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Inter-varietal hybridization in black Pepper (*Piper nigrum* L.)

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Abstract

Inter-varietal hybridization in black pepper (*Piper nigrum* L.) was carried out using var. Panniyur-1 as female parent and four cultivars of black pepper viz., Ademane pepper, Chomala, Keregadde Malligesara and Kurimale as pollen donors to get the hybrids with better quality characters like the tolerance / resistance to *Phytophthora* foot rot disease and anthracnose and also to get quality berries. The results of the initial study revealed that the cross combination of var. Panniyur-1 and cv. Keregadde Malligesara recorded better plant height, more number of leaves, leaf length, leaf breadth, internodal length and number of branches. The morphological characters in hybrid plants also differed accordingly.

Keywords: Black pepper, inter-varietal hybridization, var. panniyur-1, Ademane pepper, Chomala, Keregadde Malligesara, Kurimale

Introduction

Black pepper (*Piper nigrum*) is the perennial climber belonging to the family Piperaceae from which the black pepper of commerce is obtained. Black pepper is obtained after drying and used as spice. It is a major constituent for seasoning of culinary products. Dried and ground, dried black pepper were used since antiquity for enhancing flavour and also for preparation of traditional medicines. Its one of the world's most traded spices known for its distinctive flavour.

India is the leading producer of black pepper in the world but the productivity is relatively low due to numerous factors such as inadequate fertilizers, fluctuations in rain fall, various pests and diseases and other biotic and abiotic factors. The attack from pests and diseases are controlled using chemicals measures, however the demand from the importing countries for the clean spice, free from pesticide residues is on the rise. Therefore, there arisen the need for breeding of resistant varieties for biotic stresses which pave the way for lesser use of chemicals in crop production. In this context in order to get rid off *Phytophthora* foot rot disease and to avoid anthracnose and to get quality berries, four different cultivars were used as male parents and crossed with var. Panniyur-1.

Material and Methods

Four cultivars of black pepper viz., Ademane pepper, Chomala, Keregadde Malligesara and Kurimale were taken as pollen donors and variety Panniyur-1 was considered as female parent.

Table 1: The details of characters of each cultivar is as mentioned below

Sl. No.	Cultivar	Significant characters
1.	Ademane pepper	Tolerant to <i>Phytophthora</i> foot rot disease Spike length: 12-13 cm Yields 2.21 kg vine ⁻¹ dry berries per vine
2.	Chomala	Tolerant to <i>Pollu</i> disease Spike length: 12 cm
3.	Keregadde Malligesara	Spike length: 10-11 cm Produces spikes with compact berries
4.	Kurimale	Produces long spikes (20-22 cm) Produces compact berries with more bulk density (600g/l)
5.	Var. Panniyur-1	Tolerant to <i>Phytophthora</i> foot rot disease Spike length: 12-13 cm Yields 2.21 kg vine ⁻¹ dry berries per vine

Pollen grains were collected from male parents and repeatedly brushed onto the emasculated female spikes in order to ensure fertilization. After the maturation of berries (ripe berries), they were harvested separately and soaked in water for overnight and sown separately in polybags filled with cocopeat, sand and virgin forest soil. After regular watering and other nursery operations, seedlings were transferred to plastic pots containing the nursery media. Parental lines were left for open pollination (OP) to compare the hybrids with their parents. Different morphological and growth parameters recorded (Table 2 to 5).

Results

Total of 45 spikes were taken for study in open pollinated cultivars, 45 spikes were crossed in each cultivar for comparison (Table 2).

All the spikes of open pollinated cultivars got fertilized. About 40 spikes of var. Panniyur-1, 41 spikes of cv. Ademane pepper, 40 spikes of cv. Chomala, 42 spikes each of cv. Keregadde Malligesara and cv. Kurimale reached to maturity. About 44 spikes out of 45 spikes were fertilized when var. Panniyur-1 was crossed with cv. Keregadde Malligesara and about 41 spikes reached to maturity. The increased fertilization in the open pollinated variety might be the innate ability of the cultivar to get full fertilization. In the cross pollinated spikes, failure for increased fertilization might be either due to the low combining ability between the cultivars or lower number of matured flowers at the time of pollination. Similar results are drawn by Amiteye *et al.* (2019) ^[1] in okra where only 54 crosses got success out of 144 crosses; Nair (2018) ^[7] in turmeric, where among 57 crosses, only one crossed plant got fertilized while Reddy (2015) ^[12] recorded all the 10 crossed plants reached to maturity.

The spike setting percentage in open pollinated plants and hybrids were recorded in table 4. The spike setting percentage for the cross between var. Panniyur-1 and cv. Ademane pepper (84.44%), for var. Panniyur-1 x cv. Chomala, it was 86.67 per cent, var. Panniyur-1 x cv. Keregadde Malligesara (91.11%) and for var. Panniyur-1 x cv. Kurimale, the spike setting percentage was 88.89 per cent. This might be due to the partial failure of the endosperm owing to the genetic imbalance and might be due to the abnormal development of the endosperm that leads to failure in hybrid seed set (Prabhu and Warade, 2013) ^[11]; Nalini and Moss (1995) ^[8] in *Cajanus cajan*; Kuwada (1966) ^[6] in okra; Nunekpeku *et al.* (2012) ^[9] in cassava; Reddy (2015) ^[12] in okra obtained similar results in their study.

Among the OP cultivars and hybrids obtained, the maximum number of seeds (1769) from crossing var. Panniyur-1 and cv. Keregadde Malligesara, out of which 145 seeds were germinated with the maximum germination percentage (8.19%) (Table 2). This might be due to the high pollen

fertility that might be contributed in maximizing the germination percentage. The number of days required for first and 50 per cent germination in the parental population and the hybrids produced is depicted in the table 3. The number of days required for first germination was found to be less in the cultivar Chomala (91 days) followed by the cross combination of var. Panniyur-1 x cv. Ademane pepper; var. Panniyur-1 x cv. Keregadde Malligesara (92 days each). The minimum number of days required for germination of 50 per cent of the population was recorded in the cross combination of var. Panniyur-1 x cv. Keregadde Malligesara (139 days). This might be due to the inherent character of the cv. Chomala to germinate fast and grow into a full plant. Due to the cross pollination, the genetic recombination of the cultivar might speed up the seed to germinate in quick manner (Sasikumar *et al.*, 1999) ^[14].

Growth parameters

The height of seedlings has direct relationship with the overall growth and development of the plant. The maximum plant height (46.01 cm) in the crossed plant was obtained in the hybrid plant of var. Panniyur-1 and cv. Keregadde Malligesara showing vigorous growth of the plant and recorded the maximum heterosis of 17.43 per cent over mid parent (Table 4). This might be due to the inherent genetic qualities from both the maternal and paternal parents for the particular character that caused increase in plant height of the hybrid compared to the parents. The present result was in-line with the results obtained by Ojha *et al.* (2009) ^[10] and Sharma *et al.* (2012) ^[15] in bottle gourd and Arya and Singh (2004) ^[3] in cucumber. Comparable results were also obtained by Kuwada (1966) ^[6] in okra, where the hybrid was taller compared to both the parents in reciprocal crosses also. Since very meagre information is available on the pepper crop, the reviews of other horticultural crops were taken into account.

The number of leaves had direct relationship with the height of the plant. Since the height of the plant in the hybrid produced by crossing var. Panniyur-1 and cv. Keregadde Malligesara was more *i.e.*, the hybrid plant showed the hybrid vigour having higher values than both of the parents; number of leaves were also recorded maximum (10.13) with 62.60 per cent heterosis over mid parent and 62.08 per cent heterosis over better parent. While, leaf length (9.98 cm) and leaf breadth (8.30 cm) were also recorded maximum in the same hybrid (var. Panniyur-1 x cv. Keregadde Malligesara). This might be due to the genetic basis of the parents. This is similar to the results obtained by Azimi *et al.* (2018) ^[4] which stated more the height of the bush, more will be the number of leaves and its dimensions in case of *Iris germanica*. In the present study, the hybrid with increased plant height had recorded number of leaves and leaf dimensions.

Table 2: Spike set, germination percentage in the parental population and hybrids produced in inter-varietal hybridization

Sl. No.	Variety	No. of spikes under study	No. of spikes fertilized	No. of spikes reached maturity	Spike set percentage	No. of seeds obtained	No. of seeds germinated	Germination (%)
1	OP var. Panniyur-1	45	45	40	88.89	1529	108	7.06
2	OP cv. Ademane pepper	45	45	41	91.11	1300	82	6.31
3	OP cv. Chomala	45	45	40	88.89	1382	87	6.29
4	OP cv. Keregadde Malligesara	45	45	42	93.33	1235	97	7.85
5	OP cv. Kurimale	45	45	42	93.33	1540	97	6.29
6	Var. Panniyur-1 x cv.	45	43	38	84.44	1514	97	6.41

	Ademane pepper							
7	Var. Panniyur-1 x cv. Chomala	45	41	39	86.67	1489	99	6.65
8	Var. Panniyur-1 x cv. Keregadde Malligesara	45	44	41	91.11	1769	145	8.19
9	Var. Panniyur-1 x cv. Kurimale	45	43	40	88.89	1267	97	7.66

Where, OP: Open pollinated

Table 3: Number of days for germination in inter-varietal hybrids and parents

Sl. No.	Cultivar / cross combination	No. of days for first germination	No. of days for 50% germination
1	Var. Panniyur-1	99	158
2	Ademane pepper	97	150
3	Chomala	91	141
4	Keregadde Malligesara	95	144
5	Kurimale	97	148
6	Var. Panniyur-1 x cv. Ademane pepper	92	147
7	Var. Panniyur-1 x cv. Chomala	101	153
8	Var. Panniyur-1 x cv. Keregadde Malligesara	92	139
9	Var. Panniyur-1 x cv. Kurimale	94	143
	Mean	95.33	147
	SD	3.43	6.04
	CV (%)	3.60	4.11

The internodal length was also maximum (4.92 cm) and was more than both of the parents in the hybrid showing hybrid vigour when var. Panniyur-1 and cv. Keregadde Malligesara were crossed. This was due height of the plant, the increase in the plant height was due to the increase in internodal length of the plant. This result was in accordance to the study conducted by Kuwada (1966) [6], which stated the height of F₁ hybrid was more when compared to the parental lines in okra. Kerure *et al.* (2019) [5] in okra found that, the hybrid developed had more internodal length than the parental lines, however, in contrary, the mean plant height of hybrids was lesser than the parents in okra; Reddy *et al.* (2012) [13] opined the lower internodal length and plant height for the hybrid compared to both of the parents.

In case of the hybrid developed by crossing var. Panniyur-1 and cv. Keregadde Malligesara recorded the maximum (1.30) number of branches and this showed 4 per cent heterosis for both mid parent and better parent (Table 4). This might be due to the genetic recombination in different cultivars that might enhance production of more branches. This results confirms the earlier records of the hybrids having more number of branches than both parents in okra as studied by Kerure *et al.*,

(2018) [5] and Reddy *et al.*, (2012) [13]; Ojha *et al.* (2009) [10] and Sharma *et al.* (2012) [15] in bottle gourd; Arya and Singh (2004) [3] in cucumber.

Morphological characters

Various leaf base shape like round, cordate and acute types and leaf lamina shapes like cordate, ovate-elliptic, elliptic-lanceolate types were recorded in the parental lines and the hybrid plants obtained (Table 5, Plate 1 and Plate 2). This might be due to the genetic combination of the cultivar or variety. This is similar to the result obtained by Sasikumar *et al.* (1999) [14] which confirms that the hybrid obtained by crossing *Piper nigrum* and *P. barberi P. attenuatum*, had leaf lamina which was intermediate between both parental varieties. However, an another study by same researcher stated that when *P. nigrum* and *P. attenuatum* were crossed, the hybrid obtained had the leaf lamina similar to that of paternal parent; Vanaja *et al.* (2008) [16] in one of their studies got the leaf lamina of hybrid obtained by crossing *Piper nigrum* and *P. colubrinum* had ovate-elliptic leaf base similar to that of male parent.

Table 4: Mean values and magnitude of heterosis for growth parameters for hybrid in inter-varietal hybridization

Cross combination	Height of the plant (cm)	No. of leaves	Leaf length (cm)	Leaf breadth (cm)	Internodal length (cm)	No. of branches
1. Var. Panniyur-1 x cv. Ademane pepper						
Mean value	P ₁	38.15	6.20	7.80	7.01	3.42
	P ₂	35.38	6.00	7.52	6.13	4.44
	F ₁	36.40	7.60	8.84	7.64	4.42
Heterosis%	MP	-1.01	24.60	10.18	16.29	12.46
	BP	-4.59	22.58	13.33	8.99	-0.45
2. Var. Panniyur-1 x cv. Chomala						
Mean value	P ₁	38.15	6.20	7.80	7.01	3.42
	P ₂	36.64	6.14	8.68	7.28	4.26
	F ₁	45.08	8.88	8.96	7.29	4.50
Heterosis%	MP	17.03	43.92	8.74	1.96	17.19
	BP	18.17	43.22	3.22	0.14	5.63
3. Var. Panniyur-1 x cv. Keregadde Malligesara						
Mean value	P ₁	38.15	6.20	7.80	7.01	3.42
	P ₂	40.20	6.25	8.63	7.33	4.52
	F ₁	46.01	10.13	9.98	8.30	4.92

Heterosis%	MP	17.43	62.60	21.41	15.76	23.93	4.00
	BP	14.45	62.08	15.64	13.23	8.85	4.00
4. Var. Panniyur-1 x cv. Kurimale							
Mean value	P ₁	38.15	6.20	7.80	7.01	3.42	1.25
	P ₂	32.63	4.40	6.80	5.98	3.24	1.15
	F ₁	33.38	6.02	7.32	6.86	3.54	1.07
Heterosis%	MP	-5.68	13.58	0.27	5.54	6.31	-10.83
	BP	-12.50	-2.90	-6.15	-2.14	3.51	-14.40

Where,

P₁: Parent 1,

P₂: Parent 2,

F₁: Hybrid produced by crossing P₁ and P₂

MP: Mid parent,

BP: Better parent

Both even as well as wavy type of leaf margins were recorded in both the parents and hybrid plants. Few crosses recorded both types of leaf margin. Earlier studies also recorded even and wavy types of leaf margins (Anon., 2008) [2]. However, Vanaja *et al.* (2008) [16] noticed the leaf margin in the hybrid plants obtained by crossing *Piper nigrum* and *P. colubrinum*, in which the hybrid had wavy margin similar to the male parent.

The leaf venation in all the parents and the hybrids were campylochromous type. This might be due to the genetic makeup of the variety to produce only campylochromous type of leaf venation and seems to be very dominant character. In one of the studies, it was reported that eucamptodromous type of venation observed in *Piper barberi* meanwhile, *P. nigrum*, *P. bababudani* had acrodromous type of venation; *P. hymenophyllum* had campylochromous type of venation (Anon., 2008) [2]. The leaf texture of glabrous coriaceous was found in all the parental lines. Hence, there were no variations recorded in the hybrid produced. All the putative hybrids were

with glabrous coriaceous type of leaf texture. Since, both the parents had same kind of leaf texture, similarly, the hybrids produced had leaf texture similar to that of the parents.

The shoot tip colour varied accordingly among the parents and the hybrids produced in the study. Light purple coloured shoot tip were noted in the parental types (Table 5). Some plants in the above cross combinations recorded both light green and light purple coloured shoot tips. The colour variation in shoot tip in var. Panniyur-1 x cv. Ademane pepper and var. Panniyur-1 x cv. Keregadde malligesara is due to the interaction of genes. Since, the paternal parents had light purple coloured shoot tip, the F₁ might inherited the character from the male parent and it might be due to the dominance of paternal parent for shoot tip colour in the plant. The hybrid had majority characters of maternal parent; however, the paternal parent might transfer its character to the hybrid. This was in accordance to the studies of Vanaja *et al.* (2008) [16] where they recorded light green coloured hybrid which was similar to that of female (*Piper nigrum*) and the male parent (*P. colubrinum*) that had light purple coloured shoot tip. Sasikumar *et al.* (1999) [14] concluded that the hybrid developed by crossing *P. nigrum* and *P. barberi* had purple coloured shoot tip as that of female parent. In another study, interspecific hybrid was developed between *P. nigrum* and *P. attenuatum* and the hybrid was found to have the shoot tip of purple colour similar to that of maternal parent.

Table 5: Variability in qualitative traits in parents and hybrids in inter-varietal hybridization

Sl. No.	Trait	Character	Var. P-1	Var. P-1 x cv. Ademane pepper	Cv. Ademane pepper	Var. P-1	Var. P-1 x cv. Chomala	Cv. Chomala
1	Leaf base shape	Round	-	-	-	-	✓	-
		Cordate	✓	✓	✓	✓	✓	✓
		Acute	-	-	-	-	-	-
		Oblique	-	-	-	-	-	-
2	Leaf lamina shape	Ovate	-	-	-	-	-	-
		Ovate-elliptic	-	-	-	-	✓	-
		Ovate-lanceolate	-	-	-	-	-	-
		Elliptic-lanceolate	-	-	-	-	✓	-
3	Leaf margin	Cordate	✓	✓	✓	✓	✓	✓
		Wavy (repand)	-	-	-	-	✓	-
4	Leaf venation	Acrodromous	-	-	-	-	-	-
		Campylochromous	✓	✓	✓	✓	✓	✓
		Eucamptodromous	-	-	-	-	-	-
5	Leaf texture	Glabrous coriaceous	✓	✓	✓	✓	✓	✓
		Glabrous membranous	-	-	-	-	-	-
		Glabrous sarcous	-	-	-	-	-	-
		Downy membranous	-	-	-	-	-	-
6	Shoot tip colour	Downy along the veins	-	-	-	-	-	-
		Light green	✓	✓	-	✓	✓	✓
		Dark green	-	-	-	-	-	-
		Light purple	-	✓	✓	-	-	-
		Purple	-	-	-	-	-	-

Table 5: Contd...

Sl. No.	Trait	Character	Var. P-1	Var. P-1 x cv. Keregadde Malligesara	Cv. Keregadde Malligesara	Var. P-1	Var. P-1 x cv. Kurimale	Cv. Kurimale
1	Leaf base shape	Round	-	✓	✓	-	✓	✓
		Cordate	✓	✓	-	✓	-	
		Acute	-	✓	-	-	-	
		Oblique	-	-	-	-	-	
2	Leaf lamina shape	Ovate	-	-	-	-	✓	✓
		Ovate-elliptic	-	✓	-	-	✓	-
		Ovate-lanceolate	-	✓	-	-	-	-
		Elliptic-lanceolate	-	✓	-	-	-	-
3	Leaf margin	Cordate	✓	✓	✓	✓	✓	-
		Even (entire)	✓	✓	-	✓	✓	✓
4	Leaf venation	Wavy (repand)	-	✓	✓	-	✓	-
		Acrodromous	-	-	-	-	-	-
		Campylodromous	✓	✓	✓	✓	✓	✓
5	Leaf texture	Eucamptodromous	-	-	-	-	-	-
		Glabrous coriaceous	✓	✓	✓	✓	✓	✓
		Glabrous membranous	-	-	-	-	-	-
		Glabrous sarcous	-	-	-	-	-	-
		Downy membranous	-	-	-	-	-	-
6	Shoot tip colour	Downy along the veins	-	-	-	-	-	-
		Light green	✓	✓	-	✓	✓	✓
		Dark green	-	-	-	-	-	-
		Light purple	-	✓	✓	-	-	-
		Purple	-	-	-	-	-	-

Where Var. P-1: Var. Panniyur-1

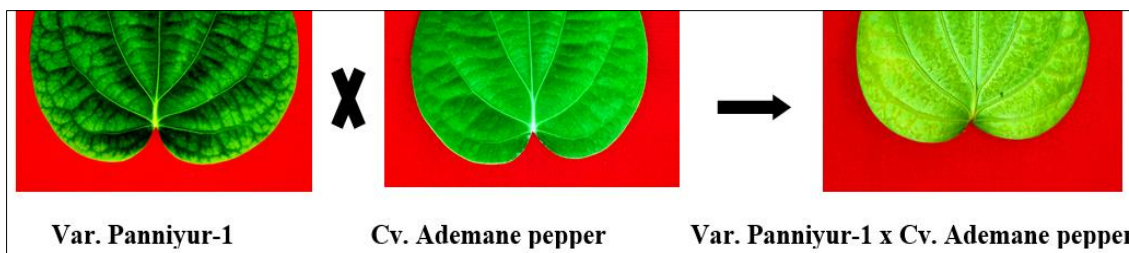


Plate 1a: Leaf base shapes of var. Panniyur-1 and cv. Ademane pepper and its hybrid after inter-varietal hybridization

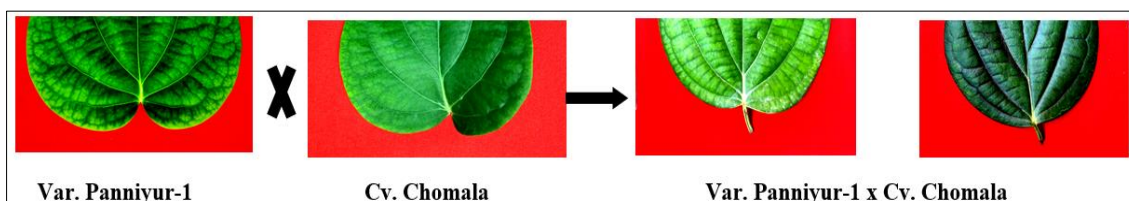


Plate 1b: Leaf base shapes of var. Panniyur-1 and cv. Chomala and its hybrid after inter-varietal hybridization

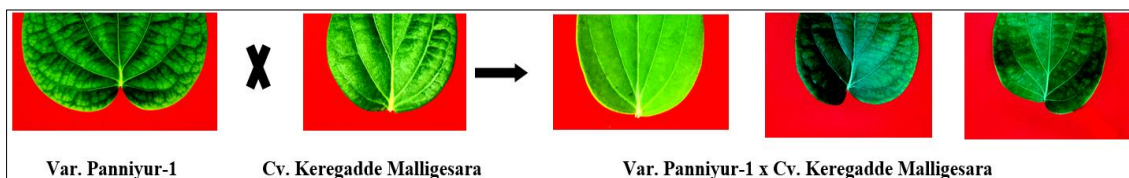


Plate 1c: Leaf base shapes of var. Panniyur-1 and cv. Keregadde Malligesara and its hybrid after inter-varietal hybridization

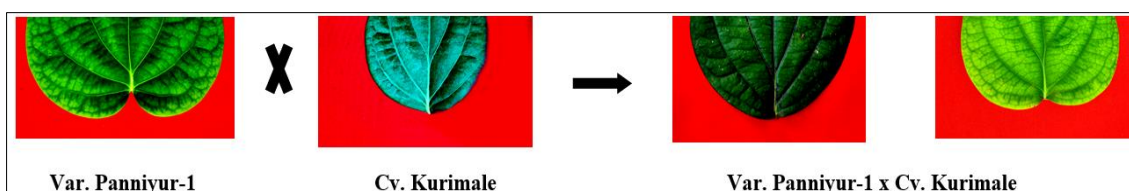


Plate 1d: Leaf base shapes of var. Panniyur-1 and cv. Kurimale and its hybrid after inter-varietal hybridization

Plate 1: Leaf base shapes of parental lines and the hybrids produced

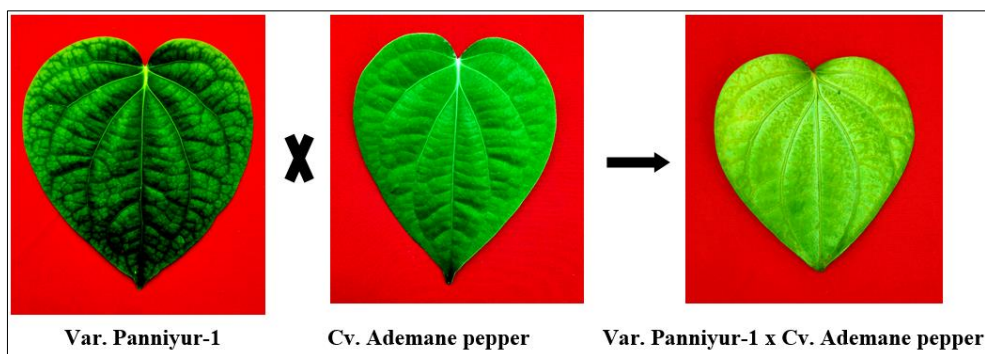


Plate 2a: Leaf lamina shapes of var. Panniyur-1 and cv. Ademane pepper and its hybrid after inter-varietal hybridization

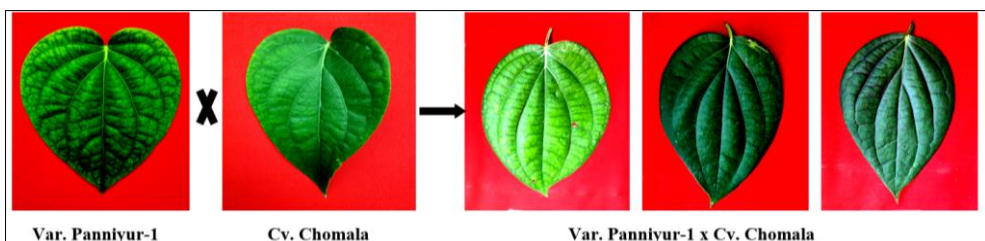


Plate 2b: Leaf lamina shapes of var. Panniyur-1 and cv. Chomala and its hybrid after inter-varietal hybridization

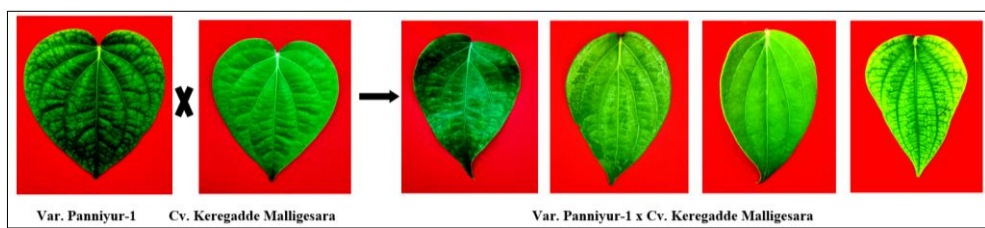


Plate 2c: Leaf lamina shapes of var. Panniyur-1 and cv. Keregadde Malligesara and its hybrid after inter-varietal hybridization

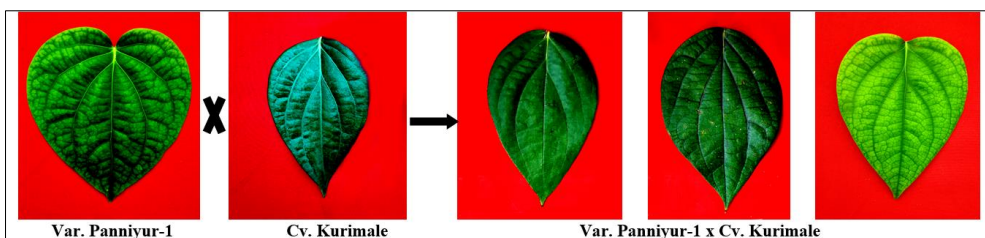


Plate 2d: Leaf lamina shapes of var. Panniyur-1 and cv. Kurimale and its hybrid after inter-varietal hybridization

Plate 2: Leaf lamina shapes of parental lines and hybrids

Conclusion

In the inter-varietal hybridization trial, among the parents or cultivars and hybrids developed by using var. Panniyur-1 as female parent and cvs. Ademane pepper, Chomala, Keregadde Malligesara and Kurimale as male parents, the least number of days for 50 per cent seed germination, maximum fruit set percentage, maximum plant (vine) height, more number of leaves, maximum leaf length, leaf breadth, internodal length and more number of branches were recorded in hybrids produced by crossing var. Panniyur-1 and cv. Keregadde Malligesara. Therefore, among the above hybrids developed, the increased length of the vine and leaf dimensions can produce better dry berry yield per vine in the hybrids produced.

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