SUMMARY

Pearl millet [Pennisetum glaucum [L.] R. Br.] is the sixth most important cereal grain in the world and the third most important food crop in India is known to be the sustenance crop of the poor. This hardy crop has several agronomic benefits and is efficiently adapted to lower input, dry land agriculture production systems of the arid and semi-arid regions which witness inadequate rainfall and poor soil conditions. Pearl millet is regarded as drought resistant and thrives well in areas that experience frequent periods of dry weather during the vegetative or reproductive phases. Due to its ability to survive and perform under harsh climatic conditions, pearl millet is projected as one of the “climate change compliant crops”. Pearl millet is the staple food for thousands of years for the poorest of the poor people in Asian and African countries particularly in the sub-tropical regions. The crop is stacked with many health benefits and is high in nutraceutical value.

India produces more than half of world’s pearl millet and currently it is grown over 10 million hectares mainly as a rain fed crop with an annual production of 9.5 million tonnes. In spite of its hardiness, pearl millet is highly susceptible to downy mildew disease which is a major constraint in pearl millet production and it greatly reduces the crop production leading to huge economic losses. Pearl millet downy mildew is caused by the biotrophic oomycete Sclerospora graminicola (Sacc.) Schroeter, whose infection causes up to 80% yield loss as the crop become chlorotic stunted at early stages and the grains are replaced by leaf-like structures in the earhead at later stages. In India, 65% of the pearl millet produced is by hybrids which succumb to the disease due to resistance breakdown caused by the highly variable S. graminicola. Therefore, management of downy mildew is vital for protecting the yield and preventing economic losses.

Downy mildew management encompasses various conventional and modern strategies, which include cultural practices, chemical management, host resistance breeding, and biological control each of which have registered several shortcomings. Cultural methods are difficult to practice to the farmer due to inconvenience in adapting the cultural practices. Chemical management is expensive for a poor man’s crop like pearl millet, the recommended chemicals are not available to farmers, and
several chemicals are toxic and have residual effects, which threaten human and environmental health. Though several downy mildew resistant sources have been identified and a large number of resistant hybrids are developed, *S. graminicola* is highly variable which rapidly changes its virulence and the host resistance is broken down very often within a few years after their wide cultivation. Biological control methods are not completely effective, highly dependent on the environment conditions, delivery methods are not optimized. Therefore, there has been a renewed interest in search for an alternative method which is safe, eco-friendly, inexpensive and also easy to deliver. One of the notable examples reflecting this trend is the induced systemic resistance (ISR) which is gaining worldwide importance and acceptance. One group of resistance inducing agents are “elicitors” which are generally capable of stimulating or triggering plant defense. Inducing systemic resistance in plants against various plant pathogens is demonstrated in a variety of crop plants. Resistance elicitors are of various types, which include virulent or avirulent pathogens, nonpathogenic fungi/ bacteria, rhizosphere microbes, cell wall fragments or peptides derived from microorganisms, plant extracts, and synthetic chemicals.

In the present study the efficacy of different plant resistance elicitors from various sources were evaluated for their efficiency in inducing resistance against downy mildew disease of pearl millet. The histological changes that take place as a result of elicitor stimulation of pearl millet innate immunity were also studied. Further, biochemical and molecular profiling of the major defense related enzymes and PR proteins and other signal compounds that are possibly involved and up/ down regulated in pearl millet plants in response to elicitor induced resistance against *S. graminicola* were also investigated.

**In the first chapter,** four different elicitors derived from different sources viz., 3,5-dichloroanthranilic acid (DCA-a synthetic elicitor), Cell Wall Glucans (CWG)-derived from the endophytic fungus *Trichoderma hamatum* UOM 13, lipopolysaccharides (LPS)-derived from the rhizobacteria *Pseudomonas fluorescens* UOM SAR 14, and Glycine betaine (N,N,N-trimethyl-glycine) (GB)-an amino acid derivative synthesized commercially from beets were evaluated for their ability to
promote pearl millet growth and also to induce resistance against downy mildew disease and the results of the present study showed that all the elicitors tested offered significant downy mildew disease protection both under greenhouse and field conditions, compared to the control, and also offered considerable growth promoting effect.

All the elicitors were initially tested for their effect on pearl millet seed germination and seedling vigor. In general, both seed germination and seedling vigor were positively influenced by the elicitor treatments. Among the four elicitors CWG treatment recorded maximum seed germination and seedling vigor, followed by GB, DCA and LPS as compared to the untreated control. This study enabled to optimize the appropriate concentration and the treatment time of the elicitor which would not negatively affect the seed parameters.

Among the treatments, undiluted CWG treatment recorded highest and significant enhancement of seed germination and seedling vigor of 97.4% and 1698 followed by GB at 30 mg/ml and LPS at 50 µg/ml recorded determined germination of 97% and 96.8% and vigor index of 1767 and 1706, respectively. Similarly, considerable percentage of seed germination and seedling vigor was recorded in 100 µM DCA treated seeds with 93.7% germination and 1510 vigor, respectively. The untreated control seeds recorded germination of 83.1% with 1249 seedling vigor. The results showed that for DCA 100 µM concentration for 3 h treatment time was optimum, for CWG undiluted concentration for 6 h treatment time was optimum, for LPS 50 µg/ml treatment for 6 h was optimum and for GB 30 mg/ml concentration for 3 h treatment time was optimum.

Subsequently, the elicitors were evaluated for their downy mildew resistance inducing ability and, in general, all the elicitor treatments significantly reduced downy mildew disease incidence in comparison to the control. However, the degree of disease reduction varied with the type of elicitor treatment. Under greenhouse conditions, highest downy mildew incidence of 97.2% was observed in control with least disease protection, whereas seed treatment with GB showed the least downy mildew incidence of 17.7% followed by LPS, CWG and DCA which showed 21.3, 27 and 32.1% downy mildew incidence, respectively as against the control which
recorded 97.2% downy mildew disease incidence. Correspondingly, GB, LPS, CWG and DCA treatments offered 81.7, 78, 72.3 and 66.9% downy mildew disease protection as against the chemical check Apron which showed 91.4% downy mildew protection. Under field conditions, significant reduction of downy mildew disease was observed in the test rows raised from seeds treated with elicitors when compared with the control rows. In general, all the elicitor treatments significantly reduced downy mildew disease incidence in comparison to the control, however, the degree of disease reduction varied with the type of elicitor treatment. Seed treatment with GB showed the least downy mildew incidence of 15.4% followed by LPS, CWG and DCA, which showed 18.6, 24.3 and 27.8% downy mildew incidence as against the control which recorded 92.5% downy mildew disease incidence. Correspondingly, GB, LPS, CWG and DCA offered 83.5, 79.8, 73.7 and 69.9% downy mildew disease protection as against the chemical check Apron which showed 94.4% downy mildew protection.

After the study of downy mildew protection ability of the elicitors, the nature and stability of the resistance induced were studied by the fungitoxic and spatiotemporal tests. The four elicitors viz., DCA, CWG, LPS and GB tested in this study were in complete compliance with these requirements of SAR. *In vitro* evaluation of the elicitors against the zoospores of *S. graminicola* did not have any fungitoxic effect. DCA, CWG, LPS and GB when tested against *S. graminicola*, there was no inhibition of the asexual sporulation as evidenced by the profuse sporulation on the abaxial side of the elicitor treated leaf bits which was on par with the control. The tested elicitors did not have any effect on the release of zoospore from sporangia, zoospore mobility and zoospore germination either.

It was shown that the nature of resistance induced by all the four elicitors was found to be systemic, which we demonstrated by maintaining spatial and temporal separation of the inducer and the challenger. A crucial time interval between application of the inducer and the onset of protection required for the plant to reach the induced state, and this transitional period varies depending on the type of the host plant, pathogen and the inducing agent. Several reports have suggested that generally it takes from a few days to a week for SAR or ISR to develop. In line with these observations our study revealed that resistance build up due to the elicitors DCA, CWG, LPS and GB all require a minimum of 3 days for the complete expression of
systemic resistance against the pearl millet downy mildew pathogen. Natural resistance mechanisms once induced due to SAR or ISR is often maintained for the lifetime of the plant and are effective against multiple pathogens. Similarly our results too indicated that resistance induced was durable since the treated plants remained disease-free throughout their life in spite of the second dose of challenge inoculation with the downy mildew pathogen.

Overall, the results of the first chapter indicated that the elicitors DCA, CWG, LPS and GB induced systemic and durable resistance against downy mildew disease. However, GB was the most efficient elicitor which offered the highest downy mildew protection, followed by LPS, CWG and DCA in comparison to the control, both under greenhouse and field conditions. A very small quantity of these inducers was effective in eliciting considerable resistance to the downy mildew pathogen and the resistance was durable. The delivery method of these elicitors was done by seed treatment which is also simple, easy and inexpensive method of application. Therefore, elicitor treatment with systemic resistance inducing agents like GB, LPS, CWG and DCA are highly recommended for the management of pearl millet downy mildew disease.

**In the second chapter**, histological investigations into mechanisms of resistance induced by immunity elicitors during pearl millet downy mildew host-pathogen interactions were carried out. Comparative analyses of the effect of different elicitors like DCA, CWG, LPS and GB on cell wall reinforcement processes including callose deposition, lignification, $\text{H}_2\text{O}_2$ generation, NO localization, HRGP cross-linking and hypersensitive response, the speed, magnitude and pattern of cell wall strengthening were evaluated with appropriate resistant and susceptible checks. The speed and magnitude of these cell wall strengthening mechanism and HR was significantly higher in elicitor treated seedlings in comparison to the controls and checks both with and without pathogen inoculation. Further, the intensity of these responses greatly varied depending on the type of elicitors. For most of these structural defense responses, resistant seedlings showed higher and quicker responses compared to the elicitor treated seedlings, whereas for some responses the elicitor treated samples showed higher intensity than the resistant seedlings. Generally, it was
observed that all the cell wall related defense responses were significantly enhanced in inoculated seedlings compared to the uninoculated seedlings.

For all the elicitors tested callose deposition, lignification and accumulation of hydrogen peroxide were significantly higher than that of the control seedlings, with or without inoculation. At all time intervals tested for all elicitor treatments, callose deposition, lignification and accumulation of hydrogen peroxide were significantly higher in inoculated seedlings compared to the uninoculated seedlings. The speed and intensity of callose deposition, lignification and accumulation of hydrogen peroxide were higher in inoculated seedlings compared to the uninoculated seedlings for all elicitor treatments. Among the elicitor treatments GB treated seedlings showed maximum callose deposition which was the highest, followed by LPS, CWG and DCA treatments compared with the untreated control. In uninoculated seedlings, CWG treated seedlings showed high callose deposition followed GB, DCA and LPS treated seedlings. Among the elicitor treatments GB treated seedlings showed highest degree of lignification followed by LPS, CWG and DCA treatment compared with the untreated control. In uninoculated seedlings, maximum lignification was recorded in LPS treated seedlings followed CWG, GB and DCA treated seedlings. Among the elicitor treatments, GB treated seedlings showed maximum H$_2$O$_2$ generation followed by LPS, CWG, and DCA treatments.

This study clearly demonstrated that the level of resistance in different categories of elicitor treated seedlings was correlated with level of HRGP cross-linking and NO localization. At all time intervals tested for all elicitor treatments, HRGPs cross-linking and NO localization were significantly higher in inoculated seedlings compared to the uninoculated seedlings. The speed and intensity of HRGPs cross-linking and NO localization were higher in inoculated seedlings compared to the uninoculated seedlings for all elicitor treatments. In inoculated samples, maximum HRGPs cross-linking was recorded in GB treated seedlings which were higher than resistant seedlings and the other elicitor treatments. In uninoculated seedlings, maximum HRGPs cross-linking was recorded in GB treated seedlings followed by LPS, CWG and DCA treated seedlings. In inoculated samples, maximum NO localization was recorded in GB treated seedlings which was even higher than the resistant check followed by LPS, CWG and DCA treatments. In general, it was
observed that elicitor treatments induced rapid NO localization with or without pathogen inoculation, and the amount of NO localization rapidly and significantly enhanced once the seedlings were challenged with the downy mildew pathogen. The present investigation reports that the intensity and rapidity of HR varied, which was more in resistant and elicitor treated seedlings compared to the susceptible control seedlings. Among all the test samples, the resistant seedlings recorded the highest HR; and among the elicitors, GB treatment showed the highest HR followed by LPS, CWG and DCA treated seedlings, which were significantly higher than that of the control seedlings.

All the elicitor treated seedlings showed comparatively higher and quicker responses in terms of cell wall strengthening processes compared to the control. Based on the degree and speed of structural changes manifested by the tested elicitors, it was inferred that GB was the most potential inducers followed by LPS and CWG whereas DCA was not a good elicitor of cell wall strengthening defense responses in pearl millet infected with *S. graminicola*. Here, it was interesting to observe that even without pathogen inoculation, elicitor treated seedlings showed significantly higher amount of callose deposition in comparison to the control indicating that the elicitor treatment itself has induced or primed the seedlings for earlier and higher expression of certain defense responses which gets further manifested after the inoculation of the pathogen. Tissue printing studies demonstrated that localization of all studied defensive proteins was by and large restricted to the regions of epidermal and vascular regions. However, the degree of expression varied with the tested elicitors and was comparatively higher in inoculated seedlings compared to the uninoculated seedlings.

Timely and coordinated expression of all the pathogen obstructing structural mechanisms along with the other biochemical changes that some of these structural substances like H$_2$O$_2$, HRGP and NO would have triggered has successfully halted the pathogen at its site of entry. Further, host plant might have picked up and transduced the signals activated by these structural defense molecules that would have manifested all the required defenses resulting in enhanced resistance against downy mildew disease. In conclusion, the results of the present investigation led to the inference that cell wall reinforcement mechanisms like the callose, lignin H$_2$O$_2$, NO, HRGPs accumulation and the HR serve as important structural/ histological markers to
ascertain the induction of systemic resistance in pearl millet downy mildew system. These parameters can be employed for screening of new elicitors of disease resistance.

In the third chapter (subchapter-A), pearl millet defense mechanism to the downy mildew pathogen: differences in expression of defense enzymes and proteins by different immunity elicitors were studied. The involvement of various defense enzymes like glucanase, phenylalanine ammonia lyase, peroxidase, polyphenoloxidase, lipoxygenase and hydroxyproline-rich glycoproteins during elicitor induced resistance, study their kinetics, temporal pattern of up or down regulation and how their activities influence the induction and development of resistance in pearl millet was elucidated.

The results of the present study clearly demonstrated that elicitor induced resistance against pearl millet downy mildew disease and several defense enzymes like glucanase, chitinase, phenylalanine ammonia lyase, peroxidase, polyphenol oxidase, lipoxygenase, catalase and defense proteins like hydroxyproline-rich glycoproteins play a major role, and the rapidity and intensity of induction and accumulation of these defense enzymes and proteins positively correlated with the degree of resistance induced by that particular elicitor. In general all the tested elicitors, i.e., GB, LPS, CWG and DCA showed earlier and higher activities of glucanase, PAL, POX, PPO, LOX and HRGPs compared to the controls. Enzyme and protein activities were significantly higher in inoculated seedlings compared to the uninoculated seedlings in all treatments and at all tested time intervals.

In resistant and elicitor treated seedlings, PAL activity peaked at 6 h in elicitor treated and resistant seedlings, whereas in susceptible seedlings PAL activity peaked at 9 h. Among the elicitor treatments, GB treated seedlings highest PAL activity, followed by CWG, LPS, and DCA treatments, which were significantly higher than that of the susceptible controls. In resistant and elicitor treated seedlings POX activity peaked at 9 h, whereas in susceptible seedlings POX activity peaked at 12 h. In pathogen inoculated seedlings, among the elicitor treatments both LPS and PA treated seedlings recorded highest POX activity followed by CWG, and DCA treatments which were significantly higher than that of the control seedlings. PPO activity
peaked at 24 h in elicitor treated, resistant and control seedlings. Among the elicitor treatments, GB treated seedlings recorded highest PPO followed by LPS, CWG, and DCA treatments. β-1,3-glucanase activity peaked at 24 h in elicitor treated, resistant and control seedlings. Among the elicitor treatments, at 24 hpi, GB treated seedlings recorded maximum β-1,3-glucanase activity, followed by LPS, CWG, and DCA treatments, which were significantly higher than that of the control seedlings. In both pathogen inoculated and uninoculated samples, LOX activity peaked at 24 h in elicitor treated, resistant and control seedlings. Among the elicitor treatments, at 24 hpi, DCA treated seedlings recorded highest LOX activity followed by CWG, LPS and GB treatments.

In both pathogen inoculated and uninoculated samples, HRGP activity gradually increased 3 h onwards and peaked at 9 h in elicitor treated, and resistant seedlings, whereas in control seedlings, HRGP activity peaked at 24h. In pathogen inoculated seedlings, HRGP activity peaked at 9 hpi and maximum HRGP activity was observed in GB treated seedlings followed by LPS, CWG, and DCA treatments, respectively. It is interesting to note that there have been no reports of induction of PAL, POX, LOX, glucanase and HRGPs induction by either DCA or GB, ours is the first study to report the same. The tested elicitors viz., GB, LPS, CWS and DCA were potential in eliciting the activities of the defense enzymes glucanase, PAL, POX, PPO, LOX and the physical defense protein HRGP, however the speed and the extent of elicitation differed. The rate at which these enzymes activities were triggered is related to the aggressiveness of that particular elicitor in imparting resistance to downy mildew in pearl millet. The level of these enzyme and protein was increased distinctly after treatment of the elicitors themselves presenting a defense priming effect, and the pathogen challenge resulted in significant further and faster increments in enzyme and protein activities. Each elicitor showed variation in the types of enzymes that it stimulated and also in their levels and temporal pattern suggesting that the underlying defense mechanisms triggered and the response mounted by the host follows different patterns and pathways for each elicitor. For most of the enzymes and proteins tested in this study, the elicitor GB promoted increase in the enzyme and protein activities in a more accentuated way and faster than other elicitors and control, implicating that GB might be a very promising elicitor of downy mildew disease
resistance in pearl millet. Taken together, it can be concluded that GB, LPS, CWS and DCA induced resistance in pearl millet against *S. graminicola* assigns important roles for the defense enzymes namely β-1,3-glucanase, PAL, POX, PPO, LOX and the defense protein HRGPs, and the rapidity and intensity of expression of these activities determine the level of downy mildew resistance imparted which is suggestive of these changes as markers for screening disease resistance elicitors.

*In the third chapter (subchapter-B)*, transcript profiling of genes involved elicitors induced defense responses against downy mildew in pearl millet was studied. The findings of the present study clearly demonstrated that during elicitor induced resistance against pearl millet downy mildew disease, several genes of the defense enzymes like phenylalanine ammonia lyase, peroxidase, polyphenoloxidase, and defense proteins like hydroxyproline-rich glycoproteins and also pathogenesis related proteins like PR-1 and PR-5 were upregulated and the speed and intensity of their induction and overexpression positively correlated with the degree of resistance induced by that particular elicitor. In general, all the tested elicitors, i.e., GB, LPS, CWG and DCA showed earlier and higher mRNA transcript accumulation of glucanase, PAL, POX, PPO, LOX, HRGPs, PR-1 and PR-5 genes compared to the controls. The results of the present study showed that constitutive level of transcripts of the defense enzymes and PR-proteins was higher in resistant and elicitor treated seedlings compared to the susceptible controls. In all sets of seedlings, gene expression was higher in inoculated samples compared to the uninoculated samples at all time points.

Among the pathogen inoculated seedlings, the expression of PAL gene was highest at 6 hpi in GB treated seedlings which was even higher than the resistant seedlings, followed by CWG, LPS and DCA treatments, which were 5.34, 5.19, 4.78 and 3.96 folds higher than that of the susceptible control seedlings, respectively. In the pathogen inoculated samples, POX gene expression gradually increased from 3 hpi and peaked at 9 hpi and decreased thereafter in resistant and elicitor treated seedlings, whereas in susceptible control seedlings POX gene expression peaked at 12 hpi. Among the elicitor treated seedlings, at 9 hpi highest POX gene expression was observed in GB treated seedlings followed by LPS, CWG and DCA treatments, which
were 7.12, 6.77, 6.88 and 6.14 folds higher than that of the susceptible control seedlings, respectively. In the pathogen inoculated samples, PPO gene expression gradually increased from 3 hpi and peaked at 24 hpi and decreased thereafter. Among the elicitor treated seedlings, highest PPO gene expression was observed in GB treated seedlings followed by LPS, CWG and DCA treatments, which were 5.36, 5.24, 4.79 and 4.04 folds higher than that of the susceptible control seedlings, respectively.

The results of the present study showed that in the pathogen inoculated samples, HRGP gene expression gradually increased from 3 hpi and peaked at 9 hpi and decreased thereafter in resistant and elicitor treated seedlings, whereas in susceptible control seedlings, HRGP gene expression peaked at 12 hpi. Among the pathogen inoculated, elicitor treated seedlings, at 9 hpi, highest HRGP gene expression was observed in GB treated seedlings followed by LPS, CWG and DCA treatments, which were 3.24, 3.06, 2.82 and 2.71 folds higher than that of the susceptible control seedlings, respectively. To our knowledge this is the first report of temporal and quantitative profiling of HRGP genes during inducer elicited systemic acquired resistance in pearl millet-downy mildew host-pathogen system.

In this study, PR-1 transcript expression level was significantly higher in resistant and GB treated seedlings compared to the other elicitor treated and susceptible controls. In the pathogen inoculated samples, PR-1 gene expression gradually increased from 3 hpi and peaked at 48 hpi and decreased thereafter. The PR-1 gene expression in GB treated seedlings was even higher than that of the resistant check at 48 hpi. Among the pathogen inoculated, elicitor treated seedlings, at 48 hpi, highest PR-1 gene expression was observed in GB treated seedlings followed by LPS, CWG and DCA treatments, which were 4.74, 4.58, 4.03 and 3.28 folds higher than that of the susceptible control seedlings, respectively. Similarly, in the pathogen inoculated samples, PR-5 gene expression gradually increased from 3 hpi and peaked at 24 hpi and decreased thereafter. The PR-5 gene expression in DCA treated seedlings was even higher than that of the resistant check at 24 hpi. Among the pathogen inoculated, elicitor treated seedlings, at 24 hpi, highest PR-5 gene expression was observed in DCA treated seedlings followed by GB, LPS, and CWG
treatments, which were 3.86, 3.71, 3.59 and 3.21 folds higher than that of the susceptible control seedlings, respectively.

To our knowledge, this is the first report of the various defense enzymes/proteins and their transcript accumulation pattern was studied during GB, LPS and DCA mediated induction of resistance, in any host pathogen system in general and a monocot-oomycete interaction in particular. Various defense enzymes and signal molecules acting co-operatively may contribute to the development of an effective mechanical and chemical defense barrier in pearl millet plants against *S. graminicola* invasion. This hypothesis is substantiated by our findings by showing that high levels of POX, PPO, PAL, HRGPs, PR-1, and PR-5 gene activities in elicitor treated pearl millet seedlings which are correlated with high levels of resistance to downy mildew disease. Measurement of mRNA accumulation demonstrates that genes encoding POX, PAL, HRGPs, PR-1 and PR-5, were induced substantially after elicitor treatment and their expression became more prominent and pronounced after challenge with *S. graminicola*, which indicates that these elicitors are good priming agents. The enzyme products of the genes examined here are predicted to be involved in the biosynthesis of defense compounds, so it is not surprising that transcripts accumulated to high levels following pathogen inoculation.

**In the fourth chapter**, two innovative holistic biological strategies were developed for management of pearl millet downy mildew disease. The first strategy included the combining different elicitors for synergized mechanisms of action against downy mildew, and the second strategy included integrating elicitors and fungicides for maximizing downy mildew suppression and minimizing fungicide dosage. In the first chapter, it was established that GB offered superior downy mildew protection in comparison to the other tested elicitors. Therefore, here GB and other previously identified effective elicitors for pearl millet downy mildew management like BTH, Chitosan, Cerebroside, BABA, *Bacillus pumilus* INR7, and *Pseudomonas fluorescens* UOM SAR 14 will be combined and evaluated against pearl millet downy mildew disease. The results showed that combination of GB+Chitosan offered significantly high downy mildew protection compared to the susceptible control and to a considerable level compared to that of the Apron treatment. Further, when GB
was first treated and after 6 h Chitosan was treated, provided the best results compared to the other treatment schemes. It was also observed that the GB+*B. pumilus INR7* and GB+*P. fluorescens* UOM SAR 14 treatments showed luxurious growth promotion in comparison to the other scheme of treatments. In this study, the second strategy tested where GB was integrated with half dosage of Apron (3g/kg seed), the combined treatment was very effective to control downy mildew disease. Prior treatment of seeds with GB followed by treatment with half dosage Apron showed DM protection which was equivalent to performance of the full dose treatment of Apron.

Overall, when the two strategies were compared, it is inferred that that combination of GB with half dosage Apron was superior to the other strategy of combining GB with different elicitors. The results of the present study, show that immunity elicitors when synergized with the other resistance inducers, bio-control agents, and chemical fungicides present a novel, eco-friendly, integrated, holistic and sustainable strategy that can be recommended for pearl millet downy mildew management in particular and other agriculturally important disease in general.

**Finally**, based on the findings at various levels, pearl millet farmers today are faced with the enormous task of devising novel sustainable strategies to control downy mildew disease and thereby prevent the yield and monetary losses incurred. A prerequisite to face this challenge, one first needs to understand the basics of *Sclerospora graminicola* pathogenicity and pearl millet innate immunity which the purpose of this study. The elicitors DCA, CWG, LPS and GB when applied as seed treatment, induced systemic and durable resistance against downy mildew disease, however, GB was the most efficient elicitor. Our results infer that cell wall reinforcement mechanisms like the callose, lignin and H$_2$O$_2$, NO, HRGPs accumulation and the HR were enhanced consequent to elicitor treatments and therefore serve as important structural markers to ascertain the induction of systemic resistance in pearl millet downy mildew system. This hypothesis is substantiated by our findings showing that high levels of PAL, POX, PPO, glucanase, LOX, HRGPs, PR-1, and PR-5 enzyme and gene activities in elicitor treated pearl millet seedlings positively correlated with high levels of resistance to downy mildew disease. The very
rapid and large changes elicitor treated seedlings, in contrast to the delayed, smaller changes in the untreated susceptible control seedlings suggests that rate and magnitude of defense gene expressions are important for the effective manifestation of defense. The combined data lead to a hypothesis that the resistance induction by CWG, LPS and GB follow SA signaling and resistance induction by DCA follows JA signaling pathway.

The most commonly used fungicides for downy mildew control have detrimental effects on human and environmental health. However, since it is unlikely to obtain absolute downy mildew control using the induced systemic strategy, integrated use of various control methods is an attractive prospect. Our results demonstrated that using the elicitor combination and fungicides, even at half-rate, can provide levels of disease control and yield increases that are equal to the best fungicide-only treatment. The results of the present study, show that immunity elicitors when synergized with the other resistance inducers, bio-control agents, and chemical fungicides present a novel, eco-friendly, integrated, holistic and sustainable strategy that can be recommended for pearl millet downy mildew management in particular and other agriculturally important disease in general.

To conclude, this study provides detailed comparative examination elicitor-responsiveness at the histological, biochemical and molecular levels and presents novel information on the changes that occur in pearl millet-downy mildew interaction. At a broader applied level, it is hoped that this research will contribute to the discovery of new elicitors for managing the destructive effects of downy mildew disease on pearl millet and other important agricultural crops.