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Breeding Approaches for Biotic Stress Resistance in Vegetables

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Abstract

In vegetables the factors for biotic stress are pests, diseases and nematodes. The damages induced by these factors reflect highly on production, productivity and quality. Although application of pesticides/fungicides and nematicides has managed these stresses, excessive use of unsafe chemicals results in environmental pollution and leave residues in vegetables which are above threshold levels and also promote the development of new races/biotypes of pests and pathogens. Therefore vegetable improvement works concentrate on high yielding varieties with multiple resistance to these biotic stresses. For such studies, the knowledge on the genetic basis of resistance and plant-pest/pathogen interactions is necessary which will in turn improve the efficiency of the breeding programmes by introducing resistant genes and result in high-yielding genetically resistant cultivars. For the development of resistant varieties and pre-breed lines, information on sources of resistance is prerequisite and serve as a backbone in the breeding programme. Further, gene action responsible for the inheritance of characters helps in the choice of suitable breeding methods for the improvement of the crop. Work has been done by using the various breeding methods and resistant varieties have been bred and they offer the cheapest means of pest/disease/nematode control. Resistant varieties obviate the use of chemicals, thus reduce environmental pollution and facilitate safe food for human consumption.

Keywords: vegetables, biotic stress, breeding methods, varieties

1. Introduction

Globally the area under vegetable cultivation is growing annually at the rate of 4.12% and production by 6.48%. The mean productivity is 15.49mt/ha which is quite low. In vegetables infestation of biotic stresses reflect highly on production productivity and quality. Application of chemicals leaves chemical residues in vegetables above threshold levels. Resistance is a relative attribute and refers to the ability of the plant to withstand the pest or pathogen. The susceptible plant shows severe symptoms due to which yield loss occur. A completely resistant plant shows nil reaction and a moderately resistant or field tolerant plant develops less disease development. Plants have selective resistance to some pests or pathogens and susceptible to others. They are species-specific or strain-specific. The rate of spread depends on the pest load or population, spore count and multiplication rate of pest/pathogen.

Improvement of cultivated plants through tapping germplasm resources depends on introducing variability through traditional and molecular breeding techniques. Wild species provides a vast gene pool for resistance development.

They have been used for decades to transfer genes of resistance or tolerance to the cultivated species. The use of wild species in breeding varieties particularly for increased vigor and resistance has been well recognized [1]. Introgression is the movement of genes or gene flow from one species into the gene pool. Inter specific hybridisation breaks the species barrier for gene transfer and makes it possible to transfer the resistant genes.

Complete exploitation of genetic variation enables the breeder to produce not only heterotic F1 hybrids but also recombinants with desirable attributes. Further, selection based on genetic nature will be highly useful to a great extent to screen out the parents and hybrids. Identification of resistance is also possible through quantifying the biochemical components present in the genotype. Further, in view of less marked host specificity, a plant breeding programme for insect resistance has to be handled separately from that of disease resistance.

In 3rd century B.C, Theophrastus observed that degree of resistance differ among varieties. It was later established in 1894, by Erikson that though pathogens are morphologically similar, they differ among each other in their ability to attack host plant. In 1911 Barrus narrated that various isolates of a pathogen differ in its ability to attack different varieties of the same plant species. This made the basis for the identification of physiological races and pathotypes. It was then called as pathogenicity *i.e* the infection of a host strain by a pathogen is genetically determined. In 1955, Flor formulated the of gene-for-gene hypothesis which denotes the relationship between host and pathogen. According to that disease resistance is determined by host and the genotype of the pathogen. The hosts differ in type of resistance while the pathogen differ in pathogenicity, but both are genetically controlled. The pathogen has the capacity to generate new variations in pathogenicity by reproduction methods and mutation. Therefore, the task of the breeder is to develop varieties resistant to the prevalent pathotypes of the pathogen and also for the new pathogen genotypes which will arise in future.

2. Genetic basis of resistance

According to the experimental results so far reported in vegetable crops, the genetic basis of insect resistance is monogenic. The resistance of muskmelon to melon aphid. The tolerance of muskmelon to western biotypes of *Aphis gossypii* in breeding line LJ 90234 was governed by a single dominant gene [2]. Inheritance studies of fruit fly resistance in pumpkin cultivar Arka Suryamukhi showed that the resistance was controlled by a dominant gene. Similar studies in water-melon also indicated that the resistance to fruit fly was governed by a single dominant gene. The work on *Cucurbita pepo* revealed that the resistance to squash bug, was controlled by at least 3 genes and gene action appeared to be additive in nature. In an interspecific cross between resistant *Cucumis callosus* and susceptible *Cucumis melo* it was revealed that the susceptibility to fruit fly was governed by two pairs of complimentary genes. While working with tomato for resistance to fruitworm Fery and Cuthbert [3] reported that the antibiotics factor present in *Lycopersicon hirsutum* appeared to be inherited recessively.

Since interest in resistant vegetable varieties started more than half a century ago work has been done on major insect pests. However, studies have shown that the Mendelian segregation has led to the identification of major genes and that the alleles for resistance were dominant over those for susceptibility in number of instances except in some where it was found to be additive or complementary gene action or recessive.

The genetics of disease resistance was first studied by Britten in 1905. Then Person and Sidhu [4] reviewed 1000 Published papers and concluded that regardless of species, resistance generally segregated in the mendelian ratios. Resistance was dominant over susceptibility. Resistance in vegetable crops have been reported to be governed by mono or oligo or polygenes and effect of genes may be additive or dominant or epistatic. The information on inheritance of various diseases of vegetables is very meager. However, some workers reported different kinds of nature of inheritance. Resistance to buck eye rot of tomato appeared to be dominant over susceptibility. Resistance to fusarium wilt of tomato was conditioned by a single dominant gene. Tomato leaf curl virus is transmitted by white fly and is most serious problem. According to Som and Chaudhary [5], resistance to TLCV was incompletely dominant and governed by polygenes.

Resistance to most of the diseases in watermelon is controlled by a single dominant gene. Walker [6] reported resistance to fusarium wilt in watermelon as recessive. Powdery mildew is a major limiting factor in the production of muskmelon in most of the parts of the world. Resistance to *Erysiphe cichoracearum* race- 1 and race-2 is monogenic dominant. A study on resistance to powdery mildew caused by *Sphaerotheca fuliginea*, in two resistant varieties campo and PMR-6 indicated that they have the same locus/loci conferring resistance. Genetic studies of resistance to *E. cichoracearum* race-2 had indicated that resistance is partly dominant and controlled by Pm-2 [7]. Resistance to downy mildew (*Pseudoperonospora cubensis*) of muskmelon in PI 124111 is controlled by two independently dominant gene [8]. Whereas resistance in PI 124112 was controlled by two partially dominant genes [9].

Antonio *et al.* [10] studied the inheritance of resistance by antixenosis for tomato leaf miner and reported that the inheritance of antixenosis resistance of genotype BGH-1497 is ruled by a polygenes in epistatic interactions, with a phenotypic proportion of 13:3 between susceptible and resistant genotypes respectively. In another experiment Gabriele Vitelli *et al.* [11] reported three transgenic eggplant lines bearing a mutagenized *Bacillus thuringiensis* Berl. gene coding for the Cry3B toxin. The fruit production was almost twice in the highly resistant lines (3-2 and 9-8). The 6-1 transgenic line showed an intermediate level of resistance. Analysis by double antibody sandwich-enzyme linked immune sorbent assay (DAS-ELISA), performed on different tissues, revealed a lower amount of Cry3B protein in the 6-1 transgenic line.

The root knot nematode, *Meloidogyne* is one of the most economically damaging plant parasitic nematode and is widely distributed throughout world [12]. The genus *Meloidogyne* is composed of 100 species, with *M. arenaria*, *M. incognita*, *M. hapla* and *M. javanica* being considered as “major” species [13]. Natural resistance genes “R-genes” are responsible for inducing resistance against nematodes in tomato. The genes Mi-1, Mi-2, Mi-3, Mi-4, Mi-5, Mi-6, Mi-7, Mi-8, Mi-9, and Mi-HT confer resistance to the root Knot nematode [14].

3. Biochemical basis of resistance

The biochemical factors are more important than morphological and physiological factors in conferring resistance. Some biochemical constituents may act as feeding stimuli for insects. Occurrence at lower concentration or total absence of such biochemical leads to non preference, a form of insect resistance [15, 16]. The biochemical constituents like glycoalkaloid (solasodine), phenols, phenolic oxidase enzymes *viz.*, polyphenol oxidase and peroxidase are available in plants and these biochemical constituents possess insect resistant properties [17]. It was also recorded that the maximum polyphenol oxidase activity is available in fruit (0.388

in fruit as changes in OD min⁻¹ g⁻¹ of sample). Several workers have reported that the biochemical constituents act as stimulants of resistance mechanism. In brinjal, Praneetha [18] and Prabhu [19] have recorded that the biochemical constituents also contribute to confer resistance to shoot and fruit borer.

Studies on the biochemical basis of resistance to *Leucinodes orbonalis* and their correlation with shoot and fruit borer damage in five selected brinjal genotypes were done during June to December 2005 and it was shown that less susceptible genotypes for both shoot and fruit borer had higher amount of polyphenol oxidase (PPO), phenylalanine ammonium lyase (PAL), lignin and lower reducing sugar. Significant negative correlation was established with per cent infestation of shoot and fruit borer and PPO, PAL and lignin, whereas it was positively correlated with reducing sugar. Negative correlation was observed with the biochemical constituents, PPO, PAL, lignin and reducing sugar but PPO was positively correlated with PAL and lignin content and vice-versa.

4. Biophysical basis of resistance

In a study Silva et al. [20, 21] evaluated ninety-nine F3 families derived from an interspecific cross using *Solanum lycopersicum* and *Solanum pimpinellifolium* 'TO-937-15' (multiple pest resistance accession with type IV glandular trichomes and acylsugar accumulation) for their resistance against the whitefly. The higher resistance levels of BTR331 were associated with a positive combination of higher type IV trichome density and higher acylsugar levels. From the breeding stand point, the genetic similarity between *S. lycopersicum* and *S. pimpinellifolium* would allow a more efficient resistance introgression by facilitating recombination and minimizing the potentially undesirable linkage drag associated with this trait.

Niranjana et al. reported that the biophysical characteristics in brinjal genotypes viz., shoot thickness at 2.5 cm below the tip, number of trichomes on lower surface of leaves, pedicel length, calyx length and diameter of fruit were correlated with the level of infestation by *L. Orbonalis*. Results revealed that the infestation in shoot was not significantly correlated with number of trichomes on leaves and positively correlated with shoot thickness. Fruit infestation was positively but not significantly correlated with length of pedicel and calyx whereas non-significant and negative correlation was recorded between fruit infestation and fruit characters viz., length and diameter of fruit. The shape and color of fruit had no significant influence on the level of infestation.

5. Categorization of resistance

According to the response of the plant to the pathogen.

- a. Susceptible:** In this the disease development is abundant and can not be checked by the plant.
- b. Resistant:** It is lesser disease development than the susceptible and it is a relative attribute. In this the plants will be infected and establishment take place but the progression in the host plant will be limited. As a result these plants exhibit minor symptoms than the susceptible ones.
- c. Tolerant:** Tolerance implies that the host is attacked by the pathogen but there is no less in biomass production or yield.

Based on number of genes governing the resistance trait as

- a. **Monogenic resistance:** Controlled by single gene. Easy to incorporate into plants by breeding. Easy to break also.
- b. **Oligogenic resistance:** Controlled by few genes
- c. **Polygenic resistance:** Controlled by many genes

Resistance is classified based on biotype reaction as.

- a. **Vertical resistance:** It is determined by one or few genes and is characterized by pathotype specificity. That is it is attacked by only one virulent pathotype. For all others, the host will be resistant. It is also called as specific resistance or race-specific resistance
- b. **Horizontal resistance:** It is determined by polygenes. Horizontal resistance does not prevent the development of symptoms of the disease, but it slows down the rate of spread of disease in the population. It is also known by race-nonspecific, partial and field resistance.

Resistance is classified based on population/Line concept

- **Pureline resistance:** Exhibited by lines which are phenotypically and genetically similar.
- **Multiline resistance:** Exhibited by lines which are phenotypically similar but genotypically dissimilar

Plants once infected by a specific pathogen become resistant to further infections by the same one. This was discovered in the beginning of 20th century. This concept is involved in viral cross-protection and induced systemic resistance. Induced systemic resistance in plants is of several types of which *Systemic Acquired Resistance (SAR)* is the most important one. It is long lasting and effective against viral, bacterial and fungal pathogens. It ranges from a oversensitive response to necrotic lesions. SAR is due to high level of salicylic acid which is essentially needed for the development of SAR. Salicylic acid acts as a phloem translocated signal that mediates SAR. It is also due to SAR genes which is different in monocotyledonous and dicotyledonous plants. In tobacco, SAR genes cover a set of non-allelic genes that can be classified on the basis of proteins they encode such as the pathogenesis related (PR) genes. These genes play an active role in the disease resistance as their expression in transgenic plants impart significant disease resistance. SAR genes in various species differ in considerable extent.

6. Sources of resistance

Plants that may be less desirable in other ways, but carry a useful disease resistance trait. Ancient known plant varieties and wild species, cultivated varieties and land races are very important to preserve because they are the most common sources of enhanced plant disease resistance. Source of resistance are available for melon aphid, striped and spotted cucumber beetles, squash bug, squash borer, pickleworm, red pumpkin beetle, fruit fly in different cucurbits, cabbage maggot

and aphid in cabbage and spinach, fruit and shoot borer in brinjal, jassids in okra, potato leaf hopper, melon fly in different beans, pea aphid and weevil. Genetic basis of insect resistance has been reported to be monogenically dominant in muskmelon aphid and in pumpkin and watermelon for fruit fly, whereas, additive gene action have been reported for resistance to both striped and spotted cucumber beetles and squash bug in squash. In interspecific crosses of muskmelon with wild melon two pairs of complimentary genes are reported to be involved for resistance to fruit fly. Maternal influence has also been indicated in inter-varietal crosses of squash for resistance to spotted cucumber beetle.

In India work on resistance of cucurbits to red pumpkin beetle and fruit fly was initiated at IARI, New Delhi as early during 1962 and at Indian Institute of Horticultural Research, Bangalore during 1969. A highly resistant source to fruit fly was obtained in pumpkin, which was utilized, in breeding a resistant variety Arka Suryamukhi. Some of the pumpkin lines were fairly resistant to red pumpkin beetle. It was observed that among the different species *Citrullus colocynthis* was highly damaged by fruit fly. In *Lycopersicon* genus *L. hirsutum* and *L. hirsutum* f. *glabratum* is resistant to fruitworm, and also indicated that since these were cross-compatible with *L. esculentum* and it was possible to transfer the resistance factor in the cultivated varieties. It was further indicated that *L. hirsutum* f. *glabratum* was also resistant to tobacco flea beetle and carmine spider mite. It was possible to incorporate resistance to more than one insect species in one genotype.

In Onion sources of resistance were identified, cause and mechanism studied and suitable varieties were developed. In Okra difference in varietal response to jassid attack was observed and fruit of the resistant line was found to have strong prickly hairs and was highly susceptible yellow vein mosaic virus. In spinach variety Manchuria was reported as resistant to aphid as early as in 1920. In Carrot resistance to fruit fly was reported and one of the amaranthus lines was observed to have high field resistance to grasshopper in Nigeria. With monogenic inheritance available for melon aphid in cantaloupe, for fruit fly in pumpkin and in watermelon it would be possible to utilize backcross method for incorporating resistant gene in commercial varieties.

7. Breeding methods

Introduction: Collections of related materials from other countries, particularly from areas where the pathogen and host species may have co-evolved, sometimes provide rich pools of resistance genes [22]. In vegetable peas, early introductions from Europe and USA were found quite successful and popular in India. These included Arkel (early maturing, dwarf type, introduction from England in 1970s) and Bonneville (main season, late maturing, tall type, introduction from USA in 1970s). These introductions were obtained at IARI, New Delhi and were released for commercial cultivation after preliminary evaluation. Early Badger a dwarf, wrinkled seeded variety introduced from USA has resistance to *Fusarium* wilt.

8. Utilization of wild species

The major bottleneck in the resistance breeding programme is the lack of resistant source in the cultivated germplasm. This has necessitated breeders to search resistance for genes in wild species that are taxonomically related and compatible. The use of wild forms in breeding crop plants, particularly to obtain vigor and

resistance has been well recognized [1]. In vegetables, several experiments involving wild species have been carried out. Selection of a genotype with high yield and resistance reduces the yield loss on one hand and increases the availability of the produce to market which is fairly free from residue on the other hand. Generally the source of genes for resistance are (i) provided by the variability within the crop species, (ii) varieties from original home of insect, (iii) varieties from centres of great insect occurrence, (iv) varieties from areas of greatest morphological diversity.

In muskmelon gene for resistance is not available in the cultivated species, but *Cucumis africanus* was found to be fairly resistant to fruit fly. Similarly, *Cucumis heptadactylis* was resistant to red pumpkin beetle. An attempt to incorporate the resistance to shoot and fruit borer in the cultivated egg plant genotypes was made in interspecific hybrid progenies of the cross *S. melongena* x *Solanum viarum* and evaluating the direct segregating progenies of such interspecific crosses so as to identify recombinant inbred plants with high yield and shoot and fruit borer resistance. The data recorded from direct F₉ generation derivatives of EP 65 x *Solanum viarum* were utilized to study thoroughly and three progenies each out of thirty in F₉ generations were selected for further studies. All these selected progenies have performed very well with respect to shoot and fruit borer resistance. From this evaluation study two hybrid derivatives were selected and designated as HD 1 and HD 2. The progenies of the culture HD1 recorded minimum shoot (7.69%) and fruit borer infestation (6.67%). The HD2 progenies recorded the minimum shoot (9.09%) and fruit borer infestation as 6.85%. The selected progenies viz., HD 1 and HD 2 showed profuse flowering and fruiting and also cluster bearing habit. The color of the fruit was bright purple while the fruit surface was smooth, glossy along with tightly packed seeds in its flesh which again act as physical barrier for mandibles of fruit borer to chew and bore into the flesh of fruits [18].

Tomato is a self pollinated crop, which is a high demand vegetable crop. The wild species are reservoir of important genes in tomato. *Solanum pimpinellifolium* is the only red-fruited wild species of tomato. Because of the close phylogenetic relationship between the two species, there is little or no difficulty in initial crosses in subsequent generations of pre-breeding and breeding activities. Nineteen accessions from seven *Lycopersicon* species were bio assayed for their resistance to *Heliothis armigera* by [23]. It was found that among the various *Lycopersicon* spp. bioassayed, accessions of *L. hirsutum* f. *glabratum* is most potential for breeding *H. armigera* resistant cultivars.

Wilt, little leaf and phomopsis blight are the serious diseases of brinjal. *Solanum incanum* is resistant to *Fusarium* wilt. In humid tropical areas brinjal is highly infected with bacterial wilt. Wild species of *Solanum* viz., *S. torvum*, *S. xanthocarpum*, *S. nigrum*, and *S. sisymbriifolium* are resistant [24]. The wild species *Solanum viarum* showed no infection and was immune, whereas the species *S. incanum* and *S. sisymbriifolium* were resistant to little leaf disease.

Pinheiro et al. [25] conducted two assays, to evaluate the resistance to root knot nematode, *M. incognita* race 1 in *Citrullus lanatus* cv. *Citroides*, *Lagenaria vulgaris*, *Sicana odorifera*, *Cucurbita facifolia*, *Cucurbita moschata*, *Cucurbita moschata* x *Cucurbita maxima*, *Luffa* sp., *Cucumis melo* and *Cucumis metuliferus* accessions. The results revealed that three accessions of *Cucumis metuliferus* ('Kino') were resistant to *M. incognita* race 1 in the first experiment. In the second experiment conducted to evaluate the reaction to nematode *M. incognita* race 1, *M. javanica* and *M. enterolobii* all the seedlings in pots were inoculated with 2nd stage (J2) juveniles and 5000 eggs of each *Meloidogyne* species. The observations on egg mass index (IMO), gall index (IG), number of eggs per gram of root (NEGR) and reproduction factor (RF) was observed on 53 and 84 days after inoculation, respectively. The melon *Cucumis metuliferus* was resistant to root-knot nematode.

9. Screening varieties

Varietal difference with regard to resistance for fruit and shoot borer was observed in brinjal. Choudhary *et al.* [26] screened eight varieties against *L. Orbonalis*. The order of susceptibility of brinjal varieties was recorded as Pant Samrat < Pant Rituraj < Manjarigota < Pusa Purple Long < Pant Brinjal-5 < Kavach < MHB-80 < BR-112 during 2014–2015 and 2015–2016, respectively. In another study, Amit *et al.* [27] screened twenty five brinjal varieties against brinjal shoot and fruit borer and red spider mite and IBH-3, IBL-116, Rajindra brinjal, KS-356, JB-24, JBH-8, IBH-02 and CHBR-1 were found tolerant. The research carried out at RARS, Jamalpur, Bangladesh showed that, the brinjal varieties Jumki-1 and Jumki-2 were highly resistant (HR), Islampuri-3, BL-34 and Muktakeshi were fairly resistant (FR), Singnath long and Singnath-4 were tolerant to brinjal shoot and fruit borer [28].

Similarly, in okra Rehman *et al.* [29] screened four varieties (SabzPari, SadaBahar, PusaSawani, Arka Anamika) and those varieties showed some degree of resistance against sucking insect pests. Okra variety Sada Bahar was less infested with jassid (1.30/leaf) and whitefly (5.36/leaf) compared to other tested varieties and resulted in maximum yield (1529.62 kg/ha). Number of fruits pods per plant was found non significantly different on all the tested okra varieties.

In another experiment, Jackson and Bohac [30] evaluated sweet potato accessions by using bio assay techniques using the adults of banded cucumber beetle and spotted cucumber beetle. A single beetle was placed on a piece of sweet potato peel that was embedded periderm-side up in plaster in a petridish. Feeding and longevity of insects on sweet potato genotypes were evaluated. Durability of feeding with respect to banded cucumber beetles on sweet potato peels ranged from 12 d for the most-resistant genotype to 123 d for a susceptible control cultivar (SC1149–19). The feeding longevity of spotted cucumber beetles was slightly shorter than banded cucumber beetles. For the highly resistant genotypes, both the species exhibited a significant delay in feeding initiation, and most beetles died before they had fed the sweet potato. Thus it was evident that both antibiosis and non preference (antixenosis) are important mechanisms of resistance in sweet potato genotypes.

Seventy seven eggplant genotypes were tested for resistance to root-knot nematode by classical testing. As a result it was determined that P29 and P52 genotypes were resistant. *Ditylenchus destructor* and *Ditylenchus dipsaci* are economically important plant-parasitic nematodes, affecting potato production mostly in temperate climates. Mwaura and Vidal [31] screened 25 potato varieties for resistance to and tolerance for *D. destructor* and *D. dipsaci* infections. Reproduction factor (RF) and relative susceptibility (RS) were used to evaluate resistance. Based on Reproduction factor, sixteen varieties were assessed as susceptible (S) and five were identified as resistant (R) to *D. destructor*. The varieties Innovator, Aveka and Spunta were identified as resistant to *Ditylenchus dipsaci*. The highly susceptible one for *D. destructor* and *D. dipsaci* in both experiments was Desiree and was used as the standard susceptible control variety for the calculation of RS. An 1–9 scale was used to assess and classify the potato varieties based on level of resistance to *D. destructor* and *D. dipsaci*, where 9 indicated the highest level of resistance. Among the varieties screened six had significantly lower relative susceptibility (RS) to *D. dipsaci* than the standard susceptible control. Few varieties were also observed to be tolerant to both the nematodes. The suitable indices for resistance and tolerance determination were relative susceptibility (RS) and external potato tuber damage.

Nayak and Pandey [32], screened one hundred fifty brinjal varieties/cultivars against root-knot nematode, only twenty varieties have shown resistant reaction with

least gall index (1.1 to 2.0) viz., Gachhabaigan, Azadkranti, Kantabaigen, Athagara Local, Kamaghara local, Solanum indicum, PBR 129-5, ARU-1, BB1-3, BB 45-C, BB-49, KS-224, Utkal madhuri, BR-112, LB-13, LB-25, LB-28, LB-30, LB-44, LB-55.

Akhter and Khan [33], screened thirty brinjal varieties for their resistance/susceptibility to root-knot nematode (*Meloidogyne incognita* race-1) infestation. Out of 30 brinjal varieties, eighteen varieties viz., Black Beauty, Brinjal 1 hybrid, Brinjal No.38, Chamak, Govinda, Green round, Nagina, Nav Kiran, Neel Kamal, Nishant, P.K-123, Prabha Kiran, Prasad, Sukhda, Surya Kiran, i9Utkal, VNR-51 and VNR-60 were highly susceptible, seven varieties (Brinjal Advance, Brinjal BSS1013, Green long, Harshit, Prapti, Shamli and Ujjwal) were susceptible, two varieties (Mahy 112 and Mahy Ruby) were tolerant, two varieties (Hybrid green and JK Kajal) were moderately resistant and only one variety Mahy 80 was resistant against *Meloidogyne incognita* race-1. Mahy 80 variety was ported to be resistant against root-knot nematode, *M. incognita* race-1 for the first time.

10. Selection

Selection is an important method for breeding of varieties resistant to biotic stresses. It is an important means of isolating or identifying sources of disease resistance. Normally the sources of resistance are available in natural populations, wild species, introductions and spontaneous mutants. In the earlier periods selection was accomplished by sequestering the resistant survivors of natural epiphytotics. Now a days advanced artificial epiphytotics are being created and selection of resistant types are being done rather than escaped susceptibles [34]. Selection of resistant plants from a commercial variety is the cheapest and quickest method of developing a resistant variety. IIHR Bangalore developed tomato varieties through pureline selection viz., Arka Alok and Arka Ahuti which showing resistant against bacterial wilt.

The production of garden pea is seriously limited by major diseases namely, wilt, powdery mildew and rust. *Fusarium* species cause root rot (*F. solani* f.sp. *pisi* and *F. avenaceum*) or wilt (*F. oxysporum* f.sp. *pisi*). Coyne *et al.* [35] developed three breeding lines (W6 26,740, W6 26,743, W6 26,745) having high level of resistance to *Fusarium* root rot caused by *F. solani* f.sp. *pisi* with acceptable agronomic traits. In melons the genotypes A19, A32, A30, JAB-11, JAB-20, JAB-3, JAB-7, C384, C67, JAB-9, JAB-18 were identified for powdery mildew resistance.

Pinheiro *et al.* [36] studied thirty seven pepper genotypes, *Capsicum chinense*, *C. annuum* and *C. frutescens*, were characterized for resistance to three root-knot nematode species (*Meloidogyne javanica*, *M. incognita* race 1 and *M. enterolobii*). Three experiments were carried out, in 2013, 2014 and 2016, in a greenhouse. Among the genotypes of *Capsicum frutescens* evaluated all were resistant or immune to *M. javanica* and *M. incognita* race 1. In *C. chinense* six accessions were susceptible. In the second experiment all genotypes of *C. chinense* and *C. annuum*, evaluated were resistant to *M. incognita*. In the third experiment, with *C. annuum* genotypes, most were susceptible to *M. incognita* race 1 while CNPH 30118 and CNPH 6144 were resistant to *M. enterolobii*, the most aggressive species. A greater degree of resistance was observed in few accessions of *C. chinense* and *C. frutescens*.

11. Hybridization

The common method used for resistance breeding is hybridization. In this the resistance is transferred by two means. In the first, by backcross method the

resistance is transferred from a wild species or a variety with undesirable horticultural attributes to a susceptible but otherwise a desirable variety. In pedigree method the resistance is combined with some desirable characters of one variety and superior characteristics of another variety.

Heterosis a complex biological phenomenon manifested in the superiority of hybrids over parental forms due to the rate of development of one or more complex traits. Heterosis values should be negative for getting tolerant hybrids through heterosis breeding. As far as pest infestation is concerned a hybrid with least incidence might be due to complementation of genes. With the genetic knowledge available on insect resistance in vegetable crops it would be necessary to employ hybridization to transfer single, multiple or additive genes into commercial varieties in the advanced filial generations. The major achievement is expected through inter specific hybridization. In order that fruit quality, yield and resistance to insect pest may preferably be incorporated in one genotype, it may possibly involve number of parents to achieve desired results.

Inter-varietal and inter-specific crosses, followed by selection, have accounted for the development of resistant hybrids. The production of inter-specific hybrids is useful for the transfer of desirable genes from wild to cultivated species. The Source, mechanism, biochemical and genetical basis of resistance in squash, muskmelon, cucumber and watermelon were studied by several authors.

By utilizing interspecific breeding technique and recombination, borer-free brinjal can be developed to protect the high yield and satisfy the preference of consumers. The parental lines EP 65 and Pusa Uttam had recorded higher yield together with lower fruit and shoot borer incidence. Both of them were found to be good combiners for the above traits. So these parents can be involved in multiple crossing programmes to transfer the resistant genes and to isolate desirable hybrids with high yield and low fruit and shoot borer incidence.

Introgression breeding has been extensively used in the genetic improvement of potato and tomato. In potato twelve traits have been introgressed from wild species viz., *S. demissum*, *S. spegazzinii*, *S. stoloniferum*, *S. chacoense*, *S. acaule*, *S. vernei* and in tomato from the wild species *S. peruvianum*, *S. cheesmanii*, *S. pennellii* and *S. chilense*. In watermelon, the F₁ (between resistant and susceptible) showed pronounced resistance to fruit fly.

Kishaba *et al.* [37] studied resistance by using several melon aphid-susceptible (MAS) recurrent parents from an initial cross of 'PMR 45' with P1 414,723. Fifteen advanced melon breeding lines with different levels of melon aphid resistance (MAR), their recurrent parents and P1 414,723 were compared in a naturally infested field test for susceptibility to feeding damage by CB. None of the MAR entries were more susceptible than their recurrent parents for fruit damage by CB. P1 414,723 was found to have a low level of resistance to seedling damage, and a high level of resistance to fruit damage from feeding by Cucumber beetle.

Hybridization was undertaken in brinjal with *Solanum viarum* to combine the resistance trait with high yield. The derivatives of the inter specific cross of *Solanum melangena* and *Solanum viarum* EP 65 (accession of *Solanum melangena*) x *Solanum viarum* were assessed till F₉ generation. As a result two recombinant progenies viz., 7 and 9 were chosen and carried to the next generation as they had recorded high marketable yield and least infestation of shoot and fruit borer. Molecular confirmation with RAPD primers was done which depicted the introgression of the genes from donor parent *Solanum viarum* to brinjal and thus hybridity was confirmed.

Backcross method: This method is widely used for incorporation of resistant gene from a wild species or any variety with undesirable traits to a susceptible variety which is good in other agronomic attributes. The parents from which the resistant gene is to transferred is called as donor parent or non-recurrent parent.

The susceptible parent in which the resistance gene is transmitted is known as the recurrent parent. The generation up to which the selection process has to be effected in a backcross program would differ according to the allelic relationship of the resistance genes *i.e.*, whether it is resistance or dominant to the allele. Normally the backcross method help in the recovery of recurrent parent phenotype along with the transfer of resistant genes.

[38] investigated hybrid progenies of late blight resistant potato somatic hybrids developed through hybridization with common potato varieties, and also linked ISSR markers with resistant parent/progenies. Potato somatic hybrids (*Solanum tuberosum* di haploid ‘C-13’ + *S. pinnatisectum*) were back-crossed with potato varieties (*S. tuberosum*) and true potato seed (TPS) were produced. TPS-raised seedlings were advanced to back-cross progenies clones (BC1-C1, BC1-C2, BC1- C3 and BC1-C4) during the five years based on tuber traits in field trials and field resistance to late blight. The BC1-C2 progenies were profiled by ISSR markers and alleles linked to late blight resistant somatic hybrid parent P8 and their progenies (P8 × Kufri Jyoti) were identified. Eight promising advanced hybrids for late blight resistance were identified. This is the first ever report in India towards widening the genetic base of potato by exploitation of interspecific somatic hybrids. The methodology followed was as follows (**Figure 1**).

Powdery mildew is a serious disease of pea and for which new good resistant donors are available, a typical backcross breeding approach as applicable to a character governed by a single recessive gene was outlined by Gritton [39].

Pedigree method: It is quite suited when the resistance is governed by horizon- tal or polygenic. In breeding for disease resistance, artificial disease epidemics are generally produced to help in selection for disease resistance. Pedigree breeding is mainly used when the resistance is governed by major genes and have higher heri- tability. If it is used for low heritability traits then the selection process will be time consuming as it takes several generations usually F5 or F6 to attain homozygosity

Figure 1.
A schematic presentation of development of interspecific potato somatic hybrids (*S. tuberosum* + *S. pinnatisectum*).

[40, 41], implemented a quantitative trait locus (QTL) mapping approach to study the inheritance of anthracnose resistance in an F2 population derived from the pedigree of *C. annuum* × *C. chinense*.

The major crops in which break through research was done and the resistant varieties developed through introgression breeding methods is tabulated below.

Crops	Diseases	Sources of resistance	Remark	References
Bottle gourd	Gummy stem blight	IIHRBGH-10 (BG 114–3 × BG 95)	Hybridization Method	IIHR, Annual report, 2019
Tomato	ToLCD+BW + EB	Arka Apeksha (ITHR 2834 X ITHR 2918)	Hybridization Method	IIHR, Annual report, 2019
		Arka Vishesh (ITHR 2834 X ITHR 2917)		
Cauliflower	Downy mildew and Alternaria leaf spot	Arka Spoorthi	Mass pedigree method	IIHR: https://www.iihr.res.in/varieties
		Arka Vimal		
Amaranthus	White rust	Arka Arunima	Pure line selection	IIHR: https://www.iihr.res.in/varieties
Garden Pea	Powdery mildew and rust	Arka Pramodh	Pedigree method of selection	IIHR: https://www.iihr.res.in/varieties
Okra	YVMV	Arka Abhay	Interspecific hybridization	IIHR: https://www.iihr.res.in/varieties
Watermelon	Powdery mildew, Downey mildew and anthracnose	Arka Manik	Mass pedigree method	IIHR: https://www.iihr.res.in/varieties
Brinjal	Bacterial wilt	Arka Anand	Hybridization Method	IIHR: https://www.iihr.res.in/varieties

12. Mutation breeding

Literature on mutation breeding of vegetable crops for resistance to insect attack is meager. In IIHR, Bangalore varietal differences, have been indicated for resistance to jassids in the M1and M 2 populations of okra (dry seed irradiated with 55 to 60 KR gamma rays), to aphid in the M population of muskmelon (30–40 KR gamma rays); and to aphid in the MQ population of watermelon (dry seed irradiated with 50 KR gamma rays). This was indicative that there was a great potential in this approach for resistance genes. It is particularly applicable in case of muskmelon to fruit fly, brinjal fruit and shoot borer, tomato fruit borer, melon fruit fly and others where useful source of resistance has not been obtained. Further in cases where one or two sources have been located in nature, it would be desirable to obtain more sources of resistance through mutation breeding. It is evident that very little experience has been gained in the use of induced mutations in resistance breeding against insect pests but the prospects of this approach are great.

Selection of spontaneous and induced mutant plants with resistance through the use of mutagenesis. In germplasm repositories sometimes natural spontaneous mutations may occur. During that occurrence some resistant types will be developed naturally. If such instances does not occur then mutations can be induced by

artificial methods. The short coming in this method is that mostly duplication of naturally occurring genes will be the outcome and induced mutants will have monofactorial behavior and the inheritance is short-lived [4]. The genetic stability has to be tested by repeated screening of mutants for the confirmation of resistance.

Potato is a major vegetable crop and is infected by many diseases which induces losses in yield and quality. Artificial mutations through irradiations were tried for developing resistant varieties against stem canker and black scurf of potato caused by *Rhizoctonia solani*. The mutants developed were resistant to these diseases. Aslı Kara and Şerife Evrim Arici [42] investigated the effect of gamma radiation on the susceptibility of the potato plant to *Rhizoctonia solani* at 22, 33, 54, 57 and 109 Gy. The best results were found with a dose of 22 Gy. The application of gamma irradiation in this study may offer a new approach for potato breeders for developing plants resistant to *R. solani*.

13. Biotechnological approaches

The Bt-brinjal has been developed by inserting cry1Ac gene from a soil bacterium called *Bacillus thuringiensis* through *Agrobacterium tumefaciens* mediated method. Bt brinjal contains three genes, cry1Ac gene, which encodes an insecticidal protein Cry1Ac, is derived from a common soil bacterium and is driven by CaMV 35S promoter (cauliflower mosaic virus 35S). It also has nptII gene (neomycin phosphotransferase-II) which contains an antibiotic resistance marker and another marker gene “aad” for amino glycoside adenyl transferase. The cry1AC protein formed in Bt brinjal is analogous in structure and function to that found in nature. The resistance against fruit and shoot borer of brinjal is provided by cry1Ac genes and it minimizes damages and facilitate for the reduction in pesticide sprays and thus it is eco-friendly. *Bacillus thuringiensis* and *B. t* var. *Kenya* (B.t.k) microbial formulations have been found to be highly specific to target insect pests, and do not have deleterious effects on non-target organisms such as beneficial insects, birds, fish, and mammals including human beings. The confirmation by ELISA revealed the presence of the Cry1Ac protein. The quantitative estimates established significant levels of Cry1Ac protein (2.46–4.33 ng ml⁻¹) in the leaf extract of the transformed plants. The expression of this insecticidal protein in high levels resulted in significant amount of mortality of larvae and also stunted the growth of any surviving larva on transformed plant tissue.

Incongruity occurs in inter-specific crosses as a result of a lack of genetic information in one partner to complete pre- and post-pollination processes in the other. After fertilization the growth of the embryo is restricted due to some post-fertilization barriers. For the developmental process an equilibrium has to be established between embryo and endosperm for sharing the nutrients. When this equilibrium in the development of the zygote is disturbed, first division is delayed and abortion of the young embryo or disintegration of endosperm happens. This abortion may occur in any stages of development of the young seed. Based on the stage of embryo abortion *in vitro* methods have been developed to overcome post-fertilization barriers in a number of plant species. Embryo rescue techniques are the oldest and most successful *in vitro* procedures.

In many instances, progeny from wild crosses of inter-varietal and inter-specific is difficult to produce owing to several barriers like pre zygotic and post-zygotic barriers. The development of young zygote may be arrested by hybrid breakdown, hybrid sterility and hybrid non viability. Post-zygotic barriers such as endosperm abortion and, at later stages, embryo degeneration are of common occurrence, leading to low fertility but these have been overcome through the use of embryo rescue.

Embryo culture is one of the earliest forms of *in vitro* culture applied to practical problems that has proven of greatest value to breeders. Among the very important strategies hybrid embryo rescue, and related applications like ovule/ovary/placental cultures through sequential embryo culture is especially useful in vegetable crops and culture of embryos has also been demonstrated in tomato, brinjal, capsicum, hot pepper, onion, potato, tomato including cucurbits for rescuing useful hybrids.

Ovule and ovary culture are more suitable than embryo culture for small seeded species or very young embryos. When abortion occurs in a very young stage and maternal tissue has no negative influence on the development of seeds, ovary culture can be applied. Ovary culture has been applied in many *Brassica* species. The ovule culture is applied in *Lycopersicon*. Depending on the genotypic combination of the inter-specific crossing, the percentage of seedlings obtained from ovule culture varied from 0.5–22.5 per cent, whereas in the *in vivo* situation on the plant no seeds could be harvested.

Embryo rescue technique was attempted in an interspecific hybridisation of tomato variety MT-3 and Kashi Amrit with wild relative *S. peruvianum* (WIR-3957). The optimum time for rescuing the embryos was standardized as twenty five days after pollination. The most effective media for germination of the immature putative hybrid embryos was Murashige and Skoog's medium supplemented with 1 mg/L GA3, 0.1 mg/L NAA and 0.5 mg/L BAP. The confirmation of hybridity of this inter-specific crosses was done using RAPD markers. Verba et al. [43] made inter-specific reciprocal crossings between *Solanum melongena*, *S. aethiopicum*.

Confirmation of resistance: The confirmation of resistance in the developed varieties/hybrids are usually undertaken by artificial screening studies.

Artificial Screening: This is artificial epidemics created by inoculation of pathogen onto the plant population. The most common methods used to inoculate *Colletotrichum* on chili plants either involve using the fruit puncture method or the spraying method in the laboratory or in the field [44]. The injection of very small amount of conidia suspension into the fruit pericarp is known as microinjection or fruit puncture method. The resistance can be known by the lesions shown at the injection or inoculation area [20, 21, 45]. Secondly, spraying method in which the conidia suspension is sprayed on plants at flowering and fruiting. But this method is not safe and risky. So the puncture of detached fruits in the laboratory has paid an outstanding development of anthracnose resistance evaluation in chili.

Different artificial screening methods are employed based on the mode of spread of the diseases as given below

- **Soil borne diseases-** Sick plots are created for testing resistance to such diseases.
- **Air borne diseases-** Spraying a suspension of spores.
- **Seed borne diseases-** dry spores are dusted on seeds or seeds may be soaked in a suspension of pathogen spores

14. Conclusion

In vegetables the biotic stresses are pests, diseases and nematodes. The damages induced by these factors affect the production, productivity and quality. Breeding of resistant varieties offer the cheapest means of pest/disease/nematode management. Resistant varieties obviate the use of chemicals, thus reduce environmental pollution. Effectiveness of resistant varieties depend upon the stability of their performance in various environmental conditions and changing climatic scenario.

However like any other concept, resistant breeding also has its own limitations. That is breakdown of resistance. However, horizontal resistance being durable but difficulty relates to an accurate & reliable assessment of the level of resistance. A variety resistant to a stress may be susceptible for other. So future planning should require far greater effort for introgression of genes from different resistant sources and develop multiple resistant varieties against several biotic stresses than that required for single.

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