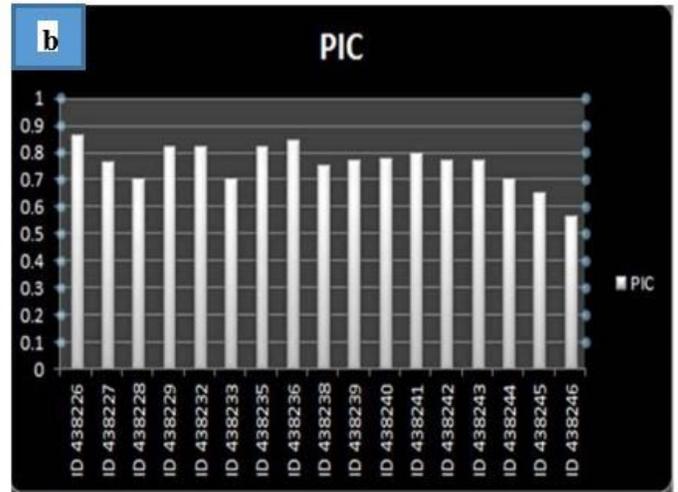
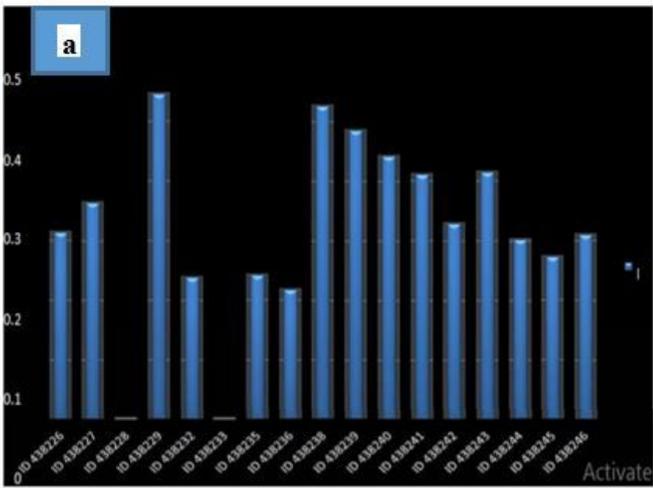


Figure 5

a and b: a) for the study of Shannon's information index and b) PIC value of primers used for the study

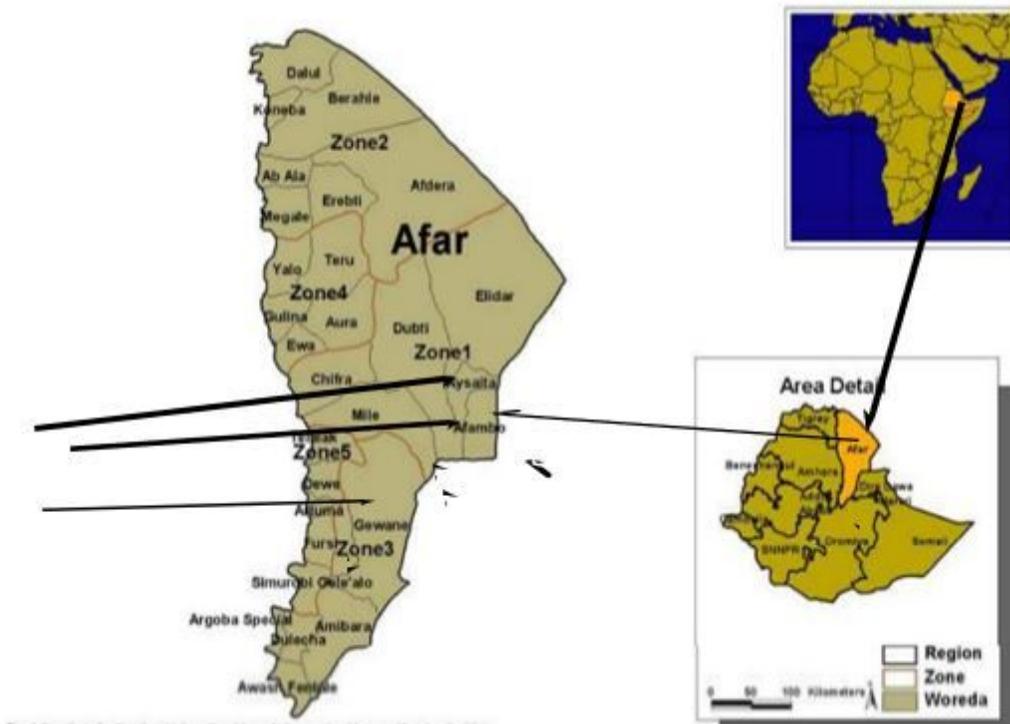


Figure 6

The sites of collected date palm samples from Afar region of Ethiopia [61]

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table3.JPG](#)
- [Table1.JPG](#)
- [Table2.JPG](#)