# ABSTRACT

Grape is the second most widely grown fruit crops of the world. It is grown under varied climatic conditions ranging from temperate to semi tropic and tropics. It is a woody perennial, cultivated in 90 countries, covering an area of about 19 million acres with Europe having the largest share (60%). Taxonomically, grapes are divided into two sub-genera, *Euvitis* Planch. (2n=38) and *Muscadinia* Planch. (2n=40). *Vitis vinifera* belongs to the sub-genera *Euvitis*. The genus *Vitis* is broadly distributed between 25° and 50° N latitude in eastern Asia, Europe, the Middle East and North America. According to an estimate, 65.5 million tons of grapes were produced world over with a value of 144 billion pounds (FAO, 2005). In India, grape is grown on an area of 60,000 ha with a production of about 1.6 million tonnes (FAO, 2005), which comprises mainly of table grapes.

Crimson Seedless, a red, table grape variety 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). It is mostly grown in California and has recently been introduced in India. The variety is favored due to its good shelf life and excellent eating characteristics like crisp and firm berries. Like most of the seedless cultivars of grapes world over, Crimson Seedless too is susceptible to various fungal diseases like mildews, anthracnose, fruit rot etc. Genetic improvement of seedless grapevine through conventional breeding is a cumbersome and time taking process. By employing appropriate regeneration system and *Agrobacterium*-mediated plant transformation method, it is possible to introduce foreign DNA into the existing genome to obtain plants with improved disease resistance (Kikkert *et al.,* 2000). Genes encoding pathogen-related proteins such as chitinase and ß-1,3-glucanase are known to be involved in the plant defense system (Legrand *et al.,*1987; Shinshi *et al.,* 1990; Kombrink *et al.,*1988).

By using a suitable plant regeneration system, *Agrobacterium tumefaciens*-mediated plant transformation offers the potential to introduce foreign DNA into the existing genome to obtain plantlets with specific traits, which have been difficult, particularly in *V. vinifera*. Development of *in vitro* propagation system has potential application in rapid multiplication of transformed plants obtained via *Agrobacterium*-mediated genetic transformation. To the best of our knowledge, there are no reports available on *in vitro* propagation, plant regeneration and transformation systems in Crimson Seedless.

The present work entitled **"*In vitro* plant regeneration and genetic transformation studies in grapevine: Crimson Seedless"** was taken up with 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.

The present investigation was carried out at the Plant Tissue Culture Division, National Chemical Laboratory, Pune, India. The objectives of the thesis are realized in the following chapters, followed by summary and bibliography.

**Chapter 1. General Introduction**

This chapter will cover the general introduction and importance of grapevine (*Vitis*) and a thorough literature survey on *in vitro* plant propagation, organogenesis, somatic embryogenesis, ovule culture, embryo recovery and genetic transformation techniques in grapevine. The aims and objectives of the study will be presented.

**Chapter 2. Materials and Methods**

The materials used and various general techniques adopted for *in vitro* plant propagation, histology and hardening of plantlets will be described.

**Chapter 3. *In vitro* propagation**

The chapter will deal with *in vitro* plant propagation system in grapevine cultivar Crimson Seedless. Results on the influence of different basal media, auxins, cytokinins on different stages of propagation *i.e.* bud break, multiple shoot induction, shoot elongation, *in vitro* and *ex vitro* rooting and plantlet establishment will be presented. Also, findings on the DNA fingerprinting of *in vitro* propagated plants of the the cultivar using molecular markers like ISSR and microsatellites will be presented.

**Chapter 4. *De novo* shoot organogenesis**

The chapter will describe the results on plant regeneration system in Crimson Seedless by shoot organogenesis in *in vitro* leaves of the cultivar. Results on the influence of basal medium, explant type and various auxins and cytokinins on direct shoot organogenesis will be presented.

**Chapter 5. Somatic embryogenesis**

The chapter will deal with the development of a plant regeneration system in Crimson Seedless via somatic embryogenesis in a variety of explants like leaf, petiole, tendril and zygotic embryo. Results on the influence of pre-bloom sprays of 4-CPPU, age of berries and growth regulators on ovule / embryo recovery and secondary embryogenesis

in zygotic and somatic embryos of the cultivar will also be presented in the chapter. Influence of three polyamines on maturation and conversion of somatic embryos derived from pro-embryonal mass (PEM) of Crimson Seedless will be described. Also, HPLC analysis of polyamine levels in PEM / somatic embryo and residual quantities in culture media and their correlation with uptake and maturation and germination stages will be described in the chapter.

**Chapter 6. Genetic transformation of grapevine using *Agrobacterium*-mediated gene transfer**

This chapter will include results on influence of various factors *viz. Agrobacterium* cell density, co-cultivation period, sonication and different anti-oxidants / anti-necrotic agents on transformation efficiency in Crimson Seedless. Binary vectors harboring *GFP* as reporter gene, *NPTII* and *HPT* as selectable markers and two anti-fungal genes, *chitinase* and *β-1,3-glucanase* will be used in the study. Confirmation of integration of these genes in Crimson Seedless genome by fluorescence microscopy and by molecular techniques like PCR, DNA sequencing and Southern blotting will be presented.

**Summary**

This section will contain salient findings on *in vitro* propagation, *de novo* shoot organogenesis, somatic embryogenesis and *Agrobacterium*-mediated gene transfer in Crimson Seedless and conclusions of the present study.