Detection and analysis of genetic variation in *Salicornieae (Chenopodiaceae)* using random amplified polymorphic DNA (RAPD) markers

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Summary

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Representatives of *Arthrocnemum, Salicornia* and *Sarcocornia* were analysed using RAPD. The methods used were optimized by testing different conditions of the parameters. The genetic characterization of the mentioned taxa proved possible, demonstrating the usefulness of RAPD techniques to determine generic limits in the *Salicornieae*.

Introduction

Salicornia L., *Sarcocornia* A. J. Scott and *Arthrocnemum* Moq. are halophyte genera belonging to the tribe *Salicornieae* Dumort. *(Chenopodiaceae)*. They are taxonomically very close and share several morphological features, such as articulate, fleshy stems and opposite scale-like leaves. Some of their ecological, caryological and palynological characteristics also coincide, but others do not. The three genera are widely distributed in coastal habitats and inland salt marshes in many parts of the world. In coastal habitats, populations of each genus may grow under very different tidal regimes, ranging from the seaward edge that is inundated twice daily to the landward fringe that is flooded only by certain spring tides (Davy & Costa, 1992). On sand and low-lying mud deposits in estuaries of south-western Spain, the different species of *Salicornia* and *Sarcocornia perennis* (Mill.) A. J. Scott are found mostly in flooded marshes (at levels between mean high tide and mean high water), whereas *Sarcocornia fruticosa* (L.) A. J. Scott and *Arthrocnemum macrostachyum* (Moric.) Moris grow in non-flooded marshes (at levels between mean high water and mean spring tides). They all play an important role in salt marsh succession (Castellanos & al., 1994), showing a clear zonation with relation to tides.

The taxonomic treatment of these genera by various authors and the delimitation and position of the species differ. The only species of *Arthrocnemum* present in Spain, *A. macrostachyum,* was included in *Salicornia* by early authors (Moricand, 1820; Lagasca, 1817), but later separated as a distinct genus based on differences in habit (*Salicornia* is annual while *Arthrocnemum* is perennial) and other characters. The two Spanish representatives of *Sarcocornia* usually recognized, *S. fruticosa* and *S. perennis,* were placed in *Salicornia* by Linnaeus (1753) and Miller (1768), respectively, then in *Arthrocnemum* by Moquin-Tandon (1840) and others, as again lately

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by Ball (1993). In current treatments, Greuter & al. (1984), Castroviejo (1990), Castroviejo & Coello (1980), and Valdés (1987) consider *Sarcocornia* as a distinct genus. Castroviejo & Lago (1992) have recently proposed a new combination, *Sarcocornia alpini* (Lag.) Castrov., thus raising the members of Spanish species to three.

Salicornia as presently understood includes annual taxa only, and can thus be easily separated from the two perennial genera *(Sarcocornia* and *Arthrocnemum).* However, the infrageneric classification presents problems. *Salicornia ramosissima* Woods and *S. europaea* L. are diploids with a wide distribution in Europe and North Africa, the latter extending to S. W. Asia. Both have been studied from a taxonomic, ecological and caryological point of view. Valdés (1987) considers them as distinct, while Valdés & Castroviejo (1990) and Castroviejo & Coello (1980) consider *S. ramosissima* as the single highly polymorphic species present in Spain. Ball & Akeroyd (1993) associated them with *S. obscura* P. W. Ball & Tutin and *S. prostrata* Pall. to form the *S. europaea* group, but state that "the specific limits within this group are obscure. Inbreeding is predominant, the taxa consisting of one or a few homozygous lines."

In this paper, we try to introduce new aspects of these three genera and, at the same time, to improve their generic and infrageneric classification.

Rapid and efficient techniques for DNA analysis are now available that allow the investigation of genotypic differences not previously noted and that can not be observed by classical methods. Detection of genetic variation is essential for a wide range of comparative genetic studies and can aid in assessing the relationship among taxa.

RAPD (random amplified polymorphic DNA) provides a very powerful technique for detecting DNA polymorphisms, as described by Williams & al. (1990). RAPD analysis is based on random amplification of genomic DNA fragments using short primers of arbitrary sequences. Amplification is achieved by PCR (polymerase chain reaction). This technique does not depend on hybridization analysis with isotopically labelled probes, requires small amounts of DNA only, and no prior knowledge of DNA sequences. The great advantage of RAPD is that it enables to determine DNA polymorphism without prior sequencing or other characterization of the genomic DNA concerned. Fingerprint polymorphism revealed by this analysis can be used for detecting genetic variation in plants at the population, species, genus or higher taxonomic level (see, e.g., Adams & Demeke, 1993; Quirós & al., 1991; Lanham & al., 1992; Dawson & al., 1993; Bucci & Menozzi, 1993; Russell & al., 1993; Wilde & al., 1992; Isabel & al., 1993, and Hadrys & al., 1992).

In this study we examine the usefulness of RAPD techniques to test generic limits in the *Salicornieae*. We also set the scene for the characterization and fingerprinting of *Salicornieae* genotypes at the specific and supraspecific levels. In order to achieve this, we had first to optimize the method by testing different conditions of DNA extraction, amplification and electrophoresis on our species.

We consider the *Salicornieae* to be an ideal group for study, because it has been well investigated taxonomically yet very few data on genotypic variability have been reported. In fact, previous studies of genotypic variation in the *Salicornieae* so far were limited to the genus *Salicornia* and made use of other techniques, such as analysis of RFLP (restriction fragment length polymorphism; Davy & al., 1990) and nuclear rDNA variation (Noble & al., 1992).

Table 1. Symbols, taxa, origins and voucher numbers of the plants studies.

Symbols	Taxa	Origin	Voucher No.
A-1	*Arthrocnemum macrostachyum*	Laguna de Don Claudio	SEV 135765
A-2	– *macrostachyum*	Isla de Saltés (North)	SEV 135766
Sarf-1	*Sarcocornia fruticosa*	Isla de Saltés (North)	SEV 135767
Sarf-2	– *fruticosa*	Isla de Saltés (South)	SEV 135768
Sarp-1	– *perennis*	Isla de Bacuta (North)	SEV 135769
Sarp-2	– *perennis*	Laguna de Don Claudio	SEV 135770
Sarp-3	– *perennis*	Laguna de Don Claudio	SEV 135775
Sali-e	*Salicornia europaea*	Isla de Saltés (South)	SEV 135771
Sali-r1	– *ramosissima*	Laguna de Don Claudio	SEV 135772
Sali-r2	– *ramosissima*	Isla de Bacuta (South)	SEV 135774
Sali-r3	– *ramosissima*	Rio Tinto High Marsh	SEV 135773

Materials and methods

Plant material. – The study used plants collected from salt marshes in the Odiel estuary (Huelva) on the Atlantic coast of S.W. Spain (Table 1). When possible, 3 to 5 samples from each population were used, but in several cases 10 plants were available. They were all stored at –80°C until used. Vouchers (Table 1) were deposited in the Herbarium of the Department of Plant Biology and Ecology, University of Sevilla (SEV).

Total genomic DNA isolation. – Total genomic DNA was extracted from stems including inflorescences, using the method described by Noble & al. (1992), with some modifications. Plant material (2 g) was ground up in liquid nitrogen with a mortar and pestle. Powdered material was transferred to a sterile tube and 5 ml of extraction buffer (50 mM Tris-HCl, pH 8.0; 10 mM EDTA; 10 % SDS; 25 mM

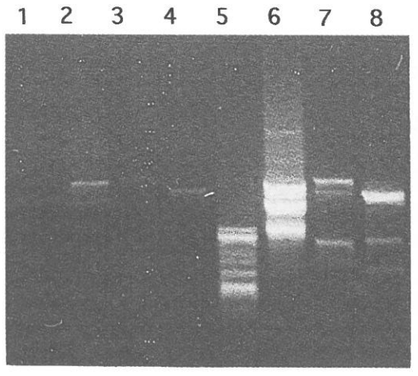

Fig. 1. Gel electrophoresis of RAPD fragments obtained with primers 11 (lanes 1-2, 5-6) and 12 (lanes 3-4, 7-8). – Lanes 1-4, amplification with 35 cycles and annealing temperature of 37°C; lanes 5-8, amplification as before followed by reamplification with 35 cycles at 37°C. – DNA from the genus *Salicornia* (Sali-r3), lanes 1, 3, 5, and 7; from the genus *Sarcocornia* (Sarp-2), lanes 2, 4, 6, and 8.

diethyldithiocarbamic acid) were gently mixed in. The mixture was incubated at 65°C for 30 min, then extracted twice with an equal volume of phenol : chloroform (1 : 1). The supernatant was incubated with DNAse-free RNAse (20 µg/ml) for 20 min at 37°C, and extracted with an equal volume of chloroform : isoamyl-alcohol (24 : 1). The supernatant was collected and the DNA precipitated by the addition of a tenth volume of 5 M NaCl and 0.54 volumes of isopropanol. Precipitated DNA was collected by centrifugation, then washed with 70 % ethanol and dried before redissolving in 500 µl of TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA, pH 8.0). The DNA was precipitated with NaCl (0.8 M final concentration) and 1 volume of PEG (Polyethylene glycol 8000) 13 % weight per volume. The DNA was purified using QIAGEN resin columns (QIAGEN-Tip 20), following the supplier instructions.

DNA amplification (RAPD). – In order to determine the optimal conditions for amplification of our material, we used the following protocol, varying only one parameter at a time (see Results).

PCR reaction mixtures (50 µl final volume) contained 0.05-5 ng/µl of genomic DNA; dATP, dCTP, dGTP, dTTP each at 200 µM final concentration, 1-2 µM primer, 1-5 mM $MgCl_2$, 1 × Taq polymerase buffer (Promega) and 1.25 units of Taq DNA polymerase (Promega). Each reaction was overlaid with 50 µl of mineral oil to prevent evaporation.

Amplification program: a preliminary step at 95°C (5 min) was followed by a set of 2, 5, 10 to 40 cycles, each consisting of a denaturation step at 95°C (1 min), an annealing step at 37°C to 43°C (1 min) and an extension step at 72°C (1 min). One final step at 72°C (10 min) was used to ensure complete final extension from the primers along the entire length of the target molecules.

When indicated, 1 µl of the above reaction mixture was used for reamplification. Reamplification program: a preliminary step at 95°C (5 min), followed by 35 cycles, each consisting in a denaturation step at 95°C (1 min), an annealing step at 37°C or 60°C (1 min) and an extension step at 72°C (1 min). One final step at 72°C (10 min) again followed.

The fourteen arbitrary primers used in this work are shown in Table 2. Amplifications were carried out in a Perkin Elmer Cetus DNA Thermal Cycler. Amplification products were analysed by agarose (1.5 %) gel electrophoresis and visualized by ethidium bromide staining and illumination with UV light. For band size determination, a 123 bp DNA ladder (Sigma) was run in each gel.

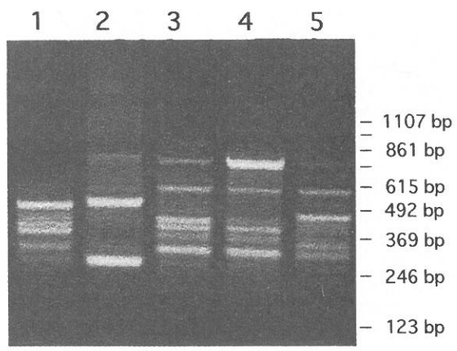

Fig. 2. Gel electrophoresis of RAPD fragments obtained with primer 2. Amplification with 35 cycles and annealing temperature of 37°C and reamplification with 35 cycles at 60°C. – *Sarcocornia,* lane 1 (Sarp-3); *Arthrocnemum,* lane 2 (A-1); *Salicornia,* lanes 3 (Sali-r1), 4 (Sali-e), and 5 (Sali-r2).

Results

Optimization of amplification parameters. – As reported by other authors (Saiki, 1989), the number and intensity of the bands are affected by several parameters, including concentration and quality of DNA, annealing temperature, $MgCl_2$ concentration and primer used (data not shown). However, the profiles of the amplification products were highly reproducible when the same amplification conditions were used.

We tested many different programs, each of them considered as optimal in the literature. When using programs of 35-40 cycles, amplification products were barely visible (Fig. 1, lanes 1-4). Programs consisting in 2 cycles at low temperature (37°C), followed without interruption by 35 cycles at 60°C, did not generate a sufficient amount of DNA. We then tested two successive programs consisting of: (a) an amplification program of 5, 10, 15 to 35 cycles at 37°C, and (b) a reamplification program of 35 cycles applied to only 1 µl of the product generated by (a). Annealing temperature for reamplification of 60°C for long primers and 37°C for shorter primers gave the best results. Consistent results were obtained when using 20 or more cycles in the amplification program (Fig. 1, lanes 5-8). With these conditions, we were able to generate fingerprints with primers from 11 to 25 nucleotides in length. When more than one primer was used, new products were obtained.

We determined the effect of the use of different annealing temperatures (37°C, 39°C, 41°C and 43°C) in the amplification programs. The results (data not shown) demonstrate that band patterns changed only slightly, although when the temperature was raised above 43°C, the amount of amplified DNA tended to decrease (data not shown).

We also tested different template concentrations in the range from 0.05 ng/µl to 5 ng/µl. The optimal concentration was found to be 0.4 to 0.6 ng/µl; higher and lower concentrations gave poorly reproducible bands and, sometimes, no bands at all (data not shown). Concentrations of $MgCl_2$ ranging from 2 mM to 5 mM produced no change in the intensity of the bands. Lower concentrations did not produce bands.

The results presented below were obtained with the following amplification conditions: PCR reaction mixtures (50 µl final volume) contained 20 ng of genomic DNA; dATP, dCTP, dGTP, dTTP each at 200 mM final concentration, 1-2 µM primer, 2.5 mM $MgCl_2$, 1 × Taq polymerase buffer and 1.25 units of Taq DNA polymerase. Amplification consisted of two consecutive programs: (a) A preliminary step at 95°C (5 min) followed by 35 cycles, each consisting of a denaturation step at 95°C (1 min), an annealing step at 37°C (1 min) and an extension step at 72°C (1 min), then a final step at 72°C (10 min); (b) Applied to 1 µl of the above reaction mixture, a preliminary step at 95°C (5 min), followed by 35 cycles, each consisting in a denaturation step at 95°C (1 min), an annealing step at 37°C or 60°C (1 min) and an extension step at 72°C (1 min), then a final step at 72°C (10 min).

Genomic fingerprinting by RAPD. – With the conditions stated above, an initial survey with 14 arbitrary primers or combinations of primers (Table 2) was carried out. The size of the amplification products varied in length from 100 to 800 bp. The

number of bands produced by each primer varies. Examples of the polymorphisms detected are shown in Fig. 2-4.

As summarized in Table 3, five primers or combination of primers (1, 2, 6, 12 [not discussed], and 14) produced banding patterns that were informative in order to analyse and identify the three genera and can, thus, be considered as genome specific markers. Nine of the primers tested gave non-useful results: four of them (4, 5, 7, and 8) gave no bands; three (3, 9, and 13) gave very faint bands and two (10 and 11) gave some lanes with faint bands and others with no bands.

As demonstrated for *Brassica* by Demeke & al. (1992), the utilization of only the faint bands or only the bright bands gave essentially the same classification. Using both gave a taxonomic representation closer to the classical relationships in *Brassica* than using either set alone. The RAPD band intensities were scored as: no band, very faint, faint, medium, and bright bands.

The genetic characterization of the genera *Arthrocnemum, Sarcocornia* and *Salicornia* (Table 4), thus obtained, can be described as follows:

With primer 1 (data not shown), two bright amplification products of c. 230 and 310 bp are constant for the three genera. In addition a different banding pattern for *Arthrocnemum* was obtained, with faint amplification products, making it possible to distinguish it from the other taxa.

Primer 2 gave different banding patterns for the three genera (Fig. 2). *Salicornia* and *Sarcocornia* present a common band of 350 bp but others differ. The *Sarcocornia* populations show one bright band of 550 bp, three faint bands of 400, 450, and 490 bp. The three *Salicornia* populations studied present in common bands of 350, 600, and 800 bp, and other bands which are characteristic for each population. *Arthrocnemum* shows a different banding pattern, with two bright bands of approximately 260 and 570 bp and one faint band of 800 bp, at a similar level as in *Salicornia* populations.

With primer 6, different banding patterns for the three genera are obtained (Fig. 3). *Salicornia* presents a very bright band of c. 300 bp and seven very faint bands at higher levels. *Sarcocornia* presents two bright bands of 220 and 350 bp and two very faint bands at higher levels, *Arthrocnemum* populations present two bright

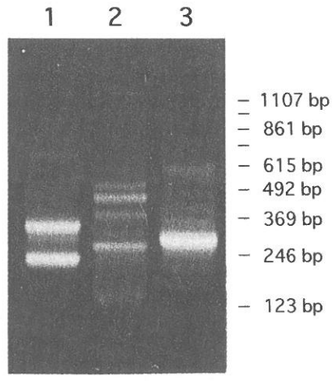

Fig. 3. Gel electrophoresis of RAPD fragments obtained with primer 6. PCR programme as in Fig. 2. – *Sarcocornia,* lane 1 (Sarp-3); *Arthrocnemum,* lane 2 (A-1); *Salicornia,* lane 3 (Sali-r1).

Table 2. Nucleotide sequences and size of the primers and primer combinations used in this work.

Primer No.	Name	Nucleotide sequence	Size (bases)
1	CP2[1]	5′ GGCGCTGTGGCTGATTTCGATAACC 3′	25
2	NS1[2]	5′ GTAGTCATATGCTTGTCTC 3′	19
3	J-2[4]	5′ TCCTCCGCTTATTGATATGT 3′	20
4	NS8[2]	5′ TCCGCAGGTTCACCTACGGA 3′	20
5	TS-1[2]	5′ TCCGTAGGTGAACCTGCGG 3′	19
6	M13-Rev[3]	5′ AACAGCTATGACCATG 3′	16
7	T3[3]	5′ ATTAACCCTCACTAAAG 3′	17
8	T7[3]	5′ AATACGACTCACTATAG 3′	17
9	M13 (-20)[3]	5′ GTAAAACGACGGCCAGT 3′	17
10	P-1[4]	5′ GCTTAATCGCT 3′	11
11	P-2[4]	5′ TGCATTCAGCT 3′	11
12	P-3[4]	5′ GATTCGACGAT 3′	11
13	P-1/P-2[4]	5′ GCTTAATCGCT 3′ / 5′TGCATTCAGCT 3′	11 / 11
14	P-2/P-3[4]	5′ TGCATTCAGCT 3′ / 5′GATTCGACGAT 3′	11 / 11

[1] Primer used for control PCR amplification (Perkin Elmer, Ref. No. N801-0055).

[2] Primers described by White & al. (1990) (synthesized by Pharmacia).

[3] Universal sequencing primers (Stratagene, Ref. No. 300304 and 300303).

[4] Primers arbitrarily designed for this work (synthesized by Pharmacia).

bands of c. 250 and 400 bp and three very faint bands, one at higher and two at intermediate levels.

Primer 14 also reveals different banding patterns for the three genera (Fig. 4): the four *Sarcocornia* populations show two common amplified products, a bright band of 480 bp and a faint band of 100 bp. The *Salicornia* populations show a banding pattern with two bright bands of 250 and 480 bp respectively, the last of them being at a similar level as one in *Sarcocornia,* and one faint band of 620 bp. On the other hand, for *Arthrocnemum* three bright bands are obtained, of approximately 300, 350, and 620 bp (the last of them at a similar level as in one of the *Salicornia* populations). A characteristic faint band of 120 bp is also obtained.

Discussion and conclusions

This paper is the first report of the use of DNA-based polymorphism to assess generic classification within the *Salicornieae*. In other groups, DNA polymorphisms, amplified using oligonucleotide primers of 9 or 10 nucleotides in length or even longer, has been used for genetic markers (Williams & al., 1990). Caetano-Anollés & al. (1991) report that DNA amplification with an arbitrary primer as short as 5 nucleotides can produce detailed and relatively complex DNA profiles, and detect DNA polymorphism, when applied to a wide variety of organisms. In the present

Table 3. Primers used in this study and results obtained.

Primers	Features obtained
1	Similar bands for *Salicornia* and *Sarcocornia*, and different banding pattern for *Arthrocnemum*
2	Different bright bands for the three genera
3	Very faint bands
4	No bands
5	No bands
6	Different bright bands for the three genera
7	No bands
8	No bands
9	Very faint bands
10	Some lanes with faint bands, others with no bands
11	Some lanes with faint bands, others with no bands
12	Polymorphisms among *Salicornia* and *Sarcocornia*
13	Very faint bands
14	Different bright bands for the three genera

study, very good results with primers of 25, 19, 16, and 11 nucleotides were obtained. The profiles of the amplification products were highly reproducible when the same amplification program was used.

RAPD is taxonomically useful. Our results demonstrate the efficiency with which it can characterize genera in the *Salicornieae*. It should be noted that some primers show considerable RAPD variation among species (Fig. 4). If those bands that vary within a genus are not considered, the remaining, more conservative bands result in a

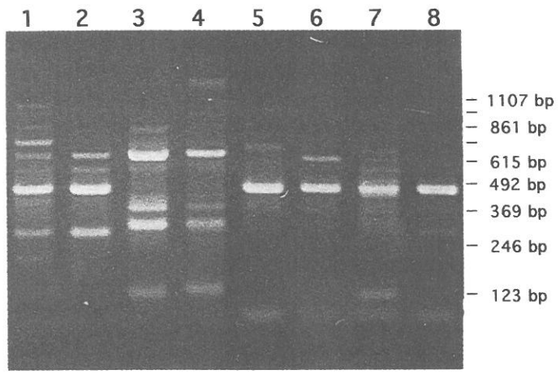

Fig. 4. Gel electrophoresis of RAPD fragments obtained with primer 14. Amplification with 35 cycles and annealing temperature of 37°C and reamplification with 35 cycles at 37°C. – *Salicornia,* lanes 1 (Sali-r1) and 2 (Sali-e); *Arthrocnemum,* lanes 3 (A-2) and 4 (A-1); *Sarcocornia,* lanes 5 (Sarp-2), 6 (Sarp-1), 7 (Sarf-2), and 8 (Sarf-1).

Table 4. Genetic characterization of the genera *Arthrocnemum, Sarcocornia,* and *Salicornia*. Only the most characteristic bands are mentioned (+, faint band; ++, bright band; –, no band).

Primer 1			
Bands (bp)	*Salicornia*	*Sarcocornia*	*Arthrocnemum*
230	++	++	++
310	++	++	++
360	–	–	+
420	–	–	+
440	–	–	+
490	–	–	+
550	–	–	+
650	–	–	+
Primer 2			
Bands (bp)	*Salicornia*	*Sarcocornia*	*Arthrocnemum*
260	–	–	++
350	++	+	–
400	–	+	–
450	–	+	–
490	–	+	–
550	–	++	–
570	–	–	++
600	++	–	–
800	++	–	+
Primer 6			
Bands (bp)	*Salicornia*	*Sarcocornia*	*Arthrocnemum*
220	–	++	–
250	–	–	++
300	++	–	–
350	–	++	–
400	–	–	++
Primer 14			
Bands (bp)	*Salicornia*	*Sarcocornia*	*Arthrocnemum*
100	–	+	–
120	–	–	+
250	++	–	–
300	–	–	++
350	–	–	++
480	++	++	–
620	+	–	++

pattern that discriminates even better between the genera. The more variable bands can in turn be used to differentiate species, and perhaps infraspecific taxa.

Our data are consistent with the separation of three genera, as indicated by Castroviejo & Coello (1980), Greuter & al. (1984), and Valdés (1987), among other authors, and contrary to Ball (1993) who includes *Sarcocornia* in *Arthrocnemum*.

However, we consider that our results are still too preliminary to assess distances among these genera.

Within *Salicornia, S. ramosissima* and *S. europaea* can be told apart with the results obtained, as can be seen in Fig. 2 and 4. We are presently investigating the polymorphism at other taxonomic levels.

In conclusion, we believe that RAPD markers offer a simple yet efficient tool for the characterization of genotypes in the *Salicornieae*. This method may have a wide range of potential applications, and may become a useful predictive tool to identify areas of maximum genetic diversity, or to monitor species in early stages of colonization and succession on marshes and sand-flats, when the identification of seedlings of *Salicornieae* is difficult (Castellanos & al., 1994; Davy & Figueroa, 1993); it can also be used to estimate levels of genetic variability in natural populations.

Acknowledgements

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Literature cited

Adams, R. P. & Demeke, T. 1993. Systematic relationships in *Juniperus* based on random amplified polymorphic DNAs (RAPD). *Taxon* 42: 553-571.

Ball, P. W. 1993. *Arthrocnemum* Moq. P. 121 *in:* Tutin, T. G., Burges, N. A., Chater, A. O., Edmondson, J. R., Heywood, V. H., Moore, D. M., Valentine, D. H., Walters, S. M. & Webb, D. A. (ed.), *Flora europaea,* ed. 2, 1. Cambridge.

– & Akeroyd, J. R. 1993. *Salicornia* L. Pp. 121-123 *in:* Tutin, T. G., Burges, N. A., Chater, A. O., Edmondson, J. R., Heywood, V. H., Moore, D. M., Valentine, D. H., Walters, S. M. & Webb, D. A. (ed.), *Flora europaea.*, ed. 2, 1. Cambridge.

Bucci, G. & Menozzi, P. 1993. Segregation analysis of random amplified polymorphic DNA (RAPD) markers in *Picea abies* Karst. *Molec. Ecol.* 2: 227-232.

Caetano-Anollés, G., Bassam, B. J. & Gresshoff, P. M. 1991. DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. *Bio/Technology* 9: 553-557.

Castellanos, E. M., Figueroa, M. E. & Davy, A. J. 1994. Nucleation and facilitation in saltmarsh succession: interactions between *Spartina maritima* and *Arthrocnemum perenne*. *J. Ecol.* 82: 239-248.

Castroviejo, S. 1990. *Arthrocnemum* Moq. Pp. 526-527 *in:* Castroviejo, S., Laínz, M., López González, G., Monserrat, P., Muñoz Garmendia, F., Paiva, J. & Villar, L. (ed.), *Flora ibérica. Plantas vasculares de la Península Ibérica e Islas Baleares,* 2. Madrid.

– & Coello, P. 1980. Datos cariológicos y taxonómicos sobre las *Salicornieae* A. J. Scott Ibéricas. *Anales Jard. Bot. Madrid* 37: 41-73.

– & Lago, E. 1992. Datos acerca de la hibridación en el género *Sarcocornia (Chenopodiaceae). Anales Jard. Bot. Madrid* 50: 163-170.

Davy, A. J. & Costa, C. S. B. 1992. Development and organization of saltmarsh communities. Pp. 157-178 *in:* Seeliger, U. (ed.), *Coastal plant communities of Latin America.* San Diego.

– & Figueroa, M. E. 1993. The colonization of strandlines. Pp. 113-131 *in:* Miles, J. & Walton, D. W. H. (ed.), *Primary succession on land.* London.

– , Noble, S. M. & Oliver, R. P. 1990. Genetic variation and adaptation to flooding in plants. *Aquatic Bot.* 38: 91-108.

Dawson, I. K., Chalmers, K. J., Waugh, R. & Powell, W. 1993. Detection and analysis of genetic variation in *Hordeum spontaneum* populations from Israel using RAPD markers. *Molec. Ecol.* 2: 151-159.

Demeke, T., Adams, R. P. & Chibbar, R. 1992. Potential taxonomic use of random amplified polymorphic DNA (RAPDs): a case study in *Brassica. Theor. Appl. Genet.* 84: 990-994.

Greuter, W., Burdet, H. M. & Long, G. 1984. *Med-Checklist*, 1. Genève & Berlin.

Hadrys, H., Balick, M. & Schierwater, B. 1992. Applications of random amplified polymorphic DNA (RAPD) in molecular Ecology. *Molec. Ecol.* 1: 55-63.

Isabel, N., Tremblay, L., Michaud, M., Tremblay, F. M. & Bousquet, J. 1993. RAPDs as an aid to evaluate the genetic integrity of somatic embryogenesis-derived populations of *Picea mariana* (Mill.) B. S. P. *Theor. Appl. Genet.* 86: 81-87.

Lagasca, M. 1817. *Memoria sobre las plantas barilleras de España.* Madrid.

Lanham, P. G., Fennel, S., Moss, J. P. & Powell, W. 1992. Detection of polymorphic loci in *Arachis* germplasm using random amplified polymorphic DNAs. *Genome* 35: 885-889.

Linnaeus, C. 1753. *Species plantarum.* Stockholm.

Miller, P. 1768. *The gardeners dictionary*, ed. 8. London.

Moquin-Tandon, C. H. B. A. 1840. *Chenopodearum monographica enumeratio.* Paris.

Moricand, M. E. 1820. *Flora veneta.* Paris.

Noble, S. M., Davy, A. J. & Oliver, R. P. 1992. Ribosomal DNA variation and population differentiation in *Salicornia* L. *New Phytol.* 122: 553-565.

Quirós, C. F., Hu, J., This, P., Chevre, A. M. & Delseny, M. 1991. Development and chromosomal localization of genome-specific markers by polymerase chain reaction in *Brassica. Theor. Appl. Genet.* 82: 627-632.

Russell, J. R., Hosein, F., Johson, E., Waugh, R. & Powell, W. 1993. Genetic differentiation of cocoa (*Theobroma cacao* L.) populations revealed by RAPD analysis. *Molec. Ecol.* 2: 89-97.

Saiki, R. K. 1989. The design and optimization of the PCR. Pp. 7-16 *in:* Erlich, H. A. (ed.), *PCR technology. Principles and applications for DNA amplification.* New York.

Valdés, B. 1987. *Sarcocornia* A. J. Scott. Pp. 186-187 *in:* Valdés, B., Talavera, S. & Galiano, E. F. (ed.), *Flora de Andalucía occidental,* 1. Barcelona.

– & Castroviejo, S. 1990. *Salicornia* L. Pp. 531-534 *in:* Castroviejo, S., Laínz, M., López González, G., Monserrat, P., Muñoz Garmendia, F., Paiva, J. & Villar, L. (ed.), *Flora ibérica. Plantas vasculares de la Península Ibérica e Islas Baleares,* 2. Madrid.

Wilde, J., Waugh, R. & Powell, W. 1992. Genetic fingerprinting of *Theobroma* clones using randomly amplified polymorphic DNA markers. *Theor. Appl. Genet.* 83: 871-877.

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. & Tingey, S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl. Acids Res.* 18: 6531-6535.