

Mango (*Mangifera indica*. L) Malformation an Unsolved Mystery

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Abstract : Mango (*Mangifera indica* L.) universally considered to be one of the finest fruits, and is an important crop in tropical and subtropical areas of the world. There are about 1500 varieties of mango in the world of which about 1200 are found in India. Among the known diseases of mango, mango malformation is the most serious disease. The etiology of malformation has not yet been discovered due to paucity of information and thus no effective control measure is known. This review summarizes the plausible cause of the etiology of this disease. [Researcher. 2009;1(5):20-36]. (ISSN: 1553-9865).

Key words: Malformation, Ethylene, ACC oxidase, cyanide,

Introduction

Mango Malformation

Mango malformation is the chief problem and a serious constraint to mango production in India and other mango growing countries (tropical and subtropical) of the world (Crane and Campbell, 1994). This disorder is widespread in flowers and vegetative shoots of mango. It has a crippling effect on mango production (Hiffny *et al.*, 1978) bringing in heavy economic losses. In spite of several decades of incessant research since its recognition in 1891, the etiology of this disease has not been established and no effective control measure is known (Ram and Yadav, 1999; Pant, 2000; Bains and Pant, 2003).

Distribution of Mango

Mango trees can withstand air temperature as low as 2⁰F (-39⁰C) for few hours with injury to leaves and small branches. Young trees may be killed at 29⁰ to 30⁰F (-1.7 to 1.1⁰C) flowers and fruits may be killed if temperature falls below 40⁰F (4.4⁰C) for few hours (Crane and Campbell, 1994). Mangoes are native to Southern Asia, especially eastern India, Burma and Andaman Islands. Mango production predominates in dry and wet tropical low land areas 23⁰26' North and South of the equator, on the Indian subcontinent, Southeast Asia and Central and South America (Litz, 1997).

India ranks first among world's mango producing countries accounting for 57.18 per cent of the total world mango production of 19.22 million tones (Negi, 2000). India's contribution to the world's mango production is the highest *i.e.*, 15,64200 mt whilst only 0.3 per cent (47,149 mt) is exported, compared to South Africa whose total

production is 38,000 mt and 32.5 per cent of it *i.e.*, 12,341mt is being exported, being the highest in terms of export among the other countries (FAO, 2002). The production share of mango was found to be next to that of banana (NBH, 2004).

Distribution of Mango Malformation

Mango malformation was reported for the first time from Darbhanga, Bihar by Maries in 1891. The disease is widespread in North India. Mango malformation though occurs all over India, incidence is more in northwest than in the northeast and South India (Mallik, 1963). Later, after Bihar, this malady was described from Bombay. Subsequently, it was observed from other mango growing provinces like Uttar Pradesh, Punjab, Maharashtra (Narasimhan, 1954), Bihar (Mallik, 1961), West Bengal (Chakrabarty and Kumar, 1997).

Malformation causes heavy damage to trees as the inflorescence fails to produce fruits. The extent of damage varies from 50 to 60 % in some cases and in severe cases the loss may be 100 per cent (Summanwar, 1967). It was reported that the intensity of disease is higher in western districts of Uttar Pradesh than eastern (Prasad *et al.*, 1965). The incidence of malformation is sporadic in southern parts (Summanwar, 1973). However, few cases have also been reported from Southern India (Kulkarni, 1979). It is also stated that the region beyond Hyderabad is free from this malady (Majumdar and Sharma, 1990). Apart from India, malformation has been reported from the Middle East, Pakistan (Khan and Khan, 1960), South Africa (Schwartz, 1968), Brazil (Flechtmann *et al.*, 1970), Central America, Mexico, USA (Malo and McMilian, 1972), Cuba

(Padron, 1983), U.A.E (Burhan, 1991) and Bangladesh (Meah and Khan, 1992).

Symptoms

Mango malformation has been broadly classified as vegetative and floral malformation (Kumar and Beniwal, 1987). However, the two classes of malformation are assumed to be symptoms of the same disease since hypertrophy of tissues is involved in both cases, and vegetative malformation appears at times on trees bearing malformed inflorescence (Tripathi, 1954; Schlosser, 1971a; Kumar and Beniwal, 1987). Further proof was obtained by grafting diseased scion onto healthy rootstocks. The diseased scion that would have produced a malformed inflorescence in on-year (flowering year) produced symptoms typical of vegetative malformation (Kumar and Beniwal, 1987).

Vegetative Malformation

Vegetative malformation is more pronounced on young seedlings (Nirvan, 1953). The seedlings produce small shoot lets bearing small scaly leaves with a bunch like appearance on the shoot apices. Apical dominance is lost in these seedlings and numerous vegetative buds sprout producing hypertrophied growth, which constitutes vegetative malformation. The multi-branching of shoot apex with scaly leaves is known as "Bunchy Top", also referred to as 'Witch's Broom' (Bhatnagar and Beniwal, 1977; Kanwar and Nijjar, 1979). The seedlings, which become malformed early, remain stunted and die young while those getting infected later resume normal growth above the malformed areas (Singh *et al.*, 1961; Kumar and Beniwal, 1992).

Floral Malformation

Floral malformation is the malformation of panicles. The primary, secondary and tertiary rachises are short, thickened and are much enlarged or hypertrophied. Such panicles are greener and heavier with increased crowded branching. These panicles have numerous flowers that remain unopened and are male and rarely bisexual (Singh *et al.*, 1961; Schlosser, 1971a; Hiffny *et al.*, 1978). The ovary of malformed bisexual flowers is exceptionally enlarged and non-functional with poor pollen viability (Mallik, 1963; Shawky *et al.*, 1980). Both healthy and malformed flowers appear on the same panicle or on the same shoot. The severity of malformation may vary on the same shoot from light to medium or heavy malformation of panicles (Varma *et al.*, 1969). The heavily malformed panicles are compact and overcrowded due to larger flowers. They continue to grow and remain as black masses of dry tissue during summer but some of them continue

to grow till the next season. They bear flowers after fruit set has taken place in normal panicles (Singh *et al.*, 1961; Varma *et al.*, 1969; Hiffny *et al.*, 1978; Shawky *et al.*, 1980) and contain brownish fluid (Prasad *et al.*, 1965; Ram and Yadav, 1999) (Figure 1.1).

Cultivar Susceptibility

Susceptibility to malformation in mango varieties is variable; the governing factors being temperature, age of the tree, time, etc. In general, late blooming varieties are less susceptible to malformation than the early blooming ones (Khurana and Gupta, 1973). The level of polyphenol oxidase (PPO) in the early years of plant growth or in the flush of vegetative growth may provide an estimate of synthesis of phenolic compounds in the plants, which may be correlated to susceptibility or resistance to floral malformation (Sharma *et al.*, 1994).

Based on polyphenol oxidase activity, phenolic content and panicle malformation 24 mango cultivars were classified into five groups.

Etiology

The etiology of malformation has remained controversial and is yet to be established. Diverse claims have been made for the cause and control of malformation. They have been ascribed to a number of biotic and abiotic factors summarized in (Figure 1.2).

Stress ethylene and mango malformation

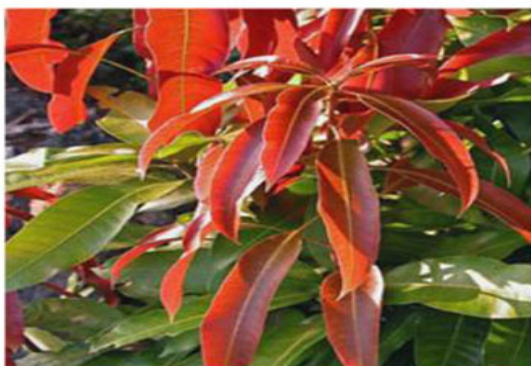
The basic phenomenon of increased ethylene production in response to stress is commonly called 'stress ethylene'. Production of stress ethylene can initiate various physiological responses, which include leaf epinasty, abscission, formation of aerenchyma etc. (Abeles, 1973).

It is proposed that mango malformation may be due to stress ethylene. The occurrence of leaf epinasty and disturbance in the natural orientation of the shoots and panicles, suppression of apical dominance, hypertrophy of lenticels and increased gummosis in trees with malformation in the same tree have been attributed to ethylene effect in malformed trees (Pant, 2000). Furthermore, the putative causal agent of mango malformation, such as excessive soil moisture, insect infestation, fungal pathogens, virus, chemical stimuli such as metal ions, herbicides and gases like SO₂ etc., seem to add to the production of stress ethylene. In the light of these facts, it was suggested that the disorder may be due to the production of 'stress ethylene' by mango plants (Pant, 2000). Studies on ethylene production in

malformed as well as healthy tissues of mango cultivars Amrapali, Khas-ul-Khas, Dashehari revealed that evolution of ethylene was maximum in the time period from 12 noon to 2 pm. Increased temperature at 12 noon to 2 pm may cause a heat

stress which increases ethylene production during this time interval (Krishnan, 2003, Nailwal *et al.*, 2006).

Healthy vegetative tissue



Healthy floral inflorescence



Malformed vegetative tissue



Malformed floral inflorescence



Figure 1.1 Types of inflorescence

Table 1.1: Classification based on polyphenol oxidase activity, phenolic content and panicle formation

Resistance/susceptibility to panicle malformation	Varieties
Highly resistant	Bhadauran and H-8-1
Moderately resistant	Dashehari, Langra, Kurukkan and Fazli
Susceptible	Sensation, Eldon, Rataul, Mallika and Alphonso
Moderately susceptible	H-31-1, Lalsundri, Totapari, Red small, Himsagar,
Highly susceptible	Neelum, Extreme, Zill, Eward and Amrapali Tommy Atkins, Chausa, Zardalu and Ratna

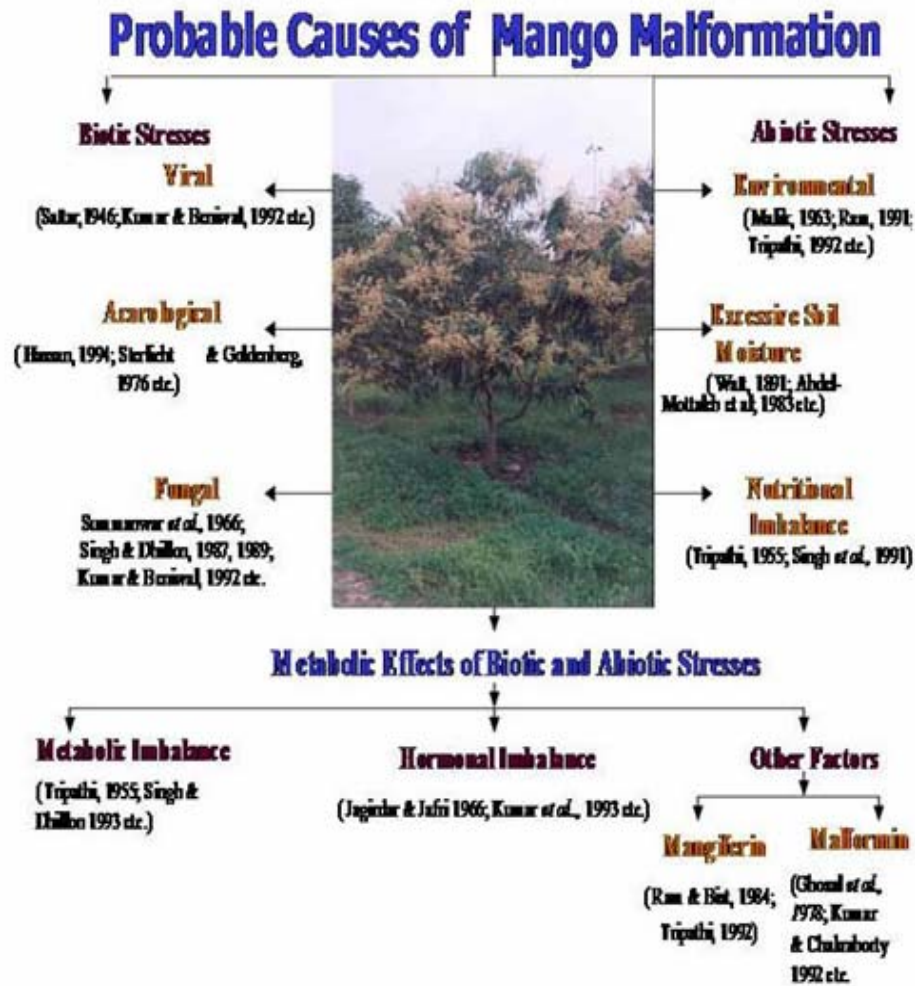


Figure 1.2 Probable causes of Mango malformation

Biosynthesis of stress ethylene

The methionine-ACC pathway has been established to operate in plants for ethylene biosynthesis and has been extensively studied (Yang and Hoffman, 1984; Miyazaki and Yang, 1987; Imaseki, 1991). S-adenosylmethionine (SAM) is synthesized from methionine and ATP. SAM is converted to 1- aminocyclopropane-1-carboxylic acid (ACC) and 5- methylthioadenosine (MTA). In the presence of air, ACC is rapidly converted to ethylene. MTA is rapidly hydrolyzed to 5-methylthioribose-1-phosphate (MTR-1-P) by means of ATP dependent phosphorylation. MTR-1-P is converted into 2-keto-4-methylthiobutyrate (KMB), which is finally transaminated into methionine. The

cyclic pathway continuously recycles the methylthio group of methionine for methionine production by utilizing ribose moiety of methionine during the synthesis of ethylene. This completes the methionine cycle. Thus, a small pool of methionine in the tissue can give rise to considerable amount of ethylene (Figure1.3). Stress ethylene is also synthesized via the methionine and ACC pathway. ACC synthase is the key enzyme and the main site of control of the biosynthetic pathway of ethylene (Imaseki, 1991). In the ethylene biosynthetic pathway, the final reaction where ethylene is produced from 1-amino-cyclopropane-1-carboxylic acid is accompanied with cyanide production on a one to one basis derived from C-1 of ACC.

The effect of cyanide on respiration and the possibility of the development of cyanide insensitive respiration in the malformed tissue cannot be ruled out (Rychter et al., 1988). Increased levels of cyanide due to 'stress ethylene' may result in the accumulation of toxic levels of cyanide resulting in the necrosis and death of malformed tissues of mango (Kukreja and Pant, 2000).

Both floral and vegetative malformations were reported to be reproducible by simply spraying spore suspension of *Fusarium* spp. (Chakrabarty and Ghosal, 1989; Ploetz and Gregory, 1993). In contrast to this, symptoms could not be produced unless the tissue was wounded prior to inoculation (Manicom, 1989). Therefore, pathogenicity for *Fusarium* in causing malformation is not clear and is yet to be proved. We ought to identify the factors that may induce biochemical and structural changes (MIP) having a potential to develop the symptoms typical of malformation, as distinct from symptoms of toxicity obtained after inoculating *Fusarium* (TP) (Kumar and Beniwal, 1992; Pant, 2000).

Temperature and growth of *Fusarium* spp.

A study of seasonal variation of populations of *Fusarium moniliforme* on mango shoots in India indicated that fungal density reaches maximum in February, when min/max temperature ranges from 8-27°C and humidity is high (85%); hotter and drier periods coincided with decline in the fungal population, Shawky et al., 1980; Campbell, 1986). *Fusarium moniliforme* var *subglutinans* has been reported to grow well at lower temperature and its growth is completely checked above 55°F (12.8°C) (Varma et al., 1971). The in vitro growth characters of the fungus were determined on different culture media, at varying temperature, light and pH conditions. Mycelial growth was better observed at temperature between 25-30°C and pH 7.0 on potato dextrose agar medium than on nine other media tested (Akhtar et al., 1999). Similar observation indicated that *Fusarium* growth was optimal on potato dextrose agar (PDA) medium at temperature between 15°C-28°C and was minimal on malt extract agar (MEA) medium at the same temperature. In 30 days old cultures, sporulation levels of *Fusarium* spp. were higher when day time temperature was 30°C versus 20°C. The high day time temperature also caused greater sporulation of macroconidia to form, and lowered the abundance of mesoconidia (Winder, 1999). The higher colonization of *Fusarium moniliforme* was observed at temperature >25°C coupled with relatively low pH (7.1-7.7), whereas low temperature during winter (<20°C) adversely affected the pathogen growth (Bisht, 2000). All these conflicting reports

suggest the need of further investigations on *Fusarium* spp. at high temperature particularly in southern part of India where malformation is rarely reported. The isolates of *Fusarium* spp. isolated from healthy and malformed tissues of mango grown at different temperatures (5-40°C) indicated that the most suited temperature for growth and development of isolates was 25°C, 30°C, 35°C and none of the spores of isolates germinated below 10°C temperature. The minimum time required to start germination was 6 hour and maximum was recorded after 24 hours (Ansari, 2004).

The enhanced promotion of ethylene is an early biochemical event in many plant-pathogen interactions. About one-third of fungi tested produced ethylene; a fact that led the scientists to conclude that ethylene is a common metabolic product of fungi (Hislop et al., 1973; Archer and Hislop, 1975; Yang and Pratt, 1978; Boller, 1982; Boller, 1990). In virus-infected plants this clearly represents 'stress ethylene' produced by plant; viruses lack the capacity to produce ethylene. However, in fungal diseases the situation is more complicated because some fungi have the capacity to produce ethylene themselves (Ilag and Curtis, 1968; Abeles et al., 1992; Lund et al., 1998; Chauge et al., 2002).

Isolates of *Fusarium* sp. from mango were observed to produce ethylene in range of 9.28 to 13.66 n mol /g dry wt/ day. Etherel was found to stimulate the germination of spores of isolates of *Fusarium* sp. obtained from mango cultivars at concentrations from 5 ppm to 100 ppm. Higher concentration of etherel was toxic for spore germination (Ansari, 2004). It was reported that significant differences were observed in the levels of cations and anions (chloride, sulfate and phosphate) in healthy and malformed tissues at, prior to full bloom and full bloom stage, but a consistent pattern was not seen. The exception was that of phosphate ion which was present in higher concentration in the malformed floral tissues at full bloom stage (Kaushik, 2002).

It is evident that the flowering period of mango that is January in the northern hemisphere and July in the Southern hemisphere, tallies closely with the temperature prevailing in the environment during that period. Roughly, two seasons of flowering in mango are apparent globally, one falling during January-March and the other during June-September (Bains and Pant, 2003).

Role of Ethylene in Mango Flowering

Ethylene mediated induction of flowering is utilized in the commercial production of mangoes. Growers in the Philippines and in India maintain smoky fires in mango orchards for several days

during a vegetative flush to induce good flowering (Valmayor, 1972). Smoke from smudged trees contains ethylene, which stimulates flowering in mango trees (Dutcher, 1972). Endogenous ethylene plays an integral role in the floral inductive process (Sen et al., 1973; Chacko et al., 1974a, b).

The involvement of endogenous ethylene in the flowering process is also supported by observations that indirectly link ethylene production to the flowering process. Symptoms such as extrusion of latex from terminal buds and epinasty of mature apical leaves, which are associated with high levels of ethylene, occur in mango plants at the time of inflorescence initiation and expansion of the panicles (Abeles, 1973; Davenport and Nunez-Elisea, 1990, 1991).

Indirect evidence of role of ethylene in flowering comes from reports of gradual increase in internal leaf ethylene production with the approach of flowering season. Inconsistent (Pandey *et al.*, 1973; Sen *et al.*, 1973) or non-responsive (Pandey and Narwadkar, 1984; Pandey, 1989) results using ethephon sprays have also been reported. Ethephon spray resulted in an elevated ethylene production in mango shoots without an accompanying floral response (Davenport and Nunez-Elisea, 1990, 1991). From these reports, it seems that the role of ethylene in flowering is still unresolved. Levels of ethylene were found to be higher in malformed vegetative and floral tissues as compared with that of healthy tissues at both prior to full bloom and full bloom stages (Pant, 2000; Bains, 2001; Kaushik, 2002; Bains *et al.*, 2003).

1-Aminocyclopropane-1-Carboxylate Synthase (ACC Synthase)

A simple and sensitive chemical assay was developed for 1-aminocyclopropane-1-carboxylate synthase (ACC synthase). The assay is based on the liberation of ethylene from ACC at pH 11.5 in the presence of pyridoxal phosphate, $MnCl_2$ and H_2O_2 . This assay was used to detect ACC in extracts of tomato fruits (*Lycopersicon esculentum* Mill.) and to measure the activity of a soluble enzyme from tomato fruit that converted S-adenosylmethionine (SAM) to ACC. The enzyme had a K_m of 13 μM for SAM, and conversion of SAM to ACC was competitively and reversibly inhibited by



An important characteristic of ACC oxidase is its requirement for ascorbic acid, Fe^{2+} and oxygen. The conversion of ACC to ethylene is accompanied by HCN production. This cyanide is produced in an

aminoethoxyvinylglycine (AVG), an analog of rhizobitoxine. The K_m value for AVG was 0.2 μM . The level of ACC-forming enzyme activity was positively correlated with content of ACC and the rate of ethylene formation in wild-type tomatoes of different developmental stages (Boller *et al.*, 1979).

The molecular mass of 1-aminocyclopropane-1-carboxylate synthase (ACC synthase) from a variety of sources was examined by high-performance gel-filtration chromatography and polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate. Enzymes used were prepared from wounded or non-wounded pericarp of ripe tomato fruits and wounded mesocarp of winter squash fruits as well as from cells of *E. coli* that had been transformed with cDNAs for the wound-induced or ripening-induced ACC synthases of tomato and the wound-induced or auxin-induced enzymes from winter squash. The enzymes from tomato tissues were isolated in a monomeric form, whereas the enzymes synthesized in *E. coli* from cDNAs for tomato ACC synthase were isolated in a dimeric form. ACC synthases of winter squash obtained either from fruit tissues or from transformed *E. coli* cells were isolated in dimeric forms (Satoh *et al.*, 1993). A multigene family encodes ACC synthase. Increased ethylene production is usually correlated with the accumulation of ACC synthase transcripts, indicating that ethylene production is controlled via the transcriptional activation of ACC synthase genes (Oetiker *et al.*, 1997).

1-Aminocyclopropane-1-Carboxylate oxidase (ACC Oxidase)

In higher plants ethylene is synthesized via methionine ACC pathway (Yang and Hoffman, 1984; Abeles and Abeles, 1972). In this pathway two crucial enzymes regulate the rate of ethylene synthesis, i.e. ACC synthase and ACC oxidase (Yang and Hoffman, 1984; Abeles *et al.*, 1972; Kende, 1993; Perscott and John, 1996). The final reaction in this pathway, which gives rise to ethylene from 1-aminocyclopropane-1-carboxylic acid (ACC) is catalyzed by ACC oxidase, previously referred to as 'ethylene forming enzyme'. The ACC oxidase reaction is summarized as follows:

amount stoichiometrically equivalent to ethylene (Peiser *et al.*, 1984) and is detoxified by conversion to β -cyanoalanine. It has been demonstrated that ACC oxidase is induced by various factors such as

fruit ripening (Ververidis and John, 1991; Dong *et al.*, 1992, Balague *et al.*, 1993, Nakatsuka *et al.*, 1998), senescence, wounding and infection by pathogen. In these studies results have shown that endogenous or exogenous ethylene participated in the regulation of ACC oxidase (Kim and Yang, 1999), further it was suggested that protein phosphorylation and dephosphorylation were responsible for the signal transduction in ethylene-mediated expression of ACC oxidase gene.

Ethylene was found to involve in the progress of senescence of post harvest broccoli florets. The induction of ACC oxidase activity rapidly occurred during senescence, which is correlated with the increased level of endogenous ethylene (Kasai *et al.*, 1998).

It was studied that a low oxygen concentration induces an increase in the ACC oxidase mRNA level, which causes an increased concentration of ACC oxidase protein. Although this protein is less active in low oxygen concentration, the increase in enzyme activity ensures that there is enough ethylene produced to stimulate petiole elongation during submergence in *Rumex palustris* (Vriezen *et al.*, 1999).

Mangiferin

Mangiferin, a non-toxic polyphenol and a normal metabolite in mango was reported to play an important role in the disease (Ghosal *et al.*, 1979). Enhanced production of mangiferin and increase in the activity of polyphenol oxidase in infected tissues were recorded. Polyphenol oxidase was considered as mangiferin degrading enzyme (Kumar and Chakraborty, 1992). Symptoms of mango

malformation induced by accumulated mangiferin (Table 1.2).

It was suggested that higher concentration of mangiferin in diseased tissues may lower the level of *Fusarium* sp. infection inside the diseased tissue. The higher concentration of mangiferin in malformed vegetative tissues and floral tissues do not clearly reveal the fact that *Fusarium* sp. infection prevents the translocation of mangiferin which results into its accumulation at the site of synthesis and do not predict an authentic correlation between *Fusarium* sp. infection level and higher concentration of mangiferin with respect to disease incidence (Ansari, 2005).

Hydrogen Cyanide

Cyanide is a harmful ion, hazardous to life, which forms a very stable complex with the active site (iron and magnesium) in enzymes, thereby inhibiting vital functions in cells such as respiration, CO₂ fixation and nitrate reduction. Cyanogenic compounds are widespread in plant species and are mostly stable compounds when conjugated to saccharides forming cyanogenic glycosides. Hydrogen cyanide is released from the cyanogenic compounds during tissue disruption, infection (Yip and Yang, 1998).

Other plant species, which don't accumulate cyanogenic glycosides but synthesize cyanohydrin from amino acids are known (Olechno *et al.*, 1984). The degradation of cyanogenic compounds (Poulton, 1990) and the oxidation of 1-aminocyclopropane-1-carboxylic acid (ACC) in ethylene biosynthesis are the two major sources by which cyanide is produced in higher plants (Yip and Yang, 1998).

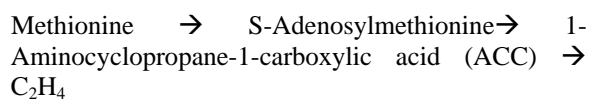
Table 1.2: Symptoms of mango malformation induced by accumulated mangiferin

<i>Effect of Mangiferin</i>	Symptoms
1. Increased IAA content	More vegetative growth
2. Increased chlorophyll content	Malformed shoots/panicles look greener.
3. Increased photosynthesis	More carbohydrate synthesis
4. Reduced respiration and amylase activity	Carbohydrate accumulation disturbed C/N ratio
5. Reduced catabolism	More longevity
6. Reduced transpiration	High moisture content

(Source: Chakarabarti and Kumar, 2002)

Cyanide – a Co product of Ethylene Biosynthesis

Ethylene biosynthesis in higher plants is established through the following pathway:



(Yang and Hoffman, 1984). By employing the ^{14}C tracer labeled at different positions of ACC, it was demonstrated that during the *in vivo* oxidation of ACC to ethylene, the carboxyl carbons, C-1 and C-2 and C-3 of ACC are metabolized into CO_2 , L-3-cyanoalanine derivatives, and ethylene, respectively. Although no free HCN was identified, it was asserted that C-1 of ACC is initially liberated as HCN, but is rapidly conjugated into L-3-cyanoalanine derivatives as soon as it is liberated. This notion was based on the observation that the metabolic fate of the C-1 of ACC during its conversion to ethylene was identical to that of administered HCN and that the amount of HCN-conjugates formed was equivalent to that of ethylene produced (Peiser *et al.*, 1984). Thus, the degradation of ACC into ethylene can be represented by the following equation:



This reaction was further confirmed in *in vitro* using the purified ACC oxidase from apple (Dong *et al.*, 1992).

Metabolism of Cyanide in Plants

Since no free HCN was detected even in plant tissues, which produced ethylene at very high rates, it was suggested that plants must have ample capacity to metabolize the HCN originating from

ACC (Peiser *et al.*, 1984). In higher plants the key enzyme to detoxify HCN is L-3-cyanoalanine synthase (EC. 4.4.1.9 (Figure 1.3).

Cyanide production in healthy and malformed tissues of Mallika, Khas-ul-Khas and Langra revealed that the change in cyanide level was highest in the time period of 12.00 noon to 2.00 pm. The cyanide levels were higher in the malformed floral tissues at all time intervals with maximum cyanide being observed at 1.0 pm. No cyanide was detectable in healthy or malformed floral tissues during early or the late light period generally (Nailwal, 2004; Nailwal *et al.*, 2006).

Beta cyanoalanine synthase

β -cyanoalanine synthase is an enzyme that catalyses the conversion of cyanide and cysteine to β -cyanoalanine (Miller and Conn, 1980). It has been detected in all plants examined but considerable variability occurs in levels of activity between species and between different tissues of the same plant. The main physiological role of CAS has been suggested to be the detoxification of cyanide produced in various stages of plants life cycle (Hendrickson and Conn, 1969; Akapyan *et al.*, 1975; Wurtele *et al.*, 1985; Manning, 1988;), such as wounding of cyanogenic plants (Poulton, 1990) and enhancing ethylene biosynthesis (Yip and Yang, 1988).

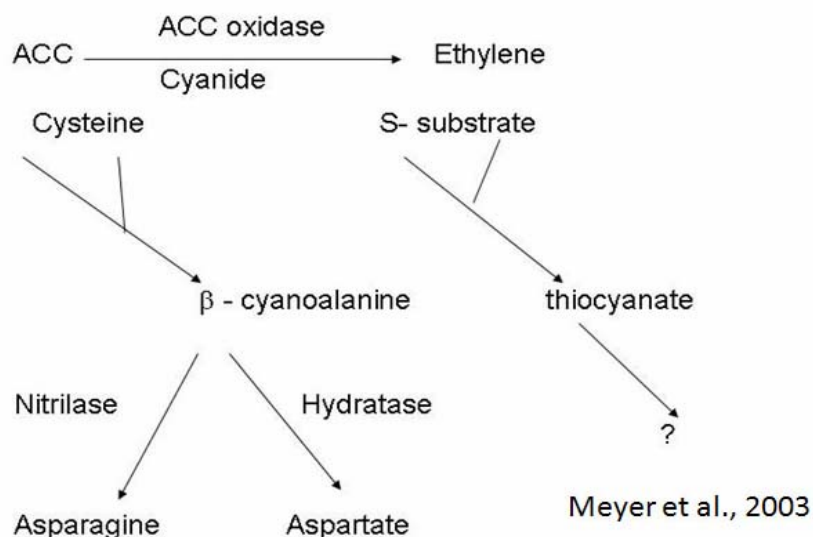
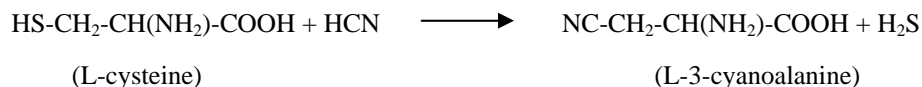


Figure 1.3 Scheme of cyanide release during ethylene formation and its putative detoxification pathways in higher plants

Beta-cyano-L-alanine-synthase or L-cysteine hydrogen sulfide lyase (adding HCN) EC (4.4.1.9), a pyridoxal dependent enzyme which catalyses the reaction between cysteine and HCN to form β -cyanoalanine (β -CA) and H_2S , was first described in 1963 (Blumenthal-Goldschmidth *et al.*,



L-3-cyanoalanine synthase is widely distributed in higher plants (Miller and Conn, 1980). L-3-cyanoalanine thus formed is further metabolized to asparagine or to γ - glutamyl-L-3-cyanoalanine (Akapyan *et al.* , 1975; Blumenthal *et al.* , 1963; Blumenthal *et al.* , 1968; Hendrickson and Conn, 1969; Manning, 1986; Peiser *et al.* , 1984; Pirrung, 1985). Thus, the cyanide is detoxified and the N is conserved as the amide of asparagines (Castric *et al.*, 1972). L-3-cyanoalanine synthase has been purified about 4000-fold from blue lupine seedlings and this enzyme is pyridoxal-dependent and can be inhibited by 2-aminoxyacetic acid (AOA) or 3-aminoxypropionic acid (Akapyan *et al.*, 1975).

Distribution of β -CAS has been observed in several plants (Miller and Conn, 1980; Peiser *et al.*, 1984). The close relationship has been found between cyanogenesis potential and CAS activity in a variety of higher plants (Miller and Conn, 1980; Peiser *et al.*, 1984 and Wen, 1997).

β -CAS is predominantly located in mitochondria (Akapyan *et al.* , 1975; Wurtele *et al.* , 1985). The ubiquitous presence of β -cyanoalanine in plants (Hendrickson and Conn, 1969; Wurtele *et al.* , 1984, 1985; Mizutani *et al.*, 1987) suggests that the enzyme is constitutive and has an important metabolic function. One plausible role, alluded to by numerous researchers, is the detoxification of cyanide produced during ethylene biosynthesis or from other sources (Blumenthal *et al.* , 1968; Miller and Conn, 1980; Wurtele *et al.* , 1984, 1985; Manning, 1986; Mizutani *et al.*, 1987).

High enzyme activities were found in tissues of cyanogenic and non-cyanogenic species and were correlated with cyanide production (Miller and Conn, 1980) and ethylene levels (Mizutani *et al.*, 1987). The capacity to metabolize cyanide is greater in older plant tissues and areas with meristematic activity (Wurtele *et al.* , 1985). Ethylene has been suggested to induce de novo synthesis of β -CAS (Manning, 1988). Since in vitro β -CAS activity in tissues that produce ethylene at high rates is quite high (1650 n mol $g^{-1} h^{-1}$ in ripe apples) and the cyanide levels are low (0.2 n mol g^{-1} fresh weight, calculated to be 0.2

1963; Floss *et al.* , 1965 and Blumenthal *et al.* , 1968). The main biological function of β -CAS is evidently β -replacement of -SH in cysteine by CN. The reaction catalyzed by the enzyme is shown below:

μM), it was Yip and Yang (1988) who concluded that plant tissues have ample capacity to detoxify HCN derived from ethylene biosynthesis by β -CAS activity. The concentration of HCN appeared to be kept below the non-toxic level of approximately 1 μM .

The K_m of β -CAS for cyanide is high; it ranges from 500 to 10,000 μM (Manning, 1988). Tissues also became cyanogenic after treatment with ACC (Yip and Yang 1988). In mungbean hypocotyls cyanide levels went up to approximately 8 μM (Grossmann, 1996).

Although CAS has been reported to be compartmentalized within the mitochondria (Wurtele *et al.* , 1985). The existence of non-mitochondrial cytoplasmic CAS in developing seeds was suggested (Ikegami *et al.* , 1989). Investigation was undertaken to demonstrate the existence of two types of CAS in germinating seeds and to determine which type is stimulated by ethylene (Hasegawa *et al.* , 1995).

Germinating seeds of many species contain two types of β -cyanoalanine synthase. One is cytoplasmic CAS (cyt-CAS) which is precipitated by 50 % to 60 % $(NH_4)_2SO_4$ and has a pH optimum of 10.5; cyt-CAS is present at high levels in dry seed and its activity does not increase during imbibitions. The activity of cyt-CAS is not affected by exogenously applied C_2H_4 except in rice (*Oryza sativa* cv. Sasanishiki). The second type of CAS found in seeds is mitochondrial CAS (mit-CAS), which is precipitated by 60 % to 70 % $(NH_4)_2SO_4$ and has an optimum pH of 9.5. Mit-CAS is present at low levels in dry seed, and its activity increases greatly during imbibition in the seeds of all species tested. Exposure to C_2H_4 stimulated mit-CAS activity in seeds of rice, barley (*Hordeum vulgare* cv. Hadakamugi), cucumber (*Cucumis sativus* cv. Kagafushinari) and cocklebur (*Xanthium pennsylvanicum*) (Hasegawa *et al.* , 1995). More and more studies have found more than one CAS isozymes in plants. Ikegami *et al.* , 1988 separated the CASs in higher plants into two classes. The CASs in lupin and barley were proposed to belong to one class. But those from spinach and

Lathyrus la tiliifolius were proposed to belong to another class, which possessed some structural similarity with Cysteine synthase (CS) in higher plants.

In blue lupine besides CAS, a CS was also found to be able to catalyze the formation of β -cyanoalanine from cyanide and cysteine (Hendrickson and Conn, 1969). Two types of CAS were also detected in cocklebur seeds (Hasegawa *et al.*, 1995). One type of CAS was attributed to a cysteine synthase (Maruyama *et al.*, 1998). Since the cytosolic CAS activity in cocklebur cotyledons was about 10 times higher than that of the mitochondrial CAS activity, CS in cytosol may be involved in cyanide metabolism in plant tissues (Maruyama *et al.*, 2000). Two of the three CAS isozymes from potato tuber slices and spinach leaves were also attributed to CSs with CAS capacity (Maruyama *et al.*, 2000; Warrilow and Hawkesford, 2000). Both CAS and CS belong to β -substituted alanine synthase family. They possess different substrate specificities and reaction efficiencies. CAS from spinach leaves showed a 10 – fold higher affinity for O-acetylserine than CS, but had a much lower substrate turnover rates than CS in Cys synthesis reaction (Warrilow and Hawkesford, 2000). In studies on the CAS and CS of potato, spinach and *Arabidopsis*, their obvious distinction was also confirmed by the immunodetection results with antibodies against CAS and CS (Maruyama *et al.*, 2000; Hatzfeld *et al.*, 2000).

Two CAS isozymes (CAS₁ and CAS₂) were observed in tobacco. Both CAS isozymes were detected in the isolated cytosol. But only CAS₁ was observed in isolated mitochondria and no CAS activity was found in the isolated chloroplast (Liang and Li, 2001). Drought stressed tobacco plants for two days did not inactivate CAS₂. Both CAS isozymes were induced and contributed to detoxify cyanide in the tobacco plants under drought stress (Liang, 2003).

CAS purified from other plant source has been reported to be a dimer (Ikegami *et al.*, 1988, 1989). CAS purified from potato tuber was composed of 34 KDa polypeptides separated by SDS-PAGE analysis, whereas the molecular mass of the native CAS was estimated to be 50 KDa by gel filtration (Maruyama *et al.*, 2000).

The β -CAS activity studied in healthy and malformed tissues of mango showed the higher levels of β -CAS activity in healthy tissues suggesting that the greater amount of HCN is being metabolized in the healthy tissues. It can be speculated that the increased level of 'stress ethylene' in the malformed tissues is responsible for the augmented levels of cyanide in the malformed tissues as compared to healthy tissues. Thus, lower levels of β -cyanoalanine

synthase in the malformed tissues coupled with augmented ethylene production may result in the accumulation of toxic quantities of cyanide resulting in necrosis of tissues (Kukreja, 2000).

Cyanide Resistant Respiration

The first observation on cyanide-resistant respiration in plants was made over 75 years ago, when Genevois found that the respiration of seedlings of *Lathyrus odo rata* (sweet pea) was resistant to cyanide. It is now well documented that cyanide-resistant respiration is a widespread phenomenon in higher plants (both monocotyledons and dicotyledons); it also occurs in a few animal species and in fungi, bacteria and algae (Henry and Nyns, 1975).

Mitochondria isolated from roots and leaves of ten species (three legumes, one C₃-monocotyledon, one C₄-monocotyledon, the rest non-leguminous C₃-dicotyledons) examined displayed cyanide-resistant oxygen uptake, which was sensitive to both SHAM and tetraethylthiuram disulfide (disulfiram). Concentrations of SHAM greater than 2 mM caused inhibition of the cytochrome path as well as of the alternative path in isolated mitochondria (Lambers *et al.*, 1983). In thermogenic spadix tissue of certain members of the family Araceae (e.g. skunk cabbage, voodoo lily) cyanide-resistant respiration contributes to respiratory rates that rival those found in insect flight muscle. This cyanide-resistant O₂ uptake takes place on the inner mitochondrial membrane and appears to contribute a pathway "alternative" to the main cyanide-sensitive cytochrome pathway by which electrons from reduced tricarboxylic acid (TCA) cycle substrates flow to the terminal electron acceptor, O₂ (Siedow and Berthold, 1986). In winter rape leaf slices grown at 5°C, the patterns of cold-induced changes in total respiratory activity and in the estimated activity of alternative pathway were found to be similar. It seemed that in leaf slices from plants grown in the cold, the cyanide-resistant, alternative pathway participates in oxygen uptake. The effect of cyanide on respiration and the possibility of the development of cyanide insensitive respiration in the malformed tissue cannot be ruled out (Rychter *et al.*, 1988).

It was proposed that activation and engagement of the alternative oxidase might keep Q reduction levels low in order to prevent harmful high levels of free radical production. Complete purification of the alternative oxidase present in the inner mitochondrial membrane has not been achieved successfully, because of its instability on solubilization (Zhang *et al.*, 1996).

Molecular characterization of Mango

Determination of male parent in mango is difficult due to small flower size and large number of flowers on a plant. The ability to distinguish true hybrids versus those resulting from self-pollination would be very useful in mango evaluation programs. A DNA based marker was evaluated for its usefulness in determining parentage among mango seedlings. Caging mature trees of the cultivars Keitt 'and Kent' produced two populations. Seedlings from each cultivar were examined by DNA analysis using RAPD markers. The Keitt' population appeared to result from self-pollination, while considerable outcrossing had occurred among the Kent' seedlings. The number of non-segregating loci in the parental cultivars was surprisingly high. This result was unexpected as mango is generally considered to be highly polymorphic. Bands amplifying in the progeny that are not present in either parent have also been found. These complications make RAPD markers less desirable for this type of analysis than other DNA molecular markers (Schnell *et al.*, 1995). Random amplification of polymorphic DNA (RAPD) markers have been extensively used to study the genetic relationship in a number of fruit crops (Ravishankar *et al.*, 2000). Apart from RAPD, other techniques like RFLP and AFLP have been used for molecular analysis of tropical and subtropical fruit trees for the identification of cultivars, somatic hybrids, chimaeras, nuclear and zygotic seedlings and in pedigree analysis, construction of genetic linkage maps and molecular marker - assisted selection (Guixin *et al.*, 2002).

DNA based RAPD markers were used to study the genetic relatedness for identification of mango cultivars (Schnell *et al.*, 1995). It was also revealed that a high degree of genetic diversity existed among the mango cultivars (Hemanth *et al.*, 2001).

The mango genotypes were grouped based on their geographical origin and the majority of mango cultivars originated from local gene pool (Lopez-Valenzuela *et al.*, 1997 and Ravishankar *et al.*, 2000).

Several rapid methods for genomic DNA extraction from plant tissues have been described (John, 1992, Kim *et al.*, 1997 and Porebski *et al.*, 1997). It was reported that isolation of genomic DNA from plants containing a high content of polyphenolics such as grape (*Vitis* spp), apple (*Malus* spp), pear (*Pyrus* spp) was difficult (Kim *et al.*, 1997).

The basic extraction protocol was slightly modified (Dellaporta *et al.*, 1983). Precipitation of DNA by 2.5 ml 5M NaCl and 10 ml ethanol was used and half volume of 5M NaCl and one volume

of isopropanol to get better yield (Ravishankar *et al.*, 2000 and Hemanth *et al.*, 2001). RAPD analysis of 29 Indian mango cultivars comprising popular landraces and some advanced cultivars generated 314 bands, 91.4% of which were polymorphic. A UPGMA dendrogram showed the majority of the cultivars from northern and eastern regions of India clustering together and separate from southern and western cultivars. Analysis of molecular variance revealed that 94.7% of the genetic diversity in mango existed within regions. However, differences among regions were significant; northern and eastern regions formed one zone and western and southern regions formed another zone of mango diversity in India (Karihaloo *et al.*, 2003).

RAPD analysis for establishing genetic variability showed that the amplified DNA fell in the range of 1400 to 350 kb. The pattern differed with each primer. Unique bands of different sizes specific to malformation were obtained with all the primers (Krishnan, 2003).

The UPGMA (Unweighted pair-group method with arithmetical averages) dendrogram revealed that healthy and malformed inflorescence of each of the varieties studied were quite distantly placed in the dendrogram further confirming genetically diverse nature of healthy and malformed inflorescence (Nailwal, 2004).

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5/11/2009