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Research Paper

Disease resistance breeding in rose: current status and potential of biotechnological tools

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Abstract

Rose (*Rosa hybrida* L.) is one of the most important commercial flowers grown its flower with cut stems, loose flowers, garden display as well as potted specimens. A wide range of pathogens viz. fungi, bacteria, viruses, nematodes and phytoplasmas attack the cultivated rose throughout the world. These pathogens cause less vegetative growth, leaf and flower mosaics, distortion, spotting, dis-coloration, necrosis and abscission resulting loss of production and poor quality of blooms. Most common diseases are – Dieback, Black Spot, Podwery / Downey Mildew, Rust, Gall besides viral disease. The paper highlights in detail how different breeding methods, bio-technological tools are successful to introduce diseases resistance in roses as along term benefit.

Key words: Disease, resistance, breeding, biotechnological tools, back cross, polyploidy, amphidiploids.

Rose (*Rosahybrida* L., Rosaceae) is one of the most important ornamental crops cultivated for its cut stems, loose flowers, garden display as bedding plants and potted specimens, as well. Roses are one of the most important ornamental crops in the world with a yearly estimated production of 18 billion cut stems,60–80 million potted roses and 220 million roses for the landscape (Roberts *et al.*, 2003).In India, total area under rose is 30,000 ha with production of 96 MT loose stems and 166 MT cut stems (NHB Database, 2013-14). More recently, value of the cut flower market of Dutch rose was estimated at $10 billion. Many of the biotic and abiotic stresses affect the quality and yield of flowers. The most important diseases caused by fungi are Black spot, Powdery mildew, Leaf rust, Grey mold and Downy mildew. Crown gall caused by bacteria and many viral diseases such as Rose rosette virus, Rose mosaic virus, Prunus necrotic ring spot virus *etc.* also cause significant damage to the crop. All these pathogens affect ornamental value of a plant by causing leaf and flower mosaics, distortion, discoloration, necrosis, development of spots and abscission. As the cosmetic appearance of ornamental plant is key to its marketability and consumer acceptance, a high degree of pest and disease control is needed.

Impact of Diseases on Rose Production:

A wide range of pathogens including fungi, bacterias, viruses, nematodes and phytoplasmas attack the cultivated rose throughout the world. These pathogens cause reduced growth and plant death as well as can drastically affect the ornamental value of a plant by causing leaf and flower mosaics, distortion, spotting, dis-coloration, necrosis and abscission. Except for a few pathogens that typically attack the roots such as nematodes, crown gall and various soil borne diseases, the major pathogens attacking roses affect the economic part of the plant *i.e.,* the flower or the leaves. As the cosmetic appearance of an ornamental plant is key to its marketability and consumer acceptance, a high degree of pest/disease control is required for the rose. In an experiment that compared rust infection of the rootstock during propagation, it was shown that a rust infection of the rootstock reduced scion growth and flower production by 50% and 25%, respectively, which drastically reduced the quality and number of saleable plants (Shattock *et al*., 1983).In a survey of rose growers, the cost for plant protection ranged from about $7000/ha/year to $32,000/ha/year with about half of this going toward disease control (Debener, 2014).Beyond the cost of the protection, issues with safety, environmental contamination and the development of pesticide resistant pathogens/pests have come to the forefront which has stimulated the development of integrated pest management protocols.

Important Diseases of Rose:

Dieback (*Diplodia rosarum*) - This is a very serious disease of roses and appears after pruning.The drying up and blackening of pruned shoots start from top to downwards. The stems become black and die. At the point where dry and healthy twigs meet, there appears brown lining and also black spots appear at these places. The conditions favourable for the prevalence of the disease include - application of large fertilizers and manure, excessive irrigation and poor drainage facility, incidence of stem borer, continuous incidence of mites, less light penetration. For its effective control, the infected portion should be dehisced and burnt and the cut ends should be painted with copper fungicide paste. Application of optimum dose of fertilizer and facilitating proper drainage followed by spraying of Copper oxychloride (50%) @ 3g / litre of water was recommended.

Black Spot (*Diplocarpon rosae*) **-** This disease occur during the humid months. Conspicuous circular black spot (less than 1 cm dia.) with fringed margins appear on either side of leaf; leaves become chlorotic, dry up and prematurely drop. It can be easily controlled by spraying Carbendazim (1g/ litre of water) or Captan (0.2%) fungicide at fortnightly intervals.

Powdery Mildew (*Sphaerothecapannosa* var. *rosae*) **-** It is a serious disease that occurs when days are warm and nights are cool. This is most common and damaging for which up to 40% of the pesticides applied on roses.Young growing shoots and leaves are covered with white powdery growth. Infected leaves turn purplish and drop. Flower buds may fail to open. It can be checked by removing all the infected leaves at the time of pruning so that the source of infection is minimized followed by dusting with 80% Sulphur or spraying 0.1% Kerathane fungicide at fortnightly interval.

Downey Mildew (*Peranospora sparsa*) - Downey mildew is very common on greenhouse roses because of the prevalence of high humidity and low temperatures. Initially the symptoms appear as pinkish-brown, irregular spots on tender leaves. The fungus causes chlorotic blotches on the upper surface of matured leaves, which ultimately become necrotic. The infection result in total defoliation and severe flower blight. The infected flowers get malformed and fail to open. The disease can be successfully controlled by spraying Metalaxyl-MZ @ 0.2%. Reducing humidity in polyhouses effectively checks the spread of the disease.

Grey Mold or Botrytis Blight (*Botrytis cinerea*) **- T**he infection of grey mold is generally seen on inner whorls of petals. In favorable conditions, the infection spreads very fast, resulting in complete blightening of flowers. Humid and wet conditions in greenhouses favour the growth and multiplication of fungus. Botrytis had developed resistance to all important groupof fungicides that were effectively used earlier. Chemicals with anti-senescent activity render the plants more tolerant. Application of growth regulators like gibberellic acid, paclobutrazol and methyl jasmonate suppresses grey mold in roses. Inhibitors of ethylene biosynthesis such as AOA, CO_2 also reduce grey mold in roses.

Rust (*Phragmidium mucronatum*) - Rose rust appears as yellow patches on the surface of leaves, with orange pustules of spores underneath the leaf. The fungus is spread by wind. Affected leaves fall prior to healthy ones and plants may be defoliated by serious infections. Spraying with Dithane Z- 78 (0.2%) helps to control the disease.

Crown Gall (*Agrobacterium tumefaciens*) - It is the most important disease of rose nurseries in many countries. About 20% of nursery stock is destroyed due to crown gall every year. The infection gradually decreases the vigour and yield of its plants. Cauliflowers like galls are produced most commonly at the crown region of the stem at the ground level. Galls are also formed on roots and on stems wounded due to harvesting,pruning and other cultural operations. Clean nursery practices and production of disease- free planting material are prerequisites for its management. Root damage by *Meloidogyne incognita* increases the incidence of crown gall. Hence care should be taken to prevent the nematode infestation of rootstocks by using rose genotypes, namely *Rosa indica, Rosa multiflora, Rosa canina* and *Rosa manetti*. A non-tumorigenic strain of *Agrobacterium radiobacter* (strain- K-84) is being successfully used in many countries to control crown gall.

Viruses:

A host of viruses have been reported in roses including Prunusnecrotic ring spot virus (PNRS), apple mosaic virus (ApMV), arabismosaic virus (ArMV), strawberry latent ring spot (SLRS), tobaccostreak virus (TSM), tobacco ringspot virus (TRSV), tomato ringspotvirus (TomRSV), rose yellow vein virus, the rose cryptic virus-1(RCV-1), and the rose rosette virus among others.Although many viruses are not lethal, infections generally reducethe vigor, flower production and flower quality. Once the plant is infected, nothing can be done in the field to eliminate the virus from the plant. Thus, the major approach to manage virus problems is to avoid the entry of the virus with the use of certification programs to ensure all planting materials are virus indexed.

Conventional Breeding Methods:

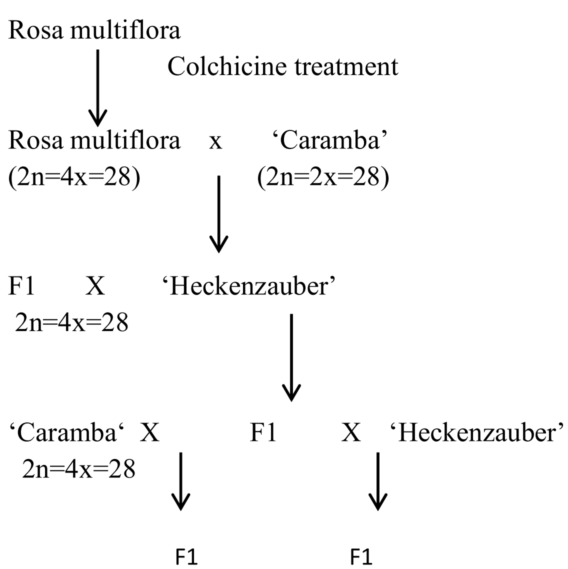
Backcross Breeding

About 130 species of roses are catalogued worldwide (Zlesak, 2006).

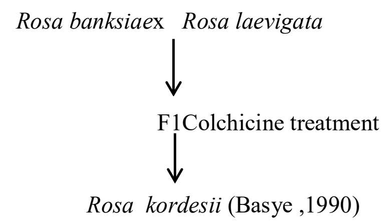
Species used in introgression of disease resistance genes	
Black spot	*Rosa multiflora, Rosa wichuriana, Rosa rugosa, Rosa spinosissima, Rosa carolina*
Powdery mildew	*Rosa multiflora, Rosa indica*
Crown gall	*Rosa multiflora, Rosa indica*

Most of the recurrent flowering cultivars are tetrasomic tetraploids (2n=4x=28) originating from 10 to 15 mostly diploid species. Various fertility barriers limit the germplasm base and restrict the size of the progeny populations in this highly heterozygous, outbreeding species complex. Since cultivated germplasm is derived from such a small proportion of known species, introgression of disease resistance triats from wild species has potential. However this involves transmission of desirable triats from the diploid to the tetraploid level. This is accomplished by several methods.

- Colchicine doubling of wild species followed by crossing with cultivated tetraploids is the most used method (Von Malek and Debener, 1998), although decreased fertility in the original 4x induced plant can occur. Furthermore, inbreeding depression of hybrids may result after successive generations of backcrossing. To avoid this effect, different cultivated varieties can be used for each backcrossing cycle rather than using single clone as the recurrent parent (Debener, 2004).



- Crossing two wild rose species and then chromosome double the resultant seedlings which are called amphidiploids. It was determined that these amphidiploids are more fertile than the original diploid hybrids, although they still have 'low to moderate' fertility.



- An alternative to chemical induction of polyploids is sexual polyploidization which has the potential to preserve the fertility and prevent inbreeding depression by maintaining heterozygosity. It is done by two methods, one is through exploitation of unreduced gametes and another method is creating Ploidy Bridge by crossing two wild species with tetraploids to generate triploids, which subsequently crossed with cultivated tetraploid germplasm to produce tetraploids.

Drawbacks of Backcross Breeding:

A significant drawback to introgression of disease resistance traits from wild species is the time frame involved. Ten or more years are needed to develop cultivars starting from roses species, as several generations of crosses are required to eliminate the wild background. A potentially quicker approach would be the detection and utilization of triats already present in cultivated germplasm. Another advantage of this approach is that polyploidization can be avoided altogether by utilizing resistant varieties. Disease resistance triats do not exist in vaccum. Flower color and form, vigor, scent, quality, and other triats are vital in commercial breeding programme. Variable offspring originated from highly heterozygous parents means that large progenies are required in order to obtain the optimal combination of triats. Since most recurrent flowering roses produce flowers as young seedlings, there is huge scope for early selection for floral triats. More resources and time required to assess disease resistance characters. Therefore, from selection for disease resistance may be very slow. In most cases, cross breeding result in integration of a lot of undesired triats. These limitations paved the way for novel approaches which are less time consuming, cost effective and quicker approaches for breeding for disease resistance.

Molecular or Biotechnological Tools:

Marker Assisted Breeding - An introgression program to transfer disease resistance genesinto a horticulturally superior ornamental plant is a long range project. More so for rose, which consists of multiple crops withina crop as there are separate breeding programs for cut-rose flowercultivars, pot roses, and garden roses. Furthermore, each major category has several subcategories for which a range of colors andflower characteristics are desired. Thus to transform the rose as acrop to be resistant to any given disease, it would involve creatinghundreds of new cultivars!

Since the early 1990s, there has been increasing work at identifying markers. Hundreds of RFLPs, AFLPs, SSRs, RGAs, PKs, CAPs, SCARs and other markers have been described and used tocreate both diploid and tetraploid map.On these mapshave been placed loci for a variety of traits including for diseaseresistance and the related RGA (resistant gene analogs) and PR (pathogenesis resistance) genes. For powdery mildew one dominant resistant gene, Rpp1, whichconditions complete resistance to race 9 of powdery mildew and multiple QTLs in various genetic sources for powdery mildewhave been reported.There have been 4 markers reported associated with *Rdr1*, one associated with *Rdr3* and one associated with *Rpp1.* In addition to these, Xu *et al*. (2005) reported markers for two QTLs for powderymildew resistance in *Rosa roxburghii*. Of these, only 3 markers associated with *Rdr1* and one associated with *Rdr3* have been tested in other germplasm from the germplasm used in their discovery. The other reported markers have not been validated in a wide range of germplasm. Thus, it appears that the markers currently available are germplasm specific and useful only when incorporating the gene from the specificgermplasm source from which the marker was discovered.

Utilization of Markers:

- Marker assisted selection could be a way to conduct early selection for resistance, concurrent with selection of floral triats.
- Marker information can be used to select for desirable genomic regions and it can also be used to select against undesirable genomic regions.
- Marker assisted background selection helpful to reduce the genetic background of wild rose species in introgression.
- We can reduce number of backcrosses required to eliminate undesirable alleles, thereby saving time and expense (Debener, 2003).

Somatic Hybridization:

Another technology for the introduction of disease resistance genes is somatic hybridization through protoplast fusion, which has the potential for transfer of resistance from species into susceptible cultivars or for introgression of nonhost resistance from other genera in the rosaceae. Protoplast fusion for the purpose of disease resistance were carried out between rose species and cultivars (Schum and Hofmann, 2001). Fused calli were obtained for hybridizations of 'Heckenzauber' + *R.wichuriana* and of 'Pariser Charme' + *R.wichuriana* by (Schum and Hofmann, 2001) which were confirmed by AFLP analysis.Squirrel *et al*. (2005) completed the next step of producing putatively intergeneric cell lines of Rosa + Prunus, which was confirmed by RAPD analysis, although regenerated plantlets contained RAPD markers from *Rosa* alone. It was suggested that asymmetric fusion, which assimilates only a part of the non-rose donor genome, might prove more successful. However, more work is needed to refine current protocols and to identify genotypes with increased capacity for regeneration.

Transgenic Approaches:

The integration of resistance via cross breeding with wild species will in most cases result in integration of a lot of undesired triats. It is possible to increase resistance against the pathogen via transformation by using elements of plant specific defense. These elements can be Pathogenesis-related proteins (PR proteins), Ribosome inactivating proteins (RIPs) or Phytoalexins. The most important PR proteins used in genetic engineering are Chitinases, Glucanases and lysozymes. These enzymes inhibit fungal growth by attacking cell walls. Ribosome inactivating proteins (RIPs) are the best- known plant toxins. They block protein biosynthesis either in the pathogen or in the infected plant cells, resulting in local cell death. Phytoalexins, e.g. stilbenes, are substances with antimicrobial effects that accumulate in plant tissue after pathogen infection.

Despite their recalcitrantnature several authors have transformed roses with antifungal proteins and quantitatively enhanced resistance to major pathogens. Marchant *et al.,* 1998 used particle bombardment of embryogenic callus of the susceptible cultivar Glad Tidings to transfer arice chitinase gene. Subsequent tests of regenerated plants carrying the transgene revealed a lower susceptibility to black spot. Another transgenic approach to generate black spot resistant rosesmade use of Agrobacterium mediated transformation. Chitinases, glucanases and ribosome-inhibiting proteins from barley were transformed into the black spot susceptible cultivars

Heckenzauber and Pariser Charme and some transformed plants displayed reductions in black spot infection of 40% as compared to control sharboring the GUS gene. Another study on transgenic roses generated via Agrobacterium transformation of an Ace-AMP1 antifungalprotein from onion reported a number of transgenic clones withenhanced resistance to powdery mildew both in detached leaf assays and in greenhouse experiments. Common to all of the above approaches is that resistance levels stayed too low to be economically significant for the ornamental industry with low tolerance levels for disease symptoms (Lee *et al.,* 2003).

Cloned Rose Genes with Putative Functions for Disease Resistance:

NBS-LRR (Nucleotide binding site-leucine rich repeats) genes is largest group of genes linked to R-gene functions. NBS-LRR are responsible for activation of apoptosis like hypersensitive cell death and innate immunity in plants (Dangl and Jones, 2001). Most of the plant genomes analyzed thus so far contain several hundred NBS-LRR genes, it is difficult to assign a particular function to individual genes of these classes without a functional assay (Dangl, 2013; Jones, 2006). The only exception so far is the isolation and first characterization of the *Rdr1* gene conferring resistance to the blackspot isolate DortE4. *Rdr1* is a TIR-NBS-LRR gene from a group of nine tightly clustered genes most probably introgressed to *Rosa hybrida* from *R. multiflora*. In the *R. multiflora* genotype from which the *Rdr1* gene was isolated, it does not recombine with its family member.

Another group of genes with putative function in disease resistance are the plant MLO genes. MLO genes encode heptahelical transmembrane proteins and occur in large gene families. Some MLO family members are needed by powdery mildews toenter the epidermal cells of the host. However, their exact biochemical function, is still elusive. It has been shown in several monocotand dicot plant systems with knock out mutants of some membersof the MLO gene family confer durable broad spectrum resistance topowdery mildews. However, whereas in barley, tomato and pea aknockout of a single MLO gene confers powdery mildew resistance, three genes have to be mutated in Arabidopsis to achieve full resistance. Naturally occurring loss of function mutations could be used in rose resistance breeding similar to the homozygous recessive recurrent blooming trait which has been successfully utilized to breed modern roses (Janik, 2006).

Conclusion:

- Cross breeding takes more time and resources, therefore, detection and utilization of disease resistance triats from cultivated germplasm would be most practical. Moreover, it avoids polyploidization also.
- Markers and map information for R genes and QTLs helps to conduct early selection for resistance as well as floral triats. Markers which are currently available are germplasm specific and marked genes itself do not ensure stable resistance.
- Transgenic approach to disease resistance could be advantageous as it could reduce number of backcrosses to obtain resistance but the resistance levels are very low.
- Genomic approaches as well as conventional strategies should progress hand in hand to meet public demand for non-chemical disease control and user friendly technologies

Future Thrusts:

- Genetic characterization of race specificity and genetic transmission of existing resistances is most important.
- Once multiple genes against any pathogen have been identified, they may be used in pyramiding strategy to gain broad resistance.
- With rapidly moving sequencing technology and an improved understanding of the molecular basis of host pathogen interaction and discovery of new marker triat associations will be more efficient.

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