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Assessment of Genetic Variability among Onion (*Allium cepa* L.) Cultivars using RAPD and SRAP Molecular Markers[1]

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Abstract: Onion (*Allium cepa* L.) is one of the most important vegetable crops worldwide, however, limited studies are available on genetic variability of onion resources. The aim of this study was to assess the genetic diversity of twelve onion cultivars which are widely grown in different regions of Sudan, using RAPD and SRAP markers. A total of 32 RAPD and 21 SRAP primers were screened. To assess variability, the data generated were used to group the cultivars using Jaccard's coefficient of similarity. Only 20, out of tested RAPD primers were producible and showed 326 different-sized DNA fragments with one or more of the tested cultivars giving an average of 27.17 alleles per cultivar. Hundred percent polymorphism was recorded for each of the primers tested with high polymorphism information content (PIC) ranging from 0.89 to 1.00. In SRAP analysis, 8 out of 21 primer pairs tested, produced bands of different sizes. A total of 66 DNA fragments were detected for the 12 cultivars with an average of 5.5 alleles per cultivar. The

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number of different fragments generated by each primer ranged from one to eight and 100 percent polymorphism was recorded for each of the primers tested with high PIC of approximately 1.00. Similarity matrices constructed using RAPD, SRAP or the combined data suggested a relatively high level of genetic diversity among the 12 onion cultivars, the most diverse cultivars were Shendi yellow and Baftaim red hybrid with 14 % similarity. The study revealed that although both molecular markers used were efficient in elucidating genetic diversity among the tested onion cultivars, SRAP markers were the most potential and could be used as an integrated approach in onion breeding programmes.

Keywords: onion, Markers, genetic diversity, polymorphism

INTRODUCTION

Onion (*Allium cepa* L.) is a herbaceous monocotyledonous biennial vegetable crop which is often grown annually for bulb production (Bassett 1986). It has a diploid chromosome number (2n=2X=16) and usually reproduces by seeds or by seed grown sets (Pinky *et al*. 2017).According to Food and Agriculture Organization (FAOSTAT 2012), 175 countries grow onions on estimated 6.7 million acres with total production of more than 98Mmetric tonnes.China, India and the USA are the leading onion producing countries in the world (FAOSTAT 2016).A total of 9,000 accessions of *Allium* were reported to be present worldwide, mostly are onion germplasm (Kik 2002).

In Sudan, onion is considered as the most economically important vegetable crops with an allocated area of 59640 hectares, production of 1.03 M tonnes and average yield of 17.4 tonnes/ha in 2011 (FAOSTAT 2012).The main states for onion production in Sudan are River Nile,Gezira, Khartoum, North Darfur, West Darfur, Kassala, Blue Nile and Northern State.In irrigated areas, onion may be grown three times a year, as an early winter crop from October to February, a late winter crop from January to May and an autumn crop from August to December (Gaffer and Abdallah 2006).

Research in onion genetics has greatly fallen behind the other major vegetable crops and it was difficult to culture, transform, and regenerate *in vitro* (Arumuganathan and Earle 1991). The knowledge of onion genetic diversity and resources is limited mainly due to a shortage of public marker and germplasm resources and the biennial habit of onion (McCallum *et al*. 2008). The number of genetic markers in onion has increased several-fold with the identification and mapping of new molecular markers (Havey *et al*. 1996; King *et al*. 1998). Several studies have reviewed the classical (Havey 1993), the biochemical (Rabinowitch 1988), and the molecular markers of onion (Peffley 1993).

PCR-based dominant marker systems, such as random-amplified polymorphic DNA (RAPD) markers are used to quickly and easily determine the genetic diversity of plant materials within the population, breeding lines, as well as general collection germplasm, and also useful in genetic analysis of the resistance to the specific diseases of vegetable crops (Cvikic *et al*. 2009, Zdravkovic *et al*. 2011). Maniruzzaman *et al*. (2010) investigated the suitability of RAPD markers for onion genetic studies. Their results indicated that 80% of theprimers werescorable. On the other hand, Kesralikar *et al*. (2017) estimated heritability and genetic variability among onion genotypes through RAPD, ISSR and SSR markers. They concluded that, ISSR and SSR markers gave diversified results than RAPD.

In spite of the fact that the multilocus marker systems such as RAPD have been used since 1990s to estimate genetic variation in plants because they produce numerous amplicons and do not require a priori sequence information for molecular characterization, but they are typically used for investigating more shallow taxonomic levels of variation (Koopman*et al*. 2008; Robarts and Wolfe 2014). Sequence-related amplified polymorphism (SRAP) markers have been developed, which are used to amplify coding regions of DNA with primers targeting open reading frames. These markers are shown to have the potential to enhance the current knowledge base of molecular tools in many fields by providing an easy-to-use, highly variable marker with inherent biological significance (Robarts and Wolfe 2014).

The objective of this research was to study the genetic variation among twelve onion cultivars of Sudan using RAPD and SRAP molecular markers.

MATERIALS AND METHODS

Plant Materials:

Seeds of 12 onion cultivars, collected from different producing localities in Sudan, were obtained from Plant Genetic Resources Unit, Agricultural Research Corporation (ARC), Gezira State, Sudan. These were: Shendi yellow, Kamleen yellow, Baftaim yellow, Dongolawhite,Elhilo, Kamleen, Baftaim red, Hudeiba red, HSD 443, Saggai, Baftaim red hybrid and Abufraiwa.

Molecular characteristics:

Genomic DNA of each cultivar was isolated by a sap-extraction method (CIMMYT 2005) from 100 mg of fresh leaf tissues.

PCR program for RAPD primers:

RAPD (32) and SRAP (21) primers were used to amplify onion extracted DNA. The amplification procedure used consisted of one cycle at 94°C for 5min, followed by 35 cycles of initial denaturation at 94°C for 1min, annealing at 32°C for 3min, extension at 72°C for 2 min and a final extension step at 72°C for 10 min.

PCR program for SRAP primers:

After several manipulations of the PCR conditions (annealing temperature, final extension step and number of cycles), the following program was found optimum for amplification of onion genomic DNA by SRAP primers: one cycle at 94°C for 5min followed by five cycles (loop1) with initial denaturation at 94°C for 1min, annealing at 35°C for 1min, extension at 72°C for 1 min. The next 35 cycles (loop2) consisted of initial denaturation at 94°C for 1min, annealing at 47°C for 1min, extension at 72°C for 2 min and a final extension step at 72°C for 10 min.

Data analysis

DNA fragments obtained by both marker types were scored as present (1) or absent (0). Polymorphism information content (PIC) values were calculated as described by Anderson *et al*. (1993) as follows:

$$PIC= 1-\sum P_{ij}^2$$

Where P_{ij} is the relative frequency of the j^{th} allele of the i^{th} locus, summed over all alleles for individual marker locus over all cultivars. A marker with a PIC value of more than 0.50 is considered as highly informative, between 0.25 and 0.50 as informative and less than 0.25 as slightly informative (Botstein *et al*. 1980). Dendrograms were constructed (PAST 3.01 software) based on Jaccard's similarity coefficients (Jaccard 1908).

RESULTS AND DISCUSSION

Results of RAPD analysis indicated that only 20, out of 32 (62.5 %), RAPD primers tested produced different-sized fragments with DNA of one or more of the tested cultivars. A total of 326 DNA fragments were detected for the 12 onion cultivars giving an average of 27.17 alleles per cultivar. The number of fragments detected for each cultivar ranged from 36 (Kamleen yellow) to 18 detected for Abufraiwa. The molecular size of the amplified fragments by all primers ranged from 100 bp to 1000 bp. It is clear in Table 1 that one to seven unique fragments with different sizes were detected for a particular cultivar but not the others. For example, seven, five and four such fragments were detected for Dongola white, Baftaim red and HSD 443/Kamleen yellow cultivars, respectively. For cultivars such as Shendi yellow, Elhilo and Baftaim yellow, two unique fragments were detected while for each of Abufraiwa and Baftaim red hybrid only one such band was detected. Bands of this kind are normally referred to as positive markers.

On the other hand, the absence of a common band in a given cultivar is referred to as a negative marker. In this study, a negative band was detected, that is the 200 bp band recorded for cultivar Shendi yellow with OPA- 09 primer. For reproducibility problems associated with RAPD primers, the use of more than one unique band may be recommended for the characterization and identification of a specific cultivar. The number of different fragments

generated by each primer ranged from one fragment, recorded for OPK- 08 and OPL- 16, to eight recorded for OPA- 09 and OPK-17 (Table 2). The results obtained demonstrated that RAPD approach have a considerable potential for identification and discrimination of different onion cultivars. Such a potentiality is also highlighted by Tanikawa *et al*. (2002), Adsul *et al*. (2009) and Pavlović *et al.* (2012).Hundred percent polymorphism was recorded for each of the primers tested with high polymorphism information content (PIC) ranging from 0.89 to 1.00. The polymorphism obtained with RAPD primers, in this study, is comparatively very high when compared with results of other similar investigations on onion; Adsul *et al.* (2009) used RAPD analysis to study the genetic diversity of 24 onion cultivars and reported 91.2 % polymorphism. Similar high level (91.24 %) was also reported for RAPD markers on onion by Kutty *et al*. (2006). Kesralikar *et al*. (2017) also used ten RAPD primers to analyze genetic diversity of onion cultivars and reported 80% polymorphism. Similarly, Adesoye *et al*. (2012) reported 64.3 % polymorphism using six RAPD primers and 14 *A. cepa* plus one *A. ascalonicum* cultivars. However, Tanikawa *et al*. (2002) reported very low level (39.8 %) polymorphism using 17 RAPD primers and seven cultivars. It is generally reported that polymorphism between cultivars can arise through nucleotide changes that prevent amplification by introducing a mismatch at one priming site, deletion of a priming site, insertions that render priming site too distant to support amplifications and insertions or deletions that change the size of amplified product (Powell *et al*. 1996). The high level of polymorphism reported here help eliminate the limitations associated with the use of morphological and biochemical markers for characterization, especially for closely related varieties (Asif *et al*. 2005).

Pair wise genetic similarities, based on RAPD markers, between cultivars were assessed using Jaccard's genetic similarity coefficients (Table 3). The grouping pattern of the cultivars in the dendrogram shown in Fig. 1 revealed five clusters; of which, cultivar Shendi yellow stands alone in a separate cluster (cluster 5) while the other cultivars were grouped in four different

clusters. The closest cultivars with a genetic similarity of 55 % were Saggai and Baftaim red hybrid, followed by Hudeiba red and Kamleen with 53% similarity. The most diverse cultivars were Shendi yellow and Baftaim red hybrid; Shendi yellow and Elhilo as well as Abufraiwa and Baftaim red hybrid, each pair with 14 % similarity (Fig. 1).

Table 1. Specific fragments (bands) for each cultivar generated by different RAPD primers

Cultivar	Primer	Specific fragment size
Dongola white	OPY – 15	900
		800
	OPL - O7	500
		400
		300
		150
		100
HSD443	OPL – 19	500
		400
	P2	450
		400
Kamleen yellow	OPK – 09	700
		650
	OPL-17	800
		700
Baftaim red	OPA – 01	800
		300
	OPK – 17	700
		600
	OPA – 11	600
Shendi yellow	OPK – 15	200
		100
Elhilo	OPL – 20	100
	P2	250
Baftaim red hybrid	OPR – 10	700
Abufraiwa	OPK- 15	500
Baftaim yellow	OPR – 10	300
	OPK – 17	900

Table 2. Percentage of polymorphism and polymorphism information content (PIC) calculated for each RAPD primer

Primer cods	Total No. of bands	No. of polymorphic bands	Polymorphism (%)	PIC
OPA – 04	7	7	100	0.93
OPA – 09	8	8	100	0.89
OPA – 11	4	4	100	1
OPA – 01	6	6	100	0.98
OPR – 10	5	5	100	1
OPY – 16	3	3	100	1
OPK – 15	5	5	100	0.99
OPY – 15	3	3	100	1
OPR – 07	3	3	100	1
OPK – 08	1	1	100	1
OPL – 19	3	3	100	1
OPL – 16	1	1	100	1
OPL – 20	2	2	100	1
OPK – 09	6	6	100	0.97
OPL – 17	4	4	100	0.99
P2	6	6	100	0.99
OPL – 07	5	5	100	1
OPR – 05	3	3	100	0.99
OPY – 18	7	7	100	0.97
OPK – 17	8	8	100	0.94
Total	90	90	100	-

In SRAP analysis, only eight out of 21 (38.1 %) primer pairs tested, produced bands of different sizes with DNA of one or more of the cultivars. A total of 66 DNA fragments were detected for the 12 cultivars with an average of 5.5 alleles per cultivar. The number of fragments detected for the cultivars ranged from zero to 11while the molecular sizes of the amplified fragments, by each cultivar, ranged from 150 bp to 750bp.A number of unique fragments of certain sizes were detected for a particular cultivar but not the others (Table 4). Five such fragments were detected for cultivar Dongola white, four for Baftaim yellow, two for HSD 443 and one fragment for each of Shendi yellow and Kamleen yellow. These unique bands together with those detected for RAPD primers may be very useful in the characterization and identification of their respective onion cultivars.

Table 3. Genetic similarity indices of RAPD (lower) and SRAP (Upper) for different onion cultivars

Cultivars	Baftaim red hybrid	Saggai	Kamleen yellow	Shendi yellow	Abufraiwa	Hudeiba red	Kamleen	Baftaim red	HSD 443	Elhilo	Baftaim yellow	Dongola white
Baftaim red hybrid		0.18	0.22	0.13	0.22	0.04	0.24	-0.08	0.00	0.04	-0.22	0.25
Saggai	0.55		0.07	-0.15	-0.08	0.39	0.48	0.34	0.00	-0.25	-0.08	-0.06
Kamleen yellow	0.26	0.44		0.48	0.48	0.03	0.35	0.24	0.00	0.03	-0.21	-0.39
Shendi yellow	0.14	0.17	0.17		0.64	0.34	0.14	-0.11	0.00	0.34	-0.11	-0.27
Abufraiwa	0.14	0.24	0.17	0.19		0.22	0.20	-0.09	0.00	0.45	-0.25	-0.18
Hudeiba red	0.19	0.41	0.38	0.20	0.38		0.24	-0.08	0.00	-0.20	0.00	0.06
Kamleen	0.24	0.32	0.27	0.21	0.41	0.53		0.69	0.00	-0.12	-0.13	0.09
Baftaim red	0.27	0.40	0.27	0.14	0.23	0.34	0.39		0.00	-0.08	-0.09	-0.13
HSD 443	0.27	0.32	0.24	0.18	0.23	0.29	0.25	0.28		0.00	0.00	0.00
Elhilo	0.16	0.26	0.23	0.14	0.27	0.38	0.29	0.34	0.35		-0.22	-0.32
Baftaim yellow	0.33	0.33	0.34	0.15	0.20	0.25	0.28	0.35	0.28	0.25		-0.18
Dongola white	0.20	0.22	0.30	0.18	0.21	0.27	0.29	0.24	0.26	0.24	0.42	

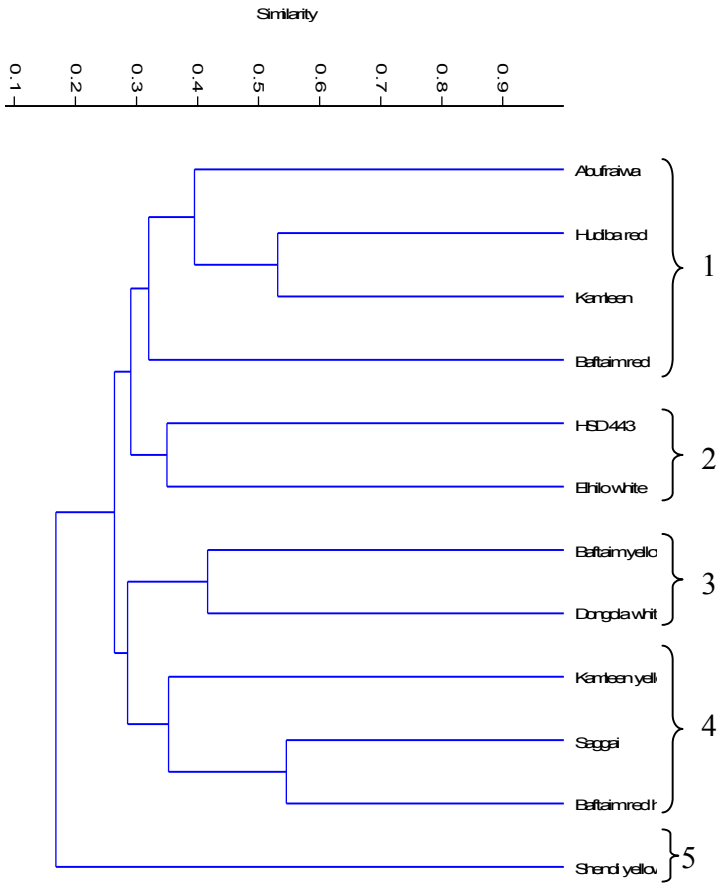

Fig.1.Dendrogram of 12 onion cultivars based on data generated from RAPD markers. Values along x-axis correspond to Jaccard's coefficient of similarity

The number of different fragments generated by each primer ranged from one (me1/em15) to 8 recorded for me13/em3 (Table 5). Hundred percent polymorphism was recorded for each of the primers tested with high PIC of approximately 1.00 (Table 5). Robarts and Wolfe (2014) reported that the application of SRAP markers has increased dramatically since their introduction in 2001, especially in the past few years, giving descriptive statistics of SRAP markers in a subset of 171 publications. Despite these, hitherto, this is the first report of using SRAP markers in the molecular analysis of *A. cepa* cultivars worldwide. However, Li *et al*. (2008) reported the successful use of SRAP and SSR markers to study the genetic diversity of *A. fistulosum*. They reported that SRAP was more accordant to reflect the morphological variability with *A. fistulosum* cultivars than did SSR markers.
Results of the clustering based on data generated from SRAP markers are shown in Table 3 and also in Fig. 2. These results suggested a relatively high level of genetic diversity among the onion cultivars. The highest genetic similarity (48 %) was shown by three pairs of the cultivars viz.Shendi yellow and Kamleen yellow, Abufraiwa and Kamleen yellow and Kamleen and Saggai. Cultivar HSD 443 showed no similarity (00.0 %) with 9 of the 12 cultivars. The 12 cultivars were clustered into 5 groups (Fig. 2), in which each of cultivars Baftaim yellow and HSD 443 formed two distinct clusters (cluster 1 and 5, respectively). Unlike in the previous clustering patterns, cultivars HSD 443 and Elhilo white were very diverse from each other (00.0 % similarity).

Table 4. Specific fragments (bands) for each cultivar generated by different SRAP primers

Cultivar	Primer	Specific fragment (band) size
Kamleen yellow	Me2/em5	250
Shendi yellow	Me2/em5	450
HSD 443	Me13/em3	700 750
Baftaim yellow	Me2/em5	250 300
	Me13/em8	250 300
	Me1/em1	400
	Me2/em3	250
Dongola white	Me13/em8	200 500
	Me3/em14	300

Table 5. Percentage of polymorphism calculated for each SRAP primer

Primer	Total No. of bands	No. of polymorphic bands	Polymorphism %	PIC
me1/em1	2	2	100	1
me1/em2	3	3	100	1
me2/em3	3	3	100	0.99
me2/em5	4	4	100	1
me1/em15	1	1	100	1
me3, em14	2	2	100	1
me13/em8	7	7	100	1
me13/em3	8	8	100	1
Total	30	30	100	-

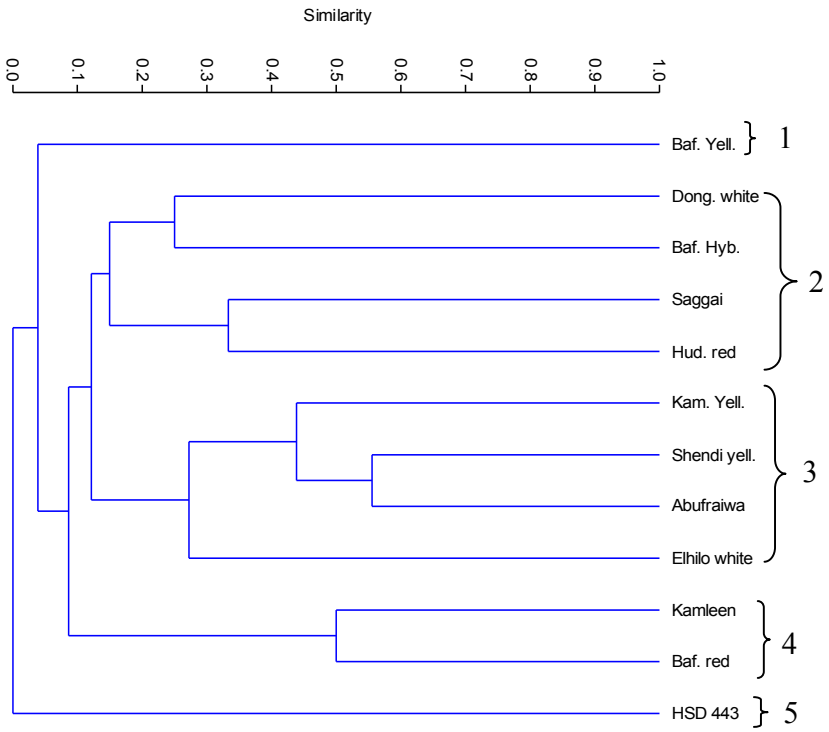

Fig. 2.Dendrogram of 12 onion cultivars based on data generated from SRAP markers. Values along x-axis correspond to Jaccard's coefficient of similarity

Similarity matrix was also constructed using the combined data generated from both RAPD and SRAP markers. Results are shown in Table 6 and Fig. 3. The similarity coefficient showed a variation from 47 % recorded for cultivars Baftaim red hybrid and Saggai as well as for Hudeiba red and Kamleen, to 11 % similarity between cultivars Shendi yellow and Baftaim red. The dendrogram based on the combined data showed that the 12

cultivars were grouped into five distinct clusters. Cluster 1 was the largest, containing four cultivars that join at 30 % similarity. Cultivars Elhilo white and HSD 443, which had 35 % similarity in the RAPD-based clustering, were again grouped in one cluster (cluster 2) with 31 % similarity. Cultivar Shendi yellow was isolated in a separate cluster (cluster 5).

CONCLUSIONS

Results of this study indicate a high degree of genetic variability among the Sudan's onion cultivars. Both RAPD and SRAP markers were efficient in elucidating genetic diversity among the tested onion cultivars, however, SRAP markers were the most potential.Genetic similarity indices of the data indicate that cultivars Kamleen and Hudeiba red were the closest to each other followed by Baftaim red hybrid and saggai and Kamleen and baftaim red. The lowest similarity indices were found between Baftaim yellow and Shendi yellow followed by Shendi yellow and Baftaim red, hence these cultivars can be used in breeding activities for introgression of desirable traits or to broaden onion genetic diversity and the potentiality of their further use in breeding programs.

Table 6. Genetic similarity indices calculated for both RAPD and SRAP markers for different onion cultivars

Cultivars	Baftaim red hybrid	Saggai	Kamleen yellow	Shendi yellow	Abufraiwa	Hudeiba red	Kamleen	Baftaim red	HSD 443	Elhilo	Baftaim yellow	Dongola white
Baftaim red hybrid	1.00											
Saggai	0.47	1.00										
Kamleen yellow	0.25	0.38	1.00									
Shendi yellow	0.15	0.14	0.23	1.00								
Abufraiwa	0.16	0.20	0.22	0.27	1.00							
Hudeiba red	0.17	0.40	0.33	0.22	0.35	1.00						
Kamleen	0.23	0.32	0.25	0.19	0.35	0.47	1.00					
Baftaim red	0.23	0.37	0.24	0.11	0.20	0.30	0.39	1.00				
HSD 443	0.24	0.27	0.20	0.15	0.20	0.26	0.24	0.27	1.00			
Elhilo	0.15	0.21	0.21	0.17	0.29	0.31	0.24	0.30	0.31	1.00		
Baftaim yellow	0.26	0.28	0.28	0.13	0.16	0.23	0.24	0.31	0.25	0.21	1.00	
Dongola white	0.21	0.20	0.23	0.15	0.18	0.24	0.25	0.19	0.21	0.18	0.33	1.00

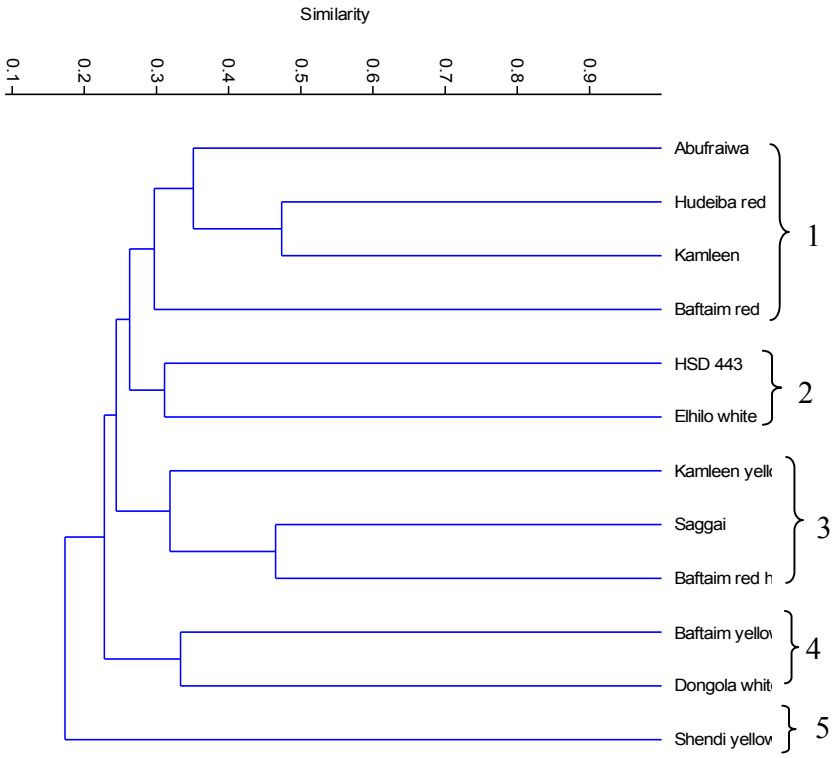

Fig. 3. Dendrogram of 12 onion cultivars based on data generated from both RAPD and SRAP markers. Values along x-axis correspond to Jaccard's coefficient of similarity

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تقويم التباين الوراثي ضمن أصناف البصل (*Allium cepa* L.) باستخدام واسمات التعدد الشكلي العشوائي (RAPD) و الواسمات الجزيئية للتعدد الشكلي المتصل بالتسلسل النيوكليوتيدي(SRAP)

المستخلص: يعتبر نبات البصل واحد من أهم محاصيل الخضر في العالم، ومع ذلك، توجد دراسات محدودة في التباين الوراثي لموارده. كان الهدف من هذه الدراسة هو تقويم التنوع الوراثي بين إثني عشر صنف من البصل والتي تزرع بتوسع في مناطق مختلفة في السودان، وذلك باستخدام واسمات التعدد الشكلي العشوائي RAPDوالتعدد الشكلي المتصل بالتسلسل النيوكليوتيديSRAP. تم مسح مجموع 32 من بادئات RAPD و21 من واسماتSRAP. لتقويم التباين، تم استخدام المعلومات الناتجة لتقسيم الأصناف باستخدام معامل جاكارد للتشابه. كان هنالك عدد 20 بادئ فقط، من بادئات الواسمات العشوائية RAPDمنتجاً وقد أظهرت 326 حزمة بأحجام مختلفة من DNA مع واحد أو أكثر من الأصناف المختبرة معطية متوسط 27.17 أليل/صنف. تم تسجيل نسبة 100% تعدد شكلي لكل واحد من البادئات المختبرة بمحتوى عال من معلومات تعدد شكلي يتراوح بين 0.89 إلى 1.00. في تحليل واسمات SRAP، أنتجت 8 من 21 من البادئات المختبرة حزم ذات أحجام مختلفة وتم تحديد مجموع 66 من حزم DNA للأصناف الـ 12 بمتوسط 5.5 أليل/صنف. كان عدد الحزم المختلفة الناتجة بكل بادئ في المدى بين واحد إلى ثمانية وتم تسجيل نسبة 100% تعدد شكلي لكل واحد من البادئات المختبرة بمحتوى عال من معلومات تعدد شكلي يساوي تقريباً 1.00. أوضحت مصفوفة التشابه المنشأة باستخدام معلومات نوعي الواسمات أو المعلومات المدمجة منهما مستوى عال من التنوع الوراثي ضمن أصناف البصل الـ12 فكان أكثر الأصناف تنوعاً هما شندي أصفر و بافطيم الأحمر الهجين بنسبة 14% تشابه. توضح هذه الدراسة أنه بالرغم من أن نوعي الواسمات الجزيئية المستخدمة كانا ذوا كفاءة في توضيح التنوع الوراثي ضمن أصناف البصل المختبرة، إلا أن واسمات SRAP كانت الأكثر قوة ويمكن أستخدامها كنهج مكمل في برامج تربية البصل.