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Section A: Featured Article

Chromosomal Pairing and Pollen Viability in *Rhizophora mangle* and *Rhizophora stylosa* Hybrids

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Abstract

Two prominent mangrove species of Fiji, Rhizophora mangle, Linn. *Rhizophora stylosa* Griff *and their puative hyubrid (R x selala) were analysed for chromosome number and pairing. Both parental species and their hybrid possess a diploid number of (2n) = 36 chromosomes. Regular 18 bivalents were observed in two species but the hybrid lacked proper chromosome pairing during meiosis. Analysis of tetrads showed normal tetrad and microspores development in parental species but very high abnormality in the hybrid. Pollen fertility determined by staining technique and pollen germination technique showed very high pollen viability in both parental species but very low pollen viability in the putative hybrid. Lack of chromosomal homology appears to be contributing to high percentage of non-viable pollen resulting in complete sterility in the putative hybrid.*

1 General Introduction

Mangroves have very special place in the lives of Pacific islanders. These trees provide wood for fire, fuel, furniture and artifact making. Their fruits (propagules) are eaten when there is shortage of food. Mangroves protect seashore from degradation and also provide sanctuary for young crustaceans. Two species of these mangroves cross in nature and produce sterile hybrids. Hybrids normally grow faster and produce better quality timber. In the present investigation two parental species and their hybrids were studied for chromosome pairing and pollen viability. This study determined the cause of sterility in hybrids, which in turn will be helpful to propagate these hybrids by making them fertile using method of chromosome doubling.

2 Introduction

Mangroves form very important plant communities, found on protected shores mostly in the tropics and subtropics (Macnae, 1966). Mangroves grow very well in regions where there is an abundance of stable silt, which receives a mixture of nutrient laden fresh water and/or oceanic salt water. In Fiji three mangrove species in the family Rhizohoraceae are found in abundance namely; *Bruguiera gymnorhiza* Linn. *Rhizophora mangle* Linn. and *Rhizophora stylosa* Griff. Two of the species – *Rhizophora mangle* and *Rhizophora stylosa* cross in nature and produce sterile hybrids (*Rx selala*). Wherever these two species occur together, hybrid trees are also found. These hybrid trees possess intermediate characteristics between two parental species. Their leaves, buds and flower can clearly be distinguished from two parental species. The hybrids flower profusely as do the parental species but do not set propagules. Therefore, it might be assumed that the hybrids between these two species are completely sterile, most probably due to failure of chromosomal pairing gamete formation. The present study was designed to test this hypothesis using chromosome analysis, tetrad formation and pollen fertility assessment in two parental species and their putative hybrid.

3 Material and Methods

The material consisted of two mangrove species: *Rhizophora mangle, Rhizophora stylosa* and their putative hybrid (*R x selala*) growing on the sea-shore near the children's park in Suva. Flower buds of a range of sizes were collected from these two mangrove species and their putative hybrids.

Flower buds were punctured then submerged and fixed in 3 parts ethyl alcohol and 1 part acetic acid (v: v). Fixed buds were kept in refrigerator for 24 hours and then washed with and stored in 70% ethanol in a refrigerator until used to determine the chromosome numbers. Snow's (1963) alcoholic hydrochloric acid carmine stain was used for chromosome analysis and counting. Staining ability of microspores from tetrads was used as an indication of normal pollen formation and fertility. Microspores were scored while still in the tetrads. Tetrads with four equal sized, stain microspores were classified normal while those with three or fewer normal-sized, poorly stained microspores, were recorded as abnormal.

To assess pollen fertility two methods were used: pollen staining and pollen germination. To score the percentage of normal pollen, pollen was stained with aceto-carmine within two hours of collection from freshly opened flowers. Pollen grains, which were round and stained with aceto-carmine scored as normal whereas, small or shriveled unstained pollen grains were scored as non-viable or sterile. To assess germination ability pollen grains were germinated on a semi-solid medium containing 1% agar (bacteriological agar Gibco), 12% W/V sucrose and 0.01% W/V boric acid. Agar was dissolved and sterilized by heating in an autoclave, then poured into sterile 85-mm diameter petri dishes to gel. Freshly collected pollen from fully opened flowers was

spread evenly with a sterile glass rod. Petri-dishes were sealed and placed in the dark at 23 (± 2) ^{o}C for 24 hours. Pollen grains were scored as germinated when the pollen tube was at least twice the pollen grain diameter (Polito and Luza 1988). In both methods at least 400 grains were counted for calculating the percentage pollen viability.

4 Results

Chromosome counts were made at prophase-I. The diploid chromosome number in two parental species and their hybrid was observed to be regularly 2n=36 (Table 1). However, the chromosomal configuration was different in the parental species and the hybrid (Figure 1).

Table 1 Chromosomal number and meiotic observation (configuration) in *Rhizophora mangle* and *Rhizophora stylosa* and their hybrid *R.x selala*.

Species/Hybrid	Chromosome Number and Configuration at Prophase – I and Metaphase - I	No. of first division segregation (Anaphase – 1) cells		
		Normal	With laggards	Total
Rhizophora mangle	18 bivalents regularly, rarely 17 bivalents and 2 univalents	50	0	50
Rhizophora stylosa	18 bivalents regularly, rarely 17 bivalents and 2 univalents	50	0	50
Hybrid (R x selala)	Only 2-5 bivalents occasionally, 26-34 univalents regularly in most of the cells	0	50	50

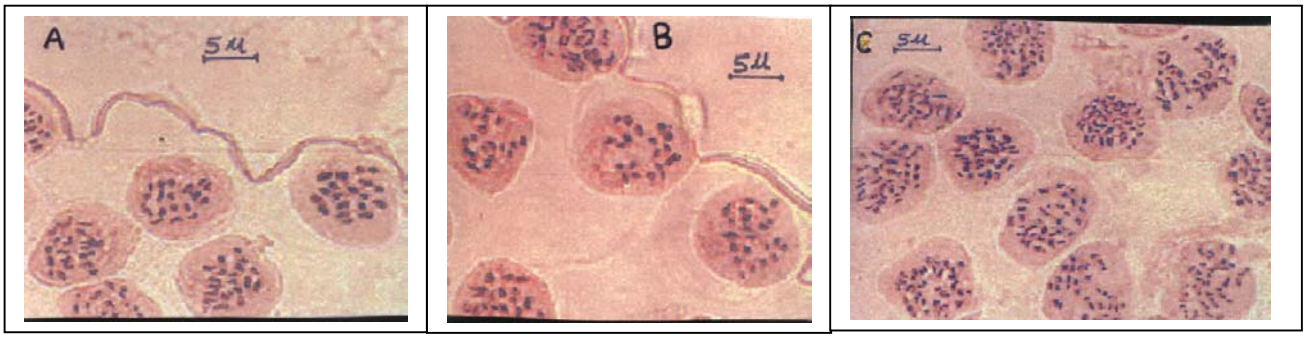

Figure 1. Chromosome spread in two mangrove species and their putative hybrid. A – *Rhizhophora stylosa*; late prophase-1: cells with regular 18 bivalents and 17 bivalents and 2 univalents. B – *Rhizophora mangle*; late prophase-1: cells with regular 18 bivalents and 17 bivalents and 2 univalents. C – *Rhizophora x selala*; late prophase-1: all cells contain mostly univalents and a few bivalents only.

Both parental species regularly showed 18 bivalent in first division of meiosis. However, in the hybrid (*R x selala*) a maximum of 5 bivalents were observed with the rest of the chromosomes were recorded as univalents (Table 1). A count of 50 meiotic-I cells showed normal chromosomal movement to opposite poles at anaphase-I in both the parental species. While parental species showed normal anaphase-I, all the anaphase-I cells in the hybrid showed lagging chromosomes.

Both parental species had a very high percentage of normal tetrads (91-94%) while hybrid possessed less than 4% normal tetrads. Almost all the tetrads in the hybrid contained three or more shriveled microspores.

Table 2 Percentage of normal and abnormal tetrads in *Rhizophora mangle*, *Rhizophora stylosa* and their hybrid *R. selala*.

Species/Hybrid	Tetrads			
	No. of cells Counted	Normal	Abnormal	% Normal
Rhizophora mangle	400	376	24	94.0
Rhizophora stylosa	400	365	38	91.3
Hybrid –(*R x selala*)	400	14	386	03.5

In both parental species very high pollen fertility and pollen germination was recorded. However a slight difference was observed between two methods (Table 3). The difference observed between two methods was not statistically significant. Pollen fertility in the hybrid was very low. It

was recorded about 5% using the staining technique and about 3% from the pollen germination method.

Table 3 Pollen fertility percentage in *Rhizophora mangle, Rhizophora stylosa* and their hybrid *R. selala*.

Species/Hybrid	Pollen Fertility Percentage			
	Staining Technique		Pollen Germination Technique	
	Range	Mean ± S.E.	Range	Mean ± S.E.
Rhizophora mangle	98.9-97.4	95.2±4.3	84.7-93.2	87.9±5.2
Rhizophora stylosa	87.3-96.2	93.8±5.1	82.9-91.2	84.6±4.2
Hybrid *(R x selala)*	4.3-7.6	5.2±1.8	2.7-6.5	3.7±1.5

5 Discussion

Both parental species of the putative *Rhizophora mangle* and *Rhizophora stylosa* showed regular chromosome pairing and segregation at meiosis-I. However, the putative hybrid completely lacked perfect chromosome pairing at meiosis-I (Figure 1). Most dividing cells of the hybrid had univalents at prophase-I and metaphase-I and lagging chromosomes during anaphase-I. Stebbins (1958) ascribed this phenomenon due to non-homology of chromosomes in two species. The two parental species are close enough to cross in nature and produce hybrids but the hybrids remain sterile due to non-homology of chromosomes. Hacker (1968) reported failure of chromosome pairing in a probable *Desmodium intortum x Desmodium sandwicense* hybrid. Due to failure of chromosome pairing during meiosis in hybrid between *Rhizophora mangle* and *Rhizophora stylosa* abnormal tetrads were formed. This led to shriveled microspores (Table 2) and extremely low pollen fertility in the hybrid. McKeller and Quesenberry (1992) reported low pollen fertility and very low viable seed production in synthetic hybrids *between Desmodium ovalifolium x Desmodium heterocarpon* crosses. However, in case of the synthetic hybrids between two *Desmodium* species, lack of chromosomal homology did not appear to be the reason for low hybrid seed production in controlled crosses (McKeller and Quesenberry, 1992). McWhiter (1962-63) reported fertile and vigorous hybrids between *Desmodium sandwicense* and *Desmodium intortum*. There are numerous examples in animal and plant kingdom where two related species cross in nature and produce viable but sterile hybrids (Arnold; 1997, Howard and Berlocher; 1998). In an earlier study Tyagi and Singh (1998) also reported low pollen viability in a putative mangrove hybrid between *Rhizophora stylosa* and *Rhizophora samoensis*. In these cases back crossing and formation of an F_2 population is extremely unlikely. Hybrid swarms and introgression between the species will be very low. Thus despite the cross compatibility the two parental species likely to remain distinct.

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