CHAPTER-1
INTRODUCTION

All plants are, in one way or other, useful to mankind. I find no better way to explain this beautiful story that emphasizes the need to preserve our ancient skills. The story tells of the legend Jivika who was the student of Bikshu Atery, a well-known professor of the University of Taxila, later became the physician of Bimbisara of Magadha during Buddha’s time. On completion of his seven years medical course of Taxila, Jivika was given the following assignment by the examiner, “Take the spade and seek around Taxila, a yojana on every side and whatever plant you see which is not medicinal, bring it to me” Jivika, so the legends goes, examined all the plants in the specified area and was forced to return to the examiner empty handed.

Since plants influenced the intellectual and the material culture of men, their references have appeared in ballads, tales, song, legends, myths, rhymes, riddles and proverbs of ancient times. Plants are known to be serving human being for thousands of years with its countless precious products which not only benefitted the society but also maintained our environment to a considerable extent. The fragrance and allusiveness of citrus fruit have made them famous scene-setters for poets throughout time and space.

Citrus is the leading tree fruit crop of the world and refers to all edible and rootstock species. The genus Citrus includes more than 162 species belonging to the Order Geraniales family Rutaceae and sub family Aurantoideae. Citrus fruits are grown throughout the world and are known for their fine flavor and quality. Citrus fruits are one of the important horticultural crops with worldwide agricultural production over 100 million metric tons per year.

The special feature of citrus fruits is the presence of juice sacs in them. Citrus fruits lack a firm pulp. It is heterozygous in nature and thus exhibits a great variability in seedling population. These elite chance seedlings possess desirable horticultural traits can be selected as variety/strains after their evaluation under particular agro-ecological zone. In India collection and conservation of citrus species started long back, however, in the middle of nineteenth century it received major emphasis. In early part, collection and conservation were primarily made for the quality fruits, while current research efforts are for collection of gene pool with distinct desirable traits, which can be utilized for improvement of cultivars.
Citrus originated from south-eastern Asia, China and the east of Indian is archipelago from at least 2000 BC (Swingle 1943; Webber et al 1967; Gmitter and Hu 1990). The fruit has been introduced to the new world via the great trade routes of Africa to the eastern Mediterranean basin by the Arab traders while the crusaders brought the fruit to Italy, Spain and Portugal around 1000 AD (1975). The fruit was introduced further to the western hemisphere by Columbus on his second voyage in 1493 (Samson 1980) and the planting material to the cape in South Africa by a Dutch merchant in 1654 (Oberholzer 1969).

Currently, Citrus is cultivated in the subtropical and tropical regions of the world between 40° north and south latitude in over 137 countries on six continents and generates about 105 billion US dollar per year in the world fruit market (Ismail and Zhang 2004). In Ethiopia, although the introduction, production on consumption of Citrus as a horticultural crop is very recent (Seifu 2003). Although the introduction of Citrus to this part of the world is fairly recent, yet it is gratifying to note that U.S.A. has developed the Citrus industry on sound footing within four countries. In India, the common Citrus fruit are mandarins, sweet orange and limes having 50, 20 and 15 per of total area (3.48 lakh hectares) under them respectively. However, they have only 10% of total area under all fruit crops in India with third rank after mango and banana. They are grown in every state of India but the leading states are Andhra Pradesh, Maharashtra, Assam and Karnataka.

Citrus fruits are most important fruit crops known by humans since antiquity and are a good source of vitamin “C” with high antioxidant potential (Gorinstein et al 2001). They contain volatile oils, limonene, alpha terpinene, alphapinene, beta pinene, citral, coumarins, bioflavanoids, vitamins and mucilage. They also act as antiseptic, antirheumatic, antibacterial and antioxidant (Rathore JS et al 2007). Citrus fruits are well known to be rich in certain phytonutrients especially flavonoids that protects humans against cancer and cardiovascular diseases (Kawaii et al 1999). They are rich in minerals that help in lowering blood pressure levels and substantially reduce the risk of stroke. The juicy pulp is either made into beverages or sucked (Thamare Kavitha et al 2008). Apart from susceptible to various biotic stresses like insects, pests and pathogens, citrus are highly sensitive to soil salinity and pH (Shelhevet J et al 1990). Though citrus are highly salt sensitive crops, differences in tolerance doing exist among species (Mass EV 1993). The best-known examples of citrus are the oranges, lemons, grapefruit, and limes.
"Citrus" is the generic term for a great number of species of fruits grown in every country having suitable soils and climates. Webber and Batchelor tell the interesting story of the spreading of Citrus culture throughout the world. The first written account of citrus fruit appears in an ancient Chinese book named "Yu Kung" or "Tribute of Yu" (an emperor who reigned from 2205 to 2197 B.C.). The book states that "The baskets were filled with woven ornamented silks. The bundles contained small oranges and pummeloes ". In 300 B.C. citrons mentioned which is probably the first genus known to the European civilization find out in Writing of Theophrastus. Citrus culture in Texas and the Gulf States dates from about 1880. The most widely grown and com-merically important species are: sweet orange (Citrus sinensis (Linn.) Osbeck), grapefruit (Citrus paradisi Macfad. or Citrus maxima Merr.), lemon (Citrus limon (Linn.) Burmann, Citrus limonia Osbeck, or Citrus limonum Risso) and lime (Citrus aurantifolia (Christm.) Swingle).

Grapefruit is important in Citrus family because of its medicinal uses and other uses.

1.1 GRAPE FRUIT

The grapefruit, Citrus paradisi Macfad. is known today to be an apomictically stabilized hybrid between the pummelo, C. grandis (L.) Osb., also known as C. maxima (Burm.) Merrill, and the sweet orange, C. sinensis (L.) Osb. (Robinson 1952; Scora et al. 1982; Scora & Kumamoto 1983). The grapefruit (Citrus paradisi) is a subtropical citrus tree known for its bitter fruit, an 18th-century hybrid first Red in Barbados (Carrington, 2003). When this new fruit was adopted into cultivation and the name grapefruit came into general circulation, American horticulturists viewed that title as so inappropriate that they endeavour Red to have it dropped in favour of "pomelo". However, it was difficult to avoid confusion with the pummelo and the name grapefruit prevailed and is in international use except in Spanish-speaking areas where the fruit is called toronja. In 1992 Florida Citrus mutual proposed changing the name to something more appealing to consumers in order to stimulate greater sales. There were so many protests from the public against a name change that the idea was abandoned. When found it was named the "forbidden fruit"; and it has also been misidentified with the pomelo or shaddock (C. maxima) one of the parents of this hybrid, the other being sweet orange (C. sinensis) (Morton JF 1987).

The first known recorded use of the word grapefruit is in Jamaica, where a fruit was grown that was commonly known as the "Barbadoes grapefruit" (Macfadyen 1830). This early name
for the fruit provides a strong clue in pursuing information on the early history of this hybrid. Today's grapefruit, however, has maliform fruits and alate petioles (Hume 1926).

1.2 ORIGIN OF GRAPEFRUIT

One ancestor of the grapefruit was the Jamaican sweet orange (Citrus sinensis), itself an ancient hybrid of Asian origin; the other was the Indonesian pomelo (C. maxima). One story of the fruit's origins is that a certain "Captain Shaddock" (Kumamoto J 1987) brought Pomelo seeds to Jamaica and breeds the first fruit. However it probably originated as a naturally-occurring hybrid (Carrington 2003). The hybrid fruit then called "the forbidden fruit" was first documented in 1750 by a Welshman, Rev. Griffith Hughes who described specimens from Barbados in The Natural History of Barbados. Currently the grapefruit is said to be one of the "Seven Wonders of Barbados (Welchman Hall Gully 2010).

The grapefruit was brought to Florida by Count Odet Philippe in 1823 in what is now known as Safety Harbor. Further crosses have produced the tangelo (1905) the Minneola tangelo (1931) and the Oroblanco (1984).

The grapefruit was known as the shaddock or shattuck until the 19th century. Its current name alludes to clusters of the fruit on the tree which often appear similar to grapes. Botanically, it was not distinguished from the pomelo until the 1830s, when it was given the name Citrus paradisi. Its true origins were not determined until the 1940s. This led to the official name being altered to Citrus paradisi, the "x" identifying its hybrid origin.

An early pioneer in the American citrus industry was Kimball Chase Atwood, a wealthy entrepreneur who founded the Atwood Grapefruit Co. in the late 19th century. The Atwood Grove became the largest grapefruit grove in the world, with a yearly output of 80,000 boxes of fruit (Herald Tribune, 2008). It was there that pink grapefruit was first discovered in 1906.

Citrus plants include large varietal collection of sweet orange (Citrus sinensis), tangerine/mandarin (Citrus reticulate), grape fruit (Citrus paradisi), lemon (Citrus limon), lime (Citrus aurantifolia) and pumelo (Citrus grandis).
1.3 VARIETIES OF GRAPE FRUIT

The following are among the most noteworthy of old and new cultivars:

'Duncan' – The fruit is round or slightly obovate; large, 3 1/2 to 5 in (9-12.5 cm) wide; peel is very light yellow (usually called "white") with large oil glands, medium-thick, highly aromatic; pulp is buff, in 12-14 segments with medium-tender membranous walls, very juicy, of fine flavor; seeds medium-large, 30-50. Tree is unusually cold-hardy. This was the leading cultivar for many years in Florida and Texas and was introduced into all the grapefruit-growing areas of the world.

'Foster' ('Foster Pink Flesh') – Fruit is oblate to round; medium-large, averaging 3 3/4 in (9.5 cm) in width; peel light-yellow blushed with pink, smooth but with large, conspicuous oil glands; albedo pink; pulp light-buff, pinkish near the centre; in 13 or 14 segments with pinkish walls, tender, juicy, of good quality despite seeds, up to 50 or even more, of medium size. In Texas it is more colorful, the pulp being entirely pinkish in hue.

'Oroblanco' – Fruit form and size similar to 'Marsh'; peel paler and thicker; pulp paler and has larger hollow in center; sections easily skinned; tender, juicy, non-bitter; has faintly astringent after-taste before full maturity or in cooler climates; seedless. Tree is vigorous, large, hardy can tolerate temperatures down to 30º F (-1.11º C); yields medium to heavy crops and may tend to alternate bearing. It seems better adapted to California's inland citrus locations than to desert sites.

'Paradisi Navel' – Fruit is oblate, smaller than a typical grapefruit. Originally very seedy but by budding onto various rootstocks and transferring from one rootstock to another over a period of years, there eventually emerged one tree bearing fruit without seeds. Budwood from this tree has produced uniformity of seedlessness regardless of rootstock.

'Star Ruby' – The seedling from one of these treated seeds was named the 'Star Ruby' and introduced into cultivation in 1971 by Richard Hensz of Texas A& I University. Several thousand trees were planted in Texas. At least 65,000 budded trees were brought into Florida in 1971 by commercial interests without proper qualifications and permits under the Division of Plant Industry. Subsequently, ringspot virus was found on one of the imported trees which had already been used as a source of budwood. Infected trees from this source were found in
a nursery and were destroyed together with all neighbouring healthy trees. The tree tends to become more chlorotic than 'Ruby Red' when sunburned or affected by poor drainage or high applications of herbicides and pesticides and it is sensitive to adverse weather conditions.

'Sweetie'—a grapefruit × pummelo hybrid released in 1984 by the Citrus Marketing Board in Israel has all the features of a typical grapefruit but the flavour is sweet.

'Thompson' (Pink Marsh)— The fruit is oblate to round, of medium size, 2 3/4 to 3 3/4 in (7-9.5 cm) wide; peel is light-yellow, smooth, with small, inconspicuous oil glands, faintly aromatic; pulp is light- to deep-buff more or less flushed with pink sometimes throughout occasionally just near the centre. There are 12 to 14 segments with abundant, colourless juice, and few seeds—usually 3 to 5.

'Triumph' - The fruit is oblate to ellipsoid, slightly flattened at both ends; of medium size; peel light-yellow, very smooth, with oil glands of medium size; medium-thick; pulp pale, tender, juicy, only faintly bitter, the flavour having a touch of orange; the centre is semi-hollow; of superior quality; 35-50 seeds. The fruit is larger than 'Marsh' grapefruit and its pummelo-like flavour is considered superior.

'Marsh' (Marsh Seedless)— The fruit is oblate to round, medium in size, 3 1/2 to 4 3/4 in (9-12 cm) wide; peel is light-yellow, very smooth, with medium-size oil glands, mildly aromatic; pulp is buff, in 12-14 segments with tender membranes, melting, extremely juicy and rich in flavour; seeds absent or 3-8, medium-sized. The leading grapefruit cultivar; grown in Florida, California, Texas, Arizona, South America, Australia, South Africa, Israel and India. A local selection, presumably of a seedling 'Marsh', in Surinam is known there as 'Hooghart'. The two are almost indistinguishable.

'Redblush' (including 'Ruby', 'Ruby Red', 'SharyRed', 'Curry Red', 'Fawcett Red', 'Red Radiance', and 'Webb' (Webb's Red blush Seedless)—It is originated as sports—lower branches—growing out of 'Thompson' trees which a Texas nursery had purchased from Glen St. Mary Nursery and sold to growers in the Rio Grande Valley and which were frozen back in 1929. All are seedless and otherwise similar to 'Thompson' but display Redder colour. 'Red blush' grapefruits have been extensively planted in Florida in the past few decades though the juice is not suitable for canning as it tends to turn brown with age. By 1950, 75% of Florida's grapefruit crop was of the pink or Red seedless type. Under the name, 'Ruby Red' a member
of this group is a standard commercial cultivar in Texas. In 1958, bud wood of 'Red blush' from California was acquired by the Regional Fruit Research Station at Abohar, India was propagated on rough lemon, and the resulting trees performed so well and showed such disease resistance that the cultivar was recommended for growing under irrigation in the and regions of the Punjab and Haryana, where it averages 250 fruits annually per tree. Probably includable in this group is 'Burgundy'. Its peel is not blushed but the pulp is intense Red throughout the season. 'Ray Ruby' and the similar if not identical 'Henderson' is branch sports propagated in Texas and introduced into Florida in the 1970's. The peel is Redder than that of 'Ruby Red' and the pulp is Red though not as intense as 'Star Ruby' throughout the season.

Plant tissue culture has emerged as a potential tool and forms the backbone of plant biotechnology. Tissue culture techniques are widely applied for the improvement of field crops, forests, horticulture and plantation crops for increased agricultural and forestry production. This technique has been commercialized globally and contributed significantly towards the enhanced production of high quality planting material.

Plant tissue culture refers to the in vitro culture of plants from plant parts (tissue, organs, embryos, single cells, protoplasts, etc.) on nutrient media under aseptic conditions (Altman 1987). In vitro cultures are now being used as tools for the study of various basic problems in plants of economic importance in large numbers by tissue culture.

Majority of the plants propagated by vegetative means contain systemic bacteria, fungi and viruses which affect the yield, quality and appearance of selected plants. Moreover majority of plants are not amenable to vegetative propagation through cuttings, budding and grafting, thus limiting multiplication of desired cultivars.

In the recent years, tissue culture has emerged as a promising technique to obtain genetically pure elite populations under in vitro conditions. In vitro propagation also called micro propagation is in fact the miniature version of conventional propagation, which is carried out under aseptic conditions. The advent of in vitro tissue culture technique has offered a new approach to the morphogenetic investigations. It allows a living system to be studied under controlled environmental conditions. This enables a study of the complex biological phenomenon in parts. Moreover these partial processes are amenable to controlled investigations.
Clonal propagation of selected phenotypes is an essential step in most of the plant breeding programs. It is a faster method of asexual reproduction in comparison to propagation through seeds. Plants raised through seeds are highly heterozygous and one has to select plants from a wide population which have the best qualities.

Tissue culture technology offers an advantage over conventional method of propagation in producing large number of true to type plants from healthy plants within a short period of time. Plant tissue culture comprises a range of technological integrated to commercial plant propagation, industries and research investigation (Smith 1983). History of plant tissue culture and their development, as useful and integrated tools of modern plant biotechnology has been reviewed and discussed by Gautheret (1983 & 1985), Thorpe (1990) and Vasil (2002). In commercial production plant tissue culture emerges as an advantages means of mass propagating economically or otherwise valuable clones and new crops with concurrent gains in production timing, product uniformity efficiency, making available clean virus free material and flexibility in (market) demand. Therefore, bearing in mind the problems associated with conventional propagation and the need to develop in vitro generation protocols for specific cultivars, the aim of the present study was to develop an efficient protocol for in vitro clonal mass multiplication of this elite citrus cultivar by inducing multiple shoots on shoot tip explants. Further multiplication was achieved using axillary shoots induced with regenerated plantlets by decapitating them.

Plant tissue culture techniques have become vitally important for pursuing a wide range of fundamental and applied problems in research and development. The techniques encompass a variety of procedures used for specific purposes.

In this perspective present investigation will be taken to standardize the protocol through micro-propagation technique in *C. paradisi* Macf. Red blush and Marsh seedless.
1.4 TAXONOMY

SCIENTIFIC CLASSIFICATION

Kingdom: Plantae

(Unranked): Angiosperms

(Unranked): Eudicots

(Unranked): Rosids

Order: Sapindales

Family: Rutaceae

Genus: Citrus

Species: paradisi

The grapefruit tree reaches 15 to 20 ft (4.5-6 m) or even 45 ft (13.7 m) with age, has a rounded top of spreading branches; the trunk may exceed 6 in (15 cm) in diameter; that of a very old tree actually attained nearly 8 ft (2.4 m) in circumference. The twigs normally bear short, supple thorns. The evergreen leaves are ovate, 3 to 6 in (7.5-15 cm) long, and 1 3/4 to 3 in (4.5-7.5 cm) wide; dark-green above, lighter beneath, with minute, rounded teeth on the margins, and dotted with tiny oil glands; the petiole has broad, oblanceolate or obviate wings Morton J, 1987. The white, 4-petalled flowers, are 1 3/4 to 2 in (4.5-5 cm) across and borne singly or in clusters in the leaf axils. The fruit is nearly round or oblate to slightly pear-shaped, 4 to 6 in (10-15 cm) wide with smooth, finely dotted peel, up to 3/8 in (1 cm) thick, pale-lemon, sometimes blushed with pink, and aromatic outwardly; white, spongy and bitter inside. The centre may be solid or semi-hollow. The pale-yellow, nearly whitish, or pink, or even deep-Red pulp is in 11 to 14 segments with thin, membranous, somewhat bitter walls; very juicy, acid to sweet-acid in flavour when fully ripe. While some fruits are seedless or nearly so, there may be up to 90 white, elliptical, pointed seeds about 1/2 in (1.25 cm) in length. Unlike those of the pummelo, grapefruit seeds are usually poly-embryonic. The 1929 US Ruby Red (of the Redblush variety) has the first grapefruit patent (Julia F 1987).
1.5 ECONOMIC IMPORTANCE

**Medicinal Uses:** An essence prepared from the flowers is taken to overcome insomnia, also as a stomachic and cardiac tonic. The pulp is considered an effective aid in the treatment of urinary disorders. Leaf extractions have shown antibiotic activity. Grapefruit has also been investigated in cancer medicine pharmaco-dynamics. Its inhibiting effect on the metabolism of some drugs may allow smaller doses to be used, which can help to Reduce costs (Gandey A 2007).

1.5.1 OTHER USES:

**Food Uses**

Grapefruit is customarily a breakfast fruit, chilled, cut in half the sections loosened from the peel and each other by a special curved knife and the pulp spooned from the "half-shell". Some consumers sweeten it with white or brown sugar, or a bit of honey. Some add cinnamon, nutmeg or cloves. As an appetizer before dinner grapefruit halves may be similarly sweetened, lightly broiled, and served hot, often topped with a maraschino cherry. The sections are commonly used in fruit cups or fruit salads in gelatines or puddings and tarts. They are commercially canned in syrup. In Australia grapefruit is commercially processed as marmalade. It may also be made into jelly.

**Grapefruit juice**

The juice is marketed as a beverage fresh canned or dehydrated as powder or concentrated and frozen. It can be made into excellent vinegar or carefully fermented as wine.

**Grapefruit peel**

It is candied and is an important source of pectin for the preservation of other fruits. The peel oil, expressed or distilled is commonly employed in soft-drink flavouring, after the removal of 50% of the monoterpenes. The maiming Radiant in the outer peel oil is nook atones. Extracted nook atones, added to grapefruit juice powder enhances the flavour of the reconstituted juice. Naringin extracted from the inner peel (albedo), is used as a bitter in "tonic" beverages, bitter chocolate ice cream and ices. It is chemically converted into a sweetener about 1,500 times sweeter than sugar. After the extraction of naringin the albedo can be reprocessed to recover pectin.
Grapefruit seed oil

It is dark and exceedingly bitter but bleached and refined, it is pale-yellow, bland, much like olive oil in flavour, and can be used similarly. Because it is an unsaturated fat its production has greatly increased since 1960.

Factory waste

The waste from grapefruit packing plants has long been converted into molasses for cattle.

Seed hulls

After oil extraction, the hulls can be used for soil conditioning or recombined of this product to pigs or poultry.

Wood

Old grapefruit trees can be salvaged for their wood the sapwood is pale-yellow or nearly white the heartwood yellow to brownish, hard fine-grained, and useful for domestic purposes.
1.6 IMPORTANCE OF THE WORK

Most of the existing *Citrus paradisi* (Red blush & Marsh Seedless) collections are conserved in field gene banks in different citrus growing countries. Such collections are vulnerable to biotic and abiotic hazards (Damania 1996). Ageing of seeds of *Citrus paradisi* (Red blush & Marsh Seedless) are recalcitrant and lose viability in a short time.

The cultivation is gaining momentum as the fruits are rich source of vitamin C and possess high medicinal values. These are known to have curative value for various diseases of bones and joints, bilious diseases, prevention of capillary bleeding, piles, dysentery, cold, influenza, habitual constipation and scurvy. (Singh N P et al 2009).

With the advent of high yielding varieties of agronomical crops, the diversity found in minor fruit crops is vanishing or at the verge of extinction due to uprooting of indigenous isolated/scattered growing citrus species in the natural habitat of the region. Importance of clonal selections in crop improvement is well recognized by several workers (Badge and Patil 1989; Badiyala et al 2004). So, it is imperative to identify superior strains of *Citrus paradisi* (Red blush & Marsh Seedless) for their collection, conservation, evaluation and utilization in the future breeding programmes. Hence, the present investigations were carried out to record the extent of genetic diversity and locate the elite genotypes possessing desirable fruit characteristics. Therefore bearing in mind the problem associated with conventional propagation and need to develop *in vitro* generation protocol for specific cultivar. The aim of the present study was to develop efficiency protocol for *in vitro* clonal mass multiplication and conservation of germplasm of this citrus cultivar by inducing multiple shoot propagation by nodal and seed culture. Further multiplication was achieved by sub culturing of these plantlets in media supplied with different plant growth regulators.

Now as the urbanisation is increasing day by day and use of drugs as medicine to cure diseases is establishing deep roots in society which have many side effects also. So it becomes necessary to introduce naturally made medicines which can assure to have a cure without any side effect. One of the best examples for this is *Citrus paradisi* Macf. (Red blush & Marsh Seedless). There are conflicting scientific demonstrations of the efficacy of grapefruit. The grapefruit has been associated with the production of primary and secondary metabolites. These are used by the plants for its purposes and activities like defence and pollination. Certain biological assays have been done in this project in order to access the phytochemical and antimicrobial potentials of grapefruit. So grapefruit can be used as a natural compound as medicine to cure many diseases without any side effects.