Breeding of okra for resistance to yellow vein mosaic virus

ABSTRACT:

Okra (*Abelmoschus esculentus* L. Moench) commonly known as Lady's finger or Bhendi is most delicious vegetable relished worldwide. The productivity of okra has been low in recent times due to various reasons *viz*., inadequate use of fertilizers, irrigation and occurrence of various pest and diseases. In India okra crop is highly susceptible to yellow vein mosaic virus (YVMV) disease probably due to warm tropical climate and intensive crop cultivation which supports the survival of whitefly population round the year. Host plant resistance to virus is one of the most practical, economical and eco-friendly strategies for reducing yield loss in okra. Identification of stable and reliable sources of resistance to viral diseases is an important step in future breeding programme. Understanding the genetics of resistance in donor parent helps in choosing suitable breeding method and attempts should be made to incorporate broad spectrum resistance through gene pyramiding and develop okra varieties with desirable resistance or tolerance to okra YVMV followed by maintenance breeding.

KEY WORDS: YVMV, Begamovirus, Leaf curl, Whitefly

INTRODUCTION:

India being the second largest producer of fruits and vegetables in the world after China, where in India stands first in the production of Mango, Banana, Papaya and Okra. India being the leading producer and exporter of okra which holds 70 per cent of foreign exchange (excluding onion) among fresh vegetables which occupies an area of 523 thousand ha, production of 6416 thousand MT (NHB 2021-22). But in recent times it is getting set back in obtaining maximum productivityin spite of its growers and consumers acceptance as well as its wide genetic diversity.

Okra (*Abelmoschus esculentus* L. Moench) previously called *Hibiscus estulentus* is one of the most important vegetable crops grown across India which belongs to family Malvaceae, mainly grown in warmer parts of the country, originated from Tropical Africa**,**

chromosome no: 2n= 130 which is an amphidiploid**.** Ten species of *Abelmoschus* known to occur in India, which are of Asiatic origin, out of which *A. esculentus*, the only cultivated species is probably of Indian origin.The cultivated okra has somatic chromosome number of 2n=130, which is an amphidiploid of *A. tuberculatus*(2n=58) and an unknown species (2n=72) and followed by doubling of chromosomes.

Uses of okra:

- Immature pods are used as vegetables, which are used fresh or canned
- Mature fruits and stem containing crude fiber, used in the paper industry
- The stem and roots of okra used for clearing cane juice in the preparation of jaggery

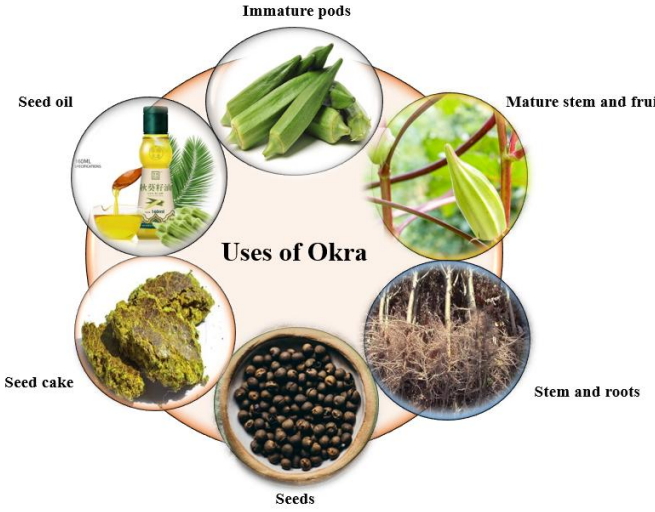

Fig 1 : Uses of Okra

- The seeds are roasted and grounded, used as substitute for coffee
- Seed cake is used as an animal feed
- Refined seed oil may be used as substitute for cotton seed oil

Some of the production constraints that inhibit okra yield and quality which includes abiotic stresses like drought and low temperature, biotic stresses like okra enation leaf curl virus, nematodes, okra fruit and shoot borer, sucking pests and okra yellow vein mosaic virus which limits its productivity. Yield loss due to these biotic stresses in okra where YVMV holds a prime place which cause yield loss ranging from 50-94 per cent depending upon the crop stage at which disease incidence takes place. Therefore, for a breeder it becomes a major concern to develop varieties/hybrids resistant to such devastating disease along with higher yield, better fruit quality, photo-thermo insensitivity and wider adoptability.

Yellow Vein Mosaic Virus (YVMV):

Viruses poses a serious constraint in the okra production and the crop is susceptible to 36 viruses across the world where 19 viruses were reported in India. Where in India okra yellow vein mosaic virus and okra enation leaf curl virus are majorly occurring in India, recently TSV (Tobacco streak virus), an Ila virus is also becoming major problem in successful okra cultivation.

- Yellow vein mosaic virus (YVMV) was first time reported by Kulkarni in 1924 during erstwhile Bombay presidency in India
- Further, Uppal *et al*., 1940 established the viral origin of the disease based on morphogenic symptoms expressed in relation with African cassava mosaic virus and named it as YVMV
- Caused by bipartite begomovirus which belongs to the family Geminiviridae
- It causes a yield loss ranging from 50-94 per cent depending on crop stage at which disease incidence takes place
- Extent of damage declines with delay in infection
- The disease is more pronounced during hot weather and high RH conditions
- It is transmitted by an insect vector Whitefly (*Bemisia tabaci*) and graft transmitted
- Distribution : Tropical (June-Sept), Temperate (Feb- June)

Table 1. Begomovirus association with yellow vein mosaic disease of okra

Disease	Viruses	Genome	Transmission	Distribution	Reference
YVMV	Bhendi yellow vein mosaic virus	Monopartite	*Bemisia tabaci*	India	Kulkarni (1924)
YVMV	Bhendi yellow vein Bhubhaneswar virus	Monopartite	*Bemisia tabaci*	India	Venkataravanappa *et al*. (2013)
YVMV	Bhendi yellow vein Haryana virus	Monopartite	*Bemisia tabaci*	India	Venkataravanappa (2008)
YVMV	Bhendi yellow vein Maharashtra virus	Monopartite	*Bemisia tabaci*	India	Venkataravanappa (2008)
YVMV	Cotton leaf curl Bangalore virus	Monopartite	*Bemisia tabaci*	India	Venkataravanappa *et al*. (2013)
YVMV	Cotton leaf curl Allahabad virus	Monopartite	*Bemisia tabaci*	India	Venkataravanappa *et al*. (2012)
YVMV	Bhendi yellow vein Delhi virus	Bipartite	*Bemisia tabaci*	India	Venkataravanappa *et al*. (2012)
YVMV	Tomato leaf curl New Delhi virus	Bipartite	*Bemisia tabaci*	India	Venkataravanappa (2008)
YVMV	Radish leaf curl virus	Monopartite	*Bemisia tabaci*	India	Kumar *et al*. (2012)
YVMV	Okra yellow vein mosaic virus	Bipartite	*Bemisia tabaci*	Pakistan	Zhou *et al*. (1998)
YVMV	Bhendi yellow vein mosaic virus	Monopartite	*Bemisia tabaci*	Pakistan	Zhou *et al*. (1998)

Genome organization of Begomovirus:

- The disease is caused by complex consisting of monopartite or bipartite begomovirus and a small satellite DNA alpha or beta components which is transmitted by an insect vector whitefly

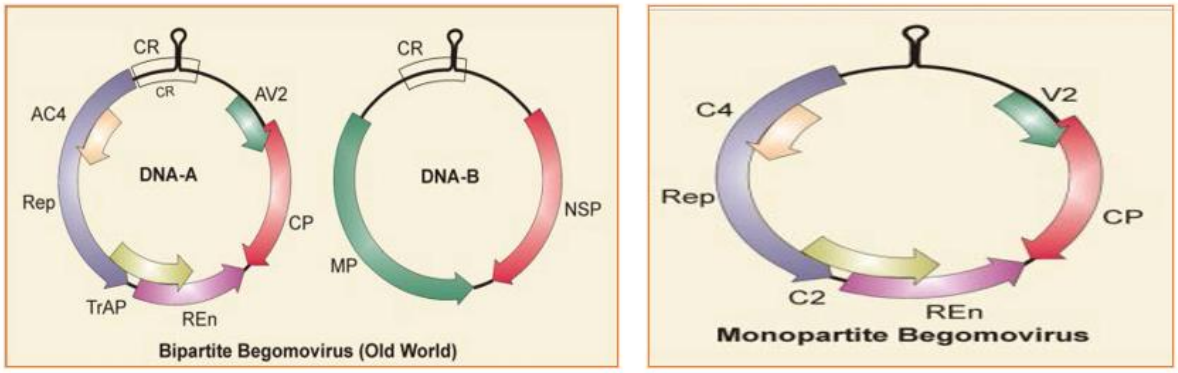

Fig 2 : Genome organization

- The bipartite genome is comprised of two similar sized DNA components (DNA A and DNA B)
- The DNA A component encodes a replicated associated protein (Rep) which is essential for DNA replication, a replication enhancer protein (REn), Coat protein (CP) and a transcription activator protein (TrAP) that controls late gene expression
- DNA B component encodes a nuclear shuttle protein (NSP) and movement protein (MP) both of which are essential for systemic infect of plants

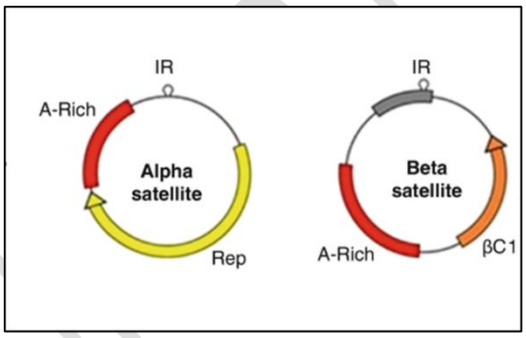

Fig 3 : Genomic changes

- Whereas majority of monopartite and bipartite begomovirus are associated with additional ssDNA molecules known as betasatellites or alphasatellites
- Betasatellites associated with monopartite virus are approximately half the size of their helper begomovirus and required to induce typical disease symptoms in the host
- These satellites depend on their helper virus for replication, movement, encapsidation and the vector transmission

- Alpha satellites are self-replicating circular ssDNA which doesn't play role in symptom induction

Replication of virus DNA

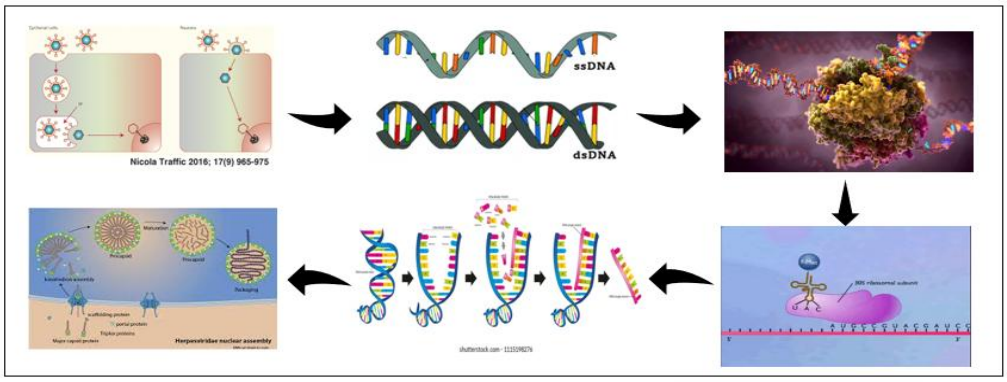

Fig 4 : Replication of DNA in Virus.

- When virus enters the host cell, ssDNA of the virus gets entered into the nucleus of the host cell, where it has to make RNA so that protein synthesis can take place. But we know that RNA polymerase cannot work on ssDNA, so it has to convert into dsDNA, so that transcription can takes and RNA can form
- So, with the help of host enzymes, it will convert into dsDNA, and use host enzymes for the transcription, as a result mRNA formed. mRNA will go out of nucleus and attach on ribosome and protein synthesis will take place
- The dsDNA with the help of DNA polymerase, ssDNA copies will be formed and which comes out of nucleus to cytoplasm and assembly of virus takes place and exact copy of virus will be formed
- So, when the insect sucks the sap from the plant, along with the sap these virus gets entered into the vector system and then insect becomes viruliferous

Symptoms of YVMV:

- Homogeneous interwoven network of yellow veins enclosing islands of the green tissues, Initially infected leaves exhibit only yellowing of the veins and veinlets but in the later stages the entire leaf turns completely yellow
- Plants remain stunted
- Fruits of the infected plants exhibit pale yellow color, become deformed, small and tough in texture

Plants undergo three types of symptoms

1. Leaves of younger plants infected very early in the season, become complete yellow and later turn brown and dry up, yield loss will be up to 70 per cent
2. In second type, infection started after flowering, upper leaves and flowers show vein clearing symptoms

   Infected plants produce some fruits but they become yellow and hard at picking stage
3. In third type, plants continue to grow in a healthy state but at the end few small young shoots appear at the basal portion which exhibit vein clearing symptoms

- If the plants within 20 days after germination – yield loss about 94 per cent
- If infected 50 and 65 days after germination yield loss of 84 and 49 per cent respectively

YVMV- Vector:

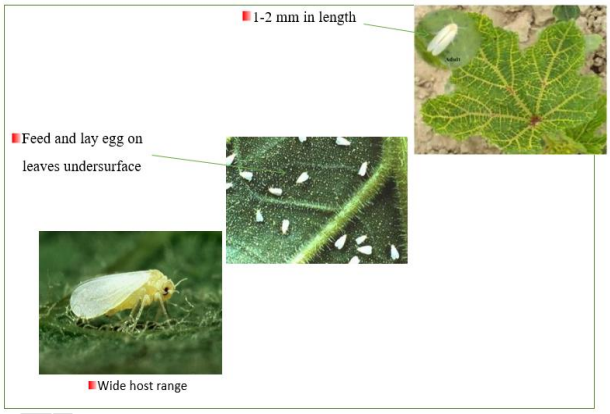

Fig 5 : YVMV vector

- Whitefly is one of the most important sucking pests that inflicts heavy damage to the crop
- Whitefly i.e., *Bemisia tabaci* which belongs to order Hemiptera and family Aleurodidae
- Adults are 1-2 mm in length, feed and lay egg on undersurface of leaves which has wide host range
- Female fly is more efficient than male fly in spread of infection
- Excess of N_2 and Deficiency of K leads to higher attack rate

- Young plants are more sensitive than older plants
- High temperature and high humidity lead in high vector population

Whitefly (*Bemisia tabaci*) : Life cycle

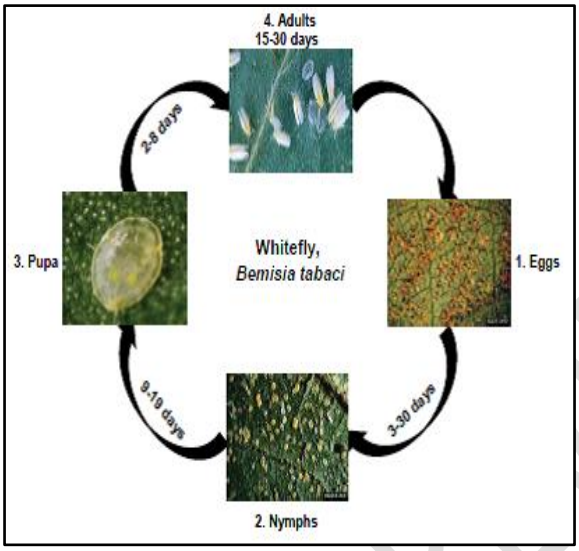

Fig 6 : Life cycle of whitefly (*Bemisia tabaci*)

Epidemiology:

- 20-30 ℃ is most favorable for disease development
- The disease severe during rainy season with high temperature and high humidity coupled with high vector population
- In South India, occurrence of YVMV is highest during June- July and less during cooler months
- Incidence increased with rise in minimum temperature and whitefly population decreased with increase in RH
- Hot and dry weather conditions favor fast spread of YVMV

Morphological and Biochemical basis of resistance:

- Resistant plants have higher trichome density, lesser leaf surface
- Resistant plants contain higher moisture, phenols, orthodihydroxy phenols and total chlorophyll content than susceptible cultivars
- Total sugar, nitrogen, reducing and non-reducing sugar were higher in infected leaves
- **At cellular level,** absence of toxin receptor sites on the host cells and detoxifying the toxins produced by the pathogen

Genetic basis of resistance:

- Inheritance of resistance to YVMV in okra was studied using three resistant (R) parents (Arka Anamika, Punjab Padmini and Arka Abhay) and three susceptible (S) parents (Pusa Sawani, Local bhendi and Pusa Makhmali)
- Cross between S×S and S×R – Two complementary dominant genes
- Cross between R×R – Two duplicate dominant genes

Sources of resistance:

1. *Abelmoschus manihot* (2x=66)
2. *A. angulosus* (2n=138)
3. *A. crinitus* (2x)
4. *A. vitifolius*
5. *A. tuberculatus* (2n=58)
6. *A. panduraeformis*
7. *A. pungens* (Amphidiploid)
8. *A. tetraphyllus* (Amphidiploid)

Table 2. Inheritance of YVMV resistance in okra

S. No.	Resistant parent (R)	Susceptible parent (S)	Gene action/ Remarks	References
1	*A. manihot* (L.) Medik and *A. manihot* (L.) Medik *ssp. manihot*	*A. esculentus cv.* Pusa Sawani	Single dominant gene	Jambhale and Nekar, 1981
2	*A. manihot ssp. manihot*		Two dominant genes (yv_1yv_2)	Sharma and Dhillon,1983
3	*A. manihot*	*A. tetraphyllus*	Single dominant gene	Dutta,1984
4	*A. manihot* (L.) Medikus *ssp. manihot*	*A. esculentus cv. Hisar Unnat*	Two complimentary dominant genes	Sharma and Sharma, 1984; Dhankhar *et al*., 2005

While working with genetics ploidy level should be considered because of the presence of chromosomal differences or variability in the ploidy level makes the crossing between wild species and cultivated species difficult and also the biggest genome size makes the breeding work more cumbersome.

Reasons for resistance breakdown:

- Even though there was development of resistant varieties like Parbhani Kranti, Punjab 7, Arka Anamika, Arka Abhay and Pusa Sawani which showed resistance against YVMV for more than two decades which later eventually became susceptible probably due to the appearance of new strains of virus or recombination/mutation of virus strain
- Emergence of polyphagous 'B' biotype of *B. tabaci*
- Increased host range of >600 plant species

Transmitting plant virus using whitefly:

The use of whitefly for transmission of plant viruses for the selection and development of resistant cultivars in breeding program is challenging where, we need to inoculate 100 per cent of plants in order to find the genotypes which posess resistant genes

There are two types of whiteflies colonies to be maintained

- Non-viruliferous whitefly colony – reared on virus free plants
- Viruliferous whitefly colony – reared on virus infected plants

1. **Whitefly colony maintenance:**These whiteflies colony should be maintained in clean and controlled growth room. Control of RH, light, photoperiod and temperature are essential. For colony that develops from egg to adult within 8-18 days. The temperature should be between 26-30 ℃, RH between 30-50%, photoperiod of 14hrs and light intensity between 800-1000foot candles and reduced dose of irrigation and fertilizer should also be provided. Whiteflies should be reared on plants inside the cages rather than freely in the growth room. The plant should be strong enough to support high insect population without collapsing.

2. **Whitefly colony establishment:** The first whitefly colonies are free from virus and insect pathogens, if whiteflies are collected from field rear them for non-host plants for about 8 weeks during which check for absence of plant symptoms. Once whiteflies are collected, they were introduced onto the plants. Every week a new cage needs to be established, during first three weeks use non host plants to establish the new cage, starting from fourth week use young adult old flies to establish new cage. During first week whiteflies will lay egg on underside of leaves, in second week immature whiteflies will occur, during third week many new adults will emerge and there will be very noticeable increase in the number of adult whiteflies, in fourth week adults should be transferred to new cage and plants were discarded.

3. **Method of inoculation to test plants:** Precise and gentle transfer is accomplished with aspiration and collecting vials. Never aspirate the whiteflies that are feeding on the crop because their stylets are embedded in the plants while feeding so pulling them off breaks their stylets. To collect the whitefly, hold the yellow color card inside the cage with one hand and gently shake the plant to encourage the whiteflies to fly, so they will get attracted to the yellow card then they can be collected using an aspirator with very gentle breath.

4. **Acquisition:** Whiteflies are placed in virus infected plants and allow to feed for 48-72 hours to acquire persistently transmitted virus.

5. **Inoculation:** Prepare a cage and place the test plants inside, collect the whiteflies by aspiration and place the vials containing whiteflies inside the cage and remove lid.
6. **Termination:** Inoculation access period is ended by killing the whiteflies with approved chemicals, apply a contact and a systemic insecticide.

Screening methods:

1. Screening in hotspots:

There are six hotspot regions identified in India for YVMV disease namely,

- Vijayawada (Andhra Pradesh)
- Raipur (Chhattisgarh)
- Jabalpur (Madhya Pradesh)
- Kalyani (West Bengal)

- Anand (Gujarat)
- Salem (Tamil Nadu)

In this method once the hotspot area is identified, field layout is designed where in border rows susceptible varieties are planted and next five lines test material should be in grown followed by susceptible varieties and so on. After sowing from 5th day onwards screening for symptom continued for 30, 60 and 120 days after screening virus indexing is done.

2. Whitefly mediated screening:

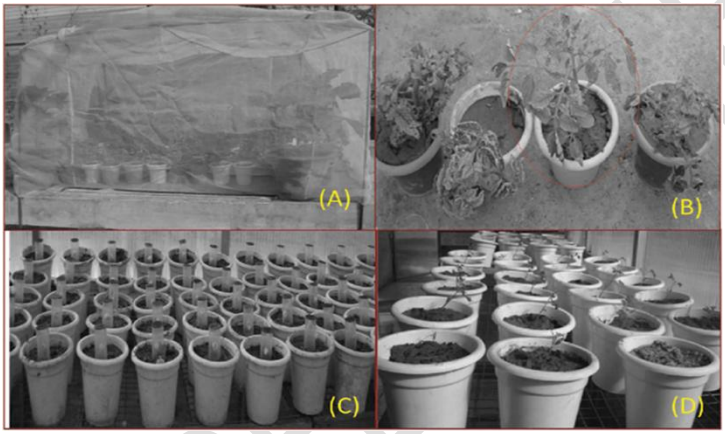

Fig 7 : Whitefly mediated screening

Followed in polyhouse or net houseswhich is done in seedling stage. Release of virulent whiteflies carrying these viruses simultaneously (10 whiteflies/seedling) or release in individual cup by which the seedlings are covered and allow whiteflies to feed on the plants overnight and take observations 20-25 days after inoculation.

Fig 8 : **Whitefly mediated phase.**

3. Screening through grafting:

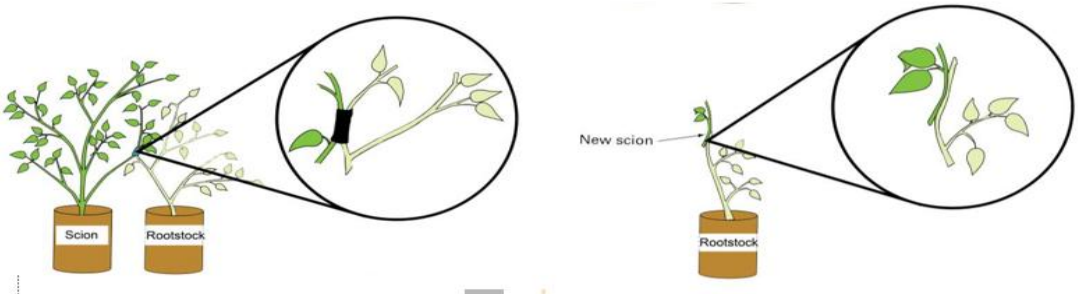

Fig 9 : Screening through grafting

To test the transmissibility of YVMV virus through graft union, pencil size thickness scion of susceptible plants was grafted onto resistant plants, using approach grafting under protected condition. Graft union takes place after 21 days and then start taking observations, if the new shoot formed on the bottom portion, if plant did not show any symptoms, then it is resistant

4. Argoinoculation based screening:

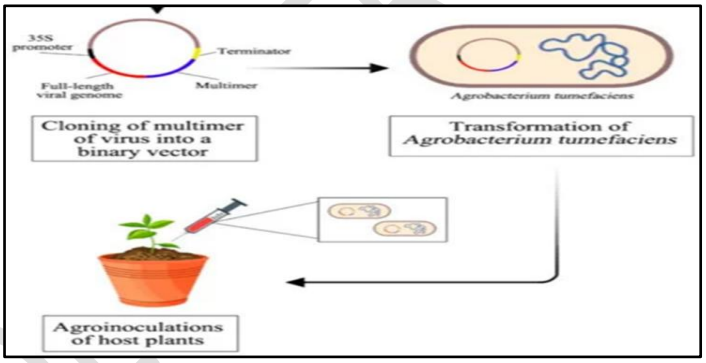

Fig 10: Argo inoculation based screening

A tandem repeat of viral genome is cloned into the T-DNA of an *Agrobacterium tumefaciens* Ti plasmid. Then the cloned bacterium is delivered into plant system through injection and then screening was done.

Virus disease indexing scale:

The virus disease indexing scale was given based on the percentage of disease incidence (%)

$$\text{PDI (\%)} \frac{\text{Number of diseased plants}}{\text{Total number of plants observed}} \times 100$$

List 1:**Virus disease indexing scale**

Grade	Reaction	PDI (%)
0	Immune	00
1	Highly resistant	1-10
2	Moderately resistant	11-25
3	Tolerant	26-50
4	Moderately susceptible	51-60
5	Susceptible	61-70
6	Highly susceptible	71-100

Resistance evaluation through sensitive assay

1. ELISA/ Nucleic acid probe: Based on the principle of antigen-antibody interaction. At first sap extract from the plant is added on the wells of microtiter plate, after incubation antibodies are added followed by washing. If the virus of interest is present in the plant, it will bind to antibodies.

2. PCR assay: The PCR is a technique that enables the specific amplification and hence detection of target DNA sequence from complex mixture of nucleic acid.

Breeding approaches:

1. Conventional methods

- Selection
- Pedigree method
- Backcross method
- Mutation breeding

2. Non-conventional methods

- Embryo rescue technique
- Post transcriptional gene silencing
- Marker assisted breeding
- Market assisted backcrossing
- Gene pyramiding

Selection method

1. CO-1 (TNAU, Coimbatore)
2. Gujarath Bhendi (AAU, Gujarath)
3. Kashi Mangai (IIVR, Varanasi)

Mutation method

1. MDU-1 : Mutant of Pusa Sawani (TNAU, Coimbatore)
2. EMS – 8 : Induced mutant of Pusa Sawani (PAU, Ludhiana)
3. Anjitha (KAU, Thrissur)
4. Manjima (KAU, Thrissur)

Pedigree method

1. Pusa Makhmali (IARI, New Delhi)
2. Pusa Sawani : IC 1542 × Pusa Makhmali
3. Varsha Uphar : Lam sel × Parbhani Kranti
4. Hissar Unnat : Sel 2-2 × Parbhani Kranti (HAU, Hissar)
5. Kashi Chaman
6. VRO-6 (Kashi Pragati)
7. VRO-5 (Kashi Vibhuti)
8. IIVR-10 (Kashi Satdhari)
9. IIVR-11 (Kashi Lila)
10. VRO-3 (Kashi Mohini)

Inter specific hybridization / Backcross breeding

1. Punjab 7- *A. esculentus* (Pusa Sawani) × *A. caillei*
2. Punjab Padmini - *A. esculentus* (Reshmi) × *A. caillei*
3. Parbhani Kranti - *A. esculentus* (Pusa Sawani) × *A. manihot*
4. Arka Anamika and Arka Abhay - *A. esculentus* (IIHR 20-31) × *A. tetraphyllus* var. *tetraphyllus*
5. Arka Nikita – GMS based hybrid

RNA mediated gene silencing:

The usual process that happens in a cell is central dogma of molecular biology

DNA ⟶ mRNA ⟶ Protein

- dsRNA has silencing effect to complementary mRNA so that protein synthesis will not take place
- dsRNA produced inside the cell leads to the production of siRNA, miRNA and shRNA by the use of Dicer, where the Dicer cleaves dsRNA into smaller fragments to produce siRNA
- Then another protein from Arganaut family binds to the siRNA and discard the sense strand of it, which later combines with other proteins like slicer from the RISC complex (RNA interfering silencing complex)

  Since it is complementary to the sense strand of mRNA, it pairs with it to inhibit translation or cleave the mRNA strand that results in formation of protein
- The technology is mainly used for gene silencing or knock down of gene

  Normally in a cell nucleus will produce mRNA which in turn produce malfunctioning protein responsible for the disease cause, so this siRNA will bind with mRNA and inhibit the production of malfunctioning protein. But after certain time again when the gene is expressed, it will produce mRNA again everytime siRNA cannot be given from outside so if we can inject a cell containing siRNA with a vector which itself produces dsRNA continuously inside the cell, which ultimately breakdown the targeted mRNA

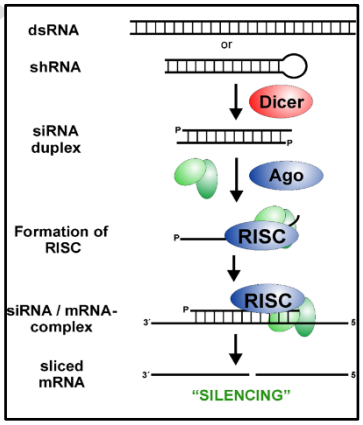

Fig 11 : Gene synthesis

Private sector hybrids:

- Shakthi, Sonal, Sarika, Singam (Nunhems)
- No.10 and No.64 (MAHYCO)
- Syngenta-152 (Syngenta)

- NS811, NS862, NS7772 and NS7778 (Namdhari seeds)
- Hyb-7315 (JK seeds)
- Avanthika (Bio seeds)
- Sahibha and Sahan (Rasi seeds)
- Janni, Navya and Radhika (Adventa seeds)

Management of YVMV:

- Select a field free of virus infected plants
- Use virus resistant varieties
- Destroy host plants such as Croton, Tomato, Brinjal and Chilli
- Spray neem oil or mustard oil @ 0.5 per cent
- Use rice straw and yellow coloured polythene as mulch
- Establish banana plants as a barrier around the okra field one or two months before planting
- *Rhizobacteria* controls the viruses through systemic defence mechanism
- Spraying acetamiprid 20% SP at 10g/l
- Application of chlorpyriphos 2.5ml/l + neem oil 2ml/l of water

Institutes working on okra:

- IIHR, Bangalore
- IARI, New Delhi
- IIVR, Varanasi
- KAU, Thrissur
- PAU, Ludhiana
- CISHAU, Hissar

Conclusion:

Identification of virus specific resistance and introgression of resistance gene from unexploited wild species will help to broaden the resistance breeding. Understanding the genetics of resistance in donor parents helps to identify suitable breeding method. Among the different sources, wild species serves as the best and they need to be exploited to develop stable virus resistant varieties/hybrids in okra.

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