Chapter 1

Introduction and review of literature
1.1 Origin, Introduction and Cultivation

Grape (Vitis spp.) is the oldest commercial fruit crop and is widely cultivated all over the world. It is supposed to have originated in Armenia between the Mediterranean basin and the Caspian Sea (Olmo 1976). An independent and recent origin of grapes is also traced to North America (Riaz et al. 2007). Leaves and seeds of grape were discovered in North America and Europe in fossil deposits of the Tertiary period of geological time. Seeds were also found in the refuse mounds of the pile dwellers of lakes in south central Europe belonging to the Bronze Age. From Armenia grapes spread westwards to Europe and Eastwards to Iran and Afghanistan. Grape was introduced into India in 1300 AD by the Mughal invaders. Grape cultivation flourished in Baluchistan and North-West Frontier Province during the 16th century. Grape cultivation declined after the fall of Mughal rulers but was reintroduced in south India (Aurangabad district of Maharashtra) by Mohammed-Bin-Tughlak and since last 50 years grape is commercially cultivated in India.

In ancient Sanskrit literature grape is mentioned in Arthashastra, Charak and Sushruta Samhita. As reported by Pillay (1968), grapes were seen flourishing in India by a Chinese Buddhist (629-645 AD), Moorish traveller Ibn? Batuta (1430) and a French traveller Thevenot (1667). Majority of commercially cultivated grapes belong to genus Vitis (species vinifera) which contains about 60 species distributed almost equally between America and Asia. The commercial varieties of grapes were introduced in India mostly by invaders of Iran and Afghanistan (Thaper 1960). Various species are found indigenously in India are V. barbata Wall. V. rugosa Wall., V. rumicisperma M. Laws. and V. parviflora Roxb., which grow in the Himalayan region, produce edible fruits and show tolerance to diseases and pests (Olmo 1970). In addition to many introduced varieties, India has developed and released varieties for commercial cultivation like Pusa Seedless and Tas-e-Ganesh,
1.2 Taxonomy

Vitaceae are woody climbers consisting of approximately 14 genera and 900 species (Wen et al. 2007) Taxonomically, grape (Vitis vinifera L.) belongs to the family Vitaceae which is divided into two sub-genera, Euvitis Planch. (2n=38) and Muscadinia Planch. (2n=40) (Einset and Pratt 1975). Grapevine is a woody, rarely evergreen climber. The permanent stem consisting of canes, possessing dormant shoots with buds or ‘eyes’ from which new growth arises after pruning. Leaves are non-lobed or 3-7 lobed irregularly toothed and glabrous or tomentose. The leaf arrangement on shoot is distichous, with leaf opposed tendrils or intermittent tendrils. The inflorescence of grapevine is called cyme, a highly branched panicle. Vitis was originally a dioecious plant and transformed to a hermaphrodite one by spontaneous mutations during the process of evolution. Many wild grapes still possess male and female flowers on separate vines. Fruits arise in bunches called panicle which consists of peduncle, pedicles, rachis and berries. Fruit is a berry, ovoid to globose and variable in size. They are greenish, red, purplish, blue or bluish black in colour. The edible part is pericarp and placenta. Wild grapes have a musky flavour which is absent in modern day cultivars.

1.3 Economic importance of Grapes: Vitis vinifera L.

1.3.1 International Scenario

Grapes are grown in more than 80 countries of the world with a total of 7,572,237 hectares devoted primarily to wine grapes, but also including table and raisin grapes and also used to produce juice, jam, concentrate, and seed oils. Grapes are extensively used worldwide for fruit and wine production. Out of the total produce, 79% is used for table purpose, 18% is used for wine making and remaining 3% is used for raisins, spirits and jam-jelly preparation.
Chapter 1: Introduction and review...

China ranks first in global market contributing 12% to international economy. While Italy ranks second followed by USA and Spain contributing 11%, 10% and 9% in International economy respectively. India ranks 18\textsuperscript{th} in grape production in global market with 1% contribution in the world economy (Table1.1, fig.1.1). On world map, there is lot of need and scope for the improvement in grape cultivation practices to increase the share of Indian grapes in world market.

\textbf{Table1.1: Major grape producing countries in the world}

<table>
<thead>
<tr>
<th>Rank</th>
<th>Country</th>
<th>Production ($1000)</th>
<th>Production (MT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>China</td>
<td>4945533</td>
<td>8651831</td>
</tr>
<tr>
<td>2</td>
<td>Italy</td>
<td>4451638</td>
<td>7787800</td>
</tr>
<tr>
<td>3</td>
<td>United States of America</td>
<td>3874265</td>
<td>6777730</td>
</tr>
<tr>
<td>4</td>
<td>Spain</td>
<td>3490979</td>
<td>6107200</td>
</tr>
<tr>
<td>5</td>
<td>France</td>
<td>3343365</td>
<td>5848960</td>
</tr>
<tr>
<td>6</td>
<td>Turkey</td>
<td>2432230</td>
<td>4255000</td>
</tr>
<tr>
<td>7</td>
<td>Chile</td>
<td>1575205</td>
<td>2755700</td>
</tr>
<tr>
<td>8</td>
<td>Argentina</td>
<td>1495698</td>
<td>2616610</td>
</tr>
<tr>
<td>9</td>
<td>Iran</td>
<td>1289379</td>
<td>2255670</td>
</tr>
<tr>
<td>10</td>
<td>Australia</td>
<td>962803</td>
<td>1684350</td>
</tr>
<tr>
<td>11</td>
<td>Egypt</td>
<td>777542</td>
<td>1360250</td>
</tr>
<tr>
<td>12</td>
<td>Brazil</td>
<td>772346</td>
<td>1351160</td>
</tr>
<tr>
<td>13</td>
<td>South Africa</td>
<td>720986</td>
<td>1261310</td>
</tr>
<tr>
<td>14</td>
<td>Germany</td>
<td>576132</td>
<td>1007900</td>
</tr>
<tr>
<td>15</td>
<td>Greece</td>
<td>573274</td>
<td>1002900</td>
</tr>
<tr>
<td>16</td>
<td>Uzbekistan</td>
<td>564357</td>
<td>987300</td>
</tr>
<tr>
<td>17</td>
<td>Portugal</td>
<td>540406</td>
<td>945400</td>
</tr>
<tr>
<td>18</td>
<td>\textbf{India}</td>
<td>\textbf{503423}</td>
<td>\textbf{880700}</td>
</tr>
<tr>
<td>19</td>
<td>Romania</td>
<td>423064</td>
<td>740118</td>
</tr>
<tr>
<td>20</td>
<td>Afghanistan</td>
<td>349440</td>
<td>611320</td>
</tr>
</tbody>
</table>

Source: FAO STAT 2010
1.3.2 Production of Grapes in India

Grape growing is one of the most remunerative farming enterprises in India, grape being one of the most important among commercially grown fruit crops. Maharashtra is the leading state in its cultivation and production.

National Horticulture Board, Govt. of India published its database in March 2010. The facts show the success of grape growing in India. Since 2001 to 2009 the area and production of grapes is continuously increasing (Table 1.2). This exhibits tremendous scope and potential in domestic market along with its export.
Table 1.2: All India, area and production of Grapes

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Year</th>
<th>Area (In ‘000’ ha)</th>
<th>Production (In ‘000’ MT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2001-2002</td>
<td>47.5</td>
<td>1184.2</td>
</tr>
<tr>
<td>2</td>
<td>2002-2003</td>
<td>52.1</td>
<td>1247.8</td>
</tr>
<tr>
<td>3</td>
<td>2003-2004</td>
<td>57.08</td>
<td>1474.8</td>
</tr>
<tr>
<td>4</td>
<td>2004-2005</td>
<td>60.5</td>
<td>1564.7</td>
</tr>
<tr>
<td>5</td>
<td>2005-2006</td>
<td>66.0</td>
<td>1649.6</td>
</tr>
<tr>
<td>6</td>
<td>2006-2007</td>
<td>65.0</td>
<td>1685.0</td>
</tr>
<tr>
<td>7</td>
<td>2007-2008</td>
<td>68.0</td>
<td>1735.0</td>
</tr>
<tr>
<td>8</td>
<td>2008-2009</td>
<td>80.0</td>
<td>1878.0</td>
</tr>
</tbody>
</table>

Source: Indian Horticulture Database 2009, Page 73

1.3.3 Maharashtra: Leading state in grape production

The state wise area and production of grapes for three years from 2006-07 to 2008-09 is presented in Table 1.3 Maharashtra is the leading state for grape production in India with about 75% of total grape production, Thus it becomes utmost essential to undertake effective steps to improve the efficiency of entire cultivation practices to improve grape production as well as to increase the contribution of India in world market.

1.3.4 Uses of grape

Grapes have many uses, they are consumed fresh, dried (as raisins) or processed (juices and liquor such as wine and champagne) or canned. In European countries, majority of the grape production is for wine compared to table purpose grapes. Other important industrial uses of grape byproducts are grape seed oil, anthocyanin pigments and ethanol production. The medicinal properties of grapes are mentioned by famous Indian medicine scholars, Sushruta and Charaka in their medical treatises entitled ‘Sushruta Samhita’ and ‘Charaka Samhita’, respectively, written during 1356-1220 BC. European folk
medicine users healed eye and skin diseases using sap of grapevines, while leaves were used to stop bleeding and inflammation of hemorrhoids. Ripe grapes and raisins were used for treating cancer, tuberculosis, smallpox, nausea, kidney and liver diseases. Resveratrol, a polyphenol present in red wines and grapes has more recently been proven to induce apoptosis of human melanoma cells (Niles et al. 2003) and prevent cancer (Yang et al. 2001), probably due to its antioxidants activity (Chanvitayapongs et al. 1997).

Table 1.3: State wise Area and Production of Grapes

<table>
<thead>
<tr>
<th>State</th>
<th>Area ('000'ha)</th>
<th>Production ('000'MT)</th>
<th>Area ('000'ha)</th>
<th>Production ('000'MT)</th>
<th>Area ('000'ha)</th>
<th>Production ('000'MT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maharashtra</td>
<td>45.4</td>
<td>1284.2</td>
<td>45.6</td>
<td>1290.0</td>
<td>55.7</td>
<td>1415.0</td>
</tr>
<tr>
<td>Karnataka</td>
<td>12.1</td>
<td>216.6</td>
<td>14.3</td>
<td>258.8</td>
<td>14.9</td>
<td>269.0</td>
</tr>
<tr>
<td>Tamilnadu</td>
<td>2.8</td>
<td>91.6</td>
<td>2.8</td>
<td>83.5</td>
<td>3.1</td>
<td>91.0</td>
</tr>
<tr>
<td>A.P.</td>
<td>2.5</td>
<td>51.8</td>
<td>2.8</td>
<td>58.0</td>
<td>3.0</td>
<td>62.2</td>
</tr>
<tr>
<td>Punjab</td>
<td>1.1</td>
<td>30.7</td>
<td>1.0</td>
<td>26.7</td>
<td>0.8</td>
<td>22.1</td>
</tr>
<tr>
<td>Others</td>
<td>1.0</td>
<td>10.4</td>
<td>1.9</td>
<td>17.6</td>
<td>2.2</td>
<td>19.2</td>
</tr>
</tbody>
</table>

A.P.: Andhra Pradesh, Source: Indian Horticulture Database 2009, Page 74

1.4 Grape Improvement

1.4.1 Conventional Breeding

As stated earlier, the primary centers of grape species diversity are North America and East Asia. Distribution of grapes is also observed mostly in America and Asia. Scion cultivars are derived chiefly from the European grape, *Vitis vinifera*, which was domesticated 6,000–10,000 years ago in the region between the Black and Caspian Seas. Grapes spread east into Asia and west into the Mediterranean region. Rootstocks were developed from North American species, including *V. riparia*, *V. rupestris*, and *V. berlandieri*. Scion breeding programs
focus on the development of cultivars adapted to biotic and abiotic stress, with high fruit quality, and time of ripening during desirable periods of market demand. Fungal disease resistance is a primary goal of many programs, while cold hardy cultivars help extend the limits of grape cultivation. Rootstock breeding focuses on providing protection against phylloxera and nematodes as well as adaptation to high/low pH, and/or water-stressed conditions. Rootstocks should propagate easily by grafting and cuttings. New cultivars are more rapidly adapted in the raisin and table grape sectors than in the wine industry, although there are several notable examples of successful wine grape cultivars developed by breeding. These cultivars are typically grown on rootstocks to resist soil-borne pests and to adapt to adverse soil conditions, but there are areas of the world where they can be grown without rootstocks. The success rate of Inter-specific breeding and scion breeding is lower so there is need to adopt alternative approaches to improve the commercially cultivated grapes and their yield practices.

1.4.2 Germplasm management through traditional identification methods

Invasion of gall-forming insect phylloxera (*Daktulosphaira vitifoliae* Fitch.) throughout the viticulture areas around the world during the nineteenth century resulted in devastation in grape and wine production. After this calamity, viticulture experts of the OIV and IPGRI declared the urgency for the establishment of the germplasm collections to account for the losses of wild forms and/or autochthonous varieties of *Vitis*. They further indicated the necessity of international co-operation in the characterization and evaluation of such invaluable genetic resources to avoid genetic erosion (Wrinkler *et al.*1974, Weaver 1976, Dettweiler 1990).

Ampelography is a scientific methodology accepted for the characterization of grapevine genotypes, based on the description of different morphological, phenological and pomological characters. This method has been standardized and extended by many scientist for more logical and accurate identification of *Vitis* materials (Galet 1985, Alleweldt and Dettweiler 1986, Dettweiler 1991).
Following the recommendations of the experts, characterization and conservation of the worldwide *Vitis* materials has been started by different researchers (Alleweldt and Dettweiler 1986; Agaoglu *et al.* 1989; Soylemezoglu *et al.* 2001, Santiago *et al.* 2007). As a consequence, the resulting *Vitis* International Variety Catalogue (VIVC) is made accessible via word wide web since 1996. This catalogue provides an inventory of the grapevine genetic resources with passport, primary and secondary descriptors, bibliography and photos.

### 1.4.3 Problems in germplasm management and Grape growing

Most of the commercially cultivated grape cultivars in India are introductions from America and European countries which have unknown interrelationships between them. The same cultivars, due to their morphological differences are known by different names leading to confused nomenclature creating problems like synonyms, homonyms and duplicates (Bowers *et al.* 1999). Ampelography experts usually know grapevine cultivars in use in their region and not familiar with those from other regions. Additionally, to achieve reproducibility and standardization of observations between distant ampelographers have been proved to be difficult (Sefc *et al.* 2001). Therefore, the long history of viticulture, vegetative propagation of cultivars and the reliance on ampelography has posed severe difficulties in accurate cultivar identification (Thomas *et al.* 1993).

Cultivar identification and management is a laborious task but is the first most essential step towards strategic planning for future breeding and for retaining sustainability of the crop. Therefore there is need to gather knowledge on genetic relationship among grape varieties for the accurate conservation of germplasm which was achieved by molecular markers (Lopes *et al.* 1999).

As majority of commercially cultivated grapes belonging to species *Vitis vinifera* are highly susceptible to various biotic and abiotic stresses; downy mildew, powdery mildew and anthracnose are the major fungal diseases while leaf eating caterpillar, chaffer beetle and flea beetle are the major pests affecting grape
production in the country. Control of these diseases by spraying fungicides and pesticides is the only remedy followed by grape grower results in 40 to 60% losses in yield (Alexander et al. 1993). These chemicals are not only expensive resulting in the increase in cost of production but also are harmful to human health and environment.

1.4.4 Need to utilize genetic diversity in Wild grapes

Plant genomes contain numerous resistance genes (R-genes) that play a role in initiating defense measures against particular pathogens. Nucleotide Binding Site/Leucine-Rich Repeat (NBS-LRR) and Serine/Threonine Kinase (STK) genes are two of the known classes of resistance (R-) genes in plants, and occur in large multigene families. (Gaspero and Cipriani 2002) The majority of the R-genes containing NBS-LRR repeats described so far belong to a super-family of genes (150–600 members) that encode proteins with a nucleotide binding site (NBS), some leucine-rich repeats (LRR) and an N-terminal domain that shows similarity to the Toll and Interleukin-1 receptors (TIR) or a N-terminal coiled-coil (CC) domain (Hans van Leeuwen 2005). The PCR clones obtained from partial NBS sequences from Grape (*Vitis aestivalis* Michx)* showed potential use as markers to differentiate resistant cultivars from the susceptible ones, a useful tool in breeding programs. (Chang et al. 2009).

Thus wild relatives of grapes carrying useful resistance genes for the biotic stresses need to be evaluated as a valuable source for improvement of commercial grapes. On similar lines, in Australia, ‘gene discovery’ project (Rosetto et al. 2001) has been initiated in grapes which aims at recognizing a potential source of useful genetic diversity for *Vitis* within the closely related taxa and identifying novel useful genes that will maximize production quality and quantity.

1.5 Biotechnological tools
1.5.1 Limitations of conventional Breeding

In 1988, INIA, the Chilean Institute for Agricultural Research started a breeding program to develop new table grape cultivars with emphasis on seedlessness, disease resistance and post-harvest life (Barticevic et al. 2004). Since seedlessness was the main objective, crosses were made among seedless cultivars followed by in vitro embryo rescue (Hewstone et al. 2006). Thus biotechnological approaches like in ovulo embryo rescue have opened new vistas in grapevine breeding.

Some workers (Cain et al. 1983, Emershad and Ramming 1984, Gray et al. 1987, Spiegel-Roy et al. 1985, Gray et al. 1990) have successfully applied embryo rescue technique and obtained hybrid plants, mainly to study the inheritance of seedlessness in the progeny. Yamashita et al. (1998) reported the production of triploid grapes from crosses between diploid (cvs. Rosario Bianco, Katta Kourgan, Sekirei and Rizamat) and tetraploid (cv. Kyoho) varieties.

By using embryo rescue, one can overcome problem of incompatibility barriers due to distant hybridizations. Interspecific hybridization along with embryo rescue thus offers a stable solution for obtaining desirable fruit quality and pest resistance in table grape varieties by crossing them with varieties possessing resistance traits. There are several seeded varieties and wild relatives of grapes in the family Vitaceae which are known to possess resistant genes against diseases.

1.5.2 Cultivar identification: DNA based markers

An early evaluation of required traits will reduce labour, time and economic aspects of a breeding programme. DNA molecular markers are not affected by environmental variations. Thus they have been proven to be an objective and viable alternative for ampelography (Thomas et al.1993) Molecular markers correlating with inheritable traits such as seedlessness and resistance allow an early evaluation of the progenies by Marker Assisted Selection (MAS).
1.5.2.1 Restriction Fragment Length Polymorphism (RFLP)

Restriction fragment length polymorphism (RFLP) analysis was used to identify grape clones, cultivars and for comparison of rootstocks (Bowers et al. 1993, Gogorcena et al. 1993, Yamamoto et al. 1991, Guerra and Meredith 1995, Barysheva et al. 2003), but this method has the disadvantage of being comparatively expensive and time consuming.

1.5.2.2 Dominant markers: AFLP, RAPD and ISSR markers

PCR based methods like Random Amplified Polymorphic DNA (RAPD) and Inter-Simple Sequence Repeat (ISSR) markers can quickly detect polymorphism and genetic analysis can be carried out at earlier stages of plant development (Powell et al. 1996a, Grando et al. 2000). The genetic relationship between different grape varieties has been assessed with the help of ISSR markers. (Bowers et al.1993, 1996, Bourquin et al. 1993, Gogorcena et al.1993, Moreno et al. 1995, Tschammer and Zyprian 1994). RAPD markers have been used for genetic analysis of progeny of the cross Cayuga White x Aurore (Lodhi and Reisch 1995) and 83-4-96 (V. quinquangularis) x Muscat Rose (V. vinifera) (Luo et al. 2002).

Similar analysis was carried out for F1 progeny derived from cross between two seedless cultivars (Ruby Seedless X Sultanina) by available RAPD and AFLP (Amplified fragment length polymorphism) markers (Mejia et al. 2007). Genetic diversity was assessed among table grapes from Brazil using RAPD markers (Patrícia Coelho et al. 2011). In this RAPD analysis closest genetic relationships among 'Italia' clones ('Italia Muscat', 'Benitaka' and 'Brasil') had been expected and results obtained were in accordance with the previous works in Brazil (Leao et al. 2009) Genetic relationships among 31 cultivated and 34 wild grape accessions originating from regions near the Caspian Sea in the Azerbaijan Republic, RAPD analysis was derived by Salayeva et al. 2010.

As reports on ISSR analysis of cultivated (seeded and seedless grapes) and wild taxa are comparatively less, the research in grape genetics can be facilitated with the recent availability of inexpensive and easy to use DNA based markers like...
Inter Simple Sequence Repeat (ISSR). So Genetic relationships between *Vitis vinifera*, Euro–American *Vitis* species and American *Vitis* species as well as seedless grapes has been initiated in China (Wu *et al* 2006, Chong-huai *et al* 2009)

**1.5.2.3 Co-dominant SSR markers**

Molecular markers based on simple sequence repeats (SSRs), or microsatellites, have been used most successfully in genetic diversity studies of fruit species, including grapes (Benjak *et al*. 2005, Agar *et al*. 2012). Microsatellites, with their high information content, ease of genotyping through polymerase chain reaction (PCR), codominant and multiallelic nature and high discriminating power, are an ideal tool for such studies (Morgante and Olivieri 1993, Powell *et al*. 1996a, Russell *et al*. 1997). In addition, only small amounts of DNA are required, and the quality of the DNA does not need to be as high as that required for most other methods (Rafalski *et al*. 1996). Microsatellites have been widely used for the construction of genetic maps, parentage determination, and population genetic structure analysis (Barreneche *et al*. 1998, Arnold *et al*. 2002, Szczys *et al*. 2005, Pedryc *et al*. 2009). Microsatellites or simple sequence repeats (SSRs) are proved to be the most efficient among DNA markers for a variety of applications such as DNA profiling/typing, mapping and breeding even in *Vitis vinifera* cultivars (Thomas *et al*. 1993, Bowers *et al*. 1996, Scott *et al*. 2000, Sefc *et al*. 1999, 2000). Microsatellite marker technology has been extensively used in grapevine biology and genetics for confirmation and definition of synonyms (Bowers *et al*. 1999) as well as germplasm management (Lopes *et al*. 1999). Grape SSRs are likely to be useful genetic tools for population investigations of Vitaceae taxa worldwide. Mapping of the *Vitis* genome have been proved to be the convenience for Viticulture research community, therefore, molecular markers were developed and the *Vitis* Microsatellite Consortium (VMC) was formed in 1997 (Sefc *et al*. 2001)
Relevance of microsatellite markers was evaluated to distinguish individual clones within each cultivar of a French collection as well as efficiently identified accessions with clonal polymorphism (Pelsy 2010). even with a small set of germplasm analyzed with SSRs wide genetic diversity was observed in Indian grape germplasm (Upadhay et al. 2010). Six duplicates/misnomers accessions were also identified. This information can be further strengthened by analyzing larger germplasm and can be used for better germplasm management and identification of core collection of cultivars with desirable traits.

Although extensive work on grape genome characterization is ongoing, there is comparatively meager work done on its wild relatives. Transferability of grape SSRs across family Vitaceae has been more successful as compared to other families (Scott et al. 2000, Rossetto et al. 2001). It has been studied that, EST derived SSRs are transferred across genera more readily (55.3%) than enrichment derived SSRs (42.7%) Two such SSRs have shown potential for assessing taxonomic studies as well as for conservation studies in Australian members of Vitaceae (Arnold et al. 2002, Scott et al. 2000).

DNA sequence is usually decoded for one individual and its application is for the whole species. The recent sequencing of the highly heterozygous Vitis vinifera L. cultivar Pinot Noir (clone ENTAV 115) genome has been useful in revealing several thousand polymorphisms and offers a good model to study the transferability of its degree of polymorphism to other individuals of the same species and within the genus (Vezzuli et al. 2008). Using SNPllex technology, the informativeness of SNP (Single Nucleotide Plymorphism) markers from the heterozygous grape cultivar Pinot Noir has been validated in V. vinifera, but it has a more limited application for wild forms of this species and has no direct application for non-vinifera Vitis species. The SNPllex technology has emerged as a robust method for rapid analysis of a limited number of SNPs on a large number of plants. Although additional SNPs could be used, the SNPs developed in this study will be very useful for accession identification and genomic research assisted breeding at the V. vinifera level.
1.5.2.4 Chloroplast diversity

Due to the conserved nature of the chloroplast genome in higher plants, the development of SSRs for non-coding regions of the chloroplast genomes has provided higher resolution tool to study the closely related taxa. Chloroplast microsatellites represent potentially useful markers to detect polymorphism at the population level (Provan et al. 2001). A set of universal primers has been developed for amplifying chloroplast SSRs (Weising and Gardner 1999). Such universal microsatellite primers were tested in 77 grapevine cultivars from Greece and other geographic origin and gene diversity was found to be high with 17 haplotypes detected in 77 grapevine cultivars (Lefort et al. 2000a, b). These microsatellite loci also revealed intra- and inter-specific length variation within the genus *Vitis* (Arroyo-Garcia et al. 2002). Genetic isolation and diffusion of wild grapevine in Italian and Spanish populations was also estimated by nuclear and chloroplast SSR analysis (Grassi et al. 2003). Putative genetic relationships between cultivated varieties and local sylvestris populations have been proposed in other regions (Grassi et al. 2003). Chloroplast genome markers are useful to study relations among cultivars growing in different regions and the haplotype distribution could help to trace geographical sites of grapevine origin (Imazio et al. 2006). The Eurasian grape (*Vitis vinifera* L.) is the most widely cultivated and economically important fruit crop in the world (Vivier and Pretorius 2002) Future genomic analyses in grape and other woody species, including the analysis of well-dated ancient DNA, should unravel the details behind domestication of these species and origin of specific cultivar. Eurasian grapevine domestication was described (Arroyo-Garcia et al. 2006) as a long-term process of selection of suitable genotypes in different locations followed by their vegetative propagation. Genetic variation would have increased during the process of domestication as a result of somatic variation and the occasional generation and propagation of spontaneous hybrids derived from crosses between cultivated plants, as
previously documented (Bowers et al. 1999) or between cultivated and *sylvestris* plants (Cunha et al.2007).

1.5.3 Taxonomic position of Vitaceae: Phylogenetic analysis

Vitaceae is a complex family characterized by leaf-opposed tendrils, which may be modified to form an inflorescence (Mabberley 1995). The small regular flowers are bisexual in most genera and the size and shape of the leaves can be variable within a single individual. The fruit is a berry 1 to 4 seeds; it can be edible but usually astringent. *Vitis, Ampelopsis* and *Parthenocissus* are mainly restricted to temperate areas while the remaining genera have predominantly tropical and subtropical distribution. The most widely recognized member of the Vitaceae and the one with the greatest economic importance is grape, *Vitis vinifera*. Being an economically important family of angiosperms whose phylogenetic placement is currently unresolved at the family level, molecular studies so far have yet to define the exact position of Vitaceae within the angiosperm phylogeny. Based on morphological characters, the Vitaceae were traditionally placed in the Rhamnales (Cronquist 1968, 1988) but recent molecular work has posed objection to such a placement. Recent phylogenetic analyses based on one to several genes (Chloroplast regions viz. *trnL* Intron, *trnL* IGS, *trnH-psb*, *atpB*, *rbcL*, *matK* and nuclear ribosomal regions viz.18S rDNA, ITS1, ITS2 and ETS) have suggested several alternative placements of this family by several workers (Taberlet et al. 1991, Soltis et al. 1999, 2000, Rossetto et al. 2001, Soejima and Wen 2006)

The non-recombinant, uniparental inheritance nature of organelle genomes makes them useful tools for evolutionary studies. The highly conserved photosynthesis related *rbcL* gene has been one of the cpDNA fragments most widely used to infer plant phylogenies (Chase et al. 1993, Soltis et al. 1999). However, at lower taxonomic levels, the non-coding regions of plastid DNA have been found to be more informative. Many of these can be successfully amplified using a series of universal primers (Taberlet et al. 1992) Sequence variation at
coding and non-coding chloroplast loci is the most widely used tool to assess phylogenetic relationships among plant taxa particularly above species level. The trnL (UAA) intron has been shown as an effective phylogenetic tool at inter and intra generic level (Gielly and Taberlet *et al.* 1994, Van Ham *et al.* 1994). The trnL (UAA) intron sequence analysis has been used to analyze the relationships among Australian genera of Vitaceae (Rossetto *et al.* 2001) and particularly within the genus *Cissus* (Rossetto *et al.* 2001, 2002). Within Australian Vitaceae the cpDNA region (trnL Intron) indicated *Tetrastigma* as a monophyletic genus (Rossetto *et al.* 2001) Similarly Monophyly of *Tetrastigma* was recently proved by Ren and workers (2011) robustly supported by a large set of species (114 samples from 53 species).

Strong sister relationship between the genera *Cayratia* and *Tetrastigma* was reported by Rossetto *et al.* 2007. The paraphyly of the genus *Cayratia* has been reported previously (Ingrouille *et al.* 2002, Rossetto *et al.* 2002, 2007, Soejima and Wen 2006, Wen *et al.* 2007) based on three chloroplast markers (trnL-F region, atpB-rbcL region, rps16 intron) and two nuclear DNA markers (ITS, GAI1). These previous workers also strongly supported a close relationship between these two genera *Cayratia* and *Tetrastigma*. In addition, the previous reports also support the close relationship between *Cayratia trilobata*, *Cayratia japonica*, *Cissus trifolia* and *Tetrastigma* (Soejima and Wen 2006, Wen *et al.* 2007, Ren *et al.* 2011).


*Cissus* is the largest genus in Vitaceae with about 350 species distributed throughout the tropics (Wen 2007a) and possesses remarkable morphological diversity (Jackes 1998, Lombardi 2007). Monophyletic nature of the genus
Cissus is still in a question as it exhibits polyphyletic behaviour (Rossetto et al. 2002, Wen 2007a, Wen et al. 2007).

1.6 Antimicrobial Activity

According to World Health Organization, traditional medicine is defined as diverse health practices, approaches, and knowledge and believes incorporating plant, animal and/or mineral-based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain well-being as well as to treat, diagnose or prevent illness (Lewington 1993, Maurya and Gupta 2006). More than 35,000 plant species are being used in various human cultures around the world for medicinal purposes (WHO, 2008). Crude drugs are usually the dried parts of medicinal plants (roots, stem wood, bark, leaves, flowers, seeds, fruits and whole plants, etc.) that form the essential raw materials for the production of traditional remedies in various systems of medicines like Ayurveda, Siddha, Unani, Homeopathy, Tibetan, etc. Several molecular approaches have been used to investigate disease resistance in plants. A particular class of proteins plays crucial role in host-pathogen interactions. These proteins contain functional domains such as NBS, LRR, TIR, cc and several kinase domains (Gaspero and Cipriani 2002, 2003). Many of the wild relatives of grape have shown resistance to diseases (Gaspero and Cipriani 2003) and have also found to be resistant to all the three major fungal diseases of grapes (Patil et al. 1990). A lot of scope has been found for biochemical characterization of these wild Vitaceae taxa for antibacterial, antiviral, anticancerous and diuretic activity. Phyto-chemical studies in three genera of Vitaceae viz. Vitis, Cissus and Cayratia revealed taxonomic significance of phytochemicals in separation and systematic relationships as well as disease resistance (Patil et al. 1998, Patil and Honrao 2000, 2002).

Cayratia trifolia L is found to be the source of an anticancerous agent in the extensive study by Kumar et al. (2011). The study reveals that methanolic extract is more potent than aqueous extract in exerting antineoplastic effect in both cell
lines as evident by a dose dependent decrease in cell growth. The effect was analyzed at different concentration levels ranging from 50 to 500 g/ml. Anthocyanin present in *Cayratia carnosa* leaves showed antiproliferative and proapoptotic properties in gastric adenocarcinoma and were also found to be protective against esophageal cancer in rodents (Rejitha and Das 2009).

### 1.7 Advanced Molecular Techniques

Grapevine molecular research was initiated in the late 1980’s and currently it involves a wide range of techniques including functional characterization of single genes to genomic approaches (Doddapaneni *et al.* 2008) including genetic mapping, physical mapping, gene discovery using ESTs (Rudd 2003, Da silva *et al.* 2005, Moser *et al.* 2005) and gene expression analysis using microarrays (Terrier *et al.* 2005, Waters *et al.* 2005).

Marker-assisted selection, primarily for disease resistance and seedlessness, is being applied in many breeding programs. Projects that focus on breeding seedless cultivars commonly use embryo rescue techniques (Tian and Wang 2008) enabling the crossing of two seedless parents, to increase the percentage of seedlings that are seedless. Genetic transformation (Hinrichsen *et al.*2004) is a routine procedure and is being used for both functional analysis of gene action as well as directly for cultivar improvement (both scions and rootstocks).

#### 1.7.1 Grape Research Coordination Network

The grape and wine industry is an international global marketplace with a competitive high-value product. The issues facing the grape and wine industry go beyond national boundaries and involve problems with berry quality, disease pressures, and climate change. In response to these global issues, the grape community developed the International Grape Genome Program (IGGP) to promote international collaboration and the placement of genomic data into the public domain to rapidly advance grape genomic studies. In June 2001 IGGP has initiated Identification and characterization of *Vitis vinifera* genes. The
understanding of their function both at the molecular and the organism level is the main goal of this effort. The main objectives of grape genome analysis include the generation of saturated genetic maps, construction of BAC libraries (Adom-Blondon et al. 2005, This et al. 2006) and a physical map and the sequence identification of all Vitis genes through both EST and genome sequencing approaches (www.vitaceae.org), the main emphasis being the genes for disease resistance, stress tolerance, nematode resistance and other traits of relevance in viticulture which are present in other Vitis species.

1.7.2 Sequencing Technology for Grape Genomics Research

The polyploidization events have important consequences in plant evolution, in particular for species radiation and adaptation and for the modulation of functional capacities. A high-quality draft of the genome sequence of grapevine (Vitis vinifera) obtained from a highly homozygous genotype. The draft sequence of the grapevine genome is the fourth one produced so far for flowering plants. Grapevine was selected because of its important place in the cultural heritage of humanity beginning during the Neolithic period. Several large expansions of gene families with roles in aromatic features are observed. The grapevine genome has not undergone recent genome duplication, thus enabling the discovery of ancestral traits and features of the genetic organization of flowering plants. This analysis reveals the contribution of three ancestral genomes to the grapevine haploid content (Jaillon et al. 2007).

The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. A 12x assembly of the grape genome has recently been produced by the French-Italian public consortium for the sequencing of the grapevine genome has produced a gene prediction (named V1) and automatic annotation of the 12X sequence assembly (Jaillon et al. 2007 ).
1.7.3 Transcriptomics

The Plant Functional Genomics Centre, located at the University of Verona, is a structure devoted to the development of cutting edge technologies for the study of functional genomics. As a member of French-Italian Consortium for the structural and functional characterization of the grape genome, the Centre is carrying on the development of the microarray reference platform for *Vitis vinifera* transcriptome analysis (Da Silva et al. 2005, Ferrarini et al. 2008)

Integration of new biotechnological tools with conventional breeding programmes opens new avenues to give transgenic grape cultivar to the world.

1.8 Genesis and structure of the present thesis

Grape is the most important among commercially grown fruit crops in India and Maharashtra is the leading state in its cultivation and production contributing 75% of total Indian grape production. Being an ancient fruit crop, more than 12000 cultivars of grape are known, although only a few hundred are actively cultivated. As grape germplasm management is a resource intensive task accurate cultivar identification is a prerequisite for effective management. To resolve the ambiguities in the identification molecular tools like DNA based markers can be effectively used for germplasm characterization which will further help the grape growers to procure correct planting material.

Majority of commercially cultivated grapes belong to species *Vitis vinifera*, which is highly susceptible to various biotic and abiotic stresses causing 40-100% yield reduction. Downy mildew, powdery mildew and anthracnose are the major fungal diseases while leaf eating caterpillar, chaffer beetle and flea beetle are the major pests affecting grape production in the country. Control of these diseases by spraying fungicides and pesticides is not advisable due to environmental and health hazards. Many of the wild relatives of grapes from the family Vitaceae carry useful resistance genes for these biotic stresses. The diverse range of environments favored by these species also suggests the potential existence of
useful resistance genes for several abiotic stresses. However, their utilization in breeding has been very limited due to failure to generate hybrid seedlings and poor fruit quality. Recent developments in biotechnology have opened up new avenues to transfer some important genes to commercially cultivated grapes. The genetic resources for *Vitis* are vast, and there is great potential to exploit this untapped potential for traits of importance to both scions and rootstocks. However, to undertake such a work, it is essential to gather sufficient information about the relationships between cultivated grapes and their wild relatives to narrow down number of resource species for such utilization.

Agharkar Research Institute is one of the centres in All India Co-ordinated Research Project on sub tropical fruits sponsored by ICAR. A large germplasm collection of grape cultivars and wild relatives of grape has been maintained under this project. The work on DNA profiling of grape varieties using molecular markers has been ongoing at the Institute since 2000. Continuing with the same line of research, the work on molecular characterization of some important grape cultivars in India as well as their wild relatives was undertaken with the following objectives:

**Objectives:**

1. To assess the genetic variability among cultivated grapes and other Vitaceae taxa using ISSR and microsatellite markers
2. To define relationships among Vitaceae taxa using nuclear ribosomal (nr) DNA and noncoding chloroplast (cp) DNA regions
3. Screening of some wild Vitaceae taxa for antimicrobial activity

**Organization of the thesis**

**Chapter 1**
Introduction and review of literature

**Chapter 2**
Assessment of the genetic relationships within cultivated grapes and selected taxa of Vitaceae using ISSR markers

Chapter 3
Molecular characterization of cultivated grapes using SSR markers and their transferability across the Vitaceae taxa

Chapter 4
Chloroplast microsatellite diversity in cultivated grapes and wild Vitaceae taxa

Chapter 5
Phylogenetic analysis of Vitaceae based on nuclear ribosomal (nr) DNA and noncoding chloroplast (cp) DNA sequences

Chapter 6
Screening of some Vitaceae taxa for antimicrobial activity

Chapter 7
Summary and future prospects

References

Annexures