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Full Length Research Paper

An assessment of the cultural capabilities of clonal propagation and molecular characterization of *Yucca elephantipes* cultivars

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This study was conducted to describe an efficient micropropagation protocol suitable for the production of clonally uniform Yucca plants through the shoot tip and lateral bud explants and compare the application and utility of TRAP and SRAP marker techniques for analysis of genetic diversity among six genetically Yucca diverse genotypes. The results indicated that the "Green" cultivar gave the significantly highest average mean value of primary and secondary shoot number (2.02 and 3.21 /explant, respectively). Medium protocol B containing MS medium + 0.2 mg/l of NAA+ 4.0 mg/l of BAP significantly gave the highest mean value for shoot multiplication derived from primary and secondary cultures (1.63 and 2.96/explant, respectively). The lateral bud explant derived propagules from the primary and secondary culture was the most effective for shoot multiplication. Molecular markers tools (SRAP and TRAP) analysis were used for detecting polymorphism among six genetically Yucca diverse genotypes. Cluster analysis using SRAP data grouped the 6 Yucca genotypes into two main clusters with Jaccard's similarity coefficient ranging from 0.40 to 0.64. The dendrogram generated from TRAP data clearly indicated two main clusters with similarity coefficient ranging from 0.40 to 0.92. SRAP and TRAP data were combined to produce a dendrogram and the similarity coefficient among the six Yucca genotypes varied from 0.39 to 0.75.

Key words: Clonal propagation**,** SRAP markers, tissue culture, TRAP markers, *Yucca elephantipes.*

INTRODUCTION

Yucca is an evergreen, monocotyledonous plant, which belongs to the Family *Agavaceae.* It is of particular interest to garden landscaping in arid and semi-arid regions for its outstanding ability to grow under these conditions. Yucca also, is a popular plant for indoor decoration. The importance of Yucca is increasing because some important steroidal saponins have been isolated from leaves and rhizomes that can be used as antiarthritic, purgative and detergent (Bahuguna and Sati, 1990; Navin et al., 1992). Propagation of Yucca by cuttings and offsets produces few plants. Micropropagation offers major production and marketing advantages over traditional propagation methods. Therefore, the present research was conducted to develop a rapid procedure for multiplication *in vitro*, rooting and acclimatization in order to facilitate the release of the used Yucca cultivars at large scale production.

Molecular markers in addition to the classical methods provide more positive identification of new cultivars. Molecular markers are a useful complement to morphological and physiological characterization of cultivars because they are plentiful, independent of tissue or environmental effects and allow cultivar identification early in plant development.

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Table 1. Components of four medium protocols used for *in vitro* propagation of Yucca cultivars.

Protocol	Protocol sequence	
	Shoot multiplication (Protocol components)	**Root induction (Protocol components)**
	MS medium	**MS medium**
A	NAA (0.1 mg/L) BAP (6.0 mg/L)	No growth regulators
B	NAA (0.2 mg/L) BAP (4.0 mg/L)	BAP (0.5 mg/L) IBA (2.0 mg/L)
C	NAA (0.2 mg/L) BAP (5.0 mg/L)	NAA (0.8 mg/L)
D	NAA (0.5 mg/L) Kinetin (5.0 mg/L)	NAA (0.2 mg/L) Kinetin (0.5 mg/L)

Molecular characterization of cultivars is also useful to evaluate potential genetic erosion, defined here as a reduction of genetic diversity in time (Al-Doss et al., 2011). Identification of varieties or breeding lines is very important in agricultural species, and is particularly interesting in Yucca when in many cases the origin of varieties is unknown. Traditionally, identification has been based on morphological characters; however the development of new techniques has allowed basing these analyses on DNA information. Recently, the new types of molecular markers, sequence-related amplified polymorphism (SRAP) and target region amplification polymorphism (TRAP), were developed and used in genetic mapping (Li and Quiros, 2001; Hu and Vick, 2003; Liu et al., 2005; Wang et al., 2005; Barakat et al., 2011).

The objectives of the present study were to (1) describe an efficient micropropagation protocol suitable for the production of clonally uniform Yucca plants through shoot tip and lateral bud explants and (2) compare the application and utility of TRAP and SRAP marker techniques for analysis of genetic diversity among six genetically Yucca diverse genotypes.

MATERIALS AND METHODS

Plant material and culture conditions

Six cultivars of *Yucca elephantipes*, that is, Albo, Green, Jewels, Mela, Silver Star and Variegata were obtained from Egypt Green Nursery, El-Mansouria, Giza and grown at the nursery of the Ornamental Research Branch, Antoniades Gardens, Alexandria, Egypt. Lateral bud and shoot tip explants of the six Yucca cultivars were surface sterilized by immersing in 70% ethanol, for a minute, followed by immersing in 0.1% mercuric chloride for 12 min., then, washed with six changes of sterile distilled water. The two explants were, aseptically, placed on four medium protocols of Murashig and Skoog (1962) based medium (MS) containing 3% sucrose varied in growth regulators combinations (Table 1) and incubated in a growth chamber at 25 ± 2°C under 16 h illumination (2000 Lux, daylight fluorescent tubes).

Agar was added after adjusting the pH to 5.8, using 1.0 M of HCl and/or 1.0 M of NaOH. The media were, then, sterilized by autoclaving. All equipments were steam-sterilized for 20 min. at 12°C (15 PSI nominal steam pressure with an extended cycle time of 30 min. was adequate).

Shoot induction, multiplication and root induction response

After six weeks of incubation, shoot induction response was recorded as primary culture and the following determinations were made: 1) Number of shoots formed from either shoot tips or the lateral buds (primary shoot number per explant), and 2) Primary shoot length (cm).

Shoots, derived from either shoot tip or the lateral bud explants were transferred to the same four medium sequences (Table 1). After six weeks, the following observations were recorded as secondary culture: - 1) Number of shoots (Secondary shoot number per explant), and 2) Secondary shoot length (cm).

Shoots with 4 to 6 leaves were transferred to the rooting medium described in Table 1 after six weeks. Regenerated plants were washed with tap water to remove agar from the roots and were transplanted to small pots filled with peat moss and perlite (1:1 v/v). The pots were incubated under moist conditions in the greenhouse for adaptation.

Molecular characterization

DNA extraction

Frozen young leaves (500 mg) of six Yucca cultivars were ground to a powder in a mortar with liquid nitrogen. The DNA extraction was done using CTAB method (Sagahi-Maroof et al., 1984).

SRAP and TRAP analysis

A total of 19 primers (Table 6) were used in SRAP analysis, and 9 primers were used in TRAP analysis (Table 7), from Pharmacia Biotech. (Amersham Pharmacia Biotech UK Limited, England HP79 NA). PCR amplification for SRAP and TRAP was carried out in a

Table 2. Analysis of variance for primary and secondary shoot number and shoot length (cm).

S. O. V.	D. F.	M. S			
		Primary shoot number[a]	Primary shoot length (cm)	Secondary shoot number[a]	Secondary shoot length (cm)
Cultivars (A)	5	5.66**	3.52**	7.55**	3.39**
Protocols(B)	3	3.86**	4.15**	6.54*	3.86**
Explants (C)	1	13.78**	0.05N.S.	43.56**	0.20N.S.
A x B	15	2.11**	1.60**	4.82**	2.20**
A x C	5	5.84**	1.12N.S.	17.18**	1.09N.S.
B x C	3	2.24N.S.	0.19N.S.	5.79N.S.	0.10N.S.
A x B x C	15	1.18N.S.	0.65N.S.	2.62N.S.	0.77N.S.
Error	240	0.96	0.68	2.24	0.81

20/l reaction mixture containing 1 x buffer, 1.5 mmol l^{-1}MgCl2, 0.1 mmol l^{-1} dNTPs, 500 nmol l^{-1} primer, 1U Taq polymerase, and 50 to 60 ng template DNA. After 5 min at 94°C, 5 cycles were performed with 1 min at 94°C, 1 min at 35°C, 1 min 40 s at 72°C, then 35 cycles the same as previous except for the annealing temperature at 50°C and a final 7 min at 72°C. Amplification products were electrophoretically resolved on 1.5% agarose gels containing 0.1 µg/ml ethidium bromides, and photographed on a UV transilluminator.

Statistical analysis

Data were statistically analyzed as a 3 – factor experiment (cultivars, medium protocols and explants) in a completely randomized design with six replicates. Shoot number was subjected to square root transformation prior to statistical analysis (Steel and Torrie, 1980). Comparisons among means were made using the Least Significant Differences test (LSD). The data were analyzed, using SAS program, version 6 (1985).

SRAP and TRAP data were scored for presence (1), absence (0) or as a missing observation, and each band was regarded as a locus. Two matrices, one for each marker, were generated. Pairwise comparisons of genotypes, based on the presence or absence of unique and shared polymorphic products, were used to determine a data matrix of pairwise similarities between cultivars, according to Jaccard coefficient (Jaccard, 1908)

All matrices based on molecular markers were used to obtain the respective dendrograms using the algorithm UPGMA (Unweighed Pair Group Method with Arithmetic Average) (Sokal and Michene, 1958) employed the SAHN (Aequential, Agglomerative, Hierarchical, and Nested clustering) from the software NTSYS.pc (Numercial Taxonomy and Multivariate Analysis System) version 1.80 (Applied Biostatics Program (Rohlf, 1993).

RESULTS AND DISCUSSION

Clonal propagation

Primary shoot formation

Statistical analysis of shoot number showed that the trait was highly significantly influenced by differences between cultivars, medium protocols and explants (Table 2). The first order interactions were highly significant except for medium protocols x explants interaction, which was insignificant. The second order interaction was, also, insignificant. The analysis of variance presented in Table 2 indicated also that shoot length was significantly influenced only due to differences among Yucca cultivars, medium protocols and their interaction.

Number of shoots varied among Yucca cultivars (Table 3). The "Green" cultivar gave, significantly the highest mean value of shoot number (2.03 shoots /explant) across the four medium protocols. On the other hand, the "Albo" cultivar gave the significantly lowest average mean of shoots number (0.96 shoots /explant) across the four medium protocols. The response for shoot number varied according to applied medium (Table 1). The medium protocol C gave significantly the lowest mean value of shoot number across the Yucca cultivars. However, the difference between the A and C medium protocols was not significant (Table 3). The significance of cultivars x medium protocols interaction indicated that cultivars showed differential response to the applied protocols (Table 3). "Green" cultivar responded favorably to protocols B and D, whereas "Variegata" cultivar gave the highest mean value of shoot number with protocols A and B. Meanwhile, the remaining cultivars showed insignificant variation for the given trait.

The results in Table 4 showed that lateral bud explant, significantly, gave the highest response of shoot number (1.64 shoots /explant) as compared with shoot tip culture (1.20 shoots /explant) across the Yucca cultivars. The interaction between cultivars and explants was significant. The cultivars varied in their response according to the explant used. The cultivars "Green" and "Variegata" gave higher mean values of shoot number (2.75 and 1.96 shoots/explant, respectively) when lateral bud explant was used compared to lower mean value of shoot number (1.29 and 0.79 shoots/explant, respectively) when shoot tip explant was used. The remaining cultivars performed statistically similar with both explants.

The results in Table 3 showed that the cultivar "Variegata" produced the highest mean value of shoot

Table 3. Means of primary shoot number (per plant) and primary shoot length (cm) as influenced by cultivars, media and their interaction.

Cultivar	Primary shoot number (per plant)				Cultivar mean	Primary shoot length (cm)				Cultivar mean
	Medium protocol					Medium protocol				
	A	B	C	D		A	B	C	D	
Green	1.66	2.67	1.25	2.50	2.03	0.91	1.34	1.16	1.40	1.20
Variegata	2.00	1.83	0.58	1.08	1.38	2.03	2.74	1.30	0.83	1.72
Albo	1.08	1.33	0.58	0.83	0.96	0.86	1.13	0.73	0.88	0.90
Mela	1.17	1.50	1.00	1.58	1.31	1.10	1.68	0.96	1.28	1.52
Jewels	1.08	1.25	1.50	1.75	1.40	0.97	1.83	1.23	1.40	1.17
Silver star	1.50	1.17	1.17	1.50	1.46	1.28	1.79	1.36	1.40	1.37
Medium mean	1.42	1.63	1.10	1.54		1.19	1.63	1.12	1.14	

L. S. D. $_{(0.05)}$ for cultivar x medium protocol interaction of primary shoot number and shoot length = 0.79 and 0.66 cm, respectively. L. S. D. $_{(0.05)}$ for cultivar means of primary shoot number and shoot length = 0.40 and 0.33 cm, respectively. L. S. D. $_{(0.05)}$ for medium means of primary shoot number and shoot length = 0. 32 and 0.27 cm, respectively.

Table 4. Means of primary and secondary shoot number (per plant) as influenced by cultivars, explants and their interaction.

Cultivar	Primary shoot number(per plant)		Secondary shoot number (per plant)	
	Shoot tip	Lateral bud	Shoot tip	Lateral bud
Green	1.29	2.75	1.83	4.58
Variegate	0.79	1.96	1.63	3.38
Albo	1.04	0.88	2.00	2.17
Mela	1.33	1.29	2.67	2.58
Jewels	1.42	1.38	2.79	2.58
Silver star	1.33	1.58	2.88	3.17
Explants mean	1.20	1.64	2.30	3.08

L.S.D. $_{(0.05)}$ for cultivar x explant interaction of primary and secondary shoot number = 0.43 and 0.85, respectively. L.S.D. $_{(0.05)}$ for explant means of primary and secondary shoot number = 0.23 and 0.35, respectively.

length (1.72 cm) across medium protocols. The medium protocol B showed the greatest potential for shoot length (1.63 cm) across cultivars and it was significantly superior to all other medium protocols. The data, also, revealed that cultivars x medium protocols interaction resulted from the magnitude of response of cultivars to the applied protocols. In "Variegata" cultivar, protocol B was significantly superior to all other protocols in shoot length. On the other hand, in "Green" and "Albo" cultivars, none of the applied protocols showed significant increase in shoot length in comparison to the other protocols. Moreover, protocol B showed significant increase in shoot length to protocols C, A and D only in "Mela", "Jewels" and "Silver Star" cultivars, respectively.

Secondary shoot formation

After assessment of shoot multiplication for primary culture, the propagules were transformed to the same fresh medium (secondary culture) and then, the shoots number and shoot length (Figure 1) were recorded after six weeks of incubation on different medium protocols.

Statistical analysis of shoot number derived from propagules of primary cultures (Table 2) revealed highly significant differences among cultivars, medium protocols and explants. All the two-way interactions were significant except medium protocols x explants interaction. However, the second order interaction (cultivar x medium protocol x explant) was not significant. The analysis of variance presented in (Table 2) indicated also that shoot length was, significantly, influenced only by differences among Yucca cultivars, medium protocols and their interaction.

The results in Table 5 showed that the cultivar "Green" produced the highest mean value of shoot number (3.21 shoots /explant) across medium protocols, which was, significantly, different from cultivars "Variegata" and "Albo". The medium protocol C showed the lowest potential for shoots number (2.28 shoots /explant) and it was, significantly lower than the B and D medium protocols. The data also revealed that there was an

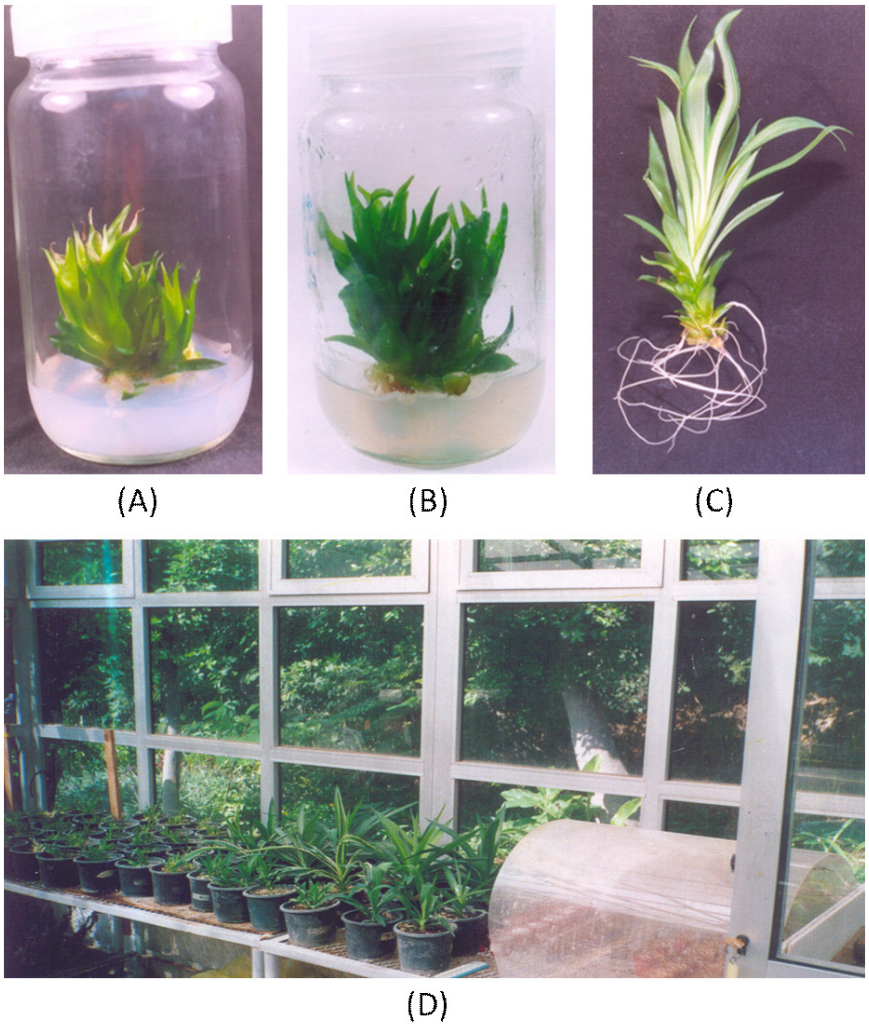

Figure 1. Micropropagation of *Yucca elephantipes* cultivars: (A) Shoot multiplication derived from Shoot tips(B) Shoot multiplication derived from Lateral buds(C) Root induction on MS medium without hormones(D)Regenerated plants established in the greenhouse.

Table 5. Means of secondary shoot number (per plant) and secondary shoot length (cm) as influenced by cultivars, media and their interaction.

Cultivar	Secondary shoot number (per plant) Medium protocol				Cultivar mean	Secondary shoot length (cm) Medium protocol				Cultivar mean
	A	B	C	D		A	B	C	D	
Green	1.33	4.25	1.50	3.75	3.21	1.55	2.20	2.01	1.98	1.93
Variegata	3.08	3.00	1.67	2.25	2.50	2.83	3.44	1.88	1.36	2.83
Albo	2.08	2.58	1.75	1.92	2.08	1.56	1.86	1.50	1.70	1.56
Mela	2.08	2.92	2.50	3.00	2.63	1.74	2.30	1.63	1.95	1.91
Jewels	2.17	2.58	2.83	3.17	2.69	1.68	1.62	2.03	2.13	1.86
Silver Star	3.17	2.42	3.42	3.08	3.02	2.09	2.62	2.42	1.86	2.25
Medium mean	2.65	2.96	2.28	2.86		1.91	2.34	1.91	1.83	

L. S. D. $_{(0.\ 05)}$ for cultivar x medium protocol interaction of secondary shoot number and shoot length = 1.2 and 0.72 cm, respectively. L. S. D. $_{(0.\ 05)}$ for cultivar means of secondary shoot number and shoot length = 0. 60 and 0.36 cm, respectively. L. S. D. $_{(0.\ 05)}$ for medium means of secondary shoot number and shoot length = 0.49 and 0.29 cm, respectively

interaction between cultivars and medium. The cultivar "Green" showed significant response to medium protocols B and D, whereas, cultivar "Variegata" responded significantly to protocols A and B. On the other hand, the remaining four cultivars showed significant variations for the tested medium protocols.

It was evident from Table 4 that the lateral bud explant appeared to be the explant choice for inducing shoot multiplication at high frequencies. The lateral bud explant derived propagules from the primary culture were the most responsive to shoot multiplication, which ranged from 4.58 (Green cultivar) to 2.17 shoots / explant (Albo cultivar). The lateral bud explant significantly gave the highest response regarding shoot number (3.08 shoots / explant) as compared with shoot tip culture (2.30 shoots / explant) across the tested Yucca cultivars. Furthermore, the data revealed that there was a significant interaction between genotypes and explants. Both "Green" and "Variegata" cultivars showed, significantly, higher shoots number with lateral bud explants than with shoot tip explants. However, the magnitude of response in "Green" cultivar was higher than that of "Variegata" cultivar. On the other hand, the remaining four cultivars performed similarly with both types of explants.

It was evident from Table 5 that the shoot length ranged from 2.83 cm for "Variegata" to 1.56 cm for "Albo" among cultivars across the four medium protocols. On the other hand, the medium protocol B significantly gave the highest mean value for shoot length (2.34 cm) across the cultivars as compared with the other three medium protocols. The results also revealed that there was an interaction between the cultivar and medium. Medium protocols A and B imposed significant increase on shoot length in "Variegata" cultivar compared to protocols C and D. On the other hand, protocol B exhibited higher mean value for shoot length compared to protocol D only in "Silver Star" cultivar. Variations in other cultivars, as influenced by the four medium protocols were insignificant.

Root induction

Root system and subsequently plants establishment *ex vitro* (Figure 1) was observed on MS basal medium without hormones with all Yucca cultivars except "Albo" cultivar, which failed to produce roots even with other MS medium containing auxins.

The results from the present investigation showed that shoot multiplication was dependent on both explant source and employed culture medium. The probable reasons of differences in morphogenetic response *in vitro* may be attributed to: a) genetic differences among the cultivars used, b) differences in the growth stimulators, c) differences in the applied growth conditions and age of the source of explants. The influences of the aforementioned factors on the morphogenetic potential of explants are well documented in the literature (Litz and Conover, 1978; Pierik and Stegmans, 1983; Kukufchanka and Kromer, 1984; Barakat and Lakany, 1992). Several investigators have suggested that both NAA and BAP must be present to induce the shoot multiplication from different explants of *Yucca* spp. (Karpov, 2000, 2004; Arce-Montoya et al., 2006). Recently, Barakat and El-samak (2011) reported that shoots were found to proliferate with increasing concentration of BA and poorer quality shoots in the presence of NAA and the absence of BA.

The results from the present investigation indicated that significant differences were found among the six Yucca cultivars evaluated for shoot multiplication. Similar results were reported by several investigators (Bentz et al., 1988; Atta-Alla et al., 1997a; Sakr et al., 1999). The previous results also showed that differences in shoot length for primary and secondary culture were observed among medium protocols and among Yucca cultivars. These results confirm those obtained by previous investigators (Bentz et al., 1988; Atta-Alla et al., 1997b).

Molecular characterization

Identification and evaluation of SRAP and TRAP markers for diversity estimates

Nineteen SRAP primers were screened for their ability to amplify the genomic DNA from 6 Yucca genotypes. The number of amplified DNA fragments ranged from 0.0 to 11.0 depending on the primer and the DNA sample with a mean value of 3.4 bands per primer (Table 6). A total of 65 fragments were produced by the 19 primers. Of these 65 amplified fragments, 27.7% were not polymorphic, while 72.3% were polymorphic among the 6 Yucca genotypes. Primer SRAP-5 generated the greatest number of amplification. (Figure 2A) shows the amplification profiles generated by primer SRAP-5 across the 6 Yucca genotypes, all of which had distinguishable banding patterns. Polymorphism between genotypes 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 sites too distant to support amplification; and insertions or deletions that change the size of the amplified product (Williams et al., 1990). SRAP is a PCR-based DNA marker system that generates multiple fragments in a single PCR reaction (Li and Quiros, 2001). SRAPs amplify several reproducible and polymorphic loci and alleles, and they may amplify functional genes since they are in related sequence. SRAP markers possess multiloci and multiallelic features, which make them potentially more efficient for genetic diversity analysis, gene mapping and fingerprinting genotypes. However, SRAP markers may not be randomly distributed across the genome (Li and Quiros, 2001).

Table 6. Number of amplification and polymorphic products, using nineteen SRAP primers in six Yucca cultivars.

Primer number	Nucleotide sequence (5` - 3`)		No. of amplification[a]	No. of polymorphic[b]	Polymorphism[b/a] (%)
	Forward primers	Reverse primers			
1	TGAGTCCAAACCGGTAG	GACTGCGTACGAATTCTG	4	3	75.0
2	TGAGTCCAAACCGGTCC	GACTGCGTACGAATTGTC	2	2	100
3	TGAGTCCAAACCGGTCA	GACTGCGTACGAATTAAT	2	1	50.0
4	TGAGTCCAAACCGGTAG	GACTGCGTACGAATTCGA	5	4	80.o
5	TGAGTCCAAACCGGTAG	GACTGCGTACGAATTCAG	11	10	90.9
6	TGAGTCCAAACCGGTAG	GACTGCGTACGAATTTGA	2	1	50.0
7	TGAGTCCAAACCGGTAG	GACTGCGTACGAATTGCA	4	3	75.0
8	TGAGTCCAAACCGGTTG	GACTGCGTACGAATTGGT	2	1	50.0
9	TGAGTCCAAACCGGTCA	GACTGCGTACGAATTCGA	2	1	50.0
10	TGAGTCCAAACCGGTGC	GACTGCGTACGAATTCAA	2	1	50.0
11	TGAGTCCAAACCGGTGC	GACTGCGTACGAATTTGC	3	2	66.6
12	TGAGTCCAAACCGGTGC	GACTGCGTACGAATTGGT	8	7	87.5
13	TGAGTCCAAACCGGTAG	GACTGCGTACGAATTGAC	0	0	0.0
14	TGAGTCCAAACCGGACC	GACTGCGTACGAATTTAG	0	0	0.0
15	TGAGTCCAAACCGGACC	GACTGCGTACGAATTCAG	2	1	50.0
16	TGAGTCCAAACCGGACC	GACTGCGTACGAATTAGC	4	3	75.0
17	TGAGTCCAAACCGGACC	GACTGCGTACGAATTTAG	2	0	0.0
18	TGAGTCCAAACCGGAAG	GACTGCGTACGAATTTGA	8	7	87.50
19	TGAGTCCAAACCGGAAG	GACTGCGTACGAATTTCG	0	0	0.0

Nine TRAP primers were used to amplify DNA segments from 6 Yucca genotypes. The number of amplified bands per primer varied between 0.0 and 19 (Table 7). A total of 65 bands were observed, with 7.2 bands per primer. 57 out of 65 bands (87.7 %) were polymorphic. An example of polymorphism is shown in (Figure 2B), which shows the amplification profiles, generated by primer TRAP-5 across the 6 Yucca genotypes, all of which had distinguishable banding patterns. Previously, Hu and Vick (2003) developed a new marker technique known as target region amplified polymorphism (TRAP), which is a rapid and efficient PCR-based technique that employs two 18-mer primers. One ‘‘fixed’’ primer is designed from a known expressed sequence tag (EST), while the other primer is arbitrary with either an AT- or GC- rich core to anneal with an intron or exon, respectively. Xu et al. (2003) used TRAPs to characterize genetic stocks of tetraploid wheat (*Triticum turgidum* L.) and found that a large number of chromosome-specific markers could be generated with this technique. The results indicated that TRAPs might be suitable for rapidly mapping the wheat genome. Recently, Liu et al. (2005) reported that TRAP markers were very efficient for rapidly generating a large number of markers scattered across the genome, which allowed linkage groups to be joined and many gaps to be filled. TRAPs also showed the same ability as SSRs to assign linkage groups to chromosomes.

Genetic diversity of molecular markers

The relationships among Yucca genotypes were estimated by a UPGMA cluster analysis of genetic similarity matrices. The composition of clusters obtained using SRAP markers alone (Figure 3A), TRAP markers alone (Figure 3B) and using both SRAP and TRAP markers together (Figure 3C) revealed similar groupings in some cases.

Cluster analysis using SRAP data grouped the 6 Yucca genotypes into two main clusters with Jaccard’s similarity coefficient ranging from 0.40 to 0.64 (Figure 3A). The highest similarity was found between Jewels and Mela (0.64) and the lowest was between Albo and Green (0.40). The first cluster included the Silver Star, Variegata and Green genotypes, while the second cluster included the Albo, Mela and Jewels.

The dendrogram generated from TRAP data clearly indicated two main clusters (Figure 3B). The Jaccard similarity coefficient ranged from 0.40 to 0.92. Maximum similarity was found between Variegata and Green. The first cluster included the Silver Star and Albo. The second cluster included the Mela, Jewels, Variegata and Green genotypes.

SRAP and TRAP data were combined to produce a dendrogram. The similarity coefficient among the six Yucca genotypes varied from 0.39 to 0.75 with the highest being between Variegata and Green genotypes, and the lowest being between Albo and Green as revealed earlier by TRAP alone. Cluster analysis revealed three main clusters (Figure 3C). The first cluster included the Silver Star alone, while the second cluster included the Mela, Jewels, Variegata and Green genotypes. The last cluster included the Albo genotype

Table 7. Number of amplification and polymorphic products, using nine TRAP primers in six Yucca cultivars.

Primer number	Nucleotide sequence (5` - 3`)		No. of Amplification[a]	No. of polymorphic[b]	Polymorphim[b/a] (%)
	Fixed primers	Arbitrary primers			
1	TGAGTCCAAACCGGT	TCACCCGCACCTTCTTCC	7	7	100.0
2	TGAGTCCAAACCGGC	CGGACAGTGGCGGAGTTA	6	6	100.0
3	TGAGTCCAAACCGGC	GGCGAACTCCGACATCTT	5	3	60.0
4	TGAGTCCAAACCGGC	GAGGAAGACGACGAGGT	10	9	90.0
5	TGAGTCCAAACCGGA	TTCTTCCTCCCGCTCATT	6	4	66.6
6	TGAGTCCAAACCGGT	CCCTCCACCAATCACAAT	3	0	0.0
7	AGTAACCCACCGCTTC	TCCTACAAACATTGCCTT	0	0	0.0
8	TGCCGCTTCCAACAAA	TCACCCGCACCTTCTTCC	19	18	94.7
9	TGAGTCCAAACCGAT	CAGGCAAGACGCAAGGG	9	8	88.9

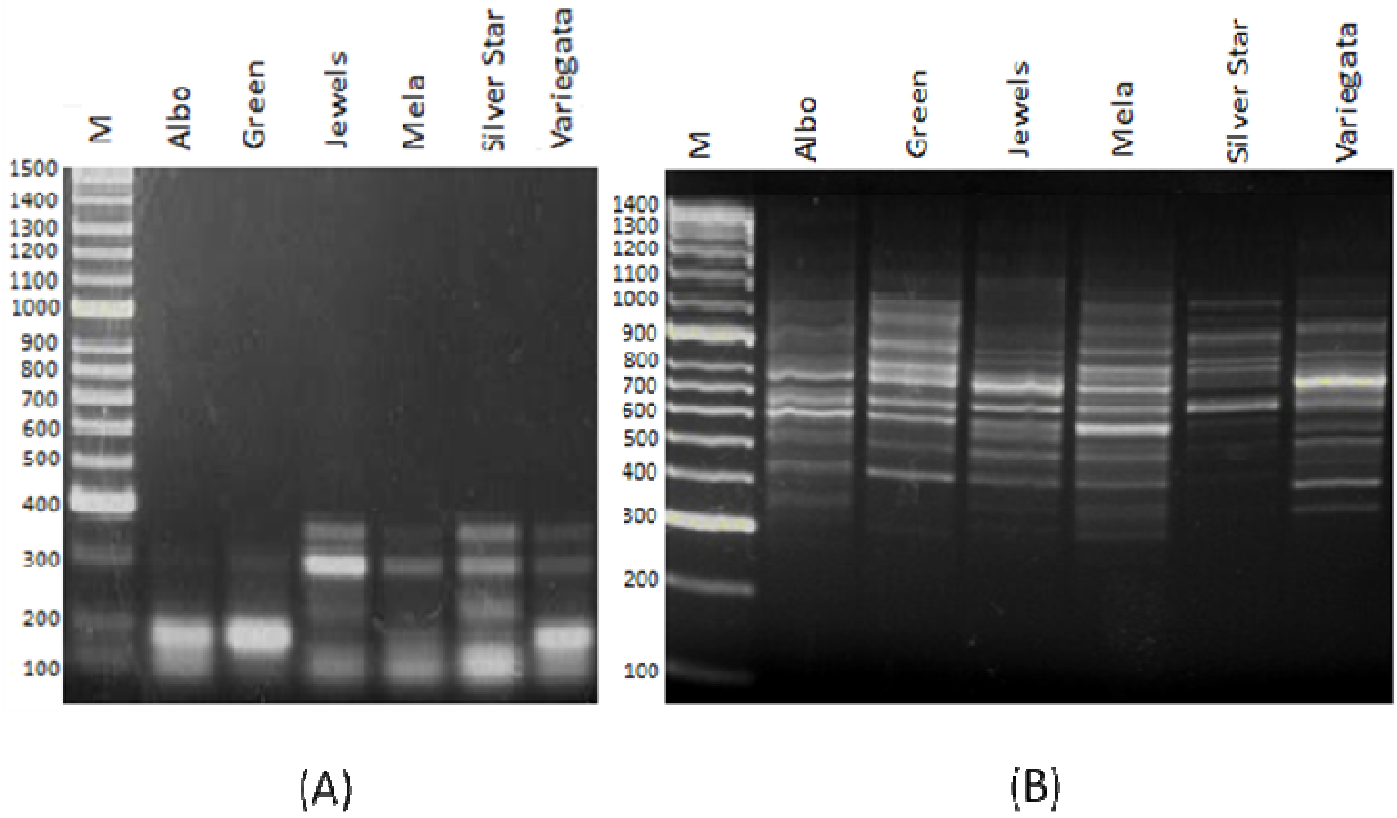

Figure 2. SRAP fragments (A), produced by primer SRAP-5 and TRAP fragments (B), produced by primer TRAP-5. M: Molecular weight followed by Yucca genotypes.

alone. Genetic diversity, relatedness and structure of parental germplasm are important for breeders to design strategy in breeding programme. Diversity analysis is important for deciphering genetic relationship including parentage and for the efficient management of germplasm and, thereby, use in breeding of improved varieties. Establishing the identity of crop variety using diversity study has assumed greater importance for protecting plant breeder's and farmer's rights. In the present study, this is the first report of using SRAP and TRAP analysis in Yuca to estimate the relationships among Yucca genotypes.

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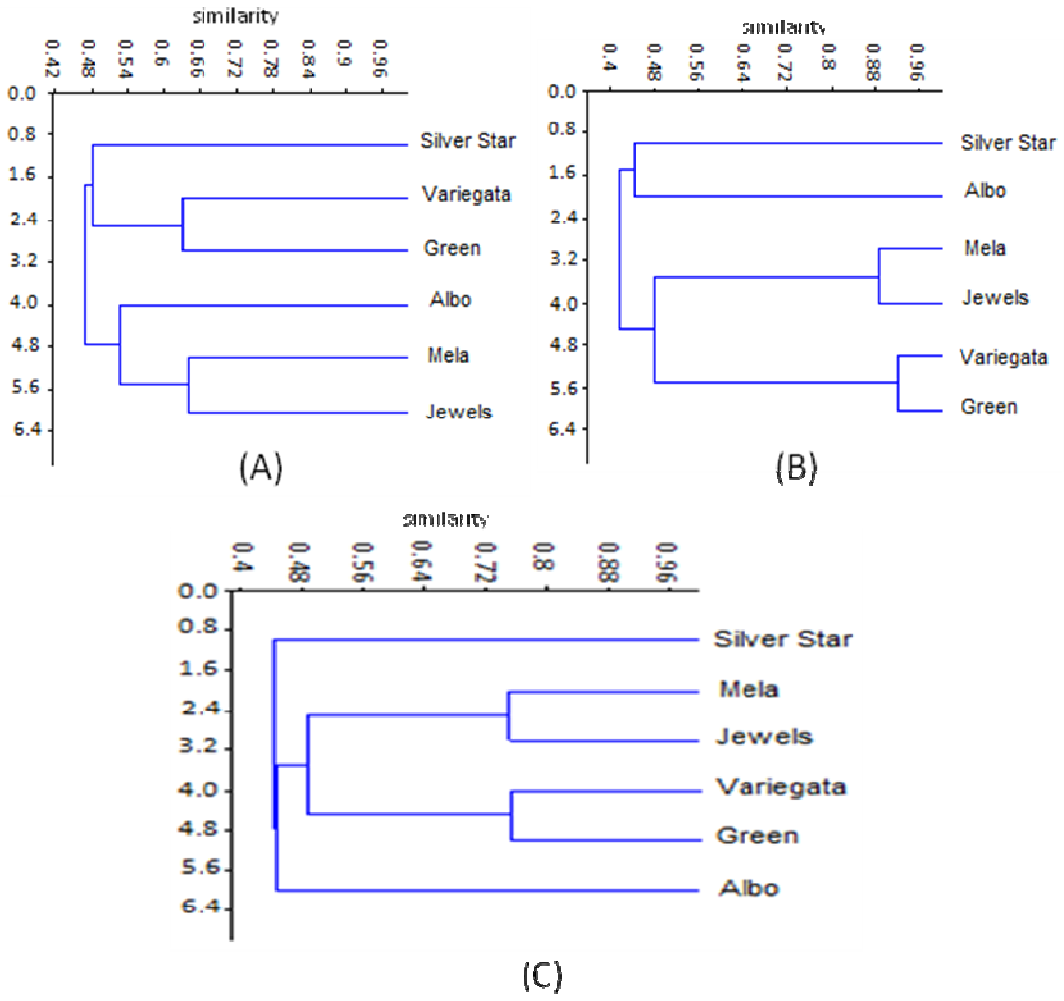

Figure 3. Dendrogram based on Jaccard similarity coefficient of 6 Yucca genotypes, generated using SRAP markers (A), TRAP markers (B) and combined SRAP and TRAP markers (C).

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REFERENCES

Al-Doss AA, Saleh M, Moustafa KA, Elshafei AA, Barakat MN (2011). Comparative analysis of diversity based on morpho-agronomic traits and molecular markers in durum wheat under heat stress. Afr. J. Biotechnol., 10(19): 3671-3681.

Arce MM, Rodriguez AM, Julio A, Gonzalez H, Ropert ML (2006). Micropropagation and field performance of *Yucca valida*. Cell Rep., 25: 777-783.

Atta AH, Van SJ (1997a). Micropropagation and establishment of *Yucca aloifolia*. Plant Cell Tiss. Organ. Cult., 48: 209-212.

Atta AH, Zaghloul M, Waly AK, Askar FM (1997b). *In vitro* shoot proliferation, rooting and establishment of *Yucca aloifolia*, *Y. filamentosa*, and *Y. filamentosa* var. Variegata. Ann. Agric. Sci. Moshtohor, 35: 915-934.

Bahuguna S, Sati OP (1990). Spirostanol saponins from *Yucca aloifolia* rhizomes. Phytochem., 29: 342-343.

Barakat MN, El-Lakany MH (1992). Clonal propagation of *Acacia saligna* by shoot tip culture. Euphytica, 59: 103-107.

Barakat MN, Al DAA, Saleh M, Moustafa KA, Elshafei AA (2011). Comparative analysis of diversity based on morpho-agronomic traits and molecular markers in durum wheat under heat stress. A. J. Biotechnol., (In Press).

Barakat MN, El SH (2011). *In vitro* culture and plant regeneration from shoot tip and lateral bud explants of *Gypsophila paniculata* L. J. Med. Plant Res., (In Press).

Bentz SE, Bruce J, Talbott HJ, Ackerman W (1988). Factors affecting *in vitro* propagation of *Yucca glauca*. Plant Cell Tiss. Org. Cult., 14: 111-120.

Hu J, Vick BA (2003). Target region amplification polymorphism: a novel marker technique for plant genotyping. Plant Mol. Biol. Rep., 21: 289-294.

Jaccard P (1908). Nouvelles rechearches sur la distribution locale. Bulletin de la Societe Vaudoise des Sciences Naturelle, 44: 223-270.

Karpov P (2000). Future of clonal propagation of Torrey Yucca. Plant Physiol. Biochem., 38: 19-24

Karpov P (2004). Clonal propagation of *Yucca aloifolia* L. Acta Univ Latvie Biol., 676: 177-182.

Kukufchanka K, Kromer KD (1984). Regeneration of Yucca sp. and propagation in culture *in vitro* Act Univ. Wratisl Pr. Bot., 30: 39 – 48.

Li G, Quiros CF (2001). Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. Theor. Appl.

Genet., 103: 455-461.
Litz RE, Conover RA (1978). Tissue culture propagation of some foliage plants. Proc Florida St. Hort. Soc., 90: 301-303.
Liu ZH, Anderson JA, Hu J, Friesen TL, Rasmussen JB, Faris JD (2005). A wheat intervarietal genetic linkage map based on microsatellite and target region amplyfied polymorphism markers and its utility for detecting quantitative trait loci. Theor. Appl. Genet., 111: 782-794.
Murashig T, Skoog F (1962). A revised medium for rapid growth and bioassay with Tobacco tissue cultures. Physiol. Plant, 15: 473-479.
Navin K, Bahuguna S, Sati OP, Sakakibara J, Kaiya T (1992). A spirostanol glycoside from *Yucca aloifolia*. Phytochem., 31: 706-707.
Pierik RL, Stegmans HM (1983). Vegetative propagation of chimerical *Yucca elephantipes* Regel *in vitro*. Sci. Hort., 21: 267-272.
Rohlf FJ (1993). NTSYS-pc numerical taxonomy and multivariate system, version 1.80 applied biostatistics Inc., New York, U.S.A.
Sagahi MMk, Soliman RJ, Allard R (1984). Ribosomal DNA spacer length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamic. Proc. Nat. Acd. Sci., 81: 8018, U.S.A.
Sakr SS, El KMA, AbdEl-Kareim AH (1999). *In vitro* production of *Yucca elephantipes*. Bull. Fac. Agric. Cairo Univ., 50: 265-282.
SAS Institute Inc (1985). SAS/STAT. Guide for personal computers. Version 6, 4th ed. Vol. 2 Cary NC, USA.
Steel RGD, Torrie JH (1980). Principles and Procedures of Statistics. A Biometrical Approach. (2 nd edition). McGraw Hill Book.
Sokal RR, Michene CD (1958). Statistical methods for evaluating systematic relationships. Uni. Kansas Sci. Bull., 38: 1409-1438.
Wang G, Pan JS, XZ LI, He HL, Wu AZ, Cai R (2005). Construction of a cucumber genetic linkage map with SRAP markers and location of the genes for lateral branch traits. Sci. China Ser. C, 48: 213-220.
Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18: 6531-6535.
Xu SS, Hu J, Faris JD (2003). Molecular characterization of Langdon durum- *Triticum dicoccoides* chromosome substitution lines using TRAP (target region amplification polymor phism) markers. In: Proc 10th Int Wheat Genet Symp, vol. 1, Institute Sperimentale per la Cerealicoltura, Rome, Italy, pp. 91-94.