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(54) Title: SUPERIOR, NOVEL, QUALITY MULBERRY SILKWORM HYBRIDS AND A METHOD THEREOF

(57) Abstract: The present invention relates to superior, novel Mulberry silkworm hybrids named Swarnandhra (APM_1 X APS_8), Kalpatharuvu (APS_9 X APS_8), and Hemavathy (APS_5 X APS_4), well adapted for all seasons and preferably adapted for tropical region, produced by crossing pureline inbreeds of Polyvoltine (APM) and Bivoltine (APS)and a reliable and consistent method of producing the hybrids, using steps comprising inbreeding, hybridizing, disease tolerance breeding, DNA fingerprinting, and selecting, wherein the said hybrids show high yield of international grade silk.

SUPERIOR, NOVEL, QUALITY MULBERRY SILKWORM HYBRIDS AND A METHOD THEREOF

Technical Field

The present invention relates to superior, novel Mulberry silkworm hybrids named Swarnandhra (APM_1 X APS $_8$), Kalpatharuvu (APS $_9$ X APS $_8$), and Hemavathy (APS_5 X APS_4), and a reliable and consistent method of producing the said hybrids.

Background Art

At present silk cocoon production in the southern Indian states of Andhra Pradesh, Karnataka and Tamil Nadu, which fall under tropical India, is mainly contributed by the traditional hybrid of polyvoltine (Pure Mysore) x bivoltine breeds (NB_4D_2), which accounts for more than 96% of the total silk produced in these states. The traditional hybrid known for consistent performance under tropical conditions but the silk produced is of inferior quality and silk cocoon productivity is considerably low.

As a result, silkworm rearers (sericulturists) and silk reelers get poor financial returns besides producing internationally ungraded silk. Non-availability of alternative productive polyvoltine x bivoltine hybrids has resulted in the over-exploitation of the existing traditional hybrid and hence, its performance with farmers is gradually declining.

Among several contributory factors, focused silkworm breeding effort directed towards silk yield contributory traits such as survival rate of silkworm larvae, weight of silk cocoon assume special importance in improving the silk productivity and silk quality. So the primary attempt that needs investment is to provide suitable breeds that can give consistent cocoon crops over a range of seasons and regions and record higher silk cocoon yield with the farmers.

This demand for better quality silk has created a supply demand gap of 7000 Mt, which is presently imported from China. To meet the changing demand pattern, the import may increase in the coming years. Besides, the silk exporting community prefers to import international grade silk for their products to meet the expectations of buyers abroad as the local silk do not meet the standard. It has been established that the bivoltine hybrids have the inherent character of producing superior quality international grade silk. Therefore it is essential to develop high silk yielding bivoltine strains to be used in tropical regions of India. This would herald a bivoltine era in tropical countries like India.

The quality of silkworm races is by far the most important input of sericulture technology and much effort should be devoted to the breeding of better races to meet the requirements. So the urgent need in the present scenario is to produce suitable bivoltine hybrids that can give consistent cocoon crop over a range of seasons and regions and record higher productivity and better returns to the farmers.

Further, it is possible to assign heredity unambiguously between parents and progeny, by showing that 50% of the bands in any individual are derived from a particular parent. This is the basis of the technique known as DNA fingerprinting.

In this backdrop of emerging demand/trend, we have focused our attention to develop silkworm hybrids more suited to tropical conditions. These hybrids give increased silk cocoon yield and gradable raw silk production. The breeding programme followed in the present invention includes integrated approaches of conventional selection and hybridization coupled with DNA marker technologies.

Objects of the present invention

The main object of the present invention is to develop superior silkworm hybrids of high yield.

Another object of the present invention is develop silkworm hybrids producing international grade silk.

Yet another object of the present invention is to develop silkworm hybrids producing silk for all seasons.

Still another object of the present invention is to develop silkworm hybrids preferably well adapted for tropical regions.

Still another object of the present invention is to develop silkworm hybrids producing silk with high reelability.

Still another object of the present invention is to develop silkworm hybrids producing silk with low renditta.

Still another object of the present invention is to produce silkworm hybrids with resistance towards virus.

Still object of the present invention is to develop silkworm hybrids with high survival rate.

Still another object of the present invention is to develop silkworm hybrids with reduced larval period.

Still another object of the present invention is to develop silkworm hybrids with desired heterozygosity

Further object of the present invention is to use DNA fingerprinting technology to evaluate the hybrid vigor of the silkworm hybrids.

Another object of the present invention is to use DNA fingerprinting to determine homozygosity of inbred lines.

Summary of the Invention

The present invention relates to superior, novel Mulberry silkworm hybrids named Swarnandhra (APM_1 X APS $_8$), Kalpatharuvu (APS_9 X APS_8), and Hemavathy (APS_5 X APS_4), well adapted for all seasons and preferably adapted for tropical region, produced by crossing pureline inbreeds of Polyvoltine and Bivoltine and a reliable and consistent method of producing the hybrids, using steps comprising inbreeding, hybridizing, disease tolerance breeding, DNA fingerprinting, and selecting, wherein the said hybrids show high yield of international grade silk.

Detailed Description of the present invention

Accordingly, the present invention relates to the superior novel Mulberry silkworm hybrid named Swarnandhra, well adapted for all seasons and preferably adapted for tropical region and the said hybrid is a cross of polyvoltine APM_1 derived from pureline inbreed of Madagascar hybrids and bivoltine APS_8 derived from pureline inbreed of Chinese hybrids, using steps comprising inbreeding, hybridization, disease tolerance breeding, DNA fingerprinting, and selection and is superior to conventional hybrids of the tropical regions mainly.

In an embodiment of the present invention, the said hybrid produces silk of superior quality and high yield.

In yet another embodiment of the present invention, silk has international grade of 2A-3A.

In still another embodiment of the present invention, silk has reelability ranging between 80 and 90%.

In still another embodiment of the present invention, silk has renditta ranging between 7.0 and 7.5 kg.

In still another embodiment of the present invention, silk has floss percentage less than 5%.

In still another embodiment of the present invention, silk has greenish yellow color.

In still another embodiment of the present invention, said Swarnandhra hybrid produces larvae of superior quality.

In still another embodiment of the present invention, larvae has survival rate ranging between 80 and 85%.

In still another embodiment of the present invention, larval period is ranging between 22 and 23 days.

In still another embodiment of the present invention, larvae is plain or without any visible markings.

In still another embodiment of the present invention, Swarnandhra hybrid produces cocoon of good yield and superior quality.

In still another embodiment of the present invention, cocoon has yield ranging between 50 and 55 per 100 dfls (kg).

In still another embodiment of the present invention, single silk cocoon weight ranging between 1.60 and 1.70-g.

In still another embodiment of the present invention, cocoon shell weight ranging between 0.290 and 0.320.

In still another embodiment of the present invention, percentage shell ratio ranging between 18 and 19.

Brief description of the accompanying drawings:

Fig 1 shows flow chart of methodology followed to develop silkworm hybrid Swarnandhra.

Fig 2 shows flow chart of methodology followed to develop and select pureline Chinese hybrids APS_5 and APS_9.

Fig 3 shows flow chart of methodology followed to develop and select pureline Japanese hybrids APS_4 and APS_8.

Fig. 4a. Silkworm eggs laid (400-500 in number) by a single female moth of a polyvoltine-inbred line, APM_1. The eggs are pigmented by pigment accumulation in serosal cells and are non-diapause in nature. The expression of pigments in the serosal cells in the non-diapause eggs is controlled by the gene, *pnd* located on chromosome 11. Usually non-diapause eggs are non-pigmented in nature and APM_1 is exception to this

Fig 4b. Silkworm eggs (450-550 in number) laid by a single female moth of bivoltine inbred line, APS_8. The eggs are pigmented by pigment accumulation in serosal cells and are diapause in nature.

Fig 4c. The silkworm eggs laid by a single female moth of hybrid, APM_1 x APS_8. The eggs are pigmented similar to the maternal parent.

Fig.5a. Fully-grown fifth instar silkworm larvae of polyvoltine inbred line, APM_1. The larvae are devoid of any visible markings on body.

Fig.5b. Fully-grown fifth instar silkworm larvae of bivoltine, APS_8. The larvae are devoid of any visible markings on body.

Fig.5c. Fully-grown fifth instar silkworm larvae of hybrid, APM_1 x APS_8. The larvae are devoid of any visible markings on body.

Fig.6a. Cocoons of APM_1. The cocoons are oval in shape and greenish yellow in color.

Fig.6b. Cocoons of APS_8. The cocoons are white in color and peanut in shape.

Fig.6c. Cocoons of the hybrid, APM_1 X APS_8 are greenish yellow in color similar to APM_1, with prominent oval shape and mild peanut constriction appearance. The greenish yellow cocoon color is dominant over white color in silkworm, *Bombyx mori* .

Fig.7a. Silk fibre reeled from the cocoons of APM_1.

Fig.7b. Silk fibre reeled from the cocoons of APS_8.

Fig.7c. Silk fibre reeled from the cocoons of the hybrid, APM_1 x APS_8. The yarn colors reflect the cocoon color of he maternal parent in the hybrid.

Fig. 8. DNA fingerprinting profiles of APM_1, APS_8 and APM_1 X APS_8. The fingerprints were generated using the Fluorescent Inter-Simple Sequence Repeat-anchored Polymerase Chain Reaction (FISSR-PCR). The PCR products were resolved on GENSCAN (Perkin Elmer). The PCR products specific to APM_1 and APS_8 are indicated by arrow and arrowheads, respectively. M: males (APS_8), F: females (APM_1); F_1: APM_1 x APS_8 hybrid (Swarnandhra).

Fig 9a. Shows silkworm eggs laid (450-550 in number) by a single female moth of inbred line, APS_5. The eggs are pigmented by pigment accumulation in serosal cells and are diapause in nature.

Fig 9b shows silkworm eggs laid (450-550 in number) by a single female moth of inbred line APS_4 . The eggs are pigmented by pigment accumulation in serosal cells and are diapause in nature.

Fig. 9c.shows silkworm eggs laid (450-550 in number) by a single female moth of inbred lines, APS_9. The eggs are pigmented by pigment accumulation in serosal cells and are diapause in nature.

Fig 9d shows silkworm eggs laid (450-550 in number) by a single female moth of inbred lines APS_8. The eggs are pigmented by pigment accumulation in serosal cells and are diapause in nature.

Fig. 9e shows silkworm egg (450-600 in number) laid by single female moths of hybrid, Kalpatharuvu (APS_9 x APS_8). The eggs are pigmented similar to the parents and are diapause in nature.

Fig. 9f shows silkworm egg (450-600 in number) laid by single female moths of hybrid, Hemavathy (APS_5 x APS_4). The eggs are pigmented similar to the parents and are diapause in nature.

Fig.10a. shows fully-grown fifth instar silkworm larvae of APS_5.

Fig. 10b. shows fully-grown fifth instar silkworm larvae of APS_4.

Fig. 10c. shows fully-grown fifth instar silkworm larvae of APS_9.

Fig. 10d. shows fully-grown fifth instar silkworm larvae of APS_8.

Fig. 10e. shows fully-grown fifth instar silkworm larvae of the hybrid, Hemavathy (APS_5 x APS_4).

Fig. 10f shows fully-grown fifth instar silkworm larvae of the hybrid, Kalpatharuvu (APS_9 x APS_8).

Fig. 11a shows cocoons of APS_5 are oval in shape and white in color.

Fig. 11b shows cocoons of APS_4 are peanut in shape and white in color.

Fig. 11c shows cocoons of APS_9 are oval in shape and white in color.

Fig. 11d shows cocoons of APS_8 are peanut in shape and white in color.

Fig. 11e shows hybrid cocoons of Hemavathy (APS_5 x APS_4) are oval in shape with peanut appearance.

Fig. 11f shows hybrid cocoons of Kalpatharuvu (APS_9 x APS_8) are also oval in shape with peanut appearance.

Fig.12a shows silk fibre reeled from the hybrids of Kalpatharuvu (APS_9 x APS_8). The yarn color is also white.

Fig.12b shows silk fibre reeled from the hybrids of Hemavathy (APS_5 x APS_4). The yarn color is also white.

Fig. 13 shows DNA fingerprinting profile of APS_4, APS_5, and hybrid APS_5 x APS_4. The fingerprints were generated using the Fluorescent Inter-Simple Sequence Repeat anchored Polymerase Chain Reaction (FISSR-PCR). The PCR products were resolved on GENSCAN (Perkin Elmer). The PCR products that are specific to the parental strains are indicated by arrows. M: males; F: females; F_1: hybrids.

Fig. 14 shows DNA fingerprinting profile of APS_9, APS_8 and the hybrid, APS_9 x APS_8. The fingerprints were generated using the Fluorescent Inter-Simple Sequence Repeat anchored Polymerase Chain Reaction (FISSR-PCR). The PCR products were resolved on GENSCAN (Perkin Elmer). The PCR products that are specific to the parental strains are indicated by arrows. M: males; F: females; F_1: hybrids.

The percentage shell ratio of the Swarnandhra fall within the range of 18 and 19 and these have been clearly illustrated in Table 1 which is given below.

TABLE 1 - COMPARATIVE YIELD PROFILE OF NEW CROSS-BREED,

SWARNANDHRA (APM_1XAPS_8)
AND THE TRADITIONAL HYBRID (PM X NB_4D_2)

Sl. No.	Yield Parameters	Traditional Hybrid (PM x NB_4D_2)	Swarnandhra (APM_1 x APS_8)**	Gain
1	Larval Period (days)[1]	24-25	22-23	2-3
2	Survival (%)[2]	60-70	80-85	15-20
3	Cocoon yield/100 dfls (Kg)[3]	40-42	50-55	8-15
4	Cocoon shell ratio (%)[4]	15-16	18-19	2-4
5	Filament length (m/cocoon)[5]	550-600	700-800	150-200
6	Reelability (%)[6]	70-75	80-90	10-20
7	Renditta[7]	9.0-10.0	7.0-7.5	2-2.5
8	Grade of Silk[8]	Ungraded	2A-3A	Intl. Grade

* based on 100070 disease free egg layings tested with 546 farmers of Andhra Pradesh and Karnataka

** based on 129505 disease free egg layings tested with 543 farmers of Andhra Pradesh and Karnataka

1 total larval duration from the time of larval eclosion from the eggs upto the onset of secretion of silk.

2 total weight of cocoons harvested from the total number of larvae used for rearing

3 total weight of cocoons harvested from the larvae raised from 100 disease free egg layings

4 the ratio of single cocoon shell weight to the weight of single silk cocoon

5 an average length of silk fibre recovered from a single silk cocoon

6 the recovery percentage of continuous silk fibre from cocoons

7 the number of kgs of cocoons required to produce 1 kg of raw silk

8 silk is graded based on international standards considering the quality, neatness and tenacity of silk fibre.

In still another embodiment of the present invention, filament length ranging between 700 and 800 m/cocoon.

In still another embodiment of the present invention, cocoon has oval shape with mild peanut constriction appearance.

In still another embodiment of the present invention, cocoon is greenish yellow color.

In still another embodiment of the present invention, eggs are pigmented, and are non-diapause in appearance.

In further embodiment of the present invention, five polyvoltine inbred lines viz., APM_1, APM_2, APM_3, APM_4 and APM_5, which were developed at this Institute are subjected for DNA Fingerprinting and established their homozygocity. (Please refer Table 2)

Table 2 - General Combining Ability (GCA) of the parents

Parents	Yield (by wt)	PR%	Cocoon Weight	Shell Weight	SR %	Filament Length
Lines:						
1. APM_2	0.427	1.893	-0.015	0.003	0.316	-10.400
2. APM_3	-0.145	-2.223	-0.058	-0.006	0.225	31.517
3. APM_4	-0.589	-0.723	-0.021	-0.006	-0.156	-25.900
4. APM_5	-1.195	-5.111	-0.042	-0.013	-0.305	-82.400
5. APM_1^*	1.502	6.164	0.136	0.023	-0.081	87.183
Testers:						
1. APS_6	-0.300	-1.096	-0.013	-0.004	-0.141	-4.767
2. APS_{10}	-0.711	-2.656	-0.018	-0.008	-0.240	59.500
3. APS_4	1.050	2.682	0.019	0.010	0.403	-35.967
4. APS_8^{**}	-0.039	1.070	0.011	0.002	-0.021	-18.767

*** => Female parent of "Swarnandhra" & ** => Male parent of "Swarnandhra"**

In still another embodiment of the present invention, all these five inbred lines were crossed each with four testers namely, APS_4, APS_6, APS_8, & APS_{10} – Peanut shape in Line x Tester design. The resultant 20 hybrids are reared in three replicates each retaining 400 larvae after third moult. Accordingly, all the 20 combinations are evaluated for their performance. The analysis is conducted for six commercially important characters viz., Cocoon yield by weight/10000 larvae, Pupation rate, Cocoon Weight, Cocoon shell weight, Cocoon shell Ratio and Filament Length. The polyvoltine inbred line APM_1 and the bivoltine-inbred line, APS_8 were found to produce the best hybrid combination. Further, combination recorded low floss% (1.30%). The analysis confirmed the superiority of APM_1 x APS_8 (Swarnandhra- please refer fig- 1). The superiority of APM_1 x APS_8 is also reconfirmed with multiple trait index method (Mano *et. al.*, 1992).

In another embodiment of the present invention, a method to produce Mulberry silkworm hybrid named Swarnandhra, preferably well adapted for tropical regions and for all seasons of the year and the said hybrid is a cross of polyvoltine APM_1, derived from pureline inbreed of Madagascar hybrids and bivoltine APS_8, derived from pureline inbreed of Chinese hybrids, and is superior to conventional hybrids of the tropical regions mainly and produces greenish yellow silk of international grade 2A – 3A, using steps comprising inbreeding, hybridization, disease tolerance breeding, DNA fingerprinting, and selection.

In yet another embodiment of the present invention, taking polyvoltine and bivoltine strains of Madagascar hybrids and Chinese hybrids respectively for separate breeding plan to generate pureline inbreeds.

In still another embodiment of the present invention, mating F_1 hybrid offsprings and rearing the progeny.

In still another embodiment of the present invention, mass mating the progeny derived from the multiple random pair up to F_4 generation.

In still another embodiment of the present invention, making outclass at F_4 generation with pure Mysore (polyvoltine) and NB_4D_2 (bivoltine) in the case of polyvoltine and bivoltine breeding plans respectively.

In still another embodiment of the present invention, mass mating up to F_6 generation, and maintaining cellular families of said generation drawn from a single pair mating in each breeding plan.

In still another embodiment of the present invention, retaining larvae from each of the cellular families and rearing the retained larvae up to cocooning.

In still another embodiment of the present invention, selecting cocoons from each of the cellular families based on several parameters comprising larval survival up to pupae stage, cocoon weight, silk content, silk filament length, cocoon shape, and floss percentage.

In still another embodiment of the present invention, selecting cellular families which register high survival rate for further inbreeding.

In still another embodiment of the present invention, selecting non-diapause egg laying from selected families.

In still another embodiment of the present invention, selecting males and females in each of the cellular families and mating the moths emerging from the cocoons of each breeding plan among themselves.

In still another embodiment of the present invention, inbreeding of the selected families up to F_8 generation and exposing F_8 generation cellular families to Bombyx mori densonucleosis virus.

In still another embodiment of the present invention, isolating F_8 generation cellular families resistant to BmDNV 1.

In an embodiment of the present invention, nine promising silkworm inbred lines including five polyvoltines and four bivoltines developed at this institute under breeding programme have been assessed at molecular level for their homozygosity within the strains and heterozygosity in between strains using five ISSR markers (Please refer Tables 3 and 4).

Table 3: ISSR- DNA markers used for the estimation of Homozygosity in the parental lines

Sl. No	ISSR- Primers
1.	CGA (ATT)4
2.	TA (CGA)4
3.	(CA)7
4.	T3 (ATT)4
5.	CT (ATT)4

Table 4. DNA band sharing percentage within the strains (Molecular marker homozygosity)

Sl. No	Silkworm strain	Male	Female	Average
1	APS_4	93	96	94.5
2	APS_6	95	96	95.5
3	APS_8	93	95	94.0
4	APS_{10}	98	97	97.5
5	APM_3	99	95	97.0
6	APM_2	97	95	96.0
7	APM_5	97	96	96.5
8	APM_4	96	94	95.0
9	APM_1	97	93	95.0

In another embodiment of the present invention, ten individuals (five males and five females) from each strain (9x10= 90) are analyzed for their homozygosity. High molecular weight genomic DNA is extracted from the pupae and purified as per standard protocols (Nagaraju and Nagaraju, 1995; Sambrook *et. al.*, 1982). DNA is amplified by PCR technique using five di and tri nucleotide repeat ISSR markers anchored with 5' degenerate nucelotides (Reddy *et.al.* 1999). For 10 ul of PCR reaction, 50 ng of genomic DNA, 1X PCR reaction buffer, 2.5 mM $MgCl_2$, 25 um each of dNTPs, and 0.5 units of Taq Gold DNA polymerase (Perkin Elmer) for dinucleotide primer and MBI Taq DNA polymerase for trinucelotide primers were used. Amplification is performed on MJ research PTC- 2000 thermal cycler and perkin Elmer Gene Amp PCR- 9600 system, With a programme of initial denaturation at 94°C for 3 min followed by 35 cycles 0f 94°C for 30 sec., 50°C for 30 sec., 72°C for 1 min and final extension at 72°C for 10 min. For Gene Amp PCR machine, the initial denaturation at 94°C is for 10 min and annealing is at 42°C and the remaining programme is same as above. The amplified DNA is separated on 2% agarose gels and generated DNA profiles are documented and analyzed for DNA band sharing levels within (homozygosity) and in between (heterozygosity) parental lines (Nei, 1978, Sharma *et. al.* 1999, Table 3 & 4).

In still another embodiment of the present invention, for patenting the hybrid Swarnandhra (APM_1 X APS_8), FISSR-PCR DNA profiling is carried out. (Please refer Table- 5)

Table 5- Molecular marker heterozygosity measured in between 9 silkworm parental lines

S No	Name	1	2	3	4	5	6	7	8	9
1	APM_3									
2	APM_2	0.600								
3	APM_5	0.738	0.667							
4	APM_4	0.727	0.683	0.447						
5	APM_1	0.780	0.658	0.486	0.459					
6	APS_8	0.750	0.703	0.853	0.667	0.758				
7	APS_4	0.846	0.833	0.879	0.857	0.813	0.548			
8	APS_6	0.852	0.760	0.826	0.808	0.821	0.662	0.651		
9	APS_{10}	0.802	0.733	0.826	0.808	0.851	0.631	0.746	0.455	

In still another embodiment of the present invention, DNA from the parents and the F1 progeny is extracted, purified and amplified by PCR technique using six different ISSR markers (Table 4), (Kumar et.al. 2001). The protocols and the PCR machines used were as described above except the addition of 5 µl of fluorescent dUTPs to the reaction mixture. The amplified DNA is separated on 5% polyacrylamide gels using Perkin Elmer gene scan Machine and the generated FISSR-PCR DNA profiles of Swarnandhra (Fig. 5) are submitted for PCT filing.(please refer Table – 6)

Table 6: FISSR Markers used for DNA fingerprinting of Silkworm Hybrid "Swarnandhra"

Sl. No.	Marker
1	(CA)7
2	(TG)7
3	T(GT)9
4	T(GA)8
5	C(GA)7
6	TA(CAG)4

In still another embodiment of the present invention, fingerprinting the DNA samples using Fluorescent-inter-simple Sequence-repeat-polymerase chain reaction (FISSR-PCR) and selecting the inbred lines showing more than 90% homozygosity based on said DNA test.

In still another embodiment of the present invention, crossing the above selected lines on a partial diallele pattern using polyvoltine as female parents and bivoltine lines as male parents.

In still another embodiment of the present invention, rearing the resultant hybrids and retaining larvae in each of the resultant hybrid after third moult.

In still another embodiment of the present invention, using DNA fingerprinting technology to test the hybrid vigor and selecting the parents giving rise to maximum hybrid vigor.

In still another embodiment of the present invention, finding cross of pureline inbreed polyvoltine (APM_1) and bivoltine (APS_8) to have maximum hybrid vigor.

In still another embodiment of the present invention, wherein bivoltine hybrids are selected from a group comprising APS_4, APS_6, APS_8, and APS_{10}.

In still another embodiment of the present invention, wherein polyvoltine hybrids are selected from a group comprising APM_1, APM_2, APM_3, APM_4, and APM_5.

In still another embodiment of the present invention, wherein bivoltine hybrids are used as tester.

In an embodiment of the present invention, as per the General Combining Ability effects, the line, APM_1 the female parent of "Swarnandhra", showed positive effects for all the six traits studied except SR %. On the other hand the line APM_5 showed negative General Combining Ability (GCA) for all characters. Among the 4 testers APS_4 followed by APS_8 showed better GCA. The tester APS_6 showed negative GCA for all the six characters studied.

In still another embodiment of the present invention, combinations APM_3 x APS_4, APM_3 x APS_8, APM_5 x APS_8 and APM_1 x APS_8 (except for SR%) showed positive Specific Combining Ability (SCA) effects for all the six traits. However, in the combination, APM_1 x APS_8, APM_1 is found to be the good combiner. (Please refer Table 7)

Table 7- Specific Combining Ability (SCA) of the hybrids

S. No.	Line x Tester	Yield (by wt)	PR%	Cocoon Weight	Shell Weight	SR %	Filament Length
1.	APM_2 x APS_6	0.867	-0.721	0.077	0.014	0.007	-16.733
2.	APM_2 x APS_{10}	0.589	3.706	0.018	-0.004	-0.434	29.667
3.	APM_2 x APS_4	-1.083	-1.598	-0.042	0.010	1.073	60.133
4.	APM_2 x APS_8	-0.372	-1.387	-0.053	-0.020	-0.646	-73.067
5.	APM_3 x APS_6	-1.961	-8.402	-0.076	-0.029	-0.955	-58.317
6.	APM_3 x APS_{10}	-0.106	2.269	-0.026	-0.008	-0.207	-126.583
7.	APM_3 x APS_4	0.926	1.594	0.065	0.017	0.317	101.217
8.	APM_3 x APS_8	1.141	4.539	0.037	0.020	0.845	83.683
9.	APM_4 x APS_6	-0.155	-1.235	0.048	0.029	1.250	32.433
10.	APM_4 x APS_{10}	1.827	10.989	-0.014	-0.011	-0.495	37.167
11.	APM_4 x APS_4	-0.052	-3.463	-0.015	-0.012	-0.564	-57.700
12.	APM_4 x APS_8	-1.620	-6.291	-0.019	-0.007	-0.190	-11.900
13.	APM_5 x APS_6	1.177	8.040	-0.006	-0.013	-0.735	48.933
14.	APM_5 x APS_{10}	-2.079	-10.843	-0.020	0.014	1.037	10.667
15.	APM_5 x APS_4	0.563	2.706	0.009	-0.006	-0.453	-59.200
16.	APM_5 x APS_8	0.338	0.097	0.017	0.006	0.151	-0.400
17.	APM_1 x APS_6	0.072	2.318	-0.043	-0.001	0.434	-6.317
18.	APM_1 x APS_{10}	-0.231	-6.121	0.041	0.009	0.099	49.083
19.	APM_1 x APS_4	-0.354	0.761	-0.016	-0.009	-0.374	-44.450
20.	APM_1 x APS_8	0.513	3.042	0.018	0.001	-0.160	1.683

In still another embodiment of the present invention, in comparison to other lines, additive gene action is predominant in the line APM_1 as evidenced from variance (gca) > variance (sca) for majority of the characters (four).

In still another embodiment of the present invention, it is evident from the SCA analysis, the tester APS_8 combines well with majority of the lines and many characters. In the hybrid combination APM_1 x APS_8 "Swarnandhra" the line APM_1 being good combiner recorded best mean performance.

Further embodiment of the present invention, Mid-Parent heterosis is an average heterosis averaged over two parents involved in the hybrid. It is the usual practice in silkworm to evaluate hybrids for mid-parental heterosis since the heterosis manifestation can be evaluated as less or more than the average performance of the parents. The survival rate or in other words cocoon yield is a typical trait that manifests with high degree of heterosis over mid-parental value when hybrids are reared under adverse conditions. (Please read Table 8).

Table 8 - Mid Parent (MP) Heterosis

S. No.	Line x Tester	Yield (by wt)	PR%	Cocoon Weight	Shell Weight	SR %	Filament Length
1.	APM_2 x APS_6	10.77	13.41*	-3.24	-3.63	0.36	-17.05**
2.	APM_2 x APS_{10}	10.48	22.07**	-2.54	-6.65	-3.65	-5.45
3.	APM_2 x APS_4	10.27	13.96*	-0.66	7.93	9.29*	0.40
4.	APM_2 x APS_8	11.13	6.77	1.55	-2.32	-3.68	-8.78
5.	APM_3 x APS_6	2.73	5.37	-5.00	-5.68	3.11	-7.66
6.	APM_3 x APS_{10}	19.98	23.94**	2.51	4.84	5.83	-9.81
7.	APM_3 x APS_4	42.27**	21.63**	14.70**	27.04**	14.10**	26.10**
8.	APM_3 x APS_8	40.23**	17.19**	16.55**	28.69**	13.32**	37.01**
9.	APM_4 x APS_6	-1.34	0.56	4.60	12.27*	10.79**	13.45
10.	APM_4 x APS_{10}	14.28	18.11**	5.62	1.54	-1.13	21.56**
11.	APM_4 x APS_4	12.55	-0.23	11.87**	13.09*	3.34	12.21
12.	APM_4 x APS_8	-3.50	-9.79	15.30**	15.07**	1.83	36.97**
13.	APM_5 x APS_6	44.13**	43.93**	10.29**	4.22	-0.83	10.89
14.	APM_5 x APS_{10}	15.11	15.13	15.04**	18.16**	7.86*	12.58
15.	APM_5 x APS_4	58.62**	36.29**	24.57**	24.31**	4.08	5.67
16.	APM_5 x APS_8	52.21**	21.91**	29.85**	29.84**	3.88	33.57**
17.	APM_1 x APS_6	9.44	9.33	2.45	-0.15	-1.80	0.92
18.	APM_1 x APS_{10}	8.93	0.83	11.97**	5.52	-5.12	13.93*
19.	APM_1 x APS_4	19.39*	9.13	14.62**	10.29*	-3.22	5.58
20.	APM_1 x APS_8	21.61*	5.22	20.37**	13.43**	-5.41	23.96**

PR: Pupation Rate; SR: Cocoon shell ratio; ** significance at 1% level

In still another embodiment of the present invention, the overall performance also confirmed the superiority of the hybrid APM_1 x APS_8. This is further substantiated by multiple trait index analysis (Mano *et.al.* 1992) with maximum average evaluation Index (60.50).

In still another embodiment of the present invention, Evaluation Index (EI) is calculated based on the following formula and the rank will be assigned on the basis of average evaluation index; Evaluation index (EI) = (A-B)/ C X 10 + 50

Where, A= Mean of particular hybrid for a trait ; B= Overall mean of all hybrids including control for a trait ; C= standard Deviation over means for a trait; 10= standard unit & 50= Base index value. (Please refer Table 9)

Table 9 - Mean Performance & Evaluation Indices (EI) (in descending order)

S. No.	Line x Tester	Yield Wt (kg)	PR (%)	Cocoon Wt. (g)	Shell Wt. (g)	S.R (%)	Filament Len. (m)	Avg. Eval. Index
1.	APM_1 x APS_8	17.16	94.00	1.89	0.335	17.67	801	60.57
2.	APM_1 x APS_4	17.38	93.33	1.87	0.334	17.88	738	59.33
3.	APM_3 x APS_4	17.01	85.78	1.75	0.332	18.87	828	58.66
4.	APM_1 x APS_{10}	15.74	81.11	1.89	0.335	17.71	927	58.23
5.	APM_1 x APS_6	16.45	91.11	1.81	0.329	18.15	807	58.10
6.	APM_3 x APS_8	16.14	87.11	1.72	0.327	18.98	827	57.10
7.	APM_2 x APS_4	15.57	86.70	1.69	0.333	19.72	745	56.57
8.	APM_2 x APS_6	16.17	83.80	1.78	0.323	18.11	699	52.87
9.	APM_4 x APS_6	14.14	86.70	1.74	0.329	18.88	733	52.16
10.	APM_2 x APS_{10}	15.48	86.70	1.71	0.302	17.57	810	56.67
11.	APM_4 x APS_{10}	15.71	91.33	1.67	0.286	17.04	802	48.64
12.	APM_4 x APS_4	15.59	82.22	1.71	0.302	17.61	611	46.11
13.	APM_5 x APS_4	15.60	84.00	1.71	0.302	17.58	553	45.43
14.	APM_2 x APS_8	15.20	85.30	1.67	0.294	17.58	629	45.25
15.	APM_5 x APS_8	14.28	79.78	1.71	0.305	17.76	629	45.12
16.	APM_3 x APS_{10}	14.22	81.11	1.62	0.289	17.71	695	43.40
17.	APM_5 x APS_6	14.86	85.56	1.67	0.280	16.75	690	43.05
18.	APM_4 x APS_8	12.93	77.78	1.70	0.299	17.56	674	42.79
19.	APM_5 x APS_{10}	11.20	65.11	1.65	0.304	18.42	719	40.01
20.	APM_3 x APS_6	12.77	72.00	1.58	0.271	17.06	699	35.94

In an embodiment of the present invention, for the development of the mulberry silkworm hybrid named Swarnandhra, a large F_2 populations contributed by the progeny from 8 – 10 pair matings of the F_1 hybrid offspring (Fig- 4c) are reared and the mass mating of progeny derived from the multiple random pair is continued up to F_4 generation.

In another embodiment of the present invention, the mass rearing without any selection is exercised to ensure desirable recombinants in the breeding population.

In yet another embodiment of the present invention, at F_4 generation an outcross is made with Pure Mysore (Polyvoltine) in case of polyvoltine breeding plan, with NB_4D_2 (bivoltine) in case of bivoltine breeding plan.

In still another embodiment of the present invention, for the next two generations, mass mating is carried out.

In still another embodiment of the present invention, after two generations of mass mating, in each breeding plan five cellular families (each cellular family is drawn from a single pair mating) are maintained.

In still another embodiment of the present invention, 400 larvae are retained and reared up to cocooning in each of the cellular families.

In still another embodiment of the present invention, the cocoons raised from each of the cellular families are selected, based on the larval survival upto pupal stage, single cocoon weight, silk content, silk filament length, cocoon shape and floss content. The cellular families, which registered higher survival rate, were only selected for further Inbreeding.

In still another embodiment of the present invention, in polyvoltine breeding plan, only non-diapause egg layings (Fig – 4a) and in bivoltine breeding plan only diapause egg layings (Fig- 4b) are selected.

In still another embodiment of the present invention, in each of the selected cellular family 25 males and 25 females are selected and the moths emerging from these cocoons are mated among themselves.

In still another embodiment of the present invention, inbreeding of the selected cellular families are continued up to F_8 generation.

In still another embodiment of the present invention, the F_8 generation families are subjected to Bombyx mori densonucleosis virus 1, to isolate cellular families for BmDNV1 resistance. The inoculation dose of 10^2 BmDNV1 dilution is used for the assay.

In still another embodiment of the present invention, at F_{10} generation, five females and five male moths from each of the lines are randomly picked and DNA is extracted by using standard protocol.

In still another embodiment of the present invention, the DNA samples are subjected to fingerprinting using Fluorescent - Inter -Simple Sequence-Repeat - Polymerase Chain Reaction method (FISSR-PCR),(Fig- 8).

In still another embodiment of the present invention, the inbred lines which show > 90% homozygosity based on DNA test are chosen for evaluation of hybrid vigor (fig 8)

In still another embodiment of the present invention, the isolated polyvoltine inbred lines (five lines) and the bivoltine-inbred lines (three lines) are crossed on a partial diallele pattern using polyvoltine as female parents and bivoltine lines as male parents. The resultant hybrids (Fig- 4c) are reared in three replicates each retaining 400 larvae after third moult.

In still another embodiment of the present invention, the hybrid performance is evaluated considering total larval duration, fifth instar larval duration, larval survival upto pupal stage, single cocoon weight, cocoon shell weight, silk filament length and floss content. (Fig – 5a, 5b, 5c)

In still another embodiment of the present invention, the parents who gave rise to the highest hybrid vigor in the hybrids are selected.

In still another embodiment of the present invention, the polyvoltine inbred line APM_1 and the bivoltine-inbred line, APS_8 are found to produce the best hybrid combination.

In still another embodiment of the present invention, the excelled hybrid combination, APM_1 x APS_8 is further field tested with the farmers during different seasons of the year in different regions of Andhra Pradesh and Karnataka, to evaluate its silk cocoon productivity, yield consistency, silk cocoon yield attributes, and quality of silk. (Fig- 7a, 7b,and 7c) (Please refer Tables 10 and 11)

TABLE 10 - SEASONAL PERFORMANCE OF SWARNANDHRA (APM_1 x APS_8) AND TRADITIONAL HYBRID (PM x NB_4D_2) WITH FARMERS OF A.P. & KARNATAKA

Season	No. of Farmers Covered	No. of dfls Tested	Total Qty. of Cocoons Harvested (Kg)	Average ± S.D. Cocoon Yield/ 100 dfls (Kg)
SWARNANDHRA (APM_1 x APS_8)				
Summer	105	27480	15222.15	23.04 ± 6.95
Rainy	203	42050	23124.20	53.82 ± 7.73
Winter	235	59975	32004.95	54.48 ± 5.78
Total / Avg.	**543**	**129505**	**70351.30**	**53.78 ± 6.82**
TRADITIONAL HYBRID (PM x NB_4D_2)				
Summer	160	24295	8338.04	34.32 ± 8.78
Rainy	185	35430	13640.55	38.5 ± 9.66
Winter	201	40345	16077.48	39.85 ± 10.65
Total / Avg.	**546**	**100070**	**38056.08**	**37.55 ± 9.87**

Table 11 - STATE-WISE PERFORMANCE OF SWARNANDHRA (APM_1 x APS_8)

Season	No. of Farmers Covered	No. of dfls Tested	Total Qty. of Cocoons Harvested (Kg)	Average ± S.D. Cocoon Yield/ 100 dfls (Kg)
Andhra Pradesh	495	109840	59109.20	53.26 ± 6.35
Karnataka	48	19665	11120.50	55.975 ± 5.81
Total / Avg.	**543**	**129505**	**70229.70**	**54.62 ± 6.08**

In still another embodiment of the present invention, the new hybrid, Swarnandhra has scored better over the ruling traditional hybrid in all the seasons of the year (Please refer Table- 3).

In still another embodiment of the present invention, the grade of silk recovered from the new hybrid is classified as 2A –3A, as compared to the ungraded silk from the ruling hybrid. The newly developed hybrid ensure higher financial returns to the farmer to an extent of 30% to 40% over the returns from the ruling traditional hybrid.

In still another embodiment of the present invention, inventors have successfully developed the elite silkworm hybrid, Swarnandhra and also are confident about higher economic returns to both silkworm rearers and silk reelers, and the silk obtained is graded 2A –3A on the basis of international standard.

In still another embodiment of the present invention, this is the first polyvoltine x bivoltine hybrid developed in India to meet the silk quality stipulated for international market.

In still another embodiment of the present invention, the hybrids of these two inbred lines are raised and reared in different seasons of the year and different regions of Andhra Pradesh to evaluate the cocoon crop stability. The check hybrid, Pure Mysore X NB_4D_2 included as control in all these studies. The performance is tabulated in Tables 9 & 10.

In still another embodiment of the present invention, said hybrid is reared and recorded an average of 53.73 kg/100 dfls. The cocoons can easily fetch Rs.30-50 more per kg of cocoons over the ruling hybrid Pure Mysore x NB_4D_2.

In still another embodiment of the present invention, the new hybrid is named as `Swarnandhra'.

In further embodiment of the present invention, Mulberry Silkworm hybrids named Kalpatharuvu and Hemavathy, preferably well adapted for tropical region and for all seasons of the year, are cross of bivoltines obtained from oval type Chinese and peanut type Japanese hybrids, using steps comprising inbreeding, hybridization, disease tolerance breeding, selection, and DNA fingerprinting, and are superior to conventional hybrids of the tropical regions mainly.

In another embodiment of the present invention, said Kalpatharuvu and Hemavathy hybrids produces silk of superior quality and high yield.

In yet another embodiment of the present invention, said silk is of international grade of 3A-4A.

In still another embodiment of the present invention, said silk has reelability ranging between 80 and 85%.

In still another embodiment of the present invention, said silk has renditta ranging between 6.5 and 7.0 kg.

In still another embodiment of the present invention, said silk is of white color.

In still another embodiment of the present invention, larvae of superior quality with survival rate ranging between 80 and 85%.

In still another embodiment of the present invention, said hybrid has larval period ranging between 22 and 23 days.

In still another embodiment of the present invention, larvae is plain or without any visible marking.

In still another embodiment of the present invention, cocoon of silkworm hybrids named Kalpatharuvu and Hemavathy has good yield and superior quality.

In still another embodiment of the present invention, said cocoon yield ranging between 60 and 65 per 100 dfls (kg).

In still another embodiment of the present invention, single silk cocoon weight ranging between 1.7 and 1.8g.

In still another embodiment of the present invention, shell weight ranging between 0.350 and 0.390g.

In still another embodiment of the present invention, percentage shell ratio ranging between 21 and 22.

In still another embodiment of the present invention, filament length ranging between 750 and 800 m/cocoon.

In still another embodiment of the present invention, oval shape with mild peanut constriction appearance.

In still another embodiment of the present invention, cocoon is white colored.

In still another embodiment of the present invention, eggs of Kalpatharuvu and Hemavathy hybrids are pigmented and has diapause appearance.

In still another embodiment of the present invention, crossing bivoltine strains of APS_9 (oval) X APS_8 (peanut) and APS_5 (oval) X APS_4 (peanut) produces Kalpatharuvu and Hemavathy respectively.

In still another embodiment of the present invention, pupation rate of Hemavathy is ranging between 92-96%.

In still another embodiment of the present invention, pupation rate of Kalpatharuvu is ranging between 85-90%.

In still another embodiment of the present invention, average evaluation Index of Hemavathy is ranging between 61-66%.

In still another embodiment of the present invention, average evaluation Index of Kalpatharuvu is ranging between 65-70%.

In still another embodiment of the present invention, ultimately, based on the overall performance and uniformity in cocoon shape two oval lines viz., APS_5 & APS_9 and two peanut lines viz., APS_4, APS_8 are selected (Fig 2 and 3)

In still another embodiment of the present invention, the selected oval lines; APS_5 & APS_9 and two peanut lines; APS_4, APS_8 lines are crossed in full diallele pattern. The resultant 16 F1s and their reciprocals along with their parents hybrids are reared in three replicates each retaining 400 larvae after third moult. The hybrids' performance is evaluated considering for six characters namely cocoon yield by weight, pupation rate, single cocoon weight, single shell weight, shell ratio and filament length. The hybrid combinations APS_9 x APS_8 (Kalpatharuvu) and APS_5 x APS_4 (Hemavathy) showed promise. The floss% in these hybrids is also found to less (APS_9 x APS_8:0.972% & APS_5 x APS_4:1.056%). Further, multiple trait evaluation index (Mano *et al.*, 1992) is calculated for the above commercially important characters and the superiority of APS_9 x APS_8 (Kalpatharuvu) and APS_5 x APS_4 (Hemavathy) is further confirmed. (Please refer Table 12)

TABLE 12. COMPARATIVE YIELD PROFILE OF NEW BIVOLTINE HYBRIDS, HEMAVATHY (APS_5 x APS_4) & KALPATHARUVU (APS_9 x APS_8) AND TRADITIONAL HYBRID (PM x NB_4D_2)

Sl. No.	Parameter	Traditional Hybrid (PM x NB_4D_2)	New Bivoltine Hybrids**	Gain
1	Larval Period (days)[1]	24-25	22-23	2-3
2	Survival (%)[2]	60-70	80-85	15-20
3	Cocoon yield/100 dfls (Kg)[3]	40-42	60-65	20-23
4	Cocoon shell ratio (%)[4]	15-16	21-22	5-6
5	Filament length (m/cocoon)[5]	550-600	750-800	150-200
6	Reelability (%)[6]	70-75	80-85	5-10
7	Renditta[7]	9-10	6.5-7	2-3
8	Grade of Silk[8]	Ungraded	3A-4A	Intl. Grade

* based on 100070 disease free egg layings tested with 546 farmers of Andhra Pradesh and Karnataka

** based on 40425 disease free egg layings tested with 191 farmers of Andhra Pradesh

1 total larval duration from the time of larval eclosion from the eggs upto the onset of secretion of silk.

2 total weight of cocoons harvested from the total number of larvae used for rearing

3 total weight of cocoons harvested from the larvae raised from 100 disease free egg layings

4 the ratio of single cocoon shell weight to the weight of single silk cocoon

5 an average length of silk fiber recovered from a single silk cocoon

6 the recovery percentage of continuous silk fibre from cocoons

7 the number of kgs of cocoons required to produce 1 kg of raw silk

8 silk is graded based on international standards considering the quality, neatness and tenacity of silk fibre.

In still another embodiment of the present invention, among the 4 parents, the parent APS_5, the female parent of "Hemavathy", is showing positive General Combining Effects (GCA) effects for all the six characters followed by APS_9, the female parent of "Kalpatharuvu", for 5 characters except Pupation Rate. While the parent APS_4, the male of "Hemavathy" showed negative effects for all the characters so also APS_8, the male parent of "Kalpatharuvu" except for Pupation Rate. (Please refer Table 13)

Table 13 - General Combining Ability (GCA) of the parents

S. No.	Parents	Yield	Pupation Rate (PR)	Cocoon Weight	Shell Weight	Shell Ratio (SR)	Filament Length
1.	APS_5	0.484	0.693	0.061	0.012	0.017	49.00
2.	APS_9	0.009	-2.366	0.007	0.004	0.101	85.42
3.	APS_4	-0.278	-0.862	-0.003	-0.008	-0.374	-63.96
4.	APS_8	-0.215	2.535	-0.065	-0.008	0.256	-70.46

In an embodiment of the present invention, a method to produce Mulberry Silkworm hybrids named Kalpatharuvu and Hemavathy, well adapted for tropical region mainly and for all seasons of the year, are cross of bivoltine, obtained from oval type Chinese and peanut type Japanese hybrids, using steps comprising hybridization, inbreeding, disease tolerance breeding, selection, and DNA fingerprinting, and are superior to conventional hybrids of the tropical regions mainly and produces white color silk of international grade 3A-4A.

In another embodiment of the present invention, taking the bivoltine strains of the Chinese and the Japanese hybrids and initiating two exclusive breeding plans to generate pureline inbreeds of high yield from both hybrids.

In yet another embodiment of the present invention, mating F_1 hybrid offsprings in each breeding plan and rearing the progeny.

In still another embodiment of the present invention, mass mating of progeny derived from the multiple random pair up to F_4 generation and without any culling to ensure desirable combinations in the breeding plan.

In still another embodiment of the present invention, making an outclass at F_4 generation with elite oval line SHOWA of Japanese origin for oval lines, and with indigenous peanut line NB_4D_2 for peanut cocoon lines.

In still another embodiment of the present invention, selecting F_5 generation on the basis of desired larval markings and cocoon shape and mass mating selected multiple lines up to F_6 generation.

In still another embodiment of the present invention, obtaining cellular families from above-mentioned separate single pair mating and thereby obtaining larvae from said cellular families.

In still another embodiment of the present invention, rearing said larvae up to cocooning in each of the cellular families.

In still another embodiment of the present invention, selecting cocoons raised from each of the cellular families on the basis of parameters comprising the larval survival upto pupation stage, single cocoon weight, silk content, silk filament length, cocoon shape, and floss content traits.

In still another embodiment of the present invention, selecting cellular families registering higher survival rate.

In still another embodiment of the present invention, selecting only diapause eggs for subsequent inbreeding and selecting males and females emerging from eggs of said cellular families.

In still another embodiment of the present invention, mating the moths emerging from the cocoons of the said families among themselves.

In still another embodiment of the present invention, inbreeding the said cellular families up to F_8 generation.

In still another embodiment of the present invention, subjecting the F_8 generation families to *Bombyx mori* densonucleosis virus1 (BmDNV1) and isolating cellular families resistant to BmDNV1.

In still another embodiment of the present invention, inbreeding the said resistant cellular families up to F_{10} generation.

In still another embodiment of the present invention, picking up female and male moths from each of the lines at F_{10} generation at random.

In still another embodiment of the present invention, extracting DNA from selected moths at F_{10} generation and subjecting isolated DNA samples to fingerprinting, using Fluorescent Inter-Simple Sequence Repeat anchored Polymerase Chain Reaction (FISSR-PCR) based analysis.

In still another embodiment of the present invention, ISSR – DNA markers for testing homozygosity are selected from a group comprising CGA(ATT)4, TA(CGA)4, (CA)7, T3(ATT)4, and CT(ATT)4.

In still another embodiment of the present invention, FISSR Markers for testing heterozygosity are selected from a group comprising (CA)7, (TG)7, T(GA)9, T(GA)8, C(GA)7, and TA(CAG)4.

In still another embodiment of the present invention, Taq polymerase is used in PCR.

In still another embodiment of the present invention, homozygosity is tested on agarose gel of concentration ranging between 1-5%.

In still another embodiment of the present invention, heterozygosity is tested on polyacrylamide gel with concentration ranging between 3-8%.

In still another embodiment of the present invention, eight promising bivoltines silkworm inbred lines developed at this institute under breeding programme have been assessed at molecular level for their homozygosity within the strains and heterozygosity in between strains using five ISSR markers (Please refer Tables 14 and 15).

Table 14 - ISSR- DNA markers used for the estimation of homozygosity in the parental lines

Sl. No	ISSR- Primers
1.	CGA (ATT)4
2.	TA (CGA)4
3.	(CA)7
4.	T3 (ATT)4
5.	CT (ATT)4

Table 15 - DNA band sharing percentage within the strains (Molecular marker homozygosity)

Sl. No	Silkworm strain	Male	Female	Average
1	APS_4	93	96	94.5
2	APS_5	92	94	93.0
3	APS_6	95	96	95.5
4	APS_7	98	97	97.5
5	APS_8	93	95	94.0
6	APS_9	96	91	93.5
7	APS_{10}	98	97	97.5
8	APS_{11}	96	96	96.0

In still another embodiment of the present invention, ten individuals (five males and five females) from each strain (8x10= 80) are analyzed for their homozygosity. High molecular weight genomic DNA is extracted from the pupae and purified as per standard protocols (Nagaraju and Nagaraju, 1995; Sambrook *et. al.*, 1982). DNA is amplified by PCR technique using five di and tri nucleotide repeat ISSR markers anchored with 5' degenerate nucelotides (Reddy *et.al.* 1999). For 10 ul of PCR reaction, 50 ng of genomic DNA, 1X PCR reaction buffer, 2.5 mM $MgCl_2$, 25 um each of dNTPs, and 0.5 units of Taq Gold DNA polymerase (Perkin Elmer) for dinucleotide primer and MBI Taq DNA polymerase for trinucelotide primers are used. Amplification is performed on MJ research PTC- 2000 thermal cycler and perkin Elmer Gene Amp PCR- 9600 system, With a programme of

initial denaturation at 94°C for 3 min followed by 35 cycles 0f 94°C for 30 sec., 50°C for 30 sec., 72°C for 1 min and final extension at 72°C for 10 min. For Gene Amp PCR machine, the initial denaturation at 94°C is for 10 min and annealing was at 42°C and the remaining programme is same as above. The amplified DNA is separated on 2% agarose gels and generated DNA profiles are documented and analyzed for DNA band sharing levels within (homozygosity) and in between (heterozygosity) parental lines (Nei, 1978, Sharma *et. al.* 1999).

In still another embodiment of the present invention, for patenting the hybrids "Kalpatharuvu" (APS_9 X APS_8) and "Hemavathy" (APS_5 X APS_4), FISSR-PCR DNA profiling is carried out. DNA from the parents and the F1 progeny is extracted, purified and amplified by PCR technique using six different ISSR markers (Please refer Table 16), (Kumar et.al. 2001).

Table 16 - Molecular marker heterozygosity measured in between 13 silkworm parental lines

S.No	Name	1	2	3	4	5	6	7
1	APS_8							
2	APS_5	0.593						
3	APS_4	0.548	0.754					
4	APS_6	0.662	0.767	0.651				
5	APS_7	0.701	0.645	0.723	0.676			
6	APS_9	0.652	0.719	0.642	0.543	0.444		
7	APS_{10}	0.631	0.800	0.746	0.455	0.618	0.543	
8	APS_{11}	0.706	0.619	0.667	0.768	0.577	0.671	0.681

In still another embodiment of the present invention, the protocols and the PCR machines used are as described above except the addition of 5 µl of fluorescent dUTPs to the reaction mixture. The amplified DNA is separated on 5% polyacrylamide gels using Perkin Elmer gene scan Machine and the generated FISSR-PCR DNA profiles of Kalpatharuvu and Hemavathy hybrids (Fig. 5, please refer Table 17) are submitted for PCT filing.

Table 17 - FISSR Markers used for DNA Fingerprinting of two Silkworm Hybrids

Sl. No.	Marker
1	(CA)7
2	(TG)7
3	T(GT)9
4	T(GA)8
5	C(GA)7
6	TA(CAG)4

In still another embodiment of the present invention, selecting the inbred lines showing >90% homozygosity based on DNA tests for evaluating hybrid vigor.

In still another embodiment of the present invention, isolating oval and peanut shaped cocoon lines showing said degree of homozygosity.

In still another embodiment of the present invention, crossing the isolated cocoons on a full diallele pattern.

In still another embodiment of the present invention, rearing the resulting hybrids and retaining larvae after third moult.

In still another embodiment of the present invention, evaluating the hybrid performance on the basis of total larval duration, fifth instar larval duration, larval survival upto pupal stage, single cocoon weight, cocoon shell weight, silk filament length, and floss content

In still another embodiment of the present invention, selecting the parents giving rise to the highest hybrid vigor in the said hybrids.

In still another embodiment of the present invention, crossing bivoltine strains of APS_9 (oval) X APS_8 (peanut) and APS_5 (oval) X APS_4 (peanut) produces the superior quality and high yield hybrids named Kalpatharuvu and Hemavathy respectively.

In still another embodiment of the present invention, among all the hybrid combinations (F1) studied APS_5 X APS_4 (Hemavathy), APS_9 X APS_8 (Kalpatharuvu) showed positive Specific Combining Ability (SCA) for all the characters studied. These hybrids involving good X poor combiners indicate that additive x dominant gene interaction were important in determining the SCA effects. (Please refer Table 18).

Table 18 - Specific Combining Ability (SCA) of the hybrids

S. No.	Hybrids (F1)	Yield by wt	PR	Cocoon Weight	Shell Weight	S.R	Filament Length
1.	APS_5x APS_9	0.441	7.121	-0.079	-0.015	0.010	27.833
2.	APS_5 x APS_4*	0.726	1.929	0.085	0.022	0.261	85.042
3.	APS_5x APS_8	0.361	0.332	-0.029	-0.002	0.198	4.375
4.	APS_9 x APS_4	-0.041	0.573	0.002	-0.007	-0.337	-18.875
5.	APS_9 x APS_8**	1.346	1.348	0.104	0.033	0.576	126.79
6.	APS_4 x APS_8	0.275	-0.614	0.034	0.002	-0.237	27.167

* => "Hemavathy" & ** => "Kalpatharuvu"

In still another embodiment of the present invention, the heterosis calculated for the F1s revealed significant to highly significant ($P \leq 0.05$ to $P \leq 0.01$) heterosis in the combination APS_5 X APS_4 (Hemavathy) for all the 6 characters and highly significant heterosis in APS_9 X APS_8 (Kalpatharuvu) for the 5 characters out of 6 characters studied. (Please refer Table 19)

Table 19 - Mid Parent (MP) Heterosis in Hybrids

S. No.	Hybrids (F1)	Yield by wt	PR	Cocoon Weight	Shell Weight	S.R	Filament Length
1.	APS_5 x APS_9	17.77**	26.15**	-6.29	-6.37	-0.13	18.21**
2.	APS_5 x APS_4	16.26**	21.31*	7.01*	15.42**	7.90**	26.21**
3.	APS_5 x APS_8	27.48**	13.70	2.43	6.12	3.43	26.07**
4.	APS_9 x APS_4	9.64	6.61	4.49	4.85	0.40	15.88**
5.	APS_9 x APS_8	30.42**	12.22	13.05**	26.65**	12.05**	50.60**
6.	APS_4 x APS_8	14.79*	1.50	10.48**	9.22	-1.03	19.04*

* => $P \leq 0.05$ & ** => $P \leq 0.01$

In still another embodiment of the present invention, Evaluation Index (EI) calculated on the basis of over all performance of the F1s also support the superiority of the hybrids APS_9 X APS_8 (Kalpatharuvu) and APS_5 X APS_4 (Hemavathy) through highest average indices (6 traits) of 67.12 and 63.15 respectively. (Please refer Table 20)

Table 20 - Mean Performance & Evaluation Indices (EI) (in descending order)

S. No	Parents	Yield wt (kg)	P.R (%)	Cocoon Weight (g)	Shell Weight (g)	Shell Ratio (%)	Filament Length (m)	Average Evaluation Index
1.	APS_9 x APS_8	19.72	87.78	2.028	0.447	22.04	1144	67.12
2.	APS_5 x APS_4	18.78	93.78	2.064	0.429	20.79	1008	63.15
3.	APS_5 x APS_8	20.02	92.22	1.918	0.387	20.14	941	57.27
4.	APS_9 x APS_5	17.35	86.00	1.958	0.396	20.22	1029	55.15
5.	APS_5 x APS_9	18.9	91.11	1.861	0.362	19.43	1080	53.67
6.	APS_5 x APS_5	16.63	75.11	2.065	0.398	19.27	900	50.06
7.	APS_9 x APS_4	17.07	79.33	1.932	0.378	19.54	941	49.34
8.	APS_4 x APS_5	17.47	75.96	2.061	0.380	18.42	862	47.99
9.	APS_8 x APS_9	16.94	81.47	1.905	0.368	19.23	923	47.90
10.	APS_4 x APS_8	17.48	84.56	1.919	0.369	19.27	768	47.84
11.	APS_8 x APS_5	15.62	81.11	1.856	0.375	20.21	809	46.56
12.	APS_8 x APS_4	16.47	83.78	1.852	0.360	19.42	801	45.61
13.	APS_4 x APS_9	16.69	81.57	1.919	0.358	18.64	848	45.29
14.	APS_9 x APS_9	15.46	69.33	1.906	0.375	19.65	927	44.51
15.	APS_4 x APS_4	15.67	79.49	1.793	0.349	19.27	698	40.35
16.	APS_8 x APS_8	14.78	87.11	1.681	0.331	19.68	593	38.19

In an embodiment of the present invention, applicants have developed two new bivoltine hybrids, APS_9 x APS_8 and APS_5 x APS_4, which are named as 'Kalpatharuvu' and 'Hemavathy' respectively.

In still another embodiment of the present invention, the exotic Chinese and Japanese hybrids are used as donor parental genetic material for breeding bivoltine inbred lines APS_4, APS_5, APS_8 and APS_9.

In still another embodiment of the present invention, the breeding is carried out by exercising selection for desired characters particularly for oval and peanut cocoon shape with uniform shape and size, hardiness of cocoon, higher pupation rate, shorter larval duration, higher single cocoon weight, higher cocoon shell weight, longer silk filament length, better reelability and overall enhancement in the quality of silk (as quantified by international silk grade).

In still another embodiment of the present invention, during the course of inbreeding, outcross is made with another qualitatively superior bivoltine breed to incorporate the beneficial characters to the newly bred inbred lines.

In still another embodiment of the present invention, during the process of inbreeding, different inbred lines are separated based on the presence/absence of markings on the larval body and shape of the cocoons (oval or peanut shaped).

In still another embodiment of the present invention, after the inbred lines attained some degree of uniformity for various quantitative traits, they are tested for homozygocity using different DNA markers. The highly inbred and homozygous lines are subjected to hybridization studies in the breeding laboratory.

In still another embodiment of the present invention, the excelled hybrid combinations, APS_9 x APS_8 and APS_5 x APS_4 are field tested during different seasons of the year in different regions of Andhra Pradesh to evaluate its silk cocoon productivity, yield consistency, silk cocoon yield attributes and quality of silk.

In still another embodiment of the present invention, the new hybrids, Kalpatharuvu and Hemavathy yielded better crop results in all the seasons of the year. The grade of silk recovered from new hybrids is tested and classified as 3A-4A.

In still another embodiment of the present invention, the overall financial returns to the farmers can be about 30-40% higher than the traditional polyvoltine x bivoltine hybrids. Thus applicants have successfully developed elite silkworm hybrids, Kalpatharuvu and Hemavathy and demonstrated that the large-scale exploitation would lead to higher economic returns to both silkworm rearers and silk reelers.

In still another embodiment of the present invention, since the silk obtained is graded as 3A-4A, which is the quality stipulated for international market. The country can benefit from the export of internationally competitive quality silk. The rearing of these hybrids in large-scale will also help to meet the industrial needs for quality silk and curbs the importing of Chinese silk.

In still another embodiment of the present invention, the new hybrids are also expected to perform better in the tropical states of Karnataka and Tamil Nadu, where there is paucity of productive bivoltine hybrids.

In still another embodiment of the present invention, two breeding plans are initiated separately using the bivoltine silkworm hybrids obtained from China and Japan as donor genetic materials. Each breeding plan is aimed at isolating Chinese (oval) type and Japanese (peanut) type of cocoons. In both the breeding plans, breeding methods involving inbreeding, hybridization, disease tolerance breeding, DNA fingerprinting, and selection for the desired traits are followed.

In still another embodiment of the present invention, silkworm rearing is conducted under standard rearing conditions. Initially, a large F_2 populations contributed by the progeny from 8-10 pair matings of the F_1 hybrid offsprings is reared. The mass mating of progeny derived from the multiple random pair is continued upto F_4 generation. The mass rearing without any culling is exercised to ensure desirable combinations in the breeding plan.

In still another embodiment of the present invention, at F_4 generation an outcross is made with elite oval for oval lines and peanut for peanut cocoon lines. At F_5 generation selection was resorted to larval markings and cocoon shape. After two generations of mass mating in each breeding plan, five cellular families (each cellular family is drawn from a single pair mating) are maintained.

In still another embodiment of the present invention, 400 larvae are retained and reared upto cocooning in each of the cellular families. The cocoons raised from each of the cellular families are selected based on the larval survival upto pupation stage. Single cocoon weight, silk content, silk filament length, cocoon shape and floss content traits are measured.

In still another embodiment of the present invention, the cellular families, which registered higher survival rate, are only selected for further inbreeding. In both the breeding plans only diapause eggs, which is characteristic feature of bivoltines, are only selected.

In still another embodiment of the present invention, in each of the selected cellular families 25 males and 25 females are selected. The moths emerged from these cocoons are mated among themselves. Inbreeding of the selected cellular families is continued upto F_{10} generation. The F_{10} generation families are also subjected to *Bombyx mori* densonucleosis virus1 (BmDNV1) to isolate cellular families for BmDNV1 resistance. The inoculation dose of 10^{-2} BmDNV1 is used for the assay.

In still another embodiment of the present invention, five females and five male moths from each of the lines are randomly picked at F_{10} generation and DNA is extracted by using the standard protocol. The DNA samples are subjected to fingerprinting using Fluorescent Inter-Simple Sequence Repeat anchored Polymerase Chain Reaction (FISSR-PCR) based analysis. The inbred lines which showed >90% homozygocity based on DNA tests are chosen for evaluation of hybrid vigor.

In still another embodiment of the present invention, the isolated oval cocoon (four lines) and peanut cocoon (four lines) lines are crossed on a full diallele pattern. The resulted hybrids are reared in three replicates each retaining 400 larvae after third moult. The hybrid performance is evaluated considering total larval duration, fifth instar larval duration, larval survival upto pupal stage, single cocoon weight, cocoon shell weight, silk filament length and floss content.

In still another embodiment of the present invention, the parents, which give rise to the highest hybrid vigor in the hybrids, are selected. The inbred line APS_9 (oval) with APS_8 (peanut) and APS_5 (oval) with APS_4 (peanut) are found to produce the best hybrid combination.

In still another embodiment of the present invention, these two promising hybrids, APS_9 x APS_8 and APS_5 x APS_4, are reared in different seasons of the year and different regions of Andhra Pradesh to evaluate the cocoon crop stability with the farmers. The performance is tabulated in Table 21.

TABLE 21 - SEASONAL PERFORMANCE OF KALPATHARUVU (APS_9 x APS_8) AND HEMAVATHY (APS_5 x APS_4) WITH FARMERS OF A.P.

Season	No. of Farmers Covered	No. of dfls Tested	Total Qty. of Cocoons Harvested (Kg)	Average ± S.D. Cocoon Yield/ 100 dfls (Kg)
KALPATHARUVU (APS_9 x APS_8)				
Summer	25	4940	2960.30	60.14 ± 7.19
Rainy	29	5395	3405.50	63.74 ± 6.95
Winter	43	10005	6305.90	62.04 ± 7.42
Total / Avg.	**97**	**20340**	**12671.70**	**61.97 ± 7.19**
HEMAVATHY (APS_5 x APS_4)				
Summer	26	5420	3238.52	60.83 ± 6.81
Rainy	39	7455	4467.90	60.21 ± 6.08
Winter	29	7210	4543.20	61.54 ± 6.78
Total / Avg.	**94**	**20085**	**12249.62**	**60.86 ± 6.56**

In still another embodiment of the present invention, the said hybrids are reared and have recorded an average of 61.43kg/100 dfls. The cocoons can easily fetch about Rs. 50-60 more per kg of cocoons over the ruling polyvoltine x bivoltine hybrid, Pure Mysore x NB_4D_2. The new hybrids, APS_9 x APS_8 and APS_5 x APS_4 are named respectively as 'Kalpatharuvu' and 'Hemavathy'.

In still another embodiment of the present invention, the bivoltine hybrids obtained from China and Japan are utilized as donor parental genetic materials in the breeding programmes. The inbred lines of oval and peanut shaped cocoons have been isolated through selection for desired metric traits and controlled progeny mating. The isolated inbred lines, which carried desired characters for larval survival, silk cocoon weight, silk content and silk quality are bred upto F_{10}-F_{11} generations. These lines are stabilized for the target traits between F_{10} and F_{11} generations.

In still another embodiment of the present invention, the DNA fingerprinting technology, which is now well proven and globally used in crop improvement programme is used to estimate the zygocity status of the developed inbred lines. Four inbred lines, APS_5 and APS_9 (oval), APS_4 and APS_8 (peanut), which, showed higher homozygocity (>90%) are chosen for further studies. These inbred lines are also screened simultaneously for *Bombyx mori* densonucleosis virus1 (BmDNV1) resistance. The highly homozygous inbred lines of oval cocoon shape are crossed systematically with similarly developed peanut shape inbred lines to analyse their hybrid vigor. The silkworm hybrid combinations, which showed higher-level heterosis, are selected for further trials with farmers.

In still another embodiment of the present invention, DNA fingerprinting is been used for the first time to test homozygosity and heterozygosity in silkworms for the first time. DNA fingerprinting reflects not genetic constitution of the hybrids but also the stability of the same. Introduction of DNA fingerprinting is a major step forward towards developing high quality silkworm hybrids. Instant application is an initiative towards achieving close association between advancement in the field of biotechnology with molecular Biology in particular and sericulture. The instant Application has overcome a major hurdle as regards uncertainty involved with random crossing of parents with hit and trials to develop desired hybrid. Applicants have made a breakthrough in the field of sericulture.

Advantages of the in present invention

1. The main advantage of the present invention is development of novel, superior silkworm hybrids.

2. Another main advantage of the present invention is development of a reliable and consistent method to develop superior silkworm hybrids.

3. Yet another advantage of the present invention is development of a silkworm hybrid producing international standard silk.

4. Still another advantage of the present invention is that silk produced by the novel hybrids is with high reelability.

5. Still another advantage of the present invention is that hybrids are resistant to viruses.

6. Still another advantage of the present invention is the use of DNA fingerprinting to develop silkworm hybrids of desired heterogeneity and traits.

7. Still another advantage of the present invention is the development of hybrids with reduced larval period.

8. Still another advantage of the present invention is the use of DNA fingerprinting to ascertain hybrid vigor in silkworms.

9. Still another advantage of the present invention is to develop hybrids with high survival rate.

Claims

1. A Mulberry silkworm hybrid named Swarnandhra, preferably well adapted for tropical region and for all seasons of the year and the said hybrid is a cross of polyvoltine APM_1 derived from pureline inbreed of Madagascar hybrids and bivoltine APS_8 derived from pureline inbreed of Chinese hybrids, said hybrid being produced using steps comprising inbreeding, hybridizing, disease tolerance breeding, DNA fingerprinting, and selecting, said hybrid is superior to conventional hybrids of the tropical regions and produces:

   (a) silk of superior quality and high yield with:
      (i) international grade of 2A-3A,
      (ii) reelability ranging between 80 and 90%,
      (iii) renditta ranging between 7.0 and 7.5 kg,
      (iv) floss percentage less than 5%, and
      (v) greenish yellow color;

   (b) larvae of superior quality with:
      (i) survival rate ranging between 80 and 85%,
      (ii) larval period ranging between 22 and 23 days, and
      (iii) plain or no visible markings;

   (c) cocoon of good yield and superior quality with:
      (i) yield ranging between 50 and 55 per 100 dfls (kg),
      (ii) single silk cocoon weight ranging between 1.60 and 1.70 g,
      (iii) cocoon shell weight ranging between 0.290 and 0.320,
      (iv) percentage shell ratio ranging between 18 and 19,
      (v) filament length ranging between 700 and 800 m/cocoon,
      (vi) oval shape with mild peanut constriction appearance, and
      (vii) greenish yellow color;

   (d) eggs with :
      (i) pigmentation, and
      (ii) non-diapause appearance.

2. A hybrid as claimed in claim 1, wherein said hybrid has average evaluation index about 60.50.

3. A hybrid as claimed in claim 1, wherein pupation rate is ranging between 92-98%.

4. A reliable and consistent method to produce Mulberry silkworm hybrid named Swarnandhra, well adapted for all seasons and preferably adapted for tropical region and the said hybrid is a cross of polyvoltine APM_1, derived from pureline inbreed of Madagascar hybrids and bivoltine APS_8, derived from pureline inbreed of Chinese hybrids, and is superior to conventional hybrids of the tropical regions mainly and produces greenish yellow silk of international grade 2A – 3A, said method comprising,

(i) mass-mating polyvoltine and bivoltine strains of Madagascar hybrids and Chinese hybrids respectively with separate breeding plan to generate pureline inbreeds up to F_4 generation,

(ii) producing outclass at F_4 generation with pure Mysore (polyvoltine) and NB_4D_2 (bivoltine) in polyvoltine and bivoltine breeding plans respectively,

(iii) mass mating outclass hybrid up to F_6 generation,

(iv) rearing larvae up to cocooning,

(v) selecting cellular families with cocoons having several characteristics comprising high larval survival up to pupae stage, cocoon weight, high silk content, silk filament length, cocoon shape, and floss percentage,

(vi) selecting non-diapause egg laying from selected families,

(vii) inbreeding selected families up to F_8 generation,

(viii) exposing F_8 generation cellular families to Bombyx mori densonucleosis virus,

(ix) isolating F_8 generation cellular families resistant to BmDNV 1,

(x) fingerprinting DNA samples of selected families using Fluorescent-inter-simple Sequence-repeat-polymerase chain reaction (FISSR-PCR) with ISSR DNA markers,

(xi) selecting inbred lines showing more than 90% homozygosity based on the DNA test,

(xii) crossing selected inbred lines on a half diallele pattern using polyvoltine as female parents and bivoltine lines as male parents,

(xiii) rearing resultant hybrids and retaining larvae after third moult,

(xiv) using the DNA fingerprinting technology to test hybrid vigor of said larvae using Fluorescent-inter-simple Sequence-repeat-polymerase chain reaction (FISSR-PCR) with FISSR DNA markers, and

(xv) selecting the parents giving rise to maximum hybrid vigor with cross of pureline inbreed polyvoltine (APM_1) and bivoltine (APS_8) having maximum hybrid vigor.

5. A method as claimed in claim 4, wherein bivoltine hybrids are selected from a group comprising APS_4, APS_6, APS_8, and APS_{10}.

6. A method as claimed in claim 4, wherein polyvoltine hybrids are selected from a group comprising APM_1, APM_2, APM_3, APM_4, and APM_5.

7. A method as claimed in claim 4, wherein bivoltine hybrids are used as tester.

8. A method as claimed in claim 4, wherein ISSR DNA markers are selected from a group comprising CGA (ATT)4, TA (CGA)4, (CA)7, T3 (ATT)4, and CT (ATT)4.

9. A method as claimed in claim 4, wherein FISSR – DNA markers are selected from a group comprising (CA)7, (TG)7, T(GT)9, T(GA)8, C(GA)7, and TA(CAG)4.

10. A method as claimed in claim 4, wherein Taq polymerase is used in PCR.

11. A method as claimed in claim 4, wherein homozygosity is tested on agarose gel of concentration ranging between 1-5%.

12. A method as claimed in claim 4, wherein heterozygosity is tested on polyacrylamide gel with concentration ranging between 3-8%.

13. Mulberry Silkworm hybrids named Kalpatharuvu and Hemavathy, preferably well adapted for tropical region and for all seasons of the year, which are cross of bivoltine strains APS_9 (oval) X APS_8 (peanut) and APS_5 (oval) X APS_4 (peanut) respectively, wherein the oval and peanut types are obtained from oval type Chinese and peanut type Japanese hybrids, said hybrids being produced by using steps comprising inbreeding, hybridization, disease tolerance breeding, selection, and DNA fingerprinting, said hybrids are superior to conventional hybrids of the tropical regions mainly and produces:

(a) silk of superior quality and high yield with:
    (i) international grade of 3A-4A,
    (ii) reelability ranging between 80 and 85%,
    (iii) floss percentage ranging between 0.9-1.1%,
    (iv) renditta ranging between 6.5 and 7.0 kg, and
    (v) white color;

(b) larvae of superior quality with:
    (i) survival rate ranging between 80 and 85%,
    (ii) larval period ranging between 22 and 23 days, and
    (iii) plain or no visible marking;

(c) cocoon of good yield and superior quality with:
    (i) yield ranging between 60 and 65 per 100 dfls(kg),
    (ii) single silk cocoon weight ranging between 1.7 and 1.8g,
    (iii) shell weight ranging between 0.350 and 0.390g,
    (iv) percentage shell ratio ranging between 21 and 22,
    (v) filament length ranging between 750 and 800 m/cocoon,
    (vi) oval shape with mild peanut constriction appearance, and
    (vii) white color;

(d) eggs with:
    (i) pigmentation, and
    (ii) diapause appearance.

14. Hybrids as claimed in claim 13, wherein pupation rate of Hemavathy is ranging between 92-96%.

15. Hybrids as claimed in claim 13, wherein pupation rate of Kalpatharuvu is ranging between 85-90%.

16. Hybrids as claimed in claim 13, wherein average evaluation Index of Hemavathy is ranging between 61-66%.

17. Hybrids as claimed in claim 13, wherein average evaluation Index of Kalpatharuvu is ranging between 65-70%.

18. A reliable and consistent method to produce Mulberry Silkworm hybrids named Kalpatharuvu and Hemavathy, well adapted for all seasons and preferably adapted for tropical region, which are cross of bivoltines strains APS_9 (oval) X APS_8 (peanut) and APS_5 (oval) X APS_4 (peanut) respectively, wherein the oval and peanut types are obtained from oval type Chinese and peanut type Japanese hybrids, and are superior to conventional hybrids of the tropical regions mainly and produces white color silk of international grade 3A-4A, and said method comprising,

(i) mass mating bivoltine strains of the Chinese and the Japanese hybrids and initiating two exclusive breeding plans to generate pureline inbreeds of high yield from both hybrids up to F_4,

(ii) Producing an outclass at F_4 generation with elite oval line SHOWA of Japanese origin for oval cocoon lines and with indigenous peanut line NB_4D_2 for peanut cocoon lines,

(iii) selecting F_5 generation on the basis of desired larval markings and cocoon shape,

(iv) mass mating selected multiple lines up to F_6 generation to obtain larvae,

(v) rearing said larvae of each cellular family up to cocooning in each of the cellular families,

(vi) selecting cocoons on the basis of parameters comprising high larval survival upto pupation stage, single cocoon weight, high silk content, silk filament length, cocoon shape, and floss content traits,

(vii) selecting only diapause eggs for subsequent inbreeding,

(viii) inbreeding moths emerging from the cocoons of the selected families up to F_8 generation,

(ix) Selecting F_8 generation families resistant to *Bombyx mori* densonucleosis virus1 (BmDNV1),

(x) inbreeding moths emerging from the cocoons of the selected families up to F_{10} generation,

(xi) extracting DNA from moths at F_{10} generation,

(xii) subjecting isolated DNA samples to fingerprinting,

(xiii) selecting inbred lines showing >90% homozygosity using Polymerase Chain Reaction (PCR),

(xiv) isolating oval and peanut shaped cocoon lines showing said degree of homozygosity,

(xv) crossing the isolated cocoons on a full diallele pattern,

(xvi) rearing resulting hybrids to retain larvae after third moult,

(xvii) evaluating the hybrid performance on the basis of total larval duration, fifth instar larval duration, larval survival upto pupal stage, single cocoon weight, cocoon shell weight, silk filament length, and floss content, and

(xviii) selecting parents giving rise to the highest hybrid vigor in the said hybrids using DNA fingerprinting technique Fluorescent Inter-Simple Sequence Repeat anchored Polymerase Chain Reaction (FISSR-PCR), with crosses of APS_9 X APS_8 and APS_5 X APS_4 showing desired characteristics.

19. A method as claimed in claim 18, wherein bivoltine hybrid strains are selected from a group comprising APS_1, APS_4, APS_5, APS_8, and APS_9.

20. A method as claimed in claim 18, wherein ISSR – DNA markers for testing homozygosity are selected from a group comprising CGA (ATT)4, TA(CGA)4, (CA)7, T3(ATT)4, and CT(ATT)4.

21. A method as claimed in claim 18, wherein FISSR Markers for testing heterozygosity are selected from a group comprising (CA)7, (TG)7, T(GA)9, T(GA)8, C(GA)7, and TA(CAG)4.

22. A method as claimed in claim 18, wherein Taq polymerase is used in PCR.

23. A method as claimed in claim 18, wherein homozygosity is tested on agarose gel of concentration ranging between 1-5%.

24. A method as claimed in claim 18, wherein heterozygosity is tested on polyacrylamide gel with concentration ranging between 3-8%.

Fig - 1

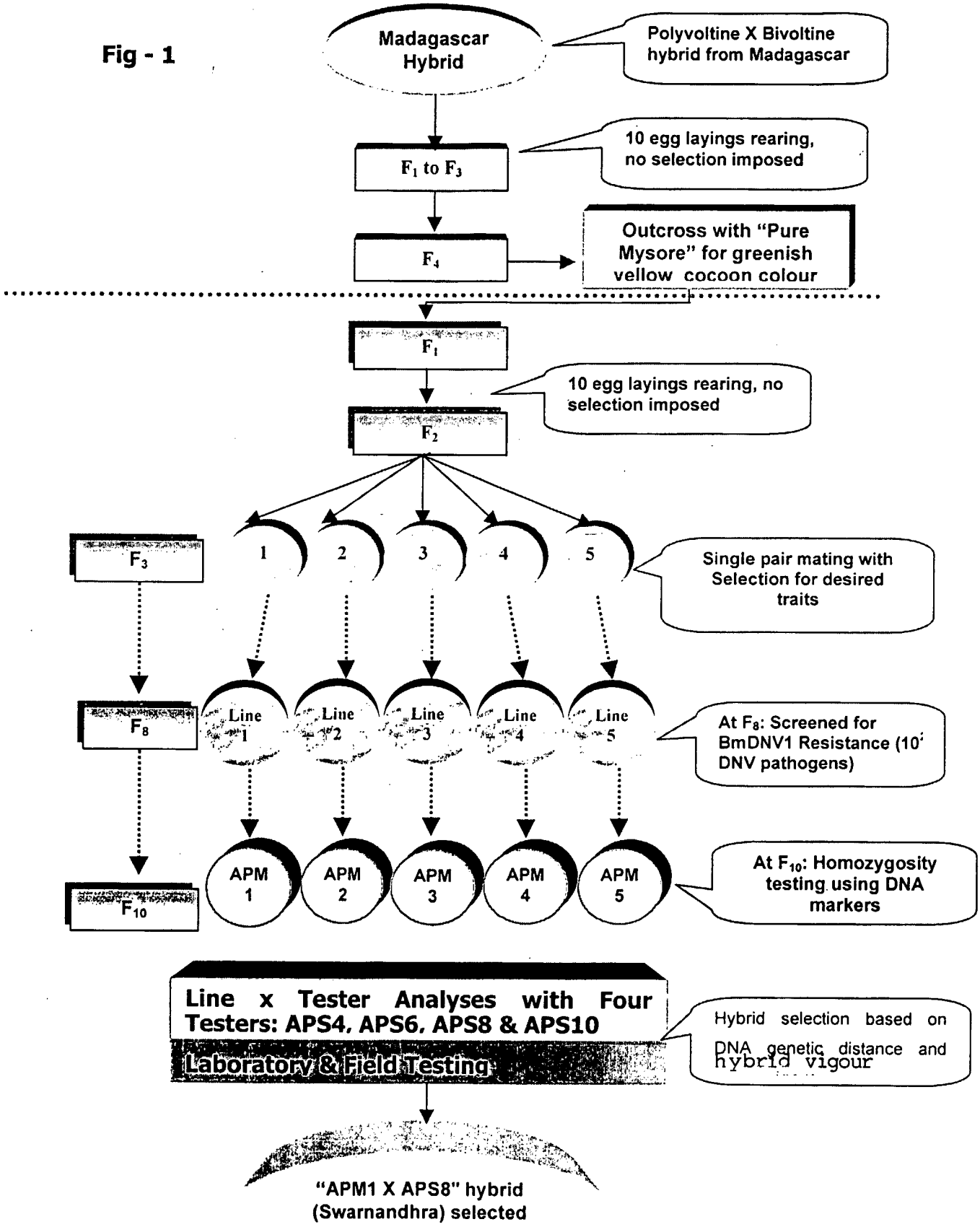

Madagascar Hybrid
Polyvoltine X Bivoltine hybrid from Madagascar
F1 to F3
10 egg layings rearing, no selection imposed
F4
Outcross with "Pure Mysore" for greenish yellow cocoon colour
F1
10 egg layings rearing, no selection imposed
F2
F3
1
2
3
4
5
Single pair mating with Selection for desired traits
F8
Line 1
Line 2
Line 3
Line 4
Line 5
At F8: Screened for BmDNV1 Resistance (10 DNV pathogens)
APM 1
APM 2
APM 3
APM 4
APM 5
F10
At F10: Homozygosity testing using DNA markers
Line x Tester Analyses with Four Testers: APS4, APS6, APS8 & APS10
Laboratory & Field Testing
Hybrid selection based on DNA genetic distance and hybrid vigour
"APM1 X APS8" hybrid (Swarnandhra) selected

Fig - 2

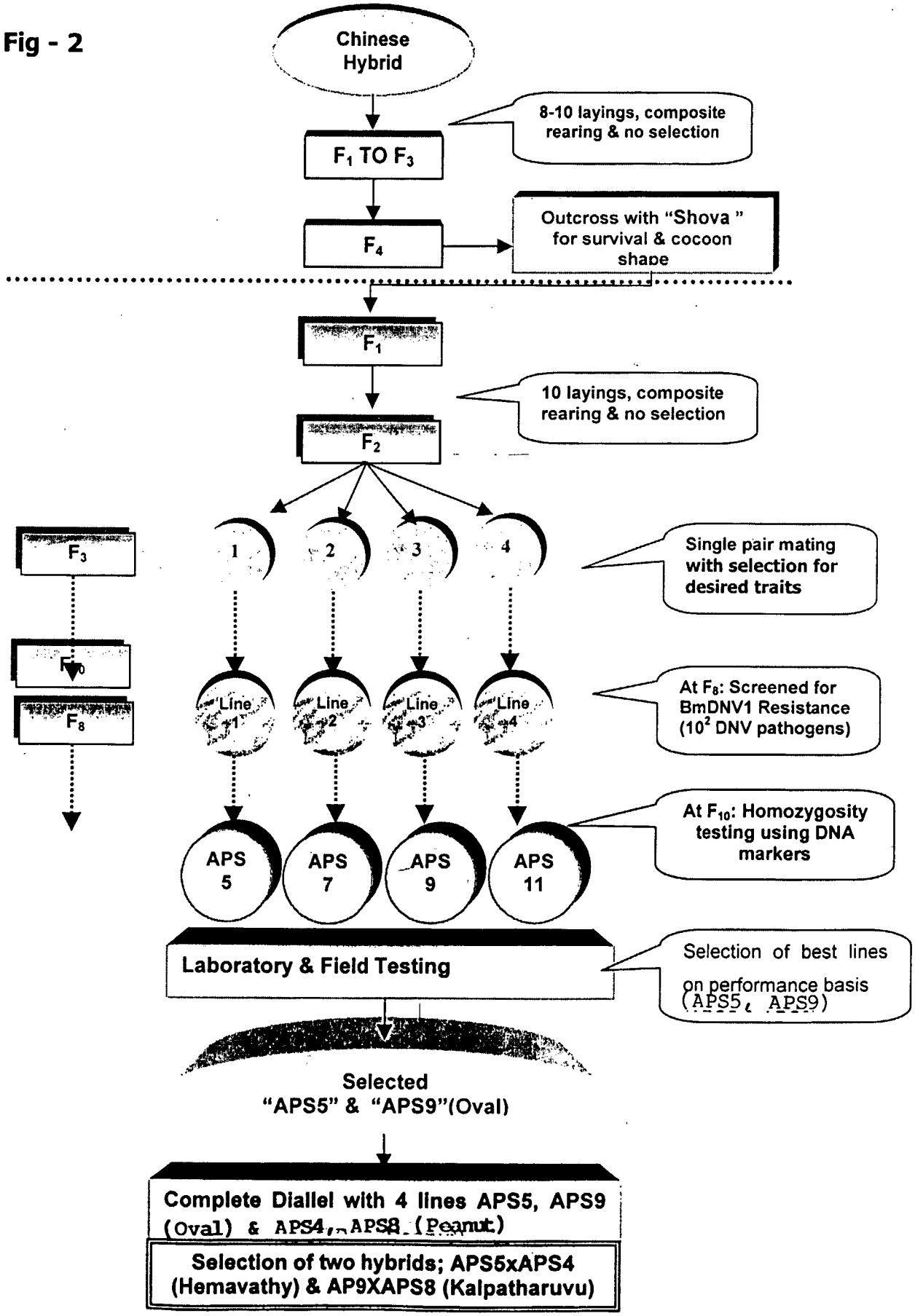
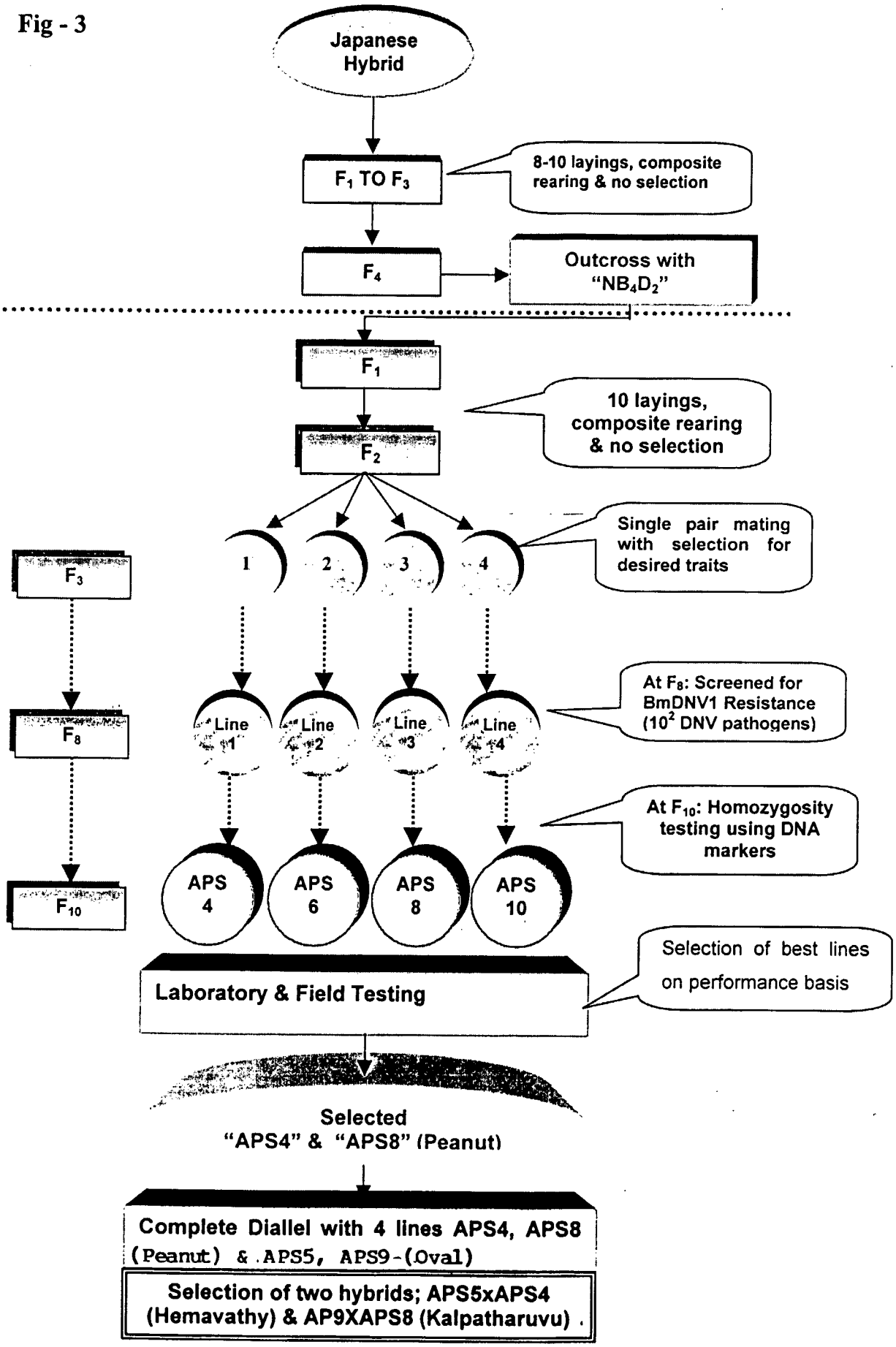

Chinese Hybrid
8-10 layings, composite rearing & no selection
F1 TO F3
F4
Outcross with "Shova " for survival & cocoon shape
F1
10 layings, composite rearing & no selection
F2
F3
1
2
3
4
Single pair mating with selection for desired traits
F9
F8
Line 1
Line 2
Line 3
Line 4
At F8: Screened for BmDNV1 Resistance (10² DNV pathogens)
APS 5
APS 7
APS 9
APS 11
At F10: Homozygosity testing using DNA markers
Laboratory & Field Testing
Selection of best lines on performance basis (APS5, APS9)
Selected "APS5" & "APS9"(Oval)
Complete Diallel with 4 lines APS5, APS9 (Oval) & APS4, APS8 (Peanut)
Selection of two hybrids; APS5xAPS4 (Hemavathy) & AP9XAPS8 (Kalpatharuvu)

Fig - 3


Japanese Hybrid
F1 TO F3
8-10 layings, composite rearing & no selection
F4
Outcross with "NB4D2"
F1
10 layings, composite rearing & no selection
F2
1
2
3
4
Single pair mating with selection for desired traits
F3
F8
Line 1
Line 2
Line 3
Line 4
At F8: Screened for BmDNV1 Resistance (10^2 DNV pathogens)
F10
APS 4
APS 6
APS 8
APS 10
At F10: Homozygosity testing using DNA markers
Laboratory & Field Testing
Selection of best lines on performance basis
Selected "APS4" & "APS8" (Peanut)
Complete Diallel with 4 lines APS4, APS8 (Peanut) & APS5, APS9-(Oval)
Selection of two hybrids; APS5xAPS4 (Hemavathy) & AP9XAPS8 (Kalpatharuvu).

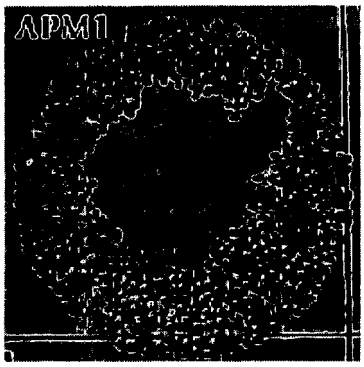


Fig. 4a.

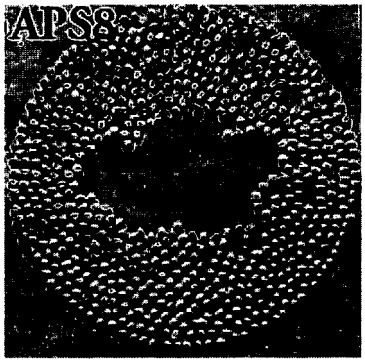


Fig 4b.

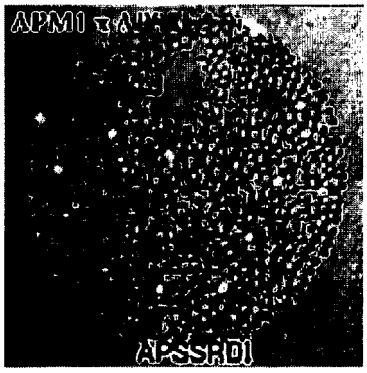


Fig 4c.

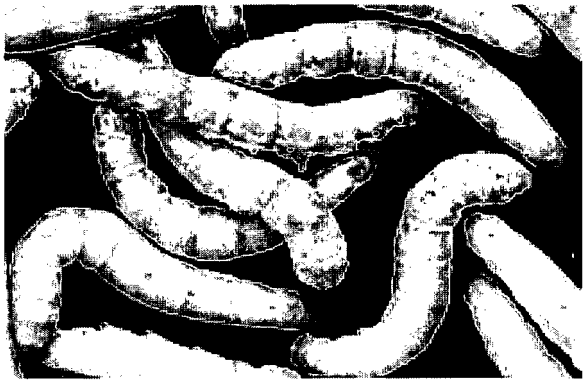


Fig.5a

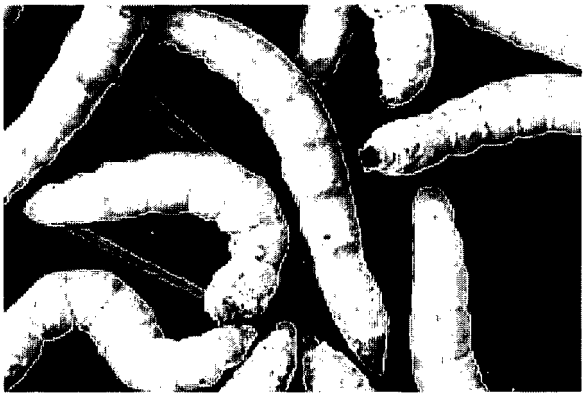


Fig.5b.

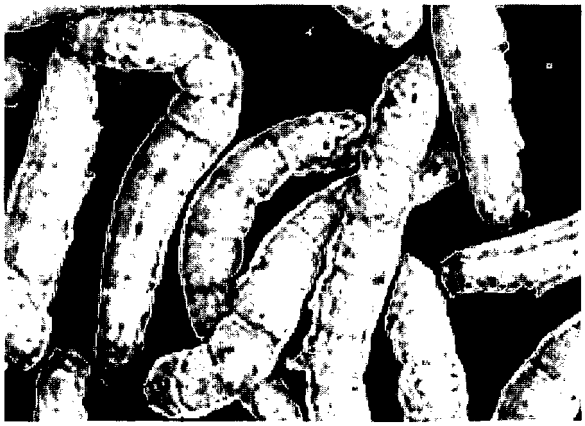


Fig.5c

Fig.6a.

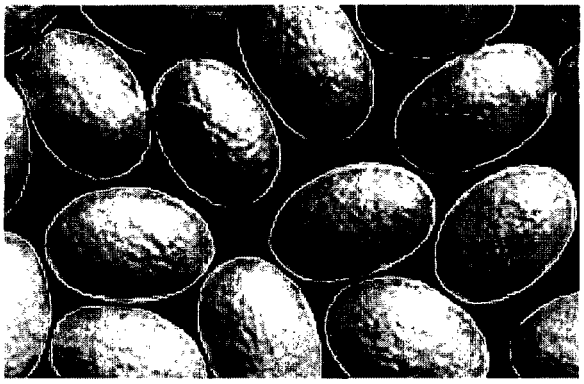


Fig.6b.

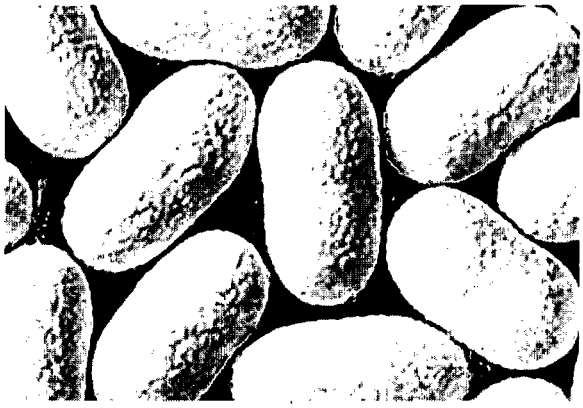


Fig.6c

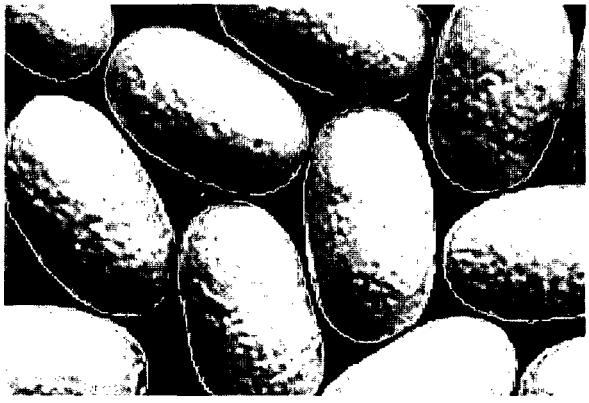


Fig.7a.

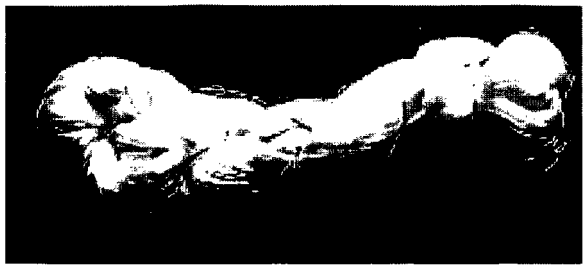


Fig.7b.

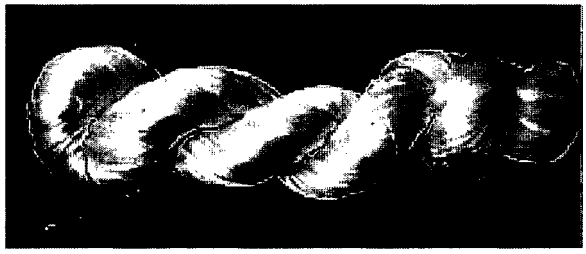


Fig.7c

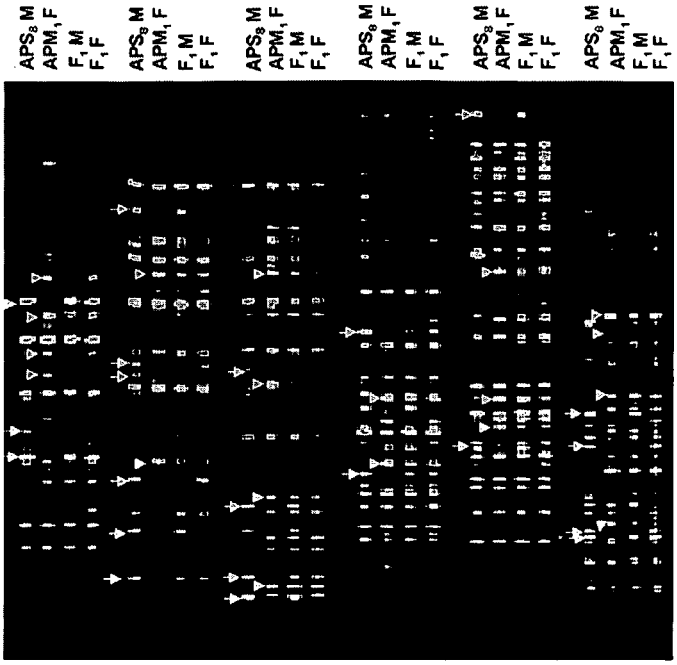


Fig. 8

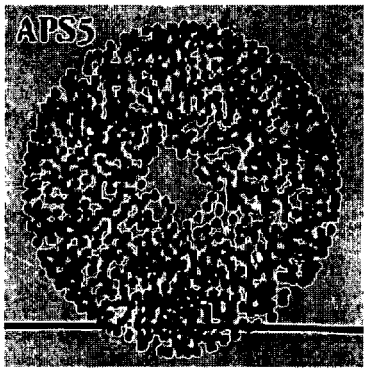


Fig. 9a

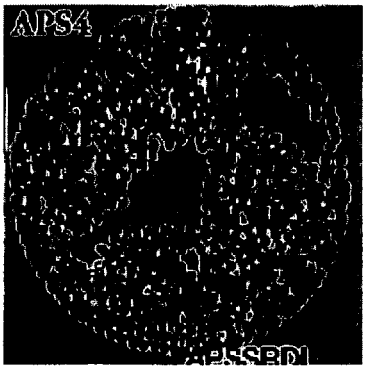


Fig 9b

Fig 9c

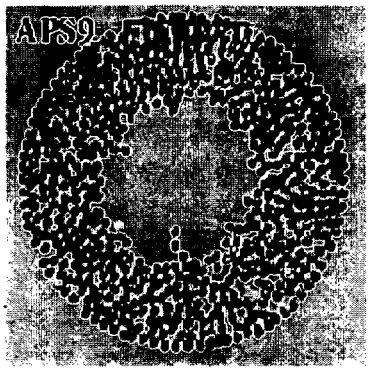
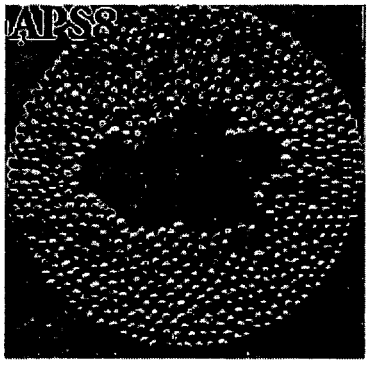


Fig 9d



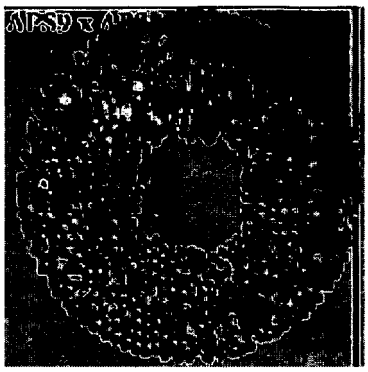


Fig. 9e

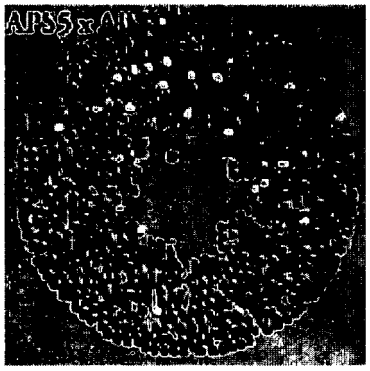


Fig 9f

Fig.10a

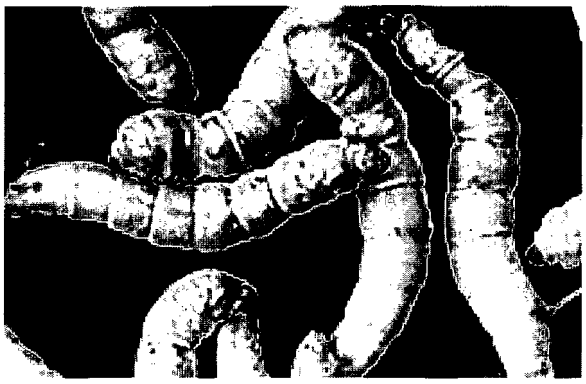


Fig 10b

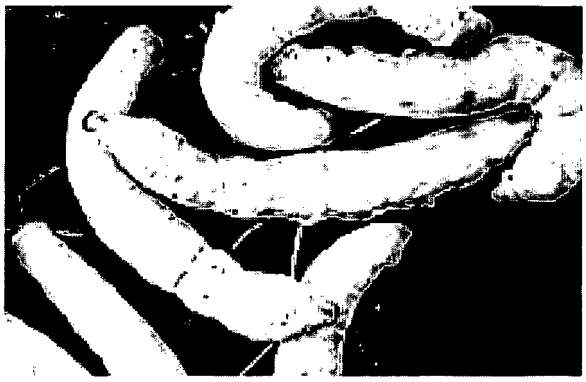
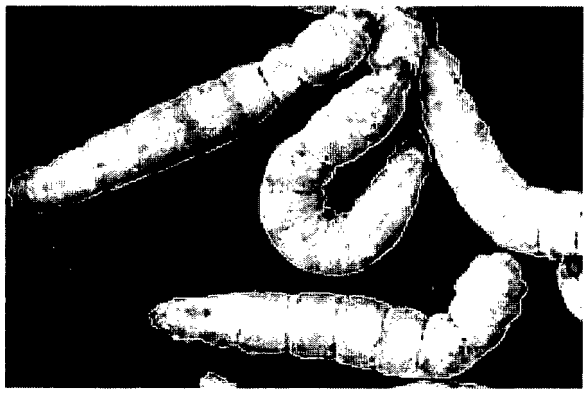


Fig. 10c



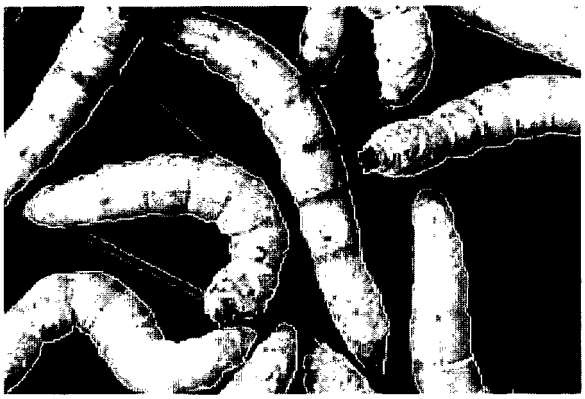


Fig. 10d

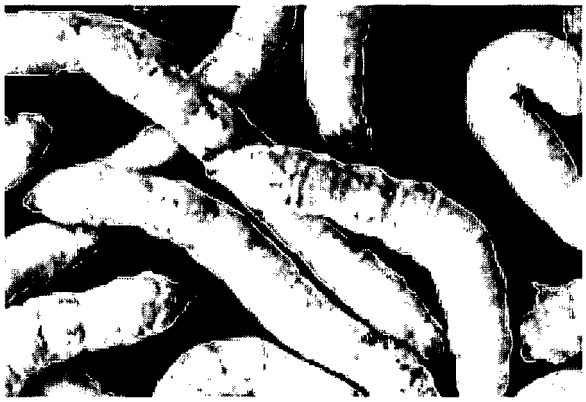


Fig. 10e.

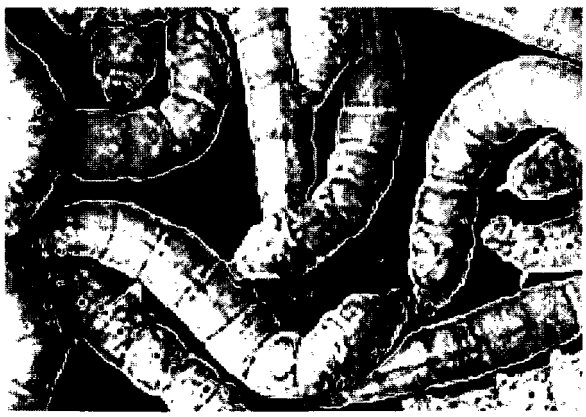


Fig 10f

Fig 11a

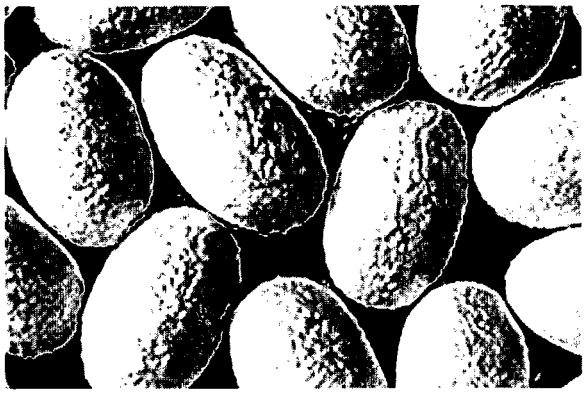


Fig 11b

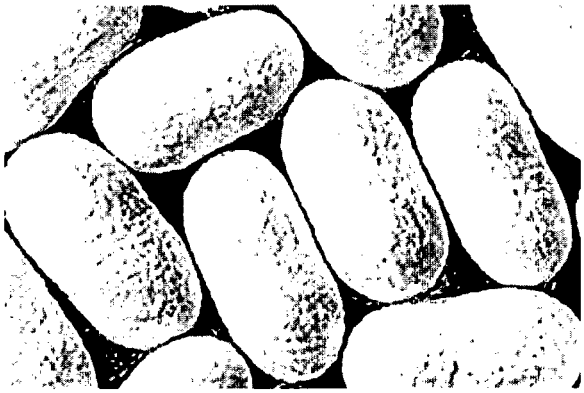


Fig 11c

Fig 11d

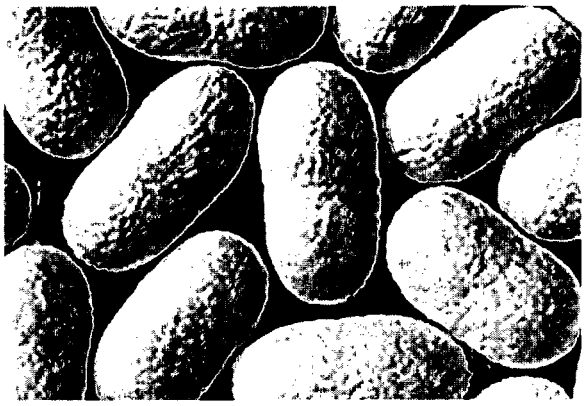


Fig 11e

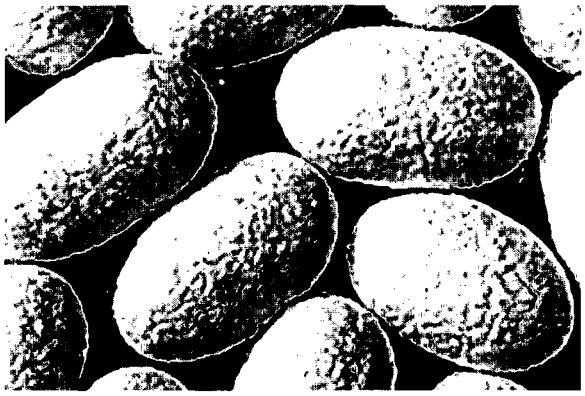


Fig 11f

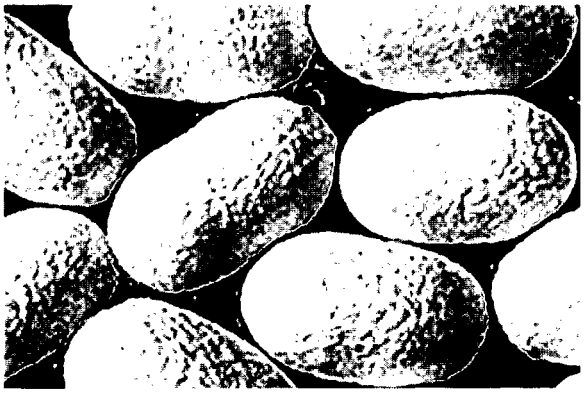


Fig 12a

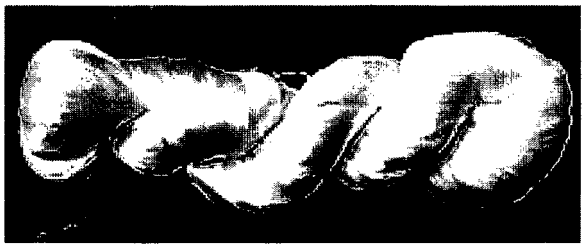


Fig 12b

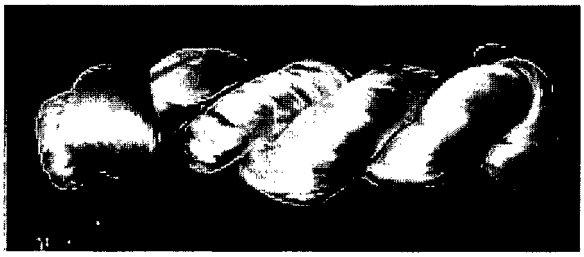


Fig 13

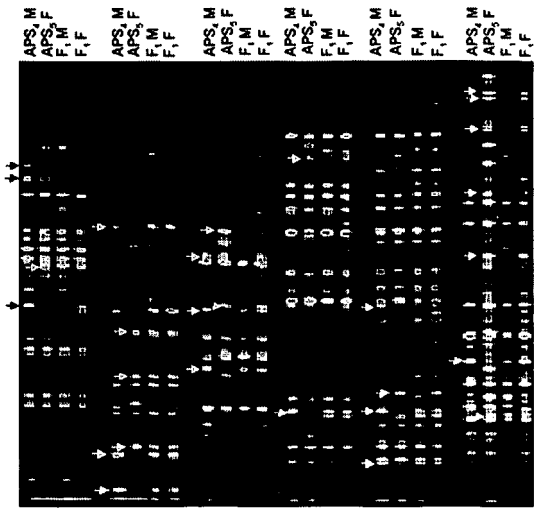


Fig 14