REVIEW OF LITERATURE
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2.1 ORIGIN AND DISTRIBUTION OF CITRUS

The genus *Citrus* is believed to be the culmination of a very long period of progressive evolution which might have begun before Australia was cut off from land connections with Asia, probably more than 20 million years ago (Swingle and Reece, 1967).

The various species of the genus *Citrus* are all considered to have evolved in the sub-tropical and tropical regions of Asia and the Malay Archipelago, Australia and tropical Africa. They are believed to have spread to other parts of the world and being cultivated from remote ages. Webber (1967) discussed in detail the origin and distribution of *Citrus*. According to Webber (1967) the history of the origin and spread of *Citrus* reads like a romance. The spread of the genus from one part of the world to another was very slow.

Webber (1967) suggested that the first member of the Citrus group to become known to European civilization was the citron, around 300 B.C. The sour orange, the lemon and the sweet orange are believed to have evolved subsequently, each centuries apart.

The citron (*Citrus medica* L.) of Indian origin was the only *Citrus* fruit known for many years in Europe.
Apparently it spread into Persia from where the Hebrews introduced it into Palestine. The Greeks and the Romans spread citron from Media and Persia, into other parts of Europe. Sour orange (Citrus aurantium L.) cultivation in Italy was depicted on some mosaic tiles in 70 A.D. Sweet orange (Citrus sinensis (L.) Osbeck) is believed to be grown in Italy during the early Christian era, but a clear evidence is not available. However, it is strongly believed that the Portuguese introduced sweet oranges into Europe from China around 1500 AD. The lime fruit (Citrus aurantifolia Swing) was known to Arabs around 1800 A.D, who probably played a major role in its spread from India. The mandarin orange (Citrus reticulata Blanco), a native of China and South Eastern Asia, was extensively planted in China and Japan at around 1100 AD. Mandarins were introduced into Europe and America from China around 1800 AD. The pummelo (Citrus grandis (L) Osbeck) is believed to have originated in the Malayan and Indian Archipelago and to have spread from there to China and India, and then to Persia, Palestine and Europe. The grape fruit (Citrus paradisi Macf.) probably originated as a mutation or sport of the pummelo around 1700 AD at Barbados from where it spread to the other parts of the world.

2.1.1 ORIGIN OF CITRUS IN INDIA

Many of the progenitors of Citrus fruits are believed to have originated in India (Bhattacharya and Dutta,
These include *Citrus ichangensis* Swing., *C. latipes* Tan., *C. macroptera* Mont., *C. assamensis* Dutta and Bhattacharya, *C. aurantium* L., *C. jambhiri* Lush., *C. limonia* Risso., *C. karna* Risso., *C. pennivesiculata* Tan., and *C. maderaspatana* Hort. ex Tan. Many of these species are wild (Bhattacharya and Dutta, 1956). Vavilov (1950) observed the presence of sweet orange (*C. sinensis* Osbeck), mandarin (*C. reticulata* Blanco), citron (*C. medica* L.) sour lime (*C. aurantifolia* Swing.), *Jenru-tenga* (*C. nobilis* Lour.), Rangpur lime (*C. jambhiri* Lush) and lemon (*C. limon* Burm. F.), both in wild and cultivated form, in North-Eastern India. Presence of Soh-Niangriang, a wild sweet orange, and *C. indica* Tan. a wild Indian mandarin furnishes a strong evidence (Bhattacharya and Dutta, 1951, 1956) that Eastern India might be a centre of origin for many citrus fruits. Tanaka (1958) also believed that sweet oranges originated in India in addition to many other citrus types.

### 2.2 Taxonomic Status of Citrus

The commonly grown citrus fruits belong to three genera, *Citrus*, *Fortunella* and *Poncirus*. All these genera are closely related and have intergeneric fertility. These genera are grouped under the subtribe *Citrinae*, tribe *Citrae*, sub family *Aurantoideae* and family *Rutaceae* (Swingle, 1948).
Taxonomic work on Citrus was started as early as in 1832 by Roxburg who recognized five species, namely *C. acida*, *C. medica, C. aurantium* (orange), *C. decumanga* (Shaddock) and *C. inermis* (Kumqurt). In 1872, Hooker recognized only four species to accommodate all the Citrus types. Brandis (1874), Watt (1889) and Kurz (1877) also grouped the citrus fruits into three or four species. This was rather unsatisfactory to accommodate the great diversity (Rhattacharya and Dutta, 1956). Bonavia (1890) critically examined almost all varieties of citrus fruits but used only native names of varieties and placed them to well defined groups to which they naturally belonged. Lushington (1910) recorded taxonomic details of nearly all the varieties of citrus which were collected and studied by Bonavia (1890) and recognized as many as 21 species. But these were not accepted by taxonomists. However Tanaka (1937a, b) recognized four species of Lushington (1910).

Divergent views on classification of *Citrus* have been expressed. Swingle (1948) and Swingle and Reece (1967) recognized 16 species, while Tanaka (1954) proposed as many as 145 and later 159 (Tanaka, 1961) species. Tanaka (1954, 1961) classified the forms of *Citrus* into two sub genera, eight sections, fifteen sub sections, nine groups, two sub - groups, two micro-groups and 159 species. Swingle's classification (Swingle, 1948, Swingle and Reece, 1967), had recognized the sub-genera Papeda (containing six species) and
Citrus (formerly Eucitrus, with 10 species). Subsequently, a large number of citrus types were studied by Hodgson (1961) and Hodgson et al. (1963a, b, c), who recognized twenty-three species of Tanaka system. All these studies were, however, based on the morphological data.

2.3 CHARACTERIZATION OF CITRUS GENOTYPES

2.3.1 CHARACTERIZATION THROUGH MORPHOLOGICAL DESCRIPTION


2.3.2 NUMERICAL CHARACTERIZATION OF MORPHOLOGICAL DATA

In view of the complication in taxonomic work in Citrus arising out of the natural hybridization, spontaneous mutations and polyembryony, morphological descriptions have been supplemented by modern techniques to characterize the genotypes.
Numerical characterization of morphological data (Sokal and Sneath, 1963) has been found eminently suitable in assessing the similarities between different Citrus species, for their classification into groups and their polygeneric relationships (Nath, 1966; Nath and Randhawa, 1969; Barret and Rhodes, 1976 and Singh and Singh, 1983). Numerical characterization has been successfully used in mango (Rhodes et al., 1970) and avocado (Rhodes et al., 1971) among other fruit crops.

2.3.3 POLYGONAL METHOD OF PRESENTATION OF MORPHOLOGICAL DATA

Objective representation of morphological data (Hutchinson, 1936) was tried in Citrus (Nath, 1966, Nath and Randhawa, 1969a and b) to clarify the position of hybrids, possibly of bispecific origin and was found useful in characterizing different Citrus types. This technique was also elegantly utilized in understanding relationship among the species and varieties of Xanthium and Empetrum (Love and Nadeau, 1961).

2.3.4 CHARACTERIZATION THROUGH OTHER METHODS

Plant chemical constituents have been proposed as additional tools in characterizing citrus genotypes. Flavonoid composition of leaves, bark and ripe fruit peel were found useful in characterizing different citrus types (Nishiura et al., 1971; Tatum et al., 1974; Dass et al.,
1977a, b; 1978a, b; Artes and Beneyto, 1978; and Gujante and Singh, 1983). Citrus leaf oil (Kesterson et al., 1964; Pieringer et al., 1964 and Score and Malik, 1970), and long chain hydrocarbons (Nordby and Nagy, 1974) were also reported to be useful.

Palynological studies were also found useful in characterizing citrus types (Ramzal and Randhawa, 1965). Scanning electron microscopy of pollen appeared to be advantageous in identifying the Citrus taxa (Kozaki and Hirai, 1982).

Cytological studies have also been used in characterizing citrus types and also in detecting the evolution of Citrus species (Sharma and Bal, 1957; Naithani and Raghuvanshi, 1958, 1962, 1963; Raghuvanshi, 1962a, b, 1969). In Citrus, all the known forms are diploid with the exception of C. latifolia Tan. The somatic chromosome number in Citrus being 2n = 18. Presence of structural hybridity in karyomorphological studies at mid pachytene suggested that citrus types might have originated as hybrids, or that, these small structural changes were accumulated over a long period of vegetative propagation coupled with reproductive nucellar embryony (Nair and Randhawa, 1969). Cytological studies in Citrus have not brought out any marked differences between the chromosomes even in the well established species. It has not brought any major changes in the classification of citrus.
and their chromosomes pair normally in meiosis (Singh and Nath, 1969).

A clear understanding of the relationship among 

*Citrus* species and genotypes may perhaps be had by morphological description coupled with biochemical analysis like electrophoretic separation of proteins and enzymes (Singh and Chadha, 1993). Numerical - morphological - chemo-
taxonomical studies have been suggested to be of a great value in grouping the *Citrus* taxa (Zhu, 1988).

## 2.4 GENETIC STUDIES

Genetic studies aimed at understanding the nature and magnitude of variability among the genotypes for different characters, the degree of transmission of characters, inter-relationships of characters, and the degree of affinity and divergence between the biological populations were reported to serve useful purposes in assessing the relationships between the genotypes and in grouping them into clusters, based on which, practical breeding strategies can be worked out.

### 2.4.1 GENETIC ANALYSIS OF VARIATION

#### 2.4.1.1 GENETIC VARIABILITY

Genotypic coefficient of variation and phenotypic coefficient of variation were estimated to study nature and magnitude of variability among the genotypes for different
characters (Burton, 1952; Allard, 1960). The degree of transmission of characters was obtained by computing the estimates of heritability. The heritable portion of the variation was indicated through heritability (Burton, 1952; Burton and De Vane, 1953; Johnson et al., 1955). Heritability estimates accompanied by estimates of genetic advance, computed as percentage of mean was found more useful than heritability alone in predicting the effects of selection based on phenotypic performance (Johnson et al., 1955). These estimates also indicate the reliability of the characters for drawing valuable conclusions.

Studies on genetic analysis were conducted extensively in field crops but very scarce in fruit crops in general and Citrus in particular. Singh et al. (1980), reported the genotypic and phenotypic variability in mandarin orange fruits.

2.4.1.2 CHARACTER ASSOCIATION

Genotypic and phenotypic coefficients of correlations have been used to infer the inter-relationship of characters (Johnson et al., 1955). Knowledge of these inter-relationships were used in the construction of selection indices and to detect some simple characters which could be useful as indicators of more complex characters.

Chakrawar and Jature (1980) had conducted correlation studies on fruits of kagzi lime strains. Prasad
and Rao (1989), Prasad et al. (1991; 1993a) conducted extensive studies on genotypic and phenotypic variation, heritability and genetic advance, phenotypic correlations and also path-coefficient analysis on some fruit morphological and biochemical characters in acid lime clones and lemon (Prasad et al., 1993b). However, no report is available on such studies covering wide range of important vegetative, floral and fruit characters.

2.4.2 GENETIC DIVERSITY AND GENOTYPIC AFFINITIES

Studies on genetic divergence, and its nature and degree, among the biological populations have been conducted extensively on several field crops to understand the relationship between genotypes of a crop, their phylogenetic affinities and to identify desirable parents for breeding programmes. Mahalanobis $D^2$ statistics of multivariate analysis (Mahalanobis, 1936; Rao, 1952) was used to quantify the degree of affinity and divergence between the biological populations.

However, studies on genetic divergence in fruit crops are very rare. Vatsala Kumari et al. (1985) studied the genetic divergence in 62 cultivars of banana and were able to assign them to eight clusters. No report is available on such studies in Citrus.
2.5 CHARACTERIZATION USING ISOZYME ELECTROPHORESIS

2.5.1 ISOZYME

The term 'isozyyme' was coined by Markert and Moller (1959) to describe different molecular forms of enzymes with the same substrate specificity occurring within the same organism. The development of the "Zymogram" technique by Hunter and Burstone (1958), employing the adaptation of Smithies (1955,1959) technique of starch gel electrophoresis, initiated the now widespread interest in isozyymes.

The occurrence of isozyyme was common and widespread in the biological world. Among the plant species examined, the number of enzymes exhibiting isozyymic forms was very large and a substantial amount of the polymorphism observed was genetic in nature (Scandalios, 1974).

The genetic mechanism in which isozyymes might arise was gene duplication with subsequent mutations at daughter and parental loci. Thus, more than one gene were reported to have contributed to the structure of any enzyme composed of more than one kind of sub-unit. Further more, two genes were capable of generating a variety of isozyyme forms if a number of multimers can be formed (Scandalios, 1974).

Isozyymes may arise through the building of a single polypeptide to varying numbers of coenzyme molecules or other prosthetic groups, by conjugation or deletion of molecules
with reactive groups such as amino, carbonyl or hydroxyl groups of the amino acid residues of the polypeptide chain. Isozymes may result from variations in the tertiary or quarternary structure of a given primary polypeptide structure (Scandalios, 1974).

2.5.2 MOLECULAR BASIS OF ISOZYME FORMATION

Two general categories were distinguished by which multiple forms of proteins (enzymatic or non-enzymatic) could be formed:

a) By mechanisms operating at the level of the genome which were then transcribed onto mRNA and their code for different polypeptides. Random assembly of subsequent peptides following translocation results in the formation of functional multimers (homo or hetero).

b) By epigenic mechanisms operating at the translational or post-translational level to modify polypeptides to varying degrees.

The two mechanisms were not mutually exclusive and infact, both were operative in most cases.

2.5.3 ISOZYME ELECTROPHORESIS

Electrophoresis is a widely used chromatographic technique for the separation of mixtures of ionic compounds. Gel electrophoresis combines elements of free boundary electrophoresis (separation based on charge) and gel filtration (separation based on size). Active enzymes could
be separated into discreet bands and their positions made visible by the use of specific enzyme stains (Simpson and Withers, 1986).

Smithies (1955) first advocated the utility of the technique as a tool for the geneticist. Proteins were attractive for direct genetic study because they were the primary products of structural genes. Changes in coding base sequence will, under many but not all circumstances, result in corresponding changes in the primary structure of proteins. If electrophoresis was conducted under natural conditions (pH - 7.0 to 8.5, and low temperature) single amino acid substitutions had marked effects on migration. Variation in banding patterns between individuals could be sorted out genetically (Shields et al., 1983).

Since amino acid sequences of proteins were determined by nucleotide sequences of coding structural gene loci, the analysis of a protein structure using electrophoresis was, to a first approximation, an analysis of a gene (Simpson and Withers, 1986).

2.5.4 METHODS OF ISOZYME ELECTROPHORESIS

Successful electrophoretic procedures allow rapid screening of plants and give band patterns which are clear and repeatable. Most studies utilize either starch or polyacrylamide gel media but numerous buffer systems, extraction procedures and enzyme stains are available and
those most suitable for a given plant tissue must be determined experimentally (Davis, 1964; Graham et al., 1964; Shields et al., 1983; Arulsekhar and Parfitt, 1986).

Polyacrylamide gels are less fragile and therefore easier to handle than starch gels, but the acrylamide monomer is more expensive than starch and highly toxic. The transparency of acrylamide gels is an additional advantage. The pore size in acrylamide gel is directly related to the concentration of acrylamide used (Simpson and Withers, 1986).

2.5.5 ADVANTAGES OF ISOZYME MARKERS

Morphological characters had several disadvantages when used as markers in plant genetic studies or breeding schemes due to likely homozygosity, epistasis and pleiotropy. The alleles (allozymes) at most isozyme loci were codominant in nature and caused no deleterious changes in plant phenotypes through recessiveness or pleiotropy. This codominance also allowed heterozygotes to be distinguished from homozygotes, an advantage shared by few morphological markers. Isozymes rarely exhibited epistatic interactions, so that theoretically a genetic stock containing an infinite number of markers could be constructed (Tanksley and Rick, 1980).

Enzyme variation within and between populations was so high that electrophoresis would be useful in the detraction of ecological races, clones, or other evolutionary
problems at the population level. Electrophoretic data might be of value to suggest evolutionary relationships among taxonomic categories (Peirce and Brewbaker, 1973).

The equipment and materials needed for screening the isozyme banding patterns (zymograms) of plants were relatively inexpensive and rapid. The process was non-destructive since only a small amount of plant tissue was needed. Virtually any plant tissue could be sampled. Often plants could be screened at seedling stage resulting in saving of time and money (Moore and Collins, 1983).

2.5.6 ISOZYMES IN HORTICULTURAL CROPS

Isozyme studies were conducted in several horticultural crops to characterize the genotypes.

Isozyme banding patterns were used to characterize the genotypes in mango (Gan et al., 1981; Degani et al., 1990, 1992), papaya (Moore and Litz, 1984), apple (Vinterhalter and James, 1982, 1986; Chyi and Weeden, 1984; Weeden and Lamb, 1985; Manganaris and Alstar, 1989), almond (Hauagge et al., 1987a, 1987b; Cerezo et al., 1989; Mowrey et al., 1990), apricot (Byrne and Littleton, 1989), avocado (Torres et al., 1978; Torres and Bergh, 1980), peach (Arulsekar et al., 1986; Messequer et al., 1987; Durhem et al., 1987), pear (Santamour and Demuth, 1980), pecan (Meilke and Wolfe, 1982), Persian walnut (Arulsekar et al., 1986),
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plum (Byrne and Littleton, 1988), grapes (Jean Schwennesen et al., 1982; Stavrakakis and Loukes, 1983; Perfit and Arulsekar, 1989), strawberry (Bringhurst et al., 1981), banana (Jarret and Litz, 1986), pineapple (Dewald et al., 1988) date palm (Al-Jibouri and Adham 1990), and citrus (Iglesias et al., 1974; Button et al., 1976; Esen and Soost, 1976; Torres et al., 1978; Soost et al., 1980; Roose and Traugh, 1988; Moore and Castle, 1988; Aspinall and Sedgley, 1988, 1989).

2.5.7 Peroxidase Polymorphism

The enzyme peroxidase (E.C.1.11.1.7) and its multiple molecular forms occur ubiquitously throughout the plant kingdom (Shannan, 1968). It has been implicated in the oxidation of indoleacetic acid (Galston et al., 1953, 1967; Siegel et al., 1967). High levels of peroxidase activity were found to be correlated with dwarfing (Cunningham, 1975; Schertz, 1971), injury to tissue (Birecha and Miller, 1974) and resistance to disease infection (Farkes and Stahmann, 1966; Johnson and Cunningham, 1972; Rudolph and Stahmann, 1964). Peroxidase enzyme is reported to be highly polymorphic and tissue specific (Hamil and Brewbaker, 1969; Siegel and Galstan, 1967) and reliable as a genetic marker in several horticultural crops like papaya (Moore and Litz, 1984), apple (Chyi and Weeden, 1984), avocado (Torres and Bergh, 1980), peach (Durham et al., 1987), pear (Santamour and Demuth,
1980), plum (Byrne and Littleton, 1988), grapes (Jean schwennesen et al., 1982), pineapple (Dewald et al., 1988), date palm and (Al-Jibarri and Adham, 1990).

In Citrus, leaf peroxidase isozyymes were useful to differentiate the zygotic and nucellar seedlings (Iglesias et al., 1974).

A rapid method for differentiating between Citrus clones as well as between zygotic and nucellar plants was developed based on root peroxidase (Button et al., 1976).

Leaf peroxidase was found to exist in polymorphic state in thirty taxa of Citrus and three related genera. Some isozyymes were common in most taxa while others were specific to certain taxa. Both qualitative and quantitative differences were found between and within taxa (Esen and Soost, 1976).