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Current status of grapevine Leafroll disease on wine grape (*Vitis vinifera L.*) Cultivar in India

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Abstract
Grapevine (*Vitis* spp.) is one of the most widely grown fruit crops in the world. Grapes are used for the production of fresh fruits, wines, juices, raisins, and in several byproducts such as jellies, vinegars, and seed oils. Grapevines are broadly classified into red- and white-berried cultivars based on their fruit skin color, although yellow, pink, crimson, dark blue, and black-berried cultivars also exist. India was the leading country in grapes production either 1st or 2nd or 3rd. But the year 2010 saw unprecedented decline in production and productivity both and ultimately lowered down its rank to 39th position in the world's list of grape. It was due to attacked by several biotic and abiotic stresses constituting the variety of pests and pathogens. Viruses (GLRaV-3 and GLRaV-1) are the two most common viruses associated with the leafroll disease of grapevine produce a wide range of symptoms in susceptible plants, modulate host metabolic pathways and cause significant losses to crop yield and quality.

Keywords: GLRaV, grapevines leafroll disease (GLD), Vitis

Introduction
Human beings' relationship with fruiting plants began during the period of their hunter gatherer lifestyle i.e. long before the origin of agriculture in 8000-10,000 BC (http://www.fruit crops.com/chapter1/). Today, fruits occupy a prime importance, in one form or other, in the dietary habits of people from mass and class both. Grapes are economically the most important fruit crop of the world and second most popular fruit after oranges (Castellarin *et al*., 2011; http://www.buzzle.com/articles/facts-aboutgrapes.html) [13]. Grape is a fruiting berry of the deciduous woody vines of the botanical genus *Vitis* and grapevine is the common name for plants of this genus. Because of it as an important global horticultural crop and its ancient historical connections with the development of human culture, grape is positioned at a unique Place. Grapes can be eaten as a refreshing snack as well as used in vegetable and fruit salad. They can be consumed raw or can be used for making various preserved products. In Indian context grape is emerging as an important fruit crop which can be witnessed from the fact that it has the third highest productivity and is the highest among fruit crops in earning foreign exchange (Adsule *et al*., 2011) [1]. The cultivation of grapevine, known as viticulture, is one of the most remunerative farming enterprises of India.

With more than 67 million tonnes (million metric tons; mMT) of global production, grape shares about 11.48 % of world's total fruit production. Worldwide, it ranks 4th in the list of fruits on the basis of quantity produced. The leading grape producing countries are China, Italy, USA, Spain, France and Turkey. During 2009-10, compared to previous years, there was the least production of grapes in India totaling around 0.88 mMT. The reduction in production and productivity may be because of several biotic and abiotic stresses operating upon the crop. Throughout the world, one of the major constraints in grape production has been the biotic stresses i.e. insect damage and plant diseases. In a recent survey conducted by Patil (2008) [25], the more incidences of pests and diseases in Indian condition have been proved to be a major constraint for grape production. Diseases are the major cause of low production and productivity of grapevine. Grapevines are susceptible to a range of fungal, viral, bacterial and phytoplasmal diseases. The common diseases of grapevine are, fungal: powdery mildew, downy mildew, Phomopsis cane and leaf spot, black rot, botrytis bunch rot, anthracnose, bitter and ripe rot, eutypa dieback; bacterial: crown gall and pierce's diseases; viral: grapevine leafroll disease (GLD), grapevine rugose disease (GRD), and nematode transmitted viral diseases such as grapevine fanleaf disease; phytoplasmal: grapevine yellows (Shaughnessy, 2012).

The Crop: Grapevine

Vitis (grapevines) is a genus of 79 accepted species of vining plants in the flowering plant family Vitaceae. The genus is made up of species predominantly from the Northern hemisphere. They are widely used for wine, table grapes, raisins, juice, and spirits; recent trends have also focused on antioxidants and healthful products derived from grapes. The study and cultivation of grapevines is called viticulture. Grapevines usually only produce fruit on shoots that came from buds that were developed during the previous growing season. Flower buds are formed late in the growing season and overwinter for blooming in spring of the next year. *Vitis* is distinguished from other genera of Vitaceae by having petals which remain joined at the tip and detach from the base to fall together as a calyptra or 'cap'. The flowers are mostly bisexual, pentamerous, with a hypogynous disk.

Grapevines are broadly classified into red- and white-berried cultivars based on their fruit skin color, although yellow, pink, crimson, dark blue, and black-berried cultivars also exist. Red berried cultivars have anthocyanin pigments in berry skin, whereas white-fruited cultivars lack this pigment since regulatory genes of the anthocyanin biosynthetic pathway are nonfunctional in these cultivars.

The genus Vitis is divided into two subgenera: Muscadinia and Euvitis (Olien, 1990) [23]. Fruiting behaviour of the vines belonging to subgenus Euvitis is of many berries borne in each cluster and thus they are known as “bunch grape” while the vines of subgenus Muscadinia are having small clusters with common name “mucadine grape”. The subgenus Muscadinia constitutes only three species and out of these three, Vitis rotundifolia, the only species with commercial value is normally referred to as the muscadine grape (Kumar *et al*., 2012) [18]. In the subgenus Euvitis, more than 60 species have been described and Vitis vinifera is the predominant commercial species cultivated worldwide (Table.1) (Kumar, 2013) [17]. It is also the only species of the genus indigenous to Eurasia and is suggested to have first appeared ~65 million years ago. The chromosome numbers of both the subgenera are 40 (2n = 40) and 38 (2n = 38) for Muscadinia and Euvitis, respectively.

Table 1: Important species of Vitis and their common names.

Sub-genera	Species	Common Name
Euvitis	*V. aestivalis*	Summer grape
	V. argentifolia	Silver leafgrape
	V. arizonica	Canyon grape
	V. baileyana	Possum grape
	V. californica	Pacific grape
	V. candicans	Mustang grape
	V. champini	Calcarie grape
	V. cinerea	Grayback grape
	V. corodifolia	Winter grape
	V. doaniana	Panhandle grape
	V. gigas	Florida Blue grape
	V. girdiana	Valley grape
	V. helleri	Round leaf grape
	V. ilex	Manatee grape
	V. labrusca	Fox grape
	V. longii	Bush grape
	V. rufotomentosa	Redshank grape
	V. lincecumii	Post-oak grape
	V. monticola	Sweet Mountain grape
	V. treleasei	Gulch grape
	V. palmate (rubra)	Cat grape
	V. rupestris	Sand grape
Muscadinia	*M. rotundifolia*	Muscadine grape
	M. munsoniana	Little Muscadine grape
	M. popenoei	Mexican Muscadine grape

Nutrition composition

One of the most popular among the regularly featuring table fruits, grapes are widely considered as the "queen of fruits" since earlier times. It has numerous health-promoting phytonutrients such as polyphenolic antioxidants, vitamins, and minerals.

- Grapes are rich in polyphenolic phytochemical compound resveratrol. Resveratrol is one of the powerful anti-oxidant which has been found to play a protective role against cancers of colon and prostate, coronary heart disease (CHD), degenerative nerve disease, Alzheimer's disease and viral/ fungal infections.
- Resveratrol reduces stroke risk by altering the molecular mechanisms inside the blood vessels. It does so, firstly by reducing the susceptibility of the blood vessel through decreased activity of angiotensin (a systemic hormone causing blood vessel constriction that would otherwise elevate blood pressure) and secondly, through increased production of vasodilator substance, nitric oxide (a beneficial compound that causes relaxation of blood vessels).
- Anthocyanins are another class of polyphenolic antioxidants present abundantly in the red grapes. These phytochemicals have been found to have an anti-allergic, anti-inflammatory, anti-microbial, as well as anti-cancer activity.
- Catechins, a type of flavonoid tannin group of anti-oxidants, discovered in the white/green varieties have also shown to possess these health-protective functions.
- Also, the berries are very low in calories. 100 g fresh grapes just provide 69 calories but zero cholesterol levels.
- Grapes are a rich source of micronutrient minerals like copper, iron, and manganese. Copper and manganese are an essential cofactor of the antioxidant enzyme, superoxide dismutase. Iron is especially concentrated in raisins. Besides, 100 g of fresh grapes contain about 191 mg of health benefiting electrolyte, potassium.
- A 2013 study published in the BMJ suggests grapes may have preventive properties when it comes to diabetes. The study looked at a large cohort of women over the course of nearly two decades and found that a greater consumption of whole fruits, particularly grapes, blueberries and apples was significantly associated with a lower risk of type 2 diabetes.
- A small-scale 2010 study published in the British Journal of Nutrition looked at 12 older adults who had memory issues but not dementia. The study found that those who drank one or two cups of Concord grape juice every day for 12 weeks showed "significant improvement of verbal learning" compared to those who did not. Another study, published in the Journal of Nutrition in 2009 reported similar findings.
- They are an also good source of vitamin-C, vitamin-A, vitamin-K, carotenes, B-complex vitamins such as pyridoxine, riboflavin, and thiamin.

Indian viti cultural scenario

The modern wine market in India is small; annual per capita consumption of wine in the country is a mere 9 milliliters, approximately 1/8000th that of France. Viticulture in India has a long history dating back to the time of the Indus Valley civilization when grapevines were believed to have been introduced from Persia. Winemaking has existed throughout most of India's history but was particularly encouraged during the time of the Portuguese and British colonization of the subcontinent. The end of the 19th century saw the phylloxera

louse take its toll on the Indian wine industry followed by religious and public opinion moving towards the prohibition of alcohol. Following the country's independence from the British Empire, the Constitution of India declared that one of the government's aims was the total prohibition of alcohol. Several states went dry and the government encouraged vineyards to convert to table grape and raisin production. In the 1980s and 1990s, a revival in the Indian wine industry took place as international influences and the growing middle class started increasing demand for the beverage. By the turn of the 21st century, demand was increasing at a rate of 20-30% a year. The city of Nashik in the state of Maharashtra is called the "Wine Capital of India.

While a large portion of the Indian subcontinent is not ideal for viticulture, the large diversity of climate and geology does cover some areas with suitable terroir for winemaking to thrive. The summer growing season in India tends to be very hot and prone to monsoons. Many of India's wine regions also fall within the tropical climate band. Vineyards are then planted at higher altitudes along slopes and hillsides to benefit from cooler air and some protection from wind. The altitude of India's vineyards typically range from around 660 ft (200 m) in Karnataka, 984 ft (300 m) in Maharashtra, 2,600 ft (800 m) along the slopes of the Sahyadri to 3,300 ft (1000 m) in Kashmir. Summertime temperature can get as hot as 113 °F (45 °C) and wintertime lows can fall to 46 °F (8 °C). During the peak growing season between June and August, rainfall averages 25–60 inches (625-1,500 mm).

According to Chadha (2008), the three climatic conditions in which grapevines are grown in India are as below:

Temperate region

Viticulture in sub-Himalayan regions of Jammu and Kashmir and Himachal Pradesh comes under this condition.

Sub-tropical region: Delhi, Punjab, Haryana and Western Uttar Pradesh are covered under it.

Tropical region

Most of the grape growing areas are confined to this region. The region comprises of mid-Maharashtra, Karnataka, Telangana region of Andhra Pradesh and Tamil Nadu.

Global production of grape shares approximately 11.48 % of world¨s total fruit production which comes more than 67 million tones. Based on the quantity produced, it is the 4 th most produced fruits at world level. China is the leading producer of grapes, producing 8.6 mM tons sharing 12.78 % of global production, followed by Italy, USA, Spain, France and Turkey (Kumar, 2013) [17].

In India the area under grape is 1.2 % of the total area of fruit crops in the country. Production is 2.8% of total fruits produced in the country. About 80% of the production comes from Maharashtra followed by Karnataka and Tamil Nadu. The Vineyards in India range from the more temperate climate of the northwestern state of Punjab down to the southern state of Tamil Nadu. Some of India's larger wine producing areas are located in Maharashtra, Karnataka near Bangalore and Telangana near Hyderabad & one winery in Titari Village of Ratlam District State Madhya Pradesh Ambi Vineyard. Within the Maharashtra region, vineyards are found on the Deccan Plateau and around Baramati, Nashik, Pune, Sangli and Solapur.

The leading countries in grapes production are China, Italy, USA, France, Spain, Chile, India, Argentina and Iran (fig.1). Global productivity of grape is 94,369 Hg/ha (hectogram per hectare; 1 Hg = 0.1Kg). India’s grapes productivity is 2590000 tonnes (FAOSTAT, 2018). Total harvested and production of grapes in India since 2010 to 2016 were increased fig.2 (FAOSTAT, 2018). Table.2 showing catchment areas of market of grapes in leading states (http://agriexchange.apeda.gov.in/market%20profile/one/grapes.aspx).

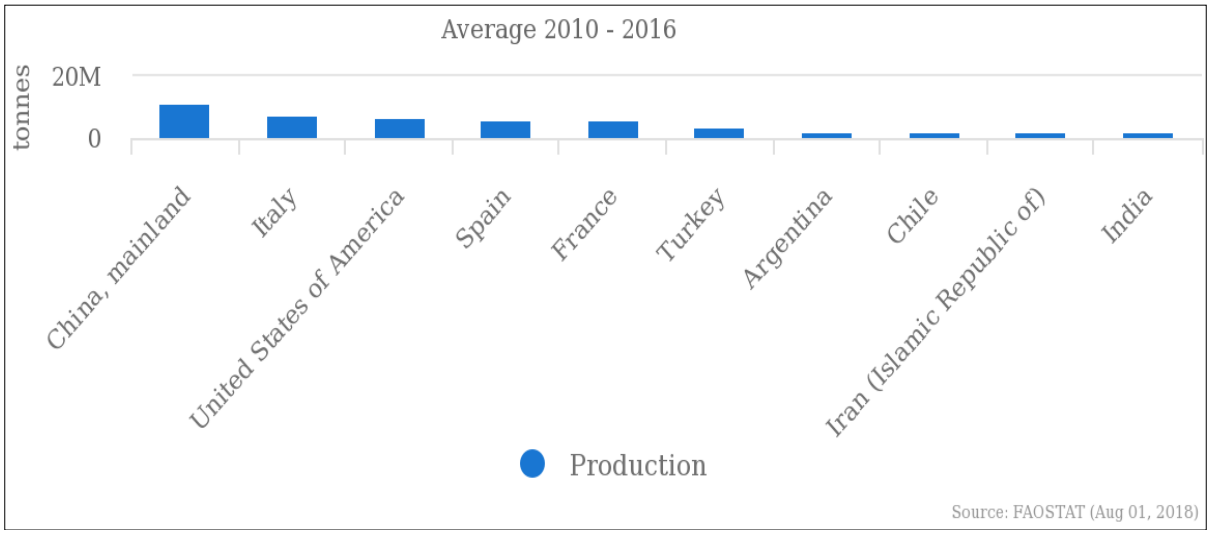

Fig 1: Leading Country in grapes production (FAOSTAT 2018).

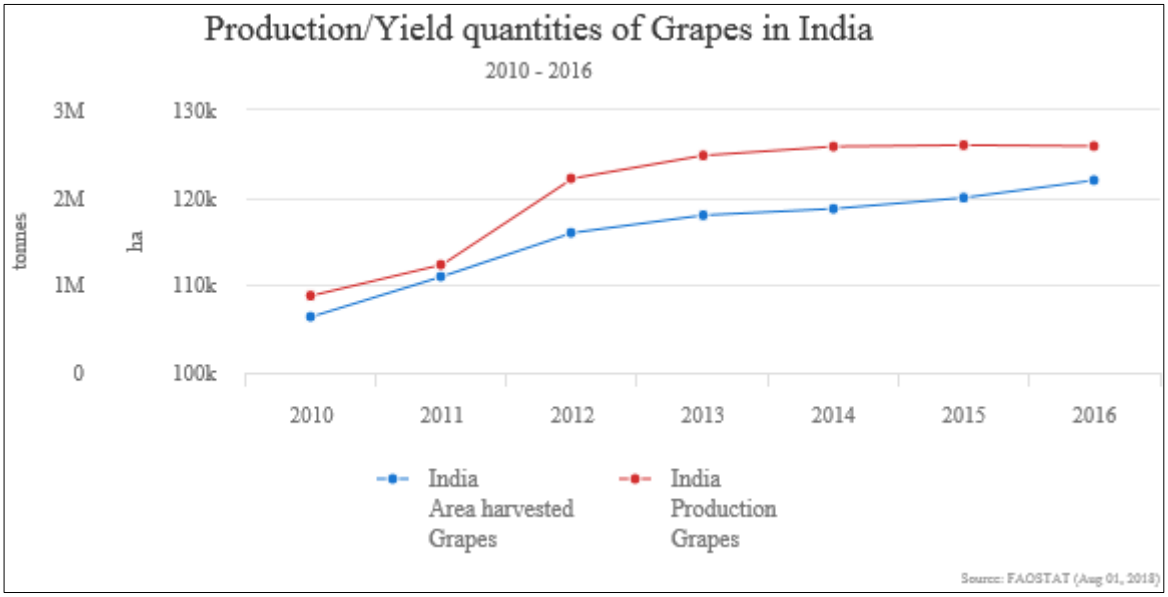

Fig 2: Total harvested and production of grapes in India (2010-2016) (FAOSTAT, 2018)

Table 2: Showing the details of catchment areas of market of grapes in leading states.

States	Districts (Market)	Blocks
Maharashtra	Solapur	Karmala, Barsi, Madha, Mohol, Mangalwedha, Sangole, Malsiras, Pandharpur, Akalkot.
	Nashik	Kalvan, Peint Igatpuri, Sinnar, Niphad, Yeola, Nandgaon, Satana, Furgana, Dindori, Melgaon.
	Sangli	Atpadi, Khanapur, Islampur, Shirala, Valva, Tasgaon, Kavathe, Mahankal, Jath, Miraj
	Ahmednagar	Srirampur, Sangamner, Akola, Rahusi, Nevasa, Parner, Pathardi, Srigonda,Karjat
	Pune	Junnar, Ambegaon, Ghod, Rajgurunagar, Wadgaonsirur, Mulshi, Welhe, Purandhar, Bhor, Baramati, Indapur, Daund, Saswad.
	Satara	Mahabaleshwar, Khandala, Wai, Phaltan, Koregaon, Khata, Patan, Karad, Vadug.
Karnataka	Bijapur	Indi, Sindgi, Basavna Bagevadi, Muddebihal, Tikota.
	Belgaum	Athni, Arkali, Chikodi, Mukeri, Bailhongal, Ramdurg, Khauapur.
	Bagalkot	Jamkhandi, Mudhol, Hungund, Badami.
	Kolar	Bagepalli, Gauribidanur, Gudibanda, Chik Ballapur, Mulbagal, Malur, Bangarapet
	Bangalore	Amekal, Sonnenahalti, Kannur, Bagalur, Nagarur, Marangondahalli, Haralur, Mantapa, Solurur, Chandapur.
Andhra Pradesh	Rangareddy	Marpali, Vikarabad, Tandur, Pargi, Ibranimpatan, Shahabad, Doma, Maisaram, Miryan, Nancherla.
	Mehbubnagar	Kollur, Keshampat, Mughalgidda, Karnul, Wanparti, Atmakur, Gadwall, Alampur, Kolhapur, Achampet, Kondangol.
Tamil Nadu	Theni	Periyakulam, Andipatti, Uttammapalayam, Bodimayakkanur, Kamban, Megamali, Vadugapatti,
	Coimbatore	Muttuppalaiyam, Avinashi, Tiruppur, Palladam, Udumallaippettai, Pollachi, Valparai.
	Dindigul	Palani, Kodaikkanal, Oddanchatran, Vedasandur, Naltam, Nilakkotai, Palaiyan
Punjab	Bhatinda	Rampura Phul, Talwani Sabo, Bhagra, Jalal, Nathana, Bandi, Jaisinghwala, Shergarh, Sangat, Malkana, Lahri, Teona.
	Ferozpur	Zira, Fazilla, Abohar, Jalalbad, Fatehgarh, Panjeke, Ladhuka, Lakhawall, Ramsara, Wahabwala.
	Muktsar	Kanianwali, Malaut, Baruwali, Sotha, Doda, Bhadar, AbulKharana, Bhagu, Waring, Khera
Haryana	Fatehabad	Ratiya, Tohana, Badalgarh, Bhattu, Sampla, Pirthala, Samiyana, Kharihajan, Bhattukalan
	Sirsa	Fatehpur, Dabwali, Ellenabad, Banwala, Salam Khera, Nathohar, Mangla, Jamal, Gushalyana.
	Hisar	Bithmara, Adampur, Narnaund, Hansi, Budak, Mangal, Umra, Sultanpur, Babal, Baria, Agroha, Kanoh, Khedar, Budak.

Production obstructions

India has always been leading in grape productivity, ranking either 1st (in years 2000, 2007, 2008 and 2009) or 2nd (in years 2001, 2002, 2004, 2005 and 2006) or 3rd (in year 2003). India's grape productivity has been either around 2.5 times (2.48 times in the year 2009) or more than 2.5 times of the world's grape productivity during the same period of years 2000 to 2009 (Kumar, 2013) [17]. In the year 2008-09 observed the highest production of 1.87 mM tons but there was slight reduction in the productivity as compared to 2007-08. But the year 2010 saw unprecedented decline in production and productivity both and ultimately lowered down its rank to 39th position in the world's list of grape. Analysis has to be carried out at states level then it can be observed that the fluctuation of grape production at national level (in the year 2008-09-10) was in fact mainly because of the similar fluctuation demonstrated by the state Maharashtra (NHB, 2012).

Abiotic and biotic stresses in their combination limit the crop growth and its production and fetch the lowered quality produce to the users. Grapevines, similar to most of the vegetatively propagated crops, are attacked by several biotic stresses constituting the variety of pests and pathogens. Indian viticulture because of mainly being in tropical region and the ever-growing nature (without any dormancy) of vines in12 this region faces more incidences of insect pests and diseases compared to temperate cultivation (Adsule *et al*., 2011) [1]. According to Martelli and Boudon-Padieu (2006), among the

variety of pests and pathogens "the infectious intracellular agents such as viruses, viroids, phloem-and xylem-limited prokaryotes constitute a major limiting factor to the development and well-being of the world grape industry, and to the quality and quantity of the crop". Among the various diseases of grapevines virus and virus like disease are the major limiting factor to the quality and quantity of the crop (Martelli and Boudon-Padieu, 2006). In Indian condition anthracnose, downy mildew, powdery mildew, bacterial leaf spot and post harvest berry rots are the important diseases of grapevines (Shikhamany, 2001; Sawant *et al*., 2007) [32, 30].

Among these infectious intracellular agents, viral infections are difficult to control producing an important impact in grapevine physiology, causing significant economic losses every year (Martelli and Walter, 1998) [21]. Viruses may also influence wine quality by causing delays in sugar accumulation, poor acid development, and poor colour development.

Viruses

Grapevines can be subject to attacks by many different pests and pathogens, including graft-transmissible agents such as viruses, viroids, and phytoplasmas (Golino, 1992) [12]. Currently, more than 60 different viruses belonging to nearly 30 different genera have been documented in grapevines (Martelli, 2012., Oliver, 2011) [20, 24]. On a worldwide basis, more viruses have been identified in grapevines than in any other woody perennial crop. All currently documented grapevine viruses are classified into four major groups based on the disease they cause or are associated with: viruses involved in the degeneration/decline disease complex, viruses associated with the leafroll disease complex, viruses associated with the rugose wood complex, and viruses associated with the fleck disease complex. Other grapevine viruses are apparently not associated with a disease and are suspected to have a minor, if any, impact.

Viruses produce a wide range of symptoms in susceptible plants, modulate host metabolic pathways and cause significant losses to crop yield and quality. (Abou Ghanem *et al*., 2010, Alabi *et al*., 2012) [2, 6]. The extent of negative impacts of viruses, however, depends on specific virus-host combinations, virulence of the virus, cultivar susceptibility. The majority of grapevine viruses have a RNA genome. Recently, two viruses with a DNA genome have been reported: First is a badnavirus associated with vein-clearing and vine decline syndrome, and second one is geminivirus associated with red blotch symptoms (Al Rwahnih *et al*., 2013, Krenz *et al*., 2012, Poojari *et al*., 2013) [5, 16, 26], with the latter emerging as an economically important constraint to grape production in the United States.

Among the virus and virus-like diseases, grapevine leafroll disease (GLD) is by far the most widespread and economically damaging viral disease of grapevines in many regions around the world. A recent economic study indicated that GLD, depending on the level of disease incidence, yield reduction, and impact on fruit quality, can cause an estimated loss of approximately $25,000 to $40,000 per hectare in the absence of any control measure (Atallah *et al*., 2012) [7]. GLD was described in Europe as early as the mid-nineteenth century and then in many other countries throughout the world. The graft-transmissibility of GLD was demonstrated in 1935, and the presence of flexuous, filamentous virus particles was reported in a leafroll-affected grapevine in 1979. A decade later, the ability of mealybugs to transmit one of the viruses associated with GLD was demonstrated (Rosciglione *et al*., 1989, Tanne et.al., 1989) [29, 33]. As improved diagnostic techniques became available, several viruses associated with GLD were characterized during the last three decades. These viruses are collectively referred to as Grapevine leafroll-associated viruses or GLRaVs.

Grapevine leafroll disease (GLD) in India

GLD is a complex disease having aetiology of eleven associated viruses with particles ranging from 1400 to 2000 nm in length and possessing a monopartite, single-stranded, positive-sense RNA genome and are indicated as grapevine leafroll-associated viruses (GLRaVs). GLRaV-1, GLRaV-3 and GLRaV-4 belong to the genus Ampelovirus, GLRaV-2 to the genus Closterovirus and GLRaV-7 to the genus Velarivirus. Strains of GLRaV-4 formerly known as GLRaV-5,-6,-9, GLRaV-Pr, GLRaV-De and GLRaV-Car are considered genetically divergent variants (or strains) of GLRaV-4 within subgroup II of the genus Ampelovirus (Rai *et al*., 2017) [27].

Grapevine leafroll-associated virus 3 and *Grapevine leafroll-associated virus 1* (GLRaV-3 and GLRaV-1) are the two most common viruses associated with the leafroll disease of grapevine (Akbas *et al*., 2009; Sharma *et al*., 2011) [4]. Though India has been one of the leading countries in grape cultivation, yet there was no record of occurrence and characterization of grapevine viruses from India till 2012 (Kumar *et al*., 2012a; Kumar *et al*., 2012b). However, a newspaper reported the presence of GLD and GRD (grapevine rugose disease) in the vineyards of Nashik and Pune regions of India (Jadhav and Sonawane, 2007) [15].

In a 2013-15 survey, typical symptoms of grapevine leafroll disease were observed in vineyards of Western and North-Eastern India. Twelve grapevine cultivars were infected with Grapevine leafroll-associated virus 4 (GLRaV-4), as shown by ELISA, RT-PCR and sequencing (Rai *et al*., 2017) [27].

Symptomatology and Impacts

Symptoms of grapevine leafroll varies in dark or red-fruited (dark or red-berried) and in light or white-fruited (light or white-berried) cultivars of grapevine. Generally, the symptoms are more expressive in red-fruited cultivars than in white-fruited cultivars of *V. vinifera*. Exhibition of red and reddish-purple discolourations in the interveinal areas of mature leaves at the basal part of the shoots in late spring or summer, depending on the climate and geographic location, is one of the early sign in dark-berried cultivars. In Indian condition typical symptoms of leafroll have been observed from months December to February. Gradually, the symptoms move upward to other leaves and the foliar discolourations enlarge so that the interveinal areas of most of the leaf surface become reddish or reddish-purple usually leaving a narrow green band along the primary and secondary veins. As the season advances, leaf blades become thick, brittle and the margins of the infected leaves roll downward. In the most severe cases, the whole leaf surface becomes deep purple (Martelli and Boudon-Padieu, 2006).

The symptoms are similar in white-berried cultivars, but the leaves become chlorotic to yellowish, instead of reddish to reddish-purple (Fig 3). Some white cultivars show no visual sign of infection (i.e. latent infection). In advanced stages of infection, the margins of the leaves of both kinds of cultivars roll downward, expressing the symptom that gives the disease its common name, i.e. "leafroll" (Martelli and Boudon-Padieu, 2006).

Physiological symptoms of GLRaV-3 infected vines include degeneration of phloem cells in leaves, stems and fruit petioles (Hoefert and Gifford, 1967) thus impairing carbohydrate translocation from foliar parenchymas.

Starch accumulates in degenerated chloroplasts causing increased thickness and brittleness of the leaf blades, and lowering of sugar content (Martelli and Boudon-Padieu, 2006). It has been proved that GLRaV-3 infection causes modulation of the flavonoid biosynthetic pathway in the infected leaves of a red-fruited wine cultivar Merlot leading to *de novo* synthesis of two classes of anthocynins namely, *cyanidin-3-glucoside* and *malvidin-3-glucoside* which finally contribute to the expression of reddish-purple colour of virus-infected grapevine leaves displaying GLD symptoms (Gutha *et al*., 2010) [14]. There occurs the depletion of potassium (K+) in the leaf blade and accumulation in the petioles (Cook and Goheen, 1961) [11]. This has a two-fold effects: first, it reduces K+ content in leaf lamina which, therefore, develop symptoms similar to those of K+ deficiency and second, it favours K+ translocation to bunches, an excess of which evokes increased level of malate and tartrate, hence of titratable acidity (Kumar, 2013) [17]. GLD symptoms vary within and among vineyards due to several factors including the variety, age of the vineyard, stage of infection, complex of virus(es) present, viticultural practices, climate and soil conditions (Martelli and Boudon-Padieu, 2006).

According to Walker *et al*., (2004) [34], the grape quality decreases as the percentage of leafroll infection increases, resulting in lower priced grapes. An experimental vineyard that was healthy when planted, but in which a number of GLRaV-3 infected plants were detected two years after grafting, was studied during its first three productive years. The sugar content of the must (freshly pressed grape juice that contains the skins, seeds, and stems of the fruit) of GLRaV-3 infected plants was an average 1° Brix lower than that of healthy plants, their titratable acidity was higher and their pH was lower, especially in years with adverse weather conditions (Cabaleiro *et al*., 1999) [9]. Overall growth and vigour of grapevines and yield of berries are detrimentally impacted by GLD. Infected vines exhibit reduced leaf area and develop weak trunks over time, which translate into decreased vineyard life span and vine productivity. The photosynthetic efficiency of infected leaves is getting reduced because of lack of chlorophyll due to leaf discolouration (Rayapati *et al*., 2008) [28]. GLRaV-3 diminishes photosynthesis by 25-65 %, depending on cultivar and environment, which directly affects growth, productivity and cropping (Cabaleiro *et al*. 1997) [8].

Fig 3: Symptoms of grapevine leafroll disease (GLD)

First and Current Report of *Grapevine leafroll-associated virus* Infecting Grapevines in India

Kumar *et al*., 2012 [18] first Report of *Grapevine leafroll-associated virus 1* Infecting Grapevines in India. They conducted survey during 2010 and 2011 in the Nashik and Pune regions of western India and reddening of interveinal areas and downward rolling, typical symptoms of leafroll disease in dark fruited cultivars, were observed, first in 2010 and subsequently in 2011. Fourteen leafroll symptomatic samples from seven cultivars of seven vineyards were collected during 2011. Samples were subjected to double antibody sandwich (DAS)-ELISA using commercially available antibodies against GLRaV-3 and GLRaV-1 (Bioreba, Reinach, Switzerland) (2). An asymptomatic sample from another cultivar of a different vineyard and samples from two plantlets of two different cultivars produced in tissue culture were used as negative controls. GLRaV-1 was detected in two cultivars, Shiraj (Nashik region) and Pinot Noir (Pune region) using DAS-ELISA. GLRaV-1 was detected either alone in cultivar Pinot Noir or as mixed infection with GLRaV-3 in cultivar Shiraj. To further confirm the presence of GLRaV-1 in these two cultivars, crude extract from petioles of these two cultivars were subjected to one step reverse transcription (RT)-PCR using GLRaV-1 specific primers pORF9F and pORF9R (GGCTCGAGATGGCGTCACTTATACCTA and CCTCTAGACACCAAATTGCTAGCGA, respectively) (3). The ˜650 bp amplicons were cloned in pGEM-T easy vector and three independent clones of each amplicon were sequenced in both directions. The cloned amplified product was 646 bp, including 630 bp of p24 protein (ORF9) of GLRaV-1. Comparative sequence analysis, using the BioEdit 7.0.3 program (http://www.mbio.ncsu.edu/BioEdit/BioEdit.html), of ORF9 of the virus under study from the cultivars Pinot Noir and Shiraj shared maximum sequence identity of 95.8 and 96.1%, respectively, at the nucleotide level with the Clatervine isolate from the United States (GenBank Accession No. HQ833477). The corresponding values of maximum identities at the amino acid level were 96.6 and 96.1%, respectively, with the same Clatervine isolate. The maximum identity between these two isolates of GLRaV-1 was 96.1% at nucleotide level and 95.7% at amino acid level. According to Kumar *et al*., 2012 [18], this study represents the first report of GLRaV-1 from India.

In 2013-14 and 2014-15, 31 grapevine samples exhibiting leafroll symptoms collected from Nashik, Baramati and Pune (Maharashtra) regions of Western India, Imphal (Manipur) of

North-Eastern India. Recently, Rai *et al*., 2017 [27] isolated twelve grapevine cultivars were infected with Grapevine leafroll-associated virus 4 (GLRaV-4) for CP, HSP70h, and the p23. Cultivars were Cabernet Sauvignon, Nana Saheb purple, Sharad Seedless, Flame Seedless, Thompson Seedless, Bangalore blue, Fantasy Seedless, Pinot noir, Krishna Seedless and Cardinal. The collected samples were tested for the presence of GLRaV-4/9 by DAS-ELISA using a commercially available kit (Bioreba, Switzerland). Mid ribs and petioles were used as a source of antigen. Samples with absorbance values at 405nm at least thrice that of the healthy control provided with the DAS-ELISA kit were considered positive for GLRaV-4/9. Out of 31 symptomatic samples, 29 reacted positively to GLRaV-4/9 antisera in DAS-ELISA. RNA were isolated from the sample followed by c-DNA were synthesized. Primer pairs specific to the CP, HSP70h, and the p23 genomic region of GLRaV-4 were designed and PCR were carried out. Only GLRaV-4 products were amplified, further PCRs were carried out using GLRaV-4 specific primers. Expected amplicons of 819bp, 1575bp and 485bp were obtained for CP, HSP70h and p23 genes. Gel-purified (QIAGEN GmbH, Hilden, Germany), cloned into a TA cloning vector (RBC, UK) and transformed in E. coli strain DH5α. Recombinant colonies were confirmed by PCR and characterized via restriction digestion using Hind III. The consensus sequences of the Indian GLRaV-4 isolates were confirmed by BLAST and assembled in BioEdit version 7.9.1. The CP gene of nine Indian isolates had 98-100% nucleotide (nt) sequence identity among themselves, and 90- 99% and 92-99% identities with GLRaV-4 isolates of other countries at the nt and amino acid (aa) levels, respectively, sharing maximum sequence identity to the LR-106 isolate from USA. Indian isolates had 68-73% and 74-83% identity with the corresponding sequences of other serologically related strains of GLRaV-4 (other members of subgroup II) at nt and aa levels, respectively. A phylogenetic analysis of the CP gene showed that the Indian isolates clustered with GLRaV-4 isolates Y252-IL (AM162279) from Israel, Y253-TK (AM176759) from Turkey, and LR106 (FJ467503) from USA with a maximum identity of 98% at the amino acid level. Thus, This type of study would help to explore the grapevines to viral infections and discriminate host-virus interactions from that of confounding factors in the field due to climate-related variables.

Summary

Among the numerous virus and virus-like diseases of grapevine, GLD is one of the most economically important diseases affecting the sustainability of the grape and wine industry in the India and in other grapevine-growing countries. GLD is a complex viral disease and produces distinct symptoms in red- and white berried *V. vinifera* cultivars. Our knowledge of different aspects of the disease and GLRaVs is still quite limited. The data on genome organization of GLRaVs indicate that these viruses make up one of the most diverse and unusually complex group of viruses infecting a single agriculturally important crop species, representing a unique virus Patho System.

Future study is required to identify the GLRaVs and other important viruses of grapevine such as *Grapevine rupestris stem pitting-associated virus* (GRSPaV), *Grapevine fanleaf virus* (GFLV), GVA (*Grapevine virus A*), GVB etc. Study will also required on variability of GLRaV-3 and -1, their full genome sequencing, mutual interaction of these two viruses and interaction of leafroll viruses with other viruses. Study on biology and epidemiology of GLD will give a better understanding of the disease for its better management in Indian conditions. Further, development of techniques and the reagents involved thereby for identification of the viruses studied will fetch an early, efficient and cost-effective management strategy.

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