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GENETIC DIVERSITY IN F_1 DESCENDENTS OF *Cereus peruvianus* Mill. (Cactaceae) SOMACLONES REGENERATED IN SOUTH REGION OF BRAZIL

[DIVERSIDAD GENÉTICA EN DESCENDENCIA F_1 DE SOMACLONES DE *Cereus peruvianus* Mill. (Cactaceae) REGENERADO EN LA REGIÓN SUR DE BRASIL]

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SUMMARY

Polymorphism in the *Cereus peruvianus* somaclones (S) and the relationships among their F1 descendents (R_1 plants) were detected and examined by RAPD markers. Sixteen primers yielded repetitive patterns for all scored bands in four somaclones (S3, S9, S48 and S71 plants) and in 14 R_1 plants of S3, 11 R_1 plants of S9, 8 R_1 plants of S48 and 14 R_1 plants of S71. The 16 primers generated 222 reproducible fragments of which 65 were polymorphic in R_0 plants and 103, 100, 80 and 108 were polymorphic in R_1 plants of S3, S9, S48 and S71, respectively. Total number of polymorphic bands for the R_1 plants from four somaclones was 124. Polymorphism in R_1 plants (55.9%) was higher than the polymorphism detected in R_0 plants (29,8%). Polymorphism between somaclones may result from genetic variability *in vitro* induced since S3, S9, S48 and S71 somaclones were independently regenerated from different callus tissues, and the polymorphism between R_1 plants may result from the genetic constitution of the male and female parent of the F1 descendents, since R_1 plants were obtained from seeds of somaclones collected from open-pollinated S3, S9, S48 and S71 plants. Dendrogram generated by Jaccard coefficient showed that there was no grouping of R_1 plants according to parental somaclone and that the similarity in R_1 plants ranged from 72.5% to 90.5%. This fact supports our hypothesis that *in vitro* induced variability may be used for broadening the genetic base of *C. peruvianus* species.

Key words: cactus, genetic diversity, mandacaru, RAPD, somaclonal variation.

RESUMEN

Se detectó y examino polimorfismos de somaclones (S) de *Cereus peruvianus* y las relaciones entre sus descendientes F_1 (R_1) por medio de marcadores RAPD. Diez y seis primers produjeron patrones repetitivos para todas las bandas examinadas en cuatro somaclones (S3, S9, S48 y S71) y en 14 R_1 para S3, 11 R_1 para S9, 8 R_1 para S48, y 14 R_1 para S71. Los diez y seis primers generaron 222 fragmentos reproducibles de los cuales 65 fueron polimórficos en plantas R_0 y 103, 100, 80 y 108 fueron polimórficos en R_1 para S3, S9, S48 y S71, respectivamente. El número total de bandas polimórficas para R_1 de los cuatro somaclones fue de 124. Polimorfismo en R_1 (55.9%) fue mayor que el polimorfismo detectado en R_0 (29.8%). Polimorfismo entre somaclones podría ser el resultado de variabilidad genética inducida *in vitro* dado que los somaclones S3, S9, S48 y S71 fueron regenerados independientemente a partir de callos de tejidos diferentes, y los polimorfismos entre plantas R_1 pudieran ser el resultado de la constitución genética de los progenitores de los F_1, dado que R_1 fueron obtenidos de semillas de los somaclones colectadas de polinización abierta de plantas S3, S9, S48 y S71. El dendrograma generado por el coeficiente de Jaccard no mostró agrupación de R_1 de acuerdso al somaclon parental y la similaridad de R_1 varió de 72.5% a 90.5%. Este hecho provee suporte a la hipótesis de que la variabilidad inducida *in vitro* pudiera ser empleada para ampliar la base genética de *C. peruvianus*.

Palabras clave: cactus, diversidad genética, mandacaru, RAPD, variación somaclonal.

INTRODUCTION

Cereus peruvianus, popularly known in Brazil as mandacaru, is a common ornamental cactus species found in gardens of tropical and subtropical countries. This species is under domestication in Israel where has been cultivated as a fruit crop (Nerd et al., 1993; Weiss et al., 1993, 1994; Mizrahi and Nerd, 1999; Nerd et al., 2002). Since *C. peruvianus* has primarily a vegetative propagation Gutman et al. (2001) have shown that this specie has only a limited genetic base and that further improvement of this crop may require the introduction of additional germplasm during breeding programs.

In vitro regenerated plants of *C. peruvianus* may contribute towards the broadening of the species genetic basis since somaclonal variation appears to be an important alternative for creation of genetic variability in crops where tissue culture plant regeneration system have been established (Chahal and Gosal, 2002). A considerable number of useful somaclonal variants have been generated from different plant species (Taji et al., 2002) and somaclonal variation has been reported in several crops for both qualitative as well as quantitative traits (Chahal and Gosal, 2002).

Regenerated plants of *C. peruvianus* obtained from callus tissues (Oliveira et al., 1995) revealed somaclonal variations. Morphological, cytological, and biochemical characters have been the main criteria by which somaclones have been identified. Atypical shoot morphologies and new isozyme patterns occurred in *C. peruvianus* somaclones (Mangolin et al., 1997; 1999; Machado et al., 2000). Consequently, a high degree of genetic diversity at DNA level may also be detected between somaclones and between the F1 descendents of somaclones. *In vitro*-induced genetic variability is important to broadening of the genetic base of *C. peruvianus* species. Thus, in this current research, RAPD markers were used to detect polymorphism in *C. peruvianus* somaclones (clonal regenerants; R_0) and examine relationships among their F_1 descendents (R_1).

MATERIAL AND METHODS

Somaclones (S), also designed clonal regenerants (R_0), were regenerated from callus culture induced using MS medium (Murashige and Skoog, 1962) containing B5 vitamins (Gamborg et al., 1968), 0.8% agar, 3% sucrose, and combinations of the growth regulators 2,4-dichlorophenoxyacetic acid (2,4-D) and N-(2-furanylmethyl)-1H-purine-6 amine (kinetin) according to the method of Oliveira et al. (1995). Callus tissue was formed from hypocotyls stalks of three seedlings of *C. peruvianus*, which grew from seeds collected from a single adult plant (grown from seed) established at the Iguatemi Experimental Farm of state University of Maringá. Callus subcultures were performed at 15-day intervals, and 18-20 weeks after culture initiation (total of 23 somaclonal generations) the friable calli produced the cactus shoots (somaclones). The somaclone population (S or R_0 plants) was planted in 1997 at the Experimental Botanic Garden of the State of University of Maringá (Maringá PR Brazil; altitude 554.9m; 23º 25'S; 51º 25' W).

F_1 descendents of *C. peruvianus* regenerated plants (R_1) were obtained from seeds of somaclones collected from open-pollinated S3, S9, S48, and S71 plants. The R_1 plants (1-3-year-old) have been maintained in vessels and within the Experimental Botanic Garden's environmental conditions.

Fresh shoot sections (100-200 mg) from R_0 and R_1 plants were individually prepared according to protocol of Aljanabi et al. (1999), with minor modifications. Shoot sections were ground to a fine powder in liquid nitrogen and homogenized in 300-600 µL buffer 200 mM Tris-HCl, pH 8.0, 50 mM EDTA, pH 8.0, 2.2 M NaCl for one-year-old R_1 plants (or 4 M NaCl for R_0 and three-year-old R_1 plants), 2% w/v CTAB, 0.06% sodium sulfite, 20% w/v CTAB, 5% w/v lauril-sarcosine, and 10% w/v PVP-40. DNA was extracted with 1 volume of phenol:chloroform: isoamyl alcohol (25:24:1), incubated for 2h with 0.1 ng/µL of RNAse (10 ng/mL) and extracted with 1 volume of chloroform:isoamyl alcohol (24:1); isopropanol (0.6 volumes) and 5 M NaCl (0.06 volumes) were used for DNA precipitation. Isolated DNA quality was determined by electrophoresis in 0.8% agar gel (Hoisington et al., 1984). UV quantification by visual comparison with known quantities of lambda DNA (Gibco-BRL) averaged about 20 ng/µL - 150 ng/µL for sample. DNA yields per sample ranged from 4 ng/mg - 30 ng/mg by Aljanabi et al. (1999) modified protocol.

As a rule, amplification reactions were undertaken according to Williams et al. (1990), with minor modifications; they were performed in aseptic chambers using volumes of 20 µL containing 25 ng of genomic DNA, 10 mM Tris-HCl, pH 8.8, 2.5 mM $MgCl_2$, 91 mM each of dATP, dGTP, dCTP, dTTP, 0.2 µL primer and 1 unit of Taq polymerase (Operon-BRL). After amplification reactions with OPA-12 and OPB-06 ten-mer primers (Operon Technologies Inc. Alameda CA USA), a total of 45 primers of kits OPA, OPB, OPC, OPP, and OPM were used in the amplification reactions. Amplifications were performed, in duplicate, with personal Eppendorf Mastercycler Gradient. PCR conditions were: denaturation for 5 min at 96°C, 44 cycles of 94°C for 30 sec, 35°C for 45 sec, and 72°C for 1 min; with a final 7 min extension of 72°C. Among the 45 primers, 16 (OPA-02, OPA-04, OPA-09, OPA-13, OPA-20, OPB-01, OPB-07, OPC-06, OPF-09, OPL-11, OPM-02, OPM-07, OPM-10, OPP-02, OPP-07, and OPP-09) were used to amplified the DNA segments after an initial primer screening using DNA of the two R_0 plants, and 16 (OPA-02, OPA-04, OPA-09, OPA-10, OPA-12, OPA-20, OPB-01, OPB-04, OPB-07, OPC-06, OPM-02, OPM-07, OPM-10, OPP-02, OPP-07, OPP-09,) were used to amplified the DNA segments after an initial primer screening using DNA of the two R_1 plants.

Amplification products were separated by electrophoresis in 1.7% agar (Invitrogen) TAE gels at

60 V for 4 hr. Gels were stained with ethidium bromide (0.5 mg/mL) and registered by image captured in a High Performance Ultraviolet Transiluminator – Edas 290, using Kodak 1D 3.5 program. 1 Kb DNA Ladder (Gibco-BRL) was used as size marker.

Fragments were analyzed by comparing RAPD profiles of each plant in terms of presence or absence of each DNA fragment. Plants' similarity was calculated by Jaccard's coefficient, while UPGMA cluster analysis was performed with NTSYS-pc software (Rohlf, 1989).

RESULTS AND DISCUSSION

The 16 primers, which yielded repetitive patterns for all scored bands, were applied to all 4 R_0 plants (S3, S9, S48, and S71) and to all 47 R_1 plants (14 R_1 plants of S3, 11 R_1 plants of S9, 8 R_1 plants of S48, and 14 R_1 plants of S71). Only one mix was prepared for each primer, which was used to compare simultaneously the individual DNA samples in the same amplification reaction. The 16 primers generated 222 reproducible fragments (Table 1) of which 65 were polymorphic in R_0 plants and 103, 100, 80, and 108 were polymorphic in R_1 plants from S3, S9, S48, and S71, respectively. Total number of polymorphic bands for R_1 plants from four somaclones amounted to 124. The number of bands for each primer varied from 6 to 17, with an average of 11.5 fragments per primer. The size of amplified products ranged from 250 to 7500 bp. In R_0 plants, primer OPM-02 generated the highest number of fragments among the tested primers while primer OPC-06 and OPP-09 generated the highest number of fragments among the tested primers in RF_1 plants. Primers OPC-06 and OPP-09 showed the greatest capacity for discriminated polymorphic fragments in R_0 and R_1 plants, respectively (Table 1).

Polymorphic banding patterns (Figure 1) reflect the genomic variability in R_1 plants from S3, S9, S48 and S71 somaclones (Table 1). Polymorphism in R_1 plants (55.9%) was higher than the polymorphism detected in R_0 plants (29,8%) and also higher than the polymorphism reported for 633 regenerated plants of *C. peruvianus* using biochemical markers of 22 isozyme loci, which was 13.6% (Mangolin et al., 1997). The level of polymorphism in R_0 and R_1 plants was also higher than RAPD-detected polymorphism among 12 clones of *C. peruvianus* (11%) from California and 13 seedlings of *C. jamacaru* (9.4%) from north-eastern Brazil (Gutman et al., 2001). The low level of genetic diversity found in specie plants has been reported as result from vegetative propagation as the predominant form for multiplication of *C. peruvianus* populations.

Table 1. Polymorphic Fragments in Somaclones (R_0) and in R_1 plants from seeds of S3, S9, S48, and S71 somaclones of *Cereus peruvianus*.

Primer	Number of Polymorphic Fragments											
	R_0		S3-R_1		S9-R_1		S48-R_1		S71-R_1		Total-R_1	
	NF	NFP	NF	NFP	NF	NFP	NF	NFP	NF	NFP	NF	NFP
OPA-02	13	5	15	8	15	8	15	9	15	10	15	10
OPA-04	14	4	15	7	14	8	15	8	15	7	15	10
OPA-09	12	2	12	3	12	3	11	1	12	2	12	3
OPA-10	-	-	13	10	12	8	12	5	12	9	13	11
OPA-12	-	-	6	2	6	1	6	1	6	1	6	1
OPA-13	12	3	-	-	-	-	-	-	-	-	-	-
OPA-20	11	05	14	9	14	9	13	4	14	9	14	11
OPB-01	15	7	11	3	11	4	11	4	11	4	11	4
OPB-04	-	-	14	8	15	8	15	7	15	9	15	9
OPB-07	15	8	13	6	13	7	12	5	13	8	13	8
OPC-06	16	9	17	11	17	6	16	7	17	7	17	11
OPF-09	13	4	-	-	-	-	-	-	-	-	-	-
OPL-11	13	3	-	-	-	-	-	-	-	-	-	-
OPP-02	12	1	14	4	14	5	13	5	13	5	14	6
OPP-07	16	0	15	3	15	3	15	0	15	3	15	3
OPP-09	15	3	17	11	17	11	17	9	17	11	17	12
OPM-02	20	3	15	9	15	8	15	6	15	9	15	9
OPM-07	7	1	14	3	14	5	14	4	14	5	14	5
OPM-10	14	2	16	6	16	6	16	5	16	9	16	9
Total	218	65	221	103	220	100	216	80	220	108	222	124

NF: Number of Fragments; NPF: Number of Polymorphic Fragments

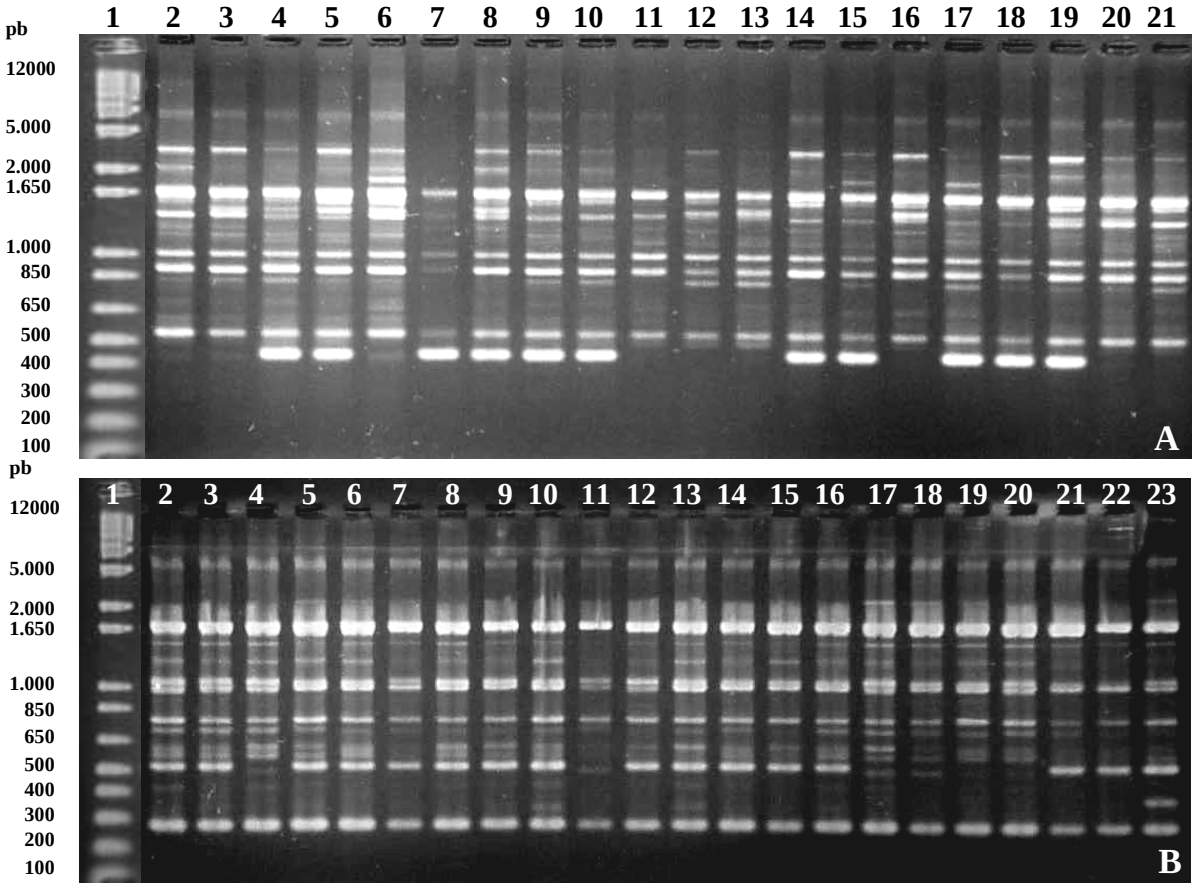

Figure 1. RAPD fingerprinting of R_1 plants from seeds of S3 and S71 somaclones of *C. peruvianus* obtained with primers OPP-09 (A: lines 2-21), OPC-06 (B: lines 2-23) from Operon Technologies Inc. (Alameda, CA) on the samples of genomic DNA. The 1 kb ladder was used as molecular weight marker.

In our research the reported polymorphism between somaclones may result from genetic variability *in vitro* induced since S3, S9, S48, and S71 somaclones were independently regenerated from different callus tissues, and the polymorphism between R_1 plants may result from the genetic constitution of the male and female parent of the F1 descendents, since R_1 plants were obtained from seeds of somaclones collected from open-pollinated S3, S9, S48, and S71 plants. As self-incompatibility has been reported for *C. peruvianus* as well as for other night-flowering columnar cactus (Weiss et al., 1993, 1994; Silva and Sazima, 1995; Casas et al., 1999; Rivera-Marchard and Ackerman, 2006), the cross-pollination system may be advantageous on R_0 plants to determine high level of genetic polymorphism. *C. peruvianus* flowers from plants maintained in southwest region of Brazil have been reported to be visited by hawkmoths *Manduca rustica* and *Agrius cingulatus* (Silva and Sazima, 1995) while flowers of *C. peruvianus* somaclones as well as from other plants of *C. peruvianus* naturally preserved in the some region of somaclones (south region of Brazil) has been reported to be visited by *Agrius spp*. and also by countless bees such as *Apis*, *Xylocopa*, and principally *Trigona spp*.(Ruvolo-Takasusuki et al., 2006). A relative abundance of floral visitors to columnar cactus have been reported, however evidence to date has demonstrated that not all visitors are pollinators. A specialized plant-pollinator system has been well documented in different cactus species (McIntosh, 2005; Rivera-Marchard and Ackerman, 2006). The relative importance of different visitors to the fecundity of the somaclones was not determined but the analysis of microsporogenesis in somaclones showed that the meiotic characteristic of somaclones is similar to the meiotic characteristic of the *C. peruvianus* natural populations (Silva et al., 2006). Thus, additional pollen donors (other plants of *C. peruvianus* naturally preserved in the same region) contributing to the open pollination may determine the higher level of polymorphism in R_1 plants.

The frequency of polymorphism was relatively higher among R_1 plants from S71 somaclone (49.1%) than that of polymorphism among the R_1 plants from S3 (46.6%), S9 (45.5%), and S48 (37%) somaclones. However, the dendrogram by Jaccard coefficient (Figure 2) showed that there was no grouping of R_1 plants according to the somaclone parental (S3, S9, S48, or S71). Dendrogram also revealed that the similarity in R_1 plants ranged from 72.5% to 90.5%. Variations among the *C. peruvianus* clones have shown genetic similarity rates ranging approximately from 89% to 99% (Gutman et al., 2001).

CONCLUSION

Evidence supports our hypothesis that the *in vitro* induced variability may be used to broaden the genetic base of *C. peruvianus* species. Therefore, seeds of R_0 and/or R_1 plants from *C. peruvianus* somaclones may be used as source available for plant propagation, development of management strategies for in situ or ex situ (seed and DNA banks) biodiversity conservation, and also for genetic improvement through breeding programms.

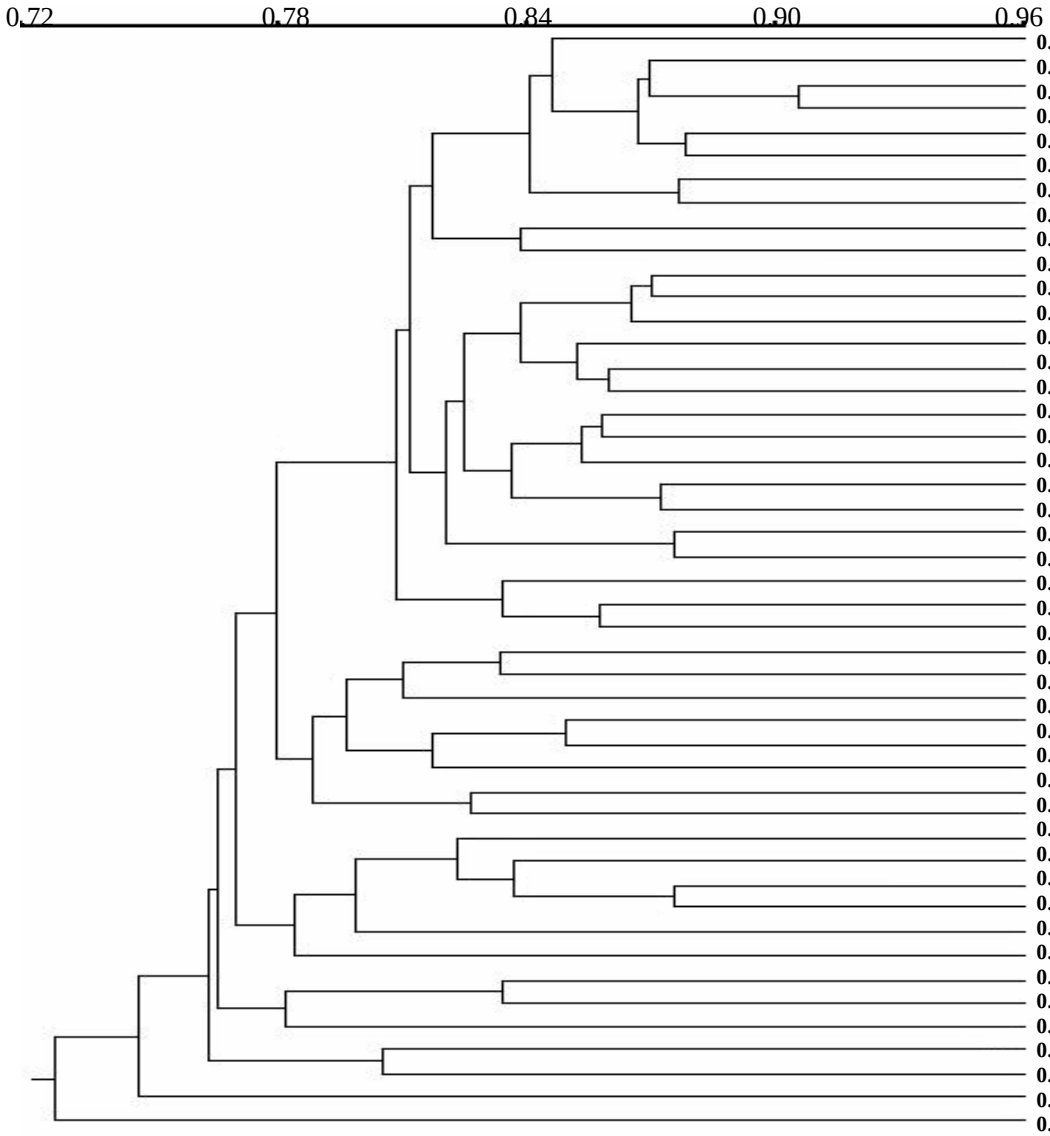

Figure 2. Dendrogram represents the relationship between the R_1 plants from seeds of S3, S9, S48, and S71 somaclones of *C. peruvianus* based on UPGMA cluster analysis of the RAPD profiles derived from 16 primers, by Jaccard's similarity coefficient.

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