

PRESENT STATUS OF VIRAL DISEASES OF GRAPEVINE (*VITIS VINIFERA* L.) AND THEIR MANAGEMENT STRATEGIES IN INDIA

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ABSTRACT

Grapevine is an important fruit crop cultivated in temperate, subtropical and tropical conditions in India. The maximum share in area, production, and productivity of grapes is governed by four states *viz.* Maharashtra, Karnataka, Tamil Nadu and Andhra Pradesh. The sudden and drastic changes in climatic conditions and the emergence of diseases have made grapevine cultivation more challenging. Fungal and bacterial diseases are the major constraints in the grapevine production. Besides, some viral diseases like Grapevine Leafroll Disease (GLD) and rupestris stem pitting associated diseases (RSPD) are reported in Indian vineyards. Although the presence of Grapevine Leafroll-Associated Viruses (GLRaVs) *viz.*, GLRaV-1, GLRaV-3, and GLRaV-4 are known, but the impact on yield and quality of the grapes is not yet studied. Similarly, the Rupestris Stem Pitting Associated Virus (RSPaV) is known to infect the Indian vineyards of different locations but a complete understanding of their overall impact on vineyards is lacking. Therefore, it is presumed that fewer or no losses occur due to GLD and RSPD diseases. There is a strong need for hours to study the impact of known viruses on physiological and yield contributing parameters. In addition to this, robust, rapid and reliable diagnostic techniques are required for the detection of known and unknown viruses. The presence of new and emerging viruses in the Indian vineyards cannot be overlooked. Further, for the management of the viruses and avoiding their spread, the availability of Disease-Free Quality Planting Material (DFQPM) is a prerequisite. To produce DFQPM, various tissue culture techniques need to be standardized for the elimination of the virus/es from the diseased vines. The Tissue Culture-Raised Quality Planting Material (TCQPM) needs to be further tested for genetic purity (true-to-type) and virus indexing to confirm DFQPM. By considering the importance of DFQPM, recently, the National Horticulture Board (NHB), Govt. of India in association with the Asian Development Bank (ADB) has initiated a National Mission on Clean Plant Programme. The implementation of this initiative is more challenging and expected to open different avenues of research and development in the production of DFQPM for the growers. This is an important and timely beginning of the production of DFQPM.

KEYWORDS: Clean plant approach, grapevine, leaf roll disease, management strategy, viroid, virus

INTRODUCTION

Grapevine (*Vitis vinifera* L.) is an important fruit crop cultivated in various regions of India, including tropical areas. It is primarily grown for fresh consumption, raisin production, juice extraction and wine processing (Ray & Chowdhury 2015; Mariappan *et al.*, 2017). In India, approximately 85 per cent of the total grape production

occurs in the form of table grapes, covering around 1,61,910 hectares of land. This contribution represents about 2.3 per cent of the global grape-growing area as of 2021-22. Notably, India exported 2,67,950.39 metric tons of grapes, valued at 313.70 million USD, during the 2021-22 period to various countries worldwide (NHB 2021-APEDA 2023). Cultivating grapevines in India presents unique challenges due to the presence of several

biotic and abiotic stresses, as well as shifting climatic conditions. Among the biotic factors, pests and diseases pose significant challenges to grapevine production, resulting in substantial financial losses for grape growers. In addition to fungal and bacterial diseases, Indian vineyards have documented the presence of viral pathogens responsible for causing GLD and Rupestris Stem Pitting disease (RSP). These viral diseases have been studied extensively, as indicated by various research references (Kumar *et al.*, 2012a, b, c; Kumar *et al.*, 2013; Kumar *et al.*, 2017; Rai *et al.*, 2018; Rai *et al.*, 2021; Sidharthan *et al.*, 2020, 2022).

Detection is the foremost step in devising disease management strategies for plant pathogens. In India, immuno-based diagnostics have been developed for three species of grapevine viruses *viz.*, GLRaV-1 (Kumar *et al.*, 2012b; Kumar *et al.*, 2018), GLRaV-3 (Kumar *et al.*, 2015; 2018; Sidharthan *et al.*, 2020, 2022) and GLRaV-4 (Rai *et al.*, 2018). Although 86 viruses are known to infect grapevine worldwide (Martelli, 2018; Fuchs, 2020), the preparedness for diagnosis of grapevine viruses exists hardly for a few viruses in India. Previously, PCR-based assays have been standardized for the detection of grapevine leafroll-associated viruses. ELISA-based diagnosis of GLRaV-1 and GLRaV-3 has been developed in India (Kumar *et al.*, 2015, 2018). Although, the presence of GLRaV-1, GLRaV-3, and GLRaV-4 is detected in the Indian vineyards systematic reports on associated losses are available due to these viruses in table grape varieties. In the recent past, virome analysis through High Throughput Sequencing (HTS) was carried out to determine the presence of viruses in the samples collected from Indian grapevine vineyards. This work revealed the presence of five grapevine viruses *viz.*, GLRaV-3, grapevine virus L (GVL), GRSPaV, grapevine geminivirus A (GVA), Grapevine Polerovirus 1 (GPoV-1), in the Indian vineyards (Sidharthan *et al.*, 2020, 2022).

Grapevine viral diseases *viz.*, leafroll, rugose wood and fleck are widespread worldwide (Martelli, 2017, 2018). These viruses belong to different families and are naturally transmitted by different insect vectors, vegetative propagation, and grafting (Martelli, 2018; Fuchs, 2020). Recently, 10 other viruses are reported to infect grapevine (Fan *et al.*, 2021; Javarvan *et al.*, 2021; Shvets *et al.*, 2022; Read *et al.*, 2022). Management of the grapevine viruses is more challenging due to graft transmission ability. At least curative management practices are known but most of them are preventive applications. Therefore,

prevention is the foremost step towards the management of grapevine viruses. The preventive approach is composed of the production of virus-free vines through tissue culture techniques and their identification through robust indexing (Golino *et al.*, 2017). The identified virus-free accession, varieties, cultivars, and genotype can be further maintained as a foundation plot. The planting material from the foundation block can be further used for propagation for production and distribution to the grape growers. Planting materials from this can be further used for the development of two subsequent blocks. These four types of vineyards are usually established and developed by nurseries (Gergerich *et al.*, 2015, Golino *et al.*, 2017). The establishment and maintenance of clean vines by the nurseries is more challenging, laborious and cost-intensive. Thus, the development of a virus-free foundation block is very important for the production of DFQPM for quality grapevine production.

MAJOR GRAPEVINE VIRAL DISEASES IN INDIA

Grapevine Leafroll Disease (GLD)

The GLD is caused by nine distinct species of Grapevine leafroll-associated virus (GLRaV) belonging to the family *Closteroviridae* and genus *Ampelovirus*. The nine distinct species of ampeloviruses are *viz.*, Blackberry vein banding-associated virus (BVBaV), GLRaV-1, GLRaV-3, GLRaV-4, little cherry virus 2 (LChV-2), pineapple mealybug wilt-associated virus 1 (PMWaV-1), pineapple mealybug wilt-associated virus 2 (PMWaV-2), pineapple mealybug wilt-associated virus 3 (PMWaV-3) and plum bark necrosis stem pitting-associated virus (PBNNSPaV) Lefkowitz *et al.* (2018). The members belonging to ampeloviruses have a single-stranded, filamentous monopartite (1400-2000 nm), positive-sense RNA genome. Ampeloviruses are transmitted efficiently in a semi-persistent manner by mealybugs and through vegetative cuttings which makes GLD management more challenging. Until 2012, there was no information available on viruses or viroid diseases of grapevine in India. The occurrence of ampeloviruses is reported more than a decade ago suggesting their infection in different grapevine varieties in India (Kumar *et al.*, 2012a, b). On 4th November 2007, before its first record from India, The Indian Express, a daily magazine published news on the

occurrence of GLD in the wine varieties of grapevines in the Nasik district of Maharashtra. Some of the farmers removed their vineyards due to the spread of GLD. Further, debates and discussions were organized among different stakeholders and the wine industry (Jadhav & Sonawane, 2007). In the year 2012, a research group from ICAR-Indian Agricultural Research Institute (IARI), New Delhi and ICAR-National Research Centre for Grapes (NRCG), Pune jointly studied the causal agent of the GLD-affected vines. The presence of GLRaV-1 and GLRaV-3 in the GLD-affected vineyards of Nashik and Pune districts of Maharashtra was confirmed (Kumar et al., 2012a, b). Among the nine ampeloviruses distinct species, only three species viz., GLRaV-1, GLRaV-3, and GLRaV-4 infecting grapevine have been reported from India. GLRaV-3 was the first ampelovirus to be recorded in India in 2012 (Kumar, 2013; Kumar et al., 2012a, b). The isolates of GLRaV-3 and GLRaV-4 are more diverse and few are the recombinant ones. In this review, a detailed study carried out on grapevine viruses and viroids has been discussed and future strategies for disease management have also been delineated.

Grapevine Leafroll-Associated Virus – 1 (GLRaV-1)

The GLRaV-1-infected grapevine exhibits leafroll symptoms. GLRaV-1 is transmitted by different species of several genera of pseudococcid mealybugs viz., *Heliooccus boemicus*, *Phenacoccus aceris*, *Planococcus ficus* and *Pseudococcus maritimus* and soft-scale insect species viz., *Pulvinaria*, *Neopulvinaria*, and *Parthenolecanium corni*. (Le Maguet et al., 2012, 2013; Fuchs et al., 2015). All instars of *Planococcus citri* transmit the virus efficiently. GLRaV-1 is not sap and seed transmissible. The use of infected planting material is the main source of virus spread. Virions are filamentous, non-enveloped, and highly flexuous with 10-13 nm in diameter and 950-2000 nm in length. In India, the information on virus-vectors of GLRaV-1 has not yet been reported and therefore needs to study virus-vector interactions, yield loss, and epidemiology of the disease.

Grapevine Leafroll-Associated Virus – 3 (GLRaV-3)

Initially, the infected vines due to GLRaV-3 exhibit downward rolling of leaf margin symptoms. In later stages in coloured varieties, the leaves develop a strong

reddening of the interveinal region and chlorosis of leaves in white varieties. The diseased vines show reduced growth, yield and sugar levels. In some green-coloured varieties, symptoms are less severe but the age of the vine, time of infection, cultivar and virus strain are the important factors for disease development (Sampol et al., 2003). GLRaV-3 is transmitted by more than nine species of mealybugs viz., *Planococcus citri*, *Planococcus ficus*, *Pseudococcus calceolariae*, *Pseudococcus longispinus*, *Pseudococcus maritimus*, *Pseudococcus viburni*, *Heliooccus boemicus* and *Phenacoccus aceris* (Fuchs et al., 2015; Blaisdell et al., 2016). Some of the soft-scale insect species viz., *Pulvinaria vitis*, *Neopulvinaria*, *Parthenolecanium corni*, *Coccus*, *Saissetia*, *Parasaissetia*, and *Ceroplastes rusci* are the reported vectors from other countries (Golino et al., 2002; Sforza et al., 2003; Bahder et al., 2013). GLRaV-3 is not sap and seed transmissible. The use of infected planting material is the main source of virus spread. Virions are filamentous and flexuous, with a size of 1400-2000 nm in length and 10-13 nm in diameter. The virus genome is monopartite, linear, single-stranded, positive sense RNA of 17,919 nucleotides (nt) long (Bester et al., 2012).

In India, the first targeted surveys for leaf roll disease were conducted during 2010–2011 in the vineyards of Nashik and Pune districts of Maharashtra which investigated the association of GLRaV-3 with seven cultivars of grapevine. Based on Coat Protein (CP) and Heat Shock Protein (HSP70h) phylogenetic analyses distinct clusters were observed and therefore, the genetic distinctiveness of Indian GLRaV-3 was confirmed (Kumar et al., 2012). Similarly, in 2011, surveys were conducted in the grapevine orchards of Himachal Pradesh which recorded the presence of mixed infections of GLRaV-1 and GLRaV-3 in 29 per cent of the analyzed leaf samples through RT-PCR (Kumar et al., 2013). Subsequently, the presence of GLRaV-3 in five grapevine cultivars was also identified based on an Immunocapture (IC) RT-PCR assay (Kumar et al., 2015). Cost-effective, robust and efficient polyclonal antiserum was developed at ICAR-Indian Agricultural Research Institute, New Delhi using *in vitro* expressed CP of GLRaV-3 isolated from grapevine cv. Cabernet Sauvignon cloned in pET28a expression vector (Kumar et al., 2018). GLRaV-3 is one of the most predominant viruses associated with GLD diseases and therefore needs much attention on virus-vector-host interactions and the development of on-site novel diagnostics for quick and early detection of the virus

presence. To study the impact of the virus on grapevine yield quality assessment of crop loss estimation needs to be worked out in Indian conditions. Grapevine cultivation for Indian farmers is one of the important sources of income generation and their livelihood. Therefore, there is a need to educate grape growers on virus symptoms, prevention measures, management strategies and the impact of the virus on crop growth. Further studies on the impact of GLD on Indian grapevine cultivars need to be worked out through systematic research efforts.

Grapevine Leafroll-Associated Virus – 4 (GLRaV-4)

Belongs to the same family *Closteroviridae* and genus *Ampelovirus* causing leafroll symptoms in vineyards (Ito *et al.*, 2013). The GLRaV-4 is mainly transmitted by two mealybug species *viz.*, *Phenacoccus aceris* and *Planococcus ficus* (Tsai *et al.*, 2010; Le Maguet *et al.*, 2012). The virus is not sap and seed transmissible. The use of infected planting material is the main source of virus spread. Virions are filamentous, not enveloped, very flexuous, 950-2000 nm long and 10-13 nm in diameter. The genome consists of a single molecule of linear, positive sense, single-stranded RNA of 13,830 nt in length (Martelli *et al.*, 2012). In India, during 2013-15 surveys were conducted in vineyards of Western and North-Eastern India. It was observed that 12 grapevine cultivars were infected with GLRaV-4 based on ELISA and RT-PCR results (Rai *et al.*, 2017). However, studies are needed in India to ascertain the actual losses caused due to single or mixed infections of associated viruses with GLD disease. From elsewhere, the impact of GLD on sugar, maturity, yield, wine quality, and productive age of the vine has been estimated (Atallah *et al.*, 2012).

Grapevine Rugose Wood Disease

Grapevine *rupestris* stem pitting-associated virus (GRSPaV) is known to cause rugose wood disease. GRSPaV belongs to the family *Betaflexiviridae* and the genus *Foveavirus* (Adams *et al.*, 2012). The virus is widespread and is characterized by narrow strips of small pits, grooves and woody cylinders (Habili *et al.*, 2006). The rugose wood is also known as a stem-pitting disease wherein affected vines show a reduction in water and nutrient movement, budburst, vigour and yield (Habili *et al.*,

2006). Scanty information is available on the natural vector GRSPaV, but the presence of the virus in pollen of the infected vines and its seed transmission have been reported (Lima *et al.*, 2006; Morelli *et al.*, 2009). The virus is not mechanically sap-transmissible. The use of virus-infected propagation material is the main source of virus spread. Virions are flexuous filamentous of approximately 723 nm in length (Petrovic *et al.*, 2003). The virus genome is a single-stranded RNA of 8744 nt in length (Lima *et al.*, 2006). The presence of GRSPaV in different grapevine cultivars grown in Maharashtra (Nashik and Pune) and Imphal, Manipur was characterized (Rai *et al.*, 2021).

MINOR VIRUSES

Grapevine Fleck Virus (GFkV): GFkV belong to the Genus *Macluravirus* (Family: *Tymoviridae*) known to cause fleck disease in grapevine. Virions isometric (icosahedral), not enveloped, 30 nm in diameter. The genome is a monopartite, linear, positive sense, and single-stranded RNA (Poojari *et al.*, 2016). The virus-infected grapevines exhibit localized clearing of veinlets and with severe strains, deformation of the leaves. The virus is not transmitted by mechanical means. The virus is transmitted by grafting and the use of infected budwood and rootstocks is the primary mode of virus spread. The virus is not transmitted through seeds (Martelli, 2017).

Grapevine Geminivirus A (GGVA): GGVA is a geminivirus that has been reported to infect grapevine. Its complete genome is monopartite and 2.9 kb in size. GGVA is assigned to the *Maldovirus* genus in the family *Geminiviridae*. Its infection was first reported in 2017 from the United States in two table grape accessions received from South Korea (Al Rwahnih *et al.*, 2017). Its infection was subsequently reported from India in the Red Globe cultivar (Sidharthan *et al.*, 2020). Recently, a crude sap-based isothermal recombinase polymerase amplification assay was developed for the simplified yet robust detection of GGVA (Kishan *et al.*, 2023).

Grapevine Polerovirus 1 (GPoV-1): GPoV-1 is a newly identified RNA virus of 5.6 kb belonging to the genus *Polerovirus*, family *Luteoviridae*. Its infection was detected in the cultivar Kishmish Chernyej from Russia (Chiaki &

Ito, 2020). In India, its infection was identified in the year 2022 (Sidharthan et al., 2022).

Grapevine Red Blotch Virus (GRBV): GRBV belongs to the family *Geminiviridae* and Genus *Grablovirus*. The virus-infected grapevine exhibits predominantly a red coloration of the leaves and a yield reduction in fruit yields. GRBV was the first geminivirus identified in grapevine and associated with Grapevine Red Blotch Disease (GRBD) in the United States in the year 2012 (Yepes et al., 2018). Its widespread occurrence has been then identified in many grapevine-growing countries. Its infection leads to blotches on leaves and reddening of primary, secondary, and tertiary veins on red varieties and chlorotic regions within leaf blades and marginal burning in different varieties. The full genome sequence comprises single-stranded DNA of 3.2 kb. Its infection from the Indian vineyard was identified as an asymptomatic infection in Punjab (Marwal et al., 2019). The virus is transmitted by the three-cornered alfalfa treehopper vector, *Spissistilus festinus*. The virus is also transmitted by grafting. The primary spread of the virus takes place through the use of infected planting material (Bahder et al., 2016).

Grapevine Virus B (GVB): It belongs to the family *Betaflexiviridae* and genus *Vitivirus*. The virus-infected grapevines exhibit symptoms of swelling and longitudinal bark cracks in young branches. In some cases, drying of the branches swelling at the graft region and precocious reddening of leaves occur. In some cultivars, symptomless infection was also recorded. The virus is transmitted by pseudococcid mealybug vectors *Planococcus ficus*, *Pseudococcus viburni*, *Pseudococcus longispinus* and *Phenacoccus aceris* in a semi-persistent manner (Kuniyuki et al., 2006; Le Maguet et al., 2012). The presence of this virus is reported in Indian vineyards (Sidharthan et al., 2022). The virus is not mechanically sap-transmissible and transmissible by grafting. The use of planting material from infected sources is the main cause of virus spread.

Grapevine Virus F (GVF): GVF is an RNA virus of the genus *Vitivirus* and family *Betaflexiviridae* which is commonly found associated with the infection of other grapevine leaf roll-associated viruses, i.e., GVA, GVB, GVD, GVE (Molenaar, 2015). Infection of vitiviruses is generally found as a mixed infection with closteroviruses and is associated with GLD. GVF is transmitted by

mealybugs and scale insects (*Pseudococcus*, *Planococcus*, *Heliococcus*, *Neopulvinaria*, *Parthenolecanium*, *Cavariella*, and *Ovatus*) in a semi-persistent manner.

DIAGNOSIS OF GRAPEVINE VIRUSES

Surveys were conducted in the grapevine orchards of Himachal Pradesh and GLD symptomatic leaf samples were collected and tested by DAS-ELISA, RT-PCR, and PCR for detection of viruses and phytoplasma. The ELISA and RT-PCR results revealed the presence of GLRaV-3 in 66.7 per cent, GLRaV-1, and GFkV in 50 per cent and Grapevine Virus B (GVB) in 12.5 per cent of symptomatic plants. Mixed infection was common and none of the plants were found virus-free (Kumar et al., 2013). Later, GLRaV-4 has been reported in the Indian vineyards, mostly as a mixed infection with GLRaV-3 and GLRaV-1. To screen the planting material, a polyclonal antiserum against the *in vitro* expressed coat protein (CP) of GLRaV-4 was produced. GLRaV-4 was detected in 54 per cent (39 out of 72) of the symptomatic grapevine samples from different grape-growing regions of India using DAS-ELISA (Rai et al., 2018). Besides 11 grapevine viruses/viroids, two mycoviruses were identified among which grapevine polerovirus 1 (GPoV-1) was reported for the first time in India. Recently, through real-time PCR assays relative titers of grapevine fleck virus and grapevine virus L were tested in two grapevine cvs. Fantasy Seedless and Manjari Medika (Sidharthan et al., 2022). The virus titer of GLRaV-3 was lower in December and higher in September and March in the symptomatic plants. Therefore, based on the results, leaf sampling needs to be done during September and March for reliable detection of grapevine viruses in tropical regions (Sidharthan et al., 2022).

In March 2022, grapevine leaf roll disease leaf samples were collected from the 45 grapevine genotypes maintained at ICAR-National Research Centre for Grapes, Pune experimental farm. Forty-five samples were subjected to DAS-ELISA (Double Antibody Sandwich - Enzyme-Linked Immuno-Sorbent Assay) using GLRaV-1 and GLRaV-3 specific commercial antibodies. Among the 45 samples, 18 samples positively reacted against GLRaV-3 antiserum whereas, 17 samples positively reacted against GLRaV-1 antiserum. Six grapevine genotypes viz., Charak-1, Charak-3, Charak-4, E29-6(BC-X-TC), Amber

green, and Aliquant Bauschet showed mixed infections of both the GLRaV-1 and GLRaV-3 viruses. The O.D. value @ 405 nm grapevine samples which showed a positive reaction against GLRaV-3 ranged from 0.819 to 3.0 as compared to healthy control value (O.D.@405 nm: 0.449). The samples that positively reacted against GLRaV-1 showed O.D @ 405 nm ranging from 0.442 to 3.0 as compared to healthy control (0.26).

A total of 254 dogridge rootstock vines have been maintained at ICAR-National Research Centre for Grapes, Pune and screened following the procedure using the same kit for three consecutive years (2020, 2021, and 2022). Out of these in the first year, 27 plants tested negative for both GLRaV-1 and GLRaV-3. In the second year, when these 27 plants were further screened, 11 plants tested negative for both viruses. On further screening, 7 plants responded as being free from GLRaV-1 and GLRaV-3. These 7 samples were subjected to HTS after constituting the pool and HTS data indicated the presence of a virus *viz.*, GVB and five viroids *viz.*, Hop Stunt Viroid (HSVd), Australian Grapevine Viroid (AGVd), Grapevine Yellow Speckle Viroid-1 (GYSVd-1), Grapevine Yellow Speckle Viroid-2 (GYSVd-2) and Citrus Exocortis Yucatan Viroid (CEVd). Through wet lab validation, it was confirmed that one sample was positive for GVB. Six plants were handed over to the nursery for further multiplication. Besides the regular screening activities, ICAR-NRCG has been instrumental in providing services to the wineries and growers to detect the viruses in their submitted samples.

MANAGEMENT STRATEGIES

Clean plant and certification

The annual impact of GLRaV-3 has been estimated around \$90 million US dollars in California (Cheon *et al.*, 2020) and \$2200 US dollars per hectare in Washington State due to grapevine red blotch virus-GRBV (Ricketts *et al.*, 2017). Management of GLD in South Africa has been achieved through the production of healthy planting material through the South African Vine Improvement Association (Engelbrecht & Schwerdtfeger, 1979), in New Zealand by New Zealand Winegrowers (NZW), in California by California Grapevine Certification and Registration (Alley & Golino, 2000). The preventive approach followed for the production of virus-free vines through tissue culture techniques and their identification through robust indexing (Golino *et al.*, 2017). The

identified virus-free genotypes were further maintained as a foundation plot and used for propagation and distribution to the grape growers (Gergerich *et al.*, 2015; Golino *et al.*, 2017). Successful elimination of grapevine fan leaf virus from three *Vitis vinifera* cultivars by somatic embryogenesis has been practiced (Gambino *et al.*, 2009). Hardwood cuttings showed a high success rate for vegetative propagation of grapevine (Singh & Chauhan, 2020; Waite *et al.*, 2015).

Production of disease-free planting material

“Prevention is better than cure” is a proverb well suited for the management of grapevine viruses. Preventive approaches should include the identification of virus-free plants through virus-indexing protocols and the development of virus-free plants through different methodologies by eliminating their presence (Golino *et al.*, 2017). Further, virus-free plants are being maintained for the development of foundation vineyards which is known as G1 block (Gergerich *et al.*, 2015; Golino *et al.*, 2017). In the G1 block only a few members of vines of each variety, cultivar, genotype, clone or selection are maintained. Planting materials from foundation blocks are used for propagation of new vineyards which is known as G2 block. From G2 block G3 and G4 are developed by distributing virus-free planting material from G2 block to the grape growers or nurseries for commercial production and from G3, G4 blocks are developed. Generally, G2, G3, and G4 blocks are developed by nurseries only (Gergerich *et al.*, 2015; Golino *et al.*, 2017). The development of virus-free clean planting material, their establishment and maintenance in the foundation block is more challenging, labour-intensive and costly. The planting material derived from foundation block has several benefits, the benefits are of economic significance, eco-friendly for enhanced and sustainable viticulture production and high fruit quality (Atallah *et al.*, 2012; Fuller *et al.*, 2019, Golino *et al.*, 2017, Ricketts *et al.*, 2015). Frequent monitoring and testing of the health status of the foundation block is essential to understanding the disease or pest attack or spread of vector-borne viruses or virus-like pathogens. Certification of the virus-free planting material has to be certified by govt. agencies based on the recommendations of testing laboratories. In India, the Government of India has announced the ‘*Atmanirbhar Clean Plant Program*’ in the union budget 2023-24 with a provision of Rs. 2200

crores for the establishment of Clean Plant Centers in India in ten major fruit crops. Among these proposed centers ICAR-National Research Centre for Grapes, Pune is one of them. Although it is in the initial stage of the implementation of the project certainly this will pave the way forward in the production of disease-free quality planting material for the nurseries and grape growers in India. This will certainly enhance the productive age of the vines, the least losses due to the various diseases, and better yield and quality of grapes.

Virus elimination through tissue culture techniques

Meristem tip culture is one of the robust and routine tissue culture techniques used for the elimination of grapevine viruses (Sim *et al.*, 2012). However, there are several challenges in practising the technique which include meristem excision, low regeneration rate, low resistance to high temperature and phytotoxicity and mutagenetic effects due to chemicals. Additionally, thermotherapy, cryotherapy and chemotherapy are practiced for enhanced virus elimination (Wang *et al.*, 2003; Bayati *et al.*, 2011). In India, work is in the process of standardization of the protocol for virus elimination through meristem-tip culture techniques in grapevine.

CONCLUSION

Grapevine cultivation in India faces significant challenges, particularly concerning the management of viral diseases. GLD and grapevine rugose wood can lead to substantial economic losses for grape growers. Detection is a crucial first step in devising effective disease management strategies. Although India has made significant progress in diagnosing some of the grapevine viruses, there is still a need for comprehensive diagnostics for all relevant viruses affecting grapevine worldwide. The management of grapevine viruses primarily relies on the production of virus-free planting material. This involves tissue culture techniques and robust indexing to ensure the virus-free status of planting material. The establishment of clean foundation blocks and their distribution to grape growers is essential for quality grapevine production. Furthermore, the Indian government's initiative to establish Clean Plant Centers in major fruit crops, including grapes, holds promise for enhancing the production of disease-free

planting material. These efforts are crucial for maintaining the health and productivity of grapevines, ultimately benefiting both growers and the grape industry in India. Additionally, ongoing research and standardization of virus elimination techniques through tissue culture are essential steps in the quest to manage grapevine viral diseases effectively.

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