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EPIGENETIC EVALUATION IN ALGERIAN OPUNTIA SPECIES UNDER SALT STRESS

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Abstract

In this study, we surveyed the epigenetic variations in salt stressed Opuntias belonging to four Algerian species; Opuntia species: O. ficus indica, O. amycleae, O. streptacantha, and O. engelmannii. Plants response to salt stress (0, 200 mM, 400 mM and 600 mM concentrations of Na Cl) was evaluated. Then, RAPD markers were used to apprehend the epigenetic variations at the DNA level. The analyses of the epigenetic variations were conducted using the random primers UBC228, UBC231 and UBC241 that generated 57 polymorphic markers. Results have shown that in comparison to the control plants, 13 markers were lacking in the control but induced by salt stress application. On the other hand, 02 markers were only present in the control plants. Interestingly, some markers were only induced at the highest salinity concentration (600 mM) and two bands were specific of the tolerant species O. engelmannii. In all, the 15 detected specific markers may be strongly involved in Marker-Assisted Selection studies against salt stress in cactus. Therefore, DNA sequencing of these interesting private markers may aid the identification of putative salt resistance genes, and genetic transformation procedures will subsequently facilitate their introgression in Opuntias to cope soil salinization.

Key words: Opuntia; Epigenetic variability; RAPD Markers; Salt stress.

INTRODUCTION

Losses of agricultural land and vegetation cover have been very high in recent years due to soil salinization, especially in arid and semi-arid regions due to moisture deficits, high groundwater evaporation and poor irrigation management (Ashraf, Oleary, 1996). In these areas, particularly in the Mediterranean basin, soil salinization is one of the major abiotic factors reducing agricultural yields of several crops. Indeed, the degradation of soil and water quality as a result of irrigation poses a serious threat to the sustainability of this land use system (Le Houérou, 1996; Badraoui et al., 1998; Boujghagh, Chajia, 2001).

Therefore, the introduction of tolerant and appropriate plants is one of the means used for exploitation of marginal soils in particular. In this context, Opuntia is a perennial plant that has the ability to adapt to various soil and climate conditions (Lallouche et al., 2017). In Algeria, different species of

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Opuntia are listed and have been an integral part of the agricultural landscape for several centuries in arid and semi-arid zones (Hadjkouider et al., 2017). These species thrive in a wide range of marginal lands characterized by the presence of salts, frequent periods of drought and low soil fertility in these areas. Indeed, several species of opuntia contribute to soil stabilization and the effective fight against the advance of the desert (Lallouche et al., 2017).

However, the choice of species adapted to each situation and type of crop would, in our opinion, be the first concern. It would therefore be interesting to select from these genetic resources the appropriate species. In addition, the study of the effects of salinity on plant growth and development and the search for stress markers represent a great importance in the selection of tolerant species. Certainly, the response of plants to saline stress is multifaceted and corresponds to a multigenic trait (Pardo, 2010). It is therefore necessary to have multiple and reliable markers to characterize tolerance behaviors.

Effective assessment of genetic diversity between species in relation to saline stress is necessary when plans for conservation of salt-infected areas are being considered. In addition, epigenetic studies allow the creation of basic collections and in situ management of these phyto-resources. Indeed, we have used here RAPD markers to study and assess the epigenetic variability of Opuntia species existing in the arid and semi-arid zones of Algeria, following the application of saline stress, in order to optimize the selection and improvement of these species under saline stress conditions.

MATERIAL AND METHOD

Plant material and salt stress application

In the present study, four prickly pear species existing in Algeria namely (*Opuntia ficus indca*, *Opuntia amycleae*, *Opuntia streptacantha*, and *Opuntia engelmannii*) were used. Cladodes were planted in plastic pots (5 L) filled with sand and put under natural growing conditions. Cladodes were irrigated weekly by potable water. At second year following culture, after the appearance of the young cladods, the plants were given four levels of salt in the form of NaCl (0, 200, 400 and 600 mM, to NaCl). RAPD analysis investigated after 60 days of salt treatment.

DNA extraction

In this study, a DNA method extraction technique for cacti which helps to overcome the difficulties caused by mucilage has been used. Thus, for each species, an external slice of the cladode was taken for analysis (Zoghlami et al., 2007).The cuticle was removed and a piece of about 1 g of the

chlorenchyma was cut using a scalpel and taking care not to include areolar meristems.

The protocol of DNA extraction used here is that of Doyle and Doyle, 1987. DNA was quantified by visual comparison with lambda DNA molecular marker on ethidim bromide stained agarose gels. Eleven primers obtained from the University of British Colombia (Table 1), were tested on four *Opuntia* species following salt stress application at four levels.

Table 1

List of RAPD primers used and quality of their amplification products

Primer	Sequence 5' = 3'	Amplification quality
UBC-226	GGGCCTCTAT	good amplification, polymorphic bands (retained)
UBC-241	GCCCGACGCG	good amplification, polymorphic bands (retained)
UBC-231	AGGGAGTTCC	good amplification, polymorphic bands (retained)
UBC-232	CGGTGACATC	low amplification, monomorphic bands (eliminated)
UBC-212	GCTGCGTGAC	low amplification, monomorphic bands (eliminated)
UBC-238	CTGTCCAGCA	low amplification, monomorphic bands (eliminated)
UBC-227	CTAGAGGTCC	low amplification, monomorphic bands (eliminated)
UBC -261	CTGGCGTGAC	low amplification, monomorphic bands (eliminated)
UBC-246	TATGGTCCGG	low amplification, monomorphic bands (eliminated)
UBC-248	GAGTAAGCGC	low amplification, monomorphic bands (eliminated)
UBC-243	GGGTGAACCG	low amplification, monomorphic bands (eliminated)

PCR reactions were performed in a 10 ml reaction mixture containing: 10 ng of template DNA, 2 µl of Go Taq buffer (Promega), 2.5 mM dNTPs (Promega), 25 mM MgCl2, 5 µM of primer and 0.2 U of Go Taq DNA polymerase (Promega). PCR products were separated on a 1.6 % agarose gel containing ethidium bromide using 1 x TAE buffer. The sizes of the amplified fragments were determined by using DNA size standards (1 kb DNA ladder, promega). DNA fragments were visualized and photographed using gel documentation system (gene Genius, Bio Imaginig System, Synaptic Group, UK). To test the reproductibility of the profiles, the reactions were repeated at least twice. The PCR was performed in a thermocycler (Genius), as described by Burrow et al., 1996. Products of the PCR were separated by electrophoresis in 1.6 % agarose gels with 1x TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) using a volt range of 3 V cm^{-1} during 3 h. Lambda DNA EcoRI/Hind III digested (Boehringer Mannheim, Germany) was used as a molecular size standard. Amplifications were performed at least twice and only reproducible (stable) products were taken into account for further data analysis.

Data analysis

The clustering of different species gives information on their similarity and dissimilarity in responses to salt stress, which facilitate the choice of the

tolerant species to be involved in future breeding programs. After, scored the polymorphic DNA bands as discrete variables. The ability of the most informative primers to differentiate between accessions was assessed by estimating their resolving power (Rp) (Prevost, Wilkinson, 1999) according to the following formula. $Rp = \Sigma Ib$, where $Ib = 1 - (2 \times |0.5 - p|)$, where p is the proportion of accessions containing the I band. Genetic distances are used for the calculation of trees following the UPGMA method (Sneath, Sokal, 1973) and the dendrogram is then constructed by Darwin software.

RESULTS AND DISCUSSION

Primers selection and resolving power

Species that were found to be highly salt tolerant, tolerant and moderately tolerant were used in the RAPD analysis. Out of 11 primers selected, the primers UBC-226, UBC-231 and UBC-241, consistently produced distinct polymorphic bands. These primers were further used in screening of Opuntias species to detect the RAPD fragments co-segregating with salt tolerance. The results showed that the three primers had an average polymorphic degree of 97.91 % (Table 2**)**.

Table 2

Primers used and number of different amplified fragments observed among four Algerian opuntia species

N°	Primer	Size	Total bands	Polymorphic bands	Resolving power (Rp)	Electrophoretic profile	Degree of polymorphism (%)
1	UBC-226	180-1600	23	23	13	14	100
2	UBC-231	200-1400	19	19	9.875	14	100
3	UBC-241	100-4000	16	15	8.5	14	93.75
Total	3		58	57	31.375	42	293.75
Average			19.33	19	10.45	14	97.91

The three informative primers were selected and used to evaluate the degree of polymorphism and genetic relationships among the genotypes under study. The different primers produce a number of amplified fragments ranging from 23 to 16, with the size of the amplified fragments ranging from 100 bp to 4000 bp. A total of 58 bands are obtained with 23 fragments for the UBC-226 primer, 19 for the UBC-231 primer, and 16 for UBC-241. It thus appears that of the 58 bands, 57 are polymorphic. The presence or absence of these bands varies according to the species and from to the level of saline stress (0, 200, 400 and 600 mM) (Table 2).

On the other hand, 42 distinct electrophoretic profiles were observed, showing a high level of genetic variability in the different species studied after the application of saline stress. The maximum numbers of fragment

bands were produced by the primers UBC-226 (23) with 100 % polymorphism (Table 2**)**.

Several bands appeared in the different species studied that did not exist in the control plants. A new fragment of size 550 bp is observed in *O. ficus indica* (Table 3), three are observed at (380 bp, 500 bp, and 1000 bp), two fragments of size 620 bp, 1600 bp for *O. streptacantha*, and five bands in *O. engelmannii* (380 bp, 800 bp, 1200 bp, and 1600 bp) (Table 3).

Table 3

Specific markers induced by the application of low, moderate and severe saline stress (200 mM, 400 mM and 600 mM NaCl) in the species studied

Species	[NaCl]	Primers UBC-226	Primers UBC-231	Primers UBC-241
O. ficus indica	200mM	-	-	3000 bp, 4000 bp
	400mM	-	700, 900, 1100, 1400 bp	3000 bp, 4000 bp
	600mM	550 bp.	700, 900, 1100, 1400 bp	-
O. amyclea	200mM	380 bp, 500 bp, 1000 bp	-	-
	400mM	380 bp, 500 bp, 1000 bp	-	-
	600mM	-	-	-
O. streptacantha	200mM	-	-	-
	400mM	-	-	-
	600mM	620 bp, 1600 bp	620, 1100 bp	-
O. engelmannii	200mM	-	-	3000 bp, 4000 bp
	400mM	380 bp	-	3000 bp, 4000 bp
	600mM	800, 1200, 1400, 1600 bp	380, 800, 1200, 1400 bp	1200, 1400, 3000, 4000bp

Thus, a total of 15 markers are induced by the application of low, moderate and severe stress (200, 400 and 600 mM NaCl) (Table 3). Of these 15 markers, five are induced by the application of the most severe saline stress (600mM NaCl). Among these five specific markers, two fragments with a size of 800 bp and 1200 bp appear in *O. engelmannii*, one marker appeared in *O. streptacantha* (620 bp), one marker is observed in *O. engelmannii* and *O. streptacantha* (1600 bp), one marker appeared in *O. ficus indica* (550 bp) (Table 3).

Our results show the synthesis of new fragments in the four stressed species. These new fragments induced by the stressed species have a direct function in increasing tolerance to salt stress. The variation in band accumulation between species may be result from a difference in gene regulation or genome organization attributed to the presence of a large number of gene copies.

Epigenetic diversity and species relationships

One weighted pair group method with arithmetic mean (UPGMA) cluster analysis based on 3 primers revealed that all the highly salt tolerant, salt tolerant and moderately tolerant plant species under study had a genetic distance between 0.4 - 0.95 (Table 4). The smallest distance values of 0.4 were observed between the specie *O. amycleae* under salt stress 400 and 600

mM NaCl (Table 4). Whereas, the maximum distance value of 0.95, suggesting great dissimilarities, were observed between the species *O. engelmannii* under salt stress 600 mM NaCl and *O. streptacantha* under salt stress 200 mM NaCl (Table 4). All the remaining accessions display different intermediate levels of similarity.

Table 4

Genetic distances calculated by pairs of genotypes of the Opuntia species studied following application of salt stress using 57 RAPD markers

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
2	0.79														
3	0.76	0.84													
4	0.82	0.91	0.56												
5	0.80	0.88	0.65	0.76											
6	0.75	0.89	0.82	0.77	0.78										
7	0.72	0.83	0.73	0.77	0.75	0.64									
8	0.73	0.84	0.80	0.78	0.76	0.79	0.73								
9	0.81	0.84	0.71	0.81	0.79	0.82	0.79	0.88							
10	0.82	0.82	0.78	0.70	0.82	0.57	0.63	0.78	0.81						
11	0.68	0.82	0.73	0.79	0.71	0.64	0.40	0.69	0.76	0.58					
12	0.75	0.73	0.74	0.83	0.81	0.76	0.76	0.70	0.77	0.73	0.69				
13	0.84	0.81	0.80	0.83	0.86	0.84	0.84	0.83	0.71	0.84	0.76	0.79			
14	0.75	0.84	0.81	0.82	0.67	0.67	0.76	0.76	0.81	0.77	0.67	0.74	0.76		
15	0.74	0.84	0.69	0.73	0.79	0.68	0.75	0.78	0.73	0.72	0.68	0.62	0.69	0.72	
16	0.88	0.86	0.89	0.91	0.94	0.89	0.91	0.95	0.89	0.92	0.92	0.90	0.87	0.89	0.89

(1, 6, 10, 14: *O. ficus indica* : 0, 200, 400 et 600 mM NaCl ; **2, 4, 7, 11**: *O. amycleae* : 0, 200, 400 et 600 mM NaCl ; **3, 8, 12, 15**: *O. streptacantha* : 0, 200, 400 et 600 mM NaCl ; **5, 9, 13 16**:*O. engelmannii* : 0, 200, 400 et 600 mM NaCl).

The RAPD based genetic distance clearly formed a very divergent group (Fig. 1) and genetic distances of these genotypes with others are presented in the table 4.

Furthermore the dendrogram constructed using neighbor joining method of cluster analysis separated all the four species of highly salt tolerant, salt tolerant and salt moderately tolerant plants into 8 main cluster (A, B, C, D, E, F, G, and H) (Figure 1). The first cluster (A) contained one salt tolerant species (*O. engelmannii* under salt stress 600 mM NaCl) (Fig. 1). (B) *O. amycleae* without salt stress (control) (Fig. 1). *O. engelmannii* under salt stress 200 and 400 mM NaCl fall in the same group (C) (Figure 1). *O. streptacantha*, and *O. amycleae* below 200 mM grouped into cluster (D) (Fig. 1). The salt tolerant species *O. engelmannii* without salt stress (control) and *O. ficus indica* under salt stress 600 mM NaCl were grouped together in

cluster (E) (Fig. 1). *O. streptacantha* under salt stress 400 and 600 mM NaCl fall in the same group (G) (Figure 1). *O. streptacantha* under salt stress 200 mM NaCl and *O. ficus indica* without salt stress (control). Cluster H consisted of two sub clusters. Cluster H1 consisted of one salt tolerant species under salt stress 200 and 400 mM (*O. amycleae*). Cluster H2 included one salt tolerant species under salt stress 200 and 400 mM (*O. ficus indica*) (Figure 1). In the eighth cluster (H) *O. amycleae* and *O. ficus indica* have the same genetic distance and seems to be close genetically.

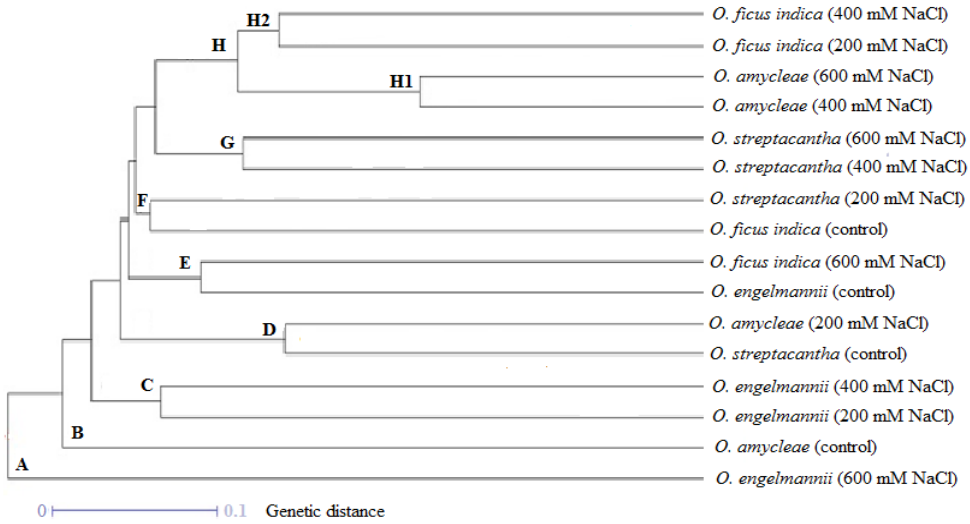

Fig. 1. Dendrogram illustrating the phylogenetic relationships between Opuntia species studied following salt stress application, established on the basis of 57 RAPD markers. (A to H: individualized groups).

Indeed species surviving under NaCl stress at 600 mM, one is grouped in group A (*O. engelmannii*), one in group E (*O. ficus indica*), one in group G (*O. streptacantha*), and *O. amyclea* in group H1. *O. engelmannii* under salt stress 600 mM NaCl (A), was significantly divergent from all tested genotypes. In fact, the aforementioned specie has been identified not only as an out group of analysis but also, as the most genetically distinct one.

The comparisons were done on the basis of alterations in RAPD profiles of NaCI treatment groups and control for Opuntia. The alterations in RAPD profiles included variations as increase in band intensities, loss and gain of bands compared with the RAPD-PCR profiles of the control plants. The improved RAPD-PCR methods enabled the determination of variation in band profiles both for exposure groups and control plants.

The RAPD Primers produced seven fragments of 180, 380, 490, 720, 800, 900 and 1400 bp in some tolerant species, but not the RAPD Primer UBC-231 produced a unique 580 bp fragments in tolerant genotypes only.

In this study, some primers generated intense, polymorphic and reproducible bands (UBC-226, UBC-231, UBC-241). Other primers generated monomorphic bands (without being able to explain their diversity) (Table 1), which, in accordance with the results obtained by some authors (Devos, Gale, 1992; Penner et al., 1993; Caetano-Anollés, 1994; Moreno et al., 1995; Valadez-Moctezuma et al., 2014), indicates that some primers are more effective than others in producing stable and reproducible profiles.

Using three universal primers tested in four Opuntia species under salt stress (0, 200; 400 and 600 mM of NaCl), we registered an average of total band of 19.33 markers per primer. This is significantly higher than one reported by Wang et al., 1998, for Opuntia accessions originating from Texas, Mexico and Chile, which is an average of 4.31; by Zoghlami et al., 2007, for Barbary fig accessions originating from Tunisia, which is an average of 4.37. Moreover, in our case the average polymorphic band is equal to 19 bands per primer. This average is much higher than those published by (Michelmore et al., 1991; Mondragón-Jacobo, 2003; Luna-Paez et al., 2007; Zoghlami et al., 2007; Silva-Ortega et al., 2008; Bendhifi et al., 2013; Bhutta, Hanif, 2013; Zarroug et al., 2015) reported: 18.3, 6.22, 18, 4.87, 6.8, 6.83, 13.7, and 5.14 whose average polymorphic markers per primer were respectively. Thus, we may assume that the Algerian opuntia is characterized by a high genetic diversity at the DNA level under salt stress.

This assumption is strongly supported with regard to the scored genetic distances among the species studied (0.40 - 0.95). In phylogenetic analyses, it has been commonly published on this kind of genetic distance (Mondragón-Jacobo, 2003; Luna-Paez et al., 2007; Zoughlami et al., 2007; Bendhifi et al., 2013; Zarroug et al., 2015) for individuals belonging to different species of genus Opuntia of the family Cactaceae.

For all primers, the values of the resolving power (Rp) ranged from 8.5 for the UBC-241 primer to 13 for the UBC-226 primer. These values reflect the high resolution of the primers used with a total Rp value of 31.375 and an average of 10.45 (Table 2). This average is much higher than that published by: Zoughlami et al., 2007; Bendhifi et al., 2013; Zarroug et al., 2015, which were 1.94, 4.02, and 2.77 respectively.

In addition, our results show the synthesis of new fragments in *O. engelmannii*, *O. sreptacantha* and *O. ficus indica*. These new NaCl induced fragments have a direct function in increasing tolerance to saline stress. It can be said that each genotype has its own salt tolerance expressed by its genes. It can be concluded that a relatively high number of polymorphic bands have been found and could be used as markers. A similar result was also reported by Iqbal et al., 2007, who pointed out that RAPD analysis provides a rich source of specific markers that can be used to characterize and cluster wheat genotypes, which will be useful in wheat breeding programmes. Khan et al.,

2013, used twenty RAPD primers to study genetic variation among ten soybean genotypes. It is concluded that the soybean cultivars closest to the group have similarity in their response to salinity tolerance. Finally, Lee et al., 2003, identified two RAPD markers in salt-tolerant rice lines, also Younis et al., 2007, developed four RAPD markers for salt tolerance in sorghum.

CONCLUSIONS

The present study highlights that RAPD markers can be used to resolve the genetic relationships among taxonomically diverse salt tolerant opuntia species. These markers can also be used to find out common DNA fragments among the salt tolerant plants and relating the amplified bands with those genetic elements that may have some role in salt tolerance. DNA sequencing of these interesting private markers may aid the identification of putative salt resistance genes, and genetic transformation procedures will subsequently facilitate their introgression in the *Opuntia* to cope soil salinization in the arid and semi-arid areas of Algeria.

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REFERENCES

1. Ashraf M., Oleary J.W., 1996, Responses of some newly developed salt-tolerant genotypes of spring wheat to salt stress: II. Water relations and photosynthetic capacity. Acta botanica neerlandica, vol.45, no.1, pp.29-39;
2. Badraoui M., Soudi B., Merzouk A., Farhat A., M'hamdi A., 1998, Changes of soil qualities under pivot irrigation in the Bahira region of Morocco: Salinization, Advances in Geoecology, vol.31, pp.503-508;
3. Bendhifi M., Baraket G., Zourgui L., Souid S., Salhi-Hannachi A., 2013, Assessment of genetic diversity of Tunisian Barbary fig (Opuntia ficus indica) cultivars by RAPD markers and morphological traits, Scientia Horticulturae, vol.158, pp.1-7;
4. Bhutta W.M., Hanif M., 2013, Identification of RAPD markers linked to salinity tolerance in wheat, African Journal of Biotechnology, vol.12, no.17;
5. Boujghagh M., Chajia L., 2001, Le cactus: outil de gestion de la sécheresse dans le Sud Marocain, Terre et vie, vol.52, pp.1-7;
6. Burow M.D., Simpson C.E., Paterson A.H., Starr J.L., 1996, Identification of peanut (Arachis hypogaea L.) RAPD markers diagnostic of root-knot nematode (Meloidogyne arenaria (Neal) Chitwood) resistance, Molecular Breeding, vol.2, no.4, pp.369-379;
7. Caetano-Anollés G., 1994, MAAP: a versatile and universal tool for genome analysis, Plant Molecular Biology, vol.25, no.6, pp.1011-1026;
8. Devos K.M., Gale M., 1992, The use of random amplified polymorphic DNA markers in wheat, Theoretical and Applied Genetics, vol.84, no.5-6, pp.567-572;

9. Doyle J.J., Doyle J.L., 1987, A rapid DNA isolation procedure for small quantities of fresh leaf tissue (No. RESEARCH).
10. Hadjkouider B., Boutekrabt A., Lallouche B., Lamine S., Zoghlami N., 2017, Polymorphism analysis in some Algerian Opuntia species using morphological and phenological UPOV descriptors, Botanical Sciences, vol.95, no.3, pp.391-400;
11. Iqbal A., Khan A.S., Khan I.A., Awan F.S., Ahmad A., Khan A.A., 2007, Study of genetic divergence among wheat genotypes through random amplified polymorphic DNA, Genet. Mol. Res, vol.6, no.3, pp.476-481;
12. Khan M.I.R., Iqbal N., Masood A., Per T.S., Khan N.A., 2013, Salicylic acid alleviates adverse effects of heat stress on photosynthesis through changes in proline production and ethylene formation, Plant Signaling & Behavior, vol.8, no.11, p.26374;
13. Lallouche B., Boutekrabt A., Hadjkouider B., Riahi L., Lamine S., Zoghlami N., 2017, Use of physio-biochemical traits to evaluate the salt tolerance of five opuntia species in the algerian steppes, Pak. J. Bot, vol.49, no.3, pp.837-845;
14. Le Houérou H.N., 1996, The role of cacti (Opuntiaspp.) in erosion control, land reclamation, rehabilitation and agricultural development in the Mediterranean Basin, Journal of Arid Environments, vol.33, no.2, pp.135-159;
15. Lee I.S., Kim D.S., Lee S.J., Song H.S., Lim Y.P., Lee, Y.I., 2003, Selection and characterizations of radiation-induced salinity-tolerant lines in rice, Breeding Science, vol.53, no.4, pp.313-318;
16. Luna-Paez A., Valadez-Moctezuma E., Barrientos-Priego A.F., Gallegos-Vazquez C., 2007, Characterization of Opuntia spp. by means of seed with RAPD and ISSR markers and its possible use for differentiation, Journal of the Professional Association for Cactus Development, vol.9, pp.43-81;
17. Michelmore R.W., Paran I., Kesseli R.V., 1991, Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations, Proceedings of the national academy of sciences, vol.88, no.21, pp.9828-9832;
18. Mondragón-Jacobo C., 2003, Molecular characterization using RAPDs of a cactus (Opuntia spp. Cactaceae) collection from central Mexico as a basis for plant breeding, Rev Chap Ser Hortic, vol.9, no.1, pp.97-114;
19. Moreno S., Gogorcena Y., Ortiz J.M., 1995, The use of RAPD markers for identification of cultivated grapevine (Vitis vinifera L.), Scientia horticulturae, vol.62, no.4, pp.237-243;
20. Pardo J.M., 2010, Biotechnology of water and salinity stress tolerance, Current Opinion in Biotechnology, vol.21, no.2, pp.185-196;
21. Penner G.A., Bush A., Wise R., Kim W., Domier L., Kasha K., Fedak G., 1993, Reproducibility of random amplified polymorphic DNA (RAPD) analysis among laboratories, Genome Research, vol.2, no.4, pp.341-345;
22. Prevost A., Wilkinson M.J., 1999, A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars, Theoretical and applied Genetics, vol.98, no.1, pp.107-112;
23. Silva-Ortega C.O., Ochoa-Alfaro A.E., Reyes-Agüero J.A., Aguado-Santacruz G.A., Jiménez-Bremont J.F., 2008. Salt stress increases the expression of p5cs gene and induces proline accumulation in cactus pear, Plant Physiology and Biochemistry, vol.46, no.1, pp.82-92;
24. Sneath P.H., Sokal R.R., 1973, Numerical taxonomy, The principles and practice of numerical classification;

25. Valadez-Moctezuma E., Ortiz-Vásquez Q., Samah, S., 2014, Molecular based assessment of genetic diversity of xoconostle accessions (Opuntia spp.), African Journal of Biotechnology, vol.13, no.2;
26. Wang X., Felker P., Burow M.D., Paterson A.H., 1998, Comparison of RAPD marker patterns to morphological and physiological data in the classification of Opuntia accessions, Journal of the Professional Association for Cactus Development, vol.3, no.1, pp.3-14;
27. Younis R.A., Ahmed M.F., El-Menshawy M.M., 2007, Molecular genetic markers associated with salt tolerance in grain sorghum, Arab J. Biotechnol, vol.10, pp.249-258;
28. Zarroug M.B., Baraket G., Zourgui L., Souid S., Hannachi, A.S., 2015, Genetic diversity and phylogenetic relationship among Tunisian cactus species (Opuntia) as revealed by random amplified microsatellite polymorphism markers, Genetics and molecular research, vol.14, no.1, pp.1423-1433;
29. Zoghlami N., Chrita I., Bouamama B., Gargouri M., Zemni H., Ghorbel A., Mliki, A., 2007, Molecular based assessment of genetic diversity within Barbary fig (Opuntia ficus indica (L.) Mill.) in Tunisia, Scientia horticulturae, vol.113, no.2, pp.134-141.

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