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Analysis of coconut cultivars and hybrids using isozyme polymorphism

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The coconut (*Cocos nucifera* L.) is one of the major oilseeds of India. In the present study, an attempt has been made to analyse coconut diversity using isozyme banding data. Cluster analysis was performed using the banding patterns obtained from polyacrylamide gel electrophoresis for 11 isozyme systems in 40 different coconut cultivars and six hybrids and their parents. The cultivars grouped mainly into six clusters. In case of hybrids and their parents, the hybrids clustered intermediate between parents.

Key words: Coconut, cultivars, hybrids, isozymes, genetic variability, cluster analysis

Introduction

The coconut (*Cocos nucifera* L.) is one of the major oilseeds of India. It belongs to the Arecaceae family, which is grown in the coastal humid tropics. This crop is considered to have originated in the Asia-Pacific region. There are two main types in the coconut, Talls and Dwarfs, and variation exists in these populations. A main requirement in any breeding programme is a knowledge of the available genetic variability in the crop. Therefore the collection, conservation and characterization of germplasm are of the utmost importance. For the characterization of germplasm, biochemical markers like isozymes can be successfully used, even though DNA markers are also used, because isozymes are co-dominant in nature, cheap and less affected by the environment. Isozyme markers have been effectively used for gene mapping in different species because of the codominant inheritance that these markers exhibit compared to other molecular markers (GUTIERREZ et al. 2001). Compared to molecular methods isozyme analysis is cheap and easy to perform.

Cluster analysis helps in the selection of parents for the production of hybrids, by selecting genetically divergent parents. Although in other plant systems cluster analysis has been conducted, in the coconut less work has been conducted, except in the case of some molecular markers (UPADHYAY et al. 2002, PERERA et al. 2000). In the present study, an attempt has been made to analyse coconut diversity using isozyme banding data.

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Materials and methods

Sampling Material

Spindle leaf extracts from the following varieties were used for the present study. The samples were collected from the Central Plantation Crops Research Institute (C.P.C.R.I.), Kasaragod, which maintains the world's largest coconut germplasm.

Tall cultivars: West Coast Tall (WCT), Java Tall (JVT), Laccadive Ordinary Tall (LCT), Philippines Ordinary Tall (PHOT), Andaman Ordinary Tall (ADOT), San Ramon Tall (SNRT), Kappadam Tall (KPDT), Laccadive Micro Tall (LMT), Strait Settlement Green Tall (SSGT), Strait Settlement Apricot Tall (SSAT), Fiji Tall (FJT), Ayiramkachi Tall (AYRT), Cochin China Tall (CCNT), Federated Malay States Tall (FMST), Andaman Giant Tall (AGT), Java Giant Tall (JVGT), Gonthembili Tall (GTBT), Seychelles Tall (SCT), Ceylon Tall (SLT), New Guinea Tall (NGAT), Benaulim Tall (BENT), Ganga Pani Tall (GPNT), Calangute Tall (CALT), Spicata Tall (SPIT), Nadora Tall (NDRT), Zanzibar Tall (ZAT) and Standard Kudat Tall (STKT).

Dwarf cultivars: Chowghat Orange Dwarf (COD), Malayan Yellow Dwarf (MYD), Chowghat Green Dwarf (CGD), Malayan Orange Dwarf (MOD), Gudanjali Dwarf (GDD), Gangabondam Dwarf (GBGD), Kenthali Dwarf (KTOD), King Coconut (RTB04), Malayan Green Dwarf (MGD), Kulasekharam Yellow Dwarf (KYD), Kulasekharam Orange Dwarf (KOD), Kulasekharam Green Dwarf (KGD) and Cameroon Red Dwarf (CRD).

Hybrids and their parents: Laccadive Ordinary Tall (LCT), Gangabondam Tall (GBGD), West Coast Tall (WCT), Strait Settlement Green Tall (SSGT), Laccadive Ordinary Tall x Gangabondam Dwarf (LCT x GBGD), West Coast Tall x Strait Settlement Green Tall (WCT x SSGT), West Coast Tall x West Coast Tall (WCT x WCT), Chowghat Orange Dwarf x Chowghat Orange Dwarf (COD x COD), West Coast Tall x Chowghat Orange Dwarf (WCT x COD) and Chowghat Orange Dwarf x West Coast Tall (COD x WCT).

Extraction of enzymes and electrophoresis

In all, 11 isozyme systems viz., Esterase (*EST*), Peroxidase (*PRX*), Glutamate Oxaloacetate Transaminase (*GOT*), Poly Phenol Oxidase (*PPO*), Malate Dehydrogenase (*MDH*), Alcohol Dehydrogenase (*ADH*), Super Oxide Dismutase (*SOD*), α-Amylase (*α-AMY*), Phosphorylase (*PHOS*) and Glucose 6-Phosphate Dehydrogenase (*G-6PDH*) were studied. Staining was done using standard protocols (VALLEJOS 1983). Extraction of samples was done using 0.1 M Tris-HCl buffer (pH 6.8) containing glycerol and β-Mercaptoethanol. Gel was run under a constant current of 25mA/ gel for 4-5 hours or until the tracking dye had migrated to the bottom of the gel at 4 °C. The apparatus was then dismantled. The top plate was prised away with a spatula and the gel was carefully removed and stained for isozyme activity.

Analysis

Cluster analysis was done on the basis of data (i.e., presence or absence of an allele) derived from the above 11 isozyme systems. The similarity index was calculated using Jaccard's coefficient (ROHLF 1993) and cluster analysis was done by the UPGMA method using NTSYS software.

Results and discussion

Similarity index and cluster analysis of coconut cultivars

The similarity index for 40 cultivars was studied using isoenzyme data (Table 1). The KPDT and LMT similarity level was 100%, followed by KPDT and ADOT, LMT and ADOT (97.2%). The similarity level was the least in SSGT and KTOD, KTOD and SCT (40.0%).

Cluster analysis of the isozyme data of 40 cultivars consisting of 13 Dwarfs and 27 Tall cultivars, showed most Dwarfs clustering together except for KOD, GBGD, GDD and CRD (Fig.1). The Dwarfs, KOD, GBGD and GDD were intermediate between Talls and Dwarfs, while CRD was more distinct and grouped along with the Talls. Among Talls, three ecotypes, SNRT, SSAT and ZAT grouped along with the Dwarfs. PHOT was intermediate between Talls and Dwarfs. Two morphologically distinct ecotypes-KPDT with very large fruits and LCT with very small fruits clustered together. GDD and GBGD in spite of being Dwarfs, grouped with the Talls, KPDT, LMT, ADOT, JVT, LCT and WCT. This clustering indicates the breeding nature of the coconut cultivars.

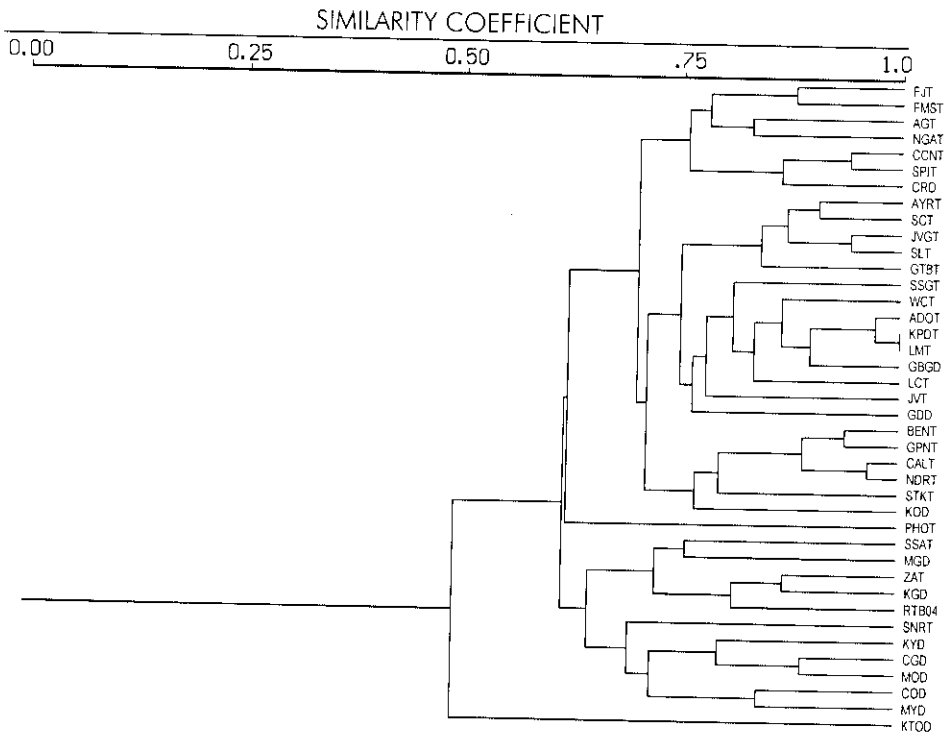

Fig. 1. Dendrogram based on Jaccard's similarity level showing the genetic relationships between accessions

Tab. 1. Similarity index of coconut cultivars

cultivar	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
1 FJT	1																																							
2 AYRT	0.714	1																																						
3 SSGT	0.692	0.789	1																																					
4 SSAT	0.629	0.553	0.667	1																																			
5 CALT	0.771	0.684	0.707	0.743	1																																		
6 EMST	0.899	0.829	0.75	0.649	0.833	1																																	
7 AGT	0.707	0.674	0.814	0.643	0.805	0.805	1																																
8 JVGT	0.771	0.882	0.795	0.605	0.737	0.833	0.682	1																															
9 GTBT	0.676	0.844	0.711	0.600	0.605	0.694	0.568	0.848	1																														
10 SCT	0.686	0.906	0.718	0.526	0.658	0.750	0.614	0.909	0.871	1																													
11 SLT	0.730	0.833	0.846	0.658	0.789	0.789	0.727	0.943	0.800	0.857	1																												
12 NGAT	0.800	0.667	0.732	0.550	0.718	0.811	0.829	0.675	0.550	0.600	0.643	1																											
13 BENT	0.676	0.73	0.795	0.694	0.833	0.737	0.762	0.784	0.649	0.703	0.848	0.634	1																										
14 GPNT	0.714	0.771	0.744	0.639	0.778	0.778	0.714	0.829	0.686	0.743	0.784	0.667	0.939	1																									
15 CALT	0.676	0.735	0.667	0.600	0.694	0.694	0.643	0.743	0.697	0.706	0.703	0.632	0.848	0.903	1																								
16 SPIT	0.722	0.641	0.667	0.742	0.941	0.784	0.805	0.692	0.564	0.615	0.744	0.675	0.833	0.778	0.694	1																							
17 NDRT	0.706	0.765	0.692	0.583	0.722	0.722	0.667	0.771	0.676	0.735	0.730	0.658	0.879	0.935	0.966	0.722	1																						
18 ZAT	0.686	0.564	0.634	0.758	0.750	0.703	0.732	0.615	0.611	0.538	0.625	0.641	0.703	0.694	0.657	0.750	0.639	1																					
19 STKT	0.706	0.667	0.650	0.583	0.632	0.676	0.628	0.722	0.583	0.639	0.684	0.658	0.771	0.818	0.781	0.632	0.813	0.686	1																				
20 WCT	0.675	0.725	0.744	0.65	0.775	0.775	0.756	0.775	0.650	0.700	0.825	0.636	0.775	0.725	0.650	0.775	0.675	0.619	0.634	1																			
21 JVT	0.600	0.682	0.778	0.543	0.652	0.689	0.75	0.727	0.614	0.659	0.773	0.638	0.727	0.682	0.614	0.652	0.636	0.587	0.636	0.841	1																		
22 LCT	0.690	0.738	0.756	0.628	0.744	0.786	0.804	0.744	0.628	0.674	0.791	0.652	0.744	0.698	0.628	0.744	0.651	0.636	0.651	0.860	0.771	1																	
23 PHOT	0.578	0.553	0.612	0.556	0.705	0.667	0.766	0.563	0.458	0.500	0.604	0.617	0.630	0.587	0.522	0.705	0.543	0.600	0.543	0.702	0.700	0.787	1																
24 ADOT	0.667	0.811	0.825	0.641	0.769	0.769	0.750	0.816	0.684	0.737	0.868	0.628	0.865	0.811	0.730	0.770	0.757	0.610	0.711	0.897	0.795	0.857	0.66	1															
25 SNRT	0.742	0.600	0.590	0.559	0.611	0.657	0.571	0.657	0.656	0.571	0.622	0.595	0.657	0.697	0.710	0.611	0.688	0.618	0.688	0.615	0.545	0.595	0.523	0.694	1														
26 KPDT	0.684	0.833	0.846	0.658	0.789	0.789	0.767	0.838	0.703	0.757	0.892	0.643	0.889	0.833	0.750	0.789	0.778	0.635	0.684	0.872	0.773	0.833	0.638	0.972	0.667	1													
27 LMT	0.684	0.833	0.846	0.658	0.789	0.789	0.767	0.838	0.703	0.757	0.892	0.643	0.889	0.833	0.750	0.789	0.778	0.625	0.684	0.872	0.773	0.833	0.638	0.972	0.667	1	1												
28 MGD	0.733	0.543	0.500	0.759	0.697	0.697	0.561	0.600	0.545	0.514	0.568	0.629	0.600	0.636	0.645	0.697	0.625	0.710	0.677	0.564	0.467	0.548	0.477	0.553	0.655	0.568	0.568	1											
29 RTB04	0.758	0.579	0.650	0.676	0.632	0.722	0.707	0.59	0.583	0.513	0.561	0.800	0.550	0.579	0.583	0.590	0.568	0.788	0.657	0.558	0.532	0.578	0.543	0.548	0.636	0.561	0.561	0.733	1										
30 KTOD	0.600	0.429	0.400	0.567	0.529	0.576	0.463	0.486	0.424	0.400	0.459	0.559	0.444	0.471	0.469	0.529	0.455	0.531	0.500	0.462	0.378	0.452	0.356	0.447	0.517	0.459	0.459	0.75	0.600	1									
31 KYD	0.645	0.559	0.553	0.667	0.571	0.571	0.500	0.618	0.613	0.529	0.583	0.514	0.667	0.710	0.724	0.571	0.700	0.677	0.759	0.538	0.477	0.524	0.422	0.611	0.741	0.629	0.629	0.731	0.645	0.578	1								
32 KOD	0.583	0.735	0.625	0.6	0.649	0.649	0.643	0.694	0.697	0.706	0.658	0.590	0.743	0.788	0.750	0.649	0.781	0.758	0.781	0.571	0.578	0.591	0.522	0.641	0.559	0.658	0.658	0.594	0.629	0.424	0.667	1							
33 KGD	0.727	0.553	0.548	0.647	0.694	0.694	0.683	0.605	0.556	0.526	0.575	0.722	0.649	0.686	0.697	0.694	0.676	0.871	0.781	0.571	0.543	0.591	0.556	0.561	0.656	0.575	0.575	0.821	0.839	0.621	0.724	0.750	1						
34 CRD	0.730	0.610	0.714	0.703	0.838	0.744	0.810	0.659	0.575	0.585	0.707	0.683	0.789	0.737	0.658	0.889	0.684	0.806	0.684	0.738	0.696	0.750	0.711	0.732	0.622	0.707	0.707	0.611	0.641	0.459	0.541	0.658	0.703	1					
35 COD	0.667	0.632	0.581	0.553	0.600	0.641	0.636	0.684	0.595	0.606	0.650	0.625	0.641	0.676	0.639	0.600	0.667	0.649	0.818	0.605	0.574	0.698	0.587	0.675	0.697	0.650	0.650	0.636	0.667	0.515	0.710	0.735	0.735	0.61	1				
36 MYD	0.629	0.513	0.512	0.514	0.564	0.564	0.568	0.564	0.514	0.487	0.537	0.550	0.561	0.595	0.556	0.564	0.583	0.611	0.673	0.535	0.511	0.591	0.522	0.600	0.719	0.575	0.575	0.594	0.629	0.469	0.667	0.600	0.647	0.575	0.844	1			
37 CGD	0.600	0.568	0.524	0.571	0.622	0.579	0.545	0.622	0.529	0.541	0.590	0.605	0.667	0.706	0.667	0.622	0.697	0.629	0.750	0.548	0.489	0.533	0.468	0.615	0.625	0.632	0.632	0.667	0.600	0.533	0.750	0.719	0.719	0.550	0.758	0.667	1		
38 MOD	0.667	0.583	0.575	0.636	0.639	0.595	0.558	0.639	0.588	0.556	0.605	0.579	0.686	0.729	0.688	0.639	0.719	0.697	0.774	0.561	0.500	0.545	0.478	0.632	0.700	0.649	0.649	0.698	0.667	0.500	0.846	0.742	0.742	0.605	0.781	0.742	0.893	1	
39 GDD	0.658	0.667	0.775	0.676	0.675	0.675	0.705	0.718	0.632	0.641	0.769	0.581	0.763	0.711	0.632	0.675	0.658	0.684	0.750	0.714	0.711	0.767	0.617	0.795	0.639	0.769	0.769	0.541	0.575	0.432	0.647	0.676	0.590	0.725	0.757	0.676	0.605	0.667	1	
40 GBGD	0.722	0.778	0.842	0.694	0.784	0.784	0.721	0.784	0.694	0.703	0.838	0.634	0.833	0.778	0.694	0.737	0.722	0.615	0.676	0.821	0.727	0.786	0.639	0.912	0.706	0.889	0.889	0.556	0.59	0.444	0.618	0.605	0.525	0.744	0.641	0.605	0.579	0.639	0.811	1

Similarity index and cluster analysis of coconut hybrids and their parents

For hybrids and their parents (Tab. 2), the similarity level was the highest in LCT x GBGD and WCT x SSGT (94.1%), while it was least in LCT x GBGD and COD (57.5%).

Clustering was done for selected hybrids and their parents (SSGT, GBGD, LCT x GBGD, WCT x SSGT, WCT, LCT, WCT x WCT, WCT x COD, COD x COD, COD x WCT and COD) as shown in Fig.2. Hybrids clustered intermediately between the parents. Another observation was that WCT x WCT was closer to the WCT x COD hybrid than WCT and COD x COD was closer to COD x WCT than COD.

The similarity index indicates the similarity between different cultivars. Cluster analysis is used to group the individuals according to their genetic variation. Individuals with similar characters are grouped together. The present study, in which 40 cultivars, 13 Dwarfs and 27 Talls were used, produced a single tree. Mainly six clusters were observed. Among Talls, SNRT, SSAT and ZAT grouped along with Dwarfs. Low intrapopulation variation was also observed for Talls like SNRT. Dwarfs also have low intrapopulation variation due to autogamy. The low intrapopulation variation observed in some Talls may be due to the fact that these cultivars have been under cultivation for centuries and must have obtained a reasonable degree of homogeneity. UPADHYAY et al. (2002) also obtained similar results. ROHDE et al. (1995) using ISTR (Inverse Sequence Tagged Repeats) markers in 21 accessions also found that some Tall accessions grouped with Dwarfs. It could be

Tab. 2. Similarity index of hybrids and their parents

	SSGT	WCT	LCT	COD	GBGD	WCT x WCT	COD x COD	WCT x COD	COD x WCT	LCT x GBGD	WCT x SSGT
SSGT	1										
WCT	0.744	1									
LCT	0.756	0.86	1								
COD	0.581	0.605	0.698	1							
GBGD	0.842	0.821	0.786	0.641	1						
WCT x WCT	0.718	0.659	0.674	0.694	0.750	1					
COD x COD	0.643	0.707	0.721	0.658	0.667	0.632	1				
WCT x COD	0.725	0.707	0.721	0.75	0.757	0.938	0.684	1			
COD x WCT	0.732	0.800	0.810	0.625	0.763	0.778	0.833	0.833	1		
LCT x GBGD	0.865	0.842	0.762	0.575	0.912	0.722	0.600	0.730	0.737	1	
WCT x SSGT	0.821	0.895	0.810	0.625	0.914	0.730	0.650	0.737	0.744	0.941	1

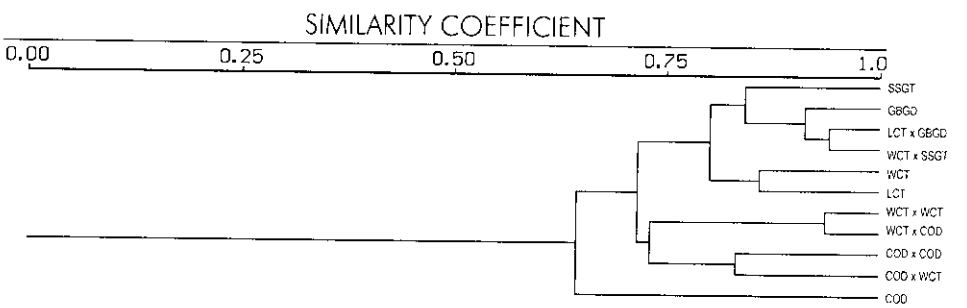

Fig. 2. Dendrogram based on Jaccard's similarity level showing the genetic relationships between hybrids and their parents

assumed that the grouping of these Tall accessions with Dwarfs in this study may be due to the fact that these accessions have some level of self-pollination due to interspadix overlapping of mature male and female phase (RATNAMBAL et al. 1995, 2000). Heterotic hybrids can be obtained by knowing the genetic diversity among parents (KHANNA and MISRA 1977, RAJANNA et al. 1977, PETER and RAI 1978). By crossing genetically distant varieties, one can get good hybrids in a scientific way. To choose divergent parents for different traits, clusters would be useful. In this study, parents like FJT, FMST, AGT and NGAT can be crossed with distantly related parents like MOD, COD, MYD and KTOD to get a good combination of hybrids. The study also showed that genetic divergence and genetic distance are not influenced by geographic origin and isolation.

Acknowledgements

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References

GUTIERREZ, J. F., VAQUERO, F., VENCES, F. J., 2001: Genetic mapping of isozyme loci in *Lathyrus sativus* L. Lthyrus Lathyrism Newsl. 2, 74-77.

KHANNA, K. R., MISRA, C. H., 1977: Divergence and heterosis in tomato. J. Soc. Advnt. Breed. Res. Asia Oceania 9, 43-50.

PERERA, L., RUSSELL, J. R., PROVAN, J., POWELL,W., 2000: Use of microsatellite DNA markers to investigate the level of genetic diversity and population genetic structure of coconut (*Cocos nucifera* L.). Genome 43, 15-21.

PETER, K.V., RAI, B., 1978: Heterosis as a function of genetic distance in tomato. Indian J. Genet. Plant Breed. 38, 173-178.

RAJANNA, A., LAL, G., PETER, K. V., 1977: Heterozygote advantage as a function of genetic divergence in tomato. Indian J. Agric. Sci. 47, 434-437.

RATNAMBAL., M. J., NAIR, M. K., MURALIDHARAN, K., KUMARAN, P. M., RAO, E. V. V. B., PILLAI, R. V., 1995: Coconut descriptor, 1. Central Plantation Crops Research Institute, Kasaragod, Kerala, India.

RATNAMBAL, M. J., NIRAL, V., KRISHNAN, M., RAVIKUMAR, N., 2000: Coconut descriptors, 2. CD-ROM, Central Plantation Crops Research Institute, Kasaragod, Kerala, India.

ROHDE, W., KULLAYA, A., RODRIGUEZ, J., RITTER, W., 1995: Genome analysis of *Cocos nucifera* L. by PCR amplification of spacer sequences separating a subset of copia-like EcoRI repetitive elements. J. Genet. Breed. 49, 179-186.

ROHLF, J. F., 1993: Numerical Taxonomy System (NTSYS). Exeter Software. Version 1.70. Applied Biostatistics Inc., New York.

UPADHYAY, A., JOSE, J., MANIMEKALAI, R., PARTHASARATHY, V. A., 2002: Molecular analysis of phylogenetic relationships among coconut accessions. In: ENGELS, J. M. M., RAMANATHA RAO, V., BROWN, A. H. D., JACKSON, M. T. (eds.), Managing plant genetic diversity, 61-66. CABI Publishers, Oxford.

VALLEJOS, C. E., 1983: Enzyme activity staining. In: TANKSLEY, S. D., ORTON, T. J. (eds.), Isozymes in plant genetics and breeding, Part A, 443-468. Elsevier, Amsterdam.

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WRISCHER, M., LJUBEŠIĆ, N., DEVIDÉ, Z., 1992: Ultrastructural studies of degradational processes in amitrol-damaged photosynthetic membranes. J. Struct. Biol. 108, 1-5.

GITTINS, R., 1981: Towards the analysis of vegetation succession. Vegetatio 46/47, 37-60.

Books:

HORVAT, I., GLAVAČ, V., ELLENBERG, H., 1974: Vegetation Südosteuropas. Geobotanica selecta Bd. IV. Gustav Fischer Verlag, Stuttgart.

Chapter in a book:

BROADWATER, S. T., SCOTT, J., 1994: Ultrastructure of unicellular red algae. In: SECKBACH, J. (ed.), Evolutionary pathways and enigmatic algae: *Cyanidium caldarium* (Rhodophyta) and related cells, 215-230. Kluwer Academic Publishers, Amsterdam.

Published proceedings paper:

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