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Pollen fertility in cultivated and wild species of cashew

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Cashew (*Anacardium occidentale* L.) is the only cultivated species in the genus and the most widely dispersed (Jhonson 1973; Ohler 1979; Mitchell and Mori, 1987). The National Cashew Field Gene Bank (NCFGB) established at Puttur, Dakshina Kannada district of Karnataka state in India has the germplasm holding of 528 accessions which also includes three wild species. Pollen fertility is very important for fruit and seed production in cashew and the knowledge of pollen fertility is essential for plant breeders and commercial growers. The pollen fertility studies on varieties of cultivated species are limited and on wild species, no reports available. Cashew is andromonoecious bearing male (staminate) and hermaphrodite (perfect) flowers on the same panicle. Hence, the present study was undertaken to determine pollen fertility status and its genetic variability in the cultivated and three wild species of cashew.

Twenty varieties of *A. occidentale* and three wild species *viz*., *A. microcarpum* Ducke., *A. othonianum* Rizz. and *A. pumilum* St. Hil (*A. humile)* available at the Directorate of Cashew Research, Puttur were used for the study. During November-February of 2012-13, when trees of all species were in flowering phase pollen grains from male and hermaphrodite flowers were assessed for pollen fertility status by using the aceto-carmine staining method. Flowers were collected randomly from three trees above 10 years old from each variety/wild species. This method of determining pollen fertility in different species of plants is comparable to other methods such as pollen germination method on artificial medium (Tyagi *et al*., 1992) and flow cytometry reactivity (Tyagi *et al*., 1995).

Pollen fertility was recorded in percentage and the data was transformed using arcsine degree transformation. The arcsine transformed data was analyzed using SAS software (9.3 version). Further, paired t-test analysis was carried out to compare the pollen fertility means between two kinds of flower used in the study. The genotypic, phenotypic and environmental coefficients of variation were calculated as per the method suggested by Burton and de Vane (1953). The heritability (broad sense) was estimated following the method given by Hanson *et al*. (1956) and genetic advance was calculated using the method given by Johnson *et al*. (1955).

It was observed that stained pollen grains were large in size and tricolpate whereas, the unstained pollen grains were smaller and round in shape in two kinds of flower in all four species studied (Fig. 1). Cashew belongs to eudicots which are more reliably categorized on the tricolpate derived poolen grains. A tricolpate pollen grain has three apertures or slits, which are spaced evenly on the surface and aligned parallel to the grain's longitudinal axis. Aperture may allow the pollen grain to contract or expand in moist environments and may also function as the site for siphonogamy during fertilization (Shmookler, 2007). The pollen grains were found to be prolate to spheroidal in shape (http://www.pollenlibrary.com/Genus/Anacardium). The smaller unstained pollen grains observed in the present study is in conformity with the report of Bhattacharya (2005) who reported that

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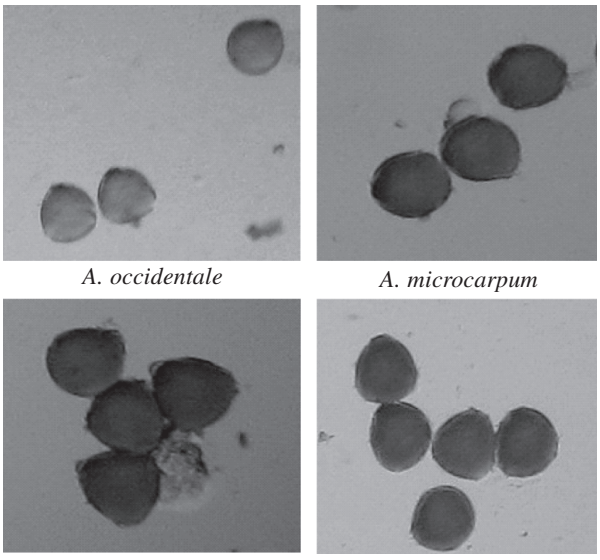

Fig. 1. Pollen grains of four species stained with aceto-carmine and observed under 10X objective (100 X magnification)

aborted pollen grains were smaller than non-aborted pollen grains using Alexander's stain in cultivated species. Wunnachit *et al*. (1992) reported that cashew produces four types of pollen from large and small stamens of the hermaphrodite and male flower (HL, HS, ML and MS) and observed similar staining characteristics for the four pollen types. The mean data on pollen fertility of different cultivated varieties and wild species of cashew with two pollen sources is presented in Table 1. There was significant difference among the varieties with pollen source from male as well as hermaphrodite flowers and wild species differed significantly only for pollen source from hermaphrodite flower. The pollen fertility means within a variety from male and hermaphrodite flowers differed significantly in two varieties *viz*., K 22-1 and Madakkathara-2 and this was also true in case of wild species *A. pumilum*.

The pollen fertility of varieties of cultivated species ranged from 53.2 per cent in a hybrid variety

Table 1. Percentage of pollen fertility from two sources of pollen in cultivated varieties and wild species of cashew

Sl. No.	Variety	Source of pollen		Paired t-test
		Male flower	Bisexual flower	
1	Ullal-3	96.42 (74.96)a	97.55 (82.00)ba	-1.99
2	Ullal-1	96.11 (74.43)a	96.08 (75.67)bac	-0.24
3	K22-1	95.29 (72.95)ba	65.99 (41.34)h	10.88*
4	Vengurla-7	94.68 (71.43)bac	92.18 (68.24)ebdgcf	0.66
5	VTH-174	94.55 (72.11)bac	84.42 (59.60)edgf	1.56
6	Vengurla-3	94.40 (71.10)bac	91.27 (71.95)ebdac	-0.08
7	Madakkathara-2	94.18 (70.91)bac	81.21 (54.75)hacgf	4.12*
8	VTH-30/4	94.17 (71.11)bac	89.33 (69.73)ebdacf	0.19
9	Bhaskara	93.42 (69.43)bac	96.60 (80.59)ba	-2.18
10	NRC-492	93.38 (69.56)bac	98.33 (85.29)a	-2.29
11	Ullal-2	93.21 (69.08)bac	96.16 (77.63)ba	-1.65
12	Ullal-4	93.17 (70.95)bac	86.41 (60.62)edgcf	2.63
13	Vridhachalam-3	92.28 (69.57)bac	84.47 (57.96)egf	2.63
14	Taliparamba-1	92.21 (67.57)bac	91.77 (71.92)ebdac	-0.48
15	NRCC Sel-2	92.04 (68.20)bac	97.70 (82.25)ba	-2.09
16	Vengurla-4	91.77 (67.67)bac	93.25 (72.41)ebd	-0.78
17	Purple Mutant	90.43 (65.42)bdc	96.89 (78.88)ba	-2.23
18	Priyanka	89.28 (63.77)dc	94.18 (74.88)bdac	-1.45
19	Kanaka	85.12 (59.30)d	98.40 (83.49)ba	-3.27
20	Dhana	53.18 (32.23)e	74.99 (52.88)hg	-2.32
	Mean	90.97	90.36	t (P<0.05): 2.77
	SEm +/-	2.15	5.59	
	CV (%)	6.50	17.83	
	LSD (P<0.05)	6.05	15.75	

Sl.No.	Species			
1	*A. pumilum*	93.84 (70.14)a	47.68 (28.54)c	16.71*
2	*A. microcarpum*	92.27 (67.56)a	97.19 (76.61)a	-3.95
3	*A. occidentale*	90.97 (65.46)a	90.36 (64.63)b	0.89
4	*A. othonianum*	90.49 (66.09)a	94.78 (76.13)a	-1.29
	Mean	91.89	82.50	t (P<0.05): 2.77
	SEm +/-	2.69	3.41	
	CV (%)	8.92	12.41	
	LSD (<0.05)	NS	10.52	

** Values in parenthesis indicate arcsine transformed values
Means with same letter do not differ significantly

Dhana to 96.4 per cent in Ullal-3 with male flower as source of pollen whereas the fertility of pollens from hermaphrodite flower, ranged from 41.3 per cent in K 22-1 to 98.4 per cent in Kanaka. The pollen fertility in wild species ranged from 90.5 per cent in *A. othonianum* to 93.8 per cent in *A. pumilum* when the pollen source was male flower. But when the pollen source was hermaphrodite flower, *A. pumilum* showed the lowest pollen fertility of 47.7 per cent and *A. microcarpum* exhibited the highest pollen fertility of 97.2 per cent. The higher pollen fertility recorded in the present study was in agreement with earlier work carried out on pollen fertility using the same aceto-carmine staining method (Damodaran *et al.*, 1966) and by staining with Alexander's staining solution (Aliyu, 2007). The low and high pollen fertility observed in hermaphrodite flowers of *A. pumilum* and *A. microcarpum* respectively were also evident from low and high fruit-set observed in these species.

GCV and PCV were found to be low for the pollen fertility in male flowers of both cultivated and wild species (Table 2) while it was moderate for the cultivated species and high for the wild species in hermaphrodite flowers. This indicates that variability of pollen fertility in cultivars and wild species is low in case of male flowers and it was moderate for perfect flowers. The heritability was found to be high for pollen fertility in male flowers and moderate in hermaphrodite flowers of cultivated species. It was low in pollen fertility in male flower and high in hermaphrodite flowers of wild species. The genetic advance was low for pollen fertility in male flower and high in hermaphrodite flowers of wild species. It was moderate for pollen fertility in two kinds of flowers of cultivated species. High heritability and high genetic advance observed for pollen fertility in hermaphrodite flowers of wild species indicated that the heritability is due to additive gene effect and selection may be effective at pollen level. Low heritability was accompanied with low genetic advance for the pollen fertility in male flower of wild species which indicated the pollen fertility is highly influenced by environmental effects and selection would be ineffective.

The present study revealed the possibility of using both male and hermaphrodite flowers as pollen source for inter-varietal hybridization. However, for those varieties with partial pollen fertility, large quantity of pollen grains needs to be collected during hybridization. Based on fertility values, it appears that in the variety K22-1, pollen from male flowers should be used whereas in Dhana

Table 3. Variability statistics for pollen fertility in cashew

Parameter	Varieties		Species	
	Male flower	Hermaphrodite flower	Male flower	Hermaphrodite flower
GCV (%)	9.69	15.08	3.60	45.19
PCV (%)	12.02	23.35	11.15	47.67
Heritability % (Broad sense)	65.02	41.71	10.44	89.86
GA as % mean	11.94	15.30	0.24	87.39

and Kanaka, pollen from hermaphrodite flowers should be used for better success of seed set in cashew. The study also revealed that all three wild species can be used as pollen donors in the inter-specific hybridization programme in which the male flowers of *A. pumilum* should be used as pollen source.

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