2. LITERATURE REVIEW

Plants in general suffer from several diseases in different seasons. They have to withstand with stress of abiotic nature as well as of biotic nature. Thus they have developed wide range of defense mechanisms to combat the stressed situations. The biotic stresses are mostly caused by fungal pathogens. Hence, plants have developed a variety of defense system which may be broadly classified as preformed and post-formed (inducible). It has been experienced that there are several signal molecules which may trigger defense against certain pathogen or pathogens of a plant. A thorough knowledge of the underlying mechanism of induced resistance at physiological, biochemical and molecular levels are now required for better understanding of the spectrum of such resistance in plants. Understandings of mechanisms of induction of different signals are also essentials for sustainable and effective management of diseases in the field.

The present study begins with an overall review of the previous scientific works that has been documented by the earlier scientist and workers. The body of literature with special reference to the present line of investigation has been presented in a selective and concise manner rather than an extensive one.

For convenience, the observations of the previous workers have been divided into six consecutive points:

- Diseases of pointed gourd
- Fungal pathogens- a threat to farming of crops
- Pathogenicity
- Detection techniques of fungal pathogens: Molecular
- Plant defense mechanism
- Defense inducers

2.1. Diseases of pointed gourd

2.1.1. Fruit and vine rot

As pointed gourd is a tropical crop, the major part of its growth phase passes through rainy season. Fruit and vine rot of pointed gourd incited by *Phytophthora melonis* Katsura appears every year in West Bengal (Saha *et al.*, 2004) and cause severe damage. The disease causes rotting of internodal region of the vine, fruit rot and leaf blight. Devastation of the entire crop is a common phenomenon in rainy season. The disease is popularly known as "Haja".

Khatua (2004) described stem rot, fruit rot and vine rot of pointed gourd. He also mentioned about leaf blight, leaf spot and marginal blight symptoms. Oozing of sticking material from the point of infection in humid condition was reported to be characteristic symptoms of the disease. In humid condition, white mycelia growth of the causal fungus with abundant sporangia was found on the infected tissues. The causal pathogen of stem and fruit rot of pointed gourd was identified as *Phytophthora cinnamomi* based on sympoidially branch sporangiophore and internal sporangial proliferation.

Guharoy *et al.* (2006) for the first time reported that *Phytophthora melonis* was a pathogen of *Trichosanthes dioica*. The disease was responsible for the devastating the cultivation of pointed gourd. They identified the pathogen by morphological and molecular tools based on the restriction fragment length polymorphism (RFLP) of non-coding internal transcribed spacer (ITS) region and ITS sequencing.

2.1.2. Net blight

Khatua, (2004) for the first time reported the presence of Net blight of pointed gourd from India. Disease was caused by very small sclerotia forming *Rhizoctonia solani* and was observed during rainy season particularly during the rainy days. He also reported that the disease initiated as water soaked angular spot, delimited by veinlets on lower

surface of the leaves and on maturity leaves dried up. Thin hyphal filaments were also observed by them on the diseased tissue.

2.1.3. Stem rot

Stem rot of pointed gourd was caused by *Sclerotium rolfsii*, as identified by Khatua in 2004. The pathogen, *Sclerotium rolfsii* infected the vines of the plant. It caused rotting of some portion of the stem, which was in close contact with soil, producing white mycelia growth on the affected tissue. Mycelia growth was also visible in the nearby soil. Later sclerotia were formed on the infected tissue and in the nearby soil. Finally, the vine was dried up. Maximum damage was reported when it attacked the roots infested with Root Knot nematode (Khatua, 2004).

2.1.4. Anthracnose

Khatua (2004) observed anthracnose disease in the field crop, grown on scaffold/macha. Individual vines were dried up due to infection on the vine. Lesions on the vine were brown in colour, 2-4 cm in length without superficial mycelia growth. Acervuli were formed on the lesion. The causal organism of anthracnose was reported to be *Colletotrichum capsici*. Acervuli of the fungus were found on stems. Acervuli were round or elongated, intra and sub epidermal. It disrupted outer epidermal cell walls of host. Setae were brown, 1–5 septate, hardly swollen at the base, slightly tapered to the paler acute apex. Conidia were hyaline, falcate with acute apex.

2.1.5. Mosaic disease of pointed gourd

Raj *et al*. (2011) detected *Ageratum enation virus,* a *Begomovirus*, from symptomatic leaf samples of the pointed gourd plants by PCR using coat protein gene-specific primers. The virus was responsible for formation of leaf mosaic symptom, leaf curl symptom, reduction of leaf lamina, deformation of fruits and dwarfing of entire plant. They reported about 20–25% disease incidence and a huge amount of crop loss. They found infestation of whiteflies (*Bemisia tabaci*) in the field. Throughout the world, several viruses from *T. dioica* have been reported (Jones *et al.*, 2000). Bhargava (1977)

reported the presence of *Watermelon mosaic virus* on *T. dioica* from India. Raj *et al.* (2011) found significant crop loss of *T. dioica* by *Trichosanthes mottle virus*.

2.1.6. Downy Mildew

Mondal *et al.* (2014) stated that during winter, pointed gourd plants are attacked by downy mildew causing fungus *Pseudoperonospora cubensis* (Berkeley & M. A. Curtis) Rostovzev, which damage the crop extensively. In West Bengal the disease appears in December and persists until March next year. Early spots were light green in appearance which later turned to chlorotic and finally became necrotic and the affected host plant died.

2.1.7. Powdery Mildew

Bharathi *et al.* (2013) reported the occurrence of powdery mildew disease caused by *Sphaerotheca fuliginea* in pointed gourd plants. They found powdery appearance on leaves along with occasional circular patches or spots on the lower surface of the leaves. The leaves gradually turned brown and after shriveled finally the plants became defoliated.

2.1.8. Sclerotinia rot of pointed gourd

Pointed gourd is a summer season crop but it is also cultivated as an early summer crop for its high market price (Khatua *et al.*, 2014). Due to such untimely cultivation practice, incidences of new diseases were also found to occur. One such disease (Sclerotinia rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary) was found to occur on pointed gourd plants of Birbhum district of West Bengal (Khatua *et al.*, 2014). Mondal *et al.* (2014) reported that Sclerotinia root rot was visible on infected pointed gourd fruit. They found prominent white mycelial growth on the fruits which became reddish-brown and the whole infected fruit rotted gradually. Abundant small to large, elliptical, circular and irregular sclerotia were discernible on the rotted fruits.

2.1.9. Root-knot nematode in pointed gourd

Root-knot nematodes (*Meloidogyne* spp.) are one of the most important and devastating polyphagous pests in agriculture. Infestation on crops greatly impact their health, yield and quality. It is one of the major biotic constraints for the profitable cultivation of pointed gourd in West Bengal (Hussey and Janssen, 2002). The nematode can cause yield loss to the extent of 44% in pointed gourd (Verma *et al.*, 2014).

2.1.10. Post harvested damage of pointed gourd

Naik *et al.* (2003) reported that in summer harvested fruits are attacked by *Fusarium solani.* The fungi form a coat of wooly mycelium on the damaged area of the fruit and appear as if wrapped in absorbent cotton. The interior tissue becomes watery, soft and the decaying matter emits a bad odor. Some other post-harvest reports are fruit rot disease of pointed gourd by *Pythium aphanidermatum* (Chattopadhay and Sengupta, 1952), Fruit rot by *P. cucurbitacearum* (Chaudhuri, 1975) and by *Fusarium equiseti* (Kritagyan *et al.*, 1980).

2.1.11. Insects and Mites causing damage to pointed gourd

Like other cucurbits, the pointed gourd is also subjected to diseases caused by a wide array of insect and non-insect pests such as fruit fly, red pumpkin beetle, leaf miner, aphids, whitefly and mites (Rajak, 2001; Jhala *et al.*, 2005; Gopal Krishnan, 2007; Kumar, 2008; Sapkota *et al.*, 2010; Tiwari *et al.*, 2012) right from the initial stages of the crop to harvest of the products in India. Such pests also attack pointed gourd in some other countries as reported by Rashid *et al.*, 2010; Haque *et al.*, 2011 and Hasan *et al.*, 2012. Patel and Karmakar (2004; 2005) reported for the first time that false spider mite (*Brevipalpus phoenicis*) was a potential pest of pointed gourd from West Bengal. Fruit fly affects pointed gourd in the districts of Malda, Murshidabad and Nadia (Jha *et al.*, 2007). The attack of acharines (Chintha *et al.*, 2002) and root-knot nematodes (Chakraborty, 2000; Khan and Banerjee, 2003) also reported to cause massive damage to the crop.

2.2. Fungal pathogens

Other than the diseases reported above several other fungal pathogens have been reported to be a threat to the farming of pointed gourd. Minor to major crop losses have been observed by the workers in the past. Some of them are being reported in the following lines.

2.2.1. *Curvularia spicifera*

Disease caused by *Curvularia spicifera* have been reported from almost all the major continents, such as Asia (India, Iraq, Pakistan, China and Iran), America (Mexico, Argentina), Europe (Greece), and Africa (Egypt, Morocco) (Golzar, 1987; Ennaffah *et al.*, 1997). According to Behdani *et al.* (2012) *C. spicifera* although show low virulence in general, but in favorable condition it can cause a significant loss of yield. *C. spicifera* has been reported as a causal agent for stem rot disease of Cynodon and Zoysia (Smiley *et al.*, 1992) and it causes leaf lesions on the date palm and ornamental palm (Forsberg *et al.*, 1985). It was also isolated from the seeds of some herbs (Han-Mo *et al.*, 2003; Domsch *et al.*, 1980). Zillinsky (1983) noted that the fungi occasionally attacks rice, wheat, and other cereals. It causes wheat crown rot and leaf spot in soft wheat and durum wheat (in India and Pakistan) and barley (in Mexico). *C. spicifera* was isolated in Iran from roots and barley leaves (Golzar, 1993), from foliar lesions of barley (Abdallah Mohamed and Ali, 2013) and from ears and wheat kernels (Razavi and Amini, 1996). Mehrian *et al.* (1994) reported this fungus as a foliar and stem rot pathogen of corn. In Morocco, *C. spicifera* was isolated from foliar lesions of rice plants (Touhami *et al.*, 2000), rice seeds (Benkirane, 1995), from *Hibiscus rosa-sinensis* (Meddah *et al.*, 2006), *Punica granatum* (Kadri *et al.*, 2011), *Citrullus lanatus* (El Mhadri *et al.*, 2009), *Ficus retusanitida* (Drider *et al.*, 2011), *Erythrina caffra* (Kachkouch *et al.*, 2011), strawberry (Mouden *et al.*, 2016) and *Musa accuminata* (Meddah *et al.*, 2010). The pathogen is also responsible for the tarnishing of rice grains (Gnancadja-André *et al.*, 2004).

2.2.2. *Fusarium equiseti*

Fusarium equiseti is a cosmopolitan pathogen (Nelson *et al*, 1983; Leslie *et al.*, 2006). Messiaen and Casini (1968) considered it as a soil-borne pathogen; commonly grows in warm temperate and subtropical areas. Joffe and Palti (1967) found this fungus to be pathogenic to cucurbits and avocado. The fungus causes root rot in winter wheat and stem rot in maize (Booth, 1971), and was also found to be associated with 'Fusarium head blight' disease in barley and wheat (Gale, 2003; Shaner, 2003). *F. equiseti* also causes diseases to pine species in forest nurseries (Bloomberg, 1981). Sanders and Cole (1981) also isolated it from bluegrass crowns that exhibited symptoms of 'Fusarium blight disease'. The pathogen *F. equiseti* was also found to be pathogenic in a number of plants such as aleppo pine of Algeria (Lazreg *et al.*, 2014), cumin of India (Ramchandra and Bhatt, 2013), cauliflower of China (Li *et al.*, 2017), wheat and heartleaf ice plant of Iraq (Lahuf *et al.*, 2018), zucchini of Morocco (Ezrari *et al.*, 2020). *F. equiseti* also cause infection in the cucurbitaceous plants *viz.* watermelon from Georgia (Li and Ji, 2015) and cantaloupe from Thailand (Nuangmek, 2018). Kritagyan, (1980) recorded its presence in pointed gourd as a fruit rot disease causing fungus.

2.2.3. *Fusarium oxysporum*

Fusarium oxysporum is a major soil borne pathogen and cause wilt diseases on a variety of crop plants (Nelson *et al.*, 1981). Some of the reports of wilt diseases are wilt of Alfaalfa (Weimer, 1928), *Gladiolus grandiflorus* (Riaz *et al.*, 2008), *Lycopersicon esculentum* (Singha *et al.*, 2011), *Chrysanthemum* (Singh and Kumar, 2014), Zunchi (Choi *et al.*, 2015), *Phaseolus vulgaris* (Toledo-Souza *et al.*, 2012), Chickpea (Jendoubi *et al.*, 2017), Banana (Dita *et al.*, 2018) and Pulses (Sinha *et al.*, 2018). It also causes Fusarium yellow of Celery (Lori *et al.*, 2008). The fungi frequently occur in strawberry plants and cause crown and root diseases (Koike *et al.*, 2009; Fang *et al.,* 2013).

2.2.4. *Colletotrichum orbiculare*

Colletotrichum orbiculare is an important pathogen which causes anthracnose of Cucurbitaceae, especially of cucumber (Vakalounakis and Williams, 1991), watermelon (Koike *et al.*, 1991; Monroe *et al.*, 1997), and muskmelon (Keinath, 2018). Farr and Rossman (2013) reported that more than 40 plant host species worldwide are affected by *C. orbiculare*. The pathogen causes lesions on stems, seedlings, petioles, leaves, and fruits of cucurbits. On fruits, circular sunken water-soaked lesions have been reported to form and that expands and turns black in moist weather. In most of the cases lesions becomes covered with pink spore masses. On leaves, lesions are pale brown to reddish, and centers may crack and fall out (Sitterly and Keinath, 1996). *C. orbiculare* also causes anthracnose in *Althaea officinalis* cultivated in Switzerland (Michel, 2005). It also affects *Nicotiana tabacum* (Shen *et al.*, 2001). Morin *et al.* (1993) reported its presence as a pathogen from *Xanthium occidentale*.

2.2.5. Curvularia lunata

Curvularia leaf spot disease, caused by *C. lunata*, is a major maize disease in northern China (Xue *et al.,* 2008; Shi-gang *et al.*, 2017). The fungus is responsible for necrotic spots in the leaves of several plant families (Dasgupta *et al.*, 2005) including Poaceae (Toledo *et al.*, 1990; Yago *et al.*, 2011; Silva *et al.*, 2014; Santos *et al.*, 2014, 2018; Kusai *et al.*, 2016). *Emblica officinalis* of Euphorbiaceae, is also affected by *C. lunata* (Ojha *et al.*, 2017). It also causes disease in *Eriobotrya japonica* in Pakistan (Abbas *et al.*, 2016). *C. lunata* is pathogenic to *Dalbergia sissoo* (Gupta *et al.*, 2017), Cassava (Msikita *et al.*, 2007), *Axonopus compressus* (Zhang, 2017), *Aloe vera* (Avasthi *et al.*, 2015), *Oryza sativa* (Kamaluddeen *et al.*, 2013; Majeed *et al.*, 2015), *Solanum melongena* (Chaudary *et al.*, 2016), Sorghum (Akram *et al.*, 2014) and *Brassica rapa* (Wonglom *et al.*, 2018).

2.2.6. *Alternaria alternata*

Alternaria alternata has been isolated from many diseased plants worldwide. The fungus induces disease symptoms mainly on leaves, fruits, stolons. The fungus has been found to infect several plants such as, *Aloe vera* (Silva and Singh, 2012), *Gerbera jamesonii* (Belle *et al.*, 2019), *Cajanus cajan* (Sharma *et al.*, 2013), *Aegle marmelos* (Maurya *et al.*, 2016), *Corylus heterophylla* (Cheng *et al.*, 2017), *Rhodiola rosea* (Liu *et al.*, 2017), *Drimia maritime* (Bagherabadi *et al.*, 2017), *Solanum lycopersicum* (Ren *et al.*, 2017), *Hydrangea paniculata* (Liu *et al.*, 2017) *Ficus carica* (Dogan *et al.*, 2018), *Avena stiva* (Raza *et al.*, 2018), *Coriandrum sativum* (Mangwende *et al.*, 2018), *Medicago sativa* (Abbasi *et al.*, 2018), *Sonchus oleraceus* (Abdessemed *et al.*, 2019), *Xanthium strumarium* (Abdessemed *et al.*, 2019), *Sonchus asper* (Akhtar, 2019), and *Rosa hybrida* (Fang *et al.*, 2020).

2.2.7. *Alternaria tenuissima*

A number of plants are infected by this fungus *viz.* potato (Jarchelou *et al.*, 2013), cabbage (Rahimloo and ghosta, 2015), *Avicennia marina* (Lin *et al.*, 2016), Raspberry (Cong *et al.*, 2016), *Picrorhiza kurroa* (Vashisht and Chauhan, 2016), *Paeonia lactiflora* (Sun and Huang, 2017), Lentil (Prasad *et al.*, 2017), Russian olive (Chen *et al.*, 2018), Black Chokeberry (Wee *et al.*, 2018), *Pittosporum tobira* (Liu *et al.*, 2018), sugar beet (Khan *et al.*, 2019), *Dioscorea polystachya* (Li *et al.*, 2019), Kiwi fruit (Li *et al.*, 2019), *Iris tectorum* (Sun *et al.*, 2019), *Nelumbo nucifera* (Zhang *et al.*, 2019), *Coreopsis lanceolata* (Li and Liu., 2019). Thus the fungus has a broad host range.

2.2.8. *Alternaria* destruens

Simmons (1998), for the first time, reported *Alternaria destruens* from *Cuscuta gronovii.* Ershad (2009) also reported *Alternaria destruens* as a pathogen infecting sunflower, eggplants, barley and mango in Iran. Again it was found as a pathogen of prunus (Hashemloo *et al.*, 2015), cabbage (Rahimloo and Ghosta, 2015) and *Ligustrum sinense* (Yang *et al.*, 2019).

2.2.9. *Curvularia aeria*

Curvularia aeria have been reported from a number of plants such as durum wheat of Argentina (Carranza and Sisterna, 1989) groundnut of India (Bansal and Mali, 1998), switchgrass of United States (Fajolu *et al.*, 2012), *Etlingera linguiformis* of India (Kithan and Daiho, 2014), *Ficus religiosa* of Pakistan (Nayab and Akhtar, 2016), *Zoysia japonica* (Nechet and Halfeld-Vieira, 2005), tomato (Iftikhar *et al.*, 2016), *Helianthus annuus* of Mexico (Valázquez-del Valle *et al.*, 2017) and lettuce of Thailand (Pornsuriya *et al.*, 2018).

2.2.10. *Curvularia verruciformis*

In 1960, wheat plants were affected by a foot-rot disease and the disease was caused by *C. verruciformis* (Agarwal and Sahni, 1963). The fungus has also been reported to cause leaf spot disease in lemongrass in India (Barua and Bordoloi, 1983).

2.2.11. *Periconia macrospinosa*

Periconia macrospinosa was previously reported as a beneficial endophyte but in some cases it was established as a pathogen such as root and crown rot of wheat and barley in Tanzania (Van Dyk, 2004).

2.2.12. *Aschochyta medicaginicola*

Aschochyta medicaginicola (Synonym: *Phoma medicaginis*) causes disease in alfalfa, lentil and chickpea (Chen *et al.*, 2015; Fan *et al.*, 2018).

2.3. Pathogenicity

A fungus needs to be assessed by some experimental process to confirm its pathogenicity of the fungus to its host. The confirmation of Koch's postulations also requires pathogenicity test of a fungus and re-isolation of the fungus from the artificially infected host tissue. For this, several techniques have been proposed by the previous workers. Some of them are being discussed here. Confirmation of a fungus as pathogen was described by Christensen *et al.* (1988). They used Petri plate test method to screen

pathogenicity of fungi from diseased seedlings, plant roots and seeds. Seedlings of *Brassica oleracea* grown axenically on water agar were inoculated with test fungi and infection was observed directly. Takahashi *et al*. (2009) developed a 'filterpaper method', for the assay of grey leaf spot in Italian ryegrass (*Lolium multiflorum*). Conidial suspension of *Pyricularia oryzae* was dropped on a filter paper. The filter paper with inoculated surface facing downward was then placed on the abaxial surface of a detached leaf placed on an agar plate. For determining the pathogenicity of *Pythium* isolates, Pettitt *et al*. in 2011, developed a detached leaf assay technique in cut-flower chrysanthemum. A slit was made on the petiole of excised young leaf where a plug of mycelium was inoculated. After incubation, the assessment of necrotic diseases was done. Necrosis was observed which indicated the pathogenicity. Giri *et al*. (2013), applied five inoculation methods namely spraying, infiltration, wounding, spore suspension drop and spore suspension drop along with agarose method for establishment of disease by artificial inoculation.

Mbadianya *et al*. (2013), studied the pathogenicity tests on susceptible eggplant variety *Solanum aethiopicum* L. by whole plant inoculation technique, using the fungal isolate *Helminthosporium infestans, Cladophialophora carrionii, Aspergillus niger, Rhizopus nigricans and Neurospora Africana.*

Guney and Guldur (2018), in their study, evaluated the effects of different inoculation methods (soil infestation with wheat bran, root dip and soil infestation with rice grain) on pathogenecity of *Fusarium oxysporum, Rhizoctonia solani*, *Macrophomina phaseolina*, and *Fusarium solani* on pepper seedlings. Pepper seedlings were inoculated and grown for three months after transplanting under growth chamber conditions.

Aregbesola *et al*. (2020), developed a detached leaf assay to assess maize resistance to Southern corn leaf blight, caused by the fungus *Bipolaris maydis.* Assessments of diverse *B. maydis* strains and host genotypes indicated that the detached leaf assay could be used to detect

both highly virulent Southern corn leaf blight strains and highly resistant maize genotypes.

2.4. Detection techniques of fungal pathogen: molecular

Molecular marker based identification of fungi is much more accurate than morphology based identification (Arbefeville *et al.*, 2017) and can be done without specific taxonomic expertise (Capote *et al.*, 2012). In recent years, several molecular methods have been applied for the recognition of fungal species. Those are polymerase chain reaction (PCR)-based methods, DNA/RNA probe-based methods, post-amplification techniques, isothermal amplification-based methods and RNA-Seq-based next-generation sequencing (Hariharan and Prasannath, 2021).

Nowadays, a number of PCR-based techniques have been used to determine the taxonomic status of fungal isolates. Molecular methods are effective to differentiate fungal species, even though their morphology is highly similar (Aoki and O' Donnell, 1999; Demeke *et al.*, 2005). In PCR, through alternate cycles of denaturation, annealing, elongation the target DNA is allowed to synthesize million copies by using specific primers (Fang and Ramasamy, 2015). By using PCR, the detection of fungal DNA is possible even before physiological symptoms are visible on plant tissue (Zhao *et al.*, 2007; Wang *et al.*, 2009). Therefore, some plant pathogens, *viz. Blumeria graminis* f. sp. *tritici* and *Zymoseptoria tritici* could be detected and identified even in a latent period without symptoms (Shetty *et al.*, 2007; Keon *et al.*, 2007; Zeng *et al.*, 2010; Fones and Gurr, 2015).

The entire internal transcribed spacer region comprising of ITS1, 5.8S and ITS2 respectively and the large subunit (nrLSU-26S or 28S), lead the way in a new era of molecular phylogenetic sequence identification in the kingdom fungi (Bruns *et al.*, 1991; Seifert *et al.*, 1995). In the course of evolution, different levels of genetic variation have been observed for these three separate regions. The small subunit region of rRNA is evolving slowly and thus possessing the lowest amount of variation among taxa. On the other hand, the ITS evolves fast and exhibits the highest variation (Bruns *et al.*, 1991; Mitchell and Zuccaro, 2006). In case of the phylogenetic

placement of a fungus at higher taxonomic levels (family, order, class, and phyla), the amplification of sequencing would be better for that kind of study (White *et al.*, 1990). In case of the identification of the intermediate taxonomic levels of fungi (*viz.* family, genera), then amplification and sequencing of the large subunit proved to be very helpful (Vilgalys *et al.*, 1990; Rehner *et al.*, 1995). The large subunit region contains the D1 and D2 hypervariable domains (Liu *et al.*, 2012) and when combined with the ITS region, it become valuable for species identification in fungi (Porras-Alfaro *et al.*, 2014; Schoch *et al.*, 2012; Schoch and Seifert, 2011). For species level identification, the ITS region is the most useful, as it is the fastest evolving portion of the rRNA cistron. Due to its widespread use, ease in amplification, and appropriately large barcode gap (i.e., the difference between interspecific and intraspecific variation), the ITS region was chosen as the official barcode for fungi (Schoch *et al.*, 2012).

Till date a number of fungal identification and barcoding have been done by sequencing of these ITS and large subunit region. For instance, Romanelli *et al.* (2010) by amplifying both the ITS and D1/D2 region, did a sequence-based identification of filamentous Basidiomycetous fungi from clinical specimens. Kelly *et al.* (2011) by using PCR and ITS specific primers tested the utility of the ITS region of nuclear ribosomal DNA for DNA barcoding in lichen-forming fungi. Tejesvi *et al.* (2011) by using fungal ITS region specific primers ITS1F and ITS4R, studied the genetic diversity of endophytic fungi in *Rhododendron tomentosum*. Kru¨ger *et al.* (2012) by using the ITS region or LSU rDNA, or a combination of both, studied the phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. Schmidt *et al.* (2012) identified the wood decay fungi in the urban trees by amplification of the ITS-rDNA.

Taverna *et al.* (2013), in their work, evaluated the ribosomal RNA gene sequencing (both the ITS region and D1/D2 region) for the identification of clinically isolated *Candida* species and compared it with a standard phenotypic method and studied the phylogenetic relationship. Horisawa *et al.* (2013), identified several wood rotting fungi by sequencing the ITS1, ITS 2, and the partial fragments of 28S rDNA region. Wang *et al.* (2014)

identified clinically important fungi and *Prototheca* species by rRNA gene sequencing. In their study, three regions of the rRNA genes were used as targets for sequencing, *viz.* the end of the LSU rRNA gene (D1/D2 region), and the ITS1 and ITS2 regions. Irinyi *et al.* (2015) analyzed the DNA barcoding of fungi causing infections in humans and animals by amplifying the ITS region. Hibbett *et al.* (2016) studied the sequence-based classification and identification of fungi by the sequencing of ITS regions. Jamali *et al.* (2016) identified eight yeast species from uncultivated soils collected from different areas of Iran, by analyzing the hypervariable D1/D2 domain of LSU region of rDNA gene sequence.

The effectiveness of ITS region and other sub-regions as DNA barcode markers for the identification of fungi (Basidiomycota) was studied by Badotti *et al.* (2017). Raja *et al.* (2017) used ITS region dependent DNA barcoding technique for identification and certification of different kind of mushrooms. Gade *et al.* (2017) in their study, demonstrated that the sequencing of 28S LSU region (D1-D2 region) of ribosomal RNA gene can be successfully used for identification genera and some species of fungi. Surženk *et al.* (2017) in their study used a combined molecular approach consisting of PCR-fingerprinting with an M13 primer to identify contaminative fungi isolated from rye breads. The identification of each genotype was done by amplification and sequencing of the ITS region and the D1/D2 region of the LSU of the 28S rDNA. Alsohaili and Bani-Hasan (2018) identified eight filamentous fungi from different environmental sources of Jordon by amplifying the ITS region. Abrego *et al.* (2018) studied the atmospheric fungal diversity by the ITS region amplification. Al-Jaradi *et al.* (2018) identified different kinds of pathogenic fungi and oomycetes associated with beans and cowpea root diseases in Oman by using PCR based identification technique. Badaluddin *et al.* (2018) in their study identified a number of isolated fungi from Kelantan and Terengganu by using ITS region specific primer pairs. Kalmer *et al.* (2018) identified the taxonomic positions of some *Melanoleuca* species as monophyletic by ITS based molecular study. Spoilage organisms of fruits and/or vegetables have

been identified by sequencing the ITS region by several workers (Koffi *et al.*, 2019; Frimpong *et al.*, 2019)

Yousefshahi *et al.* (2020) identified ectomycorrhizal fungi associated with Persian oak tree by amplifying the ITS region. Pornsuriya *et al.* (2020) identified as *Neopestalotiopsis cubana* and *N. formicarum* by amplification of ITS region. Ates *et al.* (2021) identified a number of mushroom disease causing fungi by molecular identification techniques.

In spite of the fact that the rRNA region (mainly ITS region) performs well as a suitable fungal barcoding marker, but still it has been subject to debate (Kiss, 2012). Hence, another protein coding gene, such as ACT gene (Actin gene) sequencing was taken into consideration by some workers (Donnelly *et al.*, 1999; Stielow *et al.*, 2015; Pennington *et al.*, 2016; Zhang *et al.*, 2017; Liu *et al.*, 2018).

2.5. Plant defense mechanisms

To combat the effects of pathogen attacks, plants have evolved the ability to recognize the pathogen and also to trigger an effective response immediately (Bolton, 2009). Detection of pathogen invaders by the plant is one of the most important steps of the complex host-pathogen interaction, in which resistance (R) genes play a crucial role. These host resistance mechanisms involve the recognition of a pathogen gene product called avirulence (avr) factor by a correspondent R gene. When both avr and R genes are compatible the plant will be resistant and the pathogen growth and establishment will be impaired by Hypersensitive Response (HR). HR then triggers diverse responses, including local cell death to reduce the spreading of the pathogen (Bonas and Anckerveken, 1999). Besides this local reaction, the hypersensitive responses activate a signal cascade, including hormones and pathogen related (PR) genes, which are able to establish resistance against a spectrum of different pathogen classes (Benko-Iseppon *et al.*, 2010).

Besides a local reaction, plants may also display the Induced Systemic Resistance (ISR) and Systemic Acquired Resistance (SAR). In case of ISR,

when an antagonist is present at the site of pathogen exposure, several antimicrobial substances could be synthesized by antagonist and are transported through the plant leading to inhibition of the pathogen. As a result of the induced resistance, local protection can be formed in plants. Plant Growth Promoting Rhizobacteria (PGPR) usually induces ISR. It is believed that they produce a translocatable signal which induces protection in tissues away from the roots where the pathogen is attacked. A systemic response of the plant to an elicitor molecule shows that induced resistance is taking place (van Loon *et al.*, 1998). Studies show that ISR is independent of salicylic acid, but it is mediated by jasmonic acid and ethylene, which are produced following applications of some nonpathogenic rhizobacteria (He *et al.*, 2004).

Systemic acquired resistance (SAR) is a mechanism of induced defense responses (Gajanayaka *et al.*, 2014). In case of SAR, a mobile signal is generated in the site of induction and translocated in the plant, distant from the site of exposure to the elicitor and create an induced state in tissues (van Loon *et al.*, 1998). SAR provides long-lasting protection in plants against a broad spectrum of microorganisms. Studies show that, SAR requires the signal molecule salicylic acid and it is associated with the accumulation of PR proteins and contributes a long-lasting resistance (He *et al.*, 2004). The development of SAR is related to numerous cellular defense responses, like fast changes within the cell walls, synthesis of phytoalexins and PR proteins and increased activity of varied defense-related enzymes (Durrant and Dong, 2004). After inoculation with necrotizing pathogens or application of some chemicals such as salicylic acid, SAR is induced systemically (Pieterse *et al.*, 1998; Prasannath *et al.*, 2014). Certain plant growth-promoting microorganisms can also stimulate defense activity and enhance plant resistance against soil-borne pathogens (Whipps *et al.*, 2001). The combined effects of ISR and SAR can increase the defense mechanism of plants against pathogens (Choudhary, 2007).

Resistance genes are generally classified into five categories based on their conserved domains (CD) (Bent, 1996; Hammond-Kosack and Jones,

1997; Ellis and Jones, 2000). The first class is represented by the *Pto* gene of tomato, which encodes a protein with a catalytic serine/threonine kinase (ser-thre-kinase) and a myristoylation motif in the amino terminal region (Martin *et al.*, 1993). The second class comprises many proteins that present a region rich in repetitions of leucine known as Leucine-rich repeats (LRR), a Nucleotide Binding Site (NBS) and a leucine zipper (LZ) or a coiled-coil (CC) sequences. The third class includes similar proteins as described for class II, this region shows homology to a protein domain found in the *Drosophila* Toll and human Interleukin-1 Receptor (IL - 1R), and it is called the TIR domain (Whitham *et al.*, 1994) instead of a coiled-coiled (CC) sequence at the amino terminal region (Meyers *et al.*, 1998) and these proteins are known as TIR-NBS-LRR or TNL proteins (TNL class). The proteins encoded by these three classes of genes do not have a transmembrane sequence and are therefore classified as intracellular R- proteins (Martin *et al.*, 2003). The fourth category of resistance genes belongs to the tomato Cf-family and coding similar proteins with an extracellular LRR and a short cytoplasmic tail. However, no NBS or any such kinds of recognizable domains are present (Dixon *et al.*, 1996). The fifth category includes one gene, the *Xa21* from rice that presents an extracellular LRR, a transmembrane region (TM) and a cytoplasmic ser-thre-kinase. According to Song *et al.* (1997) the structure of *Xa21* indicates an evolutionary link between different classes of plant R-genes. There is still a sixth class that presents genes with no such conserved domains, as described for the previous five classes. This group comprises the genes which do not fit the above five classes like *Hm1* gene from maize (Johal and Briggs, 1992).

Several disease resistance genes (R-genes) have been cloned till date. A list of cloned genes has been presented in the table 2.1.

2.5.1.1. Nucleotide binding site leucine rich repeats (NBS-LRR) in cucurbits (R-gene)

Several NBS-LRR disease resistance genes have been isolated from cucurbitaceae. A total of 15 homologues of NBS-LRR gene family have been

isolated from *Cucumis melo* (Brotman et. al., 2002). Harris *et al.* (2009) cloned 66 disease resistance gene analogs (RGAs) from watermelon (*Citrullus lanatus* var. *lanatus*). Wan and Chen (2010) in their study identified and cloned twenty eight NBS-type resistance genes from an extreme resistance to downy mildew introgression line from *Cucumis hystrix* ×*Cucumis sativus*. Lin *et al.* (2013) reported that sequenced genomes of watermelon, melon and cucumber had relatively a few R-genes. They mainly emphasized on the frequent loss of lineages and deficient duplications that accounted for low copy number of disease resistance genes in cucurbitaceae family. Yang *et al.* (2013) studied NBS-LRR type resistance gene homologs (RGHs) from cucumber and characterized 70 NB-containing RGHs from the Gy14 draft genome. In 2013, Wan *et al.* performed a genome-wide analysis of NBS-encoding disease resistance genes in *Cucumis sativus* and also phylogenetically studied NBS-encoding genes belonged to six species in five genera of cucurbitaceae. In 2014, González *et al.* identified 23 NBS-LRR genes in the melon genome. Andolfo *et al.* (2017) characterized disease resistance (R) gene family in *Cucurbita pepo*. Gharaei and Zamharir (2017) in their study identified a number of new NBS-LRR gene members in native types of cucurbit species in Iran. The cluster of NBS-LRR genes located in different chromosomes present in melon, cucumber and watermelon were also analyzed by Morata and Puigdomènech (2017). Wu *et al.* (2017), identified and characterized 84 R-genes (NBS-LRR) in the bottle gourd genome. Hassan *et al.* (2019) studied six R genes of NBS-LRR gene family in watermelon. Belen *et al.* (2020) reported two subfamilies (*viz.* the non-TIR-NBS-LRR and TIR-NBS-LRR) of the NBS-encoding gene family.

Table. 2.1. Cloned disease resistance genes (R genes) in plants:

Species	R-Gene	Domain	Disease	Avr Gene	Pathogen	Reference
Triticum aestivum (wheat)	*Lr10*	CNL	Leaf rust	*AvrLr10*	*Puccinia triticina*	Feuillet *et.al.*, 2003
	Lr1	CNL	Leaf rust	*Avr1*	*Puccinia triticina*	Cloutier *et.al.*, 2007
	Pm3b	CNL	Powdery mildew	*AvrPm3b*	*Blumeria graminis*	Yahiaoui *et.al.*, 2004
	Sr33	CNL	Stem rust	-	*Puccinia graminis*	Periyannan *et.al.*, 2013
	Sr35	CNL	Stem rust	-	*Puccinia graminis*	Saintenac *et.al.*, 2013
	Lr21	NL	Leaf rust	*AvrLr21*	*Puccinia triticina*	Huang *et.al.*, 2003
	Cre3	NL	Cereal cyst	-	*Heterodera avenae*	Lagudah *et.al.*, 1997
	Cre1	NL	Cereal cyst	-	*Heterodera avenae*	De Majnik *et.al.*, 2003
	Yr10	CNL	Stripe rust	-	*Puccinia striiformis*	Liu *et.al.*, 2014
	Lr34	*ABC transporter*	Leaf rust, Powdery mildew, Stripe rust	-	*Puccinia triticina; Puccinia striiformis*; *Blumeria graminis*	Krattinger *et.al.*, 2009
	Yr36	Kinase-START	Stripe rust	-	*Puccinia striiformis*	Fu *et.al.*, 2009
Hordeum vulgare (barley)	*Mla6*	CNL	Powdery mildew	*AvrMla6*	*Blumeria graminis*	Halterman *et.al.*, 2001
	Mla1	CNL	Powdery mildew	*AvrMla1*	*Blumeria graminis*	Zhou et.al., 2001
	Mla13	CNL	Powdery mildew	*AvrMla13*	*Blumeria graminis*	Halterman et.al., 2003
	Rpg1	LRR-PK	Stem rust	*Avr-Rpg1*	*Puccinia graminis*	Brueggeman et.al., 2002
	Mlo	TM	Powdery mildew	-	*Erysiphe graminis*	Buschges et.al., 1997

Table. 2.1. Cloned disease resistance genes (R genes) in plants:

Species	***R-Gene***	**Domain**	**Disease**	***Avr Gene***	***Pathogen***	**Reference**
Solanum lycopersicum (tomato)	*Prf*	CNL	Bacterial speck	*AvrPto*	*Pseudomonas syringae*	Salmeron et.al.,1996
	Mi	CNL	Root knot	-	*Meloidogyne javanica*	Milligan et.al., 1998
	I2	NL	Fusarium wilt	*Avr1*	*Fusarium oxysporum*	Ori et.al.,1997
	Ph-3	CNL	Late blight	-	*Phytophthora infestans*	Zhang et.al., 2014
	Sw-5	CNL	Tomato spotted wilt	-	*Tomato spotted wilt virus*	Brommonschenkel et.al., 1997
	Tm-2	CNL	Tobacco mosaic	-	*Tobacco mosaic virus*	Lanfermeijer et.al., 2003
Solanum tuberosum (potato)	*Cf-5*	LRR-TM	Leaf mold	*Avr5*	*Cladosporium fulvum*	Dixon *et.al.*, 1998
	Cf-9	LRR-TM	Leaf mold	*Avr9*	*Cladosporium fulvum*	Jones *et.al.*, 1994
	Ve1,2	LRR-TM	Verticillium wilt	-	*Verticillium dahliae*	Kawchuk *et.al.*, 2001
	Pto	STK	Bacterial speck	*AvrPto*	*Pseudomonas syringae*	Martin *et.al.*, 1993
	Pti1	STK	Bacterial speck	-	*Pseudomonas syringae*	Zhou *et.al.*, 1995
	Rx	CNL	PVX	-	Potato virus X	Bendahmane *et.al.*, 1999
	RB	CNL	Late blight	*Avr1*, *Ipio*, *Ipib*	*Phytophthora infestans*	Song *et.al.*, 2003
	Rx2	LZ-NL	PVX	-	Potato virus X	Bendahmane *et.al.*, 2000
	R1	LZ-NL	Late blight	*Avr1*	*Phytophthora infestans*	Ballvora *et.al.*, 2002
Lactuca sativa (lettuce)	*Rgc2 (Dm3)*	NL	Downy mildew	*Avr3*	*Bremia lactucae*	Meyers *et.al.*, 1998
Piper nigrum (black pepper)	*Bs2*	CNL	Bacterial spot	*AvrBs2*	*Xanthomonas campestris*	Tai *et.al.*, 1999

Table. 2.1. Cloned disease resistance genes (R genes) in plants:

Species	***R-Gene***	**Domain**	**Disease**	***Avr Gene***	***Pathogen***	**Reference**
Oryza sativa (rice)	*Xa1*	NL	Bacterial blight	*AvrXoo*	*Xanthomonas oryzae*	Yoshimura *et.al.*, 1998
	Pi-ta	NL	Blast	*Avr-Pita*	*Magnaporthe grisea*	Bryan *et.al.*, 2000
	Pi36	CNL	Blast	*Avr-Pi36*	*Magnaporthe grisea*	Liu *et.al.*, 2007
	Pia	CNL	Blast	*AvrPia*	*Magnaporthe grisea*	Okuyama *et.al.*, 2011
	Pi37	NL	Blast	-	*Magnaporthe grisea*	Lin *et.al.*, 2007
	Xa5	NL	Bacterial blight	*AvrXa5*	*Xanthomonas oryzae*	Iyer *et.al.*, 2004
	Xa13	SET	Bacterial blight	*AvrXa13*	*Xanthomonas oryzae*	Chu *et.al.*, 2006
	Pi54	CNL	Blast	*AvrPi54*	*Magnaporthe grisea*	Das *et.al.*, 2012
	Pi9	CNL	Blast	*AvrPi9*	*Magnaporthe grisea*	Liu *et.al.*, 2002
	Piz-t/Pi2	CNL	Blast	*AvrPiz-t*	*Magnaporthe grisea*	Zhou *et.al.*, 2006
	Rpr1	CNL	Blast	-	*Magnaporthe grisea*	Sakamoto *et.al.*, 1999
	Pid3	CNL	Blast	-	*Magnaporthe grisea*	Shang *et.al.*, 2009
	Xa3/Xa26	LRR-STK	Bacterial blight	-	*Xanthomonas oryzae*	Sun *et.al.*, 2006
	Xa10	LRR-STK	Bacterial blight	*AvrXa10*	*Xanthomonas oryzae*	Tian *et.al.*, 2014
	Xa25	TM	Bacterial blight	-	*Xanthomonas oryzae*	Liu *et.al.*, 2011
	Xa27	LRR-TM	Bacterial blight	*AvrXa27*	*Xanthomonas oryzae*	Bimolata *et.al.*, 2013
	Pi-d2	B-lectin, STK	Blast	-	*Magnaporthe grisea*	Chen *et.al.*, 2006

Table. 2.1. Cloned disease resistance genes (R genes) in plants:

Species	***R-Gene***	**Domain**	**Disease**	***Avr Gene***	***Pathogen***	**Reference**
Zea mays (maize)	*Rp1-D*	NL	Rust	-	*Puccinia sorghi*	Collins *et.al.*, 1999
	Hm1	NL	Corn leaf blight	-	*Cochliobolus carbonum*	Johal *et.al.*, 1992
Arabidopsis thaliana (*Arabidopsis*)	*RPM1*	CNL	Downy mildew	*AvrB*, *AvrRpm1*	*Pseudomonas syringae*	Grant *et.al.*, 1995
	RPS2	NL	Downy mildew	*AvrRpt2*	*Pseudomonas syringae*	Bent *et.al.*, 1994
	RPP8/ HRT	CNL	Downy mildew	*AvrRPP8*	*Peronospora parasitica*	McDowell *et.al.*, 1998
	RPP13	CNL	Downy mildew	*ATR13*	*Peronospora parasitica*	Bittner-Eddy *et.al.*, 2000
	RCY1	CNL	Mosaic type	-	Cucumber mosaic virus	Takahashi *et.al.*, 2002
	RPP1	TNL	Downy mildew	*ATR1*	*Peronospora parasitica*	Botella *et.al.*, 1998
	RPP4	TNL	Downy mildew	-	*Peronospora parasitica*	Van der Biezen *et.al.*, 2002
	RPS4	TNL	Powdery mildew	*AvrRps4*	*Pseudomonas syringae*	Gassmann *et.al.*, 1999
	RPP5	TNL	Downy mildew	*AvrRp5*	*Peronospora parasitica*	Noel *et.al.*, 1999
	RPS5	NL	Downy mildew	*AvrRphB*	*Pseudomonas syringae*	Warren *et.al.*, 1998
	RRS1	WRKY-TNL	Bacterial wilt	*AvrRRS1*	*Ralstonia solanacearum*	Deslandes *et.al.*, 2002
	RPP27	LRR-TM	Downy mildew	-	*Peronospora parasitica*	Tor *et.al.*, 2004
	RFO1	LRR-STK	Fusarium wilt	-	*Fusarium. oxysporum*	Diener *et.al.*, 2005
	PBS1	STK		*AvrPphB*	*Pseudomonas syringae*	Swiderski *et.al.*, 2001

Table. 2.1. Cloned disease resistance genes (R genes) in plants:

Species	***R-Gene***	**Domain**	**Disease**	***Avr Gene***	***Pathogen***	**Reference**
Arabidopsis thaliana (*Arabidopsis*)	*FLS2*	LRR-STK	Powdery mildew	*AvrPt*, *AvrPtoB*	*Pseudomonas syringae*	Tabata *et.al.*, 2000
	NDR1	TM		*AvrB*, *AvrRpt2*	*Pseudomonas syringae*; *Peronospora parasitica*	Century *et.al.*, 1997
	RPW8	TM-CC	Powdery mildew	-	*Erysiphe cruciferarum*	Xiao *et.al.*, 2001
Linum usitatissimum (flax)	*L6*	TNL	Rust	*AvrL6*	*Melampsora lini*	Lawrence *et.al.*, 1995
	L, L1-L11	TNL	Rust	*AvrBs3*	*Melampsora lini*	Ellis *et.al.*, 1999
	M	TNL	Rust	*AvrM*	*Melampsora lini*	Anderson *et.al.*, 1997
Beta vulgaris (sugar beet)	*Hs1pro-1*	LRR-TM	Beet cyst	-	*Heterodera schachtii*	Cai *et.al.*, 1997
Nicotiana tabacum (tobacco)	*N*	TNL	Tobacco mosaic	-	Tobacco mosaic virus	Whitham *et.al.*, 1994

2.5.1.2. Pathogenesis Related proteins

The Pathogenesis Related proteins (PR-proteins) are a group of diverse proteins that are induced by pathogens as well as some defense-related signaling molecules. Whenever, a plant faces pathogen challenge, activation of defense signaling pathways *viz.* Salicylic acid (SA) and Jasmonic acid (JA) take place, which further leads to the accumulation of PR proteins. Accumulation of PR-proteins minimizes the pathogen load or disease occurrence in uninfected plant organs.

There are two different forms of pathogens, namely, biotrophic and necrotrophic. Biotropic pathogens activate the SA pathway. The SA pathway then stimulates the transcription of NPR1 (non-expressor of pathogen-related gene 1) and leads to the activation as well as accumulation of SA related gene (PR1, PR2 and PR5) products. This entire process finally leads to the systemic acquired resistance (SAR). On the other hand, necrotrophic pathogen stimulates JA pathway. Local acquired resistance (LAR) relies on JA pathway. The JA pathway directs the activation of JA related genes (PR3, PR4 and PR12) which finally encode products that minimize pathogen load (Ali *et al.*, 2017b). The SAR provides resistance to a wide range of pathogens (Sticher *et al.*, 1997; Van Loon *et al.*, 2006; Fu and Dong, 2013). PR proteins are the widely distributed proteins in the plant domain and are present in all plant organs particularly within the leaves (Van Loon *et al.*, 1994). PR proteins are low-molecular weight proteins (5–45 kDa), resistant to proteases, thermostable and stay soluble at low pH (Van Loon *et al.*, 1994).

There are two subgroups of PR-proteins present in plants. The acidic PR protein secreted to the extracellular space and basic PR proteins containing the signal sequence are located in the C-Terminal end. The signal sequence present in the C-Terminal end of the protein helps in the transportation of the protein to the vacuole (Takeda *et al.*, 1991). PR-proteins are mainly accumulated in the apoplastic region however they are also found as vacuolar (Van Loon *et al.*, 1994). According to the results of transcriptomic studies, both biotic and abiotic stresses produce a significant amount of PR genes which lead to the development of multiple stress tolerant crop varieties (Ali *et al.*, 2017a,b, 2018; Dai *et al.*, 2016; Jiang *et al.*, 2015; Gupta *et al.*, 2013; Archambault and Strömvik, 2011; Fountain *et al.*, 2010; Seo *et al.*, 2008).

PR proteins are basically induced after pathogen attack. They are systematically classified into 17 families from PR-1 to PR-17 based on their biochemical and molecular/biological properties (van-Loon *et al.*, 2006). On the basis of the similarities among sequences and the serological or immunological properties the classification was done (van-Loon *et al.*, 1999).

Most of the PR proteins are known to have antifungal properties. The molecular mechanisms of PR2 (β-1, 3-glucanases) and PR3 (chitinases) are well known (Kitajima and Sato, 1999). PR1 is the PR protein that accumulates copiously after pathogen infection and its genes have been cloned in many plants, such as tobacco (Gaffney *et al.*, 1993), *Arabidopsis thaliana* (Metzler *et al.*, 1991) and tomato (Tornero *et al.*, 1997). PR1 gene class is considered to be a typical SAR marker (Bonasera *et al.*, 2006). PR-5 is a thaumatin like protein which has high antifungal activity. It is also expressed in overwintering monocots where it performs antifreeze activities (Hon *et al.*, 1995, Atici and Nalbantolu, 2003, Griffith and Yaish, 2004). The rest of the well-studied protein families are PR-8 (*i.e* Glycosyl hydrolase), PR-9 (*i.e.* the secretory peroxidase), PR-14 (*i.e* lipid transfer proteins), PR-15 (*i.e* oxalate oxidase) and PR-17 (*i.e* basic secretory proteins) (Nanda *et al.*, 2010). They are also involved in plant defense responses but their molecular mechanisms of action for defense are not yet known (Bolton, 2009). Under normal growth conditions, most PR genes are expressed at a minimal level, but after pathogen infection, it increase rapidly in plant cells. Environmental factors, such as osmotic, light and cold stress also regulate PR gene expression (Zeier *et al.*, 2004).

2.5.1.3. Defense-related enzymes

Proteins such as phenylalanine ammonia lyase (PAL), peroxidase, β-1,3-glucanase, chitinase and polyphenol oxidase (PPO) are responsible to induce resistance in plants (Prasannath and De Costa, 2015; Gajanayaka *et al.*, 2014; Seneviratne *et al.*, 2014). Peroxidases have been reported to involve in a range of defense-related processes, including the hypersensitive response, cross–linking of phenolics and glycoproteins, lignification, suberization and phytoalexin production (Nicholson and Hammerschmidt, 1992; Wojtaszek, 1997). Polyphenol oxidase is known to decrease the nutritional quality of food and protein digestibility by catalyzing the phenolic compounds to quinones (Felton and Duffey, 1990; Felton *et al.*, 1994). The increased production of phenolic compounds in infected plants correlates with the increase in phenylalanine ammonia lyase activity in wounded plant tissues

(Bi and Felton, 1995). Chitinase and β-1,3-glucanase are known to provide protection to plants as they are responsible for the hydrolysis of cell wall components such as chitin and β-1,3-glucans of fungus (Ebrahim *et al.*, 2011).

2.5.1.3.1. Peroxidase

Peroxidases belong to PR-9 sub family and are basically involved in strengthening of cell wall. The mode of action is quite simple. They suppress cellular spreading of infection through establishment of structural barriers or by generating reactive oxygen species, thus producing a toxic environment (Passardi *et al.*, 2005). They also produce oxidative burst which is a significant early response of host plant cells against pathogen infection (Almagro *et al.*, 2009). During the process of pathogenesis, the concentration of ethylene becomes high and peroxidase participates in this process (Tudzynski, 1997). Peroxidases are known to involve a number of physiological functions that may directly help in resistance (Thakker *et al.*, 2013). Increase in the peroxidase level by pathogen attack leads to the activation of induced systemic resistance (Prasannath *et al.*, 2014). Quick synthesis of reactive oxygen derivatives is required to fight off invading pathogens (Halfeld-Vieira *et al.*, 2006). Peroxidase leads to the bioconversion of phenolic compounds to quinones and generates hydrogen peroxide which inhibits the growth and development of the infectious agent (Agrios, 2005). Kumar *et al.* (2008) characterized the peroxidase gene from the leaves of *Ricinus communis*. Chen *et al.* (2009) characterized the Peroxidase Gene from Soybean seed coat. Simonetti *et al.* (2009) analyzed the class III peroxidase genes from the roots of susceptible and resistant wheat lines infected by *Heterodera avenae.* Fan *et al.* (2014) isolated and characterized the Class III peroxidase cDNA from Cucumber under the salt stress condition. Basha and Rao (2017) characterized the peroxidase gene from sprouted green gram roots. Wu *et al.* (2019) studied the identification, duplication, phylogeny and expression of the class III peroxidase gene family in Cassava. Li *et al.* (2020) in their study characterized the class III

peroxidase gene family and studied their expression profiles under drought stress in *Camellia sinensis.*

2.5.1.3.2. β-1,3-glucanase

The PR-2 proteins are known as β-1,3-glucanases. These are glucan endo-1,3-β-glucosidases (β-glucanases) and catalyzes the endo-type hydrolytic cleavage of the 1,3-β-D glucosidic linkages in β-1,3-glucan. The β-1,3-glucans is the major components of the cell walls of oomycetes type of fungi which do not contain chitin (Wessels *et al.*, 1981). It is proved to be effective against various fungi (Prasannath, 2017). It helps to induce defense responses against pathogen attack in plants (Smart, 1991). The enzyme work at least in two different ways: one by degrading the cell walls of the pathogen directly or by triggering the release of cell wall derived materials that can act as elicitors which help to induce defense response in plants (Bowles *et al.*, 1990). Till now a number work have been done to study the β -1,3-Glucanase gene in different plants *viz.* strawberry (Mercado *et al.*, 2010), wheat (Liu *et al.*, 2010; Gao *et al.*, 2016), *Pisum sativum* (Amian *et al.*, 2011), grapevines (Nookaraju and Agrawal, 2012), *Pyrus pyrifolia* (Liu *et al.,* 2013), *Eruca sativa* (Gupta *et al.*, 2013), *Arachis hypogaea* (Qiao *et al.*, 2014), *Brassica napus* (Kheiri *et al.*, 2014), *Podophyllum hexandrum* (Dogra and Sreenivasulu; 2014), *Gossypium* (Xu *et al.*, 2016), Sugarcane (Nayyar *et al.*, 2017), *Panax notoginseng* (Taif *et al.*, 2019), *Hevea brasiliensis* (2020), alfalfa (Hanin *et al.*, 2020).

2.5.1.3.3. Chitinase

Chitinases are a diversified group of enzymes that help in plant defense by degrading chitin, a major wall component of several fungi. They are collectively known as plant pathogenesis-related (PR) protein. It improves plant defense against pathogen having chitin as a major wall component (Jalil *et al.*, 2015). Chitinase is considered as direct defense enzyme in plants (Abeles *et al.*, 1970). In addition, Mauch *et al.* (1988) reported that chitinase and β-1,3- glucanase act synergistically to inhibit fungal growth. The mode of action of chitinase is quite simple as compared to other

enzymes. *In situ* degradation of the cell wall chitin polymers weakens the cell wall, making fungal cells osmotically sensitive (Jach *et al.*, 1995). These chitinases play a significant antifungal role against *Rhizoctonia solani* for rice sheath blight, *Alternaria* spp. responsible for rice grain discoloration, *Bipolaris oryzae* for rice brown spot, *Botrytis cinerea* for tobacco blight and *Curvularia lunata* for clover leaf spot. The enzyme also play antifungal role to several pathogens such as *Fusarium oxysporum*, *F. udum*, *Mycosphaerella arachidicola* and *Pestalotia theae* (Chu and Ng, 2005; Saikia *et al.*, 2005; Kirubakaran and Sakthivel, 2006). A number of research works have been done till now, to study the chitinase gene in different plants *viz.* grapevines (Nookaraju and Agrawal, 2012), *Casuarina equisetifolia* (Veluthakkal and Dasgupta, 2012), transgenic litchi (Das and Rahman, 2012), Sugarcane (Su *et al.*, 2015; Rahul *et al.*, 2013), cotton (Xu *et al.*, 2016), transgenic potato (Khan *et al.*, 2016), *Drosera rotundifolia* (Ďurechová *et al.*, 2013), rice (Iqbal *et al.*, 2012; Zaynab *et al.*, 2017), *Eucalyptus grandis* (Tobias *et al.*, 2017), *Brassica juncea* (Rawat *et al.*, 2017), Tobacco (Dong *et al.*, 2017), *Lilium* plants (Caceres-Gonzalez *et al.*, 2015), pepper plant (Liu *et al.*, 2017), *Brassica rapa* (Chen *et al.*, 2018), Tomato (Cao and Tan, 2019), Cucumber (Bartholomew e al., 2019), *Brassica juncea* and *Camelina sativa* (Mir *et al.*, 2019), tuber mustard (Ojaghian *et al.*, 2020).

2.5.1.3.4. Phenylalanine ammonia lyase (PAL)

PAL is one of the most extensively studied enzymes in plants due to the synthesis of various phenolic compounds as well as anthocyanin which are responsible for the resistance of plants against pathogens (Dixon *et al.*, 1995). By the rate of conversion of phenylalanine to the trans-cinnamic acid the PAL activity was measured by several workers. The cinnamic acid forms all phenylpropanoid compounds. These phenylpropanoids are responsible to induce disease resistance, crop development and mechanical support (Barber and Mitchell, 1997; Chen *et al.*, 2007; Harakava, 2005) as well as insect pest damages (War *et al.*, 2012). Cinnamic acid, the pathway product, can regulate PAL activity by modifying the expression of the PAL gene through feedback inhibition (Christensen *et al.*, 2001; Del Rio *et al.*, 2004).

Now a days, the characterization of PAL gene from different plants became a trend for many molecular biologists. Till date a number of plants have been studied for this purpose *viz. Cistanche deserticola* (Hu *et al.*, 2011), *Jatropha curcas* (Gao *et al.*, 2012), *Citrullus lanatus* (Dong and Shang, 2013), banana (Alvarez *et al.*, 2013), sugarcane (Hashemitabar *et al.*, 2014), pea plant (Okorska *et al.*, 2014), *Carthamus tinctorius* (Dehghan *et al.*, 2014), *Ginkgo biloba* (Zhang *et al.*, 2014), poplar plant (Jong *et al.*, 2015), *Solenostemon scutellarioides* (Zhu *et al.*, 2015), Salvia (Valifard *et al.*, 2015), melon and cucumber (Dong *et al.*, 2016), *Anacardium occidentale* (Sija *et al.*, 2016), *Juglans Regia* (Yan *et al.*, 2019), Rose plant (Li *et al.*, 2019) and *Corchorus olitorius* (Hossain *et al.*, 2020).

2.5.1.3.5. Polyphenol oxidase (PPO)

Polyphenol oxidase (PPO) is a group of copper-containing enzymes that catalyze the oxidation of hydroxy phenols to their quinine derivatives, which have antimicrobial activity (Chunhua *et al.*, 2001). PPO plays a major role in inducing defense against plant pathogens because of its reaction products and wound inducibility (Mayer and Harel, 1979). PPO activity increases after pathogen infection (Chen *et al.*, 2000; Deborah *et al.*, 2001). Thakker *et al.* (2007) reported an increased PPO activity in banana roots treated with *Fusarium oxysporum* derived elicitor. Increase in PPO activity was observed in banana roots treated with *Psuedomonas fluorescens* against Fusarium wilt (Sarvanan *et al.*, 2004). Several groups have also tried to correlate the protective effects of rhizosphere bacteria with the induction of defense enzymes such as PPO (Ramamoorthy *et al.*, 2002; Chen *et al.*, 2000). Li and Steffens (2002) in their study suggested that PPO-generate quinones in plant cells, which accelerate cell death. The alkylation and reduction of the bioavailability of cellular proteins to the pathogen, crosslinking of quinones with protein or other phenolics form a physical barrier for the pathogens (Jiang and Miles, 1993). There are many workers who have studied the PPO gene from different plants *viz. Camellia sinensis* (Wu *et al.*, 2010; Huang *et al.*, 2018; Ke *et al.*, 2020), Eggplant (Shetty *et al.*, 2011), poplar plant (Tran and Constabel, 2011), *Litchi Chinensis* (Wang *et al.*, 2014), fuji

apple (Liu *et al.*, 2015), *Coreopsis grandiflora* (Kaintz *et al.*, 2015), Strawberry (Jia *et al.*, 2015), *Triticum aestivum* (Hystad *et al.*, 2015), *Prunus armeniaca* (Derardja *et al.*, 2017) and *Olea europaea* (Cirilli *et al.*, 2017).

2.6. Defense inducers

Chen *et al.* (2009) studied the role of salicylic acid (SA) in plant defense against pathogens. They found endogenous SA level increased in correlation with both resistance of tobacco against tobacco mosaic virus and induction of defense-related genes (that PR-1). Conrath *et al.* (1995) also used SA to induce plant defense responses by enhancing the production of PR proteins. Chandra *et al.* (2007) studied PAL activities leading to decline in disease formation caused by *Rhizoctonia solani* following application of SA. Jendoubi *et al.* (2015) used SA to study its effect on resistance against *Fusarium oxysporum f. sp. pradicis lycopercisi* in hydroponic grown tomato plants. Zehra *et al.* (2017) investigated the collective effect of *Trichoderma harzianum*, exogenous SA and methyl jasmonate against Fusarium wilt disease in tomato plants. Umar *et al.* (2019) estimated the probable role of induced systemic acquired resistance in mungbean against *Mungbean yellow mosaic virus* (MYMV) disease. They found that, exogenous application of SA and Benzothiadiazole (BTH) triggered the SA pathway and enhanced the resistance in mungbean plants. Acharya *et al.* (2011) induced systemic resistance in *Raphanus sativus* L. by using five abiotic elicitors (arachidonic acid, cupric chloride, chitosan, isonicotinic acid and SA). Similarly, Alkahtani *et al.* (2011) studied effects of six abiotic elicitors (*viz.* Bion, Oxalic acid, SA, fungastop, potassium oxalate and photophor) for the induction study in cucumber against powdery mildew. Ullah *et al.* (2019) studied the effect of SA, Jasmonic acid (JA), and Abscisic acid (ABA) to confer resistance against *Melampsora larici-populina*, the causal agent of rust disease, in Poplar trees. They reported that, there was a strong association present among SA, flavan-3-ol biosynthesis, and rust resistance in poplars. In addition, they also showed ABA induced defense against rust infection in Popler trees. Al-Sohaibani *et al.* (2011) to combat the root rot disease of sweet basil caused by *Macrophomina phaseolina*, *Fusarium oxysporum* f. sp.

basilica and *Rhizoctonia solani* used four organic acids (oxalic acid, ABA, tannic acid, and SA); four different salts (Dipotassium phosphate, potassium chloride, disodium phosphate and sodium chloride) and two growth regulators (Indole butyric acid and Indole acetic acid). Singh *et al*. (2020) reported that among the abiotic chemicals tested, SA was best to activate plant defense against white rust of Indian mustard cultivar RH-749 under artificial epiphytotic conditions.

BTH, a salicylic acid analogue chemically known as 1,2,3-benzothiadiazole-7-thiocarboxylicacid-S-methyl-ester and commercially known as 'Actigard' is a novel chemical activator. Several scientists have used this for activation of resistance in different plants against several pathogens. In 1996, Lawton *et al*. reported that BTH conferred disease resistance in tobacco, wheat and other important agricultural plants. In their report they have shown that BTH worked by activating SAR in *Arabidopsis thaliana*. Baysal *et al*. (2003) applied three different defense activator BTH, acibenzolar-S-methyl (ASM) and Bion to induced resistance in tomato against *Clavibacter michiganensis* ssp. *michiganensis*, causal organism of bacterial canker of tomato. Perez-de-Luque *et al*. (2004) studied the effect of foliar application of three SAR activator *viz.* SA, glutathione and BTH to control the broomrape infected pea disease. Hukkanen *et al*. (2007) reported an improved resistance mechanism against powdery mildew infection in strawberry plants (under greenhouse conditions) by BTH which mediated the accumulation of soluble and cell wall bound phenolics. Cortes-Barco *et al*. (2010) worked with the anthracnose causing fungus *Colletotrichum orbiculare* to study the induction of resistance in *Nicotiana benthamiana* against it. They used two inducers for the study, *viz.* BTH and (2R, 3R)-butanediol or PC1, an isoparaffin-based mixture. After the disease assessment they concluded that, (2R, 3R)-butanediol, BTH, and PC1 reduced the number of lesions per leaf area caused by *C. orbiculare* to a significant extent. Aleandri and Reda (2010) studied the effect of three resistance inducers MeJA, BTH and K_2HPO_4 to control root rot and vine decline disease of melon caused by *Monosporascus cannonballus*. In 2013,

Dufour *et al.* reported that BTH strengthens plant defense mechanisms against pathogens. They reported the role of BTH-pretreatment in enhancing resistance against infection with various isolates of *Plasmopara viticola* and *Erysiphe necator* causing downy and powdery mildews in grapevine leaves respectively. Azami-Sardooei *et al.* (2013) investigated the effects of foliar applications of different concentrations of BTH on resistance to *B. cinerea, a* pathogen of tomato, bean and cucumber. Pye *et al.* (2013) used BTH and Tiadinil for induction of defense in tomato plants against the pathogen *Pseudomonas syringae* pv. Tomato (Pst) and *Phytophthora capsici.* Bellee *et al.* (2018) investigated the effect of a fungicide Pyrimethanil and BTH on the defense of grapevine against *Botrytis cinerea*. They reported that BTH was an efficient elicitor that induced the defense mechanism rapidly in the vineyard. Wise *et al.* (2016) suggested that the field application of BTH could effectively enhance crown rust resistance and avenanthramide production in oat by up-regulating certain metabolic pathways. Yan *et al.* (2017) studied the effect of JA and BTH to confer resistance against *Sphaerotheca pannosa* in *Rosa rugosa* 'Plena' seedlings. They were of opinion that, exogenous JA and BTH significantly improved *R. rugose* 'Plena' resistance to *S. pannosa.* Kouzai *et al.* (2018) studied the effect of BTH in the development of resistance upon infection by *Rhizoctonia solani*, causing sheath blight disease in *Brachypodium distachyon*. Cheng *et al.* (2018) suggested the role of BTH in enhancing banana plant defense responses to *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (Foc 4) infection, and demonstrated that BTH selectively affect biological processes associated with plant defenses. Frackowiak *et al.* (2019) in their work analyzed the influence of BTH and its derivatives as resistance inducers in healthy and virus-infected plants by determining the expression levels of selected resistance markers associated with the JA, SA and Ethylene pathways. From the results, they had concluded that expression of marker genes of both the SA- and JA-mediated pathways could be increased by the application of BTH and its derivatives conferring its importance in defense mechanisms in plants. Jiang *et al.* (2019) studied the effect of BTH to confer resistance against *Fusarium sulphureum*, the causal agent of fungal dry rot disease, in potato tuber. They

found that BTH treatment stimulated wound-induced suberization in harvested potato tubers and also reduced the fungal dry rot by elevating the metabolism of the phenylpropanoid pathway. López-Gresa *et al.* (2019) studied metabolic alterations in BTH-treated Money Maker and NahG tomato plants infected by citrus exocortis viroid (CEVd) and were investigated by nuclear magnetic resonance spectroscopy. Using multivariate data analysis, they identified that, defence metabolites were induced after viroid infection and BTH-treatment.

DL-β-amino butyric acid (BABA) has also got much attention by the scientists in the last two decades. According to Cohen *et al.* (2011) BABA induced both the local and systemic resistance against disease in numerous plant species. The preventive application of BABA to lettuce plants induced the resistance against downy mildew causing fungi *Bremia lactucae.* Tamm *et al.* (2011) used BABA and an aqueous extract of *Penicillium chrysogenum* (Pen) as elicitors to induce defense responses in plants against pathogen attack. Pajot *et al.* (2001) in their study, reported two elicitors BABA and Phytogard which induced systemic resistance in lettuce against downy mildew disease and protected the plants. Hassan *et al.* (2013) pre-treated tomato plants with BABA against bacterial wilt caused by *Ralstonia solanacearum*. Zeighaminejad *et al.* (2016) investigated the effects of BABA pretreatment on cucurbit powdery mildew disease in squash. Amzalek and Cohen (2007) studied the effect of BABA, BTH, INA, NaSA, AABA and GABA to induce SAR to control sunflower rust caused by *Puccinia helianthi*. Walters *et al.* (2011) reported that powdery mildew disease caused by *Blumeria graminis* f. sp. *hordei* and leaf scald disease caused by *Rhynchosporium secalis* of two spring barley varieties was controlled by the combined application of three resistance elicitors ASM, BABA and cis-jasmone in field conditions. Li *et al.* (2016) studied the effects of various chemical inducers such as BABA, BTH and INA on Huanglongbing (HLB) disease of citrus and could control HLB disease. Conrath *et al.* (1995) used 2,6-Dichloroisonicotinic acid (INA) which is a key protein involved in plant defense. A germination study was undertaken by Sharma and Sohal (2016)

to ascertain the effects of GABA on defense related enzymes *viz.* peroxidase, superoxide dismutase, total phenols and phenylalanine ammonia lyase in the hypocotyls and cotyledonary leaves of *Brassica juncea* RLM619 which showed resistance to white rust and Alternaria blight. Moosavi (2017) studied the plant defense responses and disease severity on tomato plants, by the application of abscisic acid (ABA) and gibberellin against *Meloidogyne javanica.* Thus, from literature it is evident that several chemical inducers including SA, BTH, ABA, BABA, GABA and AABA have potential to induce resistance in different plants against different pathogens.