CHAPTER 1:

GENERAL INTRODUCTION

Grape is the second most widely grown commercially important fruit crops of the world after olive. It is grown in varied climatic conditions ranging from temperate to semi tropic and tropics. It is a woody perennial vine and is cultivated on all continents except Antarctica. The genus *Vitis* is broadly distributed, largely between 25° and 50° N latitude in eastern Asia, Europe, the Middle East and North America. The worldwide distribution of grapes is coupled with the high genetic plasticity of this crop to enable its adaptation to temperate, sub-tropical and tropical regions.

1.1. Area and Production

Grape is cultivated in about 90 countries and the area under this crop was about 19 million acres in 2004 with Europe having 60% share. Three countries Italy, France and Spain each have more than one million hectares of area under grapevines. According to an estimate**,** grape production in 2004 was about 65,486,235 MT valuing 144 billion pounds (FAO, 2005).

The list of top ten grape producing countries in the world is given in the table 1.1.

Table 1.1: Top 10 grape producing countries in the world

S. No.	Country	% of world production
1.	Italy	13
2.	France	12
3.	Spain	10
4.	USA	8
5.	China	8
6.	Turkey	6
7.	Iran	4
8.	Argentina	4
9.	Australia	3
10.	Chile	3

The U.S.A with 0.47 million metric tons of fresh table grapes was the leading importing country followed by Germany (0.34 million metric tons) in 2005. Chile is the leading exporter with nearly 0.69 million metric tons of fresh grapes. The U.S.A enjoys one of the highest production efficiencies in the world - yields of 7.4 tons/acre, twice the world average, however, a greater proportion of acreage constitutes of table and raisin purpose and not wine (FAO, 2005).

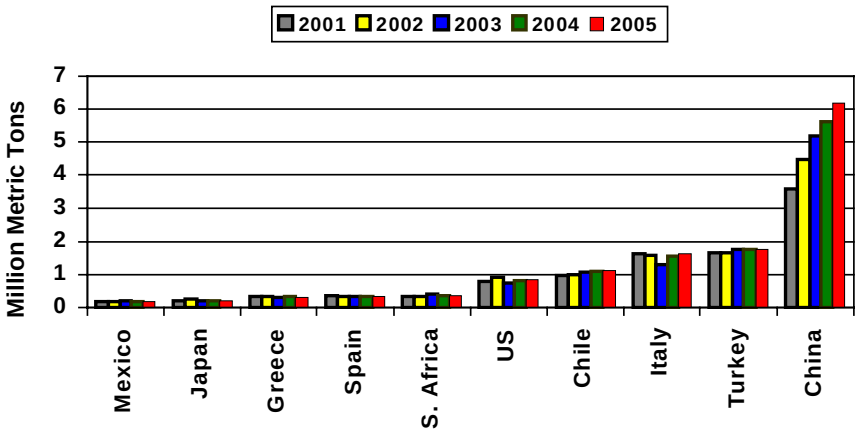

Fig. 1.1. World table grape production

Source: Foreign Agricultural Service (FAS) Annual Reports, USA.
* Data for 2005 is an estimate

Nearly a quarter of the entire world's wine is produced in Italy. The world wine market is dominated by Europe with 73.1% share followed by U.S.A (12.6%). Turkey and the U.S.A. lead in the world production of raisins, each with more than 0.3 million metric tons. Europe is only a small producer but consumes 40.6% of world production of raisins. Turkey, Iran and the U.S.A in that order are by far the leading world exporters, each with more than 1 million hundredweight exported of raisins (FAO, 2005).

1.2. Origin

Grape was one of the first fruit crops to be domesticated in the world due to its native to the region where agriculture had its origin - the Fertile Crescent; variable uses of grapes and storability in different forms like dried raisin and wines and easiness in propagation by cuttings, allowing superior selections to be cloned easily. According to De Candolle (1886), the cultivation of grape in Egypt goes back to 4000 B.C. Grape cultivation is believed to have originated in Armenia near the Caspian Sea, from where it seems to have spread westward to Europe and eastward to Iran and Afghanistan. The center of origin for *Vitis vinifera* is Asia - between and to the south of the Black and Caspian Seas.

The ancestors of present day varieties are thought to be *V. vinifera pontica*, *V. vinifera occidentalis* and *V. vinifera orientalis*. *V. labrusca* growing wild in the U.S.A., became useful as a rootstock and in breeding for phylloxera resistance in mid 19th century, when it was carried to Europe. *V. rotundifolia* is native to southeastern United States

(Virginia south through central Florida, and west to eastern Texas). Native species resembling *V. lanata* and *V. palmata* grow wild in the Himalayas, where other indigenous varieties like 'Rangspay', 'Shonltu White' and 'Shonltu Red' are grown (http://www.uga.edu/fruit/grape.html).

1.3. History

Seeds of *V. vinifera* grapes were found in excavated dwellings of the Bronze Age in South-Central Europe (3500-1000 B.C.) indicating its early movement beyond its native range *i.e.* South-Western Asia. Egyptian hieroglyphics detail the cultivation of grapevine and wine making in 2440 B.C. The Phoenicians carried wine cultivars to Greece, Rome and Southern France in 600 B.C. and Romans spread the grape throughout Europe. Grapes moved to the Far East via traders of Persia and India. Spanish missionaries brought *vinifera* grapes to California in the 1700s. Close relatives of *V. labrusca* were first seen by Viking explorers before Columbus's voyages in the mid ninetieth century and carried to Europe (http://www.uga.edu/fruit/grape.html).

Famous Indian scholars, Sasruta and Charaka in their medical treatises entitled '*Sasruta Samhita'* and *'Charaka Samhita',* respectively, written during 1356-1220 BC, mentioned the medicinal properties of grapes. Kautilya in his *'Arthashastra'* written in the fourth century B.C. mentioned the type of land suitable for grape cultivation (Shikhamany, 2001).

1.4. Taxonomy

Grape belongs to genus *Vitis* under the family *Vitaceae*. Three important species (*V. vinifera, V. labrusca* and *V. rotundifolia* Michx.) and one hybrid group comprise most of the grape production worldwide. *Vitis vinifera* is the most economically important and highly adaptable species. *Vitis* constitutes 2 subgenera: **the *Euvitis* and *Muscadinia*** (http://www.uga.edu/fruit/grape.html).

1.4.1. *Euvitis*: True or bunch grapes, characterized by elongated clusters of fruit, berries that adhere to stems at maturity, forked tendrils, loose bark that detaches in long strips, and diaphragms in pith at nodes. The *Euvitis* contains *V. vinifera* and *V. labrusca*. Diploid chromosome number (2n) is 38**.**

***V. vinifera* L.:** The European or Wine grape or Old World grape accounts for over 90% of the world's grape production. Most of the grape production is for wine making but also for table and raisins. Grape juice concentrate from *vinifera* grapes (Thompson Seedless) finds

its way into several juice blends and jellies. There are at least 5000 cultivars of *V. vinifera* grown worldwide. The most popular white wine cultivar is Chardonnay and major red wine cultivars include Cabernet Sauvignon, Merlot and Pinot Noir.

***V. labrusca* L.:** Concord or American bunch or fox grape. The species is used for sweet grape juice and associated products i.e. jelly and jam. Concord accounts for 80% of the juice production. Other important cultivars in this group comprise Niagara, Isabella, Delaware, Catawba and many seedless cultivars like Eastern Seedless.

1.4.2. *Muscadinia*: Muscadine grapes, characterized by small fruit clusters, thick-skinned fruit, berries that detach one-by-one as they mature, simple tendrils, smooth bark with lenticels, and the lack of diaphragms in pith at nodes. There are only 3 species i.e. *V. rotundifolia*, *V. munsoniana* and *V. popenoeii*. Diploid chromosome number (2n) is 40**.**

***V. rotundifolia* Michx.:** Muscadine grape is used as fresh fruit and for juice making. The species is extremely vigorous and disease tolerant compared to *vinifera* grapes, and is well adapted to the southern U.S.A. Its diploid chromosome number (2n) is 40, which makes interbreeding it with *vinifera* or Concord grapes difficult. Muscadines are not graft compatible with *Euvitis* either. This genus has two classes of cultivars: 1) pistillate or female, and 2) perfect flowered or hermaphroditic. Pistillate types are still grown with cross pollination from perfect flowered cultivars since many are of high quality. Cowart, Hunt, Noble, Jumbo, Nesbitt, and Southland are popular black cultivars, while Carlos, Higgins, Fry, Dixieland, and Summit are popular bronze-skinned cultivars. There are no seedless cultivars of muscadine grape.

French American hybrids: These are obtained through hybridization of *V. labrusca* with *V. vinifera* and have phylloxera resistance and good wine quality attributes. Cultivars such as Marechal Foch, Vidal Blanc, Chambourcin, and Seyval make good wine and allow wine grape growing in areas where pure *vinifera* grapes do not perform well, such as the eastern U.S.A. These often require cluster thinning for obtaining proper quality and have a propensity to produce higher yields than *vinifera* grapes from secondary shoots if the primary shoots are killed by frost, and are more frost tolerant.

1.5. Classification of grape species based on food usage

1.5.1. *Table grape:* These are consumed as fresh fruits. Cultivars have an attractive appearance and are generally seedless. Taste is said to be secondary, and good flavor may not be as important as production, shipping tolerance and shelf life. Thompson seedless and Perlette (white), Flame seedless and Ruby seedless (red) are the major cultivars for table

grapes. Major seeded cultivars include Emperor, Ribier, and Calmeria in USA, Italia, a white table grape in Italy and Almeria in Spain. Table grapes can include any of the three major grape species or hybrids, but *V. vinifera* is by far the most important species worldwide. Non *vinifera* table grapes include Concord (*V. labrusca*) and Scuppernong (*V. rotundifolia*).

1.5.2. *Raisin grapes:* These are seedless cultivars that obtain soft texture and pleasing flavor upon drying. Thompson Seedless is the major cultivar worldwide, and makes up 90% of raisin production in the U.S.A., while Black Corinth and Muscat of Alexandria are important in Europe.

1.5.3. *Sweet juice grapes:* Traditionally, this class was dominated by Concord. In addition to juice, jelly, jam, preserves and some wine is produced from sweet juice grapes. Recently, white grape juice concentrate from Thompson Seedless and other *vinifera* cultivars has been used extensively to blend with many other fruit juices and beverages.

1.5.4. *Wine grapes:* Wine is produced from all grape species, but the bulk of commercial production is dominated by *V. vinifera* cultivars. Several French-American hybrids also produce good quality wine. Wine cultivars vary by country and region. Adaptation and climatic requirements dictate the cultivar selection for wine making.

Wine classification: Wines are classified on the basis of alcoholic content.

Table wines: have an alcohol content of 9-14% and are further divided into still and sparkling wines.

Sparkling wine: Wines of this category derive their sparkle due to the incorporation of carbon dioxide under pressure. These show wide variations and include dry white wines and sweet sparkling red wines such as *labrusca.* 'Cold duck' is a sparkling wine made from Concord grapes. 'Brut' is a white sparkling wine typically made from a blend of Chardonnay and Pinot noir.

Still table wines: Most wines fall into this category and are further divided into white, red and rose wines (Table 1.2).

Table 1.2: Important wine cultivars from major grape countries of the world

Country	Reds	White
France	Cabernet Sauvignon, Merlot, Pinot Noir, Syrah, Cabernet franc, Gamay, Grenache	Chardonnay, Semillon, Sauvignon blanc, Chenin blanc, Aligote, Viognier,
Italy	Sangiovese, Nebbiolo, Canaiolo, Vernatsch Barbera, Lagrein, Pinot Noir, Aglianico	Trebbiano, Malvesia, Chardonnay, Vernaccia
Germany	Pinot Noir, Portugieser	Riesling, Silvaner, Muller-Thurgau, Gewurztraminer,
U.S.A.	Zinfandel, Cabernet Sauvignon, Merlot, Petite Sirah, Pinot Noir	Chardonnay, Sauvignon Blanc, Riesling, Gewurtztraminer, Chenin Blanc, Colombard
Spain	Airen, Grenache, Tempranillo, Bobal, Monastrell	Macabeo, Garnacha Blanca
Australia	Shiraz, Cabernet Sauvignon, Merlot, Pinot Noir, Malbec	Chardonnay, Sauvignon Blanc, Semillon, Rhine Riesling

Table 1.3: Classification of still wines based on colour

White wines	Red wines	Rose wines
Most are consumed with meals and are designed to have slightly acidic finish, which becomes balanced when combined with food proteins; and both can accentuate and harmonize with food flavors. Ex. Chardonnay, Riesling, Colombard, Rhine, Semillon, etc.	These are characterized by absence of detectable sweet taste due presence of bitter and astringent compounds. Ex. Merlot, Pinot noir, Airen, Shiraz, Barbera, Grenache, Cabernet Franc, Syrah, etc.	These are the most malignant group of wines. This is because of their mode of production, in which to achieve desired red colour, the grape skins are removed from juice shortly after fermentation has begun. Thus, the uptake of compounds that gives it flavor is also limited.

Fortified wines: Dessert or appetizer wines are consumed in small amounts and are completely rare. They possess high alcohol content i.e. 17–22% by volume; which limits microbial spoilage. Also their insensitivity to oxidation and marked flavor often allows them to retain their aromatic features for weeks after opening i.e. Ports, Cloroso Sherrie, Madelras and Marsalas**.**

1.6. Medicinal properties of grapes

Grapes are considered as laxative, stomachic, diuretic, demulcent and cooling and used an astringent in throat infections. They are also used in Geri forte (stress-care). The juice of *V. compressa* is used for healing in Asia. It constitutes compounds like ellagic acid, biflavonoids and phytoalexins mainly resveratrol beneficial for human health.

Ellagic acid: It has anti-cancer activity and may act as a free radical scavenger.

Biflavonoids: A good source of biflavonoid (Vitamin P), which is known to be useful in purpura, capillary bleeding in diabetes, edema and inflammation from injury, radiation damage and atherosclerosis. Catechins and anthocyanogenic tannin present in grapes possess biflavonoid activity. A valuable herbal medicine extracted from *V. vinifera* seed extract, a mixture rich in bioflavonoids specifically proanthocyanidins, enhance the activity of vitamin C through some unknown synergistic mechanism. The bioflavonoid in grape seed extract reduces the painful inflammation of swollen joints and prevents the oxidation of cholesterol in arteries. Grape seed extract enhances the antioxidant activity of vitamin C and an anti-inflammatory to treat arthritis and allergies.

Resveratrol: Processed by enzyme CYP 1B1, it converts into piceatannol, which is known for anti-cancer activity. Resveratrol belongs to a class of plant chemicals called phytoalexins, which are used by plants as a defense mechanism against attacks by fungi and insects. Resveratrol has an anti-inflammatory activity and inhibits angiogenesis. Also it inhibits the plaque build-up or clogging of arteries (atherosclerosis) by increasing the level of high density lipoproteins in the blood which carry cholesterol away from the arteries. It reduces blood platelet aggregation or clotting within blood vessels and reduces oxidative stress in nerve cells thus protecting against age related nerve changes. Enzyme activity inhibited by resveratrol is responsible for abnormal smooth muscle growth in blood vessels.

Pinot noir is the source of the highest yield of resveratrol and its level in red wines ranges from 1-46 μM, whereas in white wines it is less than 1 μM. Red wines inhibit the growth of colon carcinoma and human breast cancer.

1.7. Soil and climate requirement for *vinifera* grapes

Grapes are adapted to a wide variety of soil conditions, from high pH and slight saline to acidic and clayey soils. Grapes perform best where the soil pH is between 5 to 6. Climate has a profound influence on vine growth, productivity and quality of berries. *V. vinifera* grapes require Mediterranean climate i.e. warm, rainless summers, low humidity and mild winter temperatures. In the warmer climates raisins, sultanas, currants or lower quality bulk wines can be produced. As the temperature gets cooler, dried fruit production becomes more difficult. At the cooler limit, production of only white wines (*V. vinifera* or *vinifera* American hybrids) can occur. *V. vinifera* is a temperate species, which can not withstand extreme winter or cold. It requires warm hot summers for the maturation of its fruits. Cold hardiness is a limiting factor for *vinifera* grapes; hence these have low chilling requirement, 100 - 500 h and tend to break bud early and are frost prone in many regions.

High humidity is another limiting factor for *vinifera* grape culture due to disease susceptibility.

1.8. Vine habit

All *Vitis* are lianas or woody, climbing vines. Unlike trees, these do not expend energy to make large, self-supporting trunks, but use tendrils to attach themselves to other tall growing plants. Their shoots can extend several feet per year since most of the energy goes into growth in length, not girth. Tendrils occur opposite leaves at nodes, and automatically begin to coil when they contact another object. Grapes are generally cultivated on a trellis, fence, or other structure for support, although it is possible to develop small, freestanding vines. *V. vinifera* and American bunch grapes have loose, flaky bark on older wood, but smooth bark on one year old wood. In contrast, muscadine vines have smooth bark on wood of all ages.

Leaves vary in shape and size depending on species and cultivar. Muscadine grapes have small (2-3 inch), round, unlobed leaves with dentate margins. *V. vinifera* and American bunch grapes have large (up to 8-10 inch in width) cordate to orbicular leaves, which may be lobed. The depth and shape of the lobes and sinuses varies by cultivar. Leaf margins are dentate. Buds are compound in grapes, meaning that they have multiple growing points or meristems. In most other fruit crops, buds are simple, having only one growing point. Generally, there are three buds i.e. primary, secondary, and tertiary with primary being the largest, most well developed, and most fruitful of the three. The primary bud is usually the only bud that grows, but if it is killed, the secondary and/or tertiary buds will grow out. In American bunch grapes and French American hybrids, secondary buds can produce a crop, but *V. vinifera* grapes have very limited cropping potential generally from secondary buds.

1.9. Floral biology

Flowers are small (1/8 inch), indiscrete and green, borne in racemose panicles opposite to leaves at the base of current season's growth. There are five each of sepals, petals and stamens. Ovary is superior and contains two locules each with two ovules. The calyptra or cap is the corolla, in which petals are fused at the apex; abscises at the base of flower and pops off at anthesis. Species of *Euvitis* may have more than 100 flowers per cluster, where as muscadine grapes bear only 10 to 30 flowers per cluster. Concord and *vinifera* grapes are perfect flowered and self pollinated, where as some muscadine cultivars

have only pistillate flowers which are tiny, with non-showy petals and short reflexed stamens.

1.9.1. Pollination

Most grapes are self pollinated and do not require pollinizers; however, pistillate muscadines (Fry, Higgins, Jumbo) must be interplanted with perfect-flowered cultivars to affect pollination. Since parthenocarpy does not exist, all grapes require pollination for fruit set. Even seedless cultivars like Thompson Seedless are not parthenocarpic rather the embryos abort shortly after fertilization and fruit set. This condition being called "stenospermocarpy", which is biologically different from seedless fruit production. Pollination is accomplished by wind and to a lesser extent by insects.

1.10. Fruit

Grapes are considered a true berry because the entire pericarp is fleshy. The berries are small (<1 inch), round to oblong, with up to four seeds. Berries are often glaucous, having a fine layer of wax on the surface. Skin is thin, and is the source of the anthocyanin pigments giving rise to red, blue, purple, and black colored grapes. Thus, dark colored grapes such as ‘Zinfandel' can be made into a white or blush wine by limiting contact of the clear fruit juice with the colored skins. Green and yellow skinned cultivars are often termed white grapes. Muscadines differ from other types by having thick skin, which is sometimes bitter and tough. Fruit of muscadine grapes ripen one by one, and detach from the plant at maturity. The berries detach from the vine with a dry stem scar. While in bunch grapes, the small stem that holds the berry plugs the fruit when the berry is detached, yielding a wet stem scar. Fruit are borne in clusters, with two clusters per shoot in most cultivars, but up to five clusters per shoot in French-American hybrids. Thinning is not practiced for most types; crop load is controlled through meticulous pruning. However, French-American hybrids may require cluster thinning for development of quality and proper vine vigor.

1.11. Nutritional quality

The major food products made from grapes are reflected in the utilization data (USDA, 2002): Wine - 50-55%, Canned - < 1%, Table - 10-15%, Juice, jelly, - 6-9% and Raisins - 25-30%. The dietary and nutritional value of grapes is presented in the Table 1.4. A powerful alcoholic drink, Grappa, is distilled from fermented skins, seeds, and stems, which are left over from pressing the juice in wine making. Grappa is often used as an after-dinner drink in Italy. Many types of flavorings are added (e.g., orange or lemon

peel) to improve flavor. In addition to the fruit or its pulp, young grape shoots and leaves are edible. Grape seed oil is used as edible oil, and also for making soaps.

Table 1.4: Dietary value of the grapes (per 100 g edible portion)

	Grapes	Raisins	Wine (100 gm = 4 oz)
Water (%)	81	18	90
Calories	67	289	70
Protein (%)	0.6	2.5	Trace
Fat (%)	0.3	0.2	0
Carbohydrates (%)	17	77	1-2
Crude Fiber (%)	< 1		0
	% of US RDA*		
Vitamin A	2.0	0.4	---
Thiamin, B1	3.6	7.8	trace
Riboflavin, B2	1.9	5.0	trace
Niacin	1.7	2.8	trace
Vitamin C	9.0	2.2	0
Calcium	1.5	7.8	<1
Phosphorus	2.5	12.6	---
Iron	4.0	35	40 (red only)
Sodium	---	0.6	<1
Potassium	3.7	16	1-2

* Percentage of recommended daily allowance set by FDA, assuming a 154 lb male adult, 2700 calories per day.

1.12. Propagation

The most common method of grape propagation is bench grafting, although rooted cuttings, T-budding and layering are also used. The most common method of muscadine propagation is trench layering. Thus, muscadine vines are own-rooted, and have the advantage of coming back true from the roots if they are killed back during winter. The grape rootstocks root easily from dormant hardwood cuttings. The basic steps in bench grafting are: Dormant scion and rootstock canes are collected in late winter/early spring and grafted immediately, or collected in late fall and stored in refrigeration for one to two months. Canes are cut to 12-14 inch in length and sorted by diameter. The diameter of rootstock and scion cuttings should match. The rootstock cuttings should be disbudded to prevent formation of sucker. Grafts are usually made by machines, which make accurate, tight fitting, complementary cuts in stocks and scions. If done by hand, whip-and-tongue grafts are used. The scion is waxed by dipping in molten paraffin (and cooling in water immediately) down to the union to prevent dehydration. Vines are allowed to callus and form roots for three to four weeks at 80^{o}F in special rooms. Moist peat moss is packed

around the rootstock portion of the graft. Vines are then planted in the nursery.

1.13. Rootstocks

Rootstocks have a potential for combating soil problems and can also be a tool for manipulating vine growth and productivity. The use of rootstocks is gaining in importance in Indian orchards due to increasing problems of soil salinity, drought, nematodes and poor fruitfulness of varieties. The details of rootstocks are given below:

Table 1.5: Description of grapevine rootstocks of commercial importance

S. No.	Rootstock	Parentage	Salient features
1.	SO4	*V. berlandieri x V. riparia*	Vigorous rootstock, popular in neutral or mild alkaline soils. Good nematode and phylloxera tolerance.
2.	Kober 5 BB	*V. berlandieri x V. riparia*	Vigorous rootstock suited to areas where scion vigor is a problem. Moderate nematode resistance. Very resistant to phylloxera and perhaps has some resistance to Cotton Root Rot.
3.	99 Richter	Berlandieri x Rupestris	This rootstock is drought tolerant and performs well in acid soils. It does not tolerate salt. High resistance to phylloxera and rootknot nematodes and moderate resistance to dagger and lesion nematodes.
4.	140 Ruggeri	Berlandieri x Rupestris	Very drought tolerant, well adapted to acid soils, and resistant to salinity. Highly resistant to phylloxera. Moderate resistance to rootknot nematodes.
5.	110 Richter	*V. berlandieri x V. rupestris*	Vigorous stock that tends to delay maturity, drought tolerant and tolerant of up to 17% lime in the soils.
6.	1103 Paulsen	*V. berlandieri x V. rupestris*	Vigorous (similar to 110 R), adaptable to clay-lime soils and reported to be somewhat salt tolerant.
7.	41 B	*V. berlandieri x V. vinifera*	Moderate vigorous, imparts somewhat early fruit maturity. Possesses exceptional resistance to high-lime soils.
8.	Dogridge	*V. berlandieri x V. vinifera*	An extremely vigorous rootstock with good resistance to nematodes, moderate tolerance to phylloxera and high-lime. High level of suckering is a commercial drawback.
9.	Fercal	*V. berlandieri* and *V. vinifera*	Suitable for high lime containing European soils.
10.	Riparia Gloire de Montpelier	*V. berlandieri* and *V. vinifera*	Not appropriate for calcareous soils and dry sites. Very high resistance to phylloxera. Advances scion maturity.
11.	Rupestris Saint George	*V. berlandieri* and *V. vinifera*	Can resist drought, High resistance to phylloxera. Very sensitive to rootknot,

			sensitive to dagger, and moderately resistant to root lesion nematodes. Very susceptible to the root-rot fungi and Fanleaf degeneration.

1.14. Current status of grape in India

Grape cultivation is one of the most remunerative farming enterprises in India. In India, grape is grown on an area of 60,000 ha with a production of about 1.6 million tonnes (FAO, 2005), making a share of 1.83% of world production. Of this, nearly 78 % is used for table purpose, 17-20 % for raisin production and only 0.5 % is used for wine making (Adsule *et al.,* 2006).

The cultivated area of the different cultivars in India along with their production is presented in Table 1.6.

Table 1.6: Area and production of different varieties of grapes in India is as follows:

Variety	Area (ha)	Production (t)
Anab-e-Shahi (white, seeded)	3,000	135,000
Bangalore Blue Syn. Isabella (black, seeded)	4,500	180,000
Bhokri (white, seeded)	500	15,000
Flame Seedless (red, seedless)	500	10,000
Gulabi Syn. Muscat Hamburg (purple, seeded)	1,000	30,000
Perlette (white, seedless)	1,500	60,000
Sharad Seedless – A mutant of Kishmish Chorni (black, seedless)	1,000	20,000
Thomson Seedless and its mutants	22,000	550,000
Total	34,000	1,000,000

*Source: FAO, 2001

Approximately 85% of the total production, irrespective of the variety, is consumed fresh. About 120,000 tons of Thompson Seedless and its mutants, namely, Tas-A-Ganesh, Sonaka and Manik Chaman are dried for raisins. Some 20,000 tonnes of Bangalore Blue are crushed to make juice, and 10,000 tonnes of Bangalore Blue, Cabernet Sauvignon, Chenin Blanc, Chardonnay, Merlot, Pinot Noir and Uni Blanc are crushed to process into wine. The wild species found in India are *V. barbata*, *V. parvifolia*, *V. araneosus*, *V. indica* and *V. latifoli.*

1.15. Grape regions in India

Grape is grown under a variety of soil and climatic conditions in three distinct agro-climatic zones, namely, sub-tropical, hot tropical and mild tropical climatic regions in India.

Sub-tropical Region: This region covers the northwestern plains corresponding to 28° and 32° N latitude including Delhi; Meerut district of Uttar Pradesh; Hissar and Jind districts of Haryana; and Bhatinda, Ferozpur, Gurdaspur and Ludhiana districts of Punjab.

Tropical Region: This region covers Nashik, Sangli, Solapur, Pune, Satara, Latur and Osmanabad districts of Maharashtra; Hyderabad, Ranga Reddy, Mahbubnagar, Anantapur and Medak districts of Andhra Pradesh; and Bijapur, Bagalkot, Belgaum, Gulbarga districts of northern Karnataka lying between 15° and 20° N latitude. This is the major viticulture region accounting for 70 % of the area under grapes in the country.

Mild Tropical Region: An area covered by 10° and 15° N latitude including Bangalore and Kolar districts of Karnataka; Chittoor district of Andhra Pradesh and Coimbatore, Madurai and Theni districts of Tamil Nadu fall in this region. Maximum temperatures in a year seldom exceed 36°C, while the minimum is about 12°C. Principal varieties are Bangalore Blue (Syn. Isabella), Anab-e-Shahi, Gulabi (Syn. Muscat Hamburg), and Bhokri. Thompson Seedless is grown only with limited success. Except for Thompson Seedless, two crops are harvested in a year. *Vinifera* varieties susceptible to mildew suffer losses due to unprecedented rains during flowering and fruit set in both hot and mild tropical regions.

1.16. Factors affecting grape production

1.16.1. Biotic stresses

Diseases and pests represent a major threat to the commercial production of grapes in the world (Table 1.8). Climatic conditions are conducive to the development of several major grape diseases, including black rot, downy and powdery mildew. Each of these diseases has the potential to destroy the entire crop. Several other diseases (Phomopsis cane and leaf spot, *Botrytis* gray mold, *Eutypa* dieback and crown gall) can also result in economic losses. Most diseases occur simultaneously within the same vineyard during the growing season. Insects feeding on grapevine leaves, roots, flowers / berries and shoots are the most destructive (flea beetle, berry moth, mealy bug, army worm, mites, borers, leafhoppers, Phylloxera, nematodes). Birds, wasps, bats, bees, rats, foxes, wolves etc also are a threat to the grape industry. The excessive use of chemicals for controlling diseases or pests reduces the market value due to their residue left in fruits.

The development and implementation of Integrated Pest Management (IPM) programs for grapes has great potential for improving pest control strategies and reducing the use of pesticides. The environmental conditions during the growing season decide the pesticide. The introduction of new fungicide chemistry as well as new information related to the disease cycles of the various pathogens are providing opportunities for new disease

control strategies. Developing a disease management presents a unique challenge and should emphasize the integrated use of disease resistance, various cultural practices, knowledge of disease biology, and use of fungicides or biological control agents when necessary.

Table 1.7: Important diseases and pests of grapevine

Diseases	Causal Organism	Infected Part
Fungal		
Downy Mildew	*Plasmopara viticola* (Berk. et Curt) Berl. et De Toni	Leaves
Powdery Mildew	*Uncinula necator* (Schw.) Bur.	Berries and old branches
Gray Mold	*Botrytis cinerea* Persoon, *Botrytis vulgaris* Fr.	Leaves, clusters
Anthracnose	*Elsinoe ampelina* (de Bary) Shear	Entire vine, berries
White Rot	*Coniothyrium diplogiella* (Sperg.) Sacc.	
Bitter Rot	*Glomerella cingulata* (Ston.) Spaul. et Schr.	
Dead Arm	*Cryptosporella viticola* (Reddick) Shear	
Mould	*Cladosporium oxysporum* Berk and Curt.	Leaves, berries
Foot rot	*Rhizoctonia solani, Fusarium* sp., *Alternaria* sp.	Roots
Root rot	*Pythium debaryanum* Hessevar. *Viticolum*	Roots
Brown Spot	*Pseudocercospora vitis* (Lev) Speg.	Leaves
Berry rot	*Pestalotia menezisiana*	Berries
Axle Blotch	*Physalospora bacoae* Cavalra	
Branch wilt	*Hendersonula toruloidea* Nattrass	Leaves, branches
Bunch rot	*Diplodia natalensis*	Peduncles of bunches
Rust	*Kuehneola vitis, Phakopsora vitis*	Leaves
Black rot	*Guidnardia bidwellii* (Ellis) Viala and Ravaz, *Aspergillus niger* V. Tiegh	Leaves, berries
Dieback	*Eutypa lata*	Hardwood stems
Dead arm and wilt	*Phomopsis viticola*	Branches, leaves
Brown leaf spot	*Cercospora viticola* (Ces.) Sacc.	Leaves

Leaf blight, berry necrosis	*Alternaria alternate* (Fr.) Keissler	Leaves, berries
Green ball rot	*Cladosporium herbarum* (Pars.) Link ex Fr., *C. tenuissium* Cooke	Berries
Brown rot	*Botryodiplodia theobromae* Pat.	Bunches
Blue mould rot	*Penicillium citrinum*	
Waxy/ Yeast rot	*Geotrichum candidum* Link.	Berries
Bacterial		
Crown gall	*Agrobacterium tumefaciens*	Base of trunks
Bacterial canker	*Xanthomonas campestris pv. viticola*	Leaf blades, petioles
Bacterial leaf spot	*Pseudomonas viticola, Xanthomonas campestris* pv. *viticola* (Nayudu) Dye.	Leaves
Viral / Virus like		
Fanleaf virus		Leaves
Leaf roll virus		Leaves
Pests	**Scientific name**	**Infected Part**
Stem girdler	*Sthenias grisator* Fab. E	Canes, branches
Stem and arm borer	*Celosterna scabrator* Fbr.	Branches, leaves
Grape flea beetle	*Altica chalybea, Scelodonta strigicollis* Mots	Leaves, sprouting buds
Defoliating beetles	*Adoretus lasiopygus* Burm., *A. versutus* Harold	Leaves, berries
Leaf roller	*Sylepta lunalis* Guen.	Leaves
Grape berry moth	*Endopiza viteana*	Flower / fruit clusters
Army worm	*Spodoptera exigua*	Leaves, flowers
Mealy bugs	*Ferrisiana virgata*	Branches
Thrips	*Thrips sp*.	Leaves, berries, branches
Red spider mites	*Eotraniclus carpini*	Leaves
European red mite	*Panonychus ulmi*	Leaves
Rose chafer	*Macrodactylus subspinosus*	Fruit blossoms
Tobacco caterpillar	*Spodoptera litura* Fabr.	Leaves
Castor hairy caterpillar	*Euproctis fraternal* Moore, *E. lunata*	Leaves

	Walker	
White fly	*Aleurocanthus spiniferus* Quain	Leaves, shoots
Scale insects	*Aspidiotus lataniae* S., *A. cydoniae, Lacanium longulum* D., *Pulvinaria maxima* G.	Leaves, shoots
Bark eating caterpillar	*Indarbela* sp.	Canes
Grasshoppers	*Poecilocerus pictus* Fab.	Leaves, shoots
Asian lady beetle	*Harmonia axyridis*	Ripened fruits
Japanese beetle	*Popillia japonica*	Foliage, fruits, flowers
Grape Phylloxera	*Daktulosphaira vitifoliae*	Leaves, roots
Potato leafhopper	*Empoasca fabae*	Leaves
Eastern grape leafhopper	*Erythroneura comes*	Leaves
Three-banded leafhopper	*Erythroneura tricincta*	Leaves
Virginia creeper leafhopper	*Erythroneura ziczac*	Leaves
Horn worm	*Hippotion celerio* Linn.	Leaves
Bag worm	*Clania cramer* Westwood	Leaves, shoots
Termites	*Odontotermes* sp.	Canes
Grape root borer	*Vitacea polistiformis*	Crown, roots
Root knot nematode	*Meloidogyne incognita*	Roots
Reniform nematode	*Rotylenchulus reniformis*	Roots
Citrus nematode	*Tylenchulus semipenetrans*	Roots

1.16.2. Abiotic stresses

When environmental factors exceed their optimal conditions, grapevine undergoes stress and exhibits certain disorders. Dead arm and trunk splitting is common in pruned vines suffering from moisture stress when summer temperatures are too high. Dry spot of berries is attributed to sun-burn injury. The symptoms of salinity (high concentration of chlorides, sulphates, carbonates and bicarbonates of Ca, Mg, Na and K) injury include reduced budbreak, stunted shoot growth, shortened internodes, reduced leaf size, marginal necrosis of leaves, heavy fruiting with impaired berry development. Alkali injury is mainly due to heavy Na content in the soils, where the vines are slender with weak shoots and small leaves and short internodes. Mainly the use of the right rootstock having salinity

tolerance is adopted to get rid of the above mentioned problems coupled with soil amendments.

1.16.3. Physiological disorders

Disturbances in the normal metabolic functions of the vine created by complex factors give rise to physiological disorders. Leaf chlorosis occurs due to leaf senescence, shading, water logging, soil moisture stress or malnutrition. Cane immaturity occurs due to low temperatures or frost injury and had direct influence on fruit set. Barrenness of vines may be due to bud failure, defective training and pruning practices and inadequate care during non-bearing period. Rudimentary panicles again occur due to inadequate nourishment. The condition of berries lacking normal sugar, colour, flavor and keeping quality, referred to as water berries, can be controlled by using low N fertilizers. Shot Berries (smaller, sweeter, round and seedless as compared to normal berries) are formed due to delay in pollination and fertilization of a few flowers or due to inadequate flow of carbohydrates into the set berries. Boron deficiency, improper GA application and girdling are the known factors for shot-berry formation. Berries at the tip of the cluster shrivel, wither and remain sour even after softening / ripening called as cluster tip wilting. Chicken and hen disorder refers to the situation, when a bold berry is surrounded by many shot berries occurring due to Zn and B deficiency. Flower and berry drop may be caused due to environmental stress and inadequate C/N ratio. Blossom end rot and pink berries are due to Ca deficiency, while berry cracking and rotting is attributed to excessive rains during ripening. In recent years, pink berry disorder is a wide spread problem seen in all most all grape growing regions of the country, whose cause is unknown. But the favorable conditions for the severity of the problem is found to be sudden low temperatures coupled with long days at veraison stage.

1.17. Improvement of grapevine

1.17.1. Conventional methods

Like any other crop, grape can be improved through different methods like introduction, selection, hybridization and mutational breeding. Continuous programmes of introduction, evaluation, selection and breeding are essential for meeting the changing consumer needs, to evolve crop and cultivars to resist various abiotic and biotic stresses. There are many cultivars improved and released through various conventional methods:

1.17.1.1. Introduction and evaluation

Most of the presently cultivated grapevine cultivars in India are introductions from the grape growing regions of the world. A beginning of varietal improvement through introduction was started with the introduction of Abi (Bhokri), Fakhri and Sahebi in 1338 to Deccan. So far, nearly 1118 varieties have been introduced and evaluated in the country. The most popular varieties under cultivation in the country, Anab-e-Shahi, Bhokri, Thompson seedless and Perlette are introductions from other countries.

1.17.1.2. Selection

Selection of superior clone among the existing varieties and introduced cultivars is an age old practice for varietal improvement. Occurrence of spontaneous mutations and phenotypic variations of a genotype in different agro-climatic conditions are the basis of selection. Pusa seedless and Tas-A-Ganesh are clonal selections from Thompson seedless and Dilkus, a bud sprout from Anab-e-Shahi are some of the examples of the selection.

1.17.1.3. Hybridization

Hybridization is an advanced method of crop improvement in which varieties with specific characters can be evolved by transferring a derived character into a variety with many desirable characters. Arkavati, Arka Kanchan, Arka Shyam and Arka Hans from IIHR in 1980 and Pusa Navrang and Ousa Urvashi from IARI in 1996 are examples of hybridization.

1.17.1.4. Mutation breeding

Mutations are very important means of creating variability in the crop plants. Physical muatgens like X-rays or thermal neutrons or gamma rays and chemical mutagens like Ethyl methyl Sulphonate (EMS) or N-nitrose-N-methyl urethane or N-notrose-N-methyl-urea have been used for inducing mutations in the grapevine. Some mutants of commercial importance like seedless mutants of Catawba, Concord, emperor, Habshi and Muscat of Alexandria have been released after evaluation.

1.17.2. Biotechnological methods for grape improvement

Paradoxically, the genetic base of commercial grape varieties is rather narrow, causing vulnerability to diseases and pests, especially in the tropics and sub-tropics. Being amenable to propagation both through seed and vegetative means, there are wider options for its genetic improvement through biotechnological means. The ability to produce novel

cultivars by conventional breeding is hampered by high degree of heterozygosity, polygenic inheritance of many desired characters and the long-term juvenile period. Hence, there is a need for non-conventional methods of grapevine improvement.

There are various published reports on grapevine that have been summarized in the following sections.

1.17.2.1. Callus induction

Callus induction in grapevine was first reported by Morel (1941 and 1944), after which many researchers could obtain callus from different explants of grapevine namely stem, petiole, tendril, node, internode, flower, fruit and immature berries (Fallot, 1955; Alleweldt and Radler, 1962; Arya *et al.,* 1962; Staudt *et al.,* 1972; Hawker *et al.,* 1973; Jona and Webb, 1978). The addition of growth regulators for obtaining callus (Brezeanu *et al.,* 1980) and certain vitamins like myo-inositol for sustaining callus (Staudt, 1984) were used. Callus studies have been used in detection of phytoalexin / flavonoid accumulation in the grape tissues infected with fungal diseases (Morel, 1948; Belarbi, 1983; Dai *et al.,* 1995; Feucht *et al.,* 1996) for a better understanding on their role in the defense response. Callus cultures were used for the production of Resveratrol (Commun *et al.,* 2003; Tassoni *et al.,* 2005). Resveratrol (*trans*-3,5,4-trihydroxystilbene), a low molecular weight, constitutive and inducible phytoalexin belonging to the stilbene family (Langcake and Pryce, 1977) plays an important role in protecting plants against fungal infections (Dixon and Harrison, 1990; Hain *et al* ., 1990) and constitute the main group of phytoalexins within the family *Vitaceae* (Jeandet *et al* ., 2002).

1.17.2.2. Cell culture

Grapevine cell suspension culture offers interesting opportunities for the study of host-parasite interaction (Hoos and Blaich, 1988; Deswarte *et al.,*1996; Guillen *et al.,*1998; Morales *et al.,*1998; Colrat *et al.,*1999) and was initiated from callus culture by Hawker *et al*. (1973). Cell culture also helps in studying the production of secondary metabolites i.e. anthocyanins (substitute for food colourants) or phytoalexins (medically useful compounds) and their biosynthesis (Ambid *et al.,* 1983; Do and Cormier, 1990 and 1991; Hirasuna *et al.,* 1991; Shure and Acree, 1994; Pepin *et al.,* 1995; Decendit and Merillon, 1996). Suspension cultures (Jayasankar *et al.,*1999; Bornhoff *et al.,*2000) are convenient for gene identification and expression monitoring, providing a simple system for studying its molecular biology i.e. identification of vacuolar localization of iso-peroxidases and its significance in indole-3-acetic catabolism (Garcia-Florenciano *et al.,*1991); glutamate

dehydrogenase gene; glutamate synthetase and glutamate synthase genes (Loulakakis and Roubelakis-Angelakis, 1997); osmotin-like gene (Loulakakis, 1997); arginine decarboxylase gene and proanthocyanidin synthesis.

1.17.2.3. Organ culture

Most of the organ culture studies in grapevine were focused on inflorescence culture (Pool, 1975) so that it could provide a valuable tool for probing the mechanisms of floral induction (Lilov and Isvorska, 1978). However, *in vitro* induced flowers from tendrils lacked functional ovules and anthers (Srinivasan and Mullins, 1979). Hairy root cultures (Hemstad and Reisch, 1985; Mugnier, 1987; Gribaudo and Schubert, 1990; Guellec *et al.,* 1990; Livine, 1990; Torregrosa, 1994; Torregrosa and Bouquet, 1997) could be a powerful means of understanding the interactions between grapevine root systems and their pathogens. These could also be used to produce grapevine viruses (Lupo *et al.,* 1994) or study the efficiency against nematodes (Mugnier, 1988; Loubser and Meyer, 1990; Bavaresco and Walker, 1994) or phylloxera (Forneck *et al.,* 1998).

1.17.2.4. Direct shoot organogenesis

Production of buds, shoots and plants can also be obtained though a neo-formation process using tissues without pre-existing meristematic structures. There are two ways of well recognized regeneration systems: adventitious organogenesis giving rise to caulinary structures and somatic embryogenesis giving embryo or embryo like formations. *In vitro* somatic embryogenesis and organogenesis have been achieved from various explants of different *Vitis* species and cultivars. Organogenesis uses the ability of competent tissues to form adventitious bud-like structures either directly or via callus that develop mainly at cut surfaces. Different cultivars of grapevine were tested for their ability for organogenesis by Martinelli *et al.,* 1996. There are reports on direct shoot organogenesis from leaves and petioles of grapevine (Colby *et al.,* 1991), from hypocotyls and cotyledons of somatic embryos (Vilaplana and Mullins, 1989) and leaves of rootstocks and cultivars (Tang and Mullins, 1990). Also, adventitious bud formation from *in vitro* leaves of *Vitis* x *Muscadinia* hybrids (Torregrosa and Bouquet, 1996), improved shoot organogenesis from *in vitro* leaves of French Colombard and Thompson Seedless (Stamp *et al.,* 1990), and organogenesis from internode explants of grapevine (Rajasekharan and Mullins, 1981) have been reported.

1.17.2.5. Somatic embryogenesis

Somatic embryogenesis is the initiation of embryos from plant somatic tissues closely resembling their zygotic counterparts (Ammirato, 1983). In grapevine, it was initiated through anther culture with the aim of recovering dihaploid plants for genetic improvement programs (Gresshoff and Doy, 1974; Hirabayashi *et al.,*1976; Rajasekaran and Mullins, 1979; Mullins and Rajasekaran, 1980; Zou and Li, 1981; Bouquet *et al.,*1982; Krul and Mowbray, 1984, Krul, 1985; Mauro *et al.,*1986; Gray and Mortensen, 1987; Mullins, 1990; Gray and Meredith, 1992; Moszar and Sule, 1994; Gray, 1995; Perl *et al.,*1995; Faure *et al.,*1996a; Sefc *et al.,*1997; Nakano *et al.,*1999; Franks *et al.,*1998; Torregrosa, 1995 and 1998; Salunkhe *et al.,*1999; Martinelli *et al.,*2001; Perrin *et al.,*2001). Embryo tissues have been proved to be the best explants for transgenic plant regeneration (Martinelli, 1997). In addition somatic embryogenesis has also been proposed as a strategy aiming to introduce somaclonal variation (Kuksova *et al.,* 1997; Fallot *et al.,* 1990; Deloire and Mauro, 1991); virus elimination (Goussard *et al.,* 1991; Goussard and Wiid, 1992; Schaefers *et al.,* 1994) and synthetic seed technology for germplasm conservation (Gray and Compton, 1993). Somatic embryogenesis with somatic tissues i.e. leaves, tendrils, petioles, internodes (Krul and Worley, 1977; Stamp and Meredith, 1988a; Matsuta and Hirabayashi, 1989; Marchenko, 1991; Martinelli *et al.,*1993; Robacker, 1993; Harst, 1995; Torregrosa *et al.,*1995; Tsolova and Atanassov, 1996; Kuksova *et al.,*1997; Salunkhe *et al.,*1997; Monette, 1988; Perl and Eshdat, 1998); ovules or zygotic embryos (Mullins and Srinivasan, 1976; Stamp and Meredith, 1988b; Jayasankar *et al.,*1999) and protoplasts (Reustle *et al.,*1995; Zhu *et al.,*1997) has been achieved.

1.17.2.6. Anther culture / haploid plant production

Progress in genetic improvement of grapevine is hindered by the high heterozygosity of the genome (Alleweldt, 1997) hence availability of homozygous plants would be more interesting. Many researchers have tried to develop pure lines (Bronner and Oliveira, 1990) and haploids by anther culture but failed (Bouquet, 1978; Olmo, 1948). Though development of multinucleate pollen grains and haploid tissues was reported (Gresshoff and Doy, 1974; Rajasekaran and Mullins, 1979 and 1983; Bouquet *et al.,* 1982; Altamura *et al.,* 1992), all regenerated plants were diploid. The anther derived callus originated from somatic cells of the anther wall, connective or filament (Newton and Goussard, 1990; Perrin *et al.,* 2004). Although Zou and Li (1981) reported the production of haploid plants by anther culture, however the experiments could not be repeated. Sefc *et*

al. (1997) obtained embryoid like structures from isolated *Vitis* microspores, but could not regenerate plants.

1.17.2.7. *In ovulo* embryo rescue

The type of seedlessness occurs in grapes is called stenospermocarpy, in which fertilization occurs but seeds fail to develop completely as embryo / endosperm aborts (Stout, 1936). Now-a-days seedless grape cultivars are preferred by consumers world over for table purpose. Traditional breeding methods are based on hybridization between seeded and seedless varieties; however the proportion of seedless plants in the progenies is generally low and more dependent on the choice of the parents. Secondly, the character of seedlessness cannot be observed at an early stage hence the method is both space and time consuming. By using *in ovulo* and *in vitro* techniques it is possible to rescue viable embryos of seedless crosses (Cain *et al.,* 1983; Emershad and Ramming, 1984; Spiegel-Roy *et al.,* 1985 and 1990; Goldy and Amborn, 1987; Barlass *et al.,* 1988; Gray *et al.,* 1990; Tsolova, 1990; Bouquet and Danglot, 1996; Gribaudo *et al.,* 1993; Garcia *et al.,* 2000). This method is very efficient in obtaining progenies from seeded cultivars as seeds have very low germination ability (Bouquet, 1977) and also in obtaining triploid grapes which could offer another strategy for breeding seedless grapes as their unbalanced chromosome sets are highly sterile in nature (Yamashita *et al.,* 1998).

Embryo excision by rupturing the seed coat was effective in obtaining a higher number of embryos (Fernandez *et al.,* 1991; Aguero *et al.,* 1995; Valdez and Ulanovsky, 1997; Burger and Trautmann, 2000). Other factors affecting embryo recovery were the choice of female parent, age of the berries (Bouquet and Davis, 1989; Ponce *et al.,* 2000) and treatments with low temperatures or growth retardants (Aguero *et al.,* 1995). The hybrids obtained by seedless x seedless controlled crosses are mostly zygotic in origin (Durham *et al.,* 1989). Occurrence of multiple embryos in cultured ovules (Emershad and Ramming, 1984; Bouquet and Davis, 1989); low levels of natural polyembryony in seeds (Bouquet, 1982) and high levels of twin seedlings (Olmo, 1978) were observed. The efficiency of this technique has been improved by the use of molecular markers, which help to identify and choose the best seedless genotypes to be crossed.

1.17.2.8. Genetic Engineering / Transformation studies

Transgenic plants broadly speaking indicate those plants in which functional genes of foreign origin have been introduced in to their genome. For grape improvement the modifications by genetic transformation should leave the essential characters and identity

of the cultivar unaltered which is impossible by conventional means due to highly heterogeneous nature of grapes. Moreover, new cultivars are assigned new names contributing to their slow acceptance in the market. Novel genes cloned from any source can be targeted and introduced in the cultivar to improve traits of disease and pest resistance, product quality, production efficiency and sustainability. The successful transfer of foreign DNA into grapevine cells has been achieved by *Agrobacterium*-mediated transfer and Particle Bombardment-mediated transformation methods. Disarmed strains of *A. tumefaciens* (Huang and Mullins, 1989; Mullins *et al.,* 1990) or *A. rhizogenes* (Nakano *et al.,* 1994) have been used for introducing the foreign DNA. The disarmed strains of *A. tumefaciens* strains were used in transgenic plant production like LBA4404 (Hoekema *et al.,* 1983); GV2260 (Deblaere *et al.,* 1985) and EHA101 (Hood *et al.,* 1986). Earlier studies on *A. tumefaciens*-mediated transformation of vegetative tissues of grapevine met with limited success (Baribault *et al.,* 1990; Mullins *et al.,* 1990; Colby *et al.,* 1991). Mullins *et al*. (1990) could produce transgenic grapevines by *A. tumefaciens* co-cultivation of hypocotyls of somatic embryos. Transformation using organogenesis in grapevines (Mezzetti *et al.,* 2002) and multiple shoot induction (Manjul Dutt *et al.,* 2006) in grapevine has recently been reported.

Studies with co-cultivation of embryogenic cultures with *Agrobacterium* resulted in regeneration of transgenic grapevines (Le Gall *et al.,*1994; Martinelli and Mandolino, 1994; Nakano *et al.,*1994; Krastanova *et al.,*1995; Mauro *et al.,*1995; Scorza *et al.,*1995 and 1996; Perl *et al.,*1996 and 1999; Franks *et al.,*1998, Mozsar *et al.,*1998, Perl and Eshdat, 1998; Xue *et al.,*1999; Iocco *et al.,*2001). Kanamycin selection was mostly used earlier for selecting transformed grapevine somatic embryos (Mullins *et al.,* 1990; Colby *et al.,* 1990) compared to hygromycin selection (Thomas *et al.,* 2000). Phosphinothricin, herbicide was also used for the selection of putatively transformed tissues in grapevine (Perl *et al.,* 1996). Fluorescent assay was used for screening putative transformants for chitinolytic enzymes (Kikkert *et al.,* 2000). Variable pattern of GUS (Franks *et al.,* 1998) and GFP (Iocco *et al.,* 2001) inheritance has been reported earlier. Biolistics or microprojectile bombardment technique developed by Sanford (1993) and was successfully applied to grapevine tissues. Regenerated grapevine plants expressing the GUS marker gene (Hebert *et al.,* 1993; Franks *et al.,* 1998); chitinase genes (Kikkert *et al.,* 1996 and 2000) were obtained from embryogenic cultures of grapevine.

1.18. Rationale of the present study

Crimson Seedless, a red, table grape variety (Fig. 1.2) was developed by Ramming and Tarailo of the USDA, Fresno, California, USA as a result of cross between Emperor and C33-199 (Dokoozlian *et al.,* 1998). Nutritionally Crimson Seedless grapes have high sugar content, with half as glucose, and half as fructose. They also contribute some dietary fibre and vitamin C and contain adequate amounts of potassium and Vitamin A. These are low in Sodium. Berries are delicious, eaten raw as a snack or added to fruit salads, cheese platters, salads, crepes, cakes, tarts, sorbets, or set in jelly. Berries are equally delicious in hot dishes. Crimson Seedless mostly grown in California, USA ripens in mid-October and berries are available from late January until April. Retail trade over there has received the variety favorably due to its excellent eating characteristics like crisp and firm berries. In India, Crimson Seedless is a recent introduction; hence inadequate supply of planting material of the variety is a major constraint for a large-scale cultivation in India.

In vitro propagation offers an advantage of clonal multiplication of desired material at faster rate and on a continuous basis round the year. So far, there are very few reports describing success in micropropagation of grapevines (Sahijram *et al.,* 1996; Thomas, 2000 and Mhatre *et al.,*2000) and somatic embryogenesis in anthers and tendrils (Salunkhe *et al.,*1997 and 1999). But to the best of our knowledge, there are no reports available for *in vitro* propagation of Crimson Seedless.

Development of highly efficient methods for plant regeneration via organogenesis / embryogenesis is a prerequisite for application of tissue culture to grapevine improvement through genetic engineering. Most of the seedless varieties of grapes grown world over are susceptible to various diseases especially to fungi (mildews). The economic losses due to fungal diseases of grapevine mainly Downy and Powdery mildew are very high in a tropical country like India. There are no reports on genetic transformation of grapevine cultivar Crimson Seedless for disease resistance.

In view of the above, the present study aimed to fulfil the following objectives:

1. To develop *in vitro* plant propagation method for grape cultivar Crimson Seedless.
2. To induce organogenesis / embryogenesis in Crimson Seedless.
3. To study factors influencing *Agrobacterium*-mediated plant transformation in Crimson Seedless.

Fig. 1.2. Fruit bunch of Crimson Seedless

Fig. 1.3. Mature vine of Crimson Seedless at NRC for Grapes