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RESEARCH ARTICLE

Broadening the genetic base of the diploids with biotic resistance to accelerate banana (*Musa acuminata* L.) breeding

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Abstract

Success of banana (*Musa acuminata* L.) breeding mainly depends on the male diploid parents used in the hybridization program. Developing intermediate diploid breeding lines with fertile pollen production ability, resistance to biotic and abiotic stresses, and fruit quality through the diploid x diploid strategy is the key step in improving commercial cultivars. Till now, at the global level only a limited number of diploid accessions is being used in banana breeding programs. In the present study, through the diploid x diploid breeding strategy, the genetic diversity of diploids has been broadened by developing nine polleniferous biotic-stress-resistant diploid lines. Among these, except for two progenies (P), all were resistant to the eumusae leaf spot disease. A single progeny (P 134) showed resistance to root-lesion and root-knot nematodes, whereas P 148 showed moderate resistance to root-knot nematodes alone. Three progenies of cv. Rose x Pisang Lilin (P 427, P 428 and P 429) were moderately resistant to banana stem weevil. Irrespective of cross combinations, barring two, all other progenies exhibited resistant reactions to fusarium wilt, Foc race 1 under both hot spot and pot culture screening. Among the nine diploid progenies, P 427, P 428 and P 429 were the best prospects to contribute multiple resistance against pests (banana stem weevil) and diseases (eumusae leaf spot and Foc race 1), whereas P 134 could be used as the resistant source for root-knot nematodes. The pyramiding of resistant/defense-related alleles in the progenies has been confirmed through SSR markers associated with Foc resistance. These polleniferous resistant improved diploid progenies are the potential source for accelerating the banana breeding program for improving biotic resistances in the triploid commercial cultivars through reconstructive breeding and/or for stacking the resistant genes in other genetic backgrounds.

Keywords: Banana, improved diploids, polleniferous, biotic resistance, introgression of alleles

Introduction

Edible bananas (*Musa acuminata* L.) being triploid coupled with widespread female sterility offer very little scope for genetic improvement through classical breeding. Residual fertility among some triploid clones provides a glimmer of hope to venture into an endeavor comprising hybridization followed by selection for targeted traits. The most popular approach across the globe is to cross the triploid female with a diploid male to develop the tetraploids, which in turn would again be crossed with the diploid parent to develop the triploids. An interesting point to be noted here is that in the first cycle of hybridization, *i.e.,* for developing tetraploids, the triploid, as such passes its entire genomic content, and whatever meiotic recombinational gains are to be accrued have to come from the diploid parent only. It has been reported that economically important traits such as bunch weight, pest resistance and reduced duration are rather inherited from diploid parents than from the parents with a higher ploidy status (Tenkouano et al. 1998). Thus, diploid is used as a male parent with desirable traits in the banana breeding program (Tetraploid x Diploid, Triploid x Diploid, and Diploid x Diploid), emphasizing that selection of a compatible male parent is the main criteria for the success of breeding programs. Hence, it is empirically proven that

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to use in any genetic improvement in a cultivated triploid through conventional breeding, the genetic base of the diploid parent has to be broadened first.

Diploids are useful sources of resistance to pests and diseases (Rowe and Rosales 1993). Several *Musa acuminata* subspecies, including *banksii, burmannica, malaccensis, microcarpa, siamea* and *truncata* (AA genome) have a hypersensitive response (HR) to black leaf streak virus (BLSV) (Fouré et al. 2000) and Calcutta 4 showed resistant reaction to the banana weevil (Ortiz et al. 1995). The resistance to *Fusarium* tropical race 4 (TR4), diploid lines have also been identified in the segregating populations of *Musa acuminata* ssp. *malaccensis* (AA genome) (Ahmad et al. 2020). Though wild diploids are found to be the best sources of pest and disease resistance, the transfer of poor agronomic traits along with resistance to the cultivated species restricts their utility in the breeding program. This can be overcome by transferring the resistance to an intermediate set of materials through a pre-breeding approach. Honduras Foundation for Agricultural Research (FHIA), Honduras and the International Institute for Tropical Agriculture (IITA), Nigeria, made many possible combinations of diploid x diploid crosses that resulted in developing improved diploids with a high level of resistance to race 4 of *Fusarium* wilt and black Sigatoka such as SH-3362 by FHIA (Hamill et al. 1992) and TMB2x5105-1, TMB2x9128-3 by IITA (Tenkouano et al. 2003).

In India, nearly 30 commercial cultivars are grown in different banana-producing states but the area under their cultivation is diminishing owing to a lower yield than cv. Grand Naine. Despite the high demand in the export market for these cultivars, farmers are reluctant to cultivate in large areas because of high plant protection costs. Being a recalcitrant crop for seed set, cross introgression of the desirable gene(s) into the commercial cultivars through normal breeding techniques like pedigree selection and back cross method could not be accomplished. For consumer acceptability, the banana has to be parthenocarpic for which nature has endowed it with the ability to propagate through vegetative means. But this very selective advantage becomes an albatross that can be minimized by hybridization among the closely related diploid accessions of local commercial cultivars. Unfortunately, few Indian diploid accessions have poor pollen output, viability and germination percentage (Panda et al. 2019), which hinders their direct use in the breeding program. However, the trait of pollen fertility could be enhanced through hybridization with the diploid pollen fertile genotypes (Du et al. 1996).

At present, resistant and parthenocarpic diploid accessions are very few in India and mostly diploid accessions of International Transit Center (ITC), Belgium, are being used as resistant sources which emphasizes the need for broadening the genetic base of Indian diploids. Hence, in the present study efforts were made to widen the genetic base of diploid accessions to enhance their utilization in the improvement of Indian commercial cultivars of banana.

Materials and methods

Accessions used for diploid x diploid cross combinations

A total of ten AA diploid accessions, which includes four Indian landraces, namely, Matti, Anaikomban, Namarai, and Sanna Chenkadali, three ITC collections *viz.,* Pisang Lilin, Imbago and cv. Rose and three wild/related species, Calcutta 4, Lairawk and Calcutta 4-1, available at the ICAR-National Research Center for Banana (NRCB) farm, Trichy were used in the study. Though hybridization was initiated in all possible combinations, the seed set was not obtained in all crosses. Based on the seed set behavior among these diploid accessions, four, namely, Calcutta 4-1, Matti, Anaikomban and cv. Rose were used as both male and female parents, whereas Sanna Chenkadali was used as female and Calcutta 4, Pisang Lilin, Lairawk, Imbago and Namarai were used as male parents. The parental details are given in Table 1.

Hybridization and establishment of new diploid progenies

Male flower buds of the parent were fully covered immediately after the completion of the female/neutral phase to avoid pollen contamination. The pollen was collected from the male inflorescence, which was ready to open on that day. Individual bracts at the different nodes of the female phase of inflorescence were sequentially tied to prevent pollen from unintended sources at both pre and post-pollination stages. The bract of the female hand, which was ready to open that day was lifted and the pollen collected from the male parent was dusted over the receptive stigmatic surface between 7 to 10 AM. After the pollination, the bract over the female buds was secured again. The crossed bunches were harvested at the time of 90 to 100% maturity and the seeds were extracted after attaining full ripening.

The crossed seeds were subjected to the embryo culture technique developed at ICAR-NRCB, Trichy. (Arun et al. 2013). After attaining the third leaf stage, the seedlings were hardened and field-planted for further evaluation and multiplication. After nine months, the side suckers were collected from each progeny and planted in a new field for characterization and evaluation. These progenies (P) were evaluated for biotic resistance, parthenocarpy, fruit filling, polleniferous nature and yield-related parameters.

Evaluation of progenies for polleniferous nature

Freshly opened male flowers of improved progenies were collected and the pollen grains were gently squeezed and dispersed on a clean microslide. The whole anther image was taken by using a stereomicroscope (Olympus SZX7,

Table 1. Parental detail for their suitability in diploid breeding program

Name of the Parent	Source of the Parent	F/M	IC/ITC number	Fruit nature of the parent	Polleniferous nature of the parent	Important trait of the parent	Resistant reaction against pests and diseases				
							Root lesion nematode	Root knot nemagtode	Stem weevil	Sigatoka Leaf spot	Foc Race 1
cv. Rose	Indonesia	F/M	ITC 0712	P*	High	Aromatic pulp	S	HS	T	R	HR
Calcutta 4	India	M	ITC 0249	Se	High	Female fertile	R	HS	T	MR	HR
Pisang Lilin	India	M	IC 250648	P	Moderate	Lengthy fruit	S	R	HS	R	MR
Calcutta 4 -1	India	F/M	Nil	Se	High	Female fertile	R	HS	T	MR	HR
Lairawk	India	M	IC 634298	Se	High	Female fertile	-	-	HS	R	I
Sanna Chenkadali	India	F	IC 250654	P	Moderate	Aromatic pulp	S	S	HS	MR	R
Matti	India	F/M	IC 250635	P	Moderate	Tasty fruits	S	S	HS	S	HR
Anaikomban	India	F/M	IC 250661	P	Moderate	Lengthy fruits	R	HS	HS	S	S
TMB2 x 1928-3	Uganda	M	ITC 1437	P	Moderate	Huge bunch tasty pulp	-	-	HS	R	MR
Namarai	India	M	IC 250638	P	Moderate	Shorter duration	S	MS	HS	S	HR

F = Female; M = Male; P = Parthenocarpy, Se = Seeded; S = Susceptible; R =Resistant; T=Tolerance; HR =Highly resistant; HS= Highly susceptible; MR =Moderate resistance; I = Immune and - = Not studied.

stereomicroscopy system, Japan). A few drops of acetocarmine (commercial stain, Himedia, India) were added and covered with the glass cover slip. After three minutes, the stained mature pollen grains were examined under a light microscope (Olympus BX50 DCI microscope, Japan) (Soares et al. 2016). Two microslides were prepared for each progeny and four different places were counted. Based on the staining, the pollen grains can be classified as viable and non-viable pollen - the spherical-shaped deep red stained and unstained pollen, respectively (Dalkilic and Mestav 2011).

Evaluation of progenies for pest and disease resistance

The level of biotic stress resistance of progenies was assessed based on the field screening for eumusae leaf spot, banana stem weevil; pot culture screening for *Fusarium oxysporum* f. sp. *cubense* (Foc) race 1, root-lesion (*Pratylenchuscoffeae*) (Collingborn and Gowen 1997), root-knot nematode (*Meloidogyne incognita*) (Speijer et al. 1997) and screening at the hot spot for Foc race 1. Field screening of progenies was carried out for their resistant reaction against banana stem weevil, *Odoiporus longicollis* as per the methodology described (Padmanaban et al. 2020).

Pot culture screening against nematodes and Foc race 1

Five plants per progeny were screened under pot culture against nematodes and Foc race 1 independently. Healthy suckers of uniform size were pared, soaked in Azadirachtin 1.5% (1.5 mL/1 L water) for 30 minutes, and planted in pots containing sterilized soil, sand and vermicompost (1:1:1) mixture. One month after planting, second-stage juveniles of root-knot nematodes were inoculated @ 5000 nematodes per plant. Six months

after inoculation, the plants were carefully up-rooted and washed to remove soil free of soil. Host plant reaction was assessed by using root-knot galling index followed (Speijer and De Waele 1997) where 0 = No galling; 1 = trace infections with a few small galls; 2 = < 25% roots galled; 3 = 25 to 50% roots galled; 4 = 50 to 75% roots galled; 5 = > 75% roots galled. Similarly, for root-lesion nematode screening, one-month-old pot cultured plants were inoculated with mixed life stages of root-lesion nematodes @ 1000 nematodes per plant. Three months after inoculation, host plant reaction was assessed using reproduction ratio (Rr) = Final root nematode population (Pf) / Initial nematode inoculums (Pi), where Rr< 1 – Resistant; Rr> 1 – Susceptible (Collingborn and Gowen 1997).

True stem (corm) of the Foc race 1 infected susceptible cultivar, Grand Naine were collected from Foc race 1 hot spot area and the pathogen was isolated using a 25% strength potato dextrose agar (PDA) medium. The single spore culture of Foc race 1 was multiplied in sand and maize (19:1) medium used for pot culture evaluation. One-month-old plants were inoculated with 30g of sand maize mixed Foc race 1 inoculum containing approximately 10^6 cfu g^{-1}. Five months after inoculation the disease score was taken through a destructive method based on the percentage of discolored area in the corm on a 0-5 scale, where 0 = corm completely clean, no vascular discoloration; 1 = 1 to 5%, 2 = 6 to 25%, 3 = 26 to 50%, 4 = 51 to 75% and 5 = over 75% discoloured corm (Zuo et al. 2018).

Screening of progenies under Foc race 1 hot spot area

All the progenies were planted in five replications in a randomized block design (RBD) at the Foc race 1 hot spot area located at Muthalapuram, Theni District of Tamil Nadu, India. At the time of harvesting of the main and ratoon crops, the pathogen disease index (PDI) score was recorded based on the destructive sampling procedure described by (Zuo et al. 2018). The per cent incidence was recorded and the data was analyzed statistically.

Evaluation of progenies for yield parameters

The polleniferous diploid progenies alone were evaluated for their fruit characters in RBD in five replications with three plants per replicaion under the main and ratoon crops at ICAR-NRCB, Trichy. Data on biometric traits were recorded on both the crops for each progeny and subjected to statistical analysis. Significant differences were further subjected to Duncan's new multiple range test (DMR) as per Fonseca and Patterson (1968). The heterotic effects of progenies were estimated by calculating the percent increase or decrease of progenies over mid-parent and standard parent (Pisang Lilin) by following the formulas given below.

Heterobeltiosis = (F1-BP) /BP) *100

Standard heterosis = (F1-SP)/SP) *100

Where, F1 = Progeny; BP = Better parent; SP = Standard parent (Pisang Lilin was used as a standard parent).

Confirming the genetic diversity of diploid progenies

Genomic DNA was extracted from young leaves (cigar) of nine improved progenies, namely, P 8, P 15, P 97, P 134, P 148, P 207, P 427, P 428 and P 429 and their parents, viz., Sanna Chenkadali, Lairawk, Calcutta 4-1, Matti, Anaikomban, cv.Rose and Pisang Lilin as per the method described by Gawel and Jarnet (1991) with minor modifications. The quantity and quality were analyzed by nanodrop (Colibri Micro volume Spectrometer-Titertek/Berthol). DNA stock was further diluted and used for molecular analysis. The *in silico* polymorphic simple sequence repeats (SSR) markers (55 genes) were selected based on the stress-responsive genes containing the repeats of >20 nucleotides from the Musatrans SSRdb (http://bioinfnrcb.byethost7.com/nrcbbio/) available in the public domain (Backiyarani et al. 2019). The PCR amplification was performed in a thermocycler programmed with an initial denaturation at 95°C for 10 minutes, denaturation at 95°C for 30 secondss annealing at (55–60°C) for 30 secs, extension at 75°C for 45 seconds and final extension at 75°C for 10 minutes. The amplified products were subjected to agarose (3.5%) gel electrophoresis. PCR-amplified products of an individual genotype were treated as co-dominant markers (presence versus absence of an allele) as either present (1) or absent (0). The amplified products of each primer pair were scored for all the progenies and the data was entered in a binary form and analyzed using DARwin version 6 software (Perrier and Jacquemoud-Collet 2006). The dendrogram was constructed by cluster analysis with an unweighted pair grouping method based on arithmetic averages (UPGMA) and bootstrap analysis using the same software.

Results

Twenty two cross combinations were made using ten diploid accessions and seed set was observed in all the combinations. The progenies could be obtained from only 16 cross combinations with meager germination/regeneration efficiency (Table 2). Cultivar Matti as a female in different cross combinations resulted in the establishment of 10 progenies. Calcutta 4-1 as a female in combination with 6 different males resulted in the establishment of 14 progenies. A total of eight Anaikomban-based progenies were established using four male parents. Cv. Rose is a universal Foc resistant accession was crossed with six different male parents and 11 progenies were obtained. Sanna Chenkadali was crossed with two seeded male parents (Calcutta 4 x Lairawk) and three parthenocarpic parents (Anaikomban, Matti and Namarai), but only two progenies arising from Sanna Chenkadali x Lairawk were established. Out of 44 progenies resulting from various

Table 2. Potential of cross combinations on production of polleniferous progenies with resistance

Female parent	Male parent	Seed set	Germination	Field survival	No. of progenies	Progenies with polleniferous nature and resistance
Calcutta 4 -1	TMB2 x 1928-3	+	+	+	1	0
	Matti	+	+	+	4	1
	cv. Rose	+	+	+	4	0
	Lairawk	+	-	-	4	1
	Calcutta 4	+	+	+	1	0
Anaikomban	Lairawk	+	+	+	2	0
	Namarai	+	+	+	1	0
	Matti	+	+	+	2	1
	cv. Rose	+	+	+	3	0
Matti	cv. Rose	+	+	+	6	1
	Anaikomban	+	+	+	3	1
	Calcutta 4 -1	+	+	+	1	0
	Pisang Lilin	+	+	-	0	0
	Lairawk	+	-	-	0	0
Sanna chenkadali	Anaikomban	+	+	-	0	0
	Matti	+	-	-	0	0
	Namarai	+	-	-	0	0
	Calcutta 4-1	+	+	-	0	0
	Lairawk	+	+	+	2	1
cv.Rose	Lairawk	+	+	+	1	0
	Pisang Lilin	+	+	+	3	3
	Calcutta 4	+	+	+	7	0

+ and - Indicates the presence and absence, respectively.

diploid x diploid cross combinations, only nine progenies were polleniferous and could be used as the male parent in a further cycle of hybridization. Only one progeny, P 207 from Matti x cv. Rose combination and three progenies of cv. Rose x Pisang Lilin (P 427, P 428 and P 429); two Calcutta 4-1 based progenies (P 97 of Calcutta 4-1 x Matti; P 148 of Calcutta 4-1 x Lairawk); P 15 of Sanna Chenkadali x Lairawk; P 8 of Matti x Anaikomban and P 134 of Anaikomban x Matti were found to be polleniferous. Of these, except P 148 and P 207, all other progenies were parthenocarpic in nature. Examination of the pollen viability with acetocarmine revealed that all the progenies recorded 100% pollen viability, which confirmed that these progenies could be used as male parents in a biotic resistance breeding program.

Screening against Foc Race 1 under the hot spot area and pot culture (Table 3) revealed that among the polleniferous progenies, P 97, P 207, P 427, P 428 and P 429 showed resistance. P 134 showed resistance to *M. incognita* under pot culture screening, while P 148 showed moderate resistance against *M. incognita*. P 207 showed tolerant reaction against the stem weevil and except for P 134 and P 207 all other progenies showed a resistant reaction to eumusae leaf spot under field screening.

Analysis of the progenies for biometric traits revealed that P 15 recorded the highest value for all the traits (Table 4). Though smaller values were observed for the traits, fruits and hands per bunch in P 429, it recorded the highest bunch weight. A maximum number of hands were observed in the P 148 whereas it recorded the least bunch weight. All the progenies had positive heterbeltiosis for the trait plant height, except for cv. Rose x Pisang Lilin derived progenies and P 8, all others showed positive standard heterosis for the trait duration. Though four progenies (P15, P427, P428 and P429) recorded positive heterobeltiosis for the bunch weight, negative standard heterosis was observed for all the progenies. Similarly, P15 and P148 recorded positive heterobeltiosis and standard heterosis for the traits hands per bunch and number of fruits per bunch (Table 5).

Table 3. Reaction of banana diploid progenies for various biotic stresses under pot culture and hot spot area

Progeny	Female parent	Male parent	Fruit nature of the progeny	Pollen viability (%)	Hot spot screening			Pot culture screening		
					Eumusae leaf spot	Stem weevil	Foc race 1	Root-Knote nematode	Root lesion neamtode	Foc race 1
P 15	Sanna Chenkadali	Lairawk	Seldom seed set	100	*R	HS	MR	*S*	*S*	MR
P 97	Calcutta 4 -1	Matti	Parthenocarpy	100	R	S	R	S1	S	R
P 134	Anaikomban	Matti	No seed	100	S	HS	S	R	S	S
P 148	Calcutta 4 -1	Lairawk	Seeded	100	R	S	S	MR	S	S
P 207	Matti	cv. Rose	Seeded	100	S	HS	R	S	S	R
P 429	cv. Rose	Pisang Lilin	Parthenocarpy	100	R	T	R	S	S	R
P 427	cv. Rose	Pisang Lilin	Parthenocarpy	100	R	T	R	S	S	R
P 428	cv. Rose	Pisang Lilin	Parthenocarpy	100	R	T	R	S	S	R
P 8	Matti	Anaikomban	No seed/No pulp	100	S	S	R	HS	S	R

S = Susceptible; R = Resistant; T = Tolerant; HR = Highly resistant; HS= Highly susceptible; MR = Moderately resistant

Table 4. Mean values of different biometric traits of banana diploid progenies

Progeny	Height (cm)	Girth (cm)	Duration (Days)	Bunch weight (Kg)	Hands/bunch	Fruits /hand	Total No. of fruits / bunch
P 15	345 ^{a}	66 ^{a}	406 ^{a}	6.7 ^{a}	8.0 $^{a\,b}$	14.7 $^{a\,b}$	119 $^{a\,b}$
P 97	236 ^{cd}	64 ^{a}	397 ^{a}	3.6 $^{c\,d}$	5.3 ^{c}	15.0 ^{a}	84 ^{c}
P 115	239 ^{c}	60 ^{a}	346 ^{b}	4.8 ^{b}	6.7 $^{b\,c}$	15.3 ^{a}	104 $^{a\,bc}$
P 134	275 ^{b}	50 ^{b}	314 ^{c}	2.8 ^{de}	6.0 ^{c}	14.3 ^{ab}	81 ^{c}
P 148	258 ^{b}	51 ^{b}	348 ^{b}	2.2e	8.3 ^{a}	16.0 ^{a}	138 ^{a}
P 207	227 ^{cd}	45 ^{bc}	308 ^{c}	2.5 ^{de}	6.0 ^{c}	14.7 $^{a\,b}$	86 ^{c}
P 429	219 ^{de}	44 ^{bc}	256 ^{d}	6.8 ^{a}	6.3 ^{c}	15.0 ^{a}	88 ^{c}
P 428	191 ^{f}	41 ^{c}	259 ^{d}	5.3 ^{b}	6.0 ^{c}	12.3 ^{b}	73 ^{c}
P 427	204 ^{ef}	41 ^{c}	266 ^{d}	4.3 ^{bc}	6.0 ^{c}	12.3 ^{b}	71 ^{c}
P 8	235 ^{cd}	53.6 ^{b}	270.0 ^{d}	2.3 ^{e}	4.0 ^{d}	10.3 ^{c}	44.3 ^{d}

The same letters show no difference among means in each column

Molecular analysis of the improved progenies based on SSR marker

To confirm the genetic relationship of nine improved progenies, a phylogeny tree was constructed along with their respective parents using 55 *in-silico* polymorphic SSR markers located in resistant/defense-related genes. Overall, the phylogenetic tree was divided into four major groups (Fig. 1). The P427, P428 and P429 clustered together with their parent cv. Rose and Pisang Lilin of which P428 and P429 were found to be closer. Similarly, the P15 grouped with their parents

Sanna Chenkadali and Lairawk. P8 and P135 clustered together as they had common parents through direct and reciprocal crosses of Anaikomban and Matti. Progenies 148 and 97 were in a single cluster as they had Calcutta 4-1 as the female parent. The progeny 207 was the out-group because of the low genetic relationship with the parents, Matti and cv. Rose. It was observed that out of 55 SSR markers, the maximum male parental alleles were introgressed in the progenies 427 (18), 428 (17) and 429 (13), and the least number of male parental alleles was introgressed in the progeny 148 (6). Out of 55 genes, male parental alleles of four genes, namely NRSIP 6, 23, 70 and SRISP 9 were not introgressed in all the progenies.

Discussion

Improvement of bananas through the conventional breeding approach is highly challenging because of less

Table 5. Estimation of percent heterobeltiosis (HB) and standard heterosis (SH) for different biometric traits in banana diploid progenies

Name of the progeny	Height		girth		Duration		Bunch weight		Hands/bunch		Fruits/hand		Fruits/bunch	
	HB	SH	HB	SH	HB	SH	HB	SH	HB	SH	HB	SH	HB	SH
P15	36.7	60.5	12.1	0.0	22.4	36.1	1.3	-7.1	14.3	14.3	-7.7	-12.5	11.7	0.0
P97	4.7	9.6	17.0	-5.4	24.2	31.6	-16.1	-49.0	-3.0	-23.8	-3.2	-6.3	11.6	-27.2
P134	6.7	28.1	-21.3	-26.5	-6.3	4.0	-62.2	-59.5	-25.0	-14.3	-7.5	-10.4	-36.2	-29.9
P148	6.1	19.8	-14.7	-66.0	6.1	15.1	-62.0	-69.0	26.3	19.0	6.7	0.0	44.0	19.7
P207	6.1	5.7	-28.0	-33.3	-5.6	1.9	-61.5	-64.3	-20.0	-14.3	1.1	-8.3	-24.4	-25.5
P429	2.6	1.9	-34.3	-35.8	-15.8	-15.3	5.1	-2.4	-15.6	-9.5	0.0	-6.3	-26.4	-23.2
P427	2.6	11.2	-34.3	-39.2	-15.8	-14.1	5.1	-23.8	-15.6	-14.3	0.0	-22.9	-26.4	-3 6.8
P428	2.5	-5.0	-34.3	-39.7	-15.8	-12.0	5.1	-38.1	-15.6	-14.3	0.0	-22.9	-26.4	-38.6
P8	-9.8	8.5	-18.5	-26.9	-24.1	-11.9	-60.2	-69.0	-100.0	-75.0	-50.5	-55.3	-87.5	9.1

genetic diversity owing to imbalanced gamete formation, parthenocarpy, poor male and female fertility, seed set and germination. But the presence of residual female fertility and the polleniferous nature of some genomic groups has facilitated the banana breeders in developing many varieties (BRS 1, BRS 2, Co-1, Co-2 and Kaveri Kanchan) in India and other countries (FHIA 1, FHIA 21, NARITA 5, NARITA 8, *etc*.) through hybridization across various ploidy levels (2x, 3x and 4x) (Menon 2016). The inheritance of important traits like bunch weight and duration is mainly based on the diploid male parents used in the breeding program (Tenkouano et al. 1998). All said and done, introgression of biotic and abiotic stress resistance in the commercial varieties remains very cumbersome through the conventional approach owing to the narrow genetic base coupled with a lack of compatibility. This emphasizes the need for broadening the genetic base of diploid accessions of ancestor genomes to develop more compatible parents which might result in accelerating the improvement of conventional triploid varieties with desirable agronomic and consumer- acceptable fruit quality traits.

Irrespective of commercial or wild diploid accessions, seed set was observed in all the female parents, which revealed the presence of residual female fertility in parthenocarpic diploid landraces chosen for this study. This might be derived from their seeded ancestors since parthenocarpic accessions had proximity to the wild accessions (Durai et al. 2012). In the present study, Matti, Anaikomban, cv. Rose, Calcutta 4-1, Lairawk and Namarai were used as male parents as they are highly pollen fertile, resistant to various biotic stresses and have better fruit qualities.

The progenies established under field conditions revealed that next to the seeded accession Calcutta 4-1, the parthenocarpic accessions cv. Rose, Anaikomban and Matti were found to be the best compatible parents for developing progenies under controlled pollination. These results are in concordance with the earlier findings with respect to cultering of Matti, cv. Rose and Anaikombanas under one sub-cluster and it is revealed that they have the same ancestor/s (Sathiamoorthy and Balamohan 1993). All the cv. Rose x Pisang Lilin progenies showed resistance to Foc race 1 and were tolerant to banana stem weevil, which suggested that this trait might be inherited from the female parent, whereas the P207 of the Matti x cv. Rose cross showed a susceptible reaction to stem weevil. This speculated that a single dominant gene might not govern the stem weevil resistance and/or resistance might be to some extent due to maternal inheritance. It is interesting to note that P207 was seeded though both parents were parthenocarpic in nature, which might be due to the lack of any one/two or all three dominant independent complementary genes (Simmonds 1953). This could be confirmed by evaluating the progenies obtained through selfing (intermating of the same progeny).

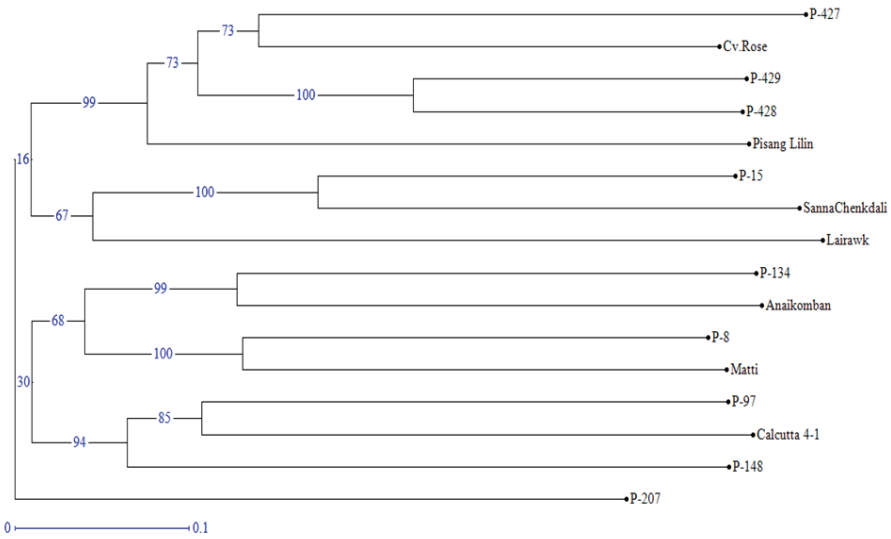

Fig. 1. Genetic relationship of diploid parents and their progenies using SSR markers with respect to defense/resistance related genes. The Neighbor-joining method of phylogenetic tree has showing the genetic relationship of between the progenies and their respective parents

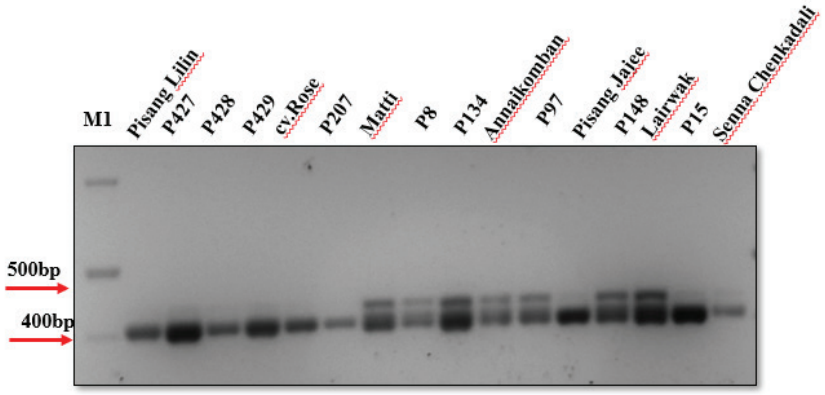

Fig. 2. Molecular screening of improved progenies for Foc race 1 resistant and susceptible with (RGA2) SSR marker. Lane: M1:1Kb plus ladder

Anaikomban is a resistant indigenous diploid source of *P. coffeae* (Damodaran et al. 2009) but its poor pollen output hinders its usage in banana breeding programme. By improving Anaikomban (diploid) for pollen fertility, resistance could be exploited for developing nematode-resistant varieties. The progeny of the direct cross of Anaikomban x Matti showed susceptible reactions to Foc race 1, while its reciprocal cross, P8 (Matti x Anaikomban) showed a resistant reaction, which indicates possibilities for the presence of a heterozygous allele for the Foc race 1 resistance gene/s or resistance being influenced by maternal inheritance. As the resistance for Foc race 1 in *Musa* spp. is governed by a single recessive gene (Ssali et al. 2013), it can be speculated that the resistant parent Matti, might have a resistance gene, whereas Anaikomban might be in the heterozygous state. It is also presumed that the resistance, it might be controlled by at least two dominant genes with epistatic interactions (Arinaitwe et al. 2019) which can be confirmed by an in-depth study on inheritance of resistance in Matti by developing a mapping population. The parthenocarpic fruits of the P134 are long in size as the female parent has only 4-5 hands instead of 7 in the female parent.

Interestingly, a triploid progeny P9 of Matti x Anaikomban produced an average of 18 kg bunch weight, which was higher than that of parents but was susceptible to both Foc race 1 and *P. coffeae*. The formation of unreduced gametes in *Musa* accessions leads to the development of parthenocarpic triploid progenies through unilateral and bilateral polyploidization (Ortiz 1997). Since this progeny was influenced by the male parent for fruit traits along with other vegetative traits resembling its female parent, the occurrence of unreduced gametes from either the male or female could not be predicted. Interestingly, P 13 of

Anaikomban x Calcutta 4-1 was found to be parthenocarpic tetraploid with no pollen output and it can be used in the tertraploid x diploid breeding programme.

Considering the seeded nature of Calcutta 4-1, artificial pollination resulted in the development of a plethora of seeds when crossed with both wild and parthenocarpic male parents. Despite the seed set, the establishment of progenies was very poor. Although, all the progenies had good pollen fertility, none of the progenies had good economic traits, especially for pulp formation. A similar kind of poor pulp formation was observed in another seeded wild type *M. acuminata* ssp. *malaccensis* progenies, when it was used as a male parent indicating the major effect of quantative inheritance. Hence, the genomic prediction model could be used to discriminate the poor fruit filling (Nyine et al. 2017, 2018). In addition, the differential expression of genes involved in fruit pulp formation causes variation in the source-to-sink capacity resulting in poor pulp filling (Nyine et al. 2019). In contradiction, in the present study P97 (Calcutta 4-1 x Matti) recorded no pulp formation and seed set for the first eight years of vegetative multiplication, however, later it developed parthenocarpic fruits and was expressed in successive multiplications. Yet another interesting case is P 148 (Calcutta 4-1 x Lairawk), where no seed set was observed in the initial years of multiplication but recorded seed set consistently in the successive cycles. The above phenomenon could be related to somatic mutation which led to genetic variation that contributes to adaptive evolution (Whitham and Slobodchikoff 1981). Thus, it is speculated that the prevalence of the somatic mutation in vegetatively propagated crops might be the reason for either fruit filling and/or seed formation over a long period and these genetic variations can be exploited with diplontic selection, thereby accelerating the accumulation of favorable alleles in clonal lineages (McKey et al. 2010). The present study revealed that Calcutta 4 -1 may not be the best female diploid parent for developing improved diploids with good fruit qualities. But owing to its other heritable traits like the good number of hands and fruits per hand along with the field resistance to eumusae leaf spot, female and male fertility, the P148 (Calcutta 4-1 x Lairawk, moderately resistant to root-knot nematode) and the parthenocarpic P 97 of Calcutta 4-1 x Matti (Foc resistant) are being used as both female and male parents in resistance breeding programme.

The aromatic parthenocarpic diploid ITC accession cv. Rose is mostly used in breeding programme at the global level because it is highly resistant to Foc race 1. The use of this diploid for the creation of potentially diverse diploids may result in broadening the resistance through the pyramiding of resistance genes for more pathogens/pests. Pisang Lilin and Calcutta 4 are the best potential sources of resistance to eumusae leaf spot; using Pisang Lilin, two leaf spot-resistant hybrids (BRS 1 and BRS 2) were developed using Pisang Lilin as the male parent. In the present study, all three progenies of cv. Rose x Pisang Lilin cross (P 427, P 428 and P 429) exhibited resistance to eumusae leaf spot, and fusarium wilt disease and also showed tolerance to banana stem weevil. Apart from being resistant to multiple biotic stresses, these hybrids were parthenocarpic and recorded high pollen fertility, which is highly encouraging to use these improved diploids as the multiple resistant male sources.

Among the crosses made in the Sanna Chenkadali, only two progenies were developed with the Lairawk as the male parent. Though fruit development is poor in both the progenies, P 15 showed a resistant reaction to leaf spot disease and was moderately resistant to fusarium wilt. Owing to its male and female fertile nature, it is also being used in breeding programs to improve biotic resistance. This progeny also recorded higher values for all biometric traits taken for this study and significant differences except for the traits' duration and hands per bunch.

Though P 148 recorded maximum hands per bunch, fruits per hand and fruits per bunch, it recorded very low bunch weight as they have less pulp with fewer seeds. Similarly, a significant difference was observed between the direct and reciprocal progenies (P 134 and P 8) of Matti and Anaikomban for the fruit traits, namely, hands per bunch and fruits per hand. Progeny 134 recorded good bunch traits than P8 which implies that these traits might be inherited from the female parent. Poor bunch weight is due to low expression of genes involved in floral cell division and differentiation and hypothesized that few QTLs govern fruit filling with major effects (Nyine et al. 2019). Overall, it was observed that apart from biotic resistance, these progenies are highly different from their respective parents for yield associated traits. Thus, yield could be improved through the gradual accumulation of favorable alleles associated with yield related traits (Ortiz 1997). Other deleterious alleles present in these highly heterozygous diploid progenies could be eliminated through recurrent selection from inter-mating among these diploid progenies.

Among the progenies, four progenies (P 15, P 427, P 428 and P 429) recorded positive heterobeltiosis (5.1) for bunch weight. The highest heterobeltiosis was recorded in the secondary triploid for bunch weight than that of the primary tetraploid and their parents using the diploid male parent (Batte et al. 2020). Hence, it is presumed that the possibility of increasing bunch weight in the secondary triploid will be more when these progenies are utilized as male parents in the tetraploid breeding programme.

Knowledge on the genetic diversity of these improved diploids will facilitate to choice of highly diverse improved diploids for further exploitation in the breeding programme. In the classificatory analysis based on SSR markers, P 427 formed a cluster with its female parent, whereas progenies

P 428 and P 429 of the same cross showed high similarity among themselves. In general, the progenies of all cross combinations showed high similarity with their respective female parents except for the P 207. It was observed that the direct and reciprocal progenies of Matti and Anaikomban crosses, namely P 8 and P 134, respectively are grouped in a single clade, which might be due to having the same parents. Overall, the clustering pattern showed that the improved diploids used in this study are highly diverse among themselves, including the improved diploids sharing common parents. This revealed that the genetic base of the diploid accessions has been broadened with resistance to various biotic stresses and they are valuable resources to the banana breeders in choosing the best compatible male parents for the improvement of banana landraces through conventional breeding approaches.

To study the contribution of male parental alleles of resistance/defense-related genes with respect to resistant reactions, the allele frequency of the progenies has been analyzed. It was observed that in progenies 134 and 8, direct and reciprocal crosses of Matti and Anaikomban, the resistant reaction to various stresses remained the same as that of the respective female parents. This revealed that the influence of male parent alleles on the resistant reaction is very meager in both crosses. Similarly, all three progenies of cv. Rose x Pisang Lilin cross showed similar kinds of resistant reactions for various biotic stresses except for *P. coffeae* and *M. incognita* as recorded in the female parent. Pisang Lilin, the male parent showed resistance reaction to *M. incognita* but was not inherited in the progenies. Irrespective of the presence of the highest number of male parental alleles in the progenies, P 428 (cross combinations; 3 4.5%), P 427 (cross combinations; 32.7%), P 429 (cross combinations; 27.2%); P8 (cross combinations; 29%) and P 134 (cross combinations; 23.6%), the non-inheritance of the resistance trait of the respective male parents were noticed. It is presumed that the resistance might be governed by maternal inheritance. Screening of a large number of segregating populations and/or in the reciprocal crosses or the male parental alleles that were integrated into the progenies, which might not have a major role for the respective stress resistance. With respect to fertility or parthenocarpy nature, no such paternal or maternal inheritance pattern was observed for either seediness or parthenocarpy nature.

In bananas, polygenic inheritance of resistance was reported for many biotic stresses, namely Foc race 1 (Li et al. 2013), TR 4 (Rowe 1991), *Radopholus similis* (Damodaran 2004) and Yellow Sigatoka (Rowe 1984). Being a vegetatively propagated polyploid crop, the introgression of polygenes in a new genetic background is cumbersome. The phenotype of a polyploidy crop is the result of multiple allelic combinations (dosages) at the heterozygous loci (Bourke et al. 2018). Thus, improved diploids with the accumulation of alleles of resistantce defense-related genes of different resistant sources developed in this study which may be a potential source to develop resistance through the stacking of desirable alleles in the commercial polyploidy banana cultivars.

Among the resistance/defense-related genes, the male parental allele of the peroxidase gene was introgressed into all progenies except for P 97 and P 148. Peroxidase plays a specific role in hypersensitive reactions against pathogens, which also involved in the lignification process. Enhanced peroxidase activity is usually associated with later stages of the infection process and the generation of hydrogen peroxides, which inhibit pathogens (Hammerschmidt et al. 1982). Higher accumulation of peroxidase in Foc resistance banana hybrids through induction of new isoforms was observed in resistant when compared with the susceptible cultivar upon Foc inoculation (Kavino et al. 2008). Similarly, male parent alleles of bHLH, MYB transcription factors and Leucine-rich repeat receptor-like kinase protein were introgressed in six progenies (P 427, P 428, P 429, P 207, P 134 and P 97). Interestingly one SSR primer belongs to the Leucine-rich repeat receptor-like kinase protein, belongs to RGA family, showed a clear difference between resistant and susceptible progenies of Foc race 1 (Fig. 2) and this could be used as the marker for early screening of the progenies for Foc race 1 resistance. Caplan et al. (2008), reported that this kind of RGAs recognizes effector proteins of the pathogen and triggers the expression of induced defence-related genes. Backiyarani et al. (2013) identified two RGAs that are closely linked to root lesion nematode resistance in banana.

The role of receptor-like kinases, transcription factors, secondary metabolites, and plant hormone-related genes in various biotic stress resistance mechanisms against Foc race 1, TR4 (Dong et al. 2020) and nematode (Backiyarani et al. 2013) has been reported which speculated that the introgression of alleles from resistant male parents might have contributed to resistance in progenies. In a nutshell, three progenies of cv. Rose and Pisang Lilin diploid parents recorded multiple resistances to various biotic stresses with desirable bunch weight and could be used in the improvement of commercial varieties. It is also suggested that the other diploid progenies could be used in (i) diploid x diploid breeding program as it is hypothesized that the heterosis or hybrid vigor will be more in the progenies obtained by hybridizing among the highly diverse improved diploid (Groose et al. 1989) (ii) in triploid x diploid or tetraploid x diploid breeding program as either the genomic dosage (Yao et al. 2013) or genome dominance (Thomas et al. 2006) and/or both play a role in heterosis. This implies that the polleniferous resistant improved diploid progenies developed in this study broaden the diploid diversity and could be an important source for accelerating the banana resistance breeding program.

Authorts' contributions

Conceptualization of research (SB); Designing of the experiments (SB); Contribution of experimental materials (SB, SU); Execution of field/lab experiments and data collection (SB, PD, RT, BP, CA, PG, SEP, MSS); Analysis of data and interpretation (SB, DKA); Preparation of the manuscript (SB, DKA, SS).

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