Final Report
Minor Research Project
F.No. 34-565/2008 (SR)

"Brassinosteroid regulated antioxidative defense system in root-knot nematode (*Meloidogyne incognita*) and host plant (*Brassica juncea*) during pathogenesis"

Submitted to
University Grants Commission

By
Puja Ohri
Department of Zoology
Guru Nanak Dev University
Amritsar-143005

Annexure - IV

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

Utilization certificate

Certified that the grant of **Rs. 1,36,300/-**(Rupees One lakh thirty six thousand and three hundred only) received from the University Grants Commission under the scheme of support for Minor Research Project entitled **"Brassinosteroid regulated antioxidative defense system in root-knot nematode (*Meloidogyne incognita*) and host plant (*Brassica juncea*) during pathogenesis"**, vide UGC letter No. F. **34-565/2008(SR)** dated **15-1-2009** has been utilized to an amount of **Rs. 1,34,993/-**(Rupees One lakh thirty four thousand and nine hundred ninety three only) for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission.

Puja Ohri

SIGNATURE OF THE
PRINCIPAL INVESTIGATOR

Dr. Puja Ohri
Principal Investigator
UGC Project
Deptt. of Zoology
G.N.D. University, Amritsar

REGISTRAR

Registrar
Guru Nanak Dev University,
Amritsar.

SIGNATURE OF THE
COINVESTIGATOR

Annexure - V

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

STATEMENT OF EXPENDITURE IN RESPECT OF MINOR RESEARCH PROJECT

1. **Name of Principal Investigator:** Dr Puja Ohri

2. **Deptt. of University:** Zoology

3. **UGC approval No. and Date:** F. No. 34-565/2008 (SR) dated 15-1-2009

4. **Title of the Research Project :** Brassinosteroid regulated antioxidative defense system in root-knot nematode (*Meloidogyne incognita*) and host plant (*Brassica juncea*) during pathogenesis

5. **Effective date of starting the project :** 1-2-2009

6. **a. Period of Expenditure:** From 1-2-2009 to 31-1-2011

b. Details of Expenditure :

S.No.	Item	Amount Approved Rs.	Expenditure Incurred Rs.
i.	Books & Journals	5000/-	5000/-
ii.	Equipment	53,000/-	51,637/-
iii.	Contingency	50,000/-	42,740/-
iv.	Field Work/Travel	2000/-	2000/-
v.	Hiring Services	--------	--------
vi.	Chemicals & Glassware	35,000/-	33,616/-
vii.	Overhead		
viii.	Any other items	**1,45,000/-**	**1,34,993/-**

c . Staff: NA

1. It is certified that the appointment(s) have been made in accordance with the terms and conditions laid down by the Commission.

2. It as a result of check or audit objective, some irregularly is noticed, later date, action will be taken to refund, adjust or regularize the objected amounts.

3. Payment @ revised rates shall be made with arrears on the availability of additional funds.

4. It is certified that the grant of Rs. **1,36,300/-** (Rupees One lakh thirty six thousand and three hundred only) received from the University Grants Commission under the scheme of support for Minor Research Project entitled **"Brassinosteroid regulated antioxidative defense system in root-knot nematode (*Meloidogyne incognita*) and host plant (*Brassica juncea*) during pathogenesis"** vide UGC letter No. F. **34-565/2008 (SR)** dated **15-1-2009** has been utilized to an amount of **Rs. 1,34,993/-**(Rupees One lakh thirty four thousand and nine hundred ninety three only) for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission.

Puja Ohri

SIGNATURE OF PRINCIPAL INVESTIGATOR

REGISTRAR

Registrar
Guru Nanak Dev University,
Amritsar.

SIGNATURE OF THE COINVESTIGATOR

Annexure - VI

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

STATEMENT OF EXPENDITURE INCURRED ON FIELD WORK

Name of the Principal Investigator: Dr Puja Ohri

Name of the Place visited	Duration of the Visit		Mode of Journey	Expenditure Incurred (Rs.)
	From	To		
Amritsar- Ludhiana	6-2-2010		Own car	2000/-

Certified that the above expenditure is in accordance with the UGC norms for Major Research Projects

Puja Ohri

SIGNATURE OF PRINCIPAL INVESTIGATOR

Dr. Puja Ohri
Principal Investigator
UGC Project
Department of Botany
Guru Nanak Dev University Amritsar

REGISTRAR
Guru Nanak Dev University,
Amritsar.

SIGNATURE OF THE CO-INVESTIGATOR

Annexure – VIII

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1. NAME AND ADDRESS OF THE PRINCIPAL INVESTIGATOR: Dr Puja Ohri, Department of Zoology, Guru Nanak Dev University, Amritsar-143005

2. NAME AND ADDRESS OF THE INSTITUTION : Guru Nanak Dev University, Amritsar-143005

3. UGC APPROVAL NO. AND DATE: F. No. 34-565/2008 (SR) dated 15-1-2009

4. DATE OF IMPLEMENTATION : 1-2-2009

5. TENURE OF THE PROJECT: 2 years

6. TOTAL GRANT ALLOCATED : Rs. 1,45,000/-

7. TOTAL GRANT RECEIVED : Rs. 1,36,300/-

8. FINAL EXPENDITURE : Rs. 1,34,993/-

9. TITLE OF THE PROJECT : Brassinosteroid regulated antioxidative defense system in root-knot nematode (*Meloidogyne incognita*) and host plant (*Brassica juncea*) during pathogenesis

10. OBJECTIVES OF THE PROJECT :

- To evaluate the role of 24-epibrassinolide on antioxidative defense system of host plant (*Brassica juncea*) during root-knot nematode (*Meloidogyne incognita*) infection
- To evaluate the role of 24-epibrassinolide on some antioxidative enzymes of *M. incognita* females.

11. WHETHER OBJECTIVES WERE ACHIEVED : Yes

- Literature of last 20 years was collected from various libraries and data bases
- Biochemical analysis was completed in nematode females under the influence of 24-epibrassinolide

- Biochemical analysis was completed in host plant during nematode infection under the influence of 24-epibrassinolide
- Data was compiled and statistically analysed
- Results were presented in a National Conference
- Results have been communicated in an International Journal

12. ACHIEVEMENTS FROM THE PROJECT

Abstract published:

Ohri, P. and Kaur, R. (2010). "Role of 24-epibrassinolide on some antioxidative enzymes of host plant during root-knot nematode infection" *In*: National Conference on Innovations in Nematological Research for Agricultural Sustainability-Challenges and a Roadmap Ahead", Tamil Nadu Agricultural University, Coimbatore, 23-25 Feb., pp 90-91.

Research paper communicated:

Ohri, P., Bhardwaj, R. and Kaur, R. (2011). 24-epibrassinolide regulated antioxidative defense system in *Brassica juncea* during root-knot nematode (*Meloidogyne incognita*) infection. *Journal of Environmental Research and Development* (communicated).

13. SUMMARY OF THE FINDINGS:

Among the various plant parasitic nematodes, the nematodes infecting plants are known as 'Phytonematodes'. Of all the known species of phytonematodes, root knot nematodes (RKNs), Meloidogyne species are of utmost importance. Among the many genera of phytonematodes (e.g Heterodera, Globodera, Pratylenchus, Tylenchus, etc.) having some economic impact, *Meloidogyne* species are responsible for large amount of damage to agricultural crops. During plant pathogen interaction, active oxygen (AO) species are produced in plants. AO species are generated at a higher rate in plants under biotic and abiotic stress, which is particularly important in defense mechanism as well as in recognition of incompatible pathogen. Therefore, nematodes infecting plants must deal not only with AO generated by their own metabolism but also with those produced by the host to their attack. For AO detoxification, plants have an efficient enzymatic system that includes catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APOX), guaiacol peroxidase (GPOX) and glutathione reductase (GR).

Plants also produce a wide array of growth regulatory compounds which have broad spectrum effects. The regulatory effects of brassinosteroids have been extensively studied in plants and have been documented in animal systems too. In the present work, *B. juncea* served as host plant which is a good source of

natural steroids. Sterilized seeds of *Brassica juncea* cv. PBR91 were treated with different concentrations (10^{-11}M, 10^{-9}M, 10^{-7}M) of 24-Epibrassinolide. Seeds were allowed to germinate and 15 days old seedlings were then inoculated with second stage juveniles ($1000J_2$s/plant) of *M. incognita*. Time course experiments were conducted in which estimations were done seven and fifteen days after inoculation. Enzyme estimation was carried out for Catalase (Aebi, 1983); Ascorbate peroxidase (Nakano and Asada, 1981); Guaiacol peroxidase (Putter, 1974), Superoxide dismutase (Kono, 1978) and Glutathione reductase (Carlberg and Mannervick, 1975) in both roots and shoots of host plant. For each experiment, treated and inoculated plants were compared with untreated, uninoculated plants (i.e.control I) as well as untreated, inoculated plants i.e. control II. The results revealed that specific activities of CAT, SOD, APOX, GPOX and GR was found enhanced in root extracts of infected plants seven and fifteen days after inoculation. After EBI treatment, the enzyme activity was enhanced in higher concentration but was suppressed in lower concentrations. However, in case of shoots, the specific activity of all enzymes was suppressed in nematode inoculated plants. Whereas, the specific activity was enhanced in almost all concentrations after EBI treatment. The enzyme activities of all enzymes in both roots and shoots of inoculated and treated plants were higher in seven DAI as compared to fifteen DAI.

To achieve the second objective, females were extracted from the cultured tomato plants and given dip treatment for two hours in three concentrations i.e. 10^{-11}M, 10^{-9}M, 10^{-7}M of EBI. Females in distilled water served as control. Enzyme estimations were carried out for Catalase (Bergmeyer, 1974); Superoxide dismutase (Kono, 1978); Glutathione-S-transferase (Chien and Dauterman, 1991) and Esterases (Katzenellenbogen and Kafatos, 1971). Protein estimations (Lowry et al., 1951) were also carried out along with the enzymatic estimations for calculating the specific activity. Catalase, GST and EST activities were enhanced in females treated with 10^{-11}M concentration and decreased in other concentrations. However, SOD activity was enhanced in two concentrations 10^{-11}M and 10^{-7}M, whereas suppressed in 10^{-9}M concentration.

14. CONTRIBUTION TO THE SOCIETY :

It is widely accepted that reactive oxygen species (ROS) are susceptible for various stress-induced damages to macromolecules and ultimately to cellular structures. Consequently, the role of antioxidative enzymes becomes very important. The increased level of antioxidative enzymes in plants following the application of EBI has been found to overcome the stress generated by nematode infection thus boosting the resistance capacity of plants. However, the present study suggests that brassinosteroids can be the suitable candidate for their application in agriculture, especially against the biotic stress.

15. WHETHER ANY PH.D. ENROLLED/PRODUCED OUT OF THE PROJECT:
NA

16. NO. OF PUBLICATIONS OUT OF THE PROJECT:

Abstract published:

Ohri, P. and Kaur, R. (2010). "Role of 24-epibrassinolide on some antioxidative enzymes of host plant during root-knot nematode infection" *In*: National Conference on Innovations in Nematological Research for Agricultural Sustainability-Challenges and a Roadmap Ahead", Tamil Nadu Agricultural University, Coimbatore, 23-25 Feb., pp 90-91.

Research paper communicated:

Ohri, P., Bhardwaj, R. and Kaur, R. (2011). 24-epibrassinolide regulated antioxidative defense system in *Brassica juncea* during root-knot nematode (*Meloidogyne incognita*) infection. *Journal of Environmental Research and Development* (communicated).

Puja Ohri

(PRINCIPAL INVESTIGATOR)

(REGISTRAR)

Guru Nanak Dev University, Amritsar.

Reem Bhardwaj

(CO-INVESTIGATOR)