Genetic Diversity of Dodder (*Cuscuta Spp.*) Collected from Khartoum and Gezira States

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Abstract- The study was carried out at the field dodder (*Cuscuta spp*.) in Khartoum and Gezira states, to study variation between 28 samples of it in two States using DNA Markers (RAPD). Four primers were used (OPAL15, OPAL18, OPB17 and OPAL20) to amplify the genomic DNA of 28 samples of *Cuscuta spp*. Two primers (OPB17, OP AL20) showed high percentage (100%) to amplify the genomic DNA polymorphism bands, while the other two primers (OPAL15, OPAL18) were produced (91.7%) polymorphism bands. UPGA analysis showed RAPD Distance Matrix range (0.10-0.98) which reflected high genetic diversity. The RAPD UPGA Tree Diagram showed high Molecular Variation within States**.** The resulting of four RAPD Markers analyses were Observed that tree diagram showed two groups A and B . Group A Consists of two sub group. As sub –group A1, and sub-group A2. Sub –group A1 have two sisters (Jebal-Ramtook and Jebal-Molokha) ,(Toti-Remet and Jebal-Amoyogha) and sub-group A2 have five sisters (Alkabash Alfalfa and Alfaki-hashim- Alfalfa) ,(Wad-Alkawahala- Onion and wada-Almajzoub Onion),(Shambat-Lime and Shambat Balloon plant),(Alalaphon Alfalfa and Omdurman- lime) and (Gezira Slang Alfalfa - and Shambat - Alfalfa).A group A and B correlated Consists variables. group B Have one sisters (Hantoob- Onion and Madina-Arab, Dahaser), (Helat-kok-Ghobash) As Sub- group A1, (Algeli - Alfalfa, Toti- Alfalfa, Khartoum- Dames, wad-Alkawahala-Onion and Algeli - Ramtook) has sub-group A2, (Shambat –Euphorbia) correlate with sub-A1, And A2 was showed sample (Alalaphon Dahaser) as out group and genetically close to each other's (sisters).

Index Terms- Cuscuta, DNA, PCR, dodder

I. INTRODUCTION

Dodder (*Cuscuta spp*.), an annual holoparasitic plant of legume crops, which belongs to family *Cuscutaeceae* ,is agenus cosmopolitan occurrence , thus *Cuscuta* species are widely distribution and colonized diversity of habitats throughout the temperate and tropical zones (Belize,1987). The most common name, dodder, possibly originates from the Old German word "dotter" which means yolk (Dawson et al. 1994). Many species of *Cuscuta* have been introduced to different parts of the world due to similarity of their seed to those of commercial crops ,especially legumes like alfalfa (*Medicago sativa L*) *Cuscuta species* ,commonly known as dodder and it is one of the most invasive weeds, (Lowe *et al*.,2001). Musslman (1984), reported seven species and described their geographical distribution in country .He reported the occurrence of *C. pedicellata* ladeb, In Khartoum, Gezira and Bahr Al Gazal state, and *C . pedniflora Ten* .In southern Darfur and Red Sea state he found *C. Hyaline* and in the Northern States, Kassala, Northern Kordofan and Khartoum States. Musslman and Bebawi, (1983) reported *C.campestris* yunker, in Shambat and Toti Island and widely spread through much of Sudan as a contaminate of Lucerne seeds**.** The uses of molecular markers are based on the naturally occurring DNA polymorphism, which forms basis for designing strategies to exploit for applied purpose. A marker must to be polymorphic i.e. it must exit in different forms so that chromosome carrying the mutant genes can be distinguished from the chromosomes with the normal gene by a marker it also carries. Genetic polymorphism is defined as the simultaneous occurrence of a trait in the same population of two discontinuous variants or genotypes. DNA markers seem to be the best candidates for efficient evaluation and selection of plant material. Unlike protein markers, DNA markers segregate as single genes and they are not affected by the environment. DNA is easily extracted from plant materials and its analysis can be cost and labor effective (Kumar, 2009).

The basis of RAPD methodology is the PCR (polymerase chain reaction)which has swept through molecular biology laboratories over the last few years. In 1991 Welsh and McClelland developed a new PCR-based genetic assay namely randomly amplified polymorphic DNA (RAPD). This procedure detects nucleotide sequence polymorphisms in DNA by using a single primer of arbitrary nucleotide sequence. In this reaction, a single species of primer anneals to the genomic DNA at two different sites on complementary strands of DNA template. If these priming sites are within an amplifiable range of each other, a discrete DNA product is formed through thermo cyclic amplification. On an average, each primer directs amplification of several discrete loci in the genome, making the assay useful for efficient screening of nucleotide sequence polymorphism between individuals (William *et al*., 1993). However, due to the

stochastic nature of DNA amplification with random sequence primers, it is important to optimize and maintain consistent reaction conditions for reproducible DNA amplification. RAPDs are DNA fragments amplified by the PCR using short synthetic primers (generally 10 bp) of random sequence. These oligonucletide serve as both forward and reverse primer, and are usually able to amplify fragments from 1–10 genomic sites simultaneously. Amplified products (usually within the 0.5–5 kb size range) are separated on agarose gels in the presence of ethidium bromide and view under ultraviolet light (Jones *et* al., 1997) and presence and absence of band will be observed. These polymorphisms are considered to be primarily due to variation in the primer annealing sites, but they can also be generated by length differences in the amplified sequence between primer annealing sites. Each product is derived from a region of the genome that contains two short segments in inverted orientation, on opposite strands that are complementary to the primer (Kumar, 2009). The aim of this study to identify *Cuscuta spp*. in Khartoum and Gezira States and determine variation between dodder samples by DNA markers.

II. Materials and Methods

1. Plant Materials

The fresh tissues of the *Cuscuta spp*. samples were carefully collected from different areas in Khartoum and Gezira States without any parts of host plant, cleaned, labeled and covered by Foil Aluminum then stored in liquid nitrogen. Samples of *Cuscuta spp.* transferred to the laboratory and frozen until use

2. DNA Extraction

DNA was extracted from fresh tissues of *Cuscuta spp*. colluded using modified CTAB method. The modification was made in intention to improve the DNA quantity and the quality. In this method the fine powdered plant materials was frozen - dried tissue powder with a pestle and mortar after frozen in liquid nitrogen. Samples immediately transferred into 15 ml Falcon Tubes containing 6 ml CTAB. the samples were then incubated in a water path at 60ºC with gentle shaking for 30 minutes. As shaking after each 5minutes and left to cool at room temperature for 10 min. Chloroform: Isoamylalcohol mixture (24:1) was added to each tube and the phases were mixed gently for 10 min at room temperature to make a homogenous mixture. The cell debris was removed by centrifugation at 4000 rpm for 15 minute and the resulted clear aqueous phases (containing DNA) were transferred to new sterile tubes. The step of the chloroform: Isoamylalcohol extraction was repeated twice. The nucleic acids in the aqueous phase were precipitated by adding equal velum of deep cooled Isopropanol let the DNA to settle down over night in Refrigerator. The contents were mixed gently and collected by centrifugation at 4000 rpm for 5 minute. The formed DNA pellet was washed twice with 70% ethanol and the ethanol was discarded. Let ethanol and DNA to dry room temperature. Then add TE buffer and store in –20^{O}C. The amount of TE to be added depends on the amount of DNA. Allow the DNA to dissolve in TE before storing in –20^{O}C. for further use DNA.

3. PCR of the RAPD Technique

The PCR reactions were carried out in 25 µl volume containing 15µl sterile distilled water, 2.5 µl 10Xbuffer, 2.5 µl (2 mM/µl) DNTPs, 1.5 µl (50 mM) $MgCl_2$, 2 µl (10 pmol/µl) primer, 0.5 µl (5u/ µl), *Taq* DNA polymerase and 1 µl (10 to30 ng/µl) template DNA, for each sample. The PCR amplification protocol was programmed for 5 min at 94°C for initial denaturation, follows by 40 cycles of 1min at 94°C, 1 min at (36°C) and 1 min at 72°C, final extension was programmed for 7min at 72°C followed by hold time at 4°C until samples were collected.

4. Documentation

The extracted DNA samples with use Ethidium Bromide and agarose gel the 1% agarose gel stained with 0.5µl (1mg/100ml .was used Electrophoresis was done at 80 Volts. The separate fragments were visualized with an ultraviolet (UV) transilluminator.As Visualized and photographed under a UV transilluminator.As. A sample without template DNA was included as a negative control in each experiment to check contamination. The sizes of DNA fragments were estimated by comparison with standard ladder.

5. RAPD Data Analysis

The number of polymorphic and monomorphic bands was determined for each primer. Genotypes were scored (1) for present band, and (0) for absent band and then entered into a data matrix. Percentage of polymorphism was calculated as the following equation: (polymorphic bands/total number of bands x 100). The tree diagram was produced by clustering the similarity data with the UPGMA method using ***STATISTICAver.10***.

III. Result and Discussion

1. DNA extraction

To isolate high quality of DNA, the CTAB-based procedure optimized in the present study, yielded good quality DNA free of phenols, which may inhibit the activity of Taq polymerase. Four primers tested for amplification of the 28 accessions (*Cuscuta spp*.) two primers showed high percentage of polymorphic bands (100%) (**OPAL20, OPB17**) the four primers were selected and used to evaluate the degree of polymorphic and genetic relationships among the genotypes under study Total of 41 amplified fragments were distinguished across the selected primers and the ***Statistical*** analysis showed 39 polymorphic bands among the accessions. The maximum number of bands were produced by primers (**OPAL15**, **OPA18**) with 91.7% polymorphism, while the minimum number of fragments' pattern produced by OPB17 (8 bands) with 100% polymorphism. RAPD fragments' pattern produced by four primers is showed in Table 1, Figure 1, 2,3and 4.

Table 1 Polymorphism and percentages of Monomorphic detected by the use of Four RAPD primers on 28 Sample *Cuscuta spp*.

Primer	Sequence	Total number of bands	Number of polymorphic	Number of monographic	Percentage of polymorphic bands	Percentage of Monomorphic bands
OPAL15	AGGGGACACC	12	11	1	.7%91	8.3%
OPAL20	AGGAGTCGGA	9	9	0	100%	0%
OPA18	AGGTGACCGT	12	11	1	91.7%	8.3%
OPB17	AGGGAACGAG	8	8	0	100%	0%
Total		41	39	2	383.4	16.6
Average		10.3	9.7	0.5	95.85	4.15

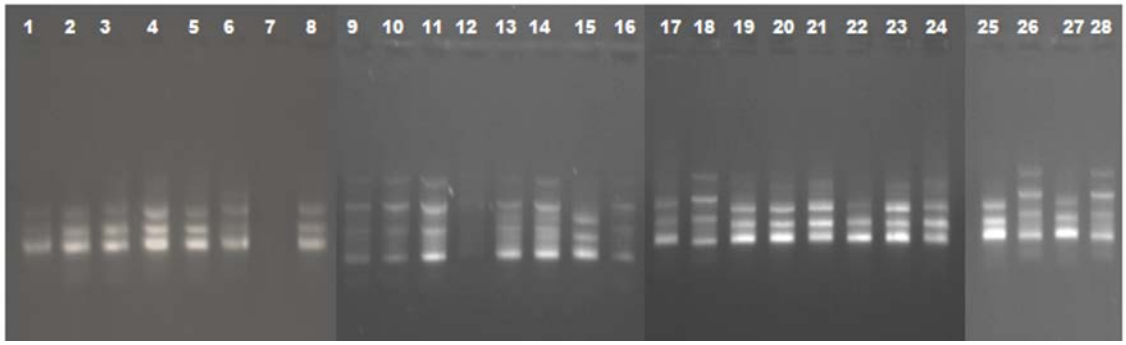

Fig. 1 RAPD analysis with primer: OPB-17 (lanes-1-8), OPB-17 (lanes-9-16), OPB-17(lanes-17-24), OPB-17 (lanes-25-28) on genomic DNA isolated from

(Ramtook ,Euphorbia ,Alkabash, Alafalfa, Remet, Amoyogha, Molokhia, Alafalfa),(Ramtook, Alafalfa, Alafalfa, Alafalfa,Dhaser Alafalfa, lime ,Damas) ,(lime ,Baloonplant ,Alafalfa, Alafalfa ,Onion, Onion , Onion, Alafalfa),(Onion, Dahasr,Lokha,Onion).

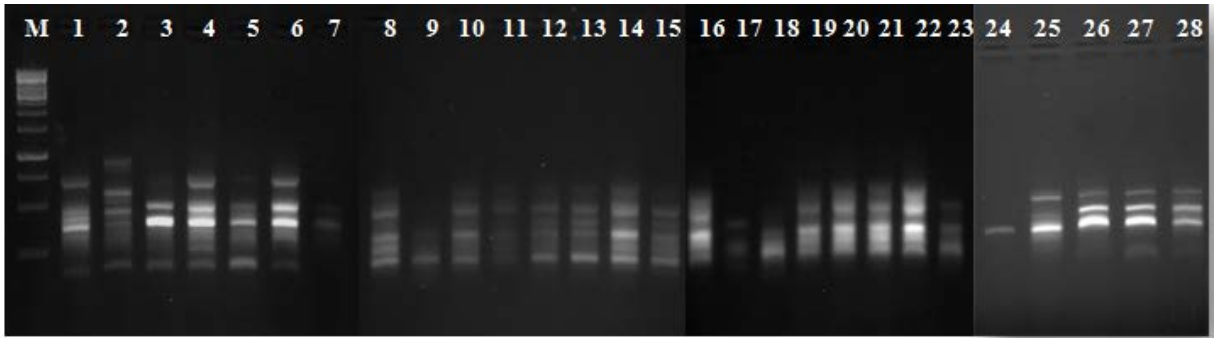

Fig. 2 RAPD analysis with primer: **OPAL-18** (lanes-1-7), **OPAL-18** (lanes-8-15**), OPAL-18** (lanes-16-23), **OPAL-18** (lanes-24-28) on genomic DNA isolated from (Ramtook ,Euphorbia ,Alghbash, Alafalfa ,Remet, Amoyogha, Molokhia),(Alafalfa, Ramtook, Alafalfa, Alafalfa, Alafalfa,Dhaser, Alafalfa,Lime),(Damas,Lime,Baloon plant, Alafalfa , Alafalfa, Onion,Onion,Onion),(Alafalfa, Onion, Dahasr,Lokha,Onion).

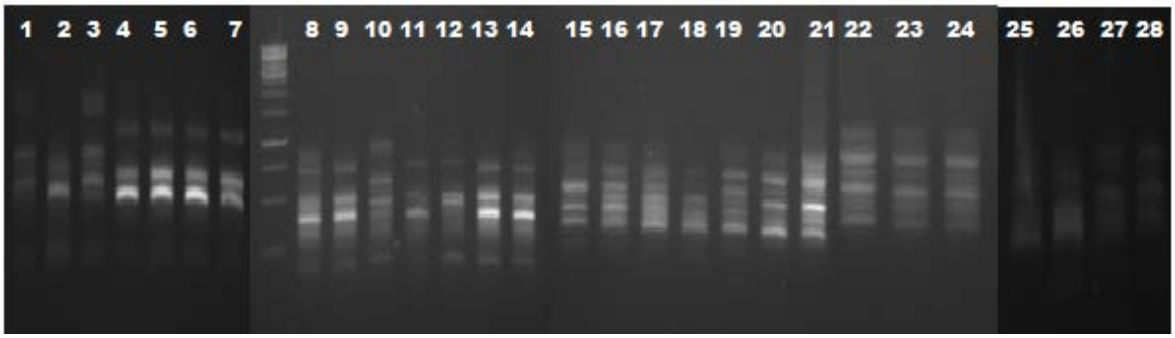

Fig. 3 RAPD analysis with primer: **OPAL 15** (lanes-1-7), **OPAL 15** (lanes-8-14**), OPAL 15** (lanes-15-21) **OPAL 15** (lanes-22-24) (lanes-25-28), on genomic DNA isolated from (Ramtook ,Euphorbia ,Alghbash, Alafalfa ,Remet, Amoyogha, Molokhia) ,(Alfalfa, Ramtook, Alafalfa, Alafalfa, Alafalfa,Dhaseir, Alafalfa),(Lime, ,Damas, Lime, Baloonplant, Alafalfa, Alafalfa,Onion),(Onion, Onion, Alfalfa),(Onion, Dahasr,Lokha,Onion).

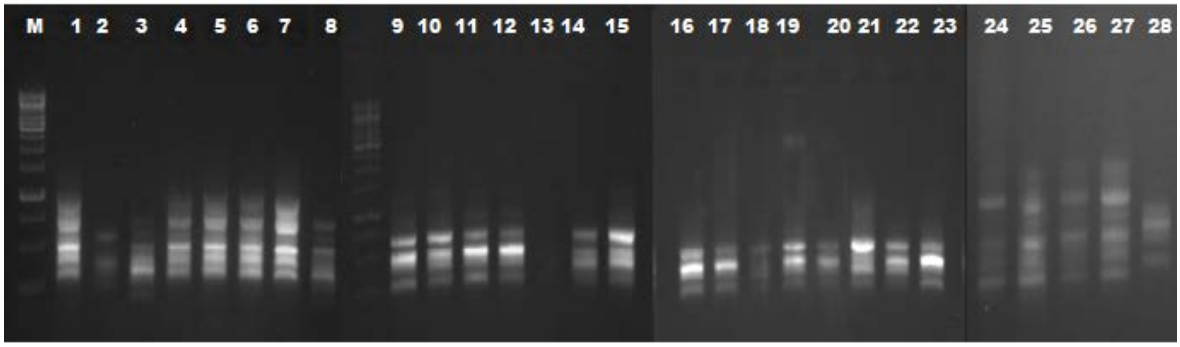

Fig. 4 RAPD analysis with primer: **OPAL-20** (lanes-1-8), **OPAL-20** (lanes-9-15**), OPAL-20** (lanes-16-23) **OPAL 20** (lanes-24-28) on genomic DNA isolated from
(Ramtook , Euphorbia ,Alghbash, Alfalfa ,Remet, Amoyogha, Molokhia), Alafalfa),(Ramtook, Alfalfa, Alfalfa, Alafalfa,Dhaseir, Alfalfa, Lime,),(Damas, Lime, ,Baloonplant, Alfalfa , Alafalfa, Onion,Onion,Onion),(Alafalfa,Onion, Damas dahaseir,Lokha,Onion).

2. The tree diagram-

The resulting of four RAPD Markers analyses were Observed that tree diagram showed tow groups A, and B .group A Consists of two sub group. As sub –group A1, and sub-group A2. Sub –group A1 have two sisters (Jebal-Ramtook and Jebal-Molokha) ,(Toti-Remet and Jebal- Amoyogha) and sub-group A2 have five sisters (Alkabash Alfalfa and Alfaki-hashim- Alfalfa) ,(Wad-Alkawahala Onion and wada-Almajzoub Onion),(Shambat-Lime and Shambat Balloon plant),(Alalaphon Alfalfa and Omdurman- lime) and (Gezira Slang Alfalfa - and Shambat - Alfalfa).A group A and B correlated Consists variables. group B Have one sisters (Hantoob- Onion and Madina-Arab, Dahaser), (Helat-kok-Ghobash) As Sub- group A1, (Algeli - Alfalfa, Toti- Alfalfa,

Khartoum- Dames, wad-Alkawahala-Onion and Algeli - Ramtook) has sub-group A2, (Shambat –Euphorbia) correlate with sub-A1, And A2 was showed sample (Alalaphon Dahaser) as out group and genetically close to each other's (sisters). **Figure 5 UPGA**

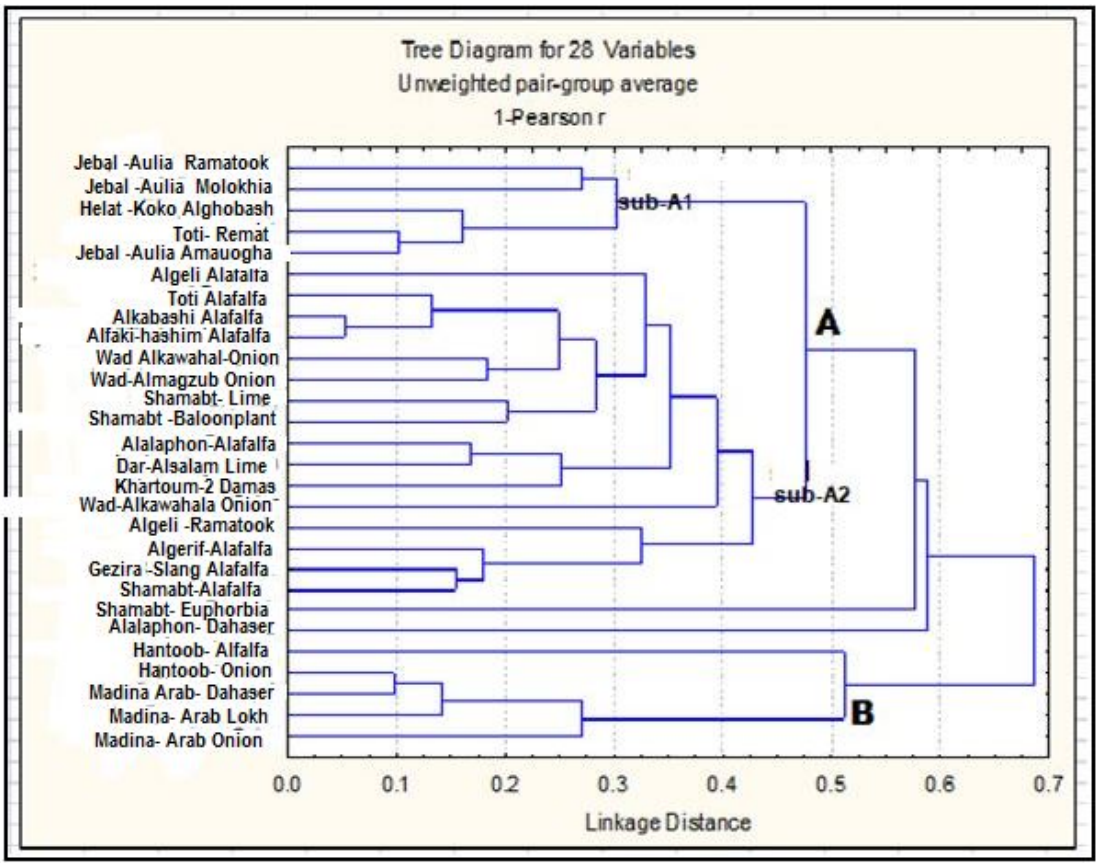

Fig.5UPGA Tree resulting from the analyses of four RAPD primers reflecting the Variation the 28 Sample of *Cuscuta spp*. (Ramtook ,Euphorbia ,Alghbash, Alafalfa ,Remet, Amoyogha, Molokhia, Alafalfa, Ramtook, Alafalfa, Alafalfa, Alafalfa,Dhaser, Alafalfa,Lime,Damas,Lime,Baloonplant, Alafalfa, Alafalfa, Onion,Onion,Onion, Alafalfa,Onion, Dahasr,Lokha,Onion)

The results was obtained genetic distance matrix values between the accessions were showed in Table (2) below. The highest similarity value of 0.98 was between accessions. (Hantoob onion and Alalaphon - Dahaser), (Madina Arab- Onion and wad Alkawahala Onion) where the genetic distance between each of the two samples mentioned Where as accessions one (Khartoum with Gezira state) and one sample (Gezira state) had the lowest similarity value of 0.10 was between accessions (Madina Arab- lokh and Madina Arab- Dahaser), (Madina Arab-Dahaser and Hantoob -Onion).

Table 2 The Genetic Distance Matrix of 28 dodder (*Cuscuta spp*.) Were tasted and analysis four RAPD primers

	2	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**
00																					
54	0.00																				
32	0.42	0.00																			
33	0.54	0.43	0.00																		
32	0.42	0.32	0.21	0.00																	
22	0.43	0.32	0.11	0.10	0.00																
27	0.50	0.40	0.38	0.29	0.27	0.00															
43	0.53	0.32	0.21	0.32	0.32	0.50	0.00														
41	0.62	0.41	0.51	0.41	0.41	0.51	0.31	0.00													
43	0.53	0.42	0.43	0.32	0.32	0.50	0.32	0.20	0.00												
51	0.52	0.41	0.41	0.41	0.41	0.51	0.41	0.41	0.20	0.00											
58	0.68	0.58	0.47	0.47	0.47	0.56	0.37	0.36	0.16	0.15	0.00										
81	0.71	0.71	0.70	0.71	0.70	0.92	0.50	0.61	0.50	0.51	0.45	0.00									
56	0.75	0.54	0.45	0.54	0.45	0.70	0.32	0.41	0.32	0.30	0.26	0.27	0.00								
62	0.71	0.37	0.51	0.48	0.51	0.64	0.26	0.35	0.37	0.35	0.31	0.31	0.17	0.00							
67	0.65	0.43	0.56	0.54	0.56	0.59	0.32	0.41	0.43	0.41	0.36	0.48	0.33	0.17	0.00						
58	0.68	0.37	0.36	0.47	0.47	0.56	0.26	0.36	0.58	0.47	0.52	0.66	0.47	0.31	0.36	0.00					
55	0.56	0.46	0.45	0.56	0.55	0.67	0.25	0.36	0.56	0.67	0.62	0.56	0.55	0.38	0.45	0.20	0.00				
49	0.59	0.27	0.38	0.37	0.38	0.55	0.16	0.36	0.37	0.46	0.42	0.55	0.38	0.22	0.27	0.31	0.29	0.00			
43	0.53	0.21	0.32	0.32	0.32	0.50	0.11	0.31	0.32	0.41	0.37	0.50	0.32	0.15	0.21	0.26	0.25	0.05	0.00		
69	0.65	0.54	0.46	0.54	0.57	0.68	0.31	0.61	0.54	0.50	0.47	0.57	0.57	0.41	0.46	0.35	0.32	0.27	0.31	0.00	
52	0.60	0.36	0.40	0.48	0.40	0.62	0.25	0.44	0.48	0.55	0.52	0.62	0.40	0.36	0.28	0.29	0.36	0.32	0.25	0.43	0.00
57	0.65	0.31	0.46	0.43	0.46	0.57	0.20	0.39	0.43	0.50	0.47	0.57	0.46	0.29	0.34	0.24	0.32	0.27	0.20	0.36	0.18
51	0.62	0.41	0.62	0.52	0.51	0.41	0.52	0.52	0.62	0.52	0.67	0.82	0.62	0.56	0.62	0.47	0.67	0.57	0.52	0.61	0.55
63	0.75	0.54	0.63	0.54	0.52	0.67	0.65	0.76	0.65	0.66	0.81	0.98	0.63	0.78	0.84	0.81	0.92	0.69	0.65	0.83	0.65
66	0.67	0.56	0.66	0.56	0.55	0.67	0.67	0.77	0.67	0.67	0.82	0.97	0.66	0.82	0.77	0.82	0.92	0.72	0.67	0.87	0.58
69	0.79	0.68	0.80	0.68	0.69	0.76	0.68	0.78	0.68	0.78	0.83	0.97	0.69	0.85	0.80	0.94	0.93	0.74	0.68	0.91	0.63
55	0.56	0.56	0.55	0.56	0.45	0.56	0.67	0.77	0.67	0.67	0.82	0.87	0.66	0.82	0.77	0.82	0.82	0.72	0.67	0.98	0.58

IV. Discussion

Investigated DNA Marker-based PCR technology using the markers is RAPD using 4 random primers where explained the relationship between species on the basis of the appearance and disappearance of the bands, specialized molecular markers use four primers OPAL15, OPAL18, OPB17, OPAL20 for the detection of the species appeared in the main bands when the general molecular weight (1-10bp) and, were used for species identification by DNA fingerprinting. Primers were used to be tested. Two primer's showed high percentage of polymorphic bands 100% showed polymorphism in dodder accessions41 polymorphic bands. While other two primers 39 polymorphic bands among the accession the maximum number of bands were produced by primers. The variation within a species in dodder the plant samples were collected from the same area was used for analysis of inter-specific variation. Overall it was considered that the tested accessions. The analysis were classified into groups A,B group observed consist two sub group, as sub group A1,and sub groupA2 the group A 1 obtained as two sisters (Jebal-Ramatook and Jebal-Molokha) , (Toti -Remat and Jab-Amoyogha) and sub group A1 was obtained five sisters(Alkabash Alfalfa and Alfaki-hashim-Alfalfa) ,(wad-Alkawahala Onion and Wada-Almajzoub Onion),(Shambat-Lime and Shambat Balloon plant),(Alalaphon Alfalfa and Dar Alslam Oum bada- lime) a group A and B with correlated consists variables a group B have one sister(Hantoob Onion and Madina-Arab Dahaser),(Helat koko Housa). Sub group A1with correlated sub group A2 .was showed sample (Alalaphon – Dahaser) as out group and genetically close to each other's (sisters). The results were findings in agreement with R. V. *et al* (2009) were found a proper method for estimation of genetic diversity and genetic relationships among different germplasm of *Jatropha curcas* L., random amplified polymorphic DNA (RAPD) technique based on polymerase chain reaction (PCR) was used for described purpose. Out of 55 decamer primers tested, 26 primers produced good amplification products. A total of 6,011 amplification products were scored from which only 1,859 bands (30.92%) were found to be polymorphic and the size

of bands ranged from 300 to 2,500 bp. Unweighted pair group method using arithmetic average cluster analysis revealed clear genetic difference among J. curcas germplasm .also agreement Khan, *et al* ., (2010) the study, the randomly amplified polymorphic DNA (RAPD) technique was employed for authentication of *Cuscuta reflexa* and its adulterant *Cuscuta chinensis*. Thirty two decamer oligonucletide primers were used to amplify the genomic DNA isolated from the dried stems as well as seeds of both the species. Out of the thirty two primers used, fourteen did not amplify, eleven gave faint and non-reproducible, while seven gave species-specific reproducible unique bands. The unique bands obtained in PCR amplification clearly discriminated the two species, having similar morphology and thus, RAPD may serve as a complementary tool for quality control. Showed the distance matrix range the results was obtained genetic distance matrix values between the accessions were shown the highest similarity value of 0.98 was between accessions Hantoob Onion and Alalaphon Dahaser, Madina Arab Onion and wad Alkawahala Onion. Where the genetic distance between each of the two samples mentioned whereas accessions one Khartoum with Gezira states, and one sample Gezira state. had the lowest similarity value of 0.10 was between accessions Madin Arab lokh and Madin Arab Dahaser. (0.10-0.98) which reflected high genetic diversity. The phylogenetic analysis indicated high genetic variation within the two States**.**

V. Conclusion

Dodders have become noxious parasitic weeds on several economically important crops in Sudan. Recent surveys (2017 - 2018) in Sudan indicated that the parasite had spread and has infested crops such as citrus spp. Additional research is needed to determine the distribution of these ecotypes and their phylogenetic analysis in other states. Furthermore, differences in species identity between these types may develop a future plan in dodder management.

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