To assess the extent of Pollen Flow from BGII-cotton to non transgenic cotton at three locations

Evaluation of cross pollination in between BGII and non-BGII cotton in the presence of honey bee pollination agents

Protocol 3 - Report

Submitted to RCGM
Date: April, 2003

MAHARASHTRA HYBRID SEEDS COMPANY, LTD
Resham Bhavan, 4th Floor, 78 Veer Nariman Road, Mumbai 400 020, India

Study title:

Pollen Flow Study on transgenic cotton Expressing *Cry1A (c)* & *Cry2A(b)* gene (BGII) at Three Locations

Objective:

To evaluate cross-pollination between BGII and non-BGII cotton in the presence of Honeybee pollination agents.

Study Conducted at :

1. Maharashtra Hybrid Seeds Co. Ltd., Jamwadi Farm, Jalna (Survey No. 198, Taluka Jalna, District Jalna, Maharash
2. DPPL Farm, Anandnagar (Survey No. 58 & 61, Mandal-Bodhan, Dist- Nizamabad, A.P.)
3. Mahyco R & D Farm (Meena winery) Village: Kavvaguda, Mandal: Shamshabad, Dist:
4. Rangareddy.S.No.446 and 447.

Duration of study:

1. 22nd July,2002 to 02nd February, 2003
2. 25th July,2002 to 15th February, 2003
3. 24th July,2002 to 08th February, 2003

Methods:

A. The approved protocol for this study is given in Annexure I-A for Jamwadi and Shamshabad locations. Transgenic BGII cotton, homozygous for the two loci was planted in a central plot measuring 20 m X 20 m, surrounded by non-transgenic cotton in 5 m X 5 m blocks in all four directions, starting from 1 meter to 55 meters from the central plot. Each of these blocks were composed of 5 rows, 1 meter apart. The BGII cotton line planted in the central plot had a visible genetic marker, i.e., normal leaf, while the non-transgenic pollen trap plants were of okra leaf phenotype. This arrangement was incorporated in the experiment in order to be able to score cross-pollination events in a grow-out-test of the seeds from the pollen trap plants. A 50 meter isolation distance was maintained for this plot.

B. A second approved protocol (Annexure I-B) was adopted for DPPL, Nizamabad location. Transgenic BGII cotton, homozygous for the two BGII loci was planted in a central plot measuring 20 m X 20 m, surrounded by non-transgenic cotton planted in concentric rows. Immediately adjacent to, i.e. 1m to 5 m from the transgenic BGII block, there will be 5 concentric 1 m apart rows planted with non transgenic line having okra leaf character. These five rows are indicated in the enclosed schematic design by solid gray fill. Additionally at a distance of 10 m to 50 m from the central transgenic block 9 concentric 5 m apart rows were grown with the same okra leaf type non transgenic cotton in accordance with the schematic design of the plot enclosed.

To facilitate cross pollination, four honey bee hives containing active colonies were placed in the four corners of the central transgenic cotton plot. Photographs of the plot taken from all four directions are given in Figures 1 to 4.

Figure 1: View towards North East of the pollen flow experimental field (Shamshabad)

Figure 2: View towards North West of the pollen flow experimental field (Shamshabad)

Figure 3: View towards South East of the of pollen flow experimental field (Shamshabad)

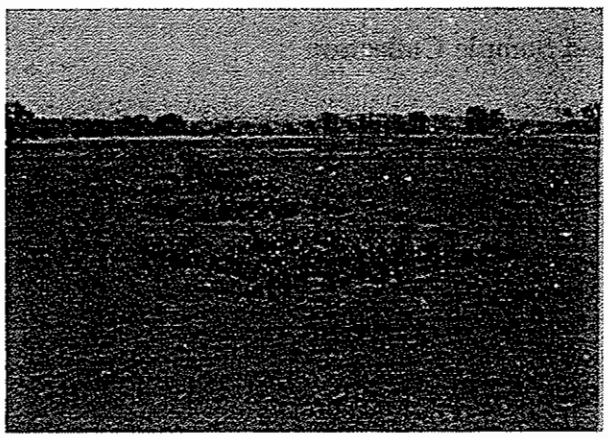


Figure 4: View towards South West side the pollen flow experimental field (Shamshabad)

Honey bee activity was periodically monitored to ensure the presence of adequate numbers of the cross-pollinator insect. Figures 5 and 6 show honey bees visiting the transgenic plot and the non-transgenic plot respectively.

Figure 5: Honeybee visiting BGII Cotton plant (Normal Leaf) in the central plot

Figure 6: Honeybee visiting okra leaf plant in a pollen trap block

Weather data was periodically recorded at the experimental site. Monthly averages of weather data for Jamwadi location is given in Annexure II.

Seed Sample Collection :

A. **Jamwadi and Shamshabad locations :** Seeds were collected seperately from each row of the first block, i.e., 1 meter to 5 meters distance from the BGII cotton plot and as single bulks from each of the distal (second to the eleventh) blocks. Three random samples were drawn from each of the above collected seed lots. Thus there were 60 samples (15 per block X 4 directions) for the first set of blocks and another 120 samples (3 per block X 10 blocks X 4 directions) for the second to eleventh set of blocks. These sample lots were subjected to a grow-out-test for scoring semi-okra leaf type plants. Also a polymerase chain reaction (PCR) analysis was done on these sample lots, to detect presence of the BGII *(cry1Ac & cry2Ab genes)* in the progeny of the pollen trap plants.

B. **DPPL location :** Each row from all four directions were harvested separately as Individual bulks and seed samples were drawn from each bulk. Each sample was tested for the assessment of pollen flow by grow out test (GOT) and BGII contamination by PCR.

Grow-out test :

Fifty seeds per sample were planted in a row and the seedlings were allowed to grow and the number of semi-okra plants that were seen in each row were counted. This gave an indication of cross pollination from the normal leaf transgenic BGII cotton plot to the okra-leaf non-transgenic pollen trap blocks.

The grow out test (GOT) was performed at the Mahyco R & D Kalegaon farm. Each sample population was planted in a single row. Thus a total of 180 rows were planted with 50 seedlings in each.

PCR analysis :

20 seeds per sample were germinated and the pooled DNA from the same was extracted. Polymerase chain reaction was performed with primers specific to the Bt *cry1Ac & cry2Ab genes.*

The nucleotide sequence of the primers used for the *cry1Ac* gene detection were:
5' GCC AAT GCC TCG TGA TTG TTC TCT GC 3' (forward primer)
5' GAT TTG CGA GGC TGG CCA GCT CCA CG 3' (reverse primer)

The nucleotide sequence of the primers used for the cry2Ab gene detection were:
5' –CGG TGT CAT CTA TGT TAC TAG ATC- 3' (forward primer)
5' –TCT TCT TTC TAT AGT GGT CTC CC –3' (reverse primer)

As a positive control, a known BGII positive cotton plant DNA mixed 1:20 with non- transgenic cotton plant DNA, was used in PCR with the same primers as the other samples. The PCR products were run in a 1% agarose gel stained with ethidium bromide and the images of the gels were recorded in a computerised gel-image documentation system. The expected amplified fragments of the *cry1Ac and cry2Ab genes* are 260 bp in length.The negative control in this case was non-transgenic cotton DNA.

Results:

The result of the grow-out-test for all the three locations are summarized in Table No.1A to 1C , and the result of the PCR analysis are shown as gel-image documents in Annexure-III and the same is summarized in Table No. 2A to 2C. Events of cross-pollination for both *cry1Ac* and *cry2Ab gene* were detected up to the third block, i.e., a distance of 15 meters, both by the grow-out-test and pooled sample PCR. The data of the latter by and large corroborated those of the former, giving credence to the sampling procedure adopted in this study.

The extent of cross pollination observed, from the BGII-cotton plot to the non-BGII cotton, ranged from averaged 0 to 2.66 % in any given row within the first 5 meters of the transgenic plot. For individual samples of 50 seeds, the range varied from 0 to as high as 4 %. The cross-pollination frequencies for the 6 to 10 meter block ranged from 0 (Jamwadi and DPPL) to 4 % (Shamshabad) for a given block, while the average for all four blocks at Shamshabad at this distance interval was 0.33% In the third block, i.e., 11 to 15 meters, the average cross pollination recorded for this distance interval was 0.33% at Shamshabad alone, the range being from 0 to 1.33 % for individual blocks.

Conclusions and Discussion :

Both the grow-out test and the PCR test on the progeny samples drawn randomly from the pollen-trap blocks, indicate a detectable level of pollen flow from the central transgenic plot upto Block 3 (Shamshabad location) of the experiment. While there are differences in the observed cross-fertilisation events among some of the direction-wise blocks, when comparing the grow-out test and the PCR test data, the distance limit of detectable dispersal is common in both these cases. These differences may be attributed to the chance presence of individuals arising out of cross-fertilisation, in the various samples. The sample size of 50 seeds X 3 samples per distance point scored, in the case of grow-out test was chosen in order to give a limit of detection approximately 0.67%. This figure is arrived at by applying the formula: 1 event out of 50 X 3 samples, for a given distance point in a particular direction sampled. All the four directions taken together lowers the limit of detection to 1 event in 600 individuals sampled, i.e., approximately 0.16%.

Based on this rationale, it may be concluded that in this particular experiment pollen flow from the transgenic BGII cotton source could be detected upto a distance of 15 meters. Beyond this distance, instances of pollen flow were not observed, subject to the 1 in 150 samples (0.66%) limit of detection for a given direction, or 1 in 600 (0.16%) samples in the case of all four directions taken together. Within the first 15 meters from the transgenic block, appreciable cross pollination took place in the first block, i.e., upto 5 meters, where-in an range of 0 to 2.66% was observed in individual rows. The average out crossing over all three locations noticed for all the four blocks taken together in the 1 meter to 5 meters distance interval was 3.0%

In the second block i.e between 6 and 10 meters, a reduced range of 0 to 4% cross-pollination frequency was observed in individual blocks over the three locations. The over all average out crossing noticed for all the four blocks taken together in the 6 to 10 meters distance interval was 0.11%. In the third block, the range of observed cross pollination frequencies was nil to 0.67% was observed in individual blocks, while the over all average for all the four blocks taken together in the 11 meter to 15 meters distance interval was 0.11% over all the locations.

Annexure I-A

Protocol 2

To assess the pollen escape in Cotton containing (BGII) expressing *Cry1A(c)* and *Cry2A(b) gene* in open environment by Honey bees.

Objective :

The trial will be conducted to evaluate cross-pollination in between BGII and non-BGII cotton in presence of Honeybee on pollination agent (Entamophilous).

Locations :

Mahyco farms located at Jalna (Maharashtra) and Shamshabad (Andhra Pradesh)

Materials and Methods :

This experiment will be conducted at two locations namely, Jalna in Maharshtra and Shamshabad, Andhra Pradesh. A compact block of BGII cotton of normal leaf in the area of 20m x 20 m will be planted in the center. On four sides of this block at a distance of five meter of each 10 blocks of 5m x 5m area will be grown with non-BGII cotton of okra leaf type, in accordance with the design of the plot enclosed. The first four square blocks indicated in the drawing by the letter A, B, C & D non BGII Cotton of okra leaf shall be planted at a distance of 1 m each in the row extending up to 5 m (5 rows) in each block. To facilitate pollen dispersal four beehives shall be installed and maintained during the course of experiment. Each block other than the first four blocks adjacent to the BGII Cotton block will be harvested separately, and seed sample will be drawn from the bulk of each block. Each drawn sample shall be tested for the assessment and extend of BGII contamination by PCR and grow out test. There will be another 40 blocks from which seeds would be sampled and tested. From each block minimum three seed samples be drawn and tested. Regarding the first four adjacent block seeds from each row will be collected and polled together during difference times of the full harvesting season. There would be five samples from each adjacent block representing each row. Three experimental samples from the boll of each row will be drawn and the extent of cry1A(c) & cry2Ab (BGII) contamination by PCR and grow out tests will be conducted. In summary there would be 20 rows in the first four adjacent square block comprising 60 samples to be tested and from the remaining 40 square blocks there would be 120 samples which are also to be tested as above. All the experimental information shall be recorded.

Observations:

Drawn samples shall be tested for the assessment and extent of BGII contamination by PCR and grow out test. In grow out test, presence of Semi-Okra plants will be indicative of cross pollination which will subsequently be confirmed by PCR method. The percentage of BGII Contamination will be determined at minimum and maximum distances.

Annexure I-B

Protocol- 3

Assessment of pollen flow from transgenic BGII Cotton containing *Cry1A(c)* and *Cry-2A(b)* genes, in open environment by honey bees.

Objective :

The trial will be conducted to evaluate cross-pollination in between BGII and non-BGII cotton in presence of Honeybee on pollination agent (Entamophilous).

Locations:

Mahyco farms located at DPPL Nizamabad (Andhra Pradesh)

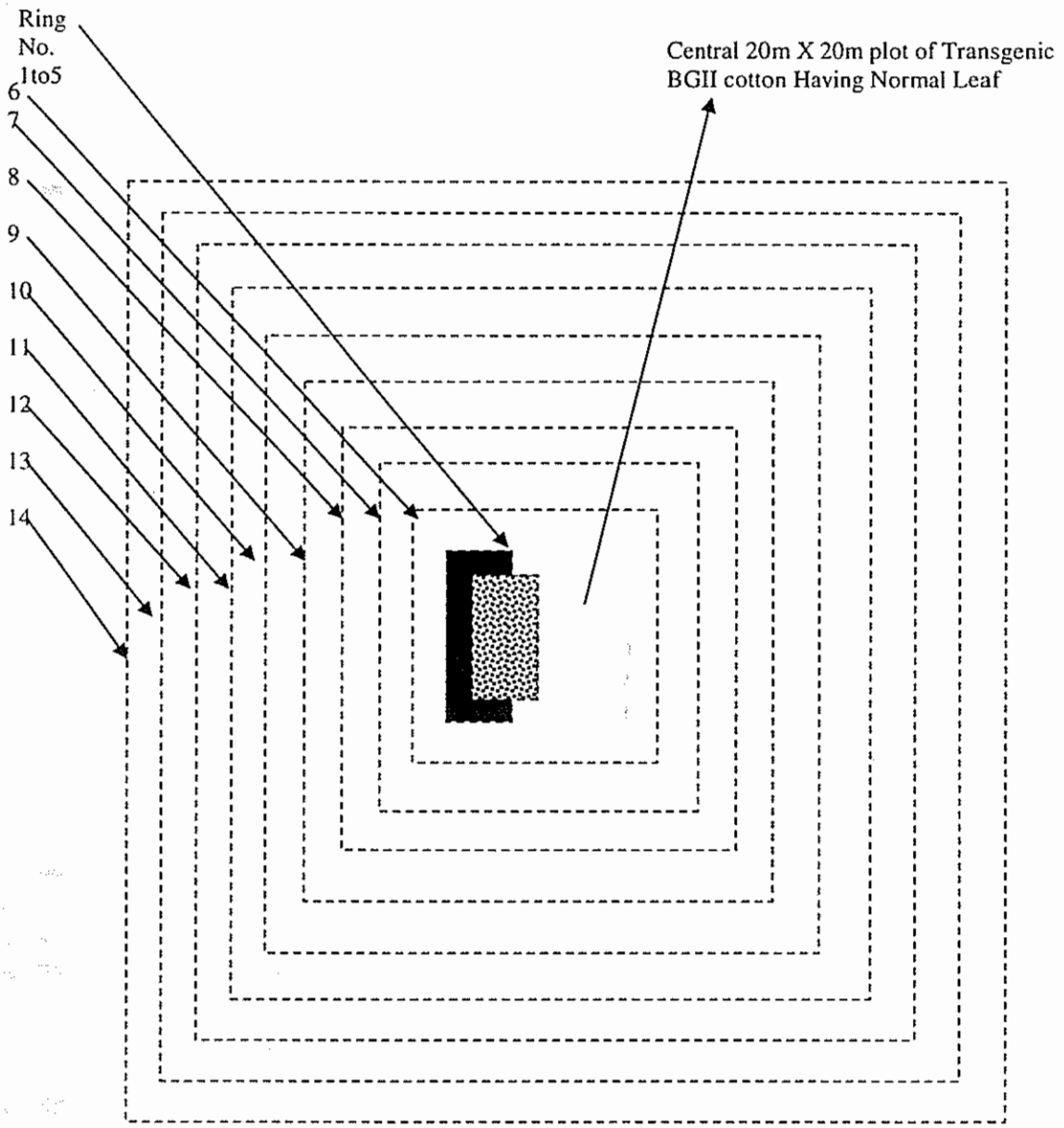
Materials and Methods :

This experimental protocol will be followed in one location, namely DPPL Nizamabad, Andhra Pradesh. A compact block of BGII cotton of normal leaf type in the area of 20m x 20m will be planted in the center. Immediately adjacent to, i.e. 1m to 5 m from the transgenic BGII block, there will be five concentric 1 meter apart rows planted with non-transgenic cotton line having Okra Leaf character. These 5 rows are indicated in the enclosed schematic design by solid gray fill. Additionally, at a distance of 10 meters to 50 meters from the central transgenic block, 9 concentric 5 meter apart rows will be grown with the same okra leaf type non- transgenic cotton, in accordance with the schematic design of the plot enclosed. To facilitate pollen dispersal, four beehives shall be installed at the four corners of the central BGII cotton plot and maintained during the course of experiment. Each row from each 4 direction will be harvested separately as individual bulks and seed sample will be drawn from each bulk. Each drawn sample shall be tested for the assessment of pollen flow by grow out test (GOT) and BGII contamination by PCR. Thus there will be 4 x 14 =54 pools from the 14 rows from which seeds would be sampled. From each of these row wise pools, three sample populations will be drawn and tested for the extent of cross pollination from the BGII plants by grow out tests and PCR. In summary there would be 60 samples to be tested from the 5 proximal rows in the first 5 meters immediately adjacent square block, and from the remaining 9 distal rows at a distance of 10 to 50 meters, there would be a total of 108 samples. All the experimental information shall be recorded.

Observations:

Drawn samples will be tested for the extent of pollen flow from the central BGII plot by grow out test, followed by PCR. In the grow out test, presence of Semi-Okra leaved plants will be indicative of cross pollination which will subsequently be confirmed by PCR method. The percentage of pollen flow will be determined for all the tested distances from the BGII cotton plot.

FIELD LAYOUT (not to scale) PLAN FOR STUDY OF POLLEN DISPERSAL BY HONEY BEES FROM CRY-X (BGII) COTTON AS PER PROTOCOL 3



Non-Transgenic Okra-Leaf Cotton Plants Forming 14 Concentric Pollen-Trap Rows:
Nos. 1to 5 are 1 meter apart and
Nos.6to14 are 5 meter apart

Location: Mahyco farm,
DPPL,Nizamabad
Andhra Pradesh

Observations :

Drawn samples shall be tested for the assessment and extent of BGII contamination by PCR and grow out test. In grow out test, presence of Semi-Okra plants will be indicative of cross pollination which will subsequently be confirmed by PCR method. The percentage of BGII. Contamination will be determined at minimum and maximum distances.

ANNEXURE II

Weather Data at Jamwadi Farm During the Pollen Flow Experiment of Kharif- 2002

Date of Sowing : 22nd July 2002.
Crop Duration :160 days

Month	Temperature Mini - Maxi	Relative Humidity(%)	Rainfall (in)
June – 02	26.04 - 32.41	67.73	9.77
July-02	25.16 - 30.97	69.9	3.13
August-02	24.02 - 27.76	82.42	8.5
September-02	24.05 - 30.96	71.6	2.32
October-02	24.22 - 32.03	52.69	2.08
November-02	20.42 - 28.79	48.83	0.06
December- 02	15.36 - 28.91	47.23	0.0
January - 03	13.95 - 28.97	46.95	0.0
February - 03	18.49 - 31.02	45.78	0.0

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MAHARASHTRA HYBRID SEEDS COMPANY, LTD
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Duration of study:

1. 22nd July,2002 to 02nd February, 2003
2. 25th July,2002 to 15th February, 2003
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Methods:

A. The approved protocol for this study is given in Annexure I-A for Jamwadi and Shamshabad locations. Transgenic BGII cotton, homozygous for the two loci was planted in a central plot measuring 20 m X 20 m, surrounded by non-transgenic cotton in 5 m X 5 m blocks in all four directions, starting from 1 meter to 55 meters from the central plot. Each of these blocks were composed of 5 rows, 1 meter apart. The BGII cotton line planted in the central plot had a visible genetic marker, i.e., normal leaf, while the non-transgenic pollen trap plants were of okra leaf phenotype. This arrangement was incorporated in the experiment in order to be able to score cross-pollination events in a grow-out-test of the seeds from the pollen trap plants. A 50 meter isolation distance was maintained for this plot.

B. A second approved protocol (Annexure I-B) was adopted for DPPL, Nizamabad location. Transgenic BGII cotton, homozygous for the two BGII loci was planted in a central plot measuring 20 m X 20 m, surrounded by non-transgenic cotton planted in concentric rows. Immediately adjacent to, i.e. 1m to 5 m from the transgenic BGII block, there will be 5 concentric 1 m apart rows planted with non transgenic line having okra leaf character. These five rows are indicated in the enclosed schematic design by solid gray fill. Additionally at a distance of 10 m to 50 m from the central transgenic block 9 concentric 5 m apart rows were grown with the same okra leaf type non transgenic cotton in accordance with the schematic design of the plot enclosed.

To facilitate cross pollination, four honey bee hives containing active colonies were placed in the four corners of the central transgenic cotton plot. Photographs of the plot taken from all four directions are given in Figures 1 to 4.

Figure 1: View towards North East of the pollen flow experimental field (Shamshabad)

Figure 2: View towards North West of the pollen flow experimental field (Shamshabad)

Figure 3: View towards South East of the of pollen flow experimental field (Shamshabad)

Figure 4: View towards South West side the pollen flow experimental field (Shamshabad)

Honey bee activity was periodically monitored to ensure the presence of adequate numbers of the cross-pollinator insect. Figures 5 and 6 show honey bees visiting the transgenic plot and the non-transgenic plot respectively.

Figure 5: Honeybee visiting BGII Cotton plant (Normal Leaf) in the central plot

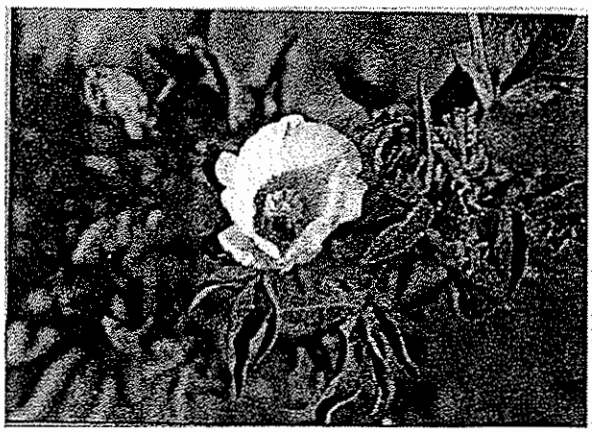

Figure 6: Honeybee visiting okra leaf plant in a pollen trap block

Weather data was periodically recorded at the experimental site. Monthly averages of weather data for Jamwadi location is given in Annexure II.

Seed Sample Collection :

A. **Jamwadi and Shamshabad locations :** Seeds were collected seperately from each row of the first block, i.e., 1 meter to 5 meters distance from the BGII cotton plot and as single bulks from each of the distal (second to the eleventh) blocks. Three random samples were drawn from each of the above collected seed lots. Thus there were 60 samples (15 per block X 4 directions) for the first set of blocks and another 120 samples (3 per block X 10 blocks X 4 directions) for the second to eleventh set of blocks. These sample lots were subjected to a grow-out-test for scoring semi-okra leaf type plants. Also a polymerase chain reaction (PCR) analysis was done on these sample lots, to detect presence of the BGII *(cry1Ac & cry2Ab genes)* in the progeny of the pollen trap plants.

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As a positive control, a known BGII positive cotton plant DNA mixed 1:20 with non- transgenic cotton plant DNA, was used in PCR with the same primers as the other samples. The PCR products were run in a 1% agarose gel stained with ethidium bromide and the images of the gels were recorded in a computerised gel-image documentation system. The expected amplified fragments of the *cry1Ac and cry2Ab genes* are 260 bp in length.The negative control in this case was non-transgenic cotton DNA.

Results:

The result of the grow-out-test for all the three locations are summarized in Table No.1A to 1C , and the result of the PCR analysis are shown as gel-image documents in Annexure-III and the same is summarized in Table No. 2A to 2C. Events of cross-pollination for both *cry1Ac* and *cry2Ab gene* were detected up to the third block, i.e., a distance of 15 meters, both by the grow-out-test and pooled sample PCR. The data of the latter by and large corroborated those of the former, giving credence to the sampling procedure adopted in this study.

The extent of cross pollination observed, from the BGII-cotton plot to the non-BGII cotton, ranged from averaged 0 to 2.66 % in any given row within the first 5 meters of the transgenic plot. For individual samples of 50 seeds, the range varied from 0 to as high as 4 %. The cross-pollination frequencies for the 6 to 10 meter block ranged from 0 (Jamwadi and DPPL) to 4 % (Shamshabad) for a given block, while the average for all four blocks at Shamshabad at this distance interval was 0.33% In the third block, i.e., 11 to 15 meters, the average cross pollination recorded for this distance interval was 0.33% at Shamshabad alone, the range being from 0 to 1.33 % for individual blocks.

Conclusions and Discussion :

Both the grow-out test and the PCR test on the progeny samples drawn randomly from the pollen-trap blocks, indicate a detectable level of pollen flow from the central transgenic plot upto Block 3 (Shamshabad location) of the experiment. While there are differences in the observed cross-fertilisation events among some of the direction-wise blocks, when comparing the grow-out test and the PCR test data, the distance limit of detectable dispersal is common in both these cases. These differences may be attributed to the chance presence of individuals arising out of cross-fertilisation, in the various samples. The sample size of 50 seeds X 3 samples per distance point scored, in the case of grow-out test was chosen in order to give a limit of detection approximately 0.67%. This figure is arrived at by applying the formula: 1 event out of 50 X 3 samples, for a given distance point in a particular direction sampled. All the four directions taken together lowers the limit of detection to 1 event in 600 individuals sampled, i.e., approximately 0.16%.

Based on this rationale, it may be concluded that in this particular experiment pollen flow from the transgenic BGII cotton source could be detected upto a distance of 15 meters. Beyond this distance, instances of pollen flow were not observed, subject to the 1 in 150 samples (0.66%) limit of detection for a given direction, or 1 in 600 (0.16%) samples in the case of all four directions taken together. Within the first 15 meters from the transgenic block, appreciable cross pollination took place in the first block, i.e., upto 5 meters, where-in an range of 0 to 2.66% was observed in individual rows. The average out crossing over all three locations noticed for all the four blocks taken together in the 1 meter to 5 meters distance interval was 3.0%

In the second block i.e between 6 and 10 meters, a reduced range of 0 to 4% cross-pollination frequency was observed in individual blocks over the three locations. The over all average out crossing noticed for all the four blocks taken together in the 6 to 10 meters distance interval was 0.11%. In the third block, the range of observed cross pollination frequencies was nil to 0.67% was observed in individual blocks, while the over all average for all the four blocks taken together in the 11 meter to 15 meters distance interval was 0.11% over all the locations.

Annexure I-A

Protocol 2

To assess the pollen escape in Cotton containing (BGII) expressing *Cry1A(c)* and *Cry2A(b)* *gene* in open environment by Honey bees.

Objective :

The trial will be conducted to evaluate cross-pollination in between BGII and non-BGII cotton in presence of Honeybee on pollination agent (Entamophilous).

Locations :

Mahyco farms located at Jalna (Maharashtra) and Shamshabad (Andhra Pradesh)

Materials and Methods :

This experiment will be conducted at two locations namely, Jalna in Maharshtra and Shamshabad, Andhra Pradesh. A compact block of BGII cotton of normal leaf in the area of 20m x 20 m will be planted in the center. On four sides of this block at a distance of five meter of each 10 blocks of 5m x 5m area will be grown with non-BGII cotton of okra leaf type, in accordance with the design of the plot enclosed. The first four square blocks indicated in the drawing by the letter A, B, C & D non BGII Cotton of okra leaf shall be planted at a distance of 1 m each in the row extending up to 5 m (5 rows) in each block. To facilitate pollen dispersal four beehives shall be installed and maintained during the course of experiment. Each block other than the first four blocks adjacent to the BGII Cotton block will be harvested separately, and seed sample will be drawn from the bulk of each block. Each drawn sample shall be tested for the assessment and extend of BGII contamination by PCR and grow out test. There will be another 40 blocks from which seeds would be sampled and tested. From each block minimum three seed samples be drawn and tested. Regarding the first four adjacent block seeds from each row will be collected and polled together during difference times of the full harvesting season. There would be five samples from each adjacent block representing each row. Three experimental samples from the boll of each row will be drawn and the extent of cry1A(c) & cry2Ab (BGII) contamination by PCR and grow out tests will be conducted. In summary there would be 20 rows in the first four adjacent square block comprising 60 samples to be tested and from the remaining 40 square blocks there would be 120 samples which are also to be tested as above. All the experimental information shall be recorded.

Observations:

Drawn samples shall be tested for the assessment and extent of BGII contamination by PCR and grow out test. In grow out test, presence of Semi-Okra plants will be indicative of cross pollination which will subsequently be confirmed by PCR method. The percentage of BGII Contamination will be determined at minimum and maximum distances.

Annexure I-B

Protocol- 3

Assessment of pollen flow from transgenic BGII Cotton containing *Cry1A(c)* and *Cry-2A(b)* genes, in open environment by honey bees.

Objective :

The trial will be conducted to evaluate cross-pollination in between BGII and non-BGII cotton in presence of Honeybee on pollination agent (Entamophilous).

Locations:

Mahyco farms located at DPPL Nizamabad (Andhra Pradesh)

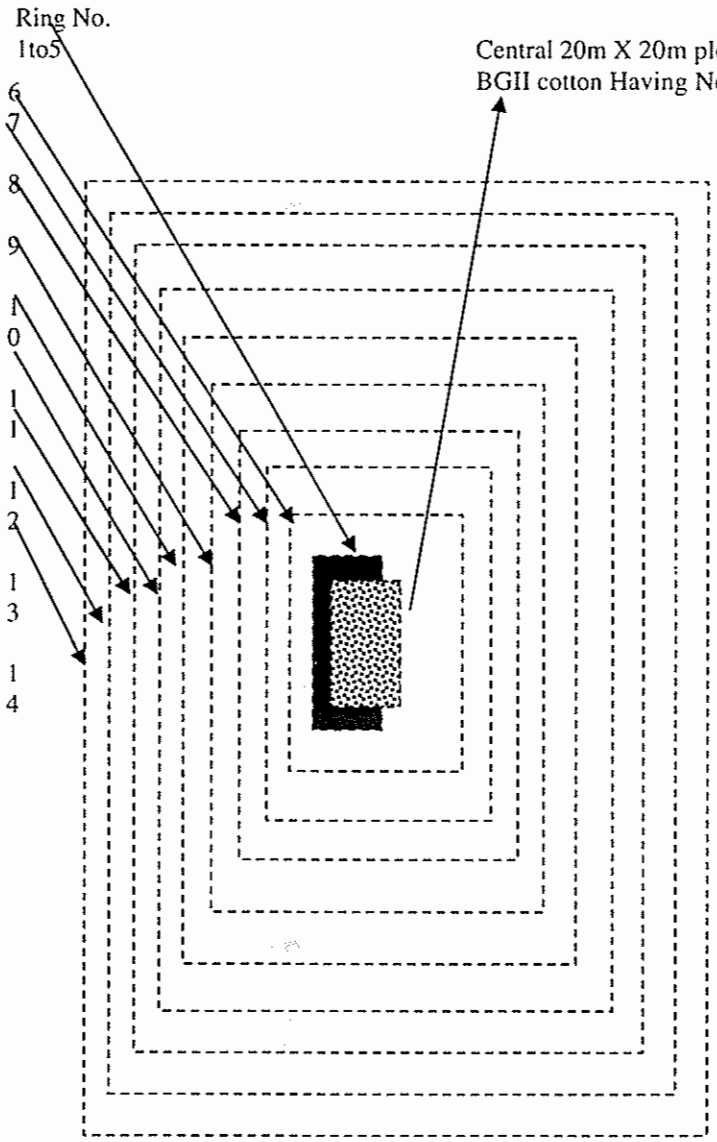
Materials and Methods :

This experimental protocol will be followed in one location, namely DPPL Nizamabad, Andhra Pradesh. A compact block of BGII cotton of normal leaf type in the area of 20m x 20m will be planted in the center. Immediately adjacent to, i.e. 1m to 5 m from the transgenic BGII block, there will be five concentric 1 meter apart rows planted with non-transgenic cotton line having Okra Leaf character. These 5 rows are indicated in the enclosed schematic design by solid gray fill. Additionally, at a distance of 10 meters to 50 meters from the central transgenic block, 9 concentric 5 meter apart rows will be grown with the same okra leaf type non- transgenic cotton, in accordance with the schematic design of the plot enclosed. To facilitate pollen dispersal, four beehives shall be installed at the four corners of the central BGII cotton plot and maintained during the course of experiment. Each row from each 4 direction will be harvested separately as individual bulks and seed sample will be drawn from each bulk. Each drawn sample shall be tested for the assessment of pollen flow by grow out test (GOT) and BGII contamination by PCR. Thus there will be 4 x 14 =54 pools from the 14 rows from which seeds would be sampled. From each of these row wise pools, three sample populations will be drawn and tested for the extent of cross pollination from the BGII plants by grow out tests and PCR. In summary there would be 60 samples to be tested from the 5 proximal rows in the first 5 meters immediately adjacent square block, and from the remaining 9 distal rows at a distance of 10 to 50 meters, there would be a total of 108 samples. All the experimental information shall be recorded.

Observations:

Drawn samples will be tested for the extent of pollen flow from the central BGII plot by grow out test, followed by PCR. In the grow out test, presence of Semi-Okra leaved plants will be indicative of cross pollination which will subsequently be confirmed by PCR method. The percentage of pollen flow will be determined for all the tested distances from the BGII cotton plot.

FIELD LAYOUT (not to scale) PLAN FOR STUDY OF POLLEN DISPERSAL BY HONEY BEES FROM CRY-X (BGII) COTTON AS PER PROTOCOL 3



Non-Transgenic Okra-Leaf Cotton Plants Forming 14 Concentric Pollen-Trap Rows:
Nos. 1to 5 are 1 meter apart and Nos.6to14 are 5 meter apart

Location: Mahyco farm,

DPPL,Nizamabad
Andhra
Pradesh

Observations :

Drawn samples shall be tested for the assessment and extent of BGII contamination by PCR and grow out test. In grow out test, presence of Semi-Okra plants will be indicative of cross pollination which will subsequently be confirmed by PCR method. The percentage of BGII. Contamination will be determined at minimum and maximum distances.

ANNEXURE II

Weather Data at Jamwadi Farm During the Pollen Flow Experiment of Kharif- 2002

Date of Sowing : 22nd July 2002.
Crop Duration :160 days

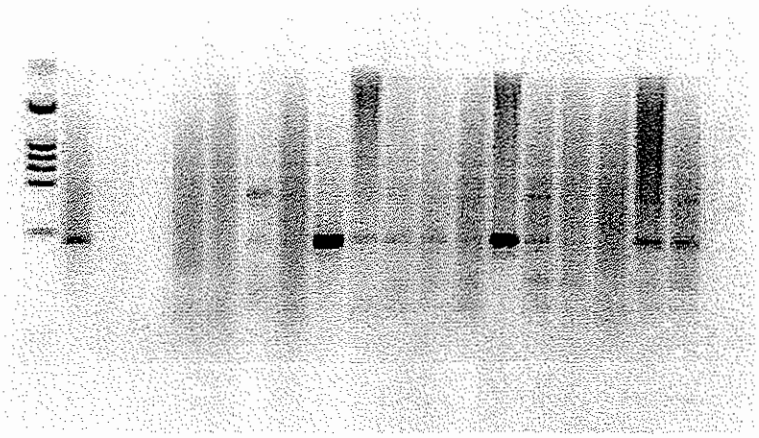
Month	Temperature Mini - Maxi	Relative Humidity(%)	Rainfall (in)
June – 02	26.04 - 32.41	67.73	9.77
July-02	25.16 - 30.97	69.9	3.13
August-02	24.02 - 27.76	82.42	8.5
September-02	24.05 - 30.96	71.6	2.32
October-02	24.22 - 32.03	52.69	2.08
November-02	20.42 - 28.79	48.83	0.06
December- 02	15.36 - 28.91	47.23	0.0
January - 03	13.95 - 28.97	46.95	0.0
February - 03	18.49 - 31.02	45.78	0.0

ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-1 (CRY 1Ac)

BLOCK A-1 JAMWADI

CONTROL

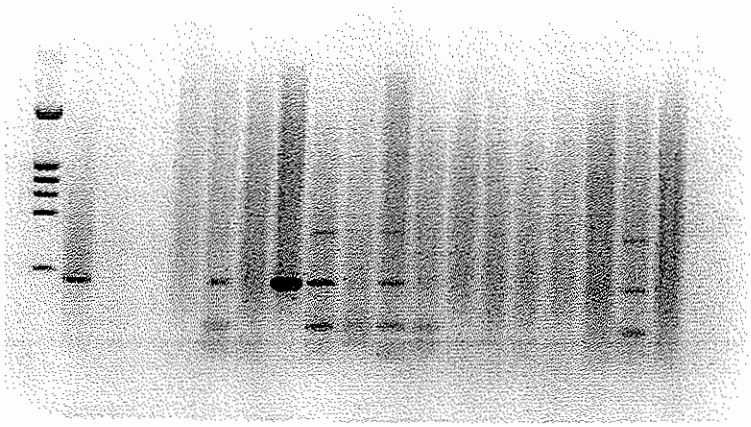
M +ve -ve w 1/1 ½ 1/3 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3



BLOCK B-1 JAMWADI

CONTROL

M +ve -ve w 1/1 ½ 1/3 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3

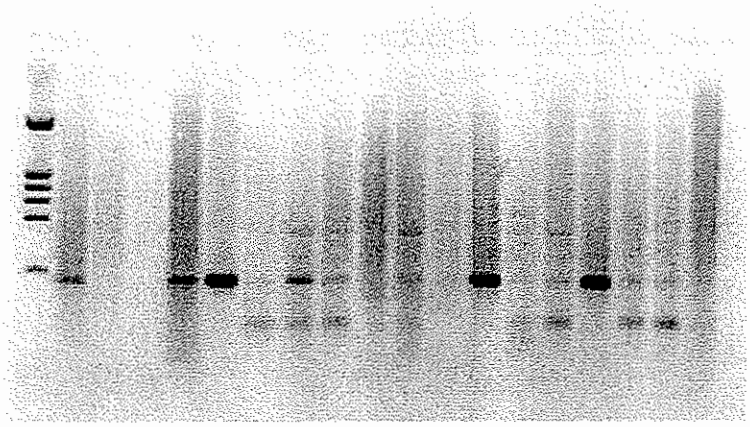


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-2 (CRY 1Ac)

BLOCK C-1 JAMWADI

CONTROL

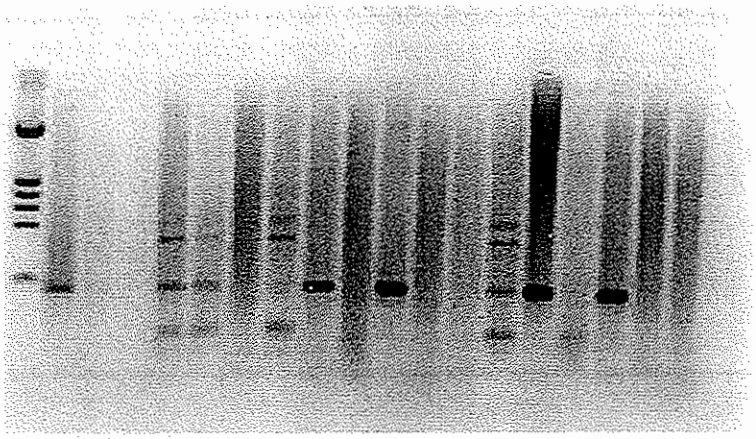
M +ve -ve w 1/1 ½ 1/3 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3



BLOCK D-1 JAMWADI

CONTROL

M +ve -ve w 1/1 ½ 1/3 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3

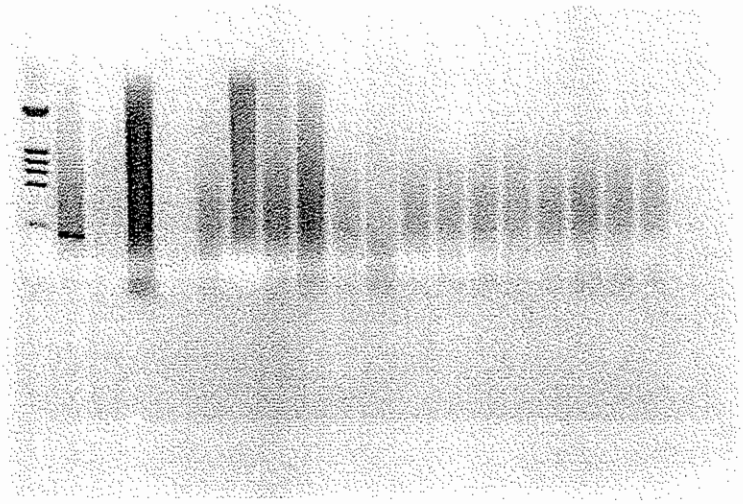


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-3 (CRY 1Ac)

BLOCK A- 2 to 7 Jamwadi .

CONTROL

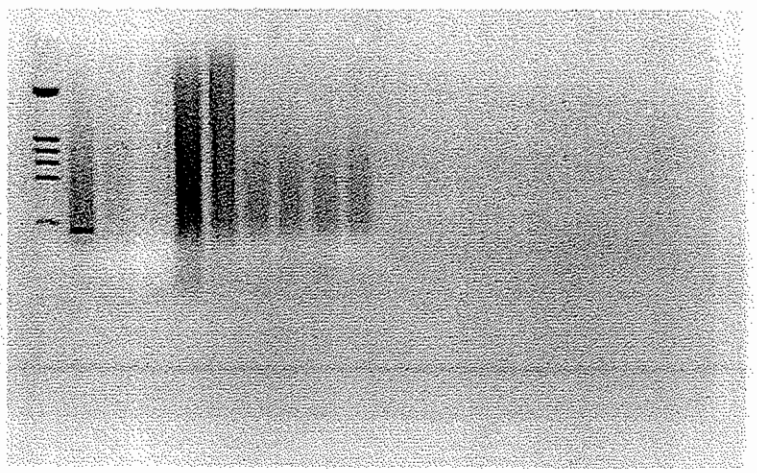
M +ve -ve w 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/3 5/1 5/2 5/3 6/1 6/2 7/1 7/2



BLOCK A- 8-9 Jamwadi

CONTROL

M +ve -ve w 7/3 8/1 8/2 8/3 9/1 9/2 9/3

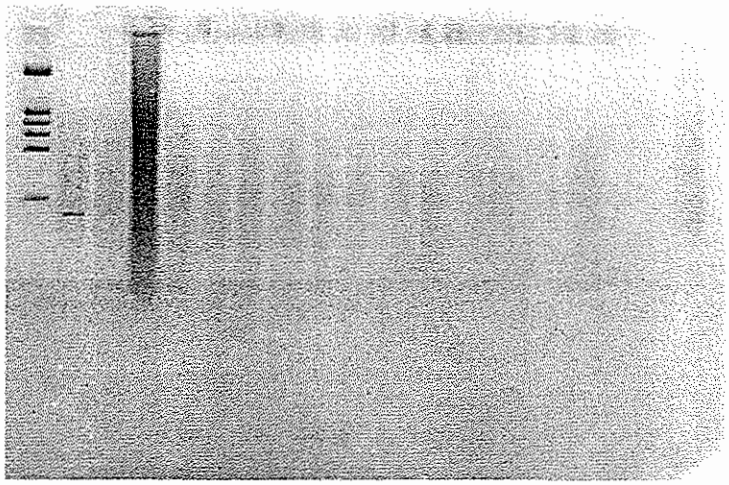


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-4 (CRY 1Ac)

BLOCK B – 2- 6 - Jamwadi

CONTROL

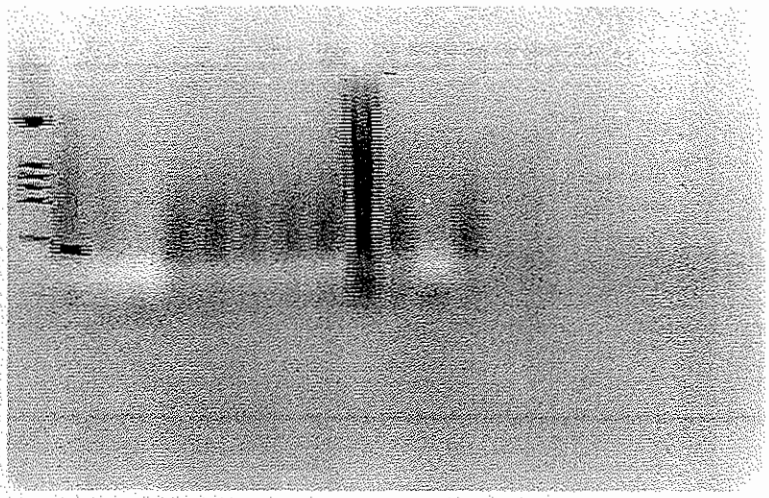
M +ve -ve w 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3 6/1 6/2 6/3



BLOCK B -7-9 Jamwadi

CONTROL

M +ve -ve w 7/1 7/2 7/3 8/1 8/2 8/3 9/1 9/2 9/3

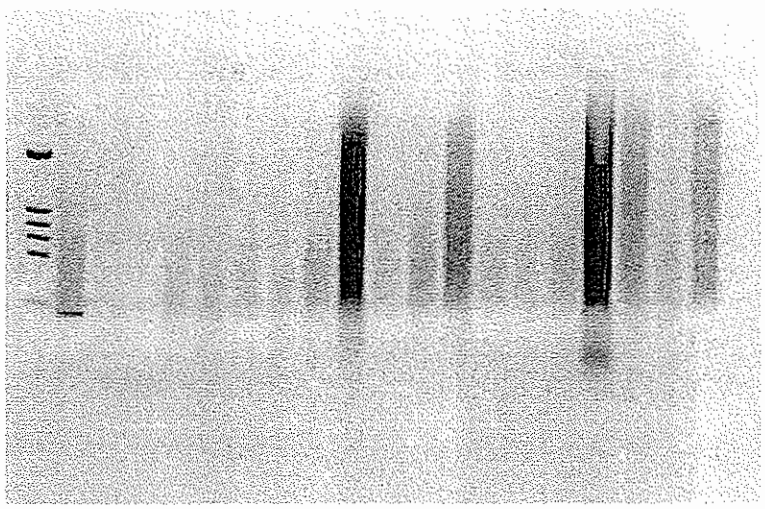


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-5 (CRY 1Ac)

BLOCK – C 2 - 7 - Jamwadi

CONTROL

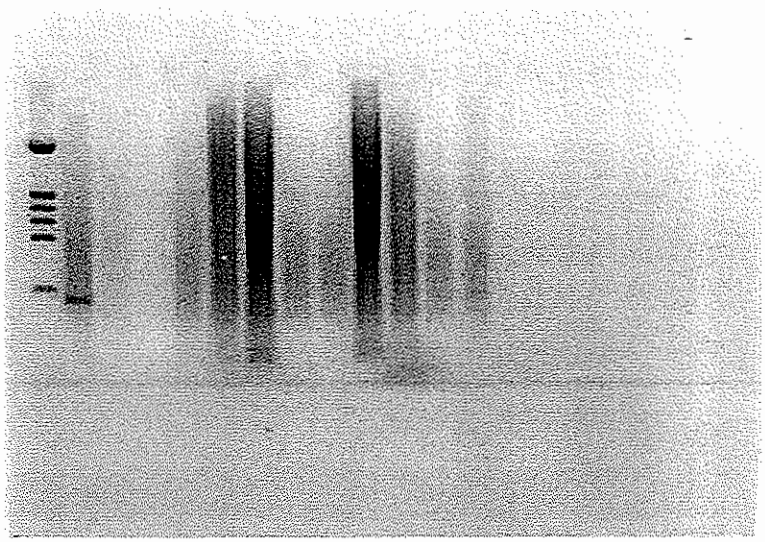
M +ve -ve w 2/1 2/2 2/3 3/1 3/3 4/1 4/2 4/3 5/1 5/2 5/3 6/1 6/2 6/3 7/1 7/2



BLOCK C – 7 -9 Jamwadi

CONTROL

M +ve -ve w 7/3 8/1 8/2 8/3 9/1 9/2 9/3

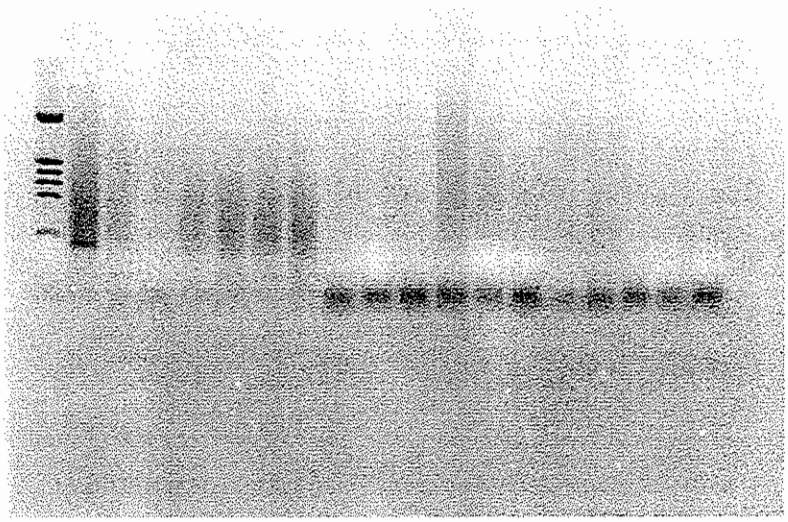


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-6 (CRY 1Ac)

BLOCK – D 2 - 7 - Jamwadi

CONTROL

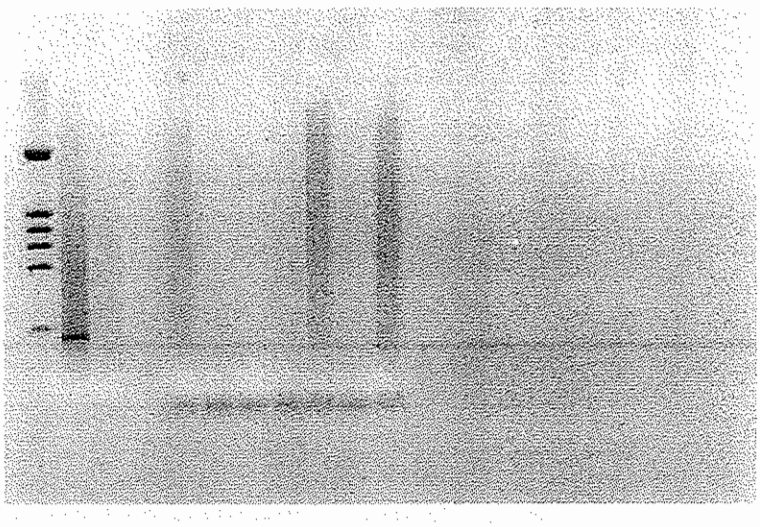
M +ve -ve w 2/1 2/2 2/3 3/1 3/3 4/1 4/2 4/3 5/1 5/2 5/3 6/1 6/2 6/3 7/1 7/2



BLOCK – D –7 - 9 Jamwadi

CONTROL

M +ve -ve w 7/3 8/1 8/2 8/3 9/1 9/2 9/3

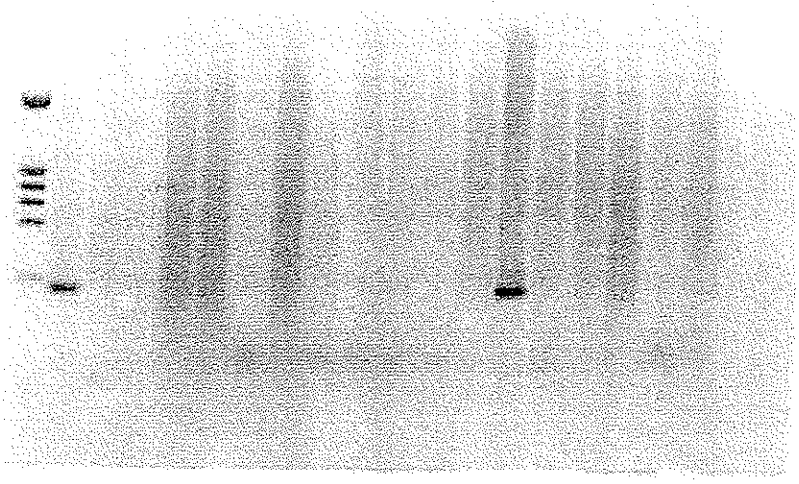


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-1 (CRY 2Ab)

BLOCK A-1 JAMWADI

CONTROL

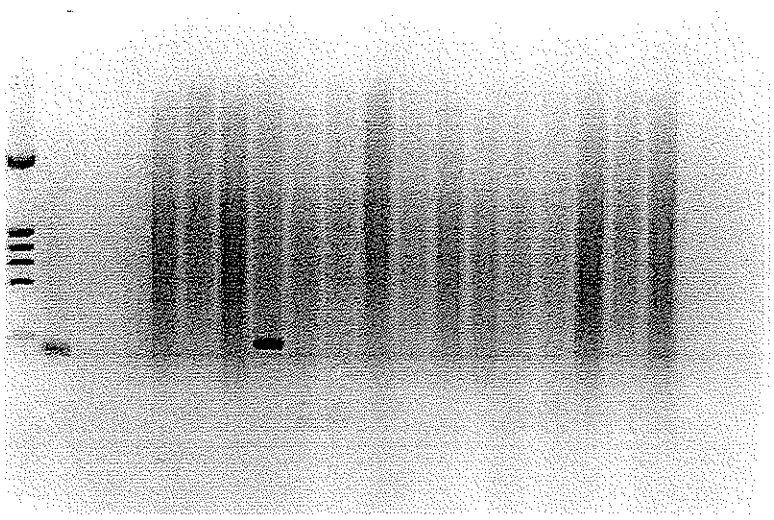
M +ve -ve w 1/1 ½ 1/3 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/



BLOCK B-1 JAMWADI

CONTROL

M +ve -ve w 1/1 ½ 1/3 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3

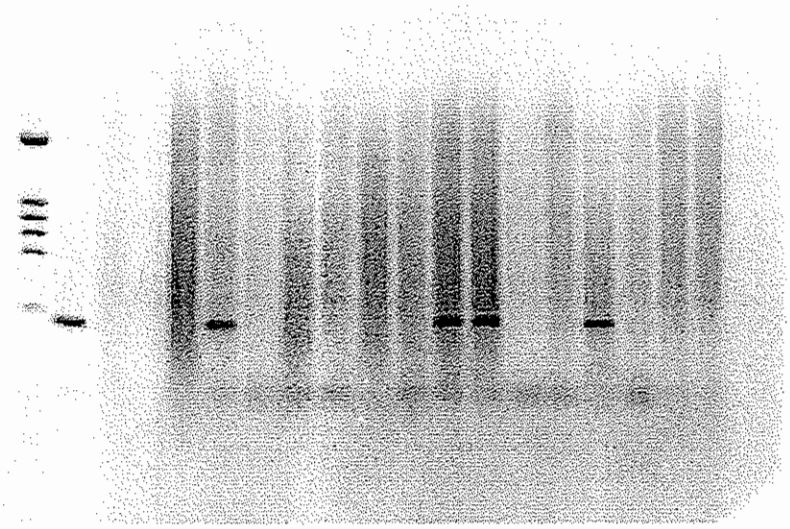


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-2 (CRY 2Ab)

BLOCK C-1 JAMWADI

CONTROL

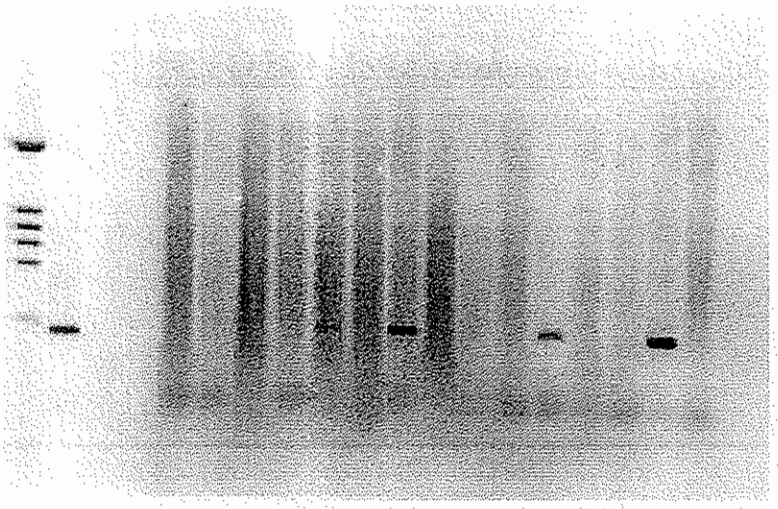
M +ve -ve w 1/1 ½ 1/3 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3



BLOCK D-1 JAMWADI

CONTROL

M +ve -ve w 1/1 ½ 1/3 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3

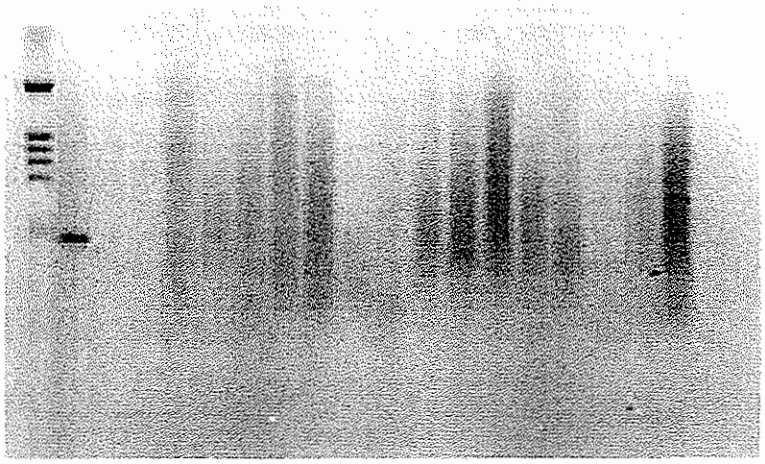


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-3 (CRY 2Ab)

BLOCK A- 2-7 - Jamwadi .

CONTROL

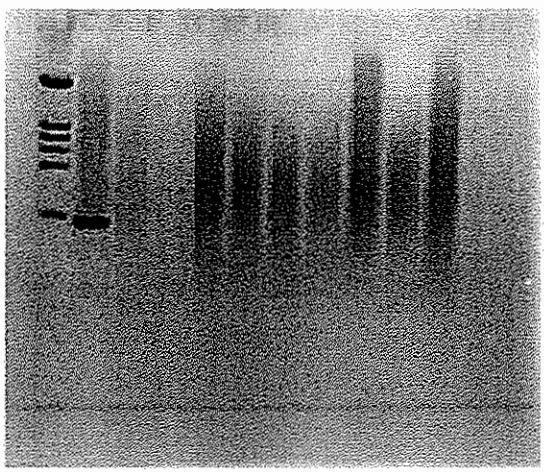
M +ve -ve w 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/3 5/1 5/2 5/3 6/1 6/2 7/1 7/2 7/3



BLOCK A- 7-9 Jamwadi

CONTROL

M +ve -ve w 7/3 8/1 8/2 8/3 9/1 9/2 9/3

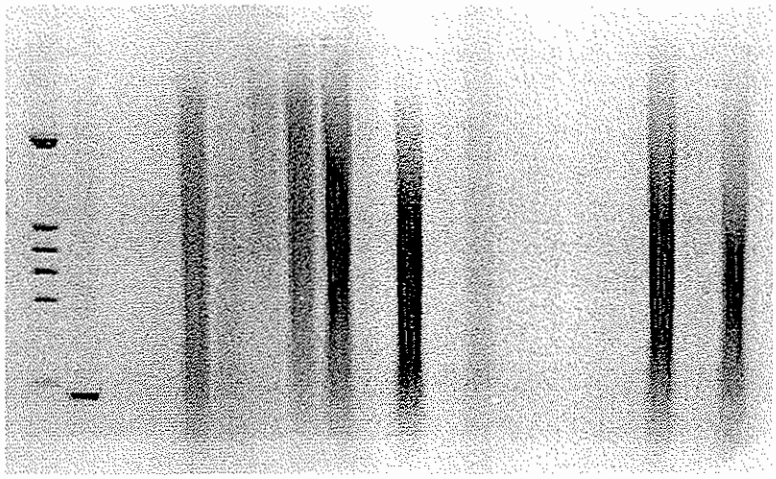


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-4 (CRY 2Ab)

BLOCK B – 2 - 7 - Jamwadi

CONTROL

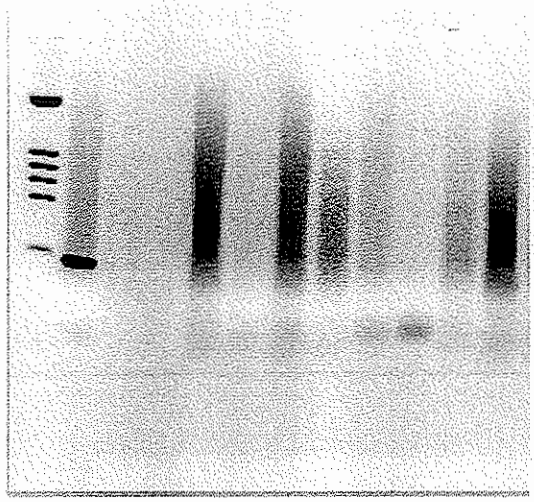
M +ve -ve w 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3 6/1 6/2 6/3 7/1



BLOCK B -7 - 9 Jamwadi

CONTROL

M +ve -ve w 7/2 7/3 8/1 8/2 8/3 9/1 9/2 9/3

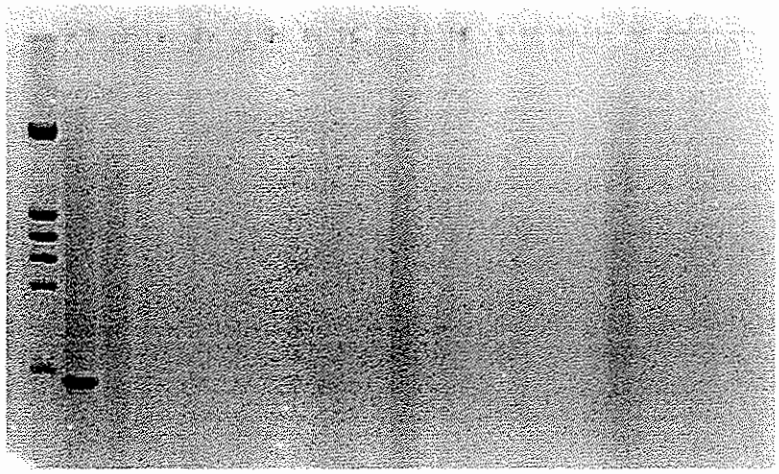


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-5 (CRY 2Ab)

BLOCK – C 2 - 7 - Jamwadi

CONTROL

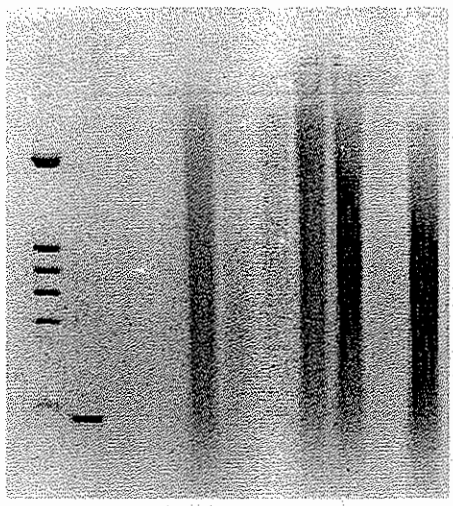
M +ve -ve w 2/1 2/2 2/3 3/1 3/3 3/1 4/2 4/3 5/1 5/2 5/3 6/1 6/2 6/3 7/1 7/2



BLOCK C – 7 -9 Jamwadi

CONTROL

M +ve -ve w 7/3 8/1 8/2 8/3 9/1 9/2 9/3

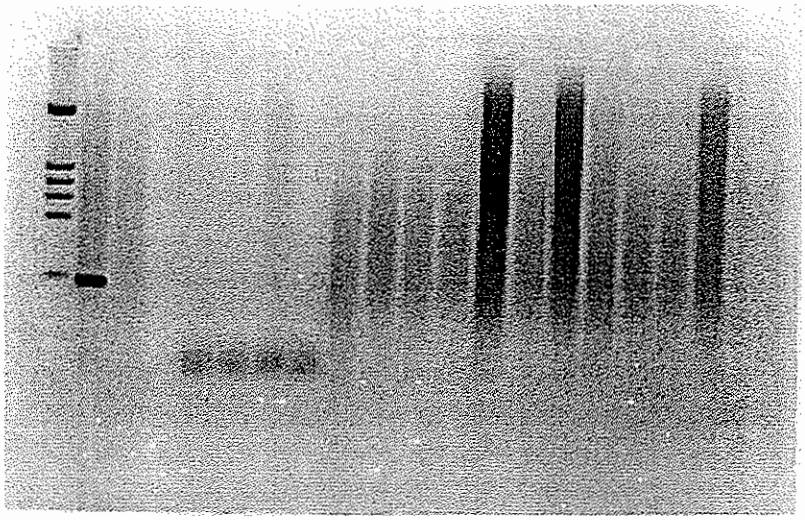


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-6 (CRY 2Ab)

BLOCK – D 2 - 7 - Jamwadi

CONTROL

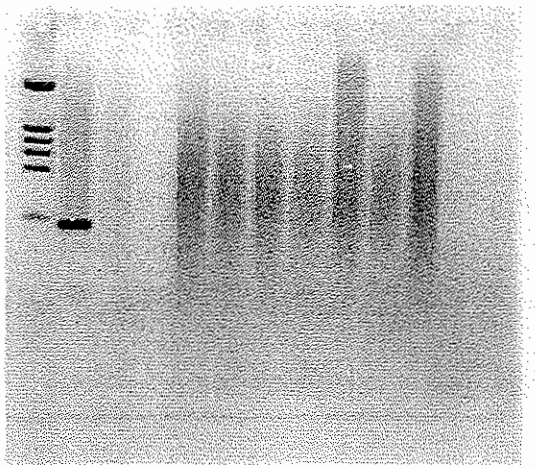
M +ve -ve w 2/1 2/2 2/3 3/1 3/3 3/1 4/2 4/3 5/1 5/2 5/3 6/1 6/2 6/3 7/1 7/2



BLOCK – D –7 - 9 Jamwadi

CONTROL

M +ve -ve w 7/3 8/1 8/2 8/3 9/1 9/2 9/3

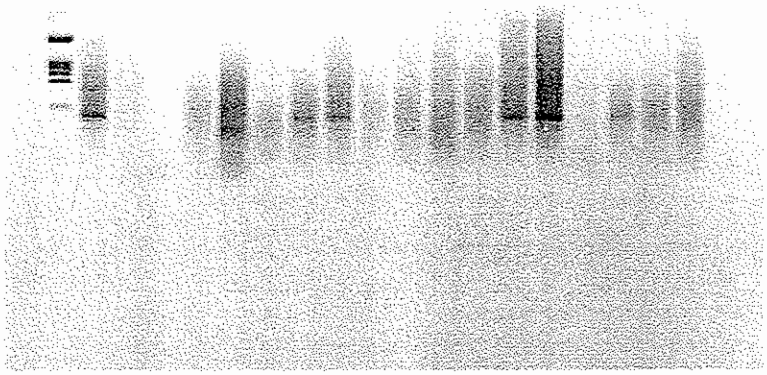


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-1 (CRY 1Ac)

BLOCK A-1 - Shamshabad

CONTROL

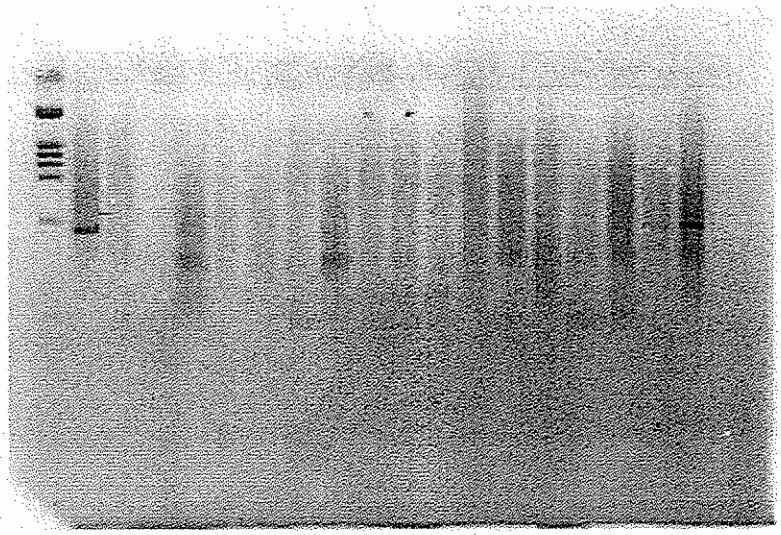
M +ve -ve w 1/1 ½ 1/3 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3



BLOCK B-1 Shamshabad

CONTROL

M +ve -ve w 1/1 ½ 1/3 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3

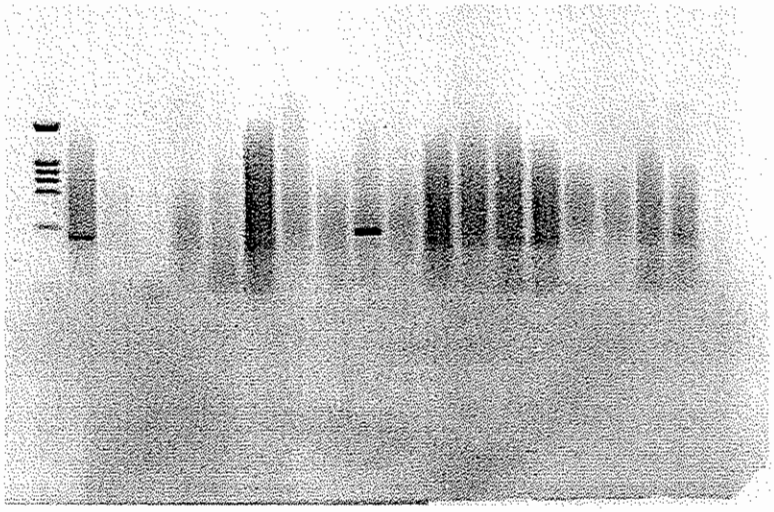


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-2 (CRY 1Ac)

BLOCK C -1 - Shamshabad

CONTROL

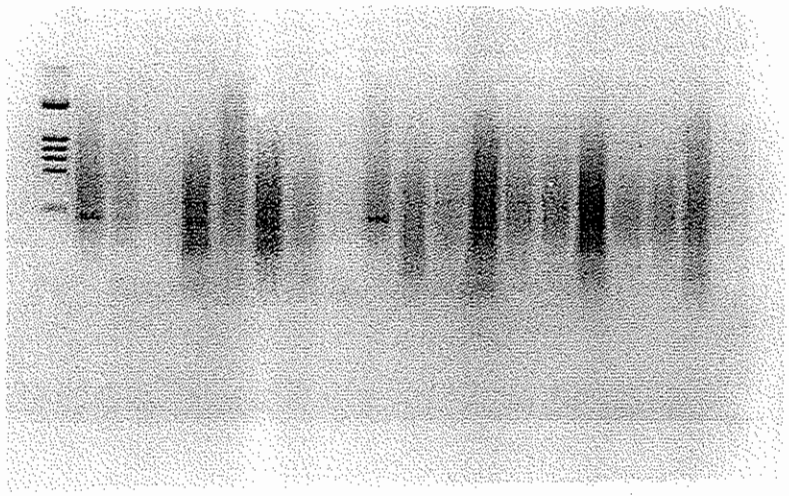
M +ve -ve w 1/1 ½ 1/3 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3



BLOCK D -1 - Shamshabad

CONTROL

M +ve -ve w 1/1 ½ 1/3 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3

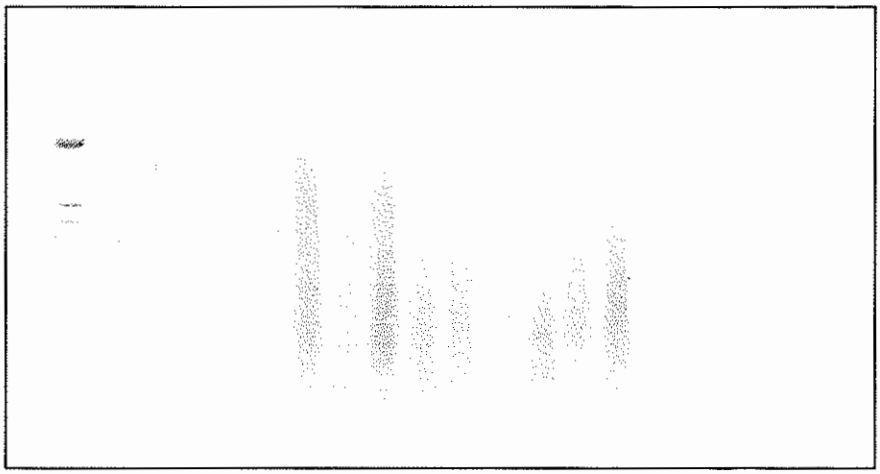


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-3 (CRY 1Ac)

BLOCK A-2-6 - Shamshabad

CONTROL

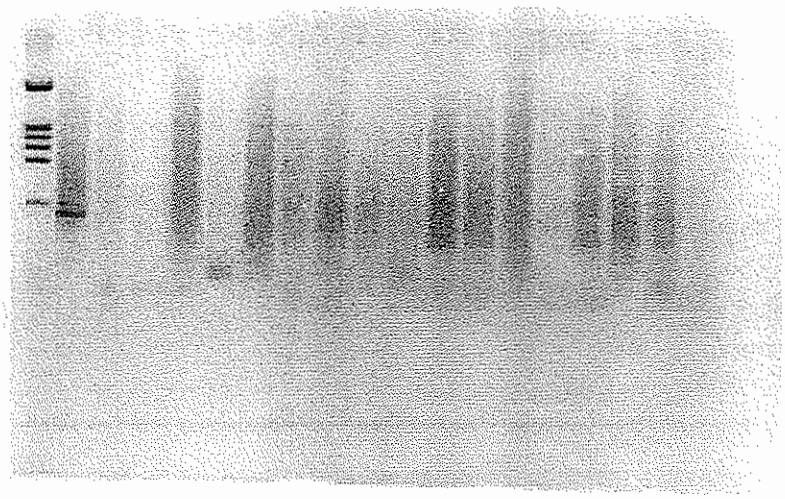
M +ve -ve w 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3 6/1 6/2 6/3



BLOCK A-7-11 Shamshabad

CONTROL

M +ve -ve w 7/1 7/2 7/3 8/2 8/3 9/1 9/2 9/3 10/1 10/2 10/3 11/1 11/2

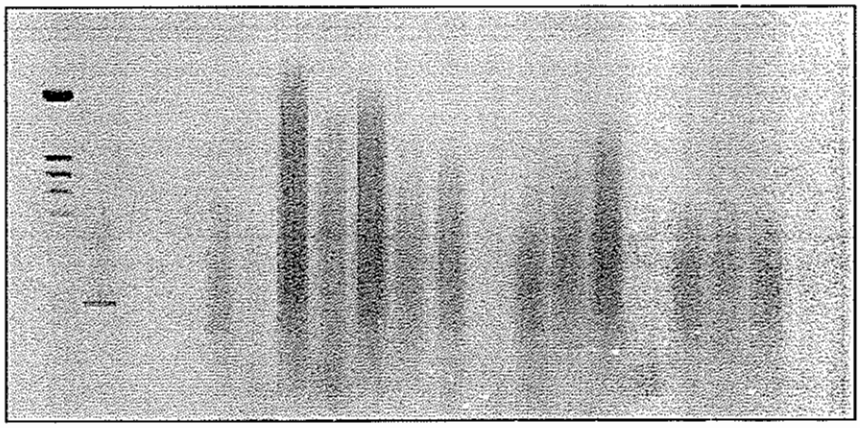


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-4 (CRY 1Ac)

BLOCK B-2-6 - Shamshabad

CONTROL

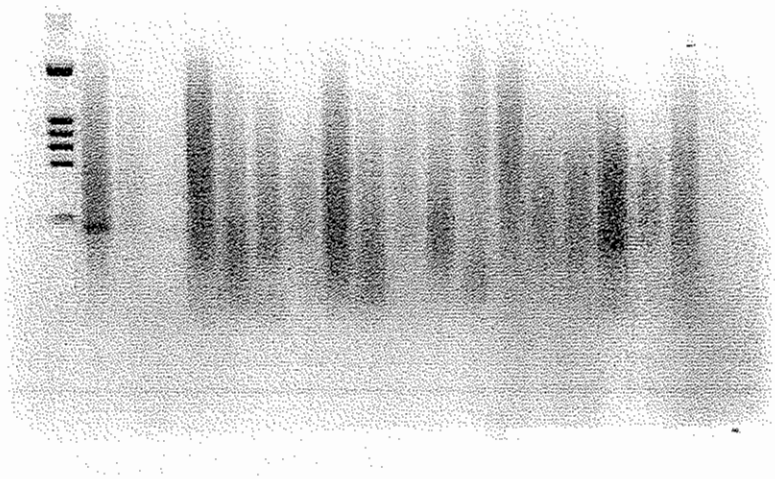
M +ve -ve w 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3 6/1 6/2 6/3



BLOCK C-7-11 Shamshabad

CONTROL

M +ve -ve w 7/1 7/2 7/3 8/1 8/2 8/3 9/1 9/2 9/3 10/1 10/2 10/3 11/1 11/2 11/3

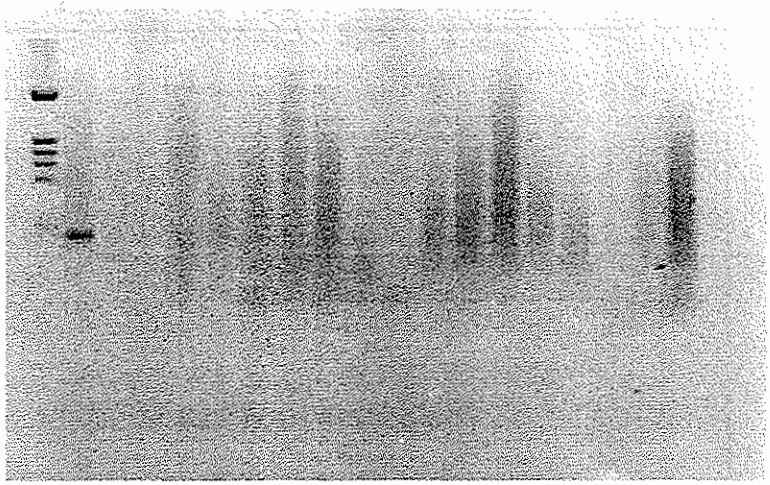


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-5 (CRY 1Ac)

BLOCK C - 2 - 6 - Shamshabad

CONTROL

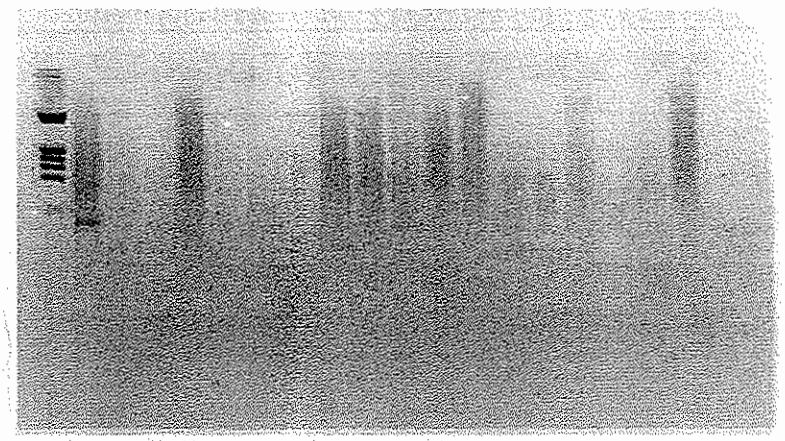
M +ve -ve w 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3 6/1 6/2 6/3



BLOCK C-7-11 Shamshabad

CONTROL

M +ve -ve w 7/1 7/2 7/3 8/1 8/2 8/3 9/1 9/2 9/3 10/1 10/2 10/3 11/1 11/2 11/3

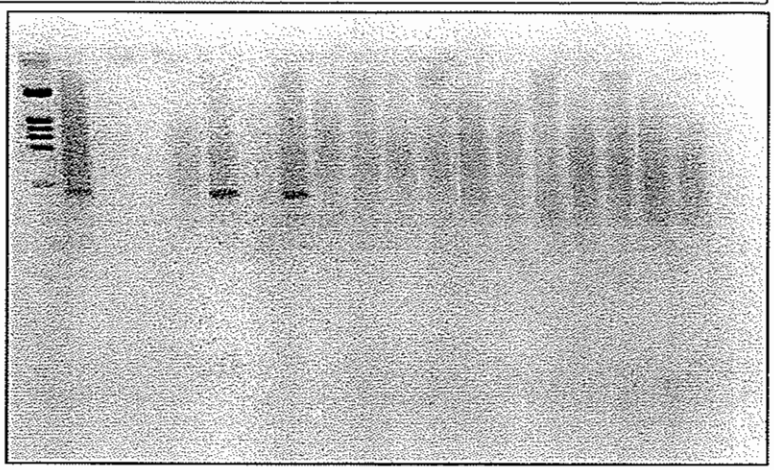


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-6 (CRY 1Ac)

BLOCK D - 2 - 6 - Shamshabad

CONTROL

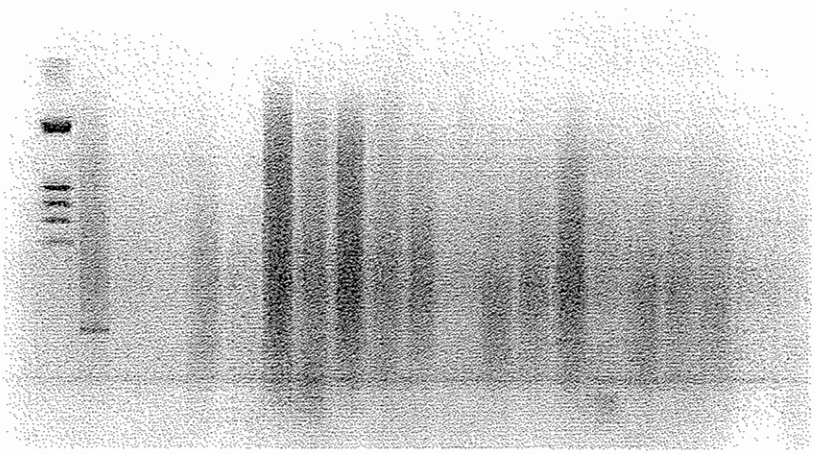
M +ve -ve w 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3 6/1 6/2 6/3



BLOCK D – 7 - 11 Shamshabad

CONTROL

M +ve -ve w 7/1 7/2 7/3 8/1 8/2 8/3 9/1 9/2 9/3 10/1 10/2 10/3 11/1 11/2 11/3

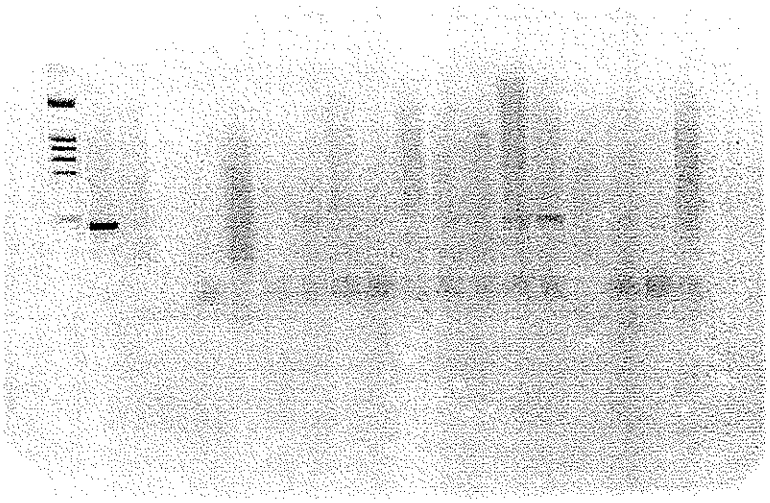


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-1 (CRY 2Ab)

BLOCK A-1 - Shamshabad

CONTROL

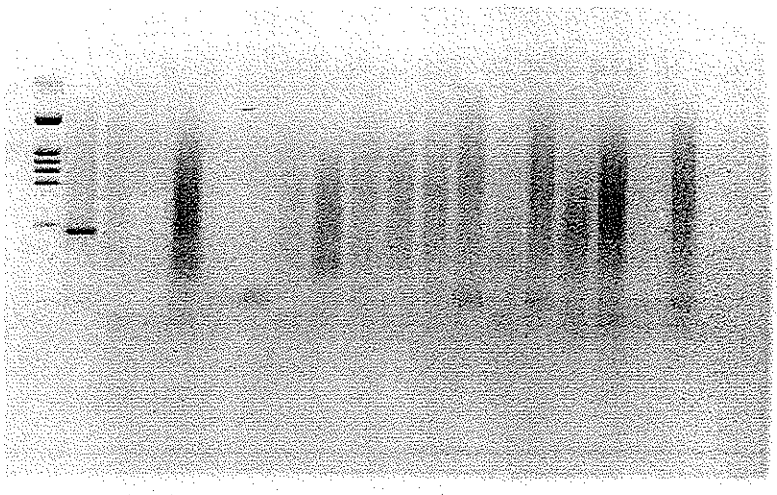
M +ve -ve w 1/1 ½ 1/3 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3



BLOCK B-1 Shamshabad

CONTROL

M +ve -ve w 1/1 ½ 1/3 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3

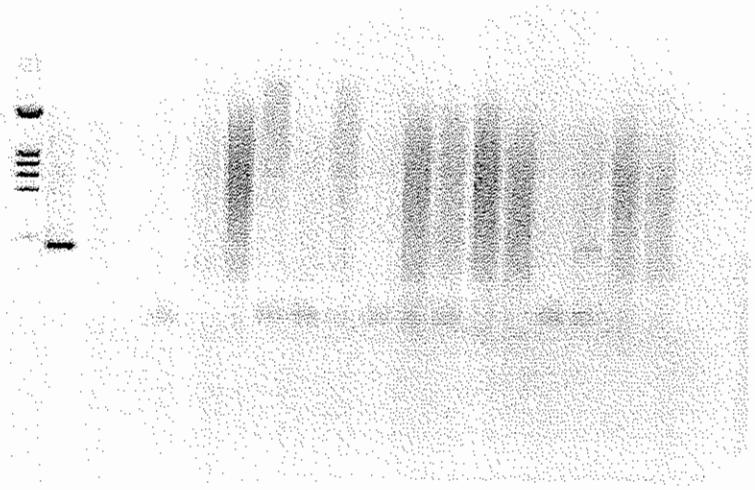


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-2 (CRY 2Ab)

BLOCK C -1 - Shamshabad

CONTROL

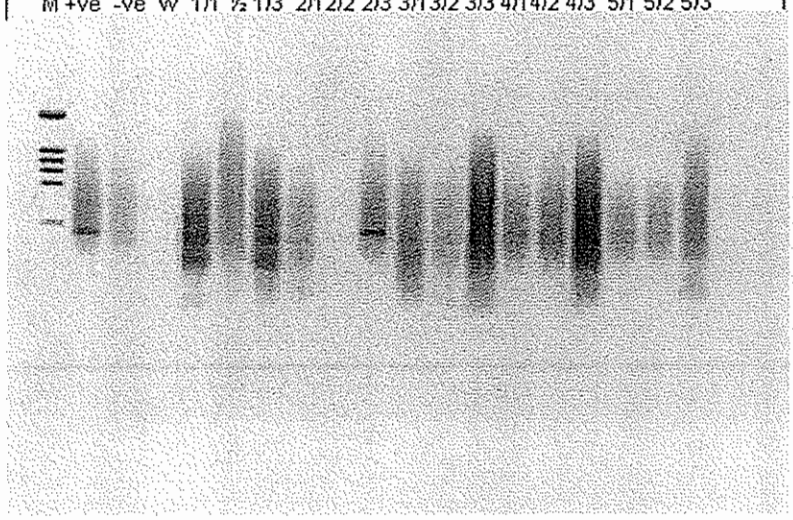
M +ve -ve w 1/1 ½ 1/3 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3



BLOCK D -1 - Shamshabad

CONTROL

M +ve -ve w 1/1 ½ 1/3 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3

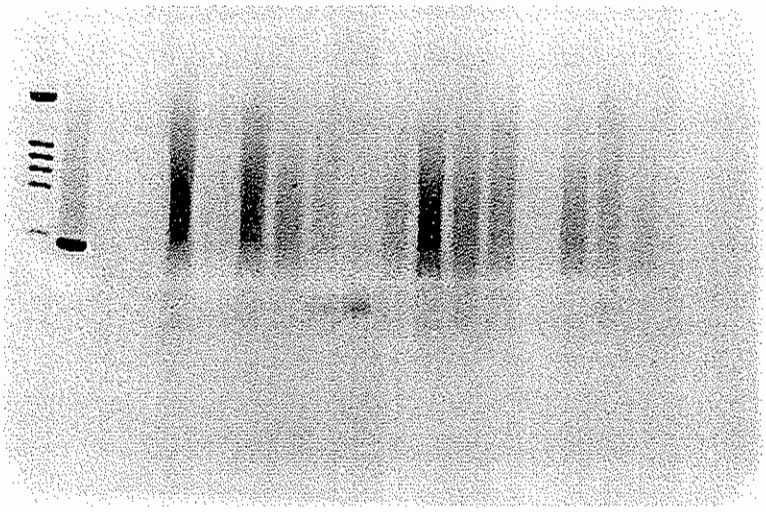


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-3 (CRY 2Ab)

BLOCK A-2-6 - Shamshabad

CONTROL

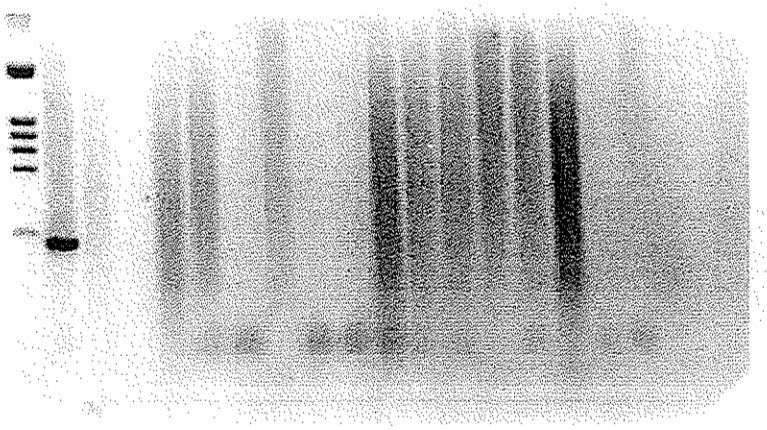
M +ve -ve w 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3 6/1 6/2 6/3



BLOCK A-7-11 Shamshabad

CONTROL

M +ve -ve w 7/1 7/2 7/3 8/2 8/3 9/1 9/2 9/3 10/1 10/2 10/3 11/1 11/2 11/3

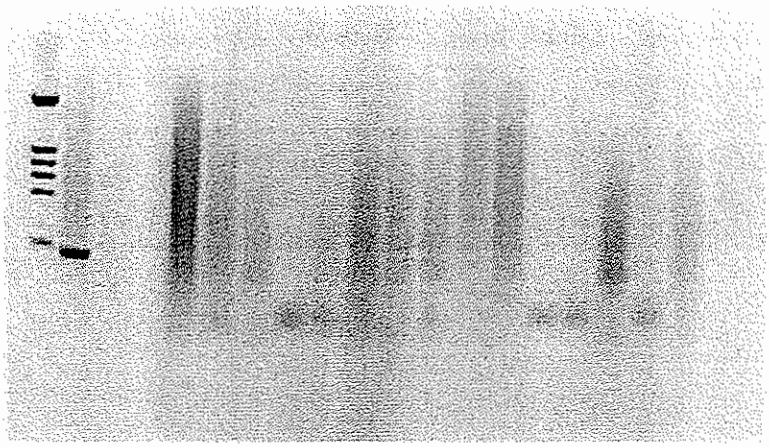


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-4 (CRY 2Ab)

BLOCK B-2-6 - Shamshabad

CONTROL

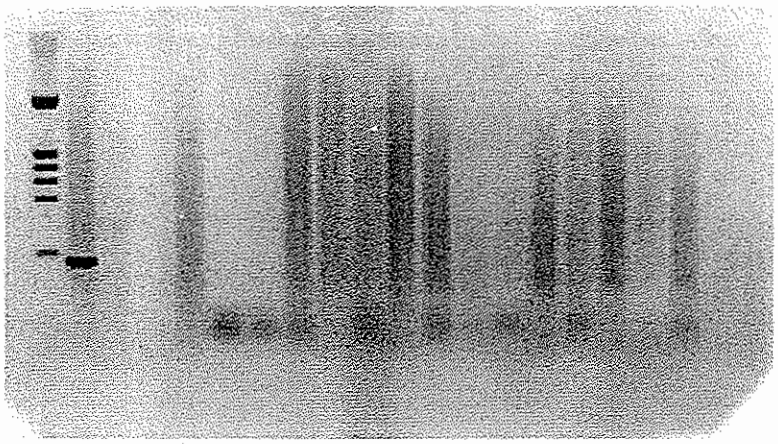
M +ve -ve w 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3 6/1 6/2 6/3



BLOCK B-7-11 Shamshabad

CONTROL

M +ve -ve w 7/1 7/2 7/3 8/1 8/2 8/3 9/1 9/2 9/3 10/1 10/2 10/3 11/1 11/2 11/3

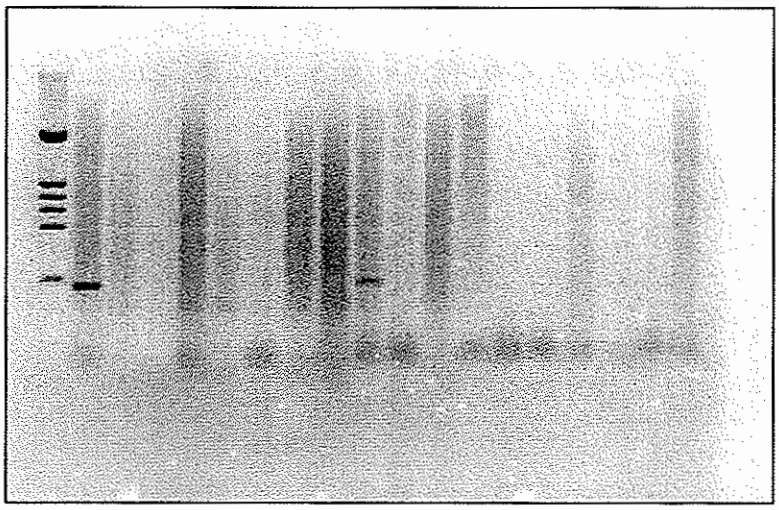


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-5 (CRY 2Ab)

BLOCK C - 2 - 6 - Shamshabad

CONTROL

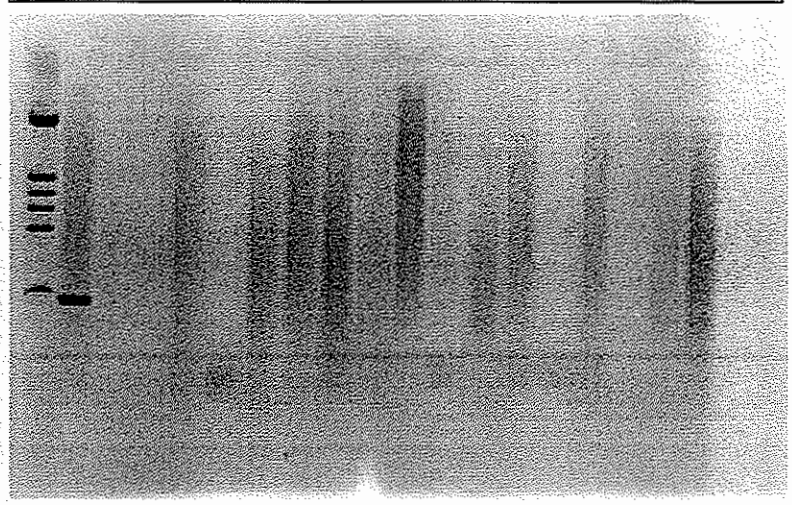
M +ve -ve w 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3 6/1 6/2 6/3



BLOCK C-7-11 Shamshabad

CONTROL

M +ve -ve w 7/1 7/2 7/3 8/1 8/2 8/3 9/1 9/2 9/3 10/1 10/2 10/3 11/1 11/2 11/3

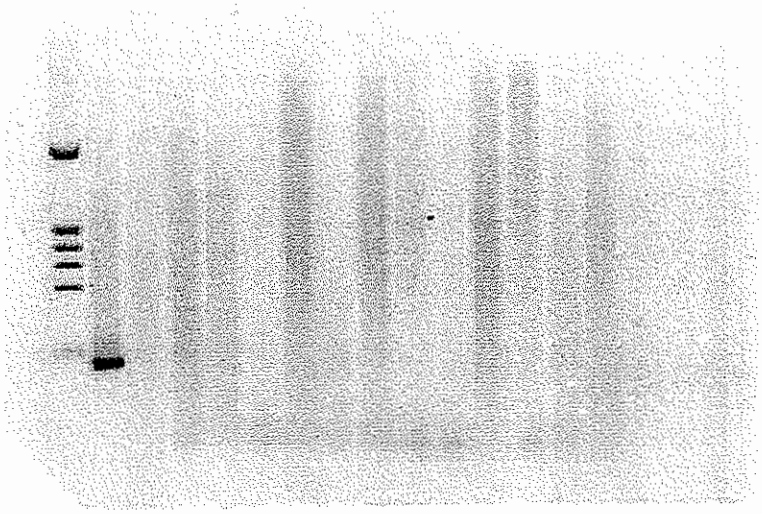


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-6 (CRY 2Ab)

BLOCK D - 2 - 6 - Shamshabad

CONTROL

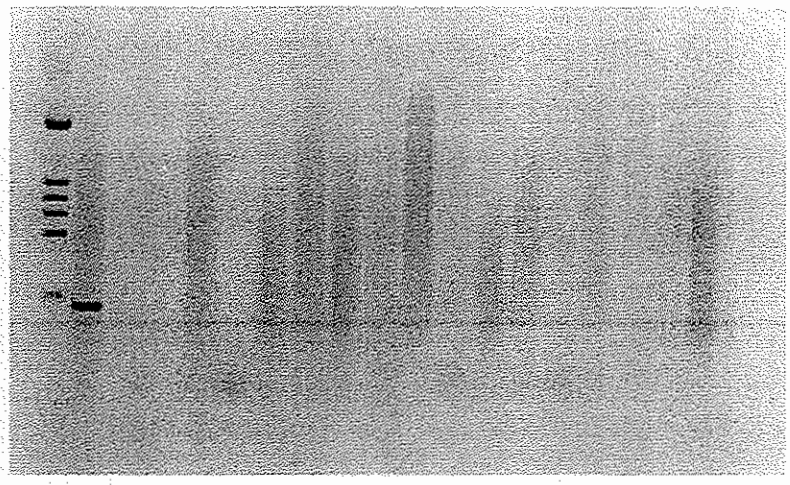
M +ve -ve w 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3 6/1 6/2 6/3



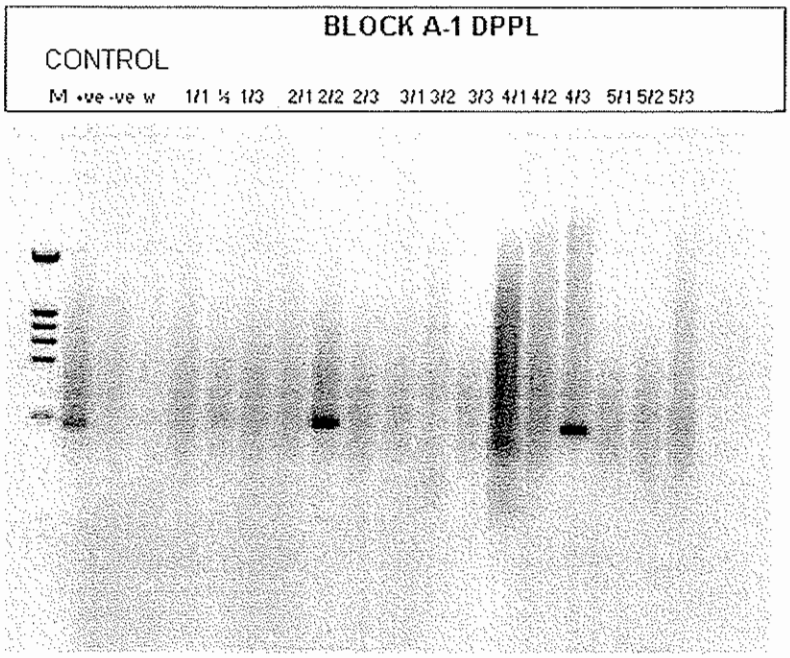
BLOCK D – 7 - 11 Shamshabad

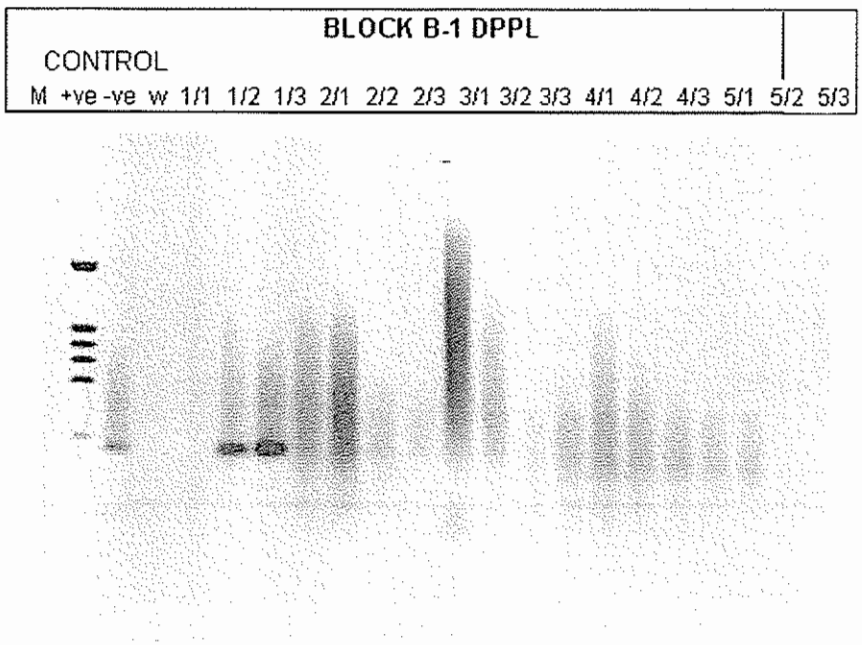
CONTROL

M +ve -ve w 7/1 7/2 7/3 8/1 8/2 8/3 9/1 9/2 9/3 10/1 10/2 10/3 11/1 11/2 11/3

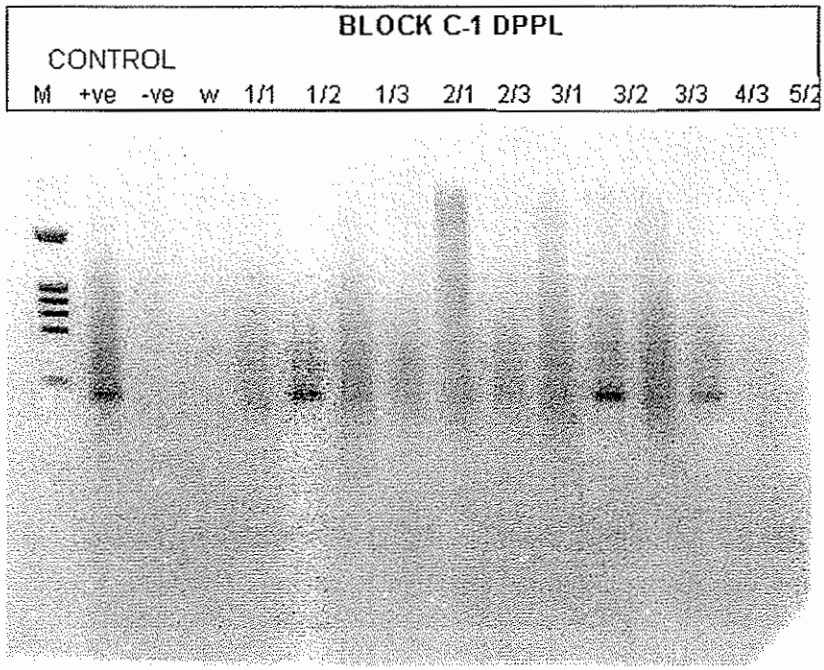


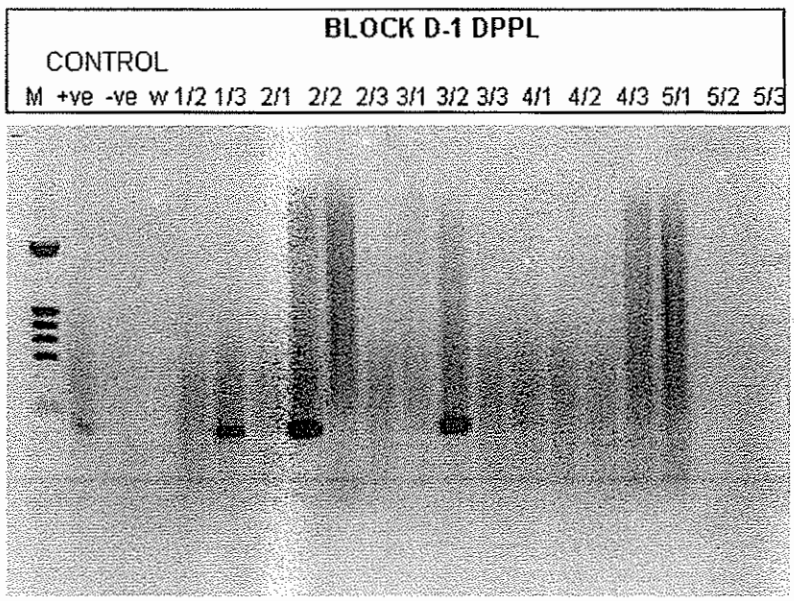
ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-1 (CRY 1Ac)





ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-2 (CRY 1Ac)



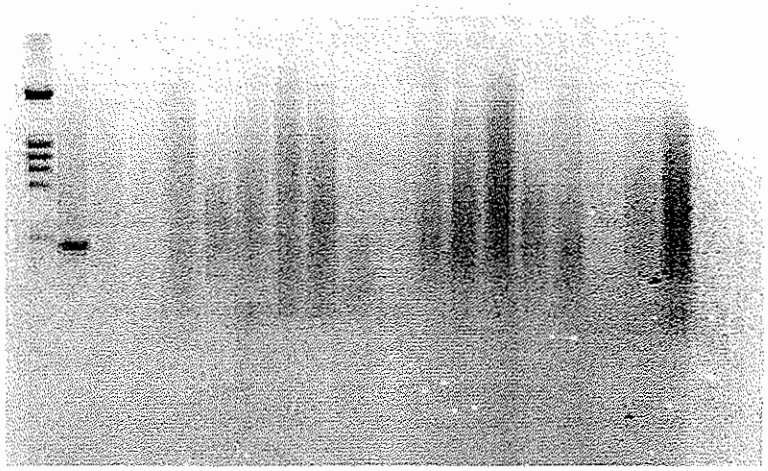


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-3 (CRY 1Ac)

BLOCK A-2-6 DPPL

CONTROL

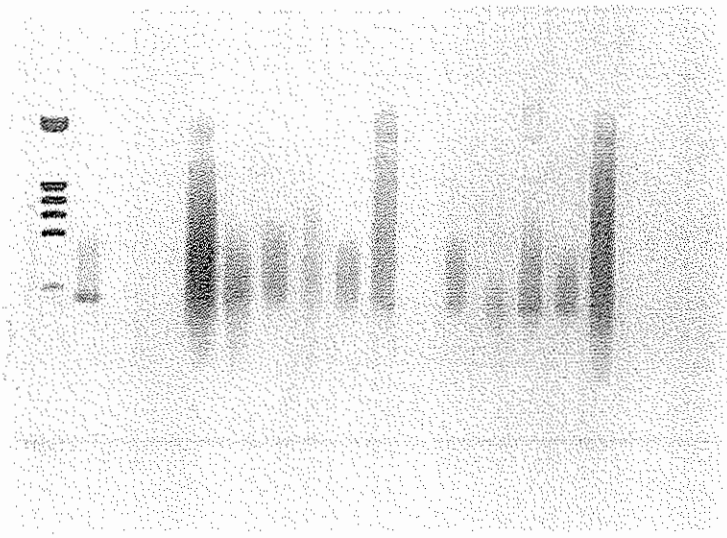
M +ve -ve w 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3 6/1 6/2 6/3



BLOCK A-7-11 DPPL

CONTROL

M +ve -ve w 7/1 7/2 7/3 8/1 8/2 8/3 9/1 9/2 9/3 10/1 10/2 10/3

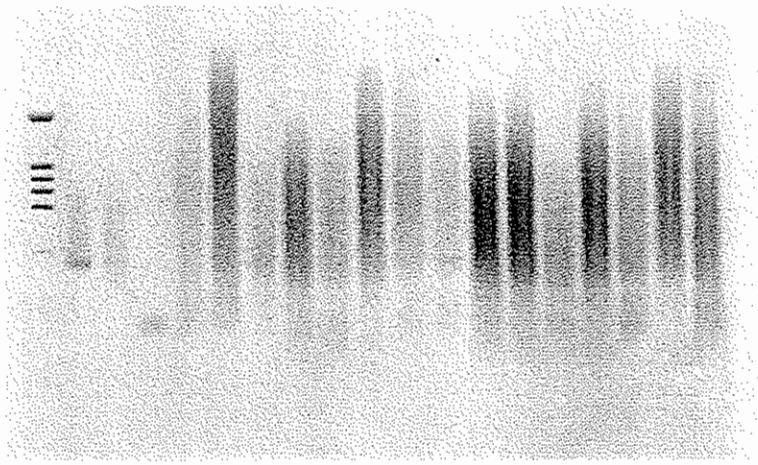


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-4 (CRY 1Ac)

BLOCK B-2-6 DPPL

CONTROL

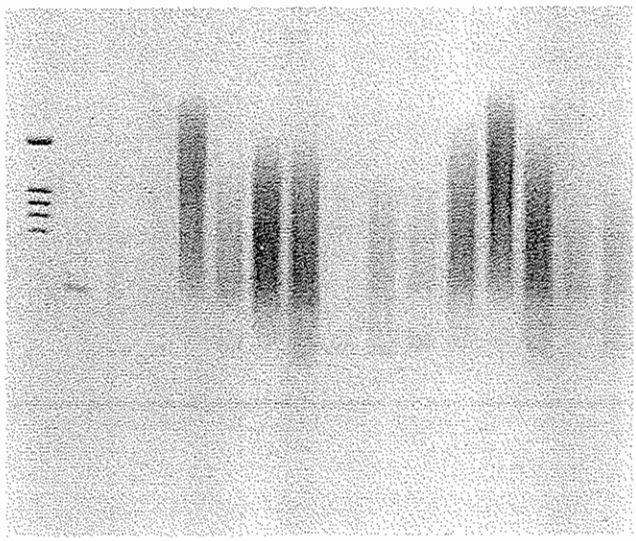
M +ve -ve w 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3 6/1 6/2 6/3



BLOCK B-7-11 DPPL

CONTROL

M +ve -ve w 7/1 7/2 7/3 8/1 8/2 8/3 9/1 9/2 9/3 10/1 10/2 10/3

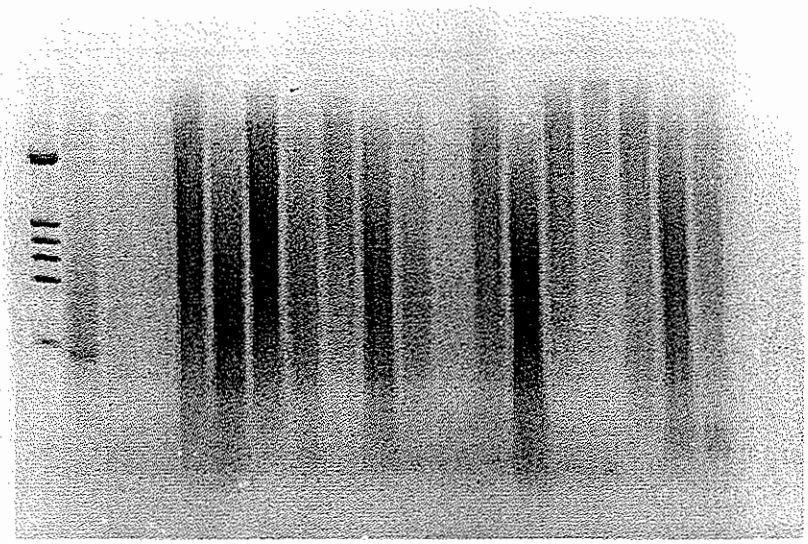


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-5 (CRY 1Ac)

BLOCK - C -2-6 DPPL

CONTROL

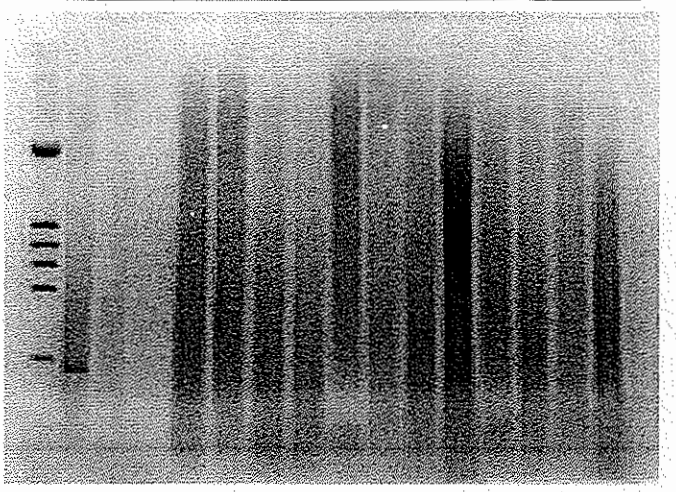
M +ve -ve w 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3 6/1 6/2 6/3



BLOCK C - 7-11 DPPL

CONTROL

M +ve -ve w 7/1 7/2 7/3 8/1 8/2 8/3 9/1 9/2 9/3 10/1 10/2 10/3

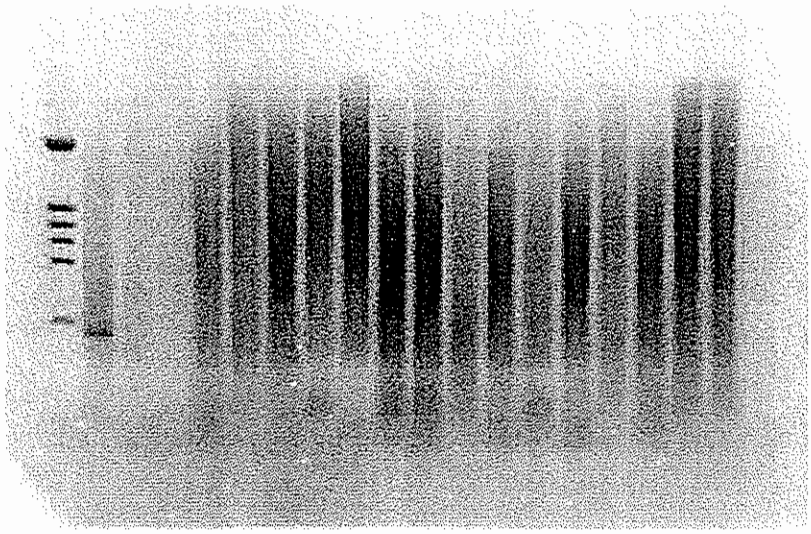


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-6 (CRY 1Ac)

BLOCK -D -2-6 DPPL

CONTROL

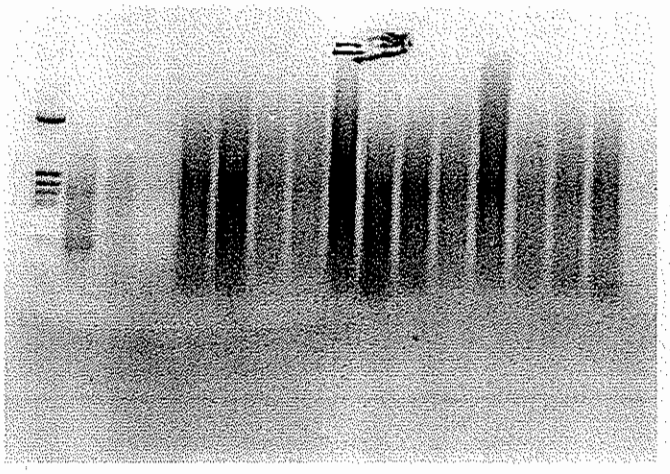
M +ve -ve w 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3 6/1 6/2 6/3



BLOCK D -7 - 11 DPPL

CONTROL

M +ve -ve w 7/1 7/2 7/3 8/1 8/2 8/3 9/1 9/2 9/3 10/1 10/2 10/3

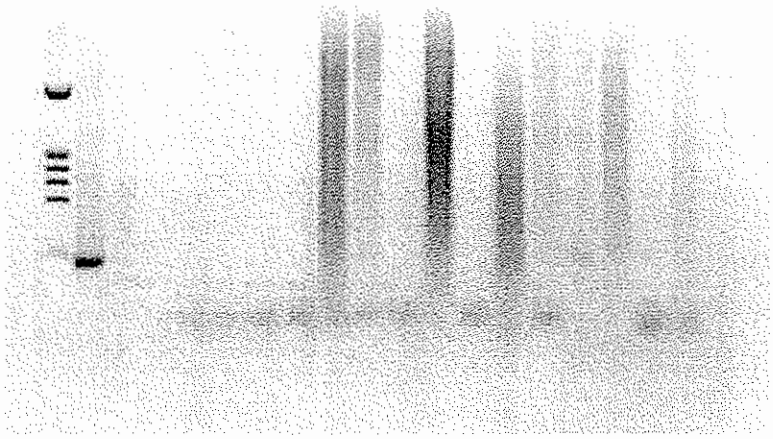


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-1 (CRY 2Ab)

BLOCK A-1 DPPL

CONTROL

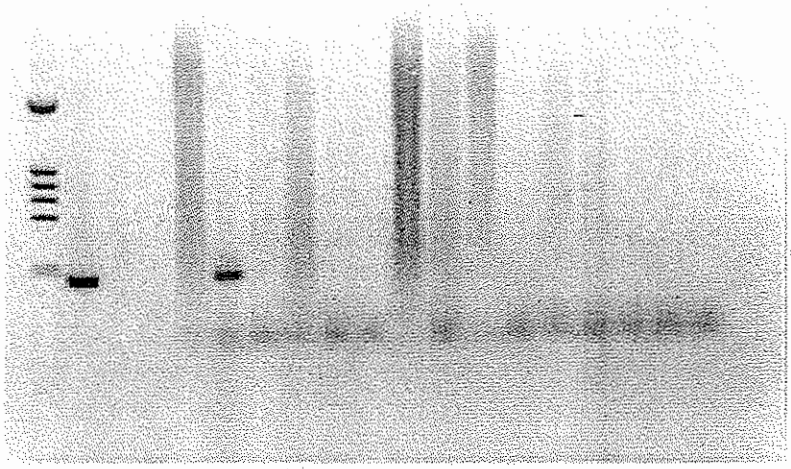
M -ve -ve w 1/1 ½ 1/3 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3



BLOCK B-1 DPPL

CONTROL

M -ve -ve w 1/1 1/2 1/3 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3

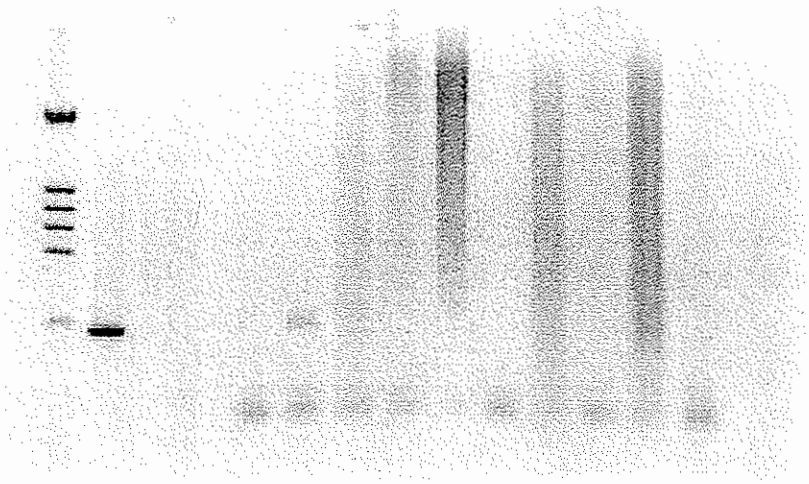


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-2 (CRY 2Ab)

BLOCK C-1 DPPL

CONTROL

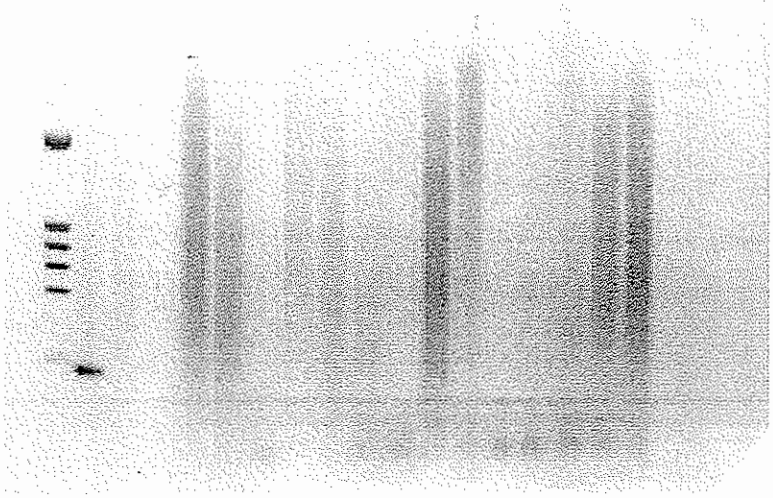
M +ve -ve w 1/1 1/2 1/3 2/1 2/3 3/1 3/2 3/3 4/3 5/2



BLOCK D-1 DPPL

CONTROL

M +ve -ve w 1/2 1/3 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3

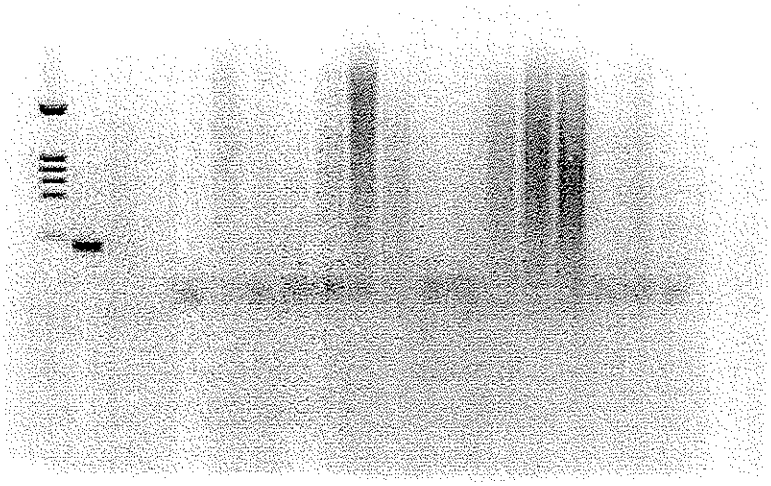


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-3 (CRY 2Ab)

BLOCK A-2-6 DPPL

CONTROL

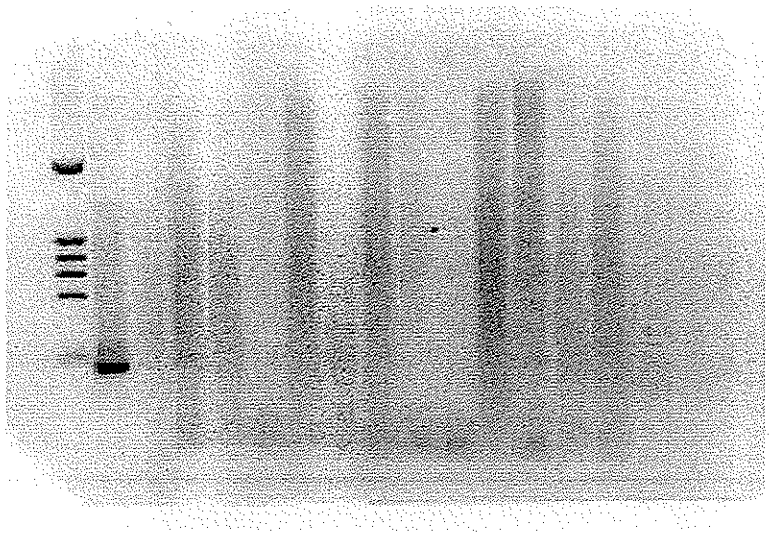
M +ve -ve w 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3 6/1 6/2 6/3



BLOCK A-7-11 DPPL

CONTROL

M +ve -ve w 7/1 7/2 7/3 8/1 8/2 8/3 9/1 9/2 9/3 10/1 10/2 10/3

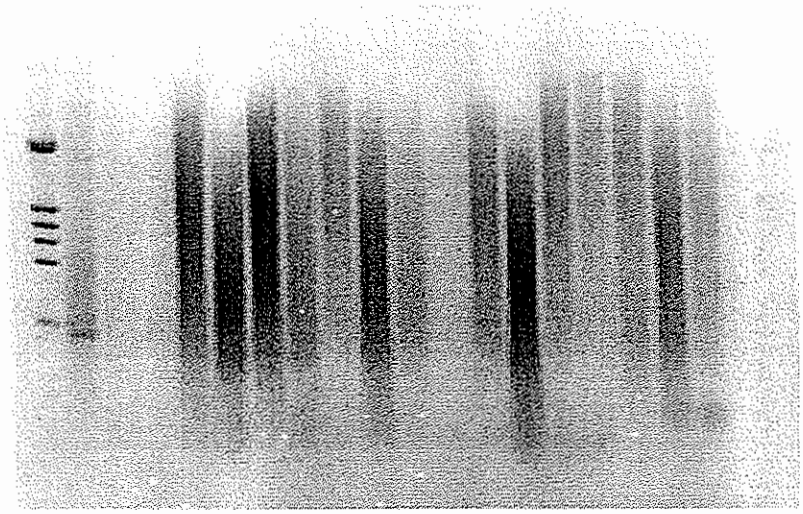


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-4 (CRY 2Ab)

BLOCK B-2-6 DPPL

CONTROL

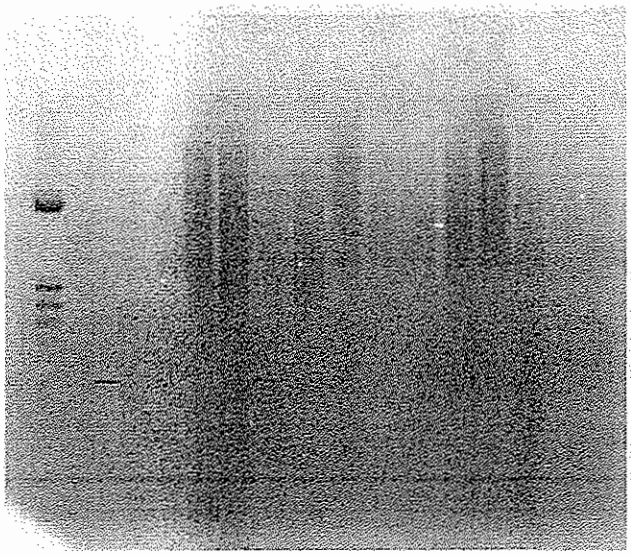
M +ve -ve w 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3 6/1 6/2 6/3



BLOCK B-7-11 DPPL

CONTROL

M +ve -ve W 7/1 7/2 7/3 8/1 8/2 8/3 9/1 9/2 9/3 10/1 10/2 10/3

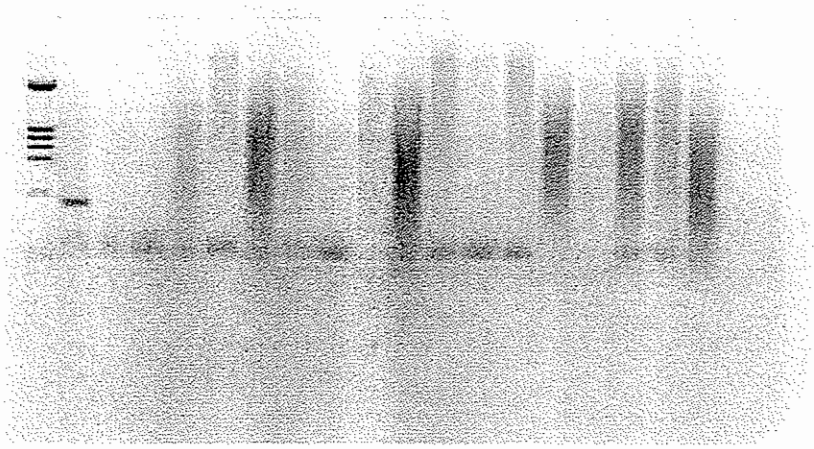


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-5 (CRY 2Ab)

BLOCK C -2-6 DPPL

CONTROL

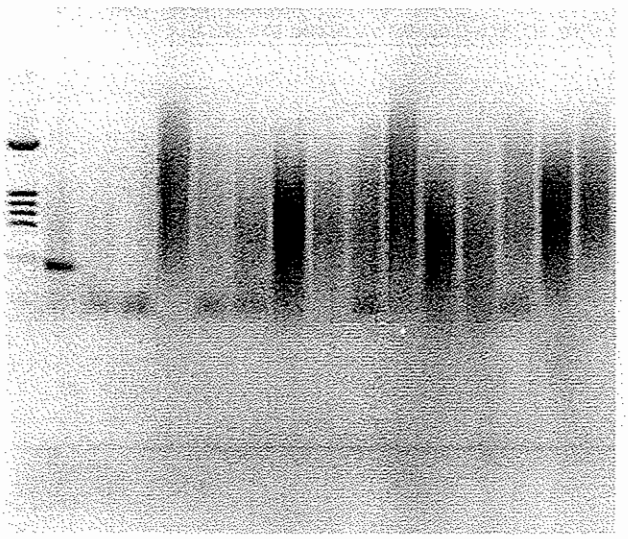
M +ve -ve w 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3 6/1 6/2 6/3



BLOCK C -7-11 DPPL

CONTROL

M +ve -ve W 7/1 7/2 7/3 8/1 8/2 8/3 9/1 9/2 9/3 10/1 10/2 10/3

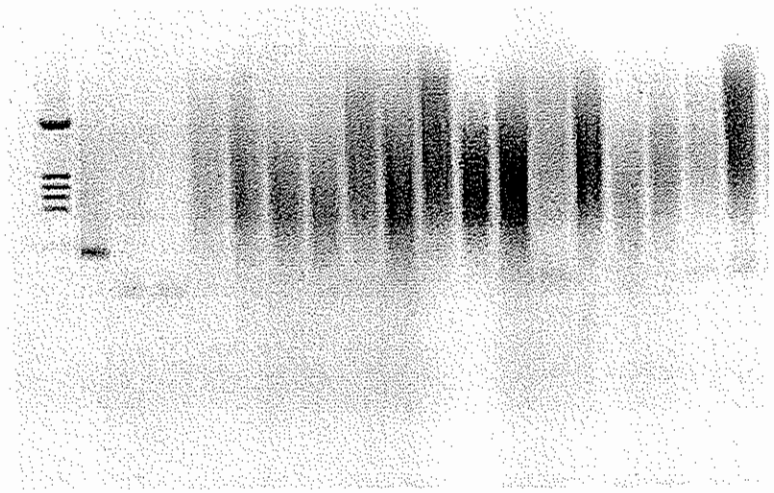


ANNEXURE-III
POLLEN FLOW EXPERIMENT ,PCR RESULTS
SHEET-6 (CRY 2Ab)

BLOCK D -2- 6 DPPL

CONTROL

M +ve -ve w 2/1 2/2 2/3 3/1 3/2 3/3 4/1 4/2 4/3 5/1 5/2 5/3 6/1 6/2 6/3



BLOCK D -7-11 DPPL

CONTROL

M +ve -ve W 7/1 7/2 7/3 8/1 8/2 8/3 9/1 9/2 9/3 10/1 10/2 10/3

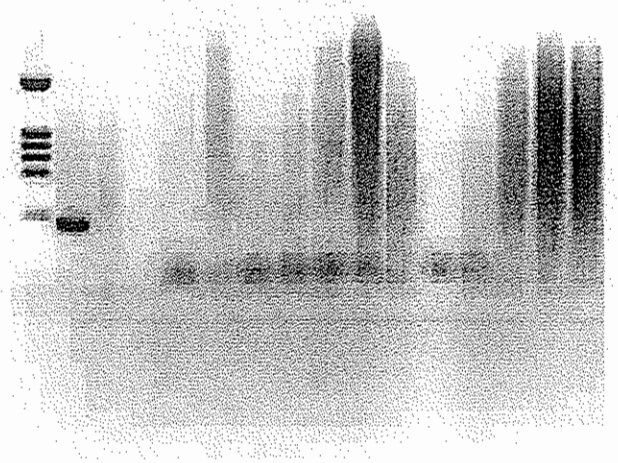


Table 1:A

SUMMARY OF POLLEN TRAP GROW-OUT-TEST

(No. of Semi-Okra individuals among the pollen trap progeny samples)

Location :Jamwadi

DIRECTION OF BLOCKS

		A (EAST)			B (SOUTH)			C (WEST)			D (NORTH)		
	Sample No.	1	2	3	1	2	3	1	2	3	1	2	3
Block No.1	Row No.1	0	0	0	0	1	0	1	0	0	2	1	0
	Row No.2	1	0	0	1	0	1	0	1	0	0	1	0
	Row No.3	0	0	1	0	0	0	2	1	1	0	0	1
	Row No.4	0	0	0	0	0	0	1	2	1	0	1	0
	Row No.5	1	1	0	1	0	0	1	0	0	0	1	0
Block No.2		0	0	0	0	0	0	0	0	0	0	0	0
Block No.3		0	0	0	0	0	0	0	0	0	0	0	0
Block No.4		0	0	0	0	0	0	0	0	0	0	0	0
Block No.5		0	0	0	0	0	0	0	0	0	0	0	0
Block No.6		0	0	0	0	0	0	0	0	0	0	0	0
Block No.7		0	0	0	0	0	0	0	0	0	0	0	0
Block No.8		0	0	0	0	0	0	0	0	0	0	0	0
Block No. 9		0	0	0	0	0	0	0	0	0	0	0	0
Block No.10		0	0	0	0	0	0	0	0	0	0	0	0
Block No.11		0	0	0	0	0	0	0	0	0	0	0	0

Table 1:B

SUMMARY OF POLLEN TRAP GROW-OUT-TEST

(No. of Semi-Okra individuals among the pollen trap progeny samples)

Location :Shamshabad

DIRECTION OF BLOCKS

		A (EAST)			B (SOUTH)			C (WEST)			D (NORTH)		
	Sample No.	1	2	3	1	2	3	1	2	3	1	2	3
Block No.1	Row No.1	0	0	0	0	0	0	0	0	0	1	0	1
	Row No.2	1	2	0	0	0	0	0	0	1	0	0	1
	Row No.3	0	0	0	0	0	0	0	0	0	0	0	0
	Row No.4	2	1	0	0	0	0	0	0	0	0	0	1
	Row No.5	1	0	0	0	1	1	2	0	0	0	0	0
Block No.2		0	0	0	0	0	0	0	0	0	0	2	0
Block No.3		0	0	0	0	0	0	0	0	0	1	0	1
Block No.4		0	0	0	0	0	0	0	0	0	0	0	0
Block No.5		0	0	0	0	0	0	0	0	0	0	0	0
Block No.6		0	0	0	0	0	0	0	0	0	0	0	0
Block No.7		0	0	0	0	0	0	0	0	0	0	0	0
Block No.8		0	0	0	0	0	0	0	0	0	0	0	0
Block No. 9		0	0	0	0	0	0	0	0	0	0	0	0
Block No.10		0	0	0	0	0	0	0	0	0	0	0	0
Block No.11		0	0	0	0	0	0	0	0	0	0	0	0

Table 1:C

SUMMARY OF POLLEN TRAP GROW-OUT-TEST

(No. of Semi-Okra individuals among the pollen trap progeny samples)

Location :DPPL(Nizamabad)

DIRECTION OF BLOCKS

		A (EAST)			B (SOUTH)			C (WEST)			D (NORTH)		
	Sample No.	1	2	3	1	2	3	1	2	3	1	2	3
Block No.1	Row No.1	0	0	0	1	2	0	0	2	0	0	0	1
	Row No.2	0	1	0	0	0	0	0	0	0	0	1	0
	Row No.3	0	0	0	0	0	0	0	1	0	0	0	1
	Row No.4	0	0	1	0	0	0	0	0	1	0	0	0
	Row No.5	0	0	0	0	0	0	0	0	0	0	0	0
Block No.2		0	0	0	0	0	0	0	0	0	0	0	0
Block No.3		0	0	0	0	0	0	0	0	0	0	0	0
Block No.4		0	0	0	0	0	0	0	0	0	0	0	0
Block No.5		0	0	0	0	0	0	0	0	0	0	0	0
Block No.6		0	0	0	0	0	0	0	0	0	0	0	0
Block No.7		0	0	0	0	0	0	0	0	0	0	0	0
Block No.8		0	0	0	0	0	0	0	0	0	0	0	0
Block No. 9		0	0	0	0	0	0	0	0	0	0	0	0
Block No.10		0	0	0	0	0	0	0	0	0	0	0	0
Block No.11		0	0	0	0	0	0	0	0	0	0	0	0

Summary Results of Pollen Trap Progeny PCR

Table 2 A: Jamwadi Location

DIRECTION OF BLOCKS

		A (EAST)			B (SOUTH)			C (WEST)			D (NORTH)		
	Sample No.	1	2	3	1	2	3	1	2	3	1	2	3
Block No.1	Row No.1	--	--	--	--	+	--	+	+*	--	+	+	--
	Row No.2	+	--	--	*	+	+	+	+	--	--	+*	--
	Row No.3	--	--	+*	--	--	--	+	*	+*	+*	--	+
	Row No.4	--	--	--	--	--	--	+	+*	+*	+	*	+
	Row No.5	+	+	--	+	--	--	+	--	--	--	*	--
Block No.2		--	--	--	--	--	--	--	--	--	--	--	--
Block No.3		--	--	--	--	--	--	--	--	--	--	--	+
Block No.4		--	--	--	--	--	--	--	--	--	--	--	--
Block No.5		--	--	--	--	--	--	--	--	--	--	--	--
Block No.6		--	--	--	--	--	--	--	--	--	--	--	--
Block No.7		--	--	--	--	--	--	--	--	--	--	--	--
Block No.8		--	--	--	--	--	--	--	--	--	--	--	--
Block No. 9		--	--	--	--	--	--	--	--	--	--	--	--
Block No.10		--	--	--	--	--	--	--	--	--	--	--	--
Block No.11		--	--	--	--	--	--	--	--	--	--	--	--

NOTE :
(a) + indicates Cry1Ac positive and * indicates Cry2Ab positive PCR reaction in the pooled DNA of a given sample
(b) – indicates PCR negative reaction in the pooled DNA of a given sample
Number of pollen trap progeny seeds per sample pool=20

Summary Results of Pollen Trap Progeny PCR

Table 2B: Shamshabad Location

DIRECTION OF BLOCKS

		A (EAST)			B (SOUTH)			C (WEST)			D (NORTH)		
	Sample No.	1	2	3	1	2	3	1	2	3	1	2	3
Block No.1	Row No.1	--	--	--	--	--	--	--	--	--	+*	--	+*
	Row No.2	+	+	--	--	--	--	--	--	+	--	--	+*
	Row No.3	--	--	--	--	--	--	--	--	--	--	--	--
	Row No.4	+*	+*	--	--	--	--	--	--	--	--	--	+
	Row No.5	+	--	--	--	+	+	*	--	--	--	--	--
Block No.2		--	--	--	--	--	--	--	--	--	--	+	--
Block No.3		--	--	--	--	--	--	--	--	--	+	--	*
Block No.4		--	--	--	--	--	--	--	--	--	--	--	--
Block No.5		--	--	--	--	--	--	--	--	--	--	--	--
Block No.6		--	--	--	--	--	--	--	--	--	--	--	--
Block No.7		--	--	--	--	--	--	--	--	--	--	--	--
Block No.8		--	--	--	--	--	--	--	--	--	--	--	--
Block No. 9		--	--	--	--	--	--	--	--	--	--	--	--
Block No.10		--	--	--	--	--	--	--	--	--	--	--	--
Block No.11		--	--	--	--	--	--	--	--	--	--	--	--

NOTE :
(a) + indicates Cry1Ac positive and * indicates Cry2Ab positive PCR reaction in the pooled DNA of a given sample
(b) – indicates PCR negative reaction in the pooled DNA of a given sample
Number of pollen trap progeny seeds per sample pool=20

Summary Results of Pollen Trap Progeny PCR

Table 2C: DPPL Location

DIRECTION OF BLOCKS

		A (EAST)			B (SOUTH)			C (WEST)			D (NORTH)		
	Sample No.	1	2	3	1	2	3	1	2	3	1	2	3
Block No.1	Row No.1	--	--	--	+	+*	--	--	+*	--	--	--	+
	Row No.2	--	+	--	--	--	--	--	--	--	--	+	--
	Row No.3	--	--	--	--	--	--	--	+	--	--	--	+
	Row No.4	--	--	+	--	--	--	--	--	+	--	--	--
	Row No.5	--	--	--	--	--	--	--	--	--	--	--	--
Block No.2		--	--	--	--	--	--	--	--	--	--	--	--
Block No.3		--	--	--	--	--	--	--	--	--	--	--	--
Block No.4		--	--	--	--	--	--	--	--	--	--	--	--
Block No.5		--	--	--	--	--	--	--	--	--	--	--	--
Block No.6		--	--	--	--	--	--	--	--	--	--	--	--
Block No.7		--	--	--	--	--	--	--	--	--	--	--	--
Block No.8		--	--	--	--	--	--	--	--	--	--	--	--
Block No. 9		--	--	--	--	--	--	--	--	--	--	--	--
Block No.10		--	--	--	--	--	--	--	--	--	--	--	--
Block No.11		--	--	--	--	--	--	--	--	--	--	--	--

NOTE :
(a) + indicates Cry1Ac positive and * indicates Cry2Ab positive PCR reaction in the pooled DNA of a given sample
(b) – indicates PCR negative reaction in the pooled DNA of a given sample
Number of pollen trap progeny seeds per sample pool=20