

Exploitation of Diploids in Indian Banana Breeding Programmes

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Abstract

Banana is a recalcitrant crop for improvement due to inherent plant-based constraints like polyploidy, parthenocarpy and male and/or female sterility. Success of banana breeding depends mainly on the use of natural diploids and/or developing superior synthetic diploids. India has nurtured the development of a number of wild and parthenocarpic diploids harbouring resistant gene sources for a number of biotic and abiotic factors. Present study on 15 diploids includes nine parthenocarpic and six wild diploid accessions of which ten are indigenous and five are exotic introductions. They have been evaluated for various traits like resistance to nematodes, weevils and borers, Fusarium wilt, leaf spot complex and reaction to drought. Based on their usefulness of traits, diploids were included in the breeding programme either directly or through development of synthetic diploids. This paper reports on the results of evaluation of these 15 diploids for their fertility status, breeding behaviour and compatibility status with other diploid and triploid parents. The paper also provides baseline information on factors affecting seed set, like temperature and relative humidity, extent of seed set in 79 diploid cross combinations, percent of good seeds with viable embryos, germination time (10-432 days), germination success (0-92%) and regeneration capacity (2.5-98.3%). This baseline information will be useful in selecting parents for future breeding programmes with better success in seed yield. The poor germination and regeneration of hybrid seeds into plantlets reiterates the need for standardised techniques for embryo rescue for better success in improvement of banana through classical breeding.

INTRODUCTION

Banana is one of the few fruit crops that lack a successful breeding history, and presently cultivated varieties are natural selections by man over years. This recalcitrance is due to polyploidy, parthenocarpy, and male and/or female sterility of most cultivated varieties. Banana has passed through a rigorous course of evolution to transform itself from seedy, non-pulpy wild progenitors to present-day parthenocarpic, edible and high-yielding bananas (Simmonds, 1962).

Banana has a number of pests and diseases that co-evolved with their host due to its long period of evolution and domestication. The genetic diversity of *Musa* found in India fails to offer any guarantee against crop losses due to pests and diseases. The almost total devastation of 'Virupakshi' (AAB, Pome) by *Banana bunchy top virus* in Tamil Nadu is a typical example. Seasonal incidence of leaf spot diseases and prevalence of Fusarium wilt (race 1 and 2) and tip-over disease are causing serious crop losses. Among the insect pests, the banana rhizome weevil, the pseudostem borer and nematodes are a great cause of concern. *Banana bunchy top virus* (BBTV), the banana streak viruses (BSV) and *Banana bract mosaic virus* (BBMV) are devastating in many regions, irrespective of the cultivars. Chemical control of insect pests, nematodes and fungal

diseases is costly and not ecofriendly. Genetic improvement of banana offers an ecologically sustainable solution for pest and disease management. Banana, with its great diversity, requires improvement adapted to the many end uses of the crop, like dessert, cooking and other processing. But due to plant-based constraints, banana breeding has long proven to be difficult, time consuming and very expensive.

As one of the centres of origin of *Musa*, India has a wide spectrum of diversity of cultivated bananas with 15-20 cultivars under commercial cultivation and 40-60 cultivars under niche cultivation as landraces (Singh and Uma, 2000). India is one of the few countries where diploid cultivars are under commercial cultivation. AA diploids, like 'Matti', 'Sanna Chenkadali', 'Anaikomban' and 'Chingan', are commercial niche cultivars in the Southern Peninsula. AB cultivars 'Ney Poovan' and 'Elakki Bale', are the basis of the banana industry in Karnataka state, while 'Kunnan' cultivars (AB) are contributing to nutritional and economic security of people in the southern tip of Tamil Nadu and Kerala. BB cultivars, especially 'Bhimkol', are contributing to therapeutic needs of locals in the vast expansion of East and North Eastern states of West Bengal, Assam, Arunachal Pradesh, etc. Diploid cultivars are thus interwoven with the tradition and commerce contributing to economic and nutritional security of niche areas.

In India, banana and plantain improvement programmes not only focus on the development of improved diploids to be used in the triploid improvement programmes. They also aim at developing superior synthetic diploids with resistance to biotic and abiotic stresses to fit into organic and marginal cultivation systems spread across traditional and non-traditional banana cultivation zones. The present study was carried out to improve three major semi-commercial diploid landraces, which combine one or more resistance traits with good horticultural characteristics. Their breeding behaviour in terms of compatibility, extent of seed set, germinability, xenia effect, rejuvenation capacity, etc., are discussed in this paper.

MATERIALS AND METHODS

Plant Materials

A total of 15 diploids were used in the studies as male and female parents. Four female diploids are discussed in detail below. The first three are parthenocarpic cultivars which have the potential to go into commercial niche cultivation; the fourth is a wild *Musa acuminata* used for developing intermediary hybrids in gene pyramiding.

'Anaikomban' is a niche cultivar of southern India and of North Eastern India in the name of Hatidat (an ecotype). It has an agronomically superior bunch (14-18 kg), long and straight fruits suitable for box packing, good male and female fertility (Uma et al., 2002), resistance to *Pratylenchus coffeae* and field tolerance to yellow Sigatoka (*Mycosphaerella musicola*) (Anon., 1997).

'Matti' is another indigenous diploid of choice in Southern India. It is highly priced for its fragrant fruits and locally valued for therapeutic purposes. It has a 20-25 kg bunch, is tolerant to yellow Sigatoka leaf spot and highly male and female fertile.

'Namarai' is another niche cultivar suited for backyard home gardens of Southern India. It is well accepted for its high suitability to marginal soil conditions and tolerance to sodic soils. It has a very short crop cycle (7-9 months) and pleasant, aromatic fruits.

'Calcutta-4' (*Musa acuminata* ssp. *burmanicoides*) is an important potential gene sources for improving commercial cultivars for resistance to the *Mycosphaerella* leaf spot complex and Fusarium wilt. In addition, it has a short crop cycle (9-10 months), is highly

polleniferous and is a female-fertile parent. It is being extensively used in banana breeding programmes across the globe. Its actual origin to at-the-time undivided India (presently Chittagong forest area of Bangladesh) is well documented (Shepherd, 1994; Uma et al., 2006). Because of its seedy nature, efforts were made at NRCB to transfer its resistance traits into agronomically superior diploids.

Pollination

The occurrence of pistillate and staminate flowers separately and sequentially makes hand pollination easy. Pollen from the anthers of desirable male parents were collected and gently spread on the receptive stigma of the female parent at its peak receptivity using a camel hair brush. The individual hands were covered with a brown paper bag to prevent cross pollination. This was done in the cooler hours of the morning between 6 and 7 am. All female flowers in the bunch were pollinated with the same male parent without leaving any hand for natural pollination.

Seed Collection and Germination

The bunch was harvested when it was fully mature, and allowed to ripe. Seeds were manually extracted from peeled fruits and the adhering pulp was washed off. The seeds were stored in small polythene pouches for immediate sowing in seed pans containing fine sand. Seeds were spread evenly in the pan and covered with a thin layer of fine sand. They were maintained in a mist chamber.

Data Collection and Analysis

The total number of days from opening of the first bract to the full maturation of the bunch was considered as duration of “bunch maturation”. The total number of seeds produced from all hands of the bunch with a single male parent was considered as the “total number of seeds”. Freshly extracted seeds were suspended in water over night. Depending on the weight of the seed, they either sink or float. Sunken seeds are usually good seeds, fully formed with seed coat, embryo and endosperm while the floating seeds had various deficiencies of these structures (Simmonds, 1952). The sunken seeds were considered as “good seeds” in this article. The normal maturation period of the crossed bunch was compared with the maturation duration of the male parent, and the difference was considered as the “deviation in maturation”. The “regeneration capacity” or the number of combinations which could be successfully germinated into seedlings with each female parent was calculated as the number of cross combinations developing into seedlings divided by the total number of combinations tried.

Pearson’s correlation coefficients were calculated to determine the relationships between total seeds and good seeds, and good seeds and duration of bunch maturation.

RESULTS AND DISCUSSION

‘Anaikomban’

‘Anaikomban’ was crossed with 12 different diploids, including parthenocarpic as well as non-parthenocarpic parents (Table 1). The highest number of seeds was observed with ‘Anaikomban’ x ‘Namarai’ with a total of 103 seeds/bunch; the lowest when ‘Pisang Mas’ was used as male parent. On average, ‘Anaikomban’ combinations produced 38.7 seeds. A maximum of 90.6% of good seeds was observed in ‘Anaikomban’ x ‘Pisang Jari Buaya’, followed by ‘Anaikomban’ x ‘Pisang Lilin’. Less than 70% good seeds were

noticed with 'Pisang Mas' followed by selfed 'Anaikomban'. But the extent of good seeds production in 'Anaikomban' was not statistically significant, irrespective of the male parent used. This suggests that 'Anaikomban' can be a good parent for developing seed progenies.

Under normal circumstances, fruit maturation in 'Anaikomban' takes an average of 115 days. In the present study, all the diploids used as male parents exhibited shorter duration for bunch maturation than 'Anaikomban'. The results revealed that in all combinations except with 'Namarai', the duration of fruit maturity in 'Anaikomban' was reduced by 6 to 37 days with an average of 18 days. This suggested a strong influence of paternal parents on bunch maturity trait on 'Anaikomban', a xenia effect. Similar findings, suggesting maternal inheritance of growth habits and paternal inheritance of productivity traits, were also reported by Ortiz et al. (1995).

Although seed set was observed in all 12 combinations involving 'Anaikomban' as a female parent, only six combinations could be successfully established in the field. The number of seeds per fruit varied among combinations, with 'Anaikomban' x 'Chengdawat' producing the highest number of seeds (10-13 seeds per fruit, and all of them were good seeds), followed by 'Anaikomban' x 'Pisang Lilin' which yielded 5-6 seeds per fruit of which more than 95% were good seeds. Though seeds produced were good in terms of basic seed structure, germination under field conditions varied drastically, ranging from 0 to 18.4%. Germination duration ranged from 44.6 to 130 days, with the shortest duration observed in the cross with 'Pisang Mas' and the longest in selfed seeds, in spite of the fact that bunch maturation remained the same. Genetic differences may partly account for the observed differences for seed duration.

Irrespective of the nature of duration of the parent (parthenocarpic/non-parthenocarpic), there was no definite trend in the relationship between good seeds to duration of bunch maturation and total seeds. Similar results were reported in 'Nendran' and 'Palayamkodan' (Krishnakumar et al., 1990).

'Matti'

'Matti' was crossed with eight parthenocarpic parents and six non-parthenocarpic parents (Table 2). The total seeds produced with 'Matti' ranged from 31.5 to 115 with an average of 63.14 seeds per bunch. In general, higher seed set was observed when 'Matti' was crossed with parthenocarpic parents (72 seeds) than with non-parthenocarpic parents (56 seeds). An output of more than 100 seeds per bunch with 'Pisang Jari Buaya' and 'Sanna Chenkadali' suggested their higher compatibility status. Although 'Matti' is a good female parent under controlled pollinations, the fertility in terms of good seeds was enhanced by non-seeded (parthenocarpic) male parents compared to seeded wild parents. Similar results were earlier reported in 'Matti' (Sathiamoorthy, 1987, 1993)

In terms of bunch maturation, 'Matti' normally takes 100-105 days. In our studies, when crossed with 11 different female parents, the maturation duration was not statistically significant with a simple increase or decrease by a maximum of 5 days. But when crossing with non-seeded parents like 'Anaikomban', 'Namarai' and 'Pisang Jari Buaya', the duration was prolonged by more than 25 days. Despite this longer bunch maturation, these combinations were promising in terms of higher percentage of good seeds. The correlation studies revealed a significant and positive relationship between total number of seeds and good seeds in all the cross combinations. With reference to the female parent 'Matti', early bunch maturation was observed in eight combinations and delayed maturation in five combinations.

Better female fertility in 'Matti' was earlier reported by Sathiamoorthy (1987) and Kumar (2006), who suggested residual fertility from its most possible ancestor *M. acuminata* ssp. *burmannica*. In terms of seed germination, not all combinations of 'Matti' germinated. Only the seeds set with 'Anaikomban', 'Kanai Bansi', 'cv. Rose', 'Lairawk', 'Pisang Jajee' and 'Pisang Lilin' germinated. Germination duration showed an unusually erratic range of 25-405 days. The range within each combination also showed great variability. 'Matti' x 'Pisang Lilin' took 23 to 94 days, whereas 'Matti' x 'cv. Rose' took 38 to 405 days. The differences in climatic condition might have also contributed to this erratic behaviour apart from the genetic difference. Similar results were noticed by Swennen et al. (1991) and Ortiz and Vuylsteke (1995) with plantains.

'Namarai'

'Namarai' was crossed with four parthenocarpic and three non-parthenocarpic diploid male parents (Table 3). The total number of seeds developed per bunch across different male parents ranged from 7.67 to 38.3 with an average seed yield of 26.38 per bunch. Parthenocarpic male parents exhibited a positive influence in terms of total seed yield (28.8) compared to non-parthenocarpic parents (21.2). The percentage of good seeds produced ranged from 7.67 to 89.17 with an average of 74.9 seeds. Like 'Matti', use of seeded male parent resulted in poor seed set, whereas the percentage of good seeds was relatively high and statistically significant. Results are strengthened by the earlier reports of Sathiamoorthy et al. (1979, 1988). Mixed effect of male parent on bunch maturity was noticed when 'Namarai' was used as female parent. 'Pisang Mas' and 'Pisang Jajee' had no influence, while use of 'Pisang Lilin' and 'Lairawk' reduced the bunch maturity period by 11 and 20 days respectively. Use of 'cv. Rose' extended the maturity period by 22 days followed by 'Lairawk'.

The relationship between the number of total seeds and good seeds was highly positive and significant in all cross combinations using 'Namarai' as female parent except with 'Pisang Lilin'. Even the number of total seeds and percentage of good seeds were lower in the 'Namarai' x 'Pisang Lilin' combination.

'Namarai' set seeds only with four different male parents, 'Calcutta-4', 'Pisang Jajee', 'cv. Rose' and 'Pisang Lilin', and seeds with 'cv. Rose' failed to germinate. Seed germination ranged from 0 to 7.6%, lower than for any other female parent. 'Namarai' x 'Pisang Jajee' produced an intermediate number of seeds per fruit but all of them (100%) were good seeds. 'Namarai' x 'Calcutta-4' produced 66% of good seeds which took 65-135 days for germination, whereas 'Namarai' x 'Pisang Lilin' produced only 45% good seeds which took 39-97 days for germination.

'Calcutta-4'

'Calcutta-4' was crossed with 10 different parthenocarpic and non-parthenocarpic diploids (Table 4). Being non-parthenocarpic in nature, 'Calcutta-4' produced a large number of seeds with all combinations. The total number of seeds produced ranged from 138 with 'Pisang Jajee' to 2009 with 'Pisang Mas', with an average output of 765 seeds per fruit. Similarly, the extent of good seeds produced was also high at more than 90% in all combinations, except with 'Pisang Jajee' (68.53%).

In general, the parthenocarpic male parents exhibited better influence in terms of production of seeds than non-parthenocarpic parents with 'Calcutta-4' as a seeded female parent. This is contradicting the results obtained by Dodds and Simmonds (1948). The results also suggested that controlled pollination influenced better seed production in

seeded female parent. Under natural conditions, only 200-250 seeds were set per bunch, while under controlled self-pollinated condition, the seed set in 'Calcutta-4' was almost double. Similar results were obtained by Simmonds (1960). This process is generally followed at NRCB for rejuvenation of 'Calcutta-4' exhibiting poor vigour and intraclonal variations clumps due to continuous vegetative propagation.

Under normal conditions, 'Calcutta-4' takes on average 90 days from shooting to fruit maturity. All the diploids used as male parents in our study exhibited higher maturation duration (295 days), except 'Chengdawt' and 'cv. Rose' (90 days). The duration of fruit maturation in crossed 'Calcutta-4' was found increased by 10 to 56 days with an average of 34 days. In general, increase in days for bunch maturation was observed in all the cross combinations, except with 'Chengdawt', 'cv. Rose' and selfing with 'Calcutta-4'. This again confirmed the strong xenia effect, maturation of the fruit being influenced by paternal parents. As with 'Anaikomban', even in 'Calcutta-4', the female fertility status (parthenocarpic/non-parthenocarpic) had no specific influence on bunch maturation. Selfing in 'Calcutta-4' also had no significant effect on bunch maturation.

As in the case of the parthenocarpic diploid 'Anaikomban', the non-parthenocarpic 'Calcutta-4' also exhibited a highly significant and positive correlation between total seeds and good seeds, irrespective of the male parent used. Number of good seeds recovered increased with an increased seed output. Irrespective of the male parent, all crosses set seeds with 'Calcutta-4'. Seed germination ranged from 28.6 to 98.3% across the combinations, while days taken for seed germination ranged from 10.0 to 94.7 days. Our field observation also suggested that 'Calcutta-4'-derived progenies established better in the field than other hybrid progenies.

Pooled Correlation of the Female Parents

Pooled correlation of female parents 'Anaikomban', 'Matti', 'Namarai' and 'Calcutta-4' with different diploid male parents was worked out. A highly significant and positive correlation was observed between total seeds produced per bunch and number of good seeds. The chances of getting a high number of good seeds increased with an increase in total number of seeds produced. A non-significant correlation was obtained between bunch maturation and total number of seeds, suggesting that the bunch maturation has no effect on the production of quality seeds, but rather depends on the maturation duration of the male parents used.

CONCLUSIONS

'Anaikomban' proved to be a good female parent for a gene pyramiding programme. It produces good seed set in most combinations with highest germination capacity in nursery. Due to xenia effect, its longer crop duration can be reduced. 'Matti' needs a careful selection of parents because of poor seed regeneration in spite of good seed set. Erratic seed germination and longer seed dormancy needs interventions through embryo culture and/or rescue. 'Namarai' is a poor female parent in terms of seed set, germination and regeneration with restricted compatibility with other male parents. 'Calcutta-4' is the most amenable female parent with maximum seed set, highest germination percent and field regeneration. This paper is a part of the compilation of baseline information pooled on 79 diploid cross combinations for their compatibility traits and factors influencing them.

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Tables

Table 1. Details of seed set and related parameters with ‘Anaikomban’ as female parent.

Male parent	Type*	Bunch maturation (days)	Total seeds/bunch	Good seeds (%)	Bunch maturity of male parent (days)	Deviation in maturity** (days)
Burmanica	NP	100	35	79	96	-19
Calcutta-4	NP	110	63	89	90	-25
Chengdawt	NP	86	38	82	90	-25
Lairawk	NP	106	26	81	95	-20
Pisang Jajee	NP	86	59	79	95	-20
cv. Rose	P	117	18	78	90	-25
Matti	P	105	21	84	105	-10
Namarai	P	129	103	82		-115
Pisang Jari Buaya	P	97	61	92	110	-5
Pisang Lilin	P	107	35	91	100	-15
Pisang Mas	P	78	12	66	100	-15
Anaikomban (selfing)	P	114	21	69	115	0
Grand Mean		102.04	38.76	81.00		
SEM		18.85	19.11	7.72		
F Value		3.45	3.77	1.98		
Sig/N.Sig		**	**	NS		
CD @ 1%		52.39	53.09			
CD @ 5%		38.75	39.27			
CV		23.54	60.36			

* Type: NP - non parthenocarpic, P - parthenocarpic.

** Bunch maturation of ‘Anaikomban’ is normally 115 days.

Table 2. Details of seed set and related parameters with ‘Matti’ as female parent.

Male parent	Type*	Bunch maturation (days)	Total seeds/bunch	Good seeds (%)	Bunch maturity of male parent (days)	Deviation in maturity** (days)
Calcutta-4	NP	107	63	84	90	-15
Chengdawt	NP	83	32	76	90	-15
Lairawk	NP	100	83	79	95	-10
M. laterita	NP	102	74	63		
Pisang Jajee	NP	106	31	44	95	-10
Anaikomban	P	78	68	79	115	+10
cv. Rose	P	104	55	74	90	-15
Matti (selfing)	P	106	92	89	105	0
Namarai	P	135	45	66	95	-10
Pisang Berlin	P	140	54	77	-	-
Pisang Jari Buaya	P	136	115	82	110	+5
Pisang Lilin	P	109	47	80	100	-5
Pisang Mas	P	107	18	10	100	-5
SCK	P	139	109	86		
Grand Mean		110.82	63.14	70.73		
SEM		12.01	29.42	12.36		
F Value		5.40	1.95	5.69		
Sig/N.Sig		**	*	**		
CD @ 1%		32.40	79.39	33.34		
CD @ 5%		24.24	59.39	24.94		

CV	15.33	65.89	24.70
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* Type: NP - non parthenocarpic, P - parthenocarpic.

** Bunch maturity of 'Matti' is normally 105 days.

Table 3. Details of seed set and related parameters with 'Namarai' as female parent.

Male parent	Type*	Bunch maturation (days)	Total seeds/bunch	Good seeds (%)	Bunch maturity of male parent (days)	Deviation in maturity** (days)
Calcutta-4	NP	110	17	71	90	-5
Lairawk	NP	75	8	89	95	0
Pisang Jajee	NP	94	44	89	95	0
cv. Rose	P	117	24	87	90	-5
Matti	P	112	29	64	105	+17
Pisang Lilin	P	85	38	36	100	+5
Pisang Mas	P	95	25	89	100	+5
Grand Mean		110	26	74.94		
SEM		14.96	9.98	15.23		
F Value		9.90	3.08	3.49		
Sig/N.Sig		**	*	*		
CD @1%		44.54	29.71	45.34		
CD @5%		32.09	21.41	32.67		
CV		16.68	46.33	24.89		

* Type: NP - non parthenocarpic, P - parthenocarpic.

** Bunch maturity of 'Namarai' is normally 95 days.

Table 4. Details of seed set and related parameters with 'Calcutta-4' as female parent.

Male parent	Type*	Bunch maturation (days)	Total seeds/bunch	Good seeds (%)	Bunch maturity of male parent (days)	Deviation in maturity** (days)
Calcutta-4 (selfing)	NP	82	524	89	90	0
Chengdawt	NP	71	475	96	90	0
Lairawk	NP	118	926	91	95	+5
cv. Rose	P	109	515	91	90	0
Matti	P	119	635	94	105	+15
Pisang Jajee	P	126	139	69	95	+5
Pisang Jari Buaya	P	100	1418	92	110	+20
Pisang Lilin	P	127	533	88	100	+10
Pisang Mas	P	145	2009	100	100	+10
SCK	P	147	480	96	100	+10
Grand Mean		114.33	765.37	90.65		
SEM		21.13	293.41	5.58		
F Value		2.72	7.09	4.70		
Sig/N.Sig		*	**	**		
CD @1%		60.13	834.89	15.88		
CD @5%		44.08	612.05	11.65		
CV		22.64	46.95	7.54		

* Type: NP - non parthenocarpic, P - parthenocarpic.

** Bunch maturity of 'Calcutta-4' is normally 90 days.

Table 5. Details on seed germination and seed dormancy among acuminate diploid parents.

Diploid female	Seed germination (%)	Germination duration	Regeneration capacity
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parents (AA)	(days)				(%)
	Min.	Max.	Min.	Max.	
Anaikomban	0	18.4	92.6	98.8	76.0
Matti	0	12.3	65.5	405	66.67
Namarai	0	7.6	60.5	184.5	42.86
Calcutta-4	28.6	98.3	10.0	94.7	90.00