CHAPTER 6

Mangifera indica L. (family Anacardiaceae) is a commercially important fruit crop of India and other countries of the world. India is the world's largest mango producer but sixth in position for productivity. The reduction of production is affected by several diseases and disorder of mango *viz*. rot, dieback, mildew, necrosis, scab, blotch, stem bleeding, wilt, spots, canker, sooty mould and floral malformation. Among them, 'mango burl' is a lesser-known or ambiguous disease and is always ignored by earlier researchers. A perusal of literature indicates that there is no much information available on its effect on the health and vigour of the trees, fruits yield loss and nutritional value of the fruits produced on severely affected trees, while no confirmed information is available on its aetiology. Keeping in view the importance of this disease, the current investigation was undertaken as "Holistic assessment of burl disease of mango germplasm".

It is an unwanted outgrowth of tumour formation on the trunk which makes it ugly. The burl shape was globose in *Langra*, *Rajapuri*, *Sukul*, *Mahuvas*, *Olour*, *Seedling*, *Tree 253*, *Krishna Bhog*, *Hybrid 10*, *Alphanso* × *Baneshan*, *Alphanso* × *Sabja*, *Banganpalli*, *Gopal Bhog*, *Prince* and *Malai* while *Mahmood Vikarabad*, *Khaja Pasand* and *Sindhu* have globose to elongated in shape. Globose to semi-elongated shape was observed in *Elite Ditla*, *Joshipura Junagadh*, *Arka Aruna*, *Arka Punit*, *Neelphanso* and *Suvarna Rekha*. 473 mango germplasm/varieties were screened throughout India for the burl disease and 34 varieties were recorded as susceptible, which accounts for nearly 7.18 % disease incidence.

In Gujarat state, 167 varieties were screened among them 20 varieties found susceptible with 12 % disease incidence. Varieties like *Arka Aruna*, *Seedling*, *Elite Ditla*, *Mankurad*, *Joshipura Junagadh*, *Mahmood Vikarabad*, *Hybrid 10*, *Badami Modal*, *Banganpalli* × *Alphanso*, *Mahuvas*, *Tree 253*, *Krishna Bhog*, *Khaja Pasand*, *Gopal Bhog*, and *Malai* showed the highest incidence (100 %). It was minimal or lowest in *Desi*, *Khodi* and *Kesar* throughout the country while no burls were observed in the mango orchards around New Delhi. In contrast, out of 167 germplasms/ varieties, nearly 20 varieties in Gujarat were susceptible and showed 100% incidence in *Arka Aruna*, *Badami Modal*, *Mankurad*, *Mahmood Vikarabad*, *Joshipura Junagadh*, *Elite Ditla*, *Banganpalli* × *Alphonso*, *Seedling*, followed by other varieties like *Langra*, *Suvarna Rekha* and *Arka Punit* while it was only 2 % in Kesar. The size of the burl was

largest in *Langra* (36.00 cm^2), followed by *Desi* (32.00 cm^2), and it was minimal in *Mankurad* (10.0 cm^2).

Since there is no unanimous opinion about the causal organism of the disease, collected diseased samples were cultured on different selective media and based on morphological characteristics of the colony, gram staining and growth on the selective media, the causative organism was provisionally identified as *Agrobacterium* for burl disease. Further confirmation of the identity was done using molecular tools and phylogenetic analysis showed a 100 % match with the *Agrobacterium tumefaciens*. The phylogenetic tree was constructed using a partial sequence of 16S rRNA using Maximum Likelihood (ML) analyses by RAxML software (Silvestro and Michalak, 2012). An ML analysis was run for 1000 bootstrap replicates under the GTR + I model to assess clade support. The isolated sequences were deposited in NCBI GenBank with accession no MK835677.

To verify the pathogenicity of the bacterial isolate obtained from burl wood were further tested using the Carrot Disk assay and by inoculating in young saplings of mango (variety *Langra*) and tomato. After one month on Carrot Disk and two months of inoculation in mango and tomato sapling, similar disease symptoms were recorded successfully in both the testing techniques. To cross-check, the pathogen was re-isolated from the inoculated saplings and checked by colony characterization and gram staining and it was confirmed as *Agrobacterium tumefaciens.*

The burl disease caused maximum fruit yield loss in *Mahmood Vikarabad* and *Langra* whereas minimum fruit yield loss was shown by the varieties like *Olour*, *Alphanso* × *Baneshan* and *Alphanso* × *Sabja*. The yield loss in *Langra* variety was also recorded throughout India and it was found highest in plants growing in Maharashtra (Rahuri) and Gujarat (Pariya, Valsad) whereas mango trees growing at New Delhi and Hisar region were found free from burl disease resulting in no yield loss. In Gujarat state, *Langra* and *Rajapuri* were selected to investigate the fruit yield losses in four different zones (*viz*. Anand, Banaskantha (Dantiwada), Junagadh and Valsad (Pariya). Both varieties showed maximum yield loss from Valsad and minimum yield loss from Junagadh.

If the development of burl is correlated with the climatic conditions, the incidence of burl formation, its size, their number per plant, and fruit yield loss, it was maximum in both the varieties growing at Pariya (Valsad) site. In contrast, it was found minimum in both varieties growing at Junagadh. Valsad district is well-known to

receive maximum rainfall in the state while Junagadh falls under arid region and shows less rainfall. At both places, the percentage of relative humidity also varied considerably which was maximal at Pariya (Valsad) and minimal at Junagadh. The incidence of burl disease when correlated with the tree age, occurrence of number of burls per individual tree upsurge with the increase in the age of the tree and its incidence was found maximum in trees more than 40-year-old while burl incidence was minimum in below 15 years old trees.

Biochemical parameters like the content of total soluble solids (TSS), total soluble sugars, reducing sugar, non-reducing sugar and ascorbic acid content was analyzed in fruits obtained from burl free and infected trees of both varieties. As compared to fruits of burl free trees, fruits of the burl affected plants of both varieties showed maximum content of TSS, total soluble sugars, reducing sugar, non-reducing sugar and ascorbic acid while the acidity was found minimum.

Similarly, wood samples of both varieties were also accessed for the content of moisture, ash, cellulose, lignin, total phenols, ascorbic acid, total sugars, non-reducing sugars and reducing sugars. The percentage of these compounds was found highest in the burl portion of the wood, which declined in the transition portion and they were minimum in healthy wood. In contrast, inverse relation was observed in xylem fibre content and starch, which was found highest in healthy wood as compared to burl affected wood of both varieties.

Histological study of burl and healthy wood showed drastic alternation in the structure and composition of the secondary xylem The secondary xylem of the burl was deformed and showed complete loss of polarity, i.e., they lost their axial orientation of the xylem elements. Morphology and dimensional details of xylem elements showed significant variation at various positions within the burl. The transverse view of the peripheral portion of the burl xylem cells showed their orientation in all three different planes *viz*. transverse, radial and tangential longitudinal plains. In this region, xylem cells showed a circular arrangement of the cells and look like circular vascular elements. In this region, xylem cells appeared like ray cells whereas xylem fibres and vessels were significantly short. Cells from the middle portion of the burl were oval to circular, lignified and thick-walled looking like ray cells. All the cell types of the xylem, *viz*. vessels, tracheids, fibres, ray cells and axial parenchyma showed the presence of tyloses. The formation of tyloses is a common feature and is correlated with compartmentalisation to protect the tissue against pathogen invasion.

In conclusion, burl disease is an important disease of mango that show an unwanted tumour like outgrowth on the main stem and lower main branches. It causes great economic loss in mango production in India and throughout the world. In India, several varieties like *Arka Aruna*, *Mahmood Vikarabad*, *Langra* etc., are highly susceptible to it while other varieties like *Desi*, *Khodi* and *Kesar* are relatively resistant *Amrapali*, *Mallika*, *Totapuri* and *Vanraj* are found free from the burl disease. In Gujarat, Valsad (Pariya) and Anand districts have favourable climatic condition for disease development whereas Dantiwada and Junagadh are non-conducive for burl development. It may be associated with a higher percentage of relative humidity and rainfall. External injury during agricultural practices may be the major source of pathogen entry in the tree. Another possible reason is the source of stock used in the grafting source may be responsible for the incidence of the disease. Possibly the stock used for grafting may be of susceptible variety. This statement may be supported by the fact that trees growing naturally in forest areas and open area wild germplasms showed no disease incidence. However, such trees bear inferior quality fruits (smaller, sour and develop a few numbers of fruit production); therefore, they were not considered in the present study. Further studies are required to check this hypothesis using stock of the wild germplasm and scion from the susceptible variety or Koch's postulate may be applied on the trees growing naturally in the forest area.

The size of the burl, its incidence and decrease in fruit yield increase significantly with the increase in the age of the trees. This disease not only changes the plant morphology but also induces considerable alteration in the stem. Higher content of the lignin burl tissue is associated with resistance, which is also reported by earlier researchers. Deformation of xylem tissue and loss of polarity in the mechanical tissue is correlated with the elevated concentration of growth hormones such as auxins and cytokinin. Auxin plays an significant role in the maintenance of the polarity of axial elements and *Agrobacterium* is already known for hormone production. The burl disease also changes the biochemical composition of fruit and stem wood of infected plants. An increase in the content of TSS, total soluble sugar, reducing sugar, non-reducing sugar and ascorbic acid while a decrease in acidity may be associated with the defence mechanism in both the species. Similarly, there is an increase in moisture content, cellulose, lignin and other parameters in the burl wood that may be associated with the modification in the structure and configuration of the secondary xylem.

Available literature also indicates that lignin plays crucial role in the defense mechanism and compartmentalization of the pathogen.

Future scope of the work: Regarding the management of disease, there is an urgent need to develop a method to treat the diseased plants. However, at present primary management methods can be established or applied to manage the disease including, proper tree management, avoiding wounding or pruning of trees from orchard may reduce impacts of the disease. Types of equipment used in the pruning of the trees should be disinfected frequently when in use because infected equipment can spread pathogen during pruning operations.

Precaution is better than cure; therefore, the use of healthy planting material in the new orchard is advisable and it will play a major role in disease management because new saplings of the mango trees are generally developed by grafting technique. Therefore, grafting material should be either resistant or infection-free. In the present study some mango varieties viz., *Mallika*, *Dashehari*, *Bombay Green* and *Amrapali* were free from this disorder; thus, non-infected scion can be used as grafting material from these varieties to stop burl infection.

The plantation of new orchard and grafting for new sapling development should also be avoided during the favourable season that supports disease development. Avoiding conducive season for pathogen season is an ideal control measure of burl and another disease of mango. The complete eradication of burl by chemical treatment is not known but farmers should remove burl from the tree trunk and apply copper oxychloride or Bordeaux paste mixed with compatible insecticide (methyl-demeton or monocrotophos) is a good way to control this disease. The fungicidal Chaubattia paste can also be applied to the wounds during the pruning operations in the first fortnight of October.