Summary and Conclusion
Moth bean (*Vigna aconitifolia*) is an economically important kharif pulse of leguminaceae family. Rajasthan is the most important moth bean growing state of the country contributing about 86% in area and 79% in production. Moth bean in arid and semiarid regions suffers from charcoal root rot disease caused by *Macrophomina phaseolina*. *M. phaseolina* (Tassi) Goid is one of the most damaging seed and soil borne fungal pathogen of moth bean growing areas in India. *M. phaseolina* has a wide host range and is responsible for causing losses on more than 500 cultivated and wild plant species.

The present study has been undertaken to understand the biochemical and molecular basis underlying defense response in 15 day and one month old moth bean plants of three varieties viz. RMO-40, CZM-3 (susceptible) and FMM-96 (resistant) inoculated with the fungal pathogen *Macrophomina phaseolina*. The analysis of various parameters such as polyphenols, flavonoids, proline, total proline content and enzyme activities such as PAL, SOD, POD, CAT, β-1,3-glucanase and chitinase were determined and found to be higher in inoculated plants as compared to control plants.

The highest polyphenol content in 15 days and one month old plants was obtained at 96 h for all the three varieties. For 15 days old plants, a consistent increase in polyphenol content was observed with a maximum of 3.15, 1.83 and 6.17 fold at 96 h after pathogen inoculation in all the three varieties, RMO-40, CZM-3 and FMM-96, respectively, as compared to control. Whereas for one month old plants, the polyphenol content is 6.13, 3.09 and 2.16 mg g⁻¹fw at 96 h for var. FMM-96, CZM-3 and RMO-40, respectively, which is 344.2, 218.5 and 272.4 % higher than the control.

Maximum flavonoid content for 15 days old plants was 1.95 fold, 1.94 fold and 3.07 fold higher than the control in var. RMO-40, CZM-3 and FMM-96, respectively, after pathogen inoculation at 72 h. In one month old plants, the maximum flavonoid content was 147.4, 160.1 and 295.4% lower in control than the inoculated plants for var. RMO-40, CZM-3 and FMM-96, respectively.

The activity of PAL, one of the main enzymes of the phenylpropanoid pathway leads to the synthesis of polyphenols and flavonoids. Any stress such as fungal pathogen inoculation will alter its level and consequently that of its product.
The PAL activity for 15 days old plants was 3.72, 3.67 and 4.29 fold higher at 2 h in
var. RMO-40, CZM-3 and FMM-96, respectively as compared to control plants. In
one month old plants the PAL activity in pathogen inoculated plants was also
obtained at 2 h which was 2.36, 2.07 and 4.97 fold higher in var. RMO-40, CZM-3
and FMM-96, respectively as compared to control plants. Beyond the maximum, no
further increase in PAL activity was observed rather a gradual decline till 168 h was
obtained.

The highest peroxidase activity in 15 days and one month old plants was
obtained at 48 h for all the three varieties. In 15 days old plants, the peroxidase
activity was 3.15, 3.10 and 3.60 fold higher in var. RMO-40, CZM-3 and FMM-96,
respectively, after pathogen inoculation as compared to control. However, the initial
increase was observed upto 48 h, thereafter a gradual decrease was observed upto
168 h in all the three varieties. In one month old plants, the maximum peroxidase
activity was 268.5, 226.7 and 331.4% lower in control than the inoculated plants for
var. RMO-40, CZM-3 and FMM-96, respectively.

The presence of H$_2$O$_2$ was also detected histochemically in control and
inoculated leaves of moth bean plants using DAB. A distinct colouration or
appearance of reddish brown patches in the inoculated leaves evidences H$_2$O$_2$
accumulation and the intensity of colouration indicated that its concentration is more
in the inoculated plants compared to the control plants. Thus, the determination of
peroxidase and detection of H$_2$O$_2$ by DAB method confirms the synthesis of H$_2$O$_2$ in
the *Vigna aconitifolia-Macrophomina phaseolina* plant-pathogen system.

The catalase activity was in general found to be higher at 24 h in the
pathogen inoculated 15 days and one month old plants of moth bean as compared to
the control plants. In 15 days old plants, the catalase activity was 1.98, 1.51 and 2.70
fold higher in var. RMO-40, CZM-3 and FMM-96, respectively, after pathogen
inoculation as compared to control. For one month old plants, the maximum catalase
activity was 2.49, 2.17 and 3.15 fold higher in var. RMO-40, CZM-3 and FMM-96,
respectively, after inoculation as compared to the control plants.

The superoxide dismutase activity was in general found to be higher at 4 h in
the pathogen inoculated 15 days and one month old plants of moth bean as
compared to the control plants. In 15 days old plants, the maximum superoxide
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dismutase activity was 2.51, 1.92 and 3.49 fold higher in var. RMO-40, CZM-3 and FMM-96, respectively, after pathogen inoculation as compared to control. For one month old plants, the maximum SOD activity at 4 h was 4.26, 4.07 and 4.88 fold higher in var. RMO-40, CZM-3 and FMM-96, respectively, as compared to control.

The total protein content and proline content was in general found to be higher at 48 h in the pathogen inoculated 15 days and one month old plants of moth bean as compared to the control plants. In 15 days old moth bean plants, the total protein content was 1.88, 2.15 and 3.30 fold lower in control plants of var. RMO-40, CZM-3 and FMM-96, respectively, as compared to pathogen inoculated plants. For one month old plants, the total protein content was 1.40, 1.92 and 2.79 fold lower in control plants of var. RMO-40, CZM-3 and FMM-96, respectively, as compared to pathogen inoculated plants.

In 15 days old moth bean plants, the proline content was 1.77, 1.92 and 3.14 fold higher in pathogen inoculated plants of var. RMO-40, CZM-3 and FMM-96, respectively, as compared to control plants. For one month old plants, the proline content was 1.76, 2.45 and 2.62 fold lower in control plants of var. RMO-40, CZM-3 and FMM-96, respectively, as compared to pathogen inoculated plants.

This study revealed the role of PR proteins (β-1,3-glucanase and chitinase) in the defense response of moth bean with respect to charcoal root rot infection. The high native activity of β-1,3-glucanase and chitinase in the resistant cultivar, the increased higher activity upon inoculation in the resistant than susceptible cultivar and the positive reaction in the western blot analysis confirmed the positive role of these enzymes in imparting disease resistance. Chitinases and β-1,3-glucanases have been purified and characterized from several of plant sources. Genes encoding these enzymes have also been cloned from a variety of plants. Currently, there is an immense interest in delineating the molecular events from pathogen recognition to the expression of these genes. In an effort to enhance the disease resistance, PR-genes have been used to transform a variety of plant sources. Based on the results obtained through our study, it can be concluded that both chitinase and β-1,3-glucanase have a distinct role in imparting disease resistance in moth bean against *Macrophomina phaseolina.*
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Research into the nature of R-genes and RGAs has been greatly accelerated in different plant species in recent times. Information on more resistance gene sequences is necessary to detect more structural motifs, which are the basis for the search of resistance gene analogs in crop plants. In this study, we report the amplification, cloning, and characterization of NBS–LRR class resistance-gene candidate sequences from *Vigna aconitifolia*, using degenerate primers based on conserved motifs of known R genes.

This is the first report on the isolation of RGAs in *Vigna aconitifolia* using a PCR-based approach. In general, the NBS-LRR class of R-genes consists of hundreds of paralogs in plant species. With the help of the identified *Vigna aconitifolia* RGAs, different primer sets can be designed for analysis of *Vigna aconitifolia* wild relatives to target novel genomic resources for the genetic improvement of *Vigna aconitifolia*. Studies on R-genes and RGAs are still explorative in nature. Information on more R-gene sequences is necessary to delineate more structural domains, which is the basis for the search of RGAs in any crop plant.

The overall response for *Vigna aconitifolia-Macrophomina phaseolina* plant-pathogen system was obtained higher in the pathogen inoculated plants as compared to control plants. Another noteworthy feature which may point to varietal differences is that in both 15 days and one month old plants of var. FMM-96, a resistant variety, shows better response to pathogen inoculation in comparison to the var. RMO-40 and CZM-3, susceptible varieties. Further, the 15 days old plants respond better to pathogen inoculation as compared to the one month old plants. This may be due to the higher metabolic rate and actively growing cells of young plants as compared to the mature plants.

From the results of present study it can be concluded that the inoculation of the fungal pathogen *Macrophomina phaseolina* in *Vigna aconitifolia* plants induces the defense response. This results in increased accumulation of polyphenols, flavonoids, proline, total proteins along with increase in the activities of PAL, peroxidases, catalase, superoxide dismutase including to PR proteins viz., β-1,3-glucanase and chitinase which are known to inhibit fungal growth.
The present study further reveals that there is a relationship between plant metabolic synthesis and fungal inoculation which support their role in plant resistance. This study can be utilized in breeding programmes for incorporation of resistant traits in promising susceptible genotypes of moth bean. Understanding resistance mechanism involved in Vigna aconitifolia-Macrophomina phaseolina interactions will aid the development of new pathogen resistant Vigna aconitifolia cultivars and allow such interaction to be used as models for other similar plant-pathogen systems. Furthermore, the identification of defense proteins in Vigna aconitifolia plants is of great importance for establishing appropriate techniques to manipulate and use these proteins through classical breeding or genetic manipulation.