CHAPTER 5

Mango is an economically important fruit crop of India and occupies 2.258 Mha of land under cultivation in India (NHB, 2018), holds first positions in mango growing countries and exports fruits to more than 40 countries all over the world (Saran *et al*., 2020a, b). If cultivated using seeds, it takes approximately 10-15 years for flowering and fruiting; moreover, there is no assurance that the fruits produced by such individuals will be of superior quality. Therefore, mango growers prefer to use saplings produced by the grafting method, which ensures the quality of the fruit and starts flowering within a short span. Nevertheless, the quality and quantity of the fruit are often affected by several biotic (microorganisms like bacteria, fungi, viruses etc.), abiotic factors (temperature, rainfall, relative humidity *etc*.) and various disorders (Prakash, 1998; Saran *et al*., 2020a, b).

Among the biotic factors, a detailed study was conducted for several fungal and bacterial diseases by various researchers (Bally, 2006; Banerjee, 2011; Knight *et al*., 2009; Litz, 2009; Kumar and Saran, 2018). That could be the possible reason that mango trees are affected by several disease and disorders. From them, tumorous outgrowth on the main stem and branches that look like a large gall is referred to as 'burl disorder' is found very frequently in most of the varieties. However, formation burl is always neglected by the researchers may be due to their occurrence on the stem and branches and are not directly associated with fruit (Saran *et al*., 2011, 2020a, b).

The occurrence of burls is known from more than 400 years ago by Malpighi (1675), available literature reveals that there is no unanimity in the usage of the terminology for this disorder. It was recognized as wood gall of mango by Prakash and Srivastava (1987), and several research workers called it as scaly wood, tumour, gall or crown gall of mango (White and Millington, 1954; Malaguti and de Reyes, 1964; Cook, 1975; Angulo and Villapudua, 1982; Garrett, 1987; Ploetz *et al.*, 1996b; Hafiz, 1986; Jiskani *et al*., 2007; Saran *et al*., 2011, 2020a, b).

It is a very well-known fact that galls are abnormal growth on leaves, twigs, flowers or roots. They are developed in response to insect association for the completion of their life cycle by laying their eggs inside these structures for defense from the predators and to provide necessary nutrition to developing larva after egg hatching (Raman, 2007). Similarly, the word "crown" is used to describe the total of an individual plant above ground organs like stem, leaves and reproductive organs. Therefore, to avoid the controversy of the terminology, in the present study burl refers to woody tumours or large-sized unwanted outgrowths on the woody stem, exposed roots and main branches (White and Millington, 1954; Saran *et al*., 2020a, b).

The occurrence of mango burls in India is documented around 60 years back by Chand and Rao (1954). Thereafter, Prakash and Srivastava (1987), also reported its presence in some species of mango. The first detailed study including morphological, histological characteristics and survey in various parts of mango burl are investigated from Uttarakhand are recorded by Saran *et al*. (2011). Though, there are sporadic reports on its occurrence all over India they are fragmentary and variety-specific data is available.

Therefore, in the current study, an extensive survey was carried out for the screening of several germplasms of mango growing throughout India. This study was undertaken to understand the alterations in the morphology of burl, its incidence, fruit yield loss, its relationship with climate, anatomical alterations in stem and to find out the causal organism. It also intends to confirm the identity of pathogen and its characterization by using traditional and molecular methods, testing of an isolated causal agent by pathogenicity tests and biochemical analysis of fruits for their nutritional value.

5.1 *Morphological characterization*:

Different mango varieties are grown in private or agricultural university orchards were screened against the incidence of burl disease in various states of India including Gujarat. White and Millington (1954), and Chand and Rao (1954), mentioned that burls are an unsolicited outgrowth of a large-sized tumour that occurs in a deformed manner and shows a distinctive woody pattern on the tree trunk. They are hard, woody, rough and darker in appearance on the tree trunk and may grow on one side or may completely encircle the tree trunk. In healthy plants, the wood grain runs parallel or slightly twisted (called spiral grain) in one direction along with the trunk of a tree and conducting elements maintain the polarity but burl infected portions of the stem showed loss of polarity and the wood is deformed appearing like wound tissue (Saran *et al*., 2020a, b, c).

During the survey, several morphological variations were observed in all the 34 mango varieties screened in the present investigation and they showed the presence of burl disease. The variation in the site of formation and burl size differs from variety to variety and region to region throughout India. Disparities were observed in its shape, colour, surface texture, the position of burl formation on the stem (upper and lower side of the stem), the total number of burls per plant, the height of the first burl from ground level and the presence of gummosis. Similar studies are also carried out by Saran *et al*. (2020b,c), and reported such observations for the Gujarat state. However, in the present study, the burl shape was recorded globose in *Alphanso* × *Baneshan*, *Alphanso* × *Sabja*, *Banganpalli*, *Gopal Bhog*, *Hybrid 10*, *Krishna Bhog*, *Langra*, *Mahuvas*, *Malai*, *Olour*, *Prince*, *Rajapuri*, *Seedling*, *Sukul*, and *Tree 253*. On the other hand, *Mahmood Vikarabad*, *Khaja Pasand* and *Sindhu* have globose to elongated shape. In contrast, it was globose to semi-elongated shape was observed in *Arka Aruna*, *Arka Punit*, *Elide Ditla*, *Joshipura*, *Junagadh*, *Neelphanso* and *Suvarna Rekha*.

Chand and Rao (1954), reported that burl shape is linear in *Mahmood Vikarabad* and covers an entire main stem of the variety. They also characterized these shapes as the main characteristic feature of the variety. White and Millington (1954), documented that burl is a superfluous outgrowth of forest trees like Redwood, which looking warty, corky with different texture and colour. According to these authors, trees may get infected with burl disease in any stage of growth and development, which may be visible as solitary or in collections with different shape, surface and colour (White and Millington, 1954; Peterson, 1961).

In the present study, it was noticed that colour of the burl changes from light brown to dark brown or black and the burl became hard and woody as it matures. Cook (1975), reported similar symptoms on '*cuarteado'* and the disease is named scaly wood from Colombia. Identical disease symptoms were also observed on mango seedlings from Hawaii (Cook *et al*., 1971). Angulo and Villapudua (1982), mentioned that the appearance of burl or its shape looks like cauliflower type and named the disease bolas' or '*Buba* of mango' from Mexico. These cauliflower-like shapes are initially light brown and later became dark brown as they mature.

In the current investigation, varieties like *Joshipura*, *Junagadh Khodi*, *Mankurad* and *Desi* showed irregular shape, with different colour and texture. In contrast, Barnard and Freeman (1982), reported that burls have globular to irregular shape in red mangroves with a rough texture and dark colour. These variations may be associated with an individual species as mangrove plants grow exclusively on the sea coast and their microclimate is also different from the areas where mangoes are under cultivation. According to Sinclair *et al*. (1993), burls are small bump to big sized globose shaped outgrowth in angiosperms.

US Department of Agriculture (USDA) Miami, from Puerto Rico, and the University of Florida in Homestead also documented that, the older galls are large, they have a rough and scaly outer surface and commonly found on the main stem of affected trees (Ploetz *et al.,* 1996b). The cracks developed on older and scaly galls may penetrate deep into the phloem and become necrotic and may be responsible for branch death. Similar reports are also available from India that trees of all ages can be affected by burl and they may occur as single or in groups of 6-10 burls per branch and trunk (Mishra and Prakash, 1999). The present study reconfirms that mango burl has a different shape, size with varying texture and may show gummosis in some cases. A similar symptom of mango stems is also reported from the USA (Ploetz and Freeman, 2009).

Saran *et al.* (2011), surveyed the mango orchards of Uttarakhand and investigated the burl disease symptoms by using various parameters. According to their investigation, burl development initiated from the junction of the main stem and branches of *Langra* and *Chausa* variety, whereas they develop solitary on the main trunk of *Gulab Jamun*. These studies also stated that in the initial stage of burl development, their shapes were globose but subsequently they became elongated. Kumar and Saran (2018), reported that burl symptoms arise on the main trunk as globose to elongate or irregular shape, later became warty and corky in its surface appearance.

On every individual tree, the number of burls varies from variety to variety; their number was more in *Langra*, *Rajapuri* and *Mahmood Vikarabad* whereas it was observed minimum in *Elite Ditla*, and *Khodi*. Previous studies by Saran *et al*. (2011) and Kumar and Saran (2018), also reported variations in the number of burls on an individual tree and is dependent on the variety and the plant parts where it occurs. In *Langra* variety, the number of burls was 1-8 per branch while the variety *Chausa* showed a presence of 1-22 per branch. In another investigation, 15,000 burls reported in angiosperms, gymnosperms and cryptogams (Meyer 1987). When the plants are young, these burls are not easily visible but subsequently, they became visible as the individual tree age increase or plants having more than 5-year age (Saran *et al*., 2011, 2020a, b; Choudhary and Rajput, 2018; Choudhary *et al*., 2020a, b, c, d).

5.2 *Burl Incidence and Size*:

In the present work, 473 mango varieties are screened against the incidence of burl disease throughout the country. Based on the survey data, almost 7.18 % of varieties grown in India show this disease incidence. All the investigated varieties of mango are prone to the disease and found affected with burl disease. Among them, varieties like *Langra*, *Mahmood Vikarabad*, *Arka Aruna* are highly susceptible while other varieties like *Neelphanso*, *Sindhu* and *Kesar* are found to be relatively less susceptible. The burl prevalence was found maximum in *Arka Aruna*, *Seedling*, *Mahmood Vikarabad*, *Hybrid 10*, *Badami Modal*, *Banganpalli* × *Alfanso*, *Mahuvas*, *Tree 253*, *Krishna Bhog*, *Khaja Pasand*, *Gopal Bhog*, *Seedling*, *Elite Ditla* and *Mankurad* while minimum incidence is observed in *Desi*, *Khodi* and *Kesar* throughout India. Gujarat state has a nearly 12 % incidence of this disease. In Gujarat state, the highest susceptible varieties recorded are *Arka Aruna*, *Seedling*, *Mahmood Vikarabad*, *Badami Modal*, *Mankurad*, *Joshipura Junagadh*, *Elite Ditla*, *Banganpalli* × *Alphonso*.

Saran *et al.* (2011), reported that the incidence of disease was not only highest in variety *Langra* followed by *Chausa* and *Gulab Jamun* from Dehradun but also showed the largest size of burl. As per their reports, variety *Langra* showed 80.3 % disease incidence with burl size 31.8 cm diameter, *Chausa* has 17.5 % incidence with burl size 16.4 cm and *Gulab Jamun* reported as 7.5 % incidence with 4.0 cm burl size. In continuation of their study, Kumar and Saran (2018), recorded 75.59 % in *Langra* followed by *Chausa* 17.14 % from the Uttarakhand region by investigating a total of 254 plants of *Langra* and 175 plants of *Chausa* among them 192 in *Langra* and 30 plants of *Chausa* were found infected with burl disease. Similarly, García-López *et al.* (2016), also reported the disease incidence ranging from 10 to 50 % in variety *Puntica* from Peravia province of Dominican Republic.

The present study showed that the size of burl is directly related with the age of the trees, which increases with the increasing age and burls were much larger in fully grown/older trees. Prakash and Shrivastava (1987), in their findings, mentioned that the size ranges from 25 to 37.5 cm in diameter and their number varies from 6 to 10 per branch. Furthermore, Prakash (1998) also observed large-sized burls on the stem and main branches whereas they were smaller but prominent on the secondary branches. A similar kind of burl behaviour i.e., size, shape and number of burls was also recorded on stems of coniferous trees by Sinclair *et al.* (1993).

Development of burl may initiate at any stage of tree growth and they may be solitary or in groups (Peterson, 1961; White and Millington, 1954, Mishra and Prakash, 1999). In aged plants, the size and shapes of burls were irregular in shape and size and their size differs from a few cm to 1 meter or more in diameter (White and Millington, 1954). The burl size is directly proportionate to the age of the trees; more the age, larger the size but at the same time newly initiating small-sized burls even can be seen on the main stem and subsequent branches of the tree (Peterson, 1961; Barnard and Freeman (1982). Available literature indicates that the largest size of the burl (i.e., 26 ft in diameter) is so far reported in *Sequoia sempervirens* that covers whole plant trunk while the second-largest burls in the world are reported from British Columbia (https://en.wikipedia.org/wiki/Burl, site visited on 10/03/2021).

5.3 *Identification of Causal Organism*:

Some of the collected samples packed in the polyethene were used to identify the causal organism based on morphological and molecular characterization. Using both approaches, the causal organism was identified as *Agrobacterium tumefaciens* for the burl disease. In the present study, pathogen isolated from the burl wood inoculated on NASA media showed red coloured colonies. Chen *et al.* (1999), used this media and isolated the *A. tumefaciens* which showed a brick red colony of bacteria. For further confirmation of the identity, samples were also inoculated on MGY and MacConkey media. Bacterial colonies appear as fluidal, white to cream coloured, yellowish colour on MGY while they were pink to reddish on MacConkey.

Holt *et al.* (1994), and Bopp *et al.* (1999), reported that *A. tumefaciens* a gram-negative bacteria showed pink to brick-red colour with smooth texture, circular, micoud, translucent and shiny appearance on MacConkey agar media. Islam *et al.* (2010), also employed MacConkey and NASA media and isolated six strains of *Agrobacterium.* Colony characters, gram-negative tests and agreement of present results of the current study coincide with the previous reports and confirmed that the causal organism of burl disease in mango is *Agrobacterium tumefaciens*.

For further confirmation of pathogen was also done by sequencing the 16S rRNA and the identity was confirmed as *A. tumefaciens*. Earlier studies also used a similar approach of 16S rRNA gene sequencing for the confirmation of the *Agrobacterium* identity by using molecular techniques (Mougel *et al.*, 2001; Puopolo *et al.*, 2007; Yang *et al.*, 2011). This method is most commonly used for its identification because it is highly conserved among different species as well as easily sequenced rapidly due to its shorter length (Woese, 1987; Coenye and Vandamme, 2003).

Moreover, inductions of tumours (burls) in response to different species of *Agrobacterium* is well known and is reported by several researchers (Ark and Schroth, 1958; Yajko and Hegman, 1971; Aloni *et al*., 1995; Veselov *et al*., 2003; Tiwary *et al*., 2007; Lee *et al*., 2009; Khan *et al*., 2016). Formation of such tumours in different plant species occurs in response to excess production of growth hormones like auxin and cytokinin that are encoded on T-DNA genes integrated into the plant genome by the *A. tumefaciens* (Veselov *et al*., 2003; Lee *et al*., 2009; Saran *et al*., 2020a).

There is no unanimous opinion about the aetiology of the disease. A review of the literature indicates that burl formation takes place due to the infection of bacteria, virus, and fungi or due to infestation by the insect, and also correlated with environmental stress or genetic mutation (Smith, 2012; Peterson, 1961; Kumar and Saran, 2018). According to Smith (2012), the formation of a burl is the result of hyperplasia, which occurs due to abnormal proliferation of parenchyma cells and deformed xylem by the vascular cambium (Veselov *et al*., 2003; Lee *et al*., 2009; Saran *et al*., 2020a). These abnormal proliferations are an outcome of the rapid division of cambial cells and hormonal changes for a long time and the resulting direction of division become asymmetrical (Ark and Schorth, 1958; Yajko and Hegman, 1971; Aloni *et al*., 1995; Veselov *et al*., 2003; Tiwary *et al*., 2007; Lee *et al*., 2009; Khan *et al*., 2016; Saran *et al*., 2020a). Peterson (1961) also reported that burls and galls are common on hardwoods which are produced due to several insect and pathogens.

Cook (1975) also examined for such type of disease from Miami and Hawaii but, the bacterium could not be identified in affected tissues. Similar to our results, the pathogen Ploetz and Prakash (1997) studied gall development on mango leaves and stems after artificial inoculation of *A. tumefaciens* and reported the development of galls. Similarly, Martin (1957-58), isolated *Agrobacterium* from soil and injected it in young plants of *Sequoia* and found the development of burl. Hartesveldt *et al.* (1975), recorded *A. tumefaciens* as a crown gall organism on *Sequoia* and coast redwood seedlings and concluded that the soil bacteria could have entered into stem through wounds caused by some kind of injuries.

Formation of tumour and gall in mango is also reported in response to *Fusarium decemcellulare* from various countries like the United States of America, Mexico and Venezuela (Malaguti and de Reyes, 1964; Angulo and Villapudua, 1982; Ploetz *et al.*, 1996b). A similar pathogen *F. decemcellulare* was also reported to cause corky wood, gall and canker disease on another woody host in the tropic and sub-tropic region

(Holliday, 1980; Farr *et al.*,1989; Alfieri *et al.*, 1994). Ploetz *et al.* (1996b), reported that *F. decemcellulare* isolates are mildly aggressive and require wounding for infection and burl formation. Based on our results using various approaches and above-cited references, it is clear that the formation of burls on mango trunk and main branches takes place in response to *A. tumefaciens* and not due to fungi, any kind of stress or genetical mutation of the genome in any variety of mango.

5.4 *Pathogenicity*:

To confirm *A. tumefaciens* as a causal organism of tumours or burls formation in mango different pathogenicity test like Koch's postulate by inoculating the isolated pathogen in young saplings of mango (variety *Langra*) and tomato plant along with carrot disc assay is also used in the present study. All these experiments gave positive results and induced burls/tumours. The symptoms appeared within 10-15 day after inoculation and complete tumour formation was observed after two months of inoculation in all three types of experiments. Other researchers employed other methods to check the pathogenicity of the isolates. Among them, carrot disc and potato disc bioassay were found to be the best (Chen *et al.*, 1999; Islam *et al.*, 2010). According to their experiments, tumour formation occurs within 8-10 days of inoculation.

Gupta *et al.* (2012), have also isolated *Agrobacterium* on Hofer's alkaline media, which showed luxurious growth of the pathogen. They also employed a pathogenicity test on the tomato plant and reported the gall development. A similar study for regeneration of the crown gall was used through artificial inoculation (using syringe, needle and wounding) on tomato (Aysan *et al.*, 2003; Chen *et al.*, 1999) and tobacco stems (Chen *et al.*, 1999; Matthysse, 2006). Matthysse (2006) also used *Bryophyllum daigremontiana* plants and observed the development of tumour after the six weeks of inoculation. Razzaq *et al.* (2011) inserted *Agrobacterium* suspension in wheat with the help of a syringe and found that nearly 26 to 27 % of the plant give a positive result. Correspondingly, Naseri *et al.* (2012), used a needle to infect the rice embryo to establish the transformation of *Agrobacterium*.

5.5 *Fruit Yield Losses*:

The present study showed fruit yield loss in burl infected plants and maximum fruit yield loss was recorded in *Mahmood Vikarabad*, *Langra* and *Arka Aruna* whereas it was minimum in *Alphanso* × *Sabja*, *Alphanso* × *Baneshan* and *Olour* variety. As compared to other varieties maximum yield loss was recorded in *Langra* variety not only in Gujarat but throughout the country indicating that *Langra* is the highly susceptible variety for burl disease. Saran *et al*. (2011), reported (162.3 kg in normal vs. 121.4 kg tree^{-1} in burl affected trees) fruit yields losses in *Langra* and (129.1 kg in normal vs. 109.5 kg tree^{-1} in affected trees), in Chausa variety. However, these authors reported contradictory results in *the Gulab Jamun* variety which showed an increase in yield (97.7 kg in normal vs. 100.2 kg tree^{-1} affected).

Kumar and Saran (2018), stated that *Langra* variety is severely affected by burl and showed a great yield loss as compared to other varieties of mango in Uttarakhand. They detected nearly 25.13% yield loss in *Langra* and 11.07 % in *Chausa* variety due to burl disease.

5.6 *Correlation of Burl and Climatic Conditions*:

We also tried to correlate disease incidence, burl size, the number of burls per plant and fruit yield loss with climatic factors like Temperature, Rainfall and Humidity of four (Pariya, Anand, Dantiwada and Junagadh) different agroclimatic zones of Gujarat state. All parameters were reported highest from Pariya (Valsad district, South Gujarat) in the presence of high rain, relative humidity and low temperature. In contrast, these parameters were lowest in the Junagadh region may be due to high temperature and low rainfall, and relative humidity. Saran *et al*. (2020b), reported the highest disease incidence, largest burl size and maximum fruit yield loss in variety *Langra* growing at Pariya location. This area is characterized by the presence of low temperature and highest rainfall and humidity as compared to other parts of the Gujarat state. On the other hand, the minimum burl incidence, burl size and fruit yield loss are recorded from the Junagadh location, which has a high temperature, low rainfall and humidity, respectively. Similar results are also recorded for the *Rajapuri* variety in the present study (Choudhary *et al*., 2021).

The highest incidence, largest burl size, highest fruit yield loss and the maximum number of burls per plant are observed at Pariya (Valsad) region, which may be associated with the highest rainfall, relative humidity and, low temperature. As compared to the Valsad region, the Junagadh region possesses the highest temperature (34.27 ^{o}C) and low relative humidity (60.59 %) which may be the possible reason for the lowest incidence of burl formation in this region. Available literature also indicates that temperature, relative humidity and precipitation are the crucial factors that enhance the growth and multiplication of any microorganisms or pathogens that consequently increase the level of symptoms (Colhoun, 1973; Agrios, 2005; Elad and Pertot, 2014). Climatic factors concerning all types of plant diseases that may alter the physiology of the plant, which indirectly weakens the plant against disease resistance (Agrios, 2005).

5.7 *Correlation of Burl Incidence with Tree Age*:

Burl disease symptoms have shown a positive correlation with the tree age. Its incidence, size, number per plant and fruit yield loss increases with the increase in the age of the trees. Burls are recorded maximum in number in more than 40 years old trees. In a confirming investigation, it was observed that burl size and fruit yield loss in varieties *Langra*, *Chausa* and *Gulab Jamun* were increased with the upsurge of tree age from more than 5 to 15 years old plants (Saran *et al*., 2011). The burl incidence, its size and fruit yield loss are directly related to the tree age, which increases with an increase in the age of the tree. A comparable relationship of age with burl size, disease incidence and fruit yield loss are also reported in several mango varieties of Gujarat region (Saran *et al*., 2020b, c). As per their observations, the disease incidence, burl size and fruit yield loss considerably increased as the tree age increases and the comparison was investigated between 25-90-year-old trees of several mango varieties.

The present study also records maximum burl incidence, maximum fruit yield loss with large burl size and the maximum number of burls per plants as compared to other age groups plants of *Rajapuri* variety. The lowest burl incidence and higher fruit yield loss were noted in 10-20 years age group trees with minimum burl size and the smaller number of burls per plant as compared to trees above forty years also observed in the current study. As mentioned earlier, burl size also enlarges with the increase in age of the tree, which also depends from variety to variety. Ploetz *et al*. (1996b), reported that the burls of mango found in range to 45 cm in diameter with rough, scaly exteriors in cv. *Langra*.

5.8 *Biochemical Analysis*:

Besides all the above observations (morphology, disease incidence, fruit yield loss, pathogen isolation and identification), a comparative biochemical analysis was also carried out for fruits collected from burl infected and healthy (burl free) tress and wood of both varieties. The investigation was carried out in fruits of *Langra* and *Rajapuri* variety to analyze the fruits for Total Soluble Solids (TSS), total sugar, reducing sugar, non-reducing sugar, ascorbic acid and acidity. For wood samples, Moisture content, Ash content, Lignin, cellulose, fibre, total sugar, reducing sugar, ascorbic acid, acidity and total phenol in stem wood was also analysed.

5.9 *Biochemical Analysis of Fruits*:

Analysis of biochemical compounds in fruits revealed that ascorbic acid, Total Soluble Solids, reducing sugar and total sugars were increased significantly in burl infected fruits of both varieties, except acidity, which was lower as compared to healthy samples. In contrast, all these compounds remained relatively less in healthy fruits except acidity, which is recorded maximum. A similar kind of study by Saran *et al.* (2020b), observed increased concentration of ascorbic acid, reducing sugar, non-reducing sugar, total sugar, and Total Soluble Solids contents in burl infected fruits of *Langra* variety, whereas all given parameters were minimum in fruits collected from burl free trees. Available literature also indicates that healthy and diseased plants show significant variations in their metabolism, which consequently alters the biochemical compounds and show an increased concentration of these compounds (Anitha and Suja, 2015; Khan *et al.*, 2017; Kore and Chavan, 1990; Saleem *et al.*, 2017).

Marmit and Sharma (2008) investigated leaf gall of mango and recorded increased concentration of α-amylase, reducing sugar, total sugars, starch, and invertase enzymes activity in affected leaves as compared to normal ones. Similarly, roots infected with *Rhizobacteria* strains significantly increase total soluble solids, total sugar and reducing sugar but showed a decrease in titratable acidity; therefore, the shelf life of burl infected fruits is reduced (Pirlak and Kose, 2009). Present results are in agreement with the previous reports and this could be due to a change in sugar metabolism and hydrolytic enzymes in response to infection.

Shad *et al.* (2002) and Saleem *et al.* (2017), worked on the content of reducing sugar, non-reducing sugar and total sugars in a quick decline disease of mango in variety *Langra*. Their findings revealed that disease affects the production and distribution of sugars metabolism in leaf and wood. According to Shaheen *et al.* (2015), pathogens play important role in the destruction of sugar metabolism in diseased mango trees; therefore, change in sugar content may be the result of the plant defence mechanism.

5.10 *Biochemical Aanalysis of Wood*:

Moisture content is an important factor for a plant's growth and development, at the same time it also helps in the development of microorganism associated with plants. In the presence of moisture, these microorganisms (bacteria and fungi) initiates root rot and leads to wood decay (Brown and Chang, 2014). However, *Agrobacterium* is not a wood-decaying organism rather it induces the formation of tumours. In the current investigation, it was observed that moisture percentage was higher in burl infected wood of both varieties (*Langra* and *Rajapuri*) as compared to burl free trees. This variation in moisture content may be associated with the alterations induced in the composition of xylem tissue, which is more parenchymatous. It is well-known fact that besides the storage of photosynthates, parenchyma cells also act as a reservoir of water (Carlquist, 2001).

Similarly, a comparative study on ash and cellulose content in both normal and affected individuals of both the varieties showed an increase in their concentration than the normal wood. Ash refers to the remains of the inorganic residue after the removal of organic components and water (Saeed *et al*., 2016). Increased concentration of ash indicates the higher content of minerals that play important role in plant development (Saeed *et al*., 2016) and might be playing crucial role in increasing the resistance of the host plant (Nafie, 2003). Cellulose is an important component of the cell wall and provides tensile strength to the primary cell wall (Alberts *et al*., 2002). Available literature indicates that cellulose deficient mutants display an increase in lignification (Malinovsky *et al*., 2014; Chamandoosti, 2017).

However, studies conducted by Malinovsky *et al*. (2014) showed an increase in lignin content in cellulose deficient *Arabidopsis*. In contrast, the present study showed an increase in cellulose content in burl wood. This increase in cellulose content may be associated with the composition of xylem tissue of the investigated species. The burl wood is a kind of tumour and is characterized by a greater number of isodiametric parenchymatous cells. Therefore, an increase in cellulose content may be associated with a greater number of parenchymatous cells (both axial and ray parenchyma) that compose the burl/tumour tissue.

The present study also exhibited a noteworthy increase in the lignin content of the burl wood of both varieties while normal wood has less lignin content. Similar trends of increase in lignin content in the infected samples are also observed by earlier researchers (Harmsen and Nissen, 1965; Hoffmann *et al.*, 1986; Kim, 1990; Blanchette, 1991). An increase in lignin content in burl wood may be attributed to infection of

Agrobacterium that is responsible for the burl induction. There are several reports that lignin plays important role in the defense mechanism against various pathogens including bacteria, fungi and gall-inducing insects (Vance *et al*., 1980; Wainhouse *et al*., 1990; Nicholson and Hammerschmidt, 1992; Lattanzio *et al.*, 2006; Gelbrich *et al.*, 2008; Bhuiyan *et al*., 2009; Xie *et al*., 2018). Deposition of lignin on cell walls not only acts as a physical barricade for pathogen invasion but also has antimicrobial activity, prevents entry of toxins released by the pathogen (Sattler and Funnell-Harris, 2013; Xie *et al*., 2018). An increase in lignin content in burl wood may be associated with the alteration in the xylem structure which is supported histologically in the present study.

Phenol is the important component for plants in response to inducing resistance (Vance *et al*., 1980; Wainhouse *et al*., 1990; Nicholson and Hammerschmidt, 1992; Gelbrich *et al.*, 2008; Xie *et al*., 2018), the phenol content was observed highest in burl infected wood and lowest in normal wood of both the varieties investigated. According to Lattanzio *et al.* (2006), phenolic contents increase in infected tissue due to the interaction of host and pathogens, whereas Gelbrich *et al.* (2008) reported that phenol, ash and sugar have no changes due to bacterial degradation of woods. Nicholson and Hammerschmidt (1992), have studied that phenol compounds (polyphenols) are toxic and their concentration increases in the host plant to enhance the resistance and mechanical strength.

Similarly, all three different sugars *viz*., reducing sugar, non-reducing sugar and total soluble sugar were found comparatively high in burl infected wood (Choudhary *et al*., 2021) than the normal wood in both varieties. Saleem *et al.* (2017), also noted a similar trend of sugar content in quick decline disease of mango in *Langra* variety. In contrast to the present study, opposite results were noticed where the low level of sugar was recorded and reducing sugar have no changes in the infected part of stem wood (Saeed *et al*., 2016). A similar tendency was reported by Saran *et al.* (2020b), which showed higher content of all three types of sugars in burl infected fruits than the fruit collected from trees free from burl disease. Further, a similar study was also conducted in *the Rajapuri* variety which showed the same trend of results. Similar to other parameters, the present study also showed elevated level of ascorbic acid content in burl infected wood than normal (healthy) wood of *Langra* and *Rajapuri* variety (Choudhary *et al*., 2021). Saran *et al.* (2020b) recorded higher content of ascorbic acid in fruits of *Langra* variety associated with burl disease.

5.11 *Burl Anatomy*:

Initiations of tumours (burls) in response to different species of *Agrobacterium* is well described by earlier researchers (Ark and Schorth, 1958; Beardsley *et al*., 1966; Yajko and Hegman, 1971; Aloni *et al*., 1995; Veselov *et al*., 2003; Lee *et al*., 2009; Saran *et al*., 2020a). A causal organism isolated from the burl tissue was identified as *Agrobacterium tumefaciens.* For confirmation, when it was inoculated using carrot disc bioassay, it showed the development of callus like meristematic tissue on the border cambial ring. Initiation of plant tumours takes place due to excessive formation of enzymes like auxin and cytokinin that are programmed on T-DNA genes combined into the plant genome by the bacterium *A. tumefaciens* (Veselov *et al*., 2003; Lee *et al*., 2009). Existing literature shows that the T-DNA on the Ti plasmid of *A. tumefaciens* C58 carries genes for auxin (iaaH and iaaM) and cytokinin (ipt) synthesis and its regulation, and for opine synthesis (Weiler and Schröder, 1987; Zambryski *et al*., 1989; Veselov *et al*., 2003; Lee *et al*., 2009). As the tree age increase, the size of the tumours also increases and differentiation of callus (looking like wound tissue) cells took place into different cell types. Therefore, lignified elements formed in the tumour became deformed and look like hazelwood.

Production of similar type hazelwood due to physical injury and at the branch junctions is known from various dicotyledons. Lev-Yadun and Aloni (1990, 1991, 1995) and Slater *et al*. (2014). Pavlovkin *et al*. (2002), reported a unique type of xylem formation in such tumours (formed in response to *A. tumefaciens*) with a greater number of xylem parenchyma and multiseriate rays with higher metabolic activity and ion absorbing properties. Such uncontrolled xylem differentiation under the influence of high ethylene concentration produced by the tumour cells was also reported earlier by Aloni *et al*. (1998). According to Aloni *et al*. (2003), the development of this type of xylem is related to competent water and nutrient supply to rapidly multiplying tumour cells. Both varieties showed an increase in the number of parenchyma cells with a smaller number of xylem fibres. Reduction in fibre production and other symptomatic uncontrolled alterations in the burl xylem such as wide rays and deformed vessels due to *A. tumefaciens* infection is reported in artificially inoculated *Ricinus* (Aloni *et al*., 1995).

Tyloses development in vessels in response to ageing or various stresses or pathogen is well-known and said to play a fundamental role in blocking the pathogen invasion (Mueller and Beckman, 1984; Dute *et al*., 1999; Micco *et al*., 2016; Saran *et* *al*., 2020a). Incidence of tyloses formation is a common phenomenon in vessels but it is a sporadic feature in tracheids of dicotyledons, gymnosperms and the xylem fibres of hardwoods (Peters, 1974; Micco *et al*., 2016; Rajput *et al*., 2017; Saran *et al*., 2020a). Tyloses most often develop in the vessels (pitted) of the metaxylem and secondary xylem whereas their occurrence is a rare feature in the protoxylem elements having annular or spiral secondary walls (Zimmermann, 1979; Rajput *et al*., 2017; Saran *et al*., 2020a). Both varieties in the present study also showed the development of tyloses in vessels and tracheids that were produced by the callus like meristem of the tumour. Such tyloses formation was also seen in the ray cells of the secondary xylem that connect normal tissue with burl tissue. As stated above, the development of tyloses is a rare feature in tracheids and fibres (Zimmermann, 1979; Rajput *et al*., 2017; Saran *et al*., 2020a) but as per our knowledge, their occurrence in ray cells is reported for the first time in the literature.