Biochem. 722

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Faisalabad

Biochem. 722 2(2-0)
RESEARCH PLANNING AND SCIENTIFIC WRITING

- **Overview** of scientific research: improvement through research; nature of scientific inquiry; application of research in industry. **Project** selection and development: Writing a research grant application; role of students & supervisors; experimental design and investigation; reviewing the literature; decision on techniques to be employed. **Methodology**: samples, controls and replications; sampling methods; use of microorganisms, animals, plants and humans in experimentation; use of pathogens in experiments; data processing. **Analysis of results**: Results interpretation; primary and secondary sources; scientific record keeping. **Scientific writing**: Compilation of a research report; publication of a research paper; selection of journal, instructions to authors, letters to editor, acknowledgement, referee's comments and suggestions, sending a revised manuscript and acceptance letter; publication of review articles. **Ownership of data**: conflict and justification of ideas; **plagiarism** and its control; filing **patent** application.

SUGGESTED READINGS

•Fink , A. G. 2004. Conducting Research Literature Reviews: From the Internet to paper. Sage Publications, London.

•Grazinao, A.M. and M.L. Raulin (2006) Research methods: A process of Inquiry. Longman. London

•Holiday, A. 2002. Doing and writing qualitative research. Sage Publications. London.

•Leedy, P.D. and J.F. Ormrod. 2009. Practical Research: Planning & Design. Publishers: Merrill.

•Lindof, T.R. 2002. Qualitative Communication Research Methods. 2nd ed. Sage Publications, London.

•Sharma, M. 2004. Research Methodology. Anmol Publications. New Delhi. India.

•Veit, R. and J. Clifford . 1985. Writing, Reading & Research Clifford. Bobbs-Merrill Educational Pubications.

RESEARCH GRANT APPLICATION FORM

COVER SHEET FOR PROPOSAL

HIGHER EDUCATION COMMISSION

H-9, ()

For HEC use only

Proposal Identification Number

A. TITLE OF PROPOSED PROJECT: **Isolation, cloning and expression of novel antimicrobial genes from medicinal plants**

B. WHETHER PROPOSED RESEARCH IS BASIC ✔ OR APPLIED ☐

C1. RESEARCH DOMAIN

✔ Sciences ☐ Engineering & Technology ☐ Social Sciences ☐ Humanities

C2. STATE FIELD OF RESEARCH AND SPECIALIZATION (For example; Major: Chemistry, Specialization: Organic)

Major **BIOCHEMISTRY** Specialization **MOLECULAR BIOLOGY**

D. PROJECT DIGEST. Describe the proposed research using (about 250) words geared to the non-specialist reader.

Fungal and bacterial infections have been increased tremendously during the past few years. One major reason is the development of resistance in pathogenic microorganisms against the available antimicrobial compounds and drugs. This may lead to fatal infections in many cases. This situation is not only a growing threat to humans, but also for plants. Therefore, there is a dire need to investigate natural sources for new antimicrobial compounds. Antifungal and antibacterial proteins and peptides have been found in many plants. We also have isolated and purified such proteins from some of the plants. However, for application of such antimicrobial compounds, large scale production is necessary. One such method is to isolate the genes corresponding to antimicrobial compounds and hyperexpress in simple systems that can give high yields. In this project we will isolate novel antimicrobial genes from some medicinal plants that are known to exhibit antimicrobial proteins/peptides. The genes will be isolated using Differential Display (DD)-PCR which is relatively a new technique for detection of novel genes under certain stress conditions. We have developed expertise and facilities for the technique in our lab. The seeds of the potential medicinal plants will be induced for the antimicrobial genes and subjected to DD-PCR for isolation of the genes. The antimicrobial genes will be expressed in *E. coli* and the corresponding proteins will be tested for antimicrobial activity. The outcome of the project will be highly beneficial for the country as it will provide an opportunity to explore novel antimicrobial proteins from our natural resources. This will also contribute towards scientific knowledge for fighting against antimicrobial infections.

E1. PRINCIPAL INVESTIGATOR NAME (full with no initials) **Dr AMER JAMIL**		E2. HIGHEST DEGREE **PhD**	E3. POSITION/TITLE **Professor**
E4. DEPARTMENT/SECTION **Chemistry and Biochemistry**	E5. UNIVERSITY/INSTITUTION **,**	E6.MAILING ADDRESS **Molecular Biochemistry Lab., Dept. of Chemistry & Biochemistry, , Faisalabad-38040**	
E7. Telephone: **041-9201104** (Area code, number and extension) Email: **amerjamil@yahoo.com**		Fax: (Area code, number)	

F: CO-PRINCIPAL INVESTIGATOR		
Name & Position **Dr Muhammad Shahid, Assistant Professor Biochemistry, ,**		Professional Address: **Protein Molecular Biology Lab., Dept of Chemistry &**
G1. PROPOSED DURATION OF PROJECT: (*in months*) **24 months**	G	H. TOTAL FUNDS REQUESTED **Rs. 3.965209 million**

CERTIFICATES

1) **Certified that the PI is a full time Faculty Member/Foreign Professor/ Eminent Scholar/Eminent Researcher of the University / Degree awarding institutes.**
2) **Certified that the equipment(s) demanded for the subject project is / are not available in the University / Institute**.
3) **Certified that the project under reference has not been submitted to any other funding agency including HEC.**
4) **Certified that No portion of the project has been funded by any other funding agency including HEC in the past.**

SIGNATURE OF PRINCIPAL INVESTIGATOR

SIGNATURE THE HEAD OF INSTITUTION

(Vice-chancellor/Rector of University, Director of Degree-awarding Institutions)

SIGNATURE OF PRINCIPAL INVESTIGATOR
Date:
SINATURE OF CO-PRINCIPAL INVESTIGATOR
Date

ENDOSEMENT OF THE HEAD OF INSTITUTION (Vice-chancellor/Rector of University, Director of Degree-awarding Institutions)
Signature & Date
Name **Prof. Dr. Iqrar A Khan** Title: **Vice Chancellor**
Address: **,**
Phone: **041-9200200** FAX: **041-9200764** E-mail: **vc@uaf.edu.pk**

PROJECT DETAILS

1. PROJECT SUMMARY

Describe the proposed research using (about 250) words.

A remarkable increase in resistance in fungal and bacterial strains against the antimicrobial compounds has been found during the last few decades. This has lead to a very serious situation in many cases especially in immunosuppressive individuals. Economic losses are also observed due to fungal infections in crops and other plants. Therefore, there is a strong need to explore new antimicrobial compounds from different sources, including medicinal plants. We have isolated and purified some antifungal and antibacterial proteins from some plants. Heterologous expression of the genes related to such proteins is an efficient way to meet the ever increasing demand of the antimicrobial compounds. Differential Display (DD)-PCR has been emerged as a very impressive and reliable technique for isolation of novel genes under different conditions from a variety of sources including plants. We have optimized this technique in our lab. The present project focuses on isolation of novel antimicrobial genes from different medicinal plants, followed by their expression in heterologous hosts. The seeds of the medicinal plants will be induced with fungal infection for expression of the antimicrobial genes. RNA from induced and non-induced samples will be isolated. First strand of cDNA will be made and subjected to PCR. The differentially expressed genes will be isolated from the gel, cloned and sequenced. The potential genes that code for antimicrobial proteins will be cloned in suitable expression vectors and expressed in *E. coli*. The expressed proteins will be tested for antimicrobial activity and molecular mass determination. The project will contribute towards new scientific knowledge for fighting against antimicrobial infections. It will also be beneficial for the country as will help explore our natural resources for isolation and expression of novel antimicrobial compounds.

2. PROPOSED GOALS/OBJECTIVES (PLEASE IDENTIFY QUANTIFIABLE GOALS)

i. If the proposed research is basic, please identify or postulate scientific hypothesis on which your proposed goal is based.
ii. If the proposed research is applied, please clearly identify the output in the form of a product or process, need or relationship to industry and also identify the end-user of your output/ product. P.I. is encouraged to make preliminary inquiries with the proposed end user and attach any certificate/ document in support of the proposed research.

HYPOTHESIS/BASIS OF RESEARCH (if basic research)

Medicinal plants express antimicrobial proteins/peptides, therefore, novel antimicrobial genes may be isolated from medicinal plants and expressed in heterologous hosts.

GOALS/OBJECTIVES (please quantify your objectives in case of Applied research)

1. **Isolation of differentially expressed genes under fungal induced conditions.**
2. **Cloning, sequencing and analysis of the genes for identification of the genes related to antimicrobial compounds.**
3. **Expression of the antimicrobial genes in heterologous hosts.**
4. **Characterization of the recombinant antimicrobial proteins.**

IDENTIFY END USER/ BENEFICIARY INDUSTRY (if applied research)

N/A

3. INTRODUCTION (NOT TO EXCEED ONE PAGE)

The introduction should consist of three paragraphs; the first paragraph should indicate the scientific hypothesis/commercial basis on which the project is based. The second paragraph should introduce the precise nature of the project, and the final paragraph should indicate the proposed objectives in the light of the first two paragraphs and explain clearly what the reader will see in the main body of the proposal.

Fungal and bacterial infections have been increased dramatically during the last few years mainly due to increased use of antibiotics, success in organ transplantation, immunosuppressive therapy, international travels, exploitation of new habitats etc. On the other hand resistance of fungal and bacterial strains against implemented antimicrobial compounds has also increased tremendously. This can lead to serious and fatal infections especially in immunosuppressive individuals. Such situation has created a great threat not only to humans, but also to crops as well. Different fungi can cause serious diseases in plants and animals. They can degrade wood, leading to economic losses; therefore, it is of growing interest to detect antifungal compounds to control the development of plant-destroying fungi (Blanchette, 1994). In this regard the researchers have directed their research focus during the last few years towards the exploration of natural sources (Yadev et al., 2007). As many of the antibiotics and other synthetic drugs have shown sensitization reactions, main thrust of research has been towards the extraction of aini-infectional compounds including antimicrobial peptides/proteins from plants, animals and microorganisms (Selitrennikoff, 2001).

Medicinal plants are highly efficient to cure diseases, and occupy a significant place in modern medicine (Bhattacharjee, 2001). These also cater the needs of people who reside in villages and remote areas. Besides the demands made by these systems as their raw material, the demands for medicinal plants made by the modern pharmaceutical industries has increased manifold (Gupta *et al*., 1999; de Lucca *et al*., 2005). Antimicrobial compounds have also been isolated and reported from plants (Theis and Stahl, 2004). Antifungal proteins from plants are organized into five major groups based on sequence analysis (van Loon, 1985) and termed Pathogenesis-related proteins: PR-1 (cystein-rich and small proteins of ~15-17 kDa), PR-2 (β-glucanases), PR-3 (chitinases), PR-4 (chitin-binding proteins), PR-5 (thaumatin-like proteins). We have investigated many plants (*Hygrophila auriculata*, *Abrus precatorius*, *Moringa oleifera*, *Croton tiglium*, *Withania somnifera*, *Solanum nigrum* and *Psoralae corylifolia*) for antimicrobial activities (Jamil *et al*., 2007). We also have isolated antifungal proteins from some plants (Jamil, 2008; Shahid *et al*., 2008). The present project has been developed based on outcome of the previous research projects completed in our lab. (Jamil, 2008; Jamil, 2009).

Novel genes expressing antimicrobial compounds need to be isolated and hyperexpressed for practical applications. In this project we will concentrate on isolation, cloning and expression of novel antimicrobial genes from plants. We will isolate the genes by differential display PCR technique that has already been optimized in our lab. The genes will be cloned in plasmids and expressed in *E. coli*. Hyperexpression of the proteins will be achieved by using strong promoter systems in the expression vectors. The recombinant antimicrobial proteins will be tested for antimicrobial activity. Apart from expression of the antifungal genes, analysis of the differentially expressed genes would also help understand the nature of host-fungal interaction as only a little information is available in this area (Sturtevant, 2000). This may lead to the development of novel antifungal drug targets. The project will contribute towards new scientific knowledge for fighting against antimicrobial infections.

4A. BACKGROUND OF THE RESEARCH PROBLEMS TO BE ADDRESSED **(Not to exceed two pages)**

i. **In case of basic research, a comprehensive and up-to-date literature survey clearly highlighting the existing gaps and what new information will be added to the existing pool of knowledge.**

ii. **In case of applied research, please also identify the industry in , which should benefit from the process/product. Please justify how the proposed research will contribute to the national economy/social sector. Please justify your claim by giving figures of import/export, present market, future trends etc. The principal Investigator is encouraged to discuss the proposed research with the proposed beneficiary and attach supporting documentation.**

Several plants have been shown to exhibit antimicrobial activities (Jamil *et al*., 2007). Alkoloidal extracts of *Zanthoxylum chiloperone* var. angustifolium have been found to exhibit antifungal activity against *Candida albicans and Asperigillus fumigatus* (Thouvenel *et al*., 2003). Similarly, *Zingiber officinale* (ginger) and *Juglans cinerea* (butternut) had pronounced antifungal activity against a variety of human pathogenic fungi (Christine *et al*., 2002). A defensin-like antifungal peptide has been reported from French bean seeds (Miyakawa *et al*., 2007; Leung *et al*., 2008). *Ginkgo biloba* seeds exhibited antifungal activity against some fungi (Sawano *et al*., 2007). Chinese cabbage (*Brassica campestris* L.) also possessed antifungal activity (Lee *et al*., 2007; Park *et al*., 2007). Antifungal activity has also been shown in the seeds of *Pouteria torta* (Boleti, 2007). Plant chitinases have also been shown to possess antifungal activities against many fungi (Kirubakaran and Sakthivel, 2007; Ho and Ng, 2007; Onaga and Taira, 2008). Athikomkulchai *et al.* (2006) reported two compounds, 3'-(4"-hydroxy-3",5"-dimethoxyphenyl)-propyl benzoate (1) and 3'-(4"-hydroxyphenyl)-propyl benzoate isolated from the branches of *Croton hutchinsonianus*. The phenylpropyl benzoates were found to exhibit antifungal activity against *Candida albicans*. Oil from the species *Croton cajucara* essential oil inhibited the growth of reference samples of *Candida albicans*. Nihei *et al.* (2005) reported different compounds from a methanol extract of *Croton jatrophoides.* Furthermore, phorbol diesters isolated from a methanolic extract of the seeds of *Croton tiglium* have been found to inhibit the growth of some microbes (Mekkawy *et al*., 2000).

Many proteins or peptides with antibacterial or antifungal activity have been isolated in recent years from various plants. Huynh *et al.* (2001) purified a protein of molecular mass of 30 kDa possessing potent and broad-spectrum antifungal activity from the leaf extracts of *Engelmannia pinnatifida.* Similarly, Cheong *et al*. (1997) purified an antifungal pathogenesis related (PR) group of 5 proteins (BFIP) with a molecular mass of 27 kDa from the floral buds of *Brassica compestris*. In another study, Casadoa *et al*. (2000) purified a 23 kDa thaumatin like protein termed as CsTL1 from mature chestnut (*Castanea sativa*) cotyledons. The purified protein had an antifungal activity against *Trichoderma viride* and *Fusarium oxysporum*. Similarly, Tonon *et al*. (2002) isolated an antifungal protein, β-1,3-glucanase (GLU-39) having a molecular mass of 39 kDa, from potato cultivar (*Solanum tuberosom* L.). A 45 kDa antifungal protein has been reported from blast fungus (*Magnaporthe grisea*)-treated rice leaves (Lee *et al*., 2007). Another 53 kDa homodimeric protein was

4B. RESEARCH PLAN: SCHEDULE/PHASING (Not to exceed one page)

The studies will be completed in two years:

Year I: Induction of antimicrobial genes in the selected medicinal plants with fungal stress followed by isolation and cloning of differentially expressed genes by DD-PCR technique

Year 2: Sequencing, characterization and expression of the isolated and cloned antimicrobial genes

Brief Methodology:

i.Medicinal plants that potentially contain antimicrobial proteins/peptides will be explored for isolation of the genes. For example, *Nigella sativa* (blackseed), *Foeniculum vulgare* (fennel), *Ricinus communis* (castor oil plant), *Cichorium intybus* (common chicory), *Capsicum frutescens* (chili pepper), *Ammi majus* (lace flower), *Trachyspermum copticum* (carom seeds), *Linum usitatissimum* (common flax), *Carthamus tinctorius* (safflower)

ii.Gene induction with fungal stress: Plant seeds, after washing, will be placed separately on Whatman filter paper in a Petri plate and incubated at 25 ˚C (Bachem, 1996). The seedlings will be inoculated with a fungus *Fusarium solani* in order to induce antifungal genes (Lee and Hwang, 2006).

iii.DD-PCR: Total RNA will be isolated by using Qiagene RNeasy plant mini (or equivalent) kit according to the manufacturer's instructions at different time intervals. DNA will be removed by DNase treatment. The integrity of the isolated sample will be checked by ethidium bromide staining through agarose gel electrophoresis (Sambrook and Russell, 2001). First strand of cDNA will be synthesized by Hminus-MMLV-reverse transcriptase kit (Fermentas) using primers anchored to oligo-dT. It will be subjected directly to PCR by using the same anchored primers and arbitrary upstream primers (Torres et al., 2006). The amplified products will be fractionated by denaturing polyacrylamide gel electrophoresis and visualized by silver staining (Deng et al., 1999).

iv.Cloning and sequencing: The gel bands of differentially expressed genes will be excised and the genes will be isolated and re-amplified by using the same set of primers as used above (Deng et al., 1999). The re-amplified products will be ligated in appropriate vector using cloneJet PCR cloning kit (Fermentas). Sequencing of the expressed genes cloned in vectors will be done from DNA sequencing facility (such as).

v.Bioinformatics tools will be employed to find out the novel genes after sequencing.

vi.Expression of the genes in *E. coli*: The genes with potential antimicrobial sequences will be cloned in expression vector (e.g., pET) and transformed in *E. coli* (Sambrook and Russell, 2001). In order to get full-length genes 3' and 5' RACE will be performed. The cloned genes will be induced under IPTG induction and the expressed proteins will be isolated and purified using different chromatographic procedures (Deutcher, 1990). Concentration of protein will be determined by method (, 1976). SDS-PAGE will be run to confirm the protein purification. The proteins will be subjected to antimicrobial assays.

vii.Antifungal assays: Fungal strains (e.g., *Aspergillus , Fusarium solani, Trichoderma harzianum, Mucor mucedo, Alterneria alterneria*) will be

4C. **REFERENCES** (CITED IN 3, 4A & 4B; NOT TO EXCEED TWO PAGES)

Asiegbu, F. O., W. Choi, G. Li, J. Nahalkova and R. A. Dean. 2003. Isolation of a novel antimicrobial peptide gene (Sp-AMP) homologue from *Pinus sylvestris* (Scots pine) following infection with the root rot fungus *Heterobasidion annosum*. FEMS Microbiol. Lett. 228: 27-31.

Athikomkulchai, S., H. Prawat, N. Thasana, N. Ruangrungsi and S. Ruchirawat. 2006. COX-1, COX-2 inhibitors and antifungal agents from *Croton hutchinsonianus*. Chem Pharm Bull. 54:262-264.

Bachem, C. W. B., R. S. van der Hoeven, S. M. de Bruijn, D. Vreugdenhil, M. Zabeau and R. G. F. Visser. 1996. Visualization of differential gene expression using a novel method of RNA fingerprinting based on AFLP: Analysis of gene expression during potato tuber development. Plant J. 9:745-753.

Bhattacharjee, S. K. 2001. Antimicrobial Peptide Can Identify Resistant Bacteria and Target Them for Destruction. Handbook of Medicinal Plants. 3rd Ed. Pointer Pub. Jaipur (India); 1-6, 377.

Blanchette, R. A. 1994. Degradation of the lignocellulosic complex in wood. Can. J. Bot. 73: S999-S1010.

5. IMPACT (OF PROPOSED RESEARCH ON TEACHING/TRAINING OF MANPOWER, INSTITUTIONAL CAPABILITY BUILDING AND ON LOCAL INDUSTRY)

The proposed project will have very positive and significant impact on different aspects of national development. It will definitely enhance the capabilities of researchers and students in the area of gene expression which is a leading field of life sciences in the developed world. We would be able to produce highly skilled manpower in this area of research of global importance. The project proposal will also be beneficial for institution of facilities and advanced techniques in the department. We will be in a better position to deliver the practical knowledge to our students. The trained graduates will be able to serve as leaders in our future research endeavors. The project will have sound impact on the economy of the country as our natural resources will be exploited for isolation and expression of novel antimicrobial genes and proteins. Any novel compound with potent antimicrobial activity will lead us to the production of the compound at large scale, catering our indigenous needs. It may have therapeutic implications after undergoing clinical trials. Furthermore, foreign exchange may be earned by patenting such compounds with international agencies. However, this would require further studies after completion of the current research project.

The project will be helpful in achieving the goal of Govt. to improve the quality, relevance, or capacity of education and research at Pakistani Institutions of higher education in science and technical fields.

6. COLLABORATING LABS

In case of collaboration with national/international research group or local industry, please identify clearly the parts of research that will be carried out in the participating laboratories and please identify complimentarity and/or justify the need for collaboration) P.I.s are encouraged to find collaborating partners within Pakistan, particularly in less developed areas. Include a letter from Collaborating agency expressing willingness to collaborate. **NA**

7. FACILITIES AND FUNDING

7A. Facilities: equipment available for the research project IN THE HOST UNIVERSITY/INSTITUTION **Electrophoresis, centrifuge machines, autoclave, orbital shakers, refrigerators, liquid nitrogen containers, weighing balances, pH meter, gel documentation and analysis system, ovens, water bath, laminar air flow cabinet.**
7B. Scientific Personnel a. Available: PI and CoPI b. Required*: One Research Associate with minimum qualification as MPhil Biochemistry (will have to enroll, preferably, PhD biochemistry program in the Dept.). Preference will be given to the candidates having research experience on DDPCR from plants. *Involvement of research students is encouraged.
7C. Other funding available for the proposed studies (if any) **Nil**

8. PRINCIPAL INVESTIGATOR

A brief resume of research accomplished in the last 05 years. Please specify title of the research proposal(s), duration, funding source(s) and award amount(s).

The research area of the PI is gene expression. Regarding the current research as proposed in this proposal, he initiated work on purification, characterization and expression of antifungal proteins and peptides from medicinal plants. Such work has been presented in HUPO conference held in (Oct., 2004), IUBMB symposium (Nov. 2005), International Symposium on Medicinal Chemistry Turkey (Aug. 2006), 55th international congress and annual meeting of the society for medicinal plant research (2007) and many national conferences, and highly appreciated by the fellow scientists. Dr. Jamil has published good quality research publications. He is also author of two chapters on gene expression and biotechnology, in foreign books. Based on his work, Dr. Jamil was awarded with TWAS (Third World Academy of Sciences) prize for Young Scientists in the South in the field of Biology for the Year 2002 and PAS (Pakistan Academy of Sciences) Gold Medal in Biochemistry for the year 2007.

He also has experience of running research projects related to gene expression work as follows:

Title of research proposal	Duration	Funding Source	Award Amount
Purification and characterization of antifungal peptides/proteins from potential medicinal plants and construction of cDNA libraries for hyperexpression.	Three years (completed)	Higher Education Commission, Govt. of .	Rs. 1.60 million
Pilot scale production, purification and characterization of xylanase from hyperexpressed mutant of *Chaetomium thermophile*	Three years (Development Project) (completed)	Higher Education Commission, Govt. of .	Rs. 11.73 million
Purfication, characterization and hyperexpression of antifungal proteins/peptides from potential medicinal plants (supportive grant to the project No. 1 above).	Two years (completed)	International Foundation for Science	US $ 6000
Hyperexpression of lysine and transfer of cellulase genes in *Brevibacterium flavum* for recycling of agro-industrial wastes.	Three years (completed)	Science Foundation	Rs. 0.754878 million
Studies on poly(A) site strength and interaction of 3'-end processing of mRNA to transcription for understanding the mechanism of gene regulation in eukaryotic systems	Three years (in progress)	Higher Education Commission, Govt. of .	Rs. 6.034800 million

1. Please attach C.V. **CV is attached** 2. Number of Publications during the last five years & page numbers on the C.V. where these publications are listed National: **11** Please see pages **6-8** of CV International: **14** Please see pages : **6-7** of CV 3. Number of research projects completed & page number where this information appears Basic: **06** Please see pages **2-3** of CV Applied: _____ Please see pages_____ of CV			

9A. ESTIMATED BUDGET FOR THE PROPOSED RESEARCH PERIOD

DESCRIPTION	% of time devoted to Project	YEAR 1	YEAR 2	YEAR 3	Amount (in million Rs.)
A. Salaries and Honorarium					
PI: One month/year of basic pay @	40	0.031	0.031		0.062
Co-PI: One month basic pay for the entire duration @	20	0.025			0.025
Research Associate @ Rs. 13000/month	100	0.156	0.156		0.312
Lab Attendant @ Rs. 6000/month	100	0.072	0.072		0.144
Office Assistant (honorarium to existing employee)	50	0.018	0.018		0.036
Subtotal:		0.302	0.277		0.579

B. Permanent Equipment (Please attach invoice/quotation and expected delivery date for items costing over Rs. 0.1 million.)				
-80^{o}C freezer with card locking system and CO_2 backup	0.79565			
Micropipettes one set of four pipettes, Gilson	0.08			
Subtotal:	0.87565			0.87565

C. Expendable supplies *(year wise quantity with full justification)*				
List attached as Annexure-I	1.418689	0.381002		1.799691
Subtotal:	1.418689	0.381002		1.799691

DESCRIPTION	YEAR 1	YEAR 2	YEAR 3	Amount (in million Rs.)
D. Others D1. Literature, documentation, information, online literature search, contingencies, postage, etc.				
	0.01	0.01		0.02
Subtotal:	0.01	0.01		0.02

D2. Local Travel (Destination and purpose with full justification)				
POL/TADA	0.01			0.01
Subtotal:	0.01			0.01

D3. Miscellaneous				
Audit Fee (Max. Rs 10,000) 0.01				0.01
Accountant Fee (Max. Rs. 10,000) 0.01				0.01
Subtotal:		0.02		0.02
Subtotal (D1 + D2 + D3):	0.02	0.03		0.05
E. Indirect cost (University overheads) 20 % of Total direct cost to meet office support, utilities, etc.	0.523268	0.1376		0.660868
Grand Total (A + B + C + D+E):	3.139607	0.825602		**3.965209**

9B. JUSTIFICATION (PLEASE JUSTIFY YOUR REQUEST IN A BACKGROUND OF THE EXISTING FACILITIES AVAILABLE AT THE HOST INSTITUTE.)

A. **Salaries & Allowances** (All positions, other than PI and Co-PI, must be fully justified. Please give qualifications/requirements of each of the new full-time positions requested for in the Proposal.)

•**Research Associate** will be the person mainly responsible for the conduct of research work in the project. A full time researcher who can devote 100 percent time to the project work is absolutely necessary for achieving the targets. Minimum qualification of the researcher would be MPhil Biochemistry preferably with research experience on DDPCR from plant samples.

•**Lab Attendant** is highly necessary for the project. The person should be well aware of general lab. equipment and procedures such as autoclave, water bath, glassware cleaning, storage of chemicals etc. He will also assist in sampling of plants from the filed. Moreover, he will facilitate the purchases and other requirements of the project. No such person is available from the department/university. Execution of the project is not possible without such person.

1.Office Assistant/typist is demanded on part time basis for preparation and submission of project bills and maintaining the project record. Already employed persons from the dept. will be engaged in the project and honorarium will be given to such person.

B. **Permanent Equipment** (Please identify major items (over Rs. 25,000). Major pieces of equipment costing over Rs. 0.1 million must be fully justified. Minor items (under Rs. 25,000) may be lumped into one.)

1. **-80^{o}C freezer with card locking system and CO_2 backup** is needed to preserve the plant and RNA samples. RNA is degraded very rapidly at high temperatures. The best recommended temperature is -**80^{o}C**. Time course experiments have to be conducted in the project, therefore, a large number of samples will have to be preserved. The samples will be placed in liquid nitrogen followed by their storage in the freezer. Moreover, bacterial competent cells and strains also demand this temperature. The long term storage of fungal spores is also done at low temperatures. The freezer with **CO_2 backup** is especially demanded due to the electricity problem in the country. The backup system will help in keeping the temperature to -80 ^{o}C even if electricity fails for more than 24 hours. It is also needed due to intermittent power failure throughout the day. No such facility is available in the department. Success of the project is very much dependent upon this equipment, otherwise there are chances of sample and strain losses.

2. **Micropipettes one set of four pipettes** is demanded as dedicated pipettes are needed for RNA work due to its rapid degradation. The project work involves extensive work on RNA, therefore, a set of four micropipettes covering the whole range is requested.

C. **Expendable supplies** (With full justification and details of **quantity required** for the project)

1. Glassware/disposables: Nuclease-free glassware and plastiware is required for molecular biology work proposed in the project, which is highly expensive.

2. The chemicals and kits needed for molecular biology work are exclusive and very expensive.

The year wise list of expendable supplies with their potential use is given as Annexure-I.

D. **Other Costs.** (Travel must be justified.)

A very small amount for POL and TA/DA is demanded in the proposal as seed and fungal samples have to be collected. Moreover, the PI and researcher have to travel for meetings regarding the project.

Annexure-I List of Expendable Supplies

Items	Catalog #	Company	Packing	Unit Price	Qty Year 1	Price	Qty Year 2	Price	Total Price	Use
Top vision LEGQ agarose	R0491	Fermentas	1x100g	18500	0	0	1	18500	18500	DNA separation

THANK YOU