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Commentary

Insight into virus disease challenges in food legumes in Genomic era

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With more than 35 years of research experience on characterization, diagnosis, comparative and functional genomics of whitefly transmitted geminiviruses infecting grain legumes, cotton and tomato, Dr V.G. Malathi has developed and deposited more than 100 full length genomic clones of 20 begamovirus, one mastrevirus deposited with the Clone Bank at Advanced Centre for Plant Virology, IARI, New Delhi, developed Partial tandem dimeric infectious constructs of 14 begamovirus and complete tandem repeat construct of one mastrevirus deposited with Clone Bank at Advanced centre for Plant Virology, IARI, and several RNA I constructs targeting the geminivirus rep gene, coat protein and intergenic regions. She has to her credit more than 200 NCBI sequence submissions and more than a hundred publications. She was nominated as a Member in the Study Group on Geminiviruses in the International Committee on the taxonomy of viruses (2010-2015). She is also recipient of several awards like ICAR Best women scientist award 2010 and Khwarizmi International Award 2007, Islamic Republic of Iran to name a few.

Food legumes have been cultivated from time immemorial and constitute the most valuable ingredient in vegetarian diet providing full complement of proteins. They are cultivated in both tropical and subtropical regions in nearly 200 million hectares. Their adaptation to diverse climatic conditions makes them more vulnerable to biotic constraints, especially viral diseases. From perusal of data available in International Committee on Taxonomy of Viruses (ICTV report, 2020) it is evident that about 145 viruses belonging to 27 groups and 31 unclassified viruses naturally infect legumes. The genome of legume viruses include positive, negative and ambisense RNA, and both single stranded and double stranded DNA viruses.

The puzzling phenomenon about legume viruses is the contrast observed in their distribution. While some viruses such as *Groundnut bud necrosis virus*, *Pigeonpea sterility mosaic virus* and whitefly transmitted yellow mosaic viruses are endemic and occur only in Asia, viruses like *Bean common mosaic virus, soybean mosaic virus and cucumber mosaic virus* are wide spread and infect wide range of leguminous hosts worldover. Novel insights gained in the turn of millennium on legume viruses will be briefly discussed in this communication.

Great advancements in next generation sequencing and high throughput genotyping technologies led to the establishment of etiological agents of many devastating diseases. The etiology of sterility mosaic disease remained a mystery for well over 70 years despite extensive efforts and finally the full genome was revealed by deep sequencing of double-stranded RNA preparations, from SMD-affected genotype ICP8863 from ICRISAT-Patancheru (India) and was shown to contain five RNA segments (Elbeaino et al., 2014) The virus was designated as new species, *Pigeonpea sterility mosaic virus* (PPSMV). It had a very high homology with *European mountain ash ringspot-associated virus* (EMARaV), *Fig mosaic virus* (FMV) and *Rose rosette virus* (RRV), members grouped in the genus *Emaravirus*. Differential reaction of pigeonpea genotypes across different locations in India suggested probable occurrence of several variants of PPSMV. Soon it was resolved that in an another variant (PPSMV 2) sequences corresponding to RNA-3 and RNA-4 share less than 60% homology at the nucleotide level with PPSMV1 and probably could explain the differential response of genotypes in Karnataka and Tamil Nadu (Patil and Lavakumar, 2015). Deep sequencing of RNA and small RNA led to discovery of arrays of virome in legumes in wild eco system and agrosystem . From a study on virome using NGS in pea in Germany, thirty-five different viruses were detected in addition to nine associated nucleic acids. From these viruses, 25 are classified as either new viruses, novel strains or viruses that have not been reported previously from Germany. One of the new viruses was a member of Emaravirus (Gaafar, et al., 2020). Similar studies in cowpea revealed presence of new polerovirus in Burkino Faso, new enamoviruses and rhabdoviruses in wild legumes in different countries. In India association of cowpea mild mottle virus was demonstrated in leaf crinkle affected blackgram. From NGS data the possibility of multiple infection of the hosts by different groups of viruses has been established. However why only one or two viruses give rise to symptom expression and others remain as cryptic needs to be studied. It is essential that at least few elite genotypes of legume hosts and highly prevalent wild legumes in cultivation area are subjected to NGS so that we are fully geared up for any emergence of new diseases. NGS also has opened up opportunities for development and application of highly specific, sensitive diagnostics which may help in robust resistance screening.

The discovery of new virus group Endornaviridae infecting wide range of eukaryotes and crops such as rice, faba bean, common bean and winged bean is another interesting development. Endornaviruses are naked single stranded positive sense RNA of 9 to 19 kb length, present in low copy number. They are highly seed transmitted and increasingly getting detected in many plants. We may have to see how these RNA will interfere with viral pathogenesis.

The grey area in legume virology is the highest seed transmitted viruses recorded in legumes. Despite high rate of transmission and quarantine issues this problem has not been addressed as intensely as it deserves to be. In addition to RNA viruses, in recent years, whitefly transmitted yellow mosaic viruses also were shown to be seed borne. Even for RNA viruses the mechanism of seed transmission, movement of virus or virome from sperm mother cells to embryo is not understood. For example, Maule et al., (2003) suggested, that discontinuities or pore like structures in the suspensor sheath at the base of suspensor at the endosperm- suspensor boundary may allow the virus to pass through *en route* to the embryo. They also hypothesized that at some point symplastic movement may be taking place. Using *in situ* hybridization labelling studies sugar transporter proteins (SWEET) have been studied in soybean and *Arabidopsis*. It is demonstrated in *Arabidopsis* that there is exchange of solutes between apoplast and symplast whereby there is flow of nutrients from mother cells to embryo. We should look for such transporters in our legumes, characterize it and see its interaction with viral movement proteins.

Management of viral diseases continue to be enigmatic, as virus diversity, lack of genetic variability and absence of robust protocols for resistance screening specifically against one virus. Exploitation of genetic resources, deployment of resistance gene, use of molecular diagnostics for detection are looked upon as reliable strategy for mitigation of virus diseases. In that direction valuable information have been generated on the basis of genome sequencing of soybean, chickpea, groundnut and pigeonpea. Resistance genes conferring resistance to sterility mosaic disease have been characterized and markers developed and introgression into elite cultivar is in progress.

The pathogen derived resistance approach has been attempted either through coat protein or si RNA. However till date only success story is the genetically engineered bean cultivar Olathe with hairpin construct targeting Rep gene of the the begomovirus *Bean golden mosaic virus*. Very high level of resistance has been observed in field even under mixed infection with *Bean common mosaic necrosis and Bean rugose mosaic* as RNA defense system of plants have been triggered. Alternatively, the approach gaining impetus is to identify host factors facilitating replication and movement of viruses, and try to knock them out. For example eukarotic elongation factor (eEF1A)in cowpea and soybean play a major role in replication of soybean mosaic virus in soybean and turnip mosaic virus in cowpea. Genome editing of sequences by using artificial zinc finger proteins would prevent the replication of virus resulting in resistance.

Instead of transgenic route which is difficult in legumes and time consuming, spraying of double stranded RNA targeting either viral sequence or proviral factors (spray induced gene silencing, SIGS) is an emerging new strategy. One interesting research showed that about 73% reduction in symptoms was achieved by delivering ds RNA constructs against Asian rust through *Bean pod mottle virus.*

Molecular docking study of small molecules of microbial or plant origin has helped in identifying peptide aptamers, cationic peptides that would destabilize assembly, movement and replication of viruses. Application of catinic peptide Melittin has already been shown to work against *Tomato spotted wilt virus.* With lot of hopes consortia of microbes to trigger PAMP mediated immunity are being developed

The legumes/viruses is a complex biological system. In some cases both the hosts and viruses are so confined to a region and highly different that knowledge gained in other systems cannot be applied directly. Perhaps this exclusiveness also is advantageous as it opens up plethora of opportunities to researchers. The resilient nature of these crops combined with knowledge on genomic resources and innovative technologies like genome editing and marker assisted selection of trait should help us to improve productivity and sustainable food security.