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Fertilizing Ability of Cryopreserved Pollen in Inter-Intra Specific Crosses in *Carica papaya* L.

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Abstract

Fertilizing ability of cryopreserved pollen for intra and inter specific crossing in *Carica* species was investigated. Pollen of *Carica papaya* cultivar 'Washington' and *Carica cauliflora* were cryostored for six and eight years and viability tested using hanging drop method. Cryopreserved pollen was placed onto compatible stigmas of *C. papaya* cultivar 'Solo'. Fruit and seed formation from these crosses were assessed as a function of fertilizing ability of cryopreserved pollen. Seeds recovered from both intra and inter specific crosses were also assessed for germination. In *Carica papaya* 'Washington' and *Carica cauliflora*, pollen cryostored after six and eight years showed germination of pollen tubes comparable to controls. No changes in vigour parameters were observed after cryostorage for six and eight years. 'Washington' pollen stored at -196°C for eight years can still be effective in pollination and brought about fruit set and seed development to the extent of 80 % and 86 % respectively. In the case of *Carica cauliflora*, the results are 85 % and 76 % respectively. Seed set with six- and eight-year cryostored pollen germinated to the extent of about 82% and 83 % respectively over the control. The study indicates the possibility of establishing a pollen bank for *Carica* species to improve breeding efficiency, heterosis breeding and hybrid seed production as well as conserving nuclear genetic diversity (NGD).

INTRODUCTION

Long-term storage of pollen in papaya was initially investigated for breeding and conservation of nuclear genetic diversity (Ganeshan, 1986) resulting in the possibility of establishing a cryobank for *Carica* species. Pollen under cryogenic (–196°C) conditions retains its viability and fertilizing ability (Kobayashi et al., 1978; Towill, 1981; Parfitt and Almehdi, 1983, 1984a, 1984b). Pollen at ultra low temperature undergoes negligible changes in term of physiological and biological processes (Towill, 1985), which otherwise is rendered non viable under other storage conditions, where temperature and humidity fluctuations during storage accelerate loss of viability (Stanley and Linskens, 1974). Earlier investigations on papaya pollen storage under low temperatures resulted in reduction in viability and fertilizing ability of pollen (Singh, 1960; Allan, 1963; Sharma and Bajpai, 1969; Ganeshan, 1985). The present study examines the effects of cryostored pollen of *Carica papaya* cultivar 'Washington' and *Carica cauliflora* on fruit and seed set in inter and intra specific crosses.

MATERIALS AND METHODS

Carica papaya and *Carica cauliflora* pollen were collected from freshly opened male flowers on a bright sunny day. Pollen was dried and an adequate quantity was transferred to gelatin capsules, which were then sealed in aluminium pouches. Samples were directly plunged into liquid nitrogen containers after stacking the pollen containing pouches in the stainless steel canisters. Pollen samples were retrieved from cryobiological containers post storage and tested for viability and fertility after thawing to room temperature.

Viability was tested using 15 % sucrose with Brewbaker and Kwack salts

(Brewbaker and Kwack, 1963) by hanging drop method (Brewbaker, 1959). The slides were incubated for six hours. Germinated pollen was stained with Alexander's stain (Alexander, 1980) and three slide replicates were counted for germinated, ungerminated and aborted pollen in ten microscopic fields under a compound microscope. Per cent germination was obtained in pollen stored for six and eight year as well as fresh pollen (control).

Fertility assessment of pollen in 'Washington' and *Carica cauliflora* was tested on female flowers of *Carica papaya* cultivar 'Solo'. Pollen stored for six and eight years were used in field pollination by transferring adequate quantities of pollen on to the receptive stigma of flowers, bagged one day prior to pollination. The flowers were immediately rebagged after crossing to avoid stray pollination. For control, pollination was carried out using fresh pollen. Fruit set and fruit drop were recorded for pollination with stored as well as fresh pollen. The seed set was recorded after harvesting the mature ripe fruit. Number of healthy and aborted seeds recovered was recorded and percentage calculated. Healthy seed obtained from all crosses were tested for seed germination.

RESULTS AND DISCUSSION

'Washington' pollen stored for six and eight years resulted in germination to the extent of Ca. 94 % and 91 % over the control respectively while the results for *Carica cauliflora* pollen were Ca. 98 % and 102 % respectively (Table 1). For eight-year cryostored pollen, evaluation of vigour in terms of pollen tube length showed a reduction of 51µm in 'Washington' but an increase of 8µm in *Carica cauliflora* over the control (Table 2). Abnormal pollen tubes, manifested as branching were observed in 'Washington' after cryogenic storage of eight years.

Female flowers of *Carica papaya* cultivar 'Solo' set fruit and seed to the extent of 80 % and 86 % respectively when pollinated with 'Washington' pollen stored for eight years. In *Carica cauliflora*, the fruit and seed set was 85 % and 76 % respectively (Table 3). Seeds obtained from crossing with 'Washington' and *Carica cauliflora* pollen, showed germination of 82 % and 83 % respectively over the control (Table 4).

The ability of papaya pollen to retain viability and fertility under cryogenic temperature was reported earlier (Ganeshan, 1986) for pollen stored for 485 days and fertility assessed after 300 days. This study shows that papaya pollen can even be stored much longer without adverse effects under cryogenic conditions. Mazur (1970) also showed the ability of pollen to retain its function under cryogenic temperature for several years with negligible changes in biological material. The fertilizing ability of pollen stored over eight years has immense value in hybrid seed production where pollen or male genetic resources can be conserved over a long period. It also demonstrates the effective conservation of nuclear genetic diversity for an extended duration with negligible changes in viability and fertility. The results of this study can be applied for conservation of nuclear genetic diversity in *Carica* species and the possibility of establishing a pollen genebank to provide male genetic resources in a sustainable manner, for papaya breeding programs.

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Tables

Table 1. Percentage germination of fresh pollen and after cryostorage of 'Washington' and *C. cauliflora* for six and eight years.

Duration of storage in liquid nitrogen (Years)	Replication	*Carica papaya* 'Washington'		*Carica cauliflora*	
		Stored pollen	Fresh pollen	Stored pollen	Fresh pollen
Six	R1	62.06	68.44	87.81	90.00
	R2	68.37	80.80	86.01	91.04
	R3	76.64	72.00	90.54	87.99
	Average	68.24	72.60	87.51	89.30
Eight	R1	63.65	70.00	86.64	84.20
	R2	59.35	66.98	88.87	84.01
	R3	61.22	73.69	86.37	84.49
	Average	62.24	68.40	86.49	84.80
Means		65.22	71.61	87.53	86.98

Table 2. Pollen tube length (μm) of fresh pollen and after cryostorage of 'Washington' and *C. cauliflora* for six and eight years.

Duration of Storage in liquid nitrogen (Years)	Replication	*Carica papaya* ' Washington'		*Carica cauliflora*	
		Stored pollen	Fresh pollen	Stored pollen	Fresh pollen
Six	R1	241	296	368	386
	R2	264	245	345	328
	R3	289	312	369	359
	Average	264	284	360	357
Eight	R1	264	341	397	368
	R2	285	352	368	357
	R3	298	308	356	372
	Average	282	333	373	365
Means		273	309	367	361

Table 3. Post-storage fertility of 'Washington' and *C. cauliflora* pollen stored at liquid nitrogen for six and eight years.

Male x female parent	Storage duration	Repli-cation	No. flowers crossed	Number of fruit set		Number of flower/fruit drop		Number of fruit recovered		Average number of healthy seed recovered		% fruit set	
				Stored pollen	Fresh pollen	Stored pollen	Fresh pollen	Stored pollen	Fresh pollen	Stored pollen	Fresh pollen	Stored pollen	Fresh pollen
C. Califlora x 'Solo'	6 years	Average	25	17	20	2	1.67	15	18.67	317.00	383.33	60.00	74.67
	8 years	Average	25	17	17	4	2	13	15.67	303.67	391.67	53.33	62.67
'Washington' x 'Solo'	6 years	Average	25	15	18	1.33	2.33	14	16	730.67	842.33	56.00	64.00
	8 years	Average	25	17	21	2	2.33	15.33	19	617.67	715.67	61.33	76.00
		Means	25	16	19	2.31	2.06	14.38	17.31	492.25	583.25	57.63	69.31

Table 4. Per cent germination of seed recovered from crosses with pollen of 'Washington' and *C. cauliflora* stored at liquid nitrogen for six and eight years.

Male x female parent	Storage duration	Replication	Number of seed sown	Number of seed germinated		Per cent seed germination		Per cent seed germination over control
				Stored	Control	Stored	Control	
C. cauliflora x 'Solo'	6 years	R1	50	41	45	82	90	91.11
		R2	50	32	42	64	84	76.19
		R3	50	34	41	68	82	82.93
		Average	50	35.67	42.67	71.33	85.33	83.41
	8 years	R1	50	38	43	76	86	88.37
		R2	50	41	42	82	84	97.62
		R3	50	35	45	70	90	77.78
		Average	50	38	43.33	76	86.67	87.92
'Washington' x 'Solo'	6 years	R1	50	36	41	72	82	87.80
		R2	50	32	44	64	88	72.73
		R3	50	37	42	74	84	88.10
		Average	50	35	42.33	70	84.67	82.88
	8 years	R1	50	38	46	76	92	82.61
		R2	50	32	40	64	80	80.00
		R3	50	33	39	66	78	84.62
		Average	50	34.33	41.67	68.67	83.33	82.41
Means			50	35.75	42.50	71.50	85.00	84.13

Papaya Breeding for Tolerance to Bacterial Decline (*Erwinia* sp.) in the Caribbean Region

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Abstract

Papaya has a great potential for agricultural diversification in the Caribbean region, because of its rusticity, rapid production, high yields and the wide demand on local and international markets. At the end of the 1960s, a disease caused by *Erwinia* sp. appeared in the West Indies. This 'bacterial decline' decimated all the papaya fields in the area, where the cultivar 'Solo' was grown for its high yields and good quality. The disease is found from Virgin Islands to Venezuela. Tolerant accessions have been previously identified in the regional germplasm, however tolerance sources generally showed poor fruit quality and dioecy. A breeding project has been implemented by CIRAD in Guadeloupe to select good quality cultivars with improved level of tolerance. The first step was analyzing morphological and genetic diversity of regional germplasm, as compared with some commercial cultivars. Morphological diversity is important, but shows no particular structure, while isozyme diversity is more limited but displays some geographic structure. The level of tolerance to *Erwinia* strains appeared variable in relation with the tested papaya families, the *Erwinia* strains and the environmental conditions. However the rank for tolerance of the families remains the same whatever the strains and the environmental conditions. Tolerance appears to be transmitted in a co-dominant way in F1 hybrids between cultivar Solo and tolerant accessions. Selection of tolerant hermaphrodite lines is going on through backcross and endogamy strategies.

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INTRODUCTION

The production of papaya (*Carica papaya* L.) is particularly attractive for fruit crop diversification in the West Indies because of its yield potential, rusticity, high demand on local market, and potential for export. However, the cultivation of papayas responding to modern export standards has been made impossible because of the extremely severe impact of a bacterial canker caused by an *Erwinia* species (Persad, 1978; Webb, 1985; Frossard, 1985; Prior, 1985). This disease is present in most of the West Indies, from the Virgin Islands to Venezuela (Guevara et al., 1993), with the exception of the Greater Antilles. Similar bacterial diseases have been observed in Java, Taiwan and in the Mariana Islands (Trujillo and Schroth, 1982). The *Erwinia* strains of Antillean papayas display an important diversity, by their biochemical characteristics as well as by their genomic constitution (de Lapeyre et al., 1996; Gardan et al., 2004). They are different of the well-known *Erwinia* species. The most related one, according to biochemical characteristics and molecular hybridization (ADN/ADN), seems to be *E. mallotivora* (Gardan et al., 2004). Chemical control is not efficient (Frossard, 1985) while genetic control is more promising, as some local populations have shown high levels of resistance/tolerance, particularly in Guadeloupe, Venezuela, Granada, and Trinidad (Webb, 1983; de Lapeyre and Lyannaz, 1992). However, their heterogeneity does not allow a uniform production and their pomological and organoleptic characteristics are not adapted to the sweet fruit market. A breeding program to create cultivars combining resistance to the bacteriosis with good fruit quality appeared

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