International Journal of Microbiology Research
ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 10, Issue 4, 2018, pp.-1166-1170.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000234

Review Article

MOLECULAR ASPECTS OF *SCLEROTINIA* STEM ROT DISEASE MANAGEMENT IN OILSEED CROPS

GUPTA N.C.*[1], RAO MAHESH[1] AND SHARMA PANKAJ[2]
[1]ICAR-National Research Centre on Plant Biotechnology, Pusa, New Delhi, Delhi 110012, India
[2]ICAR-Directorate of Rapeseed Mustard Research, Sewar, Bharatpur, 321303, Rajasthan, India
*Corresponding Author: Email - navinbtc@gmail.com

Received: April 17, 2018; Revised: April 21, 2018; Accepted: April 24, 2018; Published: April 30, 2018

Abstract- The pathogens aggression with endless evolutionary pressure refines their molecular strategies to achieve the successful pathogenesis. *Sclerotinia sclerotiorum* (Lib) de Bary, a necrotrophic phytopathogen is ubiquitously distributed worldwide and affecting the large number of host species. Several control measures like fungicides application, cultural practices, crop rotation are the usual practices available to the farmers are in use. But, despite its success, these processes are quite expensive and indistinctness of fungicides doses and time of application are the major hurdle in their routine use. Although, partial resistance/tolerance has already been reported in *B. napus* and *B. carinata* but not a single source *in B. juncea*, which hinders the resistance breeding program. However, the recent advancement in biotechnological interventions are observed more promising in developing the alternatives like fungal growth inhibition, defense response activation, detoxification of the virulence factors, and RNAi or HIGS for engineered resistance to the *Sclerotinia* stem rot disease.

Keywords- *Sclerotinia*, Stem rot, Oilseed, Oxalic acid, Anti-microbial peptides

Citation: Gupta N.C., *et al*., (2018) Molecular Aspects of *Sclerotinia* Stem Rot Disease Management in Oilseed Crops. International Journal of Microbiology Research, ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 10, Issue 4, pp.-1166-1170. DOI: http://dx.doi.org/10.9735/0975-5276.10.4.1166-1170

Introduction

Sclerotinia sclerotiorum (Lib.) de Bary is worldwide distributed plant pathogenic fungus which has the widest host range of any known plant pathogen. It nearly affects more than 400 species of 275 genera which includes cruciferous vegetables like cabbage and cauliflower, tomato, potato, sunflower, soybean, lettuce and also predominantly affects major oilseed crops such as canola (*B. rapa* and *B. napus*) and Indian mustard (*Brassica juncea*). In global perspectives, India ranks third in acreage (19.3 %) after Canada and China and in production (11.12%) after China and Canada [1]. *S. sclerotiorum* pathogen is a menace for the oilseed crop right from seedling stage to the end of the flowering stage that accounts for 10-100% economic damage to the crop based on disease severity in field condition. Out of various ways, one means of mitigating the deficit between production and demand of the oilseed is to reduce the damage caused by the pathogen to the crop [2]. Resistance breeding program for the stem rot disease is mainly hindered because of the non-availability of resistant source especially in B. juncea or the presence of partial resistance/tolerance in very few germplasms of *B. napus* and *B. carinata*. Moreover, limited germplasm availability is the major hurdle that hampers the scale of screening for identifying the resistance source in the existing gene pool.

Recent advances in agricultural biotechnology interface have significantly enhanced the fundamental understanding of host-pathogen interaction and processes of pathogenesis that substantially helps in devising alternative strategies for getting wider adoption among the oilseed growers to assist the sustainable farming system. Therefore, consideration for developing alternative strategy contrary to the existing one has drastically shifted towards more promising one based on the recent advances made towards the understanding of molecular biology of the of host-pathogen interaction for controlling the pathogen invasion [3]. The invention and utilization of molecular markers in classical breeding have considerably facilitated the rapid selection procedure of the desired trait in a population that forms the basis for marker-assisted selection (MAS) in the molecular breeding program. Transformation methodologies and genome editing technologies have also been well devised for generating the fertile transgenic and genome edited oilseed crops, respectively. This review insights the current molecular aspects and advances in devising the alternative approaches for controlling the *Sclerotinia* stem rot disease, a problem of major importance to the *Brassica* growing farmers.

The pathogen, *Sclerotinia sclerotiorum*, and Mode of infection

Sclerotinia sclerotiorum (Lib.) de Bary pathogen has been known since from late 19th century and categorised into the family *Sclerotinia*ceae, order Heliotales, and the phylum *Ascomycota*. As usual feature of the genus, *S. sclerotiorum* produces melanized hyphal aggregates (sclerotia) as a resting body [4] at the end of the infection cycle and survive in the form of sclerotia inside the diseased stem or in stubbles fell on the ground for prolonged period [5]. *S. sclerotiorum* is a necrotrophic, polyphagous fungal plant pathogen which completes their life cycle in four different stages namely, sclerotia, apothecium, ascospores, and mycelium [6]. Under favorable conditions, the resting bodies i.e., sclerotia germinates and infect the host plants either by myceliogenically or carpogenically [7] depending on the temperature of the environment. Sclerotial germination was observed stopped below 10ºC and partially inhibited at 15ºC temperatures [8]. Differential pattern of sclerotial germination determine the means of infection to the host plants in field condition. The carpogenically germinating sclerotia involves the production of apothecia (fruiting bodies) that harbors the ascospores and infects the aerial portion of the plant through air current whereas myceliogenically germinating sclerotia infects the ground level tissues through mycelium production [6]. Ascospores usually infect the senescing tissues of the plant [9] especially petals that fell over the leaf lamina or at leaf axils facilitate the infection process as reported in case of an outbreak of stem rot disease in *B. napus* [10,11].

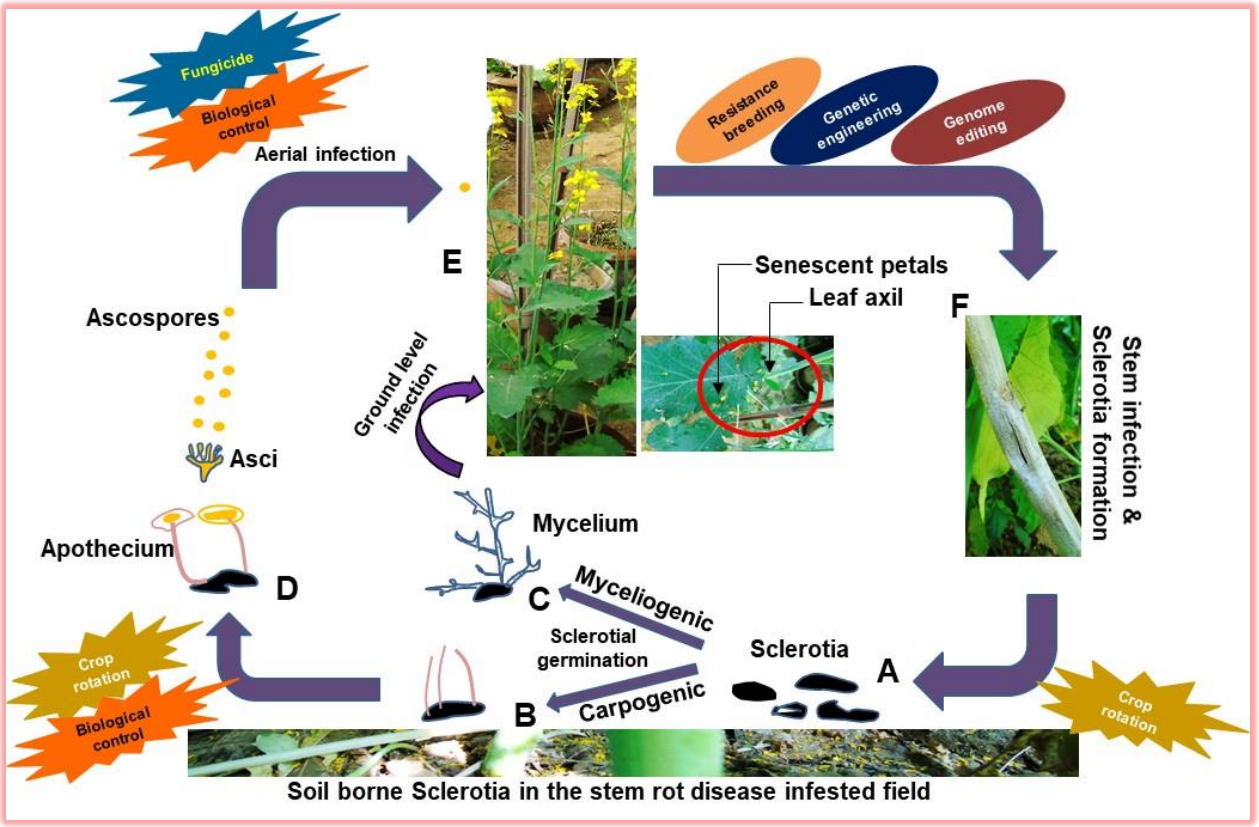

Fig-1 Infection cycle of the pathogen *S. sclerotiorum* with potential targets for the disease control in oilseed crop. (A) Sclerotia of *S. Sclerotinia* from infected stem survives in soil, (B & C) Under favourable condition *Sclerotia* germinates either carpogenically or myceliogenically, (D) Carpogenically germinated *Sclerotia* produce apothecia and ascospores, (E) Ascospores through air current infects senescing petals and aerial part of the stem, (F) infection spreads from leaf axil to the stem and produces sclerotia at maturity. Potential targets for disease control are depicted in colored boxes, some of them are in use while rest of them are under development. Myceliogenic germination leads to ground level infection.

The colonization of the senescent petals promotes the growth of the fungal pathogen as it serves as a nutritional source to them [12]. The production of appressoria, a penetrating structure produced by fungus from its hyphal tips is also called as infection cushion helps in infecting the young tissues of the plant [9]. Oxalic acid, a strong virulence factor produced by the pathogen after interacting with the host tissue has been observed facilitates the spread of the disease by their biochemical and physiological influence over host and pathogen [13]. In the host, acidic nature of the oxalic acid lowers the pH of the middle lamella that resulted in induce seizure of the Ca_2+ ions that affects stomatal closure by excessive accumulation of the K+ ions and increased rate of the starch hydrolysis. In the pathogen, oxalic acid enhances the activity of pectolytic and cellulolytic enzymes that promote the degradation of host tissues. Brown to white necrotic lesion forms at the site of infection and as the fungus spreads from the infection site lesion length also increases and forms sclerotia inside the infected portion of the stem. The major yield loss occurred by the disease is due to the lodging of the disease infested plants as the pathogen girdles the infected stem and weaken their rigidity as reported in case of *B. napus* [5]. There are several measures some of them are being used and some are under development for the control of *S. sclerotiorum* infection targeting the various steps of the infection cycle [Fig-1]. The control measures those are being used by farmers involves cultural practices, crop rotations, fungicide applications, biological agents and resistance breeding but genetic modification approach is still being at the infancy stage and needs a momentum to provide an alternative to the existing practices. The disease symptoms appear after infestation by this devastating necrotrophic pathogen are preferably called as white mold/*Sclerotinia* stem rot/stem rot, watery soft rot, middle stalk rot, head rot, *Sclerotinia* wilt/root rot, depending on the host it infects. In winters it affects the plants directly from mycelia and forms hyphal aggregates called sclerotia in stem and soil (long-term survival structures). During spring, once the conditions become favorable the sclerotia germinate to form apothecia to produce ascus and releases ascospores at the end of spring or in early summer.

The mode of infection of this fungus involves invading through the stomata and sub-stomatal chamber of plants by either ascospores or mycelium which progresses rapidly through the leaf tissues. Pathogen infestation has also been found from the early falling of petals that serves as a nutrient source for the pathogen and subsequently mounts the infection pressure significantly with the weather conditions. Stem rot disease Infection in oilseed crops begins after the seedling emergence and prolonged up to maturation stage. Hence, because of the wider disease infestation arena of the *S. sclerotiorum* pathogen, results in severe yield losses ranging from 10-100% based on the disease severity in oilseed crops has emerged prominently in few years. This has grabbed much more attention of the *Brassica* breeders and molecular biologists to explore the molecular aspects of the pathogenesis and also look into the host susceptibility factors for devising new strategies to counter the *S. sclerotiorum* borne diseases.

Biotechnological interventions as a potential solution

Uncertainty in *Sclerotinia* stem rot disease outbreak and lack of resistant germplasm for this disease provides an open window for implementing the genetic engineering approach to tackle the *Sclerotinia* problem in oilseed crop. However, transgenes that aimed to tackle the pathogen infection must be tested for its efficacy for the target trait in particular crop and their overall impact on environment and consumers before release for its field cultivation. It is always advisable to use such gene that targets the key aspects of the cellular processes of the pathogen in more specific ways. A plethora of research has been done on understanding the molecular basis of host-pathogen interaction that facilitated identification of the potential candidate genes for countering the fungal disease including *S. sclerotiorum* in *Brassica* species. Some of the identified genes have already been used to tackle the stem rot disease through genetic engineering approach by transgenics development and number of diverse candidate genes and effector candidates are under investigation for targeting the range of necrotrophic fungi.

The transgenic strategies mainly aimed to counter the pathogenicity factor Oxalic acid (OA) in case of *S. sclerotiorum*, induction of innate immunity by activating the inbuilt defense pathways, and engineering anti-fungal protein machinery in plants for inhibiting the *S. sclerotiorum* growth after infection would be the best-suited alternatives to the existing control practices [14]. The following section discusses the various transgenes tested for the pathogen resistance and that could also be employed in oilseed *Brassica* improvement program. Further, the other potential strategies for engineering *S. sclerotiorum* resistance are also discussed.

Oxalic acid degrading enzymes confers *Sclerotinia* resistance

In host-pathogen interaction, secretions play a crucial role and serve primary signal molecules in establishing the biological linkages. Oxalic acid (OA), a known pathogenicity factor of the *S. sclerotiorum* [4], secretion and synthesis in pathogen after host infection and its association with virulence determination have been known for very long [15-20]. Several studies have demonstrated that heterologous expression of the oxalic acid degrading enzymes, confers resistance to *S. sclerotiorum* in susceptible hosts including oilseed (*B. napus*) [21,22], sunflower [23-25], tomato (*Solanum lycopersicum*) [26], and soybean (*Glycine max*) [27,28].

Oxalic acid oxidases (OxOs) enzymes present in plants are basically members of germin family of proteins and a subset of cupin superfamily [29-32]. Germin-like proteins (GLPs) is ubiquitously present throughout the plant kingdom including dicot and monocots whereas cupins are present in all kingdoms including bacteria, fungi, and plants. However, not all GLPs are having the OxO activity but the germins isolated from true cereals as referred by Hill [33] including rice, wheat, oat, barley, rye, maize, and pine were known to have OxO activity y [34,35]. Cupins of microbial origin with OxO activity have also been reported from bacteria and fungi [36] and their enzymatic activity degrades the oxalic acid into carbon dioxide and a lethal by-product formic acid that might be toxic to the plant cells. Hence, OxO which degrades oxalate into CO_2 and H_2O_2 [37] like the cupins present in wheat and barley are preferred in making transgenics for *Sclerotinia* stem rot disease resistance. The produced H_2O_2 on catalysis of oxalate plays a crucial role in basal defense response through signal cascading and provides rigidity to the cell wall during lignification of the infested tissues by cross-linking the cell wall constituents and apart from this it is also having the direct role in pathogen impairment [38].

For the very first OxO from Barley root were expressed in *B. napus* and observed it contributes resistance to wilting induced by the oxalic acid application [21] but none of the transgenics were found resistant to *Sclerotinia* stem rot disease in field condition. The overexpressing *OxO gene* (gf-2.8) from wheat in transgenic lines of soybean [27] has shown a great reduction in lesion length development and disease progression in the inoculated cotyledon and stem indicating that the ectopic expression of the *OxO gene* even in heterologous species imparted resistance to the *Sclerotinia* stem rot disease. Furthermore, Wheat *OxO gene* (gf-2.8) overexpressing lines of sunflower plants were also shown the decrease in disease and lesion length progression [25]. The other wheat *OxO gene* (gf-2.1) derived transgenics in *B. napus* has shown a 44% reduction in lesion size in vitro challenged leaves whereas about 80% reduction in disease reported in stem assay under field condition [22]. Interestingly, in case of tree species, the overexpression of the germin protein of wheat in hybrid poplar tree species reported conferring resistance to the Septoria musiva, an oxalic acid producing pathogen [39]. In addition to it, the OxO-transgenic maize exhibited enhanced insect resistance [40]. These results indicate that the germin family OxO proteins generating H_2O_2 after oxalate degradation are remarkable best suited and have wider applicability in developing transgenics for developing resistance against the continuum of insect-pests and pathogens in plants.

Antifungal proteins mediated approach

Antimicrobial peptides or proteins (AMPs) [41] are an integral part of the innate immunity in all the living organism that determines resistance/tolerance to varied microbes. These AMPs are usually cysteine-rich, small sized (<50 aa) proteins and most of them have shown broad-spectrum activity against both prokaryotic and eukaryotic microbes and some are specific against the fungal pathogen [42]. The effectiveness of AMPs is solely depended on the nature of the microbes with respect to membrane composition and protein structure of the organism [43].

A range of AMPs isolated from various organism was expressed in *S. sclerotiorum* susceptible oilseed *Brassica* species and tested for their efficacy towards delivering resistance to the targeted pathogen. Overexpression of the AMPs from White pine ($PmAMP_1$) in *B. napus* has shown ~80% reduction in lesion length after *S. sclerotiorum* inoculation and surprisingly the developed transgenic lines were also found resistant to other major pathogens *Alternaria Brassicae* and *Leptosphaeria maculans* [44]. In connotation with the previous findings, the transgenic *B. juncea* lines expressing $PmAMP_1$ has harbored the enhanced resistance to *A. Brassicae*, a major fungal pathogen of oilseed crops [45]. The multifacet role of the AMPs in imparting resistance/tolerance to a number of. the pest and pathogens entail its broad-spectrum utility for developing inherent immunity in susceptible host plants. In addition to AMPs, plant-specific lipid transfer proteins (LTPs) has also been observed contributing *Sclerotinia* resistance. These LTPs are basically involved in the transfer of phospholipid between the cell membrane and functions both in cellular constituent biosynthesis as well as in defense response pathways against invading pathogen [46]. The recent studies on LTP transgene expression in *B. napus* showed the enhanced level of resistance to SR disease. The ectopic expression of an LTP from Brazilian upland rice has demonstrated a reduced level of lesion size on infected leaves and a greater percentage of germination as compared to susceptible control plants [47]. Contrary to this, the ectopic expression of motherwort LTP resulted in a comparatively lesser reduction in lesion size over the infected leaves. In both the cases plant defense mechanism involved H_2O_2 synthesis and increased level of PR-2 synthesis in transgenic lines [48]. The other set of transgenes encoding proteins of antifungal nature, chitinases are also known to directly inhibit the fungal growth by breaking down the chitin present in their cell wall. Although it plays an important role in defense response in many cases it has been found the induction of chitinase doesn't respond equally effective in defying the pathogen invasion. The overexpression of chitinases from tomato and *Nicotiana benthamiana* in *B. napus* resulted in resistance to *S. sclerotiorum* and several pathogens in variable manners [49]. The significant resistance to stem rot disease was observed in transgenic *B. napus* lines carrying overexpressing gene construct for the chitinase gene from the chitin depleting fungus *Trichoderma viride* [50].

By modulating the host cell physiology

Programmed cell death (PCD) is the intricate feature in most of the host-pathogen interactions and it determines the susceptibility or resistance associated with the necrotrophic or biotrophic nature of the invading pathogens respectively [51]. Oxalic acid (OA) in case of *Sclerotinia* pathogenesis is thought to be a major elicitor for the apoptosis that leads to host susceptibility. Hence the one way to stop *S. sclerotiorum* pathogenesis is to either inhibit the apoptosis-promoting genes induced after infection or ectopically express the genes that prevent apoptosis in host cells. The effect of ectopic expression of the cell death regulatory genes in heterologous species was first demonstrated by Dickman, *et al*., [52] in *N. benthamiana* by expressing individually four different cell death regulatory genes isolated from *Caenorhabditis elegans* (*ced-9*), humans (*bcl-2* and *bcl-xl*), and *Orgyia pseudotsugata* multiple nucleopolyhedroviruses (*op-iap*) and observed the transgenic plants were resistant to *S. sclerotiorum* and other necrotrophic fungal pathogens. The reported apoptotic genes are further needed to be tested in oilseed crop to check their efficacy against various pathogens and pests for yield improvement in oilseed *Brassica* crop.

Host-induced gene silencing (HIGS) approach

The building of natural resistance to *S. sclerotiorum* in transgenic *Brassica* lines through genetic engineering means is an alternative strategy in contrast to the existing methods of control measures for stem rot disease in oilseed crops. The HIGS (host-induced gene silencing) strategy [53] will be served as more exciting means to promote the innate immunity in transgenic lines. HIGS concept involves the incorporation of precursor miRNA construct into host plant and in planta expression of that in association with host RNAi machinery leads to the production of processed miRNA that suppresses the specific target gene in the fungal pathogen.

Usually, the HIGS approach incorporates the nucleic acid-based fungicide within the plant system. A recent study involved the in-planta expression of micro RNA for chitinase genes of *S. sclerotiorum* in tobacco leaves exhibited 87% reduction in disease severity [54]. In the other study targeting of *cyp 51*, a conserved gene in *Fusarium* species by HIGS resulted in complete immunization to *F. graminearum* [55]. Indeed, a gene-specific feature of this technology involves targeting the lifesaving gene of the pathogen as most of the fungicides targeting the product of the same gene and act very efficiently.

Conclusion

There are numerous control measures like fungicides application, crop rotation, and cultural practices have been in use to counter the *Sclerotinia* stem rot disease in oilseed *Brassica*. But due to one and other reason despite their effectiveness farmers are looking for a new and sophisticated approach that significantly reduces the crop damage from this devastating pathogen.

An alternative to the existing practices will meet the grower demands in terms of devising insightful novel ideas by incorporating the natural resistance or innate immunity in plants to *S. sclerotiorum* through genetic engineering approach in developing the resistant transgenic oilseed lines. Furthermore, the fundamental understandings about molecular aspects of *S. sclerotiorum* pathogenesis process are much more needed for its effective utilization in more applied research.

Application of review: The current effort of advanced genomics approach will certainly benefit the oilseed researchers with a new set of genes and effector candidates to combat the *Sclerotinia* stem rot disease in near future

Review Category: Molecular Genetics of Oilseed Crops

Abbreviations:

SR: Stem Rot
RNAi: RNA interference
HIGS: Host-induced gene silencing

Acknowledgement / Funding: The authors are grateful to Indian Council for Agricultural Research (ICAR), New Delhi, India and ICAR-National Research Centre on Plant Biotechnology for providing the financial support and facilities to conduct the research work on Stem rot disease in oilseed crop.

***Principle Investigator: Dr N.C. Gupta**

Institute: ICAR-National Research Centre on Plant Biotechnology, Pusa, New Delhi, Delhi 110012

Research project name or number: Study on *Sclerotinia sclerotiorum* with emphasis on management of *Sclerotinia* rot in *Brassica*; F.No. CS/18(15)/2015-O&P

Author contributions: NCG and PS conceived the project and obtained funding from ICAR-EMR project for *Sclerotinia* work in Indian mustard. NCG and MR wrote the manuscript and PS has edited the manuscript.

Author statement: All authors red, reviewed, agreed and approved the final manuscript

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

References

[1] FAOSTAT (2016) *http://faostat3.fao.org/browse/Q/QC/E. Accessed March* 2016.
[2] Campbell M.A., Fitzgerald H.A., Ronald P.C. (2002) *Transgenic Res*. 11, 599-613.
[3] Durante M., Vannozzi G.P., Pugliesi C., Bernardi R. (2002) *Proceedings of the Fifth European Conference on Sunflower Biotechnology, San Giuliana Terme, Pisa, Italy*, 25, 1-28.
[4] Bolton M.D., Thomma B.P., Nelson B.D. (2006) *Molecular Plant Pathology*, 7, 1-16.
[5] Khangura R., Beard C. (2015) *Australian Government. [https://www.agric.wa.gov.au/canola/managing-Sclerotinia-stem-rot-canola]*.
[6] Purdy L.H. (1979) *Phytopathology*, 69, 875-80.
[7] Bardin S.D., Huang H.C., (2001) *Canadian Journal of Plant Pathology*, 23, 88-98.
[8] Jones D., Gray E.G. (1973) *Transactions of the British Mycological Society,* 60, 495-500.
[9] Jamaux I., Gelie B., Lamarque C. (1995) *Plant Pathology*, 44, 22-30.
[10] Bom M., Boland G.J. (2000) *Canadian Journal of Plant Science*, 80, 889-98.
[11] Clarkson J.P., Phelps K., Whipps J.A., Young C.S., Smith J.A., Watling M. (2007) *Phytopathology*, 97, 621-31.
[12] Garg H., Li H., Sivasithamparam K., Kuo J., Barbetti M.J. (2010c) *Annals of Botany*, 106, 897-908.
[13] Hegedus D.D., Rimmer S.R. (2005) *FEMS Microbiology Letters*, 251, 177-84.
[14] Derbyshire, M.C., Denton-giles, M. (2016) *Plant pathology*, 65, 859-77.
[15] Maxwell D.P., Lumsden R.D. (1970) *Phytopathology*, 60,1395-98.
[16] Noyes R.D., Hancock J.G. (1981) *Physiological Plant Pathology*, 18, 123-32.
[17] Marciano P., Dilenna P., Magro P. (1983) *Physiological Plant Pathology*, 22, 339-45.
[18] Magro P., Marciano P., Dilenna P. (1984) *FEMS Microbiology Letters*, 24, 9-12.
[19] Tu J.C. (1985) *Physiological Plant Pathology*, 26, 111-7.
[20] Godoy G., Steadman J.R., Dickman M.B., Dam R. (1990) *Physiol. Mol. Plant Pathol*., 37, 179-191.
[21] Thompson C., Dunwell J.M., Johnstone C.E., *et al*., (1995*) Euphytica*, 85, 169-72.
[22] Dong X.B., Ji R.Q., Guo X.L. *et al*., (2008) *Planta*, 228, 331-40.
[23] Lu G., Bidney D., Bao Z., Hu X., Wang J., Vortherms T., Scelonge C., Wang L., Bruce W., Duvick J. (2000) *The Proceedings of 15th International Sunflower Conference. Toulouse, France, June* 2000, K72-7.
[24] Scelonge C., Wang L., Bidney D., Lu G., Hastings C., Cole G., Mancl M., D'Hautefeuille J.-L., Sosa-Dominguez G., Coughlan S. (2000) The *Proceedings of 15th International Sunflower Conference. Toulouse, France. June* 2000, K66-71.
[25] Hu X., Bidney D.L., Yalpani N. *et al*., (2003) *Plant Physiology*, 133, 170-81.
[26] Walz A., Zingen-Sell I., Loeffler M., Sauer M. (2008) *Plant Pathology*, 57, 453-8.
[27] Donaldson P.A., Anderson T., Lane B.G., Davidson A.L., Simmonds D.H. (2001) *Physiological and Molecular Plant Pathology*, 59, 297-307.
[28] Calla B., Blahut-Beatty L., Koziol L. *et al*., (2014*) Molecular Plant Pathology,* 15, 563-75.
[29] Lane B.G., Dunwell J.M., Ray J.A., Schmitt M.R., Cuming A.C. (1993) *J. Biol. Chem*., 268,12239-42.
[30] Kotsira V.P., Clonis Y.D. (1997) *Arch Biochem. Biophys*. 340, 239-49.
[31] Dunwell J.M. (1998) *Biotechnology & Genetic Engineering Reviews*, 15, 1-32.
[32] Davidson R.M., Reeves P.A., Manosalva P.M., Leach J.E. (2009) *Plant Science*, 177, 499-510.
[33] Hill A., (1937) *Economic Botany. New York, USA, McGraw-Hill*.
[34] Dunwell J.M., Khuri S., Gane P.J. (2000) *Microbiol. Mol. Biol., Rev*, 64, 153-79.
[35] Lane B.G. (2000) *Biochem. J*., 349, 309-321.
[36] Escutia M.R., Bowater L., Edwards A. *et al*., (2005) *Applied and*

Environmental Microbiology, 71, 3608–16.
[37] Dumas B., Cheviet J.P., Sailland A., Freyssinet G. (1993) *In: Fritig BM, Legrand M, eds. Mechanisms of Plant Defense Responses. Dordrecht, Netherlands: Springer*, 451.
[38] Dumas B., Freyssinet G., Pallett K.E. (1995) *Plant Physiology*, 107, 1091-6.
[39] Liang H., Maynard C.A., Allen R.D., Powell W.A. (2001) *Plant Mol. Biol*. 45, 619-629.
[40] Ramputh A.I., Arnason J.T., Cass L., Simmonds J.A. (2002) *Plant Sci.,* 162, 431-440.
[41] Ganz T. (2003) *Integrative and Comparative Biology*, 43, 300-4.
[42] Rao, A.G. (1995) *Mol Plant Microbe Interact*, 8, 6-13.
[43] Broekaert W.F., Cammue B.P.A., DeBolle M.F.C., Thevissen K., DeSamblanx G.W., Osborn R.W. (1997) *Critical Reviews in Plant Science*, 16, 297-323.
[44] Verma S.S., Yajima W.R., Rahman M.H. *et al*., (2012) *Plant Molecular Biology*, 79, 61-74.
[45] Rustagi A., Kumar D., Shekhar S., Yusuf M.A., Misra S., Sarin N.B. (2014) *Molecular Biotechnology*, 56, 535-45.
[46] Garcia-Olmedo F., Molina A., Segura A., Moreno M. (1995) *Trends in Microbiology*, 3, 72-4.
[47] Fan Y., Du K., Gao Y. *et al*., (2013) *Russian Journal of Genetics*, 49, 380-7.
[48] Jiang Y.Z., Fu X.L., Wen M.L. *et al*., (2013b) *Physiological and Molecular Plant Pathology*, 82, 81-7.
[49] Grison R., Grezes-Besset B., Schneider M. *et al*., (1996) Nature Biotechnology, 14, 643-6.
[50] Solgi T., Moradyar M., Zamani M.R., Motallebi M. (2015) *Plant Protection Science*, 51, 6-12.
[51] Deller S., Hammond-Kosack K.E., Rudd J.J. (2011) *Journal of Plant Physiology*, 168, 63-71.
[52] Dickman M.B., Park Y.K., Oltersdorf T., Li W., Clemente T., French R. (2001) *Proceedings of the National Academy of Sciences, USA*, 98, 6957-62.
[53] Koch A., Kogel K.H. (2014) *Plant Biotechnology Journal*, 12, 821-31.
[54] Andrade C.M., Tinoco M.L.P., Rieth A.F., Maia F.C.O., Arag~ao F.J.L. (2015) *Plant Pathology*, doi: 10.1111/ppa.12447.
[55] Koch A., Kumar N., Weber L., Keller H., Imani J., Kogel K.H. (2013) *Proceedings of the National Academy of Sciences, USA*, 110, 19324-9.