



SDI Review Form 1.6

Journal Name:	International Journal of Biochemistry Research & Review
Manuscript Number:	Ms_IJBCRR_41986
Title of the Manuscript:	Temporal and spatial changes in host defense enzymes in response to Fusarium wilt in Pigeonpea (Cajuns cajan (L.) Millspaugh)
Type of the Article	Original Research Article

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound. To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

(<http://www.sciencedomain.org/page.php?id=sdi-general-editorial-policy#Peer-Review-Guideline>)



SDI Review Form 1.6

PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	Abstract Abstract matches the rest of the article, informative and the aim is clear. Line 19: Please replace the word "established resistance markers" with "biochemical markers for resistance". Introduction The research questions were clearly laid out and the background information is collected well. What is missing? Line 23, Paragraph 1: States that there is heavy loss in yield? Is it economic yield loss? Please mention clearly the quantity of loss in terms of produce as well as in economic terms of evaluating loss. Line #46, Paragraph#2: Please correct the sentence" Higher activity of enzyme in highly resistant cultivars and it lower activity in susceptible ones...[9]. 2. Materials and Methods Lines # 75-77, Section 2.1 : Please divide into two sentences. Line # 81, Sentence #1: Please replace "fields" with "Plants". Line 81-83, Sentence 2, Paragraph 1, Section 2.2: Please rewrite the sentence into two meaningful simple sentences. For example, "The pathogenic fungal isolates were collected from fourth-node stem sections of wilted pigeonpea plants according to Tullu et al. [15]. These fungi were colonized on filter paper and dried in the transfer hood. The colonized filter paper was aseptically cut into small pieces." Line 86, paragraph 2: Spore suspension was already pelleted. The following sentence is stating again "conidia were washed to adjust the spore suspension...", conidia are the structures that hold spores in fungi. Please check the continuity of the process along with the terms used. Please correct the sentences in lines 86 and 87. Line 91: Please add "was" before "pathogenicity" Line 93: Please check for the sterilisation in parenthesis? Why are these sterilisation required after the usage of 2k sterile soil for filling the pots. Line 94: Please mention the amount of spore suspension used for inoculation in parenthesis after "14 day old culture". 2.3 Extraction of antioxidant enzymes Line 103:is missing information of type of tissue samples. Please insert "root, shoot and leaf" after "The". It is not required to edit at all if the author includes, the isolation part in sentence of lines 96-98, where the type of tissue collected is written. Please include a simple sentence saying"These tissue samples of leaf, root and shoot were subjected to isolation of major biomolecules and assesing their activity." Line 107:2.4. Isolation and activity assay of peroxidase (EC 1.11.1.7) What does this EC1.11.1.7 refers to? Please use the reference of company for product that was used for the assay along with catalogue number and the year it was purchased, in the text procedure in the materials section. Line 110: Please check for the formula of Pyrogallol,It is an organic compound with the formula $C_6H_3(OH)_3$. Line 115: Please check for the "one unit of peroxidase"expression units "U", is it a standardized SI unit. Lines 108-116: This is the description given at the sigma Aldrich website for this assay. Please check your procedure for any mistakes. If the modifications were done, kindly explain why was the modification necessarily required? Is the pathogen different from the one that was used to standardise the protocol by Kar and Mishra. This procedure is for the determination of Peroxidase enzymatic activity using Pyrogallol as the substrate. The continuous spectrophotometric rate determination	Replaced as per reviewer's suggestion Data on economic loss provided Corrected Complied Replaced as per reviewer's suggestion Rewritten as per reviewer's suggestion Corrected Added Microbial spores may escape/survive single sterilization effect hence, it was done thrice Mentioned Sentence reframed to include reviewer's suggestion It is the IUB enzyme code of the enzyme examined and not the company product code or catalogue number hence be not confused with any company product It is the same compound and chemical formula suggested by reviewer is incorporated now in the text It is expressed as per Kar and Mishra (1976) protocol, is not a SI unit Kar and Mishra (1976) standardized the protocols for enzyme assay during senescence in rice leaves. The protocol of Kar and Mishra (1976) very reliable and has been used for enzymatic studies during abiotic and bioc stress in plants in 1074 published papers in reputed journals



SDI Review Form 1.6

	(A420, Light path = 1 cm) is based on the following reaction: $H_2O_2 + \text{pyrogallol(donor)} \xrightarrow{\text{enzyme peroxidase}} 2H_2O + \text{Purpurgallin (oxidized donor)}$. Unit Definition: One unit of peroxidase will form 1.0 milligram of purpurogallin from pyrogallol in 20 seconds at pH 6.0 at 20 °C. This unit is equivalent to ~18 µM units per minute at 25 °C." Line 118: Please correct the subhead 2.5. by deleting "Isolation and" and capitalise the "a" in "Activity". Lines 120-124: It is a repetition of the same as stated in 2.4. It was already mentioned that procedure is same 2.4 in the first sentence. Therefore, these lines can be deleted. Line 126, subhead 2.6, please do the same as stated above by deleting "Isolation and". State the reasons for modification. Lines 137 and 144: The procedure is saying author followed sigma product to do the activity analysis, if it is true, the absorbance should be measured at 270nm. But the procedure followed is written differently with modification to the method followed by previous scientists as cited "Zucker,[18]". ****"Throughout out the methodology and procedures laid out, follow only one style of what has been done. Please do not include Sigma catalogue numbers for the products used, as the protocol followed were different for most of the activity assays. Please make corrections as necessary by thoroughly editing the document. Please clarify with your explanation for including both information". Over all The process of subject selection is clear, variables defined and measured appropriately and the study methods are valid and reliable. Results The data is presented in an appropriate way, with relevant figures and are clearly presented with appropriate units. The titles, X and Y axis were labelled correctly and clearly with colored legends indicating each category and cultivars Vs data were all grouped appropriately. There is no repetitive data or the text., or data in text. The entire text is written with lot of effort and is excellent contribution with practically meaningful results. Entire data analysis and comparisons made were completely based on Standard Error of mean, Author studied only the increase or decrease in the enzymatic activity during early, mid and late infection stages of various plant tissue samples. Therefore, author did not say clearly about what is a statistically significant result? Inclusion of tables with SE means, CV and CD will be excellent in place of bar graphs. Please include atleast one table with over all mean data for all the variables and traits studied. Discussion and Conclusion Line 315: please replace "other workers" with "previous researchers" and delete "as well". Line 321-323: Please make two simple sentences out of single huge sentence. Line 325: What is meant by "induction"? is the induction means "PPO level increased after infection with fungi"? Please clarify. Line 330: Please replace "conform" with "confirm". Line 337: Please write the expanded version of "P. syringae" as Plasmodium syringae". Line 351: Is H_2O_2 is involved in disease resistance? Lines 384-386: Please clearly explain term "fold" that was used in the text to compare within and between different tissues and among resistant and susceptible cultivars. Please correct the entire document for the term "fold increase" by incorporating observed quantitative data next to the traits compared in parenthesis. Visually, it is clear from the figure with bar charts, that there is increase and decrease in the level of enzymatic activity. Numerical/quantitative data is not presented in tabular form anywhere showing significant and non significant results obtained. Line 390-393: The sentence has too much information, please divide it into two simple meaningful sentences. Line 393: Please remove "s" from "hypocotyls" to make singular form "hypocotyl". Line 395: Please add "in beta1,3-glucanase activity" after "similar increases" Line 396: Please replace "have been" with "were"	Corrected Deleted Wave length used is based on the method adopted by other scientists for study of PR proteins No sigma catalogue numbers are used and it actually IUB enzyme code, hence no clarification required The figures have been replaced with tables. One table with over all mean data for all the variables and traits studied can not be provided due to loss of primary data. We have processed data (tables and figures) with us at the moment. Replaced Divided into two sentences Induction refers to change in the parameter due to presence of pathogen Replaced Expanded version written yes It is fold increase (times change in comparison to control) Figures are now replaced with tables in the revised manuscript Divided into two sentences Removed
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SDI Review Form 1.6

	Line 460" Please replace "as well as" with "and". Lines 464-466: The future strategy from the findings is good. It needs sentence correction as rephrased here, "The presented results are very important for future studies focusing on the transgenic pigeonpea development by isolation and transfer of disease resistance genes from resistant cultivar to susceptible cultivar during the stage of their potential expression in the tissues, upon challenging with pathogen. This will be an integrated management strategy to protect the crop plants from further pathogen attack." Over all The discussion was well written with supportive literature and explanation. The conclusions made were answering the aim of the study for all the enzymatic assays conducted for various tissue samples upon challenging with Fud pathogen. There are future opportunities that were informed by the authors for future research References Except Datta and Lal, 2012 remaining references are all old, ranging from 1950s to 2003. The references are relevant. Author can eliminate some of the references which are related to other fungi, unless there is no supportive literature from fusarium in any other crops. All references are cited correctly and key studies were partly included. The challenging plant tissues will express the infection while finding way to recover from infection by seing various inbuilt biochemical pathways and by producing enzymes (proteins) that combat the fungal attack. The reasons for resistance in resistant cultivars, the pathways involved were not clearly mentioned for these five enzymes studied Overall statement or summary The article is written with a sustained effort and hard work in collecting huge data from the samples and laborious work in biochemical analysis of the tissue samples. It is consistent within itself, study design answered the aim and the author knows about the study. It needs thorough revision by adding data and tables as necessary in the results section and in Discussion, the observations need supportive literature from recent findings from biochemical pathways involved in disease resistance for the specific enzymes/proteins studied for specific fungal resistance. Strengths of the article: Excellent contribution to the science and agriculture field crops Weaknesses of the article : Needs revision The study is interesting, but lacks proper presentation skills of what has done. Needs thorough revision in concising the text and eliminating the repetition of the results throughout the text., inclusion of tables with information on significance. No Specific comments	Added Replaced Replaced Sentence corrected as per reviewer's suggestion
Minor REVISION comments		
Optional/General comments		