

**BEFORE THE KHYBER PAKHTUNKHWA SERVICE TRIBUNAL, PESHAWAR**

**SERVICE APPEAL NO. 894/2018**

Date of institution ... 16.07.2018

Date of judgment ... 11.09.2019

Dr. Muhammad Waqas, Agriculture Officer (BPS-17)  
Agriculture Extension, Gagra, District Buner.

... (Appellant)

**VERSUS**

1. Government of Khyber Pakhtunkhwa through Chief Secretary Khyber Pakhtunkhwa, Civil Secretariat, Peshawar.
2. The Secretary, Agriculture Livestock & Cooperative Department, Civil Secretariat, Khyber Pakhtunkhwa, Peshawar.
3. The Director General Agriculture Extension, Khyber Pakhtunkhwa, Peshawar.
4. The District Director, Agriculture Extension Department, District Buner.

... (Respondents)

*M Amin*  
*11.9.2019*

APPEAL UNDER SECTION-4 OF THE KHYBER PAKHTUNKHWA SERVICE TRIBUNAL ACT, 1974 AGAINST THE IMPUGNED ORDER DATED 20.02.2018 WHEREBY MINOR PENALTY OF WITHHOLDING/STOPPAGE OF TWO ANNUAL INCREMENTS FOR A PERIOD OF TWO YEARS HAS BEEN IMPOSED ON THE APPELLANT IN UTTER VIOLATION OF LAW AND RULES AND AGAINST THE APPELLATE ORDER DATED 25.06.2018 WHEREBY THE DEPARTMENTAL APPEAL OF THE APPELLANT HAS BEEN REGRETTEED ON NO GOOD GROUNDS.

Mr. Noor Muhammad Khattak, Advocate.

.. For appellant.

Mr. Riaz Ahmad Paindakheil, Assistant Advocate General

.. For respondents.

Mr. MUHAMMAD AMIN KHAN KUNDI

.. MEMBER (JUDICIAL)

MR. AHMAD HASSAN

.. MEMBER (EXECUTIVE)

**JUDGMENT**

MUHAMMAD AMIN KHAN KUNDI, MEMBER: - Counsel for the appellant present. Mr. Riaz Ahmad Paindakheil, Assistant Advocate General alongwith Mr. Muhammad Nawaz, Assistant Agriculture Engineer for the respondents present. Arguments heard and record perused.

2. Brief facts of the case as per present service appeal are that the appellant was serving in Agriculture Department as Agriculture Officer (BPS-17). He was imposed minor penalty of withholding of two increments for a period of two years vide order dated 20.02.2018 on the allegation that he was granted study leave on half pay with effect from 01.03.2011 to 28.02.2015 and was required to report for duty on 01.03.2015 but could not join the duty and willfully absented himself up to 13.04.2017. The appellant filed departmental appeal on 26.02.2018 but the same was ~~not decided~~ <sup>rejected on 25.6.2018 W</sup> hence, the present service appeal on 16.07.2018.

3. Respondents were summoned who contested the appeal by filing of written reply/comments.

4. Learned counsel for the appellant contended that the appellant was granted study leave for a period of 1460 days i.e four years. It was further contended that after completion of PHD Degree from Kyungpock National University was placed in a joint research project between the academic Department of the above mentioned university and Ministry of Environment (South Korea) which was mandatory for the purpose of awarding PHD Degree to a student and according to the schedule the period of completion of the said project was February 2017. It was further contended that prior to the completion of his 1460 days leave on 28.02.2015, the appellant applied for extension of his Ex-Pakistan study leave for another period of three years due to the abovementioned reason as such the same was properly corresponded between the respondent department and Establishment Department of Khyber Pakhtunkhwa but no reply regarding acceptance or rejection was communicated to the appellant. It was further contended that after completion of his study leave, the appellant arrived in Pakistan on 12.04.2017 and also submitted his arrival report on the next day but the respondent-department initiated disciplinary proceeding against the appellant and ultimately the appellant was

*M. Amin*  
11.9.2019

imposed aforesaid penalty. It was further contended that the absence of the appellant was not intentional but he had already applied to the department for another period of three years for study leave. It was further contended that the departmental proceeding was not conducted in accordance with law as neither the appellant was provided opportunity of personal hearing nor he was provided opportunity of defence therefore, the impugned order is illegal and liable to be set-aside.

5. On the other hand, learned Assistant AG for the respondents opposed the contention of learned counsel for the appellant and contended that the appellant was granted study leave for four years and he was required to report for duty on 01.03.2015 but he willfully remained absent up to 13.04.2017 for a period of more than two years. It was further contended that the appellant was imposed minor penalty after fulfilling all the codal formalities by the respondent department and the appellant could not satisfy the department for the aforesaid absence period of two years. It was further contended that the respondent-department has already taken linnet view of minor punishment therefore, the appeal has no force and prayed for dismissal of appeal.

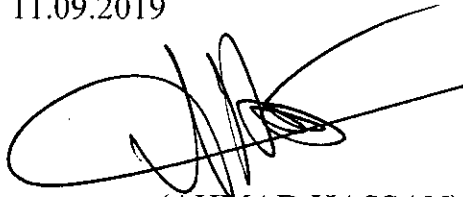
6. Perusal of the record reveals that the appellant was granted study leave for a period of four years and he was required to report for duty on 01.03.2015 but he could not join the duty and willfully absented himself up to 13.04.2017 for a period of more than two years. Though the appellant has claimed that he had already applied for extension of Ex-Pakistan study leave for another period of three years but admittedly the said application was not accepted by the respondent-department and the appellant remained absent without the permission of the lawful authority for a period of two years from duty. Moreover, the record also reveals that all the codal formalities have been fulfilled before imposing the minor penalty to the appellant. Furthermore, the respondent-department has already taken linnet view of imposing minor penalty

*M. Amin*  
11.9.2019

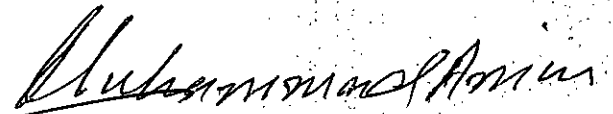
of withholding of two increments for a period of two years therefore, the appeal has no force which is hereby dismissed. Parties are left to bear their own costs.

File be consigned to the record room.

ANNOUNCED  
11.09.2019



(AHMAD HASSAN)  
MEMBER



(MUHAMMAD AMIN KHAN KUNDI)  
MEMBER

Service Appeal No. 894/2018

11.09.2019

Counsel for the appellant present. Mr. Riaz Ahmad Paindakheil, Assistant Advocate General alongwith Mr. Muhammad Nawaz, Assistant Agriculture Engineer for the respondents present. Arguments heard and record perused.

Vide our detailed judgment of today consisting of four pages placed on file, the appeal has no force which is hereby dismissed. Parties are left to bear their own costs. File be consigned to the record room.

ANNOUNCED

11.09.2019

  
(AHMAD HASSAN)  
MEMBER

  
(MUHAMMAD AMIN KHAN KUNDI)  
MEMBER

07.05.2019

Counsel for the appellant and Mr. Ziaullah, DDA for the respondents present.

Rejoinder in response to the reply of the respondents submitted which is placed on record. To come up for arguments on 02.07.2019 before the D.B

  
Member

  
Chairman

02.07.2019. Due to general strike on the call of Khyber Pakhtunkhwa Bar Council, learned counsel for the appellant is not available today. Mr. Zia Ullah Learned Deputy District Attorney for the respondents present. Adjourned. To come up for arguments on 28.08.2019 before D.B


  
(Hussain Shah)  
Member

  
(M. Amin Khan Kundi)  
Member

28.08.2019

Counsel for the appellant present. Mr. Ziaullah, DDA for respondents present. Learned counsel for the appellant seeks adjournment. Adjourn. Case to come up for arguments on ~~12.9~~ 2019 before D.B.

  
Member

  
Member

07.12.2018

Nemo for appellant. Mr. Muhammad Jan, DDA alongwith Aizad Ali, Agriculture Officer for the respondents present.


Representative of the respondents requests for further time for submission of reply/comments. Adjourned for the needful to 16.01.2019 before S.B.

  
Chairman

16.1.2019

Counsel for the appellant and Addl. AG alongwith Jalalud Din, Agronomist for the respondents present.

Parawise comments have been submitted on behalf of all the respondents. To come up for arguments before D.B on 15.03.2019. The appellant may furnish rejoinder within a fortnight, if so advised.

  
Chairman

15.03.2019

Junior to counsel for the appellant and Mr. Kabir Ullah Khattak learned Additional Advocate General present. Junior to counsel for the appellant seeks adjournment as senior counsel for the appellant is not in attendance. Adjourn. To come up for arguments on 07.05.2019 before D.B

  
Member

  
Member

20.08.2018

Appellant with counsel present. Learned counsel for the appellant argued that disciplinary proceedings were initiated against him and upon culmination minor penalty of withholding of two increments for a period of two years was imposed on him vide impugned order dated 20.02.2018. Feeling aggrieved, he filed departmental appeal on 27.02.2018, which was rejected on 29.05.2018 communicated to the appellant through endorsement dated 25.06.2018 followed by instant service appeal on 16.07.2018. The appellant has not been treated according to law and rules.

Points urged need consideration. The appeal is admitted for regular hearing subject to deposit of security and process fee within 10 days thereafter, notice be issued to the respondents for written reply/comments for 23.10.2018 before S.B.

Appellant Deposited  
Security & Process Fee

(Ahmad Hassan)  
Member

23-10-2018

*Due to retirement of Honorable  
Chairman the Tribunal is non  
functional therefore the case is  
adjourned to come up for the same on  
7-12-2018*



*Special  
Reader*



Form- A  
FORM OF ORDER SHEET

Court of \_\_\_\_\_

Case No. 894/2018

S.No.	Date of order proceedings	Order or other proceedings with signature of judge
1	2	3
1-	16/07/2018  18-7-2018	<p>The appeal of Mr. Muhammad Waqas presented today by Mr. Noor Muhammad Khattak Advocate may be entered in the Institution Register and put up to the Worthy Chairman for proper order please:</p> <p style="text-align: right;"> REGISTRAR</p> <p>This case is entrusted to S. Bench for preliminary hearing to be put up there on <u>20-8-2018</u></p> <p style="text-align: right;"> CHAIRMAN</p>

**BEFORE THE KHYBER PAKHTUNKHWA SERVICE**  
**TRIBUNAL PESHAWAR**

APPEAL NO. 894 /2018

**MUHAMMAD WAQAS VS Govt. of KP & OTHERS**

**INDEX**

<b>S.NO.</b>	<b>DOCUMENTS</b>	<b>ANNEXURE</b>	<b>PAGE</b>
1	Memo of appeal	.....	1- 4.
2	Letter dated 18-02-2011	<b>A</b>	4.
3	Application & Correspondence	<b>B</b>	5 – 11
4	Return Ticket/Boarding Pass	<b>C</b>	12 – 14
5	Charge Sheet + Reply	<b>D</b>	15 – 22
6	Statement of Allegation + Reply	<b>E</b>	23 – 127
7	Show Cause Notice	<b>F</b>	128 – 130
8	Reply	<b>G</b>	131 – 132
9	Impugned Order	<b>H</b>	133
10	Departmental Appeal	<b>I</b>	134 – 135
11	Appellate Order dated 25-06-2018	<b>J</b>	136
12	Vakalatnama	-----	137

**APPELLANT**

Through:   
**NOOR MOHAMMAD KHATTAK,**  
Advocate High Court, Peshawar.  
0345-9383141

**BEFORE THE KHYBER PAKHTUNKHWA SERVICE TRIBUNAL**  
**PESHAWAR**

APPEAL NO. 894 /2018 Khyber Pakhtunkhwa  
Service Tribunal

Dr. Muhammad Waqas, Agriculture Officer (BPS-17),  
Agriculture Extension, Gagra, District Buner.

Diary No. 1163  
Dated 16-7-2018

..... APPELLANT

**VERSUS**

- 1- Government of Khyber Pakhtunkhwa through Chief Secretary Khyber Pakhtunkhwa, Civil Secretariat, Peshawar.
- 2- The Secretary, Agriculture Livestock & Cooperative Department, Civil Secretariat, Khyber Pakhtunkhwa, Peshawar.
- 3- The Director General <sup>Agriculture</sup> Extension, Khyber Pakhtunkhwa, Peshawar.
- 4- The District Director, Agriculture Extension Department, District Buner.

..... RESPONDENTS

**APPEAL UNDER SECTION-4 OF THE KHYBER**  
**PAKHTUNKHWA SERVICE TRIBUNAL ACT 1974**  
**AGAINST THE IMPUGNED ORDER DATED 20-02-2018**  
**WHEREBY MINOR PENALTY OF**  
**WITHHOLDING/STOPPAGE OF TWO ANNUAL**  
**INCREMENTS FOR A PERIOD OF TWO YEARS HAS BEEN**  
**IMPOSED ON THE APPELLANT IN UTTER VIOLATION OF**  
**LAW AND RULES AND AGAINST THE APPELLATE ORDER**  
**DATED 25-06-2018 WHEREBY THE DEPARTMENTAL**  
**APPEAL OF THE APPELLANT HAS BEEN REGRETTEED ON**  
**NO GOOD GROUNDS**

**PRAYER:**

That on acceptance of this appeal the impugned orders dated 20-02-2018 and 25-06-2018 may very kindly be set aside and the respondents may be directed to restore the two Annual Increments of the appellant with all back benefits. Any other remedy which this august Tribunal deems fit that may also be awarded in favor of the appellant.

**R/SHEWETH:**

**ON FACTS:**

- 1- That appellant is the employee of the respondent Department and is working as Agriculture Extension Officer (BPS-17) under the administrative control of respondents. That right from appointment till date the appellant has served the Department

Filed to day  
*[Signature]*  
Registrar

quite efficiently, honestly and up to the entire satisfaction of his high ups.

- 2- That the appellant while performing his duty in the respondent Department applied for higher studies of Ph.D in the Kyungpock National University of South Korea. That the said University allowed/granted scholarship to the appellant and accordingly the appellant applied for study leave and as such the same was sanctioned/granted to the appellant for 1460 Days i.e. 4 Years vide order dated 18-02-2011. Copy of the letter is attached as Annexure ..... **A.**
- 3- That the appellant after completing his PhD Degree from Kyungpock National University was placed in a joint research project between the academic Department of the above mentioned University and Ministry of Environment (South Korea) which was mandatory for the purpose of awarding PhD degree to a student and according to the schedule the period of completion of the said project was February 2017.
- 4- That the appellant prior to completion of his 1460 days leave on 28-02-2015 applied for extension of his Ex-Pakistan study leave for another period of 3 years due to the above mentioned reason and as such the same was properly corresponded between the respondent Department and establishment Department of Khyber Pakhtunkhwa. Copy of application and correspondence is attached as Annexure ..... **B.**
- 5- That it is pertinent to mention that no reply regarding acceptance or rejection of his application was communicated to the appellant, therefore the appellant continued his research study in the said University. That the appellant after completion of the assigned task of joint research at Korea arrived in Pakistan on 12-04-2017 and on the very next day submitted his arrival report (forenoon) for duty in the respondent Department by forwarding his arrival report to Director General Agriculture Extension which was properly forwarded to Secretary Agriculture, Livestock & Cooperative Department on 02-05-2017. Copies of the relevant record are attached as annexure ..... **C.**
- 6- That after arrival report the appellant started performing his duty quite efficiently and up to the entire satisfaction of his superiors. That astonishingly the appellant was served with Charge Sheet & statement of allegations vide Notification dated 21-11-2017 which was properly replied by the appellant through documentary proof. Copies of charge sheet and statement of allegation and reply along with record are attached as annexure ..... **D & E.**

- 7- That finally the appellant was issued with show cause notice through letter dated 04-01-2018 received through respondent no. 3 which was also properly replied by the appellant. That finally the respondent No.1 issued the impugned order dated 20-02-2018 whereby minor penalty of "Withholding of two increments for a period of two years" has been imposed on the appellant in utter violation of law and Rules. Copies of the show cause, reply and impugned order dated 20.2.2018 are attached as annexure ..... **F, G & H.**
- 8- That feeling aggrieved from the impugned order dated 20-02-2018 the appellant filed Departmental Appeal dated 27-02-2018 before the appellate authority which was rejected on no good grounds by the appellate authority vide appellate order dated 25-06-2018. Copies of Departmental Appeal & Appellate order are attached as annexure ..... **I & J.**
- 9- That appellant feeling aggrieved and having no other remedy preferred the present appeal on the following grounds amongst the others.

**GROUND:**

- A- That the impugned orders dated 20-02-2018 and 25-06-2018 are against the law, facts, norms of natural justice and materials on the record hence not tenable and liable to be set aside.
- B- That the appellant has not been treated by the respondent Department in accordance with law and rules on the subject noted above and as such the respondents have violated Article 4 & 25 of the Constitution of Islamic Republic of Pakistan 1973.
- C- That the impugned orders dated 20-02-2018 and 25-06-2018 has not been issued by the respondents in accordance with law and Rules.
- D- That no chance of personal hearing/defense has been given to the appellant while issuing the impugned orders dated 20.2.2018 and 25.6.2018 by the respondents.
- E- That no regular inquiry has been conducted in the matter which is as per Supreme Court Judgments is necessary in punitive actions against the Civil servants.
- F- That, the appellant has been discriminated in the matter while issuing the impugned orders dated 20-02-2018 and 25-06-2018.

- G-** That the stoppage of annual increments with cumulative effect is not permissible under the law and Rules and as such the same is violative of FR-29 of the Fundamental and Supplementary Rules.
- H-** That the issue of absentia had become past and closed transaction after the acceptance of arrival report, therefore the impugned orders dated 20.2.2018 and 25.6.2018 are not tenable and liable to be set aside.
- I-** That the appellant seeks permission to advance other grounds and proofs at the time of hearing.

It is therefore most humbly prayed that the appeal of the appellant may be accepted as prayed for.

Dated: 9-07-2018

APPELLANT

  
**DR. MUHAMMAD WAQAS**

THROUGH:

  
**NOOR MOHAMMAD KHATTAK**

&

**MUHAMMAD MAAZ MADNI  
ADVOCATES,  
HIGH COURT, PESHAWAR**

A-4

GOVT: OF KHYBER PAKHTUNKHWA  
FINANCE DEPARTMENT

(REGULATION WING)

NO SO(FR)/FD/6-9/2010 Vol-II  
Dated Peshawar, the 18-02-2011

To /

The Secretary to Govt. of Khyber Pakhtunkhwa,  
Agriculture, Livestock & Cooperative Department.

Subject

GRANT OF STUDY LEAVE/ NOC FOR HIGHER STUDIES.

Dear Sir,

I am directed to refer to your letter No. SOE(AD)21-578/2010 dated 09-02-2011 on the subject noted above and to state that Finance Department agrees to grant 04 years (1460 days) study leave on half pay with effect from the date of availing in respect of Mr. Muhammad Waqas, Agriculture Officer, Gagra District Buner for Ph.D in Kyungpook National University, South Korea under the provision of FR-84 read with Appendix 9-1 (A) of FR & SR Vol-I & II, subject to the condition that internal/ local arrangement will be made by the Administrative Department and no contract employee will be appointed during the entire leave of the applicant concerned.

2. Audit copy may be prepared and sent to this department for authentication.

Yours faithfully,

  
(ANDEEB NAZ)  
SECTION OFFICER (FR)

ATTESTED



0937  
574288

B-5

(1) (2)

The Secretary  
Agriculture, Livestock and Cooperative Department  
Govt. of Khyber Pakhtunkhwa

Through: Proper channel

Subject: Extension of study leave/NOC for higher studies

Dear Sir

I Mr. Muhammad Waqas, Agriculture Officer, Gagra District Buner was granted 04 year study leave/NOC for higher studies in Kyungpook National University, South Korea order No. SO(FR)/FD/6-9/2010 Vol-II dated Peshawar the 18-2-2011. I have successfully completed my higher studies and awarded with Ph.D degree in 2014. According to the university rules, during the course of my studies, I was placed in a joint research project between our academic department and Ministry of Environment (Government of South Korea) to complete and fulfill the requirements for the awarding of degree. It is also mandatory to complete the research project and service tenure (06 year), even after getting the degree due to experience gained in this particular research area. The reason behind this is that a new comer due to lack of experience cannot complete the remaining part of the investigation being made in just remaining two years.

Therefore it is kindly requested to extend my study leave for two year more with or without pay. It will be counted toward my post-doctorate experience without any financial burden on Government of Pakistan, as usually Higher Education Commission of Pakistan spend fifteen to twenty thousand US dollars on single person post-doctorate research in foreign countries for just only nine months. This opportunity will enable me to further increase my experience and publish more research papers. This will also increase the impact of my department in the form of highly skilled human resource.

Thanks in anticipation

Muhammad Waqas (Ph.D)

Agriculture Officer

Department of Agriculture Extension  
Gagra, Buner, Khyber Pakhtunkhwa

ATTESTED



6

Atta-ur-Rahman

6



**GOVERNMENT OF KHYBER PAKHTUNKHWA**  
**FINANCE DEPARTMENT**  
**(REGULATION WING)**  
No. SO(FR)/FD/S-9/2012(A)  
Dated Peshawar, the 12-02-2015

To,

The Secretary to Govt. of Khyber Pakhtunkhwa,  
Agriculture, Livestock & Cooperative Department.

Subject: - EXTENSION IN EX-PAKISTAN LEAVE / NOC FOR HIGHER STUDIES

Dear Sir,

I am directed to refer to your department letter No. SOE(AD)21-578/2010 dated 26-01-2015 on the subject and to state that extension in study leave beyond 04 years is not admissible under the rule, however request for grant of 03 years Extra Ordinary Leave may be decided by the Administrative Department at their own level in the prescribed manner / per rules in vogue.

Yours faithfully,

(MURAD AHMED)  
SECTION OFFICER (FR)

ATTESTED

B

7

10

46



GOVERNMENT OF  
KHYBER PAKHTUNKHWA  
AGRICULTURE LIVESTOCK & COOPERATIVE  
DEPARTMENT

NO.SOE(AD)21-578/2010  
Dated Peshawar, the February 24, 2015

To

The Director General,  
Agriculture Extension,  
Khyber Pakhtunkhwa Peshawar

SUBJECT:- EXTENSION IN EX-PAKISTAN LEAVE/NOC FOR HIGHER STUDIES

I am directed to enclose herewith a copy of application filed by Mr. Muhammad Waqas Agriculture Officer, Agriculture Extension, alongwith copy of Government of Khyber Pakhtunkhwa, Finance Department letter No.SO(FR)/FD:5-7/2012 (A) dated 12.2.2015 which is self-explicit with request to verify the study of the officer and furnish progress report with your comments.

Encl: As above.

*(Signature)*  
(DAULAT KHAN)  
SECTION OFFICER-ESTT.

DIRECTORATE GENERAL AGRICULTURE (EXTENSION) KHYBER PAKHTUNKHWA,  
PESHAWAR

Dist. No. 12/675/Estt/ 4603 /DL Dated Peshawar, the 9/03 2015

*along with its enclosures.*  
Copy forwarded to the District Director Agriculture, Buner for information and

necessary action

*(Signature)*  
SUPERINTENDENT (ESTT)  
HQ Office

ATTESTED  
*(Signature)*

8

Agriculture Extension Department District Buner  
GOVERNMENT OF KHYBER PAKHTUNKHWA  
Address: Tehsil Coloney Daggan District Buner  
Phone and Fax: (0939)510315&510470 Fax: (0939)510315



NO. 620 DDA (Ext) Buner  
Dated 17 /04/2015

8

To: Director General,  
Agriculture Extension Khyber Pakhtunkhwa,  
Peshawar.  
Attention: Superintendent (Establishment) HQ

W

Subject: EXTENSION IN EX PAKISTAN LEAVE/NOC FOR HIGHER STUDIES

Memo. Kindly reference your office endost No: 4603 dated 09-03-2015 On the subject as noted above. The documents are resubmitted along with application form along verified by the District account officer Buner. The officer had submitted application through post and had not submitted arrival report for duty after expiry of his leave on 28-02-2015.

Submitted for further necessary action please.

DISTRICT DIRECTOR  
AGRICULTURE EXTENSION DEPARTMENT  
DISTRICT BUNER

ATTESTED

9

APPLICATION FOR LEAVE

Notes. Item 1 to 9 must be filled in by all applicants. Item 10 applies only in the case of Government servants and above.

Name of applicant: MUHAMMAD WAQAS  
 Leave Rules applicable:  
 Post held: AGRICULTURE OFFICER (BPS-11)  
 Department in office: DEPARTMENT OF AGRICULTURE EXTENSION  
 Pay: 11600/-  
 House Rent Allowance / conveyance allowance or other compulsory allowances drawn in the past:  
 (a) Nature of leave applied for: EXTRA ORDINARY LEAVE (LWG) WITHOUT PAY  
 (b) Period of leave in days: 1093 DAYS  
 (c) Date of commencement: 01-03-2015  
 Particular of rules under which leave is admissible:  
 (a) Date of return from last leave: 20/2/2015  
 (b) Nature of leave:  
 (c) Period of leave in days: 1160 DAYS on half pay  
 Signature of the applicant: Muhammad Waqas  
 10. Remarks and recommendation of the Controlling officer:  
 11. Certified that leave applied for is admissible under Rule..... and necessary conditions are:

Dated:

12. Report of the officer

*verified that the officer concerned having completed 5 months i.e. 310 year for which he is entitled to 5 months leave without pay. Subject to the condition*

Signature  
 Designation  
 District In-charge

Signature  
 Designation

13. Orders of the sanctioning authority certifying that on the expiry of leave the applicant is likely to resume post having the compensatory allowances being drawn by him.

ATTESTED

Dated

Signature  
Designation

To  
The District Director  
Agriculture Extension Department  
District Buner, Govt. of Khyber Pakhtunkhwa

10

Subject: Extension of study leave/NOC for higher studies/ Absence from Duty

Respected Sir

I have the honor to refer to your office letter No. 2067/DDA (Ext) Buner dated 16-09-2015 on the subject noted above and to say,

That, I (Mr. Muhammad Waqas), Agriculture Officer, Gagra District Buner was granted 04 years study leave/NOC for higher studies in Kyungpook National University, South Korea vide office order No. SO(FR)/FD/6-9/2010 Vol-II dated 18-2-2011.

That I successfully completed my higher studies and awarded to me PhD degree in the year 2014 by the Kyungpook National University, South Korea (copy of Ph. D Degree attached as Annex-A).

That according to the university rules, I was placed in a joint research project between our academic department and Ministry of Environment (Government of South Korea (copy attached as Annex-B) to complete and fulfill the requirements for awarding of my degree during the course of my studies, which is mandatory to complete the research project tenure (07 year), even after getting the degree for the experience gained in the particular research area.

That before the expiry of my study leave, I had already applied for extension of extraordinary leave without pay for a period of 3 years, which was forwarded by the honorable Secretary to Govt. of Khyber Pakhtunkhwa, Agriculture, Livestock and Cooperative Department vide his letter No. SOE (AD)21-578/2010 dated 26-01-2015.

That after receiving favorable comments of the Finance department vide his letter No. SO(FR)/FD/5-9/2012(A) dated Peshawar the 12-02-2015 my application was routed through proper channel vide Directorate General Agriculture (Extension) Khyber Pakhtunkhwa Endst.

ATTESTED

*[Handwritten signature]*

Agriculture Livestock & Cooperative  
Government of Khyber Pakhtunkhwa  
Peshawar,

11

Through: Proper channel

Subject: ABSENCE FROM DUTY

Dear Sir,

Please refer to your letter No. SOE(AD)21-S-578/2010, DATED 23.06.2016 on above cited subject.

In this regard it is intimated that after completion of my Ph D Degree in the year 2014 from the Kyungpook National University, South Korea (copy enclosed) I was placed in a joint research project between our academic department and Ministry of Environment (South Korea), which is mandatory for the purpose. This research will be completed on 20<sup>th</sup> February, 2017, as is Evident from the University Certificate (copy enclosed). As reflected in the certificate, I have to stay at least in the university till the final completion of the project till 20<sup>th</sup> February, 2017. Resultantly, I had no other choice but to apply for extension of leave to the District Director, Agriculture Extension Department, Buner (copy of leave application is enclosed).

My case for admissibility or otherwise of leave was processed both in the Agricultural Extension and Establishment Departments and was simultaneously forwarded to the Finance Department, KPK, for advice. The Finance Department reply was also very encouraging (copy enclosed). A letter to the District Director, Agriculture Extension Department, Daggar, District Buner was sent for confirmation of leave and the reply from this quarter was also positive.

It is reiterated that the South Korean is spending a huge amount on my study the ultimate beneficiary of which will be my own country. To leave the study incomplete will spoil both the amount of scholarship spent on my study abroad and my career as well. It is the procedure of the university which compelled me to request for extension in leave otherwise I had no pre-plan to stay in Korea beyond the permitted period. If leave is not sanctioned to me it will spoil my career beside a great loss to my country and the Korean government in term of spending the amount of scholarship.

It is, therefore, requested that since all the indicators goes in my favour, therefore extension in the leave either with or without pay as per rules in vogue, may kindly be granted to me till 30 April, 2017 in the public interest enabling me to complete my research.

Yours faithfully,

*Muhammad Waqas*  
Muhammad Waqas (PhD)

Agriculture Officer,

Department of Agriculture Extension,

Gagra, Buner, Khyber Pakhtunkhwa

ATTESTED  
*A*

C - 12

Confirmation for reservation Waqas, Busan, 12 April.

Message

flightrsvn@thaiairways.com <net.flightrsvn@thaiairways.com>  
agronomist89@gmail.com

4 April 2017 at 11:03

Confirmation for reservation

Thank you for choosing ROYAL e-booking to make your travel reservation. You can review your reservation information below.  
Your trip reservation

Reservation Code: 6WKOPB  
Reservation status: Confirmed

We recommend you to make a note of the booking reservation number or print this mail.

Traveller information

Doctor Muhammad Waqas

Contact Information

E-Mail: agronomist89@gmail.com  
Home phone: +82 01020075709  
Emergency contact: MUHAMMAD UZAIR - 937872113

Your flight selection:

Busan to Islamabad

Flight 1 Wednesday, April 12, 2017  
confirmed Departure: 08:30 Busan, Korea (Republic of) - Gimhae International, terminal  
Arrival: 11:50 Bangkok, Thailand - Suvarnabhumi International  
Airline Thai Airways Aircraft: Airbus Industrie A330  
International TG 651  
Fare type: Economy Restricted

Change of plane required. Time between flights : 7:10.

Flight 2 Wednesday, April 12, 2017  
confirmed Departure: 19:00 Bangkok, Thailand - Suvarnabhumi International  
Arrival: 22:10 Islamabad, Pakistan - Benazir Bhutto International  
Airline Thai Airways Aircraft: Airbus Industrie A330  
International TG 349  
Fare type: Economy Restricted

Flight payment and ticket  
64,700 KRW Total for all travellers  
Payment 464,700 KRW Contact THAI office  
Ticket Electronic ticket

Flight Notes

Not all seat and meal options are offered on all flights.  
Specific rules and restrictions may apply to this fare.  
Taxes are included except where local airport taxes are collected at check-in time.

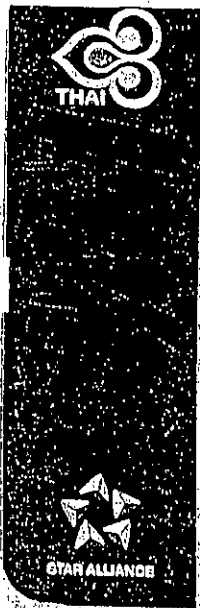
ATTESTED  
*[Signature]*

Services

Traveller 1: Muhammad Waqas

Busan - Islamabad

- Flight 1: Busan - Bangkok
- Seat 38K



BOARDING PASS

ECONOMY

14

NAME OF PASSENGER

FLIGHT

API

DATE

SEAT

WAQAS MUHAMMAD DR

TG651

12APR

38K

FROM BUSAN / PUS  
TO BANGKOK / BKK

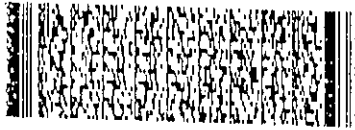
Y

GATE 5

BOARDING TIME 07:50

ETKT 217231815358001

SEQ 0146



GATE CLOSES 10 MINUTES BEFORE DEPARTURE

PUS822924



BOARDING PASS

ECONOMY

NAME OF PASSENGER

FLIGHT

API

DATE

SEAT

WAQAS MUHAMMAD DR

TG349

12APR

38K

FROM BANGKOK / BKK  
TO ISLAMABAD / ISB

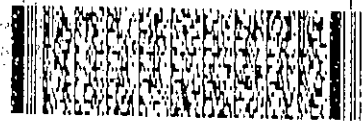
Y

GATE

BOARDING TIME 18:20

ETKT 217231815358002

SEQ 0067



GATE CLOSES 10 MINUTES BEFORE DEPARTURE

PUS822924

ATTESTED

*[Handwritten signature]*





GOVERNMENT OF KHYBER PAKHTUNKHWA  
AGRICULTURE LIVESTOCK & COOPERATIVE  
DEPARTMENT

88

D-15

**CHARGE SHEET**

I, Muhammad Azam Khan, Chief Secretary, Khyber Pakhtunkhwa, as Competent Authority, hereby charge you, Mr. Muhammad Waqas, Agriculture Officer (BS-17) Office of District Director Agriculture, Buner as follow:-

That you, while posted as Agriculture Officer, Buner O/o the District Director Agriculture, Buner committed the following irregularities:

- a) Granted 1460 days study leave on half pay w.e.f 1.3.2011 to 28.02.2015, and was required to report for duty on 01.03.2015 but could not join duty and wilfully absented yourself upto 13.04.2017.
2. By reasons of the above, you appear to be guilty of misconduct under rule-3 (d) of the Khyber Pakhtunkhwa Government Servants (Efficiency and Discipline) Rules, 2011 and have rendered yourself liable to all or any of the penalties specified in section-4 of the Rules ibid.
3. You are, therefore, required to submit your written defense within seven days of the receipt of this Charge Sheet to the Inquiry Officer/Committee. as the case may be.
4. Your written defense, if any, should reach the Inquiry officer/Committee within the specified period, failing which it shall be presumed that you have no defence to put in and in that case, ex parte action shall follow against you.
5. Intimate whether you desire to be heard in person.
6. A statement of allegations is enclosed.

  
(MUHAMMAD AZAM KHAN)

CHIEF SECRETARY,  
KHYBER PAKHTUNKHWA

(COMPETENT AUTHORITY)

ATTESTED  


16

To

The Deputy Secretary (Home)/Inquiry Officer

Home & Tribal Affairs Department, Khyber Pakhtunkhwa, Peshawar

**Subject: REPLY TO THE CHARGE SHEET AND STATEMENT OF ALLEGATION SERVED ON ME VIDE NOTIFICATION NO. DS(J)/HD/2017/Vol-I/2 DATED PESHAWAR THE 21.11.2017. / RELEVANCY OF RESEARCH OUTPUT IN THE DEVELOPMENT OF CROP PRODUCTION IN KHYBER PAKHTUNKHWA**

**Respected Sir**

I have the honor to refer to your office Notification No. DS(J)/HD/2017/Vol-I/2 Dated Peshawar the 21-11-2017 on the subject noted above and to say as follow,

Below please find an overview of my PhD/ Post Doctorate/ Contract Professorship research and then its relevancy/ applicability for the development of crop production in Khyber Pakhtunkhwa/Pakistan particularly in current scenario of climate change and, in last my future research planning.

**Overview of PhD/ Post Doctorate Research:** Rationale; Global climate change has created adverse situation in the form of biotic/abiotic stresses and therefore became the principal limiting factors for decline in agricultural productivity. Food and Agriculture Organization reported in 2007, that only 3.5% of the total global land area has left unaffected by any kind of environmental constraint and among the remaining global land area, water deficit/ drought has affected 64%, flood 13%, salinity 6%, mineral deficiency 9%, acidic/heavy metal 1.5% and, cold 57%. Keeping in view this challenge for agriculture sector on global scale in general and for my Homeland in particular, my supervisory committee advised to work on dominant biotic/ abiotic stresses comprised of insect/ pest/ pathogens attack, drought, low/high temperature, salinity, flooding, nutrient starvation and heavy metal.

Overview; My specialization is Agronomy with major in Crop Physiology and conducted PhD thesis research under the title of "Plant Growth Regulatory Effects of Endophytic Fungi under Diverse Environmental Stresses and Identification of the Effective Substances". During

ATTESTED

5/22

17

PhD/Post-Doctorate/ Contract Professorship, series of studies/research were taken to understand the underlying plant physiological mechanisms at molecular and metabolomic levels on most important agronomic (rice, soybean, sunflower, mungbean, maize), medicinal (*Panax ginseng*, *Solanum nigrum*) and horticulture (tomato, lettuce, cucumber, pepper, sponge gourd) crops under diverse biotic/abiotic stresses. Along with this, beneficial microorganisms (fungi/bacteria), which are the most important natural dwellers of diverse environments, having the enormous metabolic capabilities to mitigate biotic/abiotic stresses were studied. The purpose of the studies was to define and interpret plant-microbe relationships in terms of protection against biotic/abiotic stresses under the continuous pressure of increasing climatic alterations. Plant-microbe interactions comprises of complex mechanisms and biochemical, molecular and physiological studies were carried out to understand this complex integrated processes within plant system to generate deeper insights into the stress-mitigating mechanisms in crop plants for their translation in higher productivity. Further, we focused on the role and function of plant hormonal regulation (abscisic acid, jasmonic acid, salicylic acid, ethylene, and gibberellins) and their signaling during plant-microbes interaction at biochemical and transcriptomic levels under diverse stresses. It was assessed and elucidated that beneficial microbes can counteract the adverse impacts of these stresses in major agronomic and horticultural crops. On the basis of our findings, it was concluded that microbial interactions with plants are an integral part of the living ecosystem, and is the only natural partners that can modulate local and systemic mechanisms in plants to offer defense under adverse external conditions in eco-friendly and sustainable manner. The results were quite interesting and have been published in international reputed and peer reviewed journals indexed by SCI/SCIE and presented in various international conferences.

Expertise gained; I have gained broad experience in experimental designing, and execution ranging from lab analysis to growth chamber, green house and open field conditions. Further, I have successfully operated and worked on high-technology analytical equipments that includes High Performance Liquid Chromatography (HPLC), Gas Chromatography-Mass Spectrometry (GC-MS), Spectrophotometer, Photosynthesis Analyzer, SPAD Machine, PCR, RT-PCR, Ultra High Centrifuge Machine, Amino Acid Analyzer, Elemental Analyzer and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in my experiments. I used these equipments for extraction and quantification of various oxidative stress signaling enzymes (like POD, PPO, CAT, SOD,

ATTESTED

6/22



18

APX), antioxidants (like GSH, MDA), total polyphenols, amino acids, chlorophyll contents, nutrients, photosynthesis, plant hormones and genes expression at transcriptomic level. Additionally, determination of phytohormones in microbes and plants remained my area of active research.

**Applicability of Research in the Development of Crop Production in Khyber**

**Pakhtunkhwa/ Pakistan:** Agriculture in our Province faces various challenges, such as loss of soil fertility, fluctuating climatic factors and increasing pathogen/ pest and abiotic stress attacks. Few years back, there was rhythm in seasonal changes of our Province/ Country, however, due to the unbalanced anthropogenic activities on world wide scale has disturbed this regular pattern of seasonal changes. As a consequence climatic change had occurred in our Province/Country with direct effects on both qualitative and quantitative loss in our crop production system. That is why; next era is the big challenge for us in agriculture sector to ward off the negative effects of climate change on our crop production system more wisely in sustainable manner. Though, our Provincial Government is making efforts but no any solid strategy has been achieved until now and so development of stable crop production technology is urgently needed. Based on PhD/ Post Doctorate/ Contract Professorship research, I can best serve my Province/Country in this scenario. My main area of research, as stated earlier is Plant-Microbe Interaction/ uses of plant growth regulators under diverse biotic/abiotic stresses. Few of the salient points regarding the applicability of my research/ expertise in the development of crop production in Khyber Pakhtunkhwa are mentioned below:

1. Microbes with their potential intrinsic metabolic and genetic capabilities, contribute to alleviate abiotic stresses in the plants and is recently the area of great concern. Microbes contain useful variation for tolerating abiotic stresses like extremes of temperature, pH, salinity and drought; heavy metal and pesticide pollution. Seeking such tolerant microbes is expected to offer enhanced plant growth and yield even under a combination of stresses. The selection, screening and application of these stress tolerant microorganisms by making plants tougher toward abiotic stresses, therefore, could be viable options to help overcome productivity limitations of crop plants in stress-prone areas of our Province/Country.

ATTESTED  
[Signature]

19

2. Sustainability and environmental safety of agricultural production relies on eco-friendly approaches. The multiplicity of beneficial plant-microbe interaction emphasizes the need for their use in modern agriculture. These microbes increase plant growth, accelerate seed germination, improve seedling emergence in response to external stress factors, protect plants from multiple stresses, and promote root growth using different strategies. These different strategies comprise, (1) direct mechanisms that include synthesis of organic compounds which facilitate uptake of essential nutrients and micronutrients from the soil along with the production of plant growth regulators, e.g., iron and zinc sequestration, siderophore production, phosphorus and potassium solubilisation (mobilization and/or production of nutrients), plant hormone production (phytostimulant compounds), and atmospheric nitrogen fixation (2) on the other hand, indirect mechanisms involve antagonistic activity toward plant pathogenic organisms, production of HCN and antifungal compounds and generally tolerance against biotic stresses.
3. Plant-microorganism associations have long been studied, but their exploitation in our agriculture system for partially or fully replacing fertilizers/ pesticides is very low. Unluckily the indiscriminate use of chemicals on crops, vegetables, and fruits is still unchecked in our Country/ Pakistan, for example, the official statistics for the years 1993, 2009 and 2011 of Pakistan show that the usage of fertilizer has been increased from 92 Kg in 1990-91 to 183 Kg per hectare in 2011 and pesticides usage increased from 0.93 Kg in 1990- 91 to 3.15 Kg per cropped hectare in 2010-11. Particularly, the use of chemicals is alarmingly high on farms involved in off season vegetable production. This demand for agri-chemicals in our agriculture has historically been influenced by interrelated factors such as population growth, economic growth, and agricultural production. However, effective microorganism can be used to grow crops through its application as a bio-fertilizers and bio-pesticides to minimize fertilizers or pesticides usage. Further, the use of beneficial microbes can cut down price of crop production by minimizing the use of agri-chemicals in more eco-friendly way as recently the cost of crop production has alarmingly increased in our country due to the high prices of agri-chemicals. Interest in the use of microbes that promote plant growth and yield has increased in advanced countries due to reasons as discussed, e.g. nitrogen fertilizers are expensive and can damage the environment through water contamination with nitrates, acidification of soils

ATTACHED

8/22

20

and greenhouse-gas emissions. The nitrogen provided by microbes is less prone to leaching, volatilization and denitrification. Moreover, plants can only use a small amount of phosphate from chemical sources, because 75-90% of the added P is precipitated through metal-cation complexes and rapidly becomes fixed in soils. Adding to this, even the application of fertilizer is not rewarding due to high pH of our soil and continuous use of chemical fertilizers has deteriorated the health conditions of our soil, while the uses of these microbes not only improve the health condition but also maintain the fertility of soil.

4. Similarly, plant growth regulators like Gibberellins (GAs), Salicylic Acid (SA), Glycinebetaine (GB), Indole-3-Acetic Acid (IAA) enhance abiotic stress resistance in various crops. For example, drought stress in sunflower reduces achene- and oil yields, however, application of GB and SA improve yield of sunflower under the same stress. Similarly application of GB at foliar stage in super basmati rice improves salinity and heat stress resistance and reduces their adverse effects. The GAs are considered to be responsible for mitigating impact of drought stress and confer drought tolerance in wheat enabling utilization of soil moisture of lower profiles through more proliferated roots.

To sum up, so far no comprehensive program has been adopted for the use of beneficial microbes and plant growth regulators that decreases crop production cost and manage plant against climatic change stresses. I want to initiate work on the same program for the capacity building of officers in the Department of Agriculture Extension and Research and to impart know how about the advance agricultural technologies currently adopted for improved crop production. On the basis of my experience/ skills related with plant-microbe interaction, plant growth regulators and physiology of biotic/ abiotic stress in crops, I can independently conduct research in these areas of interest which may significantly contribute towards the improvement of crop production under climate change scenario in Khyber Pakhtunkhwa. Furthermore, I am sure to attract funding from national and international organizations for conducting these experiments/ projects to develop Eco-Friendly Technologies.

**Future Research Planning:** To further improve the crop production under climate change conditions and identify the factors other than this that could potentially decrease the crop production area, I have following interest for research in future:

ATTESTED

9/22

21

1. Identification of High Water Use Efficient Varieties in Major Crops:

As the agriculture in Pakistan is predominantly irrigated (90 percent) due to the country's arid and semi-arid climate and consumes about 95% of annual available surface water. However, organizations e.g. International Monetary Fund (IMF) has reported that Pakistan is among the top 36 countries facing water scarcity and it is predicted that the situation will become worse by the mid of century making Pakistan its position to top 20 due to changing weather patterns and frequent occurrence of extreme weather events. Keeping in view this aspect, my future comprehensive research plan on immediate basis is to develop/identify high water use efficient varieties of major crops such as rice, sugarcane, tobacco and wheat to counter the negative impact of climate change on our crop production and food security.

2. Identification of Climate Clever Clovers Varieties and Management Practices in Rice:

Agriculture and livestock is recognized as the second largest contributor to total greenhouse gas (GHGs) emissions in Pakistan. Methane is one of the most potent heat trapping gasses among GHGs emit into the atmosphere particularly due to agriculture and livestock practices. To reduce GHGs particularly methane emissions from rice field, the following improved management practices should be promoted: reduction in use of chemicals, conservation of water, promotion of no-till farming and better water management.

As mitigating methane production by ruminants is a significant challenge to global livestock production. The response to direct selection of animals for low methanogenic potential in the rumen is likely to be slow, so another strategy the identification of climate clever clovers varieties offer a new paradigm to reduce methane emissions from ruminants.

3. Future Urban Land Expansion and Implications for Croplands in Our Province/Country:

Most of the irrigated croplands are located near urban areas in our Province and rapid urban expansion has been occurred on these croplands, emphasizing the potential competition for land between agricultural and urban uses. Very few studies have been conducted and show that high rates of urban expansion over the last decades have resulted in the loss of cropland in our Province/ Country. Although cropland loss has become a significant concern in terms of food production and livelihoods for us, there is

ATTESTED  
A

10/22

22

very little scientific understanding of how future urban expansion will affect our croplands. In this regard, I have planned to work on related very important questions such as: Where are croplands most vulnerable to conversion due to future urban expansion in our Province? What is the magnitude of cropland loss, especially of prime cropland, due to future urban expansion in our Province? and how will the loss of croplands affect total cropland area and relative economic importance of agriculture in our Province?

Thanking you in anticipation Sir.

Date: 27-11-2017

Sincerely yours

*Muhammad Waqas*

D. Muhammad Waqas

(PhD, Post Doc.)

Agriculture Officer

Department of Agriculture Extension

Gagra, Buner, Khyber Pakhtunkhwa

ATTESTED

*[Signature]*





GOVERNMENT OF KHYBER PAKHTUNKHWA  
AGRICULTURE LIVESTOCK & COOPERATIVE  
DEPARTMENT

DISCIPLINARY ACTION

E -

(48) (23) (23)

I, Muhammad Azam Khan, Chief Secretary, Khyber Pakhtunkhwa as Competent Authority, am of the opinion that Mr. Muhammad Waqas Agriculture Officer (BS-17) O/o District Director Agriculture, Buner has rendered himself liable to be proceeded against, as he committed the following acts/omissions within the meaning of section-3 of the Khyber Pakhtunkhwa Government Servants (Efficiency and Discipline) Rules, 2011.

STATEMENT OF ALLEGATIONS

1. He was granted 1460 days study leave on half pay w.e.f 1.3.2011 to 28.02.2015, he was required to report for duty on 01.03.2015 but could not join duty and wilfully absented himself upto 13.04.2017.
2. For the purpose of inquiry against the said accused with reference to the above allegations, an Inquiry Officer/Committee, consisting of the following, is constituted under rule 10(1)(a) of the ibid rules:
  - i. Mr. Noor Wali Khan
  - ii. D.S. Home department
3. The inquiry officer/committee shall, in accordance with the provisions of the ibid rules, provide reasonable opportunity of hearing to the accused, record its findings and make, within thirty (30) days of the receipt of this order, recommendations as to punishment or other appropriate against the accused.
4. The accused and a well conversant representative of the department shall join the proceedings on the date, time and place fixed by the Inquiry Officer/Committee.

ATTESTED  
*[Signature]*

(MUHAMMAD AZAM KHAN)

CHIEF SECRETARY,  
KHYBER PAKHTUNKHWA

(COMPETENT AUTHORITY)

24

24

To

The Deputy Secretary (Home)/Inquiry Officer

Home & Tribal Affairs Department, Khyber Pakhtunkhwa, Peshawar

**Subject: REPLY TO THE CHARGE SHEET AND STATEMENT OF ALLEGATION SERVED ON ME VIDE NOTIFICATION NO. DS(J)/HD/2017/Vol-I/2 DATED PESHAWAR THE 21.11.2017 / PROVISION OF RESEARCH ARTICLES**

**Respected Sir**

I have the honor to refer to your office Notification No. DS(J)/HD/2017/Vol-I/2 Dated Peshawar the 21-11-2017 on the subject noted above, below given is the list of my selected research articles published till date in International and National reputed journals / conference proceedings. It is also submitted that the information regarding those research articles currently submitted/ under review process has also been listed. In addition, more than ten research articles are under preparation and will be submitted in near future to the international journals:

Note: Research articles/conference Proceedings with below given numbering in chronological order and the sequence of their respective copies attached as annexures are same.

**Research Articles/ Conference Proceedings Published in Year 2007 and 2008**

1. Arif M, Munsif F, Waqas M, Khalil IA, Ali K. 2007. Effect of tillage on weeds and economics of fodder maize production. Pak. J. Weed Sci. Res. 13(3-4): 167-175.
2. Arif M, Waqas M, Nawab K, Shahid M. Effect of Seed Priming in Zinc Solutions on Chick Pea and Wheat. 8th African Crop Science Conference Proceedings. El-Minia, Egypt, 27-31 October 2007. Vol. 8. pp 237-240, Part-I.
3. Evaluation of Zinc Application Methods in Maize. 12th Congress of Soil Science Society. October 20-23, 2008. NWFP Agricultural University Peshawar, Pakistan.
4. Impact of Nitrogen and Potassium Combinations and Source Sink Removal on Yield and Yield Components of Wheat. 12th Congress of Soil Science Society: October 20-23, 2008. NWFP Agricultural University Peshawar, Pakistan.

ATTESTED

*[Handwritten signature]*

25

**Research Articles/ Conference Proceedings Published in Year 2011**

5. Munsif F, Arif M, Khan N, Hussain Z, **Waqas M**, Ali K. 2011. Effect of tillage system on early seedling growth, fodder yield and economics value of maize. *Int. J. Bio. Biotech.* 8(2): 233-237.
6. Saifullah, Jan A, Munsif F, Arif M, Khan H, Ali K, **Waqas M**, Ali A. 2011. Performance of millet varieties under different irrigation levels. *Sarhad J. Agric.* 27(1): 1-7.
7. Arif M, **Waqas M**, Munsif F, Khan N, Ali A, Hussain Z, Samad A. 2011. Seed priming in Zn solution enhances emergence and yield of chickpea. *Int. J. Bio. Biotech.* 8(2): 295-298.
8. Ali K, Khalil SK, Hussain Z, Munsif F, Din IU, **Waqas M**, Wagma. 2011. Effect of various tillage methods and nitrogen management on weeds and maize performance. *Pak. J. Weed Sci. Res.* 17(3): 253-262.
9. Khan AL, Hamayun M, Ahmad N, Hussain J, Kang SM, Kim YH, Adnan M, Tang H, **Waqas M**, Radhakrishnan R, Park ES, Lee JJ. 2011. Salinity stress resistance offered by endophytic fungal interaction between *Penicillium minioluteum* LHL09 and *Glycine max.* *L. J. Microbiol. Biotechnol.* 21(9): 893-902.
10. Khan AL, Hamayun M, Ahmad N, **Waqas M**, Kang SM, Kim YH, Lee JJ. 2011. *Exophiala* sp. LHL08 reprograms *Cucumis sativus* to higher growth under abiotic stresses. *Physiol Plant.* 143: 329-343.

**Research Articles/ Conference Proceedings Published in Year 2012**

11. **Waqas M**, Khan AL, Kamran M, Hamayun M, Kang SM, Kim YH, Lee JJ. 2012. Endophytic fungi produce gibberellins and indoleacetic acid and promotes host-plant growth during stress. *Molecules.* 7;17(9):10754-73.
12. **Waqas M**, Khan AL, Hamayun M, Kamran M, Kang SM, Kim YH, Lee JJ. 2012. Assessment of endophytic fungi cultural filtrate on soybean seed germination. *Afr. J. Biotechnol.* 11(85): 15135-15143.
13. Khan AL, Hamayun M, Radhakrishnan R, **Waqas M**, Kang SM, Kim. Y.H, Shin J.H, Choo Y.S, Kim J.G and I.J. Lee. 2012. Mutualistic association of endophyte *Paecilomyces formosus* LHL10 offered membrane stability and thermotolerance to *Cucumis sativus*. *Antonie Van Leeuwenhoek* 101: 267-279.

ATTESTED



14. Kim YH, Khan AL, Shinwari ZK, Kim DK, **Waqas M**, Kamran M, Lee IJ. 2012. Silicon treatment to Rice (*Oryza sativa* L. cv 'Gopumbyeo') plants during different growth periods and its effects on growth and grain yield. Pak. J. Bot. 44 (3): 891-897.
15. Khan AL, Hamayun M, **Waqas M**, Kang SM, Kim YH, Kim DH, Lee IJ. 2012. *Exophiala* sp. LHL08 association gives heat stress tolerance by avoiding oxidative damage to cucumber plants. Biol. Fert. Soils. 48(5): 519-529.
16. Khan AL, Shinwari ZK, Kim YH, **Waqas M**, Hamayun M, Kamran M, Lee IJ. 2012. Role of endophyte *Chaetomium globosum* LK4 in growth of *Capsicum Annuum* by production of gibberellins and indole acetic acid. Pak. J. Bot. 44:1601-1607.
17. Kang SM, Khan AL, Hussain J, Ali L, Kamran M, **Waqas M**, Lee IJ. 2012. Rhizonin A from *Burkholderia* sp. KCTC11096 and its growth promoting role in lettuce seed germination. Molecules 17, 7980-7988.

#### Research Articles/ Conference Proceedings Published in Year 2013

18. **Waqas M**, Khan AL, Ali L, Kang SM, Kim YH, Lee IJ. 2013. Seed germination-influencing bioactive secondary metabolites secreted by the endophyte *Cladosporium cladosporioides* LWL5. Molecules. 18, 15519-15530.
19. AL Khan, **Waqas M**, Khan AR, Hussain J, Kang SM, Gilani SA, Hamayun M, Shin JH, Kamran M, Al-Harrasi.A, Yun BW, Adnan M, IJ Lee. 2013. Fungal endophyte *Penicillium janthinellum* LK5 improves growth of ABA-deficient tomato under salinity. World Journal of Microbiology and Biotechnology. 29(11):2133-44.
20. Khan AL, **Waqas M**, Hamayun M, Al-Harrasi A, Al-Rawahi A, Lee IJ. 2013. Co-synergism of endophyte *Penicillium resedanum* LK6 with salicylic acid helped *Capsicum annuum* in biomass recovery and osmotic stress mitigation. BMC Microbiology. 2013 13:51.
21. Ullah I, Khan AR, Park GS, Lim JH, **Waqas M**, Lee IJ, Shin JH. 2013. Analysis of phytohormones and phosphate solubilization in *Photobacterium* sp. Food Science and Biotechnology. 22(1) 25-31.

ATTESTED



16

- 22. Khan AL, Waqas M, Hussain J, Al-Harrasi A, Lee IJ. 2013. Fungal endophyte *Penicillium janthinellum* LK5 can reduce cadmium toxicity in *Solanum lycopersicum* (Sitiens and Rhe). *Biol Fertil Soils*. 50, (1), 75-85.
- 23. Ali K, Arif M, Khan Z, Tariq M, Waqas M, Gul B, Bibi S, Din Z, Ali M, Shafi B, Adnan M. 2013. Effect of cutting on productivity and associated weeds of canola. *Pak. J. Weed Sci. Res.* 19(4): 393-401.
- 24. M Tariq, Khan Z, Arif M, Ali K, Waqas M, N Khalid, Ali M, MA Khan, Shafi B, Adnan M. 2013. Effect of nitrogen application timings on the seed yield of Brassica cultivars and associated weeds. *Pak. J. Weed Sci. Res.* 19(4): 493-502.
- 25. Kamran M, Khan AL, Waqas M, Kang SM, Kim J, Khan AR, Imran QM and Lee IJ. 2013. An Eco-Friendly approach for control of *Echinochloa crus-galli* (Barnyard grass). International Conference on Crop Management in Changing Climate, February 11-13, Faisal Abad, Pakistan. Page-102
- 26. Kamran M, Khan AR, Khan AL, Waqas M, Kang SM, Shin JH and Lee IJ. 2013. Plant derived smoke reprograms *Glycine max* L. to higher growth under salt stress by modifying antioxidants, abscisic acid, salicylic acid and jasmonic acid signals. International Conference on Crop Management in Changing Climate, February 11-13, Faisal Abad, Pakistan. Page-103
- 27. Kim YH, Kamran M, Ahn IK, Khan AL, Lee JS, Waqas M, Jang SW and Lee IJ. 2013. Regulation of endogenous gibberellins and abscisic acid levels during different seed collection periods of *Panax ginseng* C.A Meyer (Korean ginseng) in the Republic of Korea. International Conference on Crop Management in Changing Climate, February 11-13, Faisal Abad, Pakistan. Page-118

**Research Articles/ Conference Proceedings Published in Year 2014**

- 28. Waqas M, Khan AL, Kang SM, Kim YH, Lee IJ. 2014. Phytohormone-producing fungal endophytes and hardwood-derived biochar interact to ameliorate heavy metal stress in soybeans. *Biology and Fertility of Soils*. 50, (7), 1155-1167.

ATTESTED

29. Waqas M, Ahmad B, Arif M, Munsif F, Khan AL, Amin M, Kang SM, Kim YH, Lee IJ. 2014. Evaluation of humic acid application methods for yield and yield components of mungbean. American Journal of Plant Sciences. 5, 2269-2276.
30. Waqas M, Khan AL, Lee IJ. 2014. Bioactive chemical constituents produced by endophytes and effects on rice plant growth. Journal of plant interactions: 9(1), 478-487.
31. Kim YH, Khan AL, Waqas M, Shim JK, Kim DH, Lee KY, Lee IJ. 2014. Silicon application to rice root zone influenced the phytohormonal and antioxidants responses under salinity stress. Journal of Plant growth regulation. 33(2) 137-149.
32. Kim Y-H, Khan AL, Kim D-H, Lee S-Y, Kim K-M, Waqas M, Jung H-Y, Shin J-H, Kim J-G, Lee I-J. 2014. Silicon mitigates heavy metal stress by regulating P-type heavy metal ATPases, *Oryza sativa* low silicon genes, and endogenous phytohormones. BMC Plant Biology. 14:13.
33. Kamran M, Khan AL, Waqas M, Imran QM, Hamayun M, Kang S-M, Kim Y-H, Kim M-J, Lee I-J. 2014. Effects of plant-derived smoke on the growth dynamics of Barnyard Grass (*Echinochloa crus-galli*). Acta Agriculturae Scandinavica, Section B - Soil & Plant Science. 64 (2), 121-128.
34. Kang SM, Khan AL, Waqas M, You YH, Kim JH, Kim JG, Hamayun M, Lee IJ. 2014. Plant Growth Promoting Rhizobacterias reduces adverse effects of salinity and osmotic stress by regulating phytohormones and antioxidants in *Cucumis sativus*. Journal of plant interactions. 9(1), 673-682.
35. Kim YH, Ahn IO, Khan AL, Kamran M, Waqas M, Lee JS, Kim DH, Jang SW, Lee IJ. 2014. Regulation of endogenous gibberellins and abscisic acid levels during different seed collection periods of *Panax ginseng* c. a. meyer in Republic of Korea. Horticulture, Environment, and Biotechnology. 55(3):166-174.
36. Kim YH, Khan AL, Waqas M, Jeong HJ, Kim DH, Shin JS, Kim JG, Yeon MH, Lee IJ. 2014. Regulation of jasmonic acid biosynthesis by silicon application during physical injury to *Oryza sativa* L. Journal of Plant Research. 127(4):525-32.
37. Khan AL, Waqas M, Kang SM, Al-Harrasi A, Hussain J, Al-Rawahi A, Al-Khiziri S, Ullah I, Ali L, Jung H Y, Lee IJ. 2014. Bacterial endophyte *Sphingomonas* sp. LK11 produces gibberellins and IAA and promotes tomato plant growth. Journal of Microbiology. 52(8), 689-695.

ATTESTED

20

38. Khan AL, **Waqas M**, Hussain J, Al-Harrasi A, Al-Rawahi A, Al-Hosni K, Kim M-J, Adnan M, Lee I-J. 2014. Endophytes *Aspergillus caespitosus* LK12 and *Phoma* sp. LK13 of *Moringa peregrina* produce gibberellins and improve rice plant growth. *Journal of Plant Interactions*, 9(1), 731-737.

39. Khan AR, Ullah I, Khan AL, Hong SJ, **Waqas M**, Park GS, Kwak Y, Choi JB, Jung BK, Park M, Lee IJ, Shin JH. 2014. Phytostabilization and physicochemical responses of Korean ecotype *Solanum nigrum* L. to cadmium contamination. *Water Air Soil Pollut*. 225:2147.

40. Khan AL, **Waqas M**, Lee IJ. 2014. Resilience of *Penicillium resedanum* LK6 and exogenous gibberellin in improving *Capsicum annuum* L growth under abiotic stresses. *J Plant Res*. 128(2):259-68.

41. Kang SM, **Waqas M**, Khan AL, Lee IJ. 2014. Plant growth promoting rhizobacteria: Potential candidates for gibberellins production and crop growth promotion. In Miransari M, editor. *Use of microbes for the alleviation of soil stresses*, volume 1. Springer, New York, pp. 1-19.

42. Ullah I, Khan AL, Ali L, Khan AR, **Waqas M**, Lee IJ, Shin JH. 2014. An insecticidal compound produced by an insect-pathogenic bacterium suppresses host defenses through phenoloxidase inhibition. *Molecules*. 19: 20913-20928.

43. Shahzad R, Khan J, Gurmani AR, **Waqas M**, Hamayun M, Khan AL, Kang SM, Lee IJ. 2014. Seed priming with gibberellic acid (GA<sub>3</sub>) in sponge-gourd modulated high salinity stress. *Pakhtunkhwa J. Life Sci*. 02(01): 75-86.

44. Shahzad R, Khan J, Gurmani A R, **Waqas M**, Park J M, Lee SK, Kang SM, Kim YH, Lee IJ. 2014. Seed priming with gibberellic acid 3 in sponge gourd modulated salinity stress. *The Korean Society of Crop Science Conference (Improvement of Crop Productivity Response to Climate Change)*, April 17-18, Global Plaza KNU, South Korea. *Korean Journal of Crop Science*. P. 53.

45. Park JM, Kim YH, Kang SM, Kim DK, Kim MJ, Lee SK, **Waqas M**, Radhakrishnan R, Shahzad R, Lee IJ. Effect of Indole acetic acid producing *Enterbacteria* sp. I-3 on lettuce growth. *The Korean Society of Crop Science Conference (Improvement of Crop Productivity Response to Climate Change)*, April 17-18, Global Plaza KNU, South Korea. *Korean Journal of Crop Science*. P. 53.

ATTESTED



Research Articles/ Conference Proceedings Published in Year 2015

46. **Waqas M**, Khan AL, Shahzad R, Ullah I, Khan AR, Lee I-J. 2015. Mutualistic fungal endophytes produce phytohormones and organic acids that promote japonica rice plant growth under prolonged heat stress. *Journal of Zhejiang University-SCIENCE B*. 16(12); 1011-1018.

47. **Waqas M**, Khan AL, Hamayun M, Shahzad R, Kang S-M, Kim J-G, In-Jung Lee. 2015. Endophytic fungi promote plant growth and mitigate the adverse effects of stem rot: An example of *Penicillium citrinum* and *Aspergillus terreus*. *Journal of Plant Interactions*. 10(1), 280-287.

48. **Waqas M**, Khan AL, Hamayun M, Shahzad R, Kim YH, Choi KS, Lee IJ. 2015. Endophytic infection alleviates biotic stress in sunflower through regulation of defence hormones, antioxidants and functional amino acids. *Eur J Plant Pathol*. 141(4), 803-824.

49. Ullah I, Khan AL, Ali L, Khan AR, **Waqas M**, Hussain J, Lee IJ, Shin JH. 2015. Benzaldehyde as an insecticidal, antimicrobial, and antioxidant compound produced by *Photorhabdus temperata* M1021. *Journal of Microbiology*. 53(2) 127-133.

50. Khan SA, Lee JH, Hamayun M, Hussain A, Khan AL, **Waqas M**, Lee IJ, Kim JG. 2015. Alteration in the gene expression of *Glehnia littoralis* seedlings exposed to culture filtrate of *Penicillium citrinum* KACC43900. *Journal of Plant Interactions*, 10(1) 51-58.

51. Kang SM, Khan AL, **Waqas M**, You YH, Hamayun M, Joo GJ, Shahzad R, Choi KS, Lee IJ. 2015. Gibberellin-producing *Serratia nematodiphila* PEJ1011 ameliorates low temperature stress in *Capsicum annuum* L. *European Journal of Soil Biology*. 68, 85-93.

52. Halo BA, Khan AL, **Waqas M**, Al-Harrasi A, Hussain J, Ali L, Adnan M, Lee IJ. 2015. Endophytic bacteria (*Sphingomonas* sp. LK11) and Gibberellin can improve *Solanum lycopersicum* growth and oxidative stress under salinity. *Journal of Plant Interactions*. 10(1), 117-125.

53. Khan AL, **Waqas M**, Hussain J, Al-Harrasi A, Hamayun M, Lee IJ. 2015. Phytohormones enabled endophytic fungal symbiosis improve aluminum phytoextraction in tolerant *Solanum lycopersicum*: an examples of *Penicillium janthinellum* LK5 and comparison with exogenous GA3. *Journal of Hazardous Materials*. 295, 70-79.

54. Khan AR, Ullah I, Khan AL, Park GS, **Waqas M**, Hong SJ, Jung BK, Kwak Y, Lee IJ,

ATTESTED





20

Shin JH. 2015. Improvement in phytoremediation potential of *Solanum nigrum* under cadmium contamination through endophytic-assisted *Serratia* sp. RSC-14 inoculation. Environmental Science and Pollution Research. 22(18):14032-42.

55. Ali K, Arif M, Jan MT, Khan MJ, Yaseen T, **Waqas M**, Munsif F. 2015. Biochar: a novel tool to enhance wheat productivity and soil fertility on sustainable basis under wheat-maize-wheat cropping pattern. Pak. J. Bot. 47(3): 2015.

56. Khan AR, Ullah I, **Waqas M**, Shahzad R, Hong SJ, Park GS, Jung BK, Lee IJ, Shin JH. 2015. Plant growth promoting potential of endophytic fungi isolated from *Solanum nigrum* leaves. World J. Microbiol. Biotechnol. 31(9):1461-1466.

57. Hamayun M, Hussain A, Khan SA, Irshad M, Khan AL, **Waqas M**, Shahzad R, Iqbal A, Ullah N, Rehman G, Kim H-Y and Lee I-J. 2015. Kinetin modulates physio-hormonal attributes and isoflavone contents of Soybean grown under salinity stress. Front. Plant Sci. 6:377.

58. Kim Y, Hwang S, **Waqas M**, Khan AL, Lee J, Lee J, Nguyen HT and Lee I. 2015. Comparative analysis of endogenous hormones level in two soybean (*Glycine max* L.) lines differing in waterlogging tolerance. Front. Plant Sci. 6:714.

59. Shahzad R, **Waqas M**, Khan AL, Hamayun M, Kang S-M, Lee I-J. 2015. Foliar application of Methyl Jasmonate induced physio-hormonal changes in *Pisum sativum* under diverse temperature regimes. Plant Physiology and Biochemistry. 96, 406-416.

60. **Waqas M**, Hamayun M, Khan AL, Shahzad R, Lee I-J. 2015. Endophytic fungi promotes growth and reprograms the adverse affect of stem rot by regulating systemic acquired resistance in Sunflower. 18th International Plant Protection Congress. (August 24-27). Berlin, Germany. PP- 467.

**Research Articles/ Conference Proceedings Published in Year 2016 and 2017**

61. **Waqas M**, Shahzad R, Khan AL, Asaf S, Kim Y-H, Kang S-M, Bilal S, Hamayun M, Lee I-J. 2016. Salvaging effect of triacontanol on plant growth, thermotolerance, macro-nutrient content, amino acid concentration and modulation of defense hormonal levels under heat stress. Plant Physiology and Biochemistry. 99, 118-125.

62. Kim YH, Khan AL, **Waqas M**, Shahzad R, Lee IJ. 2016. Silicon-mediated mitigation of

ATTESTED  
*[Signature]*



wounding stress acts by up-regulating the rice antioxidant system. *Cereal Research Communications*. 44(1), 111-121.

63. Iqbal A., Shah F., Hamayun M., Ahmad A., Hussain A., **Waqas M.**, Kang S., & Lee IJ. 2016. Allergens of *Arachis hypogaea* and the effect of processing on their detection by ELISA. *Food & Nutrition Research*. 60:28945.

64. Shahzad R, **Waqas M**, Khan AL, Al-Hosni K, Kang S-M, Seo C-W, Lee I-J. 2017. Indoleacetic acid production and plant growth promoting potential of bacterial endophytes isolated from rice (*Oryza sativa* L.) seeds. *Acta Biologica Hungarica*. 68(2), 175-186.

65. Kang S-M, **Waqas M**, Shahzad R, You Y-H, Asaf S, Khan MA, Lee K-E, Kim S-J, Joo G-J, Lee I-J. 2017. Isolation and characterization of a novel silicate-solubilizing bacterial strain *Burkholderia eburnea* CS4-2 that promotes growth of rice. *Soil Science and Plant Nutrition*. 63(3), 233-241.

66. Khan AR, Ullah I, **Waqas M**, Park GS, Khan AL, Hong SJ, Ali R, Jung BK, Park CE, Ur-Rehman S, Lee IJ, Shin JH. 2017. Host plant growth promotion and cadmium detoxification in *Solanum nigrum*, mediated by endophytic fungi. *Ecotoxicology and Environmental Safety*. 136, 180-188.

67. Hamayun M, Hussain A, Khan SA, Kim H-Y, Khan AL, **Waqas M**, Irshad M, Iqbal A, Rehman G, Jan S and Lee I-J. 2017. Gibberellins producing endophytic fungus *Porostereum spadiceum* AGH786 rescues growth of salt affected soybean. *Front. Microbiol*. 8:686.

68. Kamran M, Khan AL, Ali L, Hussain J, **Waqas M**, Al-Harrasi A, Imran QM, Kim Y-H, Kang S-M, Yun B-W, Lee I-J. 2017. Hydroquinone; A novel bioactive compound from plant-derived smoke can cue seed germination of lettuce. *Front. Chem*. 5:30.

69. Asaf S, Khan AL, Khan MA, **Waqas M**, Kang S-M, Yun B-W, Lee I-J. 2017. Chloroplast genomes of *Arabidopsis halleri* ssp. *gemmaifera* and *Arabidopsis lyrata* ssp. *petraea*: Structures and comparative analysis. *Scientific Reports*. 7: 7556.

70. Kang S-M, **Waqas M**, Hamayun M, Asaf S, Khan AL, Kim A-Y, Park Y-G, Lee I-J. 2017. Gibberellins and indole-3-acetic acid producing rhizospheric bacterium *Leifsonia xyli*

ATTACHED  
*[Handwritten signature]*

SE134 mitigates the adverse effects of copper-mediated stress on tomato. *Journal of Plant Interactions*, 12:1, 373-380.

71. Ullah I, Waqas M, Khan MA, Lee IJ, Kim WC. 2017. Exogenous ascorbic acid mitigates flood stress damages of *Vigna angularis*. *Appl Biol Chem*. 60:6, 603-614.
72. Khan AL, Bilal S, Halo BA, Al-Harrasi A, Khan AR, Waqas M, Al-Thani GS, Al-Amri I, Al-Rawahi A, Lee I-J. 2017. *Bacillus amyloliquefaciens* BSL16 improves phytoremediation potential of *Solanum lycopersicum* during copper stress, *Journal of Plant Interactions*, 12:1, 550-559.

#### Research Articles currently under peer review process in International Journals

73. Biochar amendment changes jasmonic acid levels in two rice varieties and alters their resistance to herbivory. Submitted and under review process in PLOS One
74. Indole acidic acid producing endophytic fungi (*Yarrowia lipolytica* FH1) reprograms maize growth through hormonal regulation under saline environment. Submitted and under review process in Frontier in Plant Science
75. Integrated phytohormone production by PGPR *Bacillus tequilensis* SSB07 induced thermotolerance in soybean. Submitted and under review process in Journal of Plant Physiology and Biochemistry.
76. Regulation of reactive oxygen and nitrogen species by salicylic acid in rice plants under salinity stress conditions. Submitted and under review process in PLOS One
77. Proteomic profiling of endophytic *Bacillus amyloliquefaciens* RWL-1 under methanol utilization. Submitted and under review process in Microbial Cell Factories
78. Halo-tolerant rhizospheric *Arthrobacter wohnwensis* AK1 mitigates salt stress and induces physio-hormonal changes in soybean. Submitted and under review process in Saudi Journal of Biological Sciences
79. The Importance of Non-Timber Forest Products for Food Security in Jordan. Submitted and under review process in Forests, Trees and Livelihoods

ATTESTED

80. Agronomic approaches to improve rice production under abiotic stress: Book Chapter  
under preparation

Thanking you in anticipation Sir.

Enclosures: (As above)

Date: 27-11-2017

Sincerely,

*Muhammad Waqas*  
Dr. Muhammad Waqas

(PhD, Post Doc.)

Agriculture Officer

Department of Agriculture Extension

Gagra, Buner, Khyber Pakhtunkhwa

ATTESTED  
*[Signature]*

## EFFECT OF TILLAGE ON WEEDS AND ECONOMICS OF FODDER MAIZE PRODUCTION

Muhammad Arif<sup>1</sup>, Fazal Munsif<sup>1</sup>, Muhammad Waqas<sup>1</sup>, Ibni Amin Khalil<sup>2</sup> and Kawsar Ali<sup>1</sup>

### ABSTRACT

An experiment was conducted at Agricultural Research Farm, NWFP Agricultural University Peshawar during spring 2007. The experiment was laid out in randomized complete block design having four replications. The tillage systems consisted of no-till (NT), conventional tillage (CT) and deep tillage (DT). Maize type sweet corn (Swat local) was sown on April 23, 2007. The net plot size of 30 x 20 m<sup>2</sup> was used. The crop was sown as broadcast with seed rate of 60 kg ha<sup>-1</sup>. Phosphorus and nitrogen were applied at the rate of 90 and 120 kg ha<sup>-1</sup>, respectively. Weed density and diversity were significantly higher for NT followed by RT and DT. Higher fresh and dry weights of weeds were also noted in NT followed by RT and DT. RT resulted in higher fresh fodder yield followed by DT and NT. Similarly, RT resulted in higher gross income and gross margin. However, comparison showed that NT with less fuel consumption could be a viable economical alternative when the efficiency coefficients are taken into account which was 1:4 for NT.

**Key words:** Spring maize, tillage, weeds, fodder

### INTRODUCTION

Maize (*Zea mays* L.) is the most important cereal and fodder crop of NWFP and Pakistan. It is a dominant crop in the farming system because it is a staple food crop for most of the rural population as well as fodder for their animals. Maize is cultivated both in spring and summer season in NWFP as a dual purpose crop. Staggered planting from February to September helps cope with the fodder scarcity problems faced in May-June and October-November. Its nutritious fodder is relished by all livestock, especially milch animals. The green fodder of maize contains 1.56% protein, 0.30% fat, and

<sup>1</sup> Department of Agronomy, NWFP Agricultural University Peshawar 25130, Pakistan  
<sup>2</sup> Department of Plant Breeding and Genetics, NWFP Agricultural University Peshawar 25130, Pakistan

ATTESTED  


## Effect of seed priming in Zn solutions on chickpea and wheat

MUHAMMAD ARIF<sup>1</sup>, MUHAMMAD WAQAS<sup>1</sup>, KHALID NAWAB<sup>2</sup> & MUHAMMAD SHAHID<sup>1</sup>

<sup>1</sup>Department of Agronomy, <sup>2</sup>Department of Agricultural Extension Education and Communication, NWFP Agricultural University Peshawar, Pakistan, marifkhan75@yahoo.com

**Abstract:** In order to study the effect of seed priming in different concentration of Zinc solutions on chickpea and wheat, experiments were conducted at Agricultural Research Farm of NWFP Agricultural University Peshawar during Rabi 2002-2003. The experiments were laid out in Randomized Complete Block design with four replications. The seed of chickpea variety Karak-1 was primed in water, 0.05% and 0.075% Zn solutions while the seed of wheat variety Saleem-2000 was primed in 0.1%, 0.2%, 0.3% and 0.4% Zn solutions. Dry seed (non primed) was used as control treatment in both crops. A plot size of 15m by 4 m with rows distance of 30 cm was used for chickpea while a plot size of 2.5m by 3.5m with rows distance of 30 cm was used for wheat. Analysis of the data indicated that seed priming significantly affected emergence m<sup>-2</sup>, grain and biological yields of chickpea. Seed primed in 0.05% Zn solution resulted in the highest emergence m<sup>-2</sup>, grain and biological yields of chickpea. In case of wheat, seed priming significantly affected gains spike<sup>-1</sup>, thousand grain weight, grain yield, biological yield. Seed primed in 0.4% Zn solution resulted in higher grains spike<sup>-1</sup>, thousand grain weight and biological yield while seed primed in 0.1% Zn solution resulted in the maximum grain yield of wheat. It is concluded that seed priming in Zn solutions improves grain yield of chickpea and wheat.

**Key words:** Seed priming, Chickpea, Wheat, Yield

### Introduction

Wheat is the most important winter crop of Pakistan as well as of the world. It ranks first on the basis of area and production in Pakistan. Likewise, chickpea is also the important pulse crop which is mostly sown on marginal and rainfed areas of the country.

Crop production is affected by a low chemical availability of P and Zn (Ryan, 1997). Especially during the early growth stages, a lack of P and Zn retards seedling growth, rendering the young plantlets particularly sensitive to the frequently encountered dry spells (Jones and Wahbi, 1992). A rapid establishment of healthy seedlings and a sufficient supply with P and Zn are prerogative to reduce the risk of crop failure (Brown *et al.*, 1987). Zinc is essential for the synthesis of plant growth regulators like auxins, also act as a metal activator of several enzymes, involved in the synthesis of protein and nucleic acids in plants. Zinc deficiency occurs in alkaline soil, and is unavailable due to the formation of zinc hydroxide and oxides. N and P also affect zinc uptake by the plants (Khalil and Jan, 2002). Khattak and Parveen (1986) reported that out of 320 soil samples collected from NWFP, 23% were deficient in Zn. This study included samples from forest soils but district-wise data revealed that nearly 50% of samples were Zn deficient in Karak district and 100% deficient in soils of the Peshawar valley. Based on extensive research on micronutrients in soil and crops, it has been estimated that now about 70% of the cultivated area of the country is considered zinc deficient, and Zn deficiency is the third most serious crop nutrition problem in the country after N and P deficiency (Rashid, 1996).

Seed priming comprises the soaking of seed in water and drying back to the storage moisture until use. The

soaking induces a range of biochemical changes in the seed that are required to start the germination process (breaking of dormancy, hydrolysis or metabolism of inhibitors, imbibition and enzyme activation). Some or all of these processes that precede the germination are triggered by priming and persist following the redessiccation of seeds (Asgedom and Becker, 2001). Thus upon seeding, primed seed can rapidly imbibe and revive the seed metabolism, resulting in a higher germination rate and a reduction in the inherent physiological heterogeneity in germination (Rowse, 1995). Nutrient priming has been proposed as a novel technique that combines the positive effects of seed priming with an improved nutrient supply (Al-Mudaris and Jutzi, 1999).

In nutrient priming, seeds are pretreated (primed) in solutions containing the limiting nutrients instead of being soaked simply in water (Arif *et al.*, 2005). Seed priming has been shown to be effective in improving stand establishment and crop vigor in range of crops (Musa *et al.*, 2001; Harris *et al.*, 2001). Osmo-conditioned seeds may have improved germination and uniformity, especially under adverse seedbed condition such as low temperature (Pill and Finch, 1988; Stoffela *et al.*, 1988).

The present experiment was initiated to study the effect of seed priming with various zinc concentration on yield and yield components of chickpea and wheat.

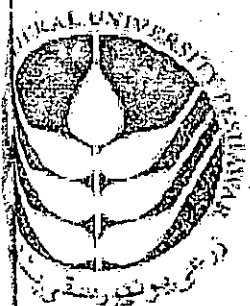
### Materials and Methods

#### Experimental site

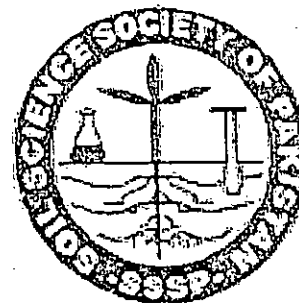
The experiment was conducted at Agricultural Research Farm, NWFP Agricultural University Peshawar, Pakistan. Peshawar is located about 1600 km north of Indian Ocean and thus has a continental climate. The experimental site is

ATTESTED

# 12<sup>TH</sup> CONGRESS OF SOIL SCIENCE



OCTOBER 20-23, 2008



## ABSTRACTS

*Theme*

ACHIEVING MILLENNIUM DEVELOPMENT GOALS  
THROUGH WISE SOIL MANAGEMENT

*at*

*NWFP Agricultural University, Peshawar*

*Major Sponsors*

*Higher Education Commission, Islamabad  
Pakistan Science Foundation, Islamabad*

ATTESTED

**SOIL SCIENCE SOCIETY OF PAKISTAN**

EFFECT OF TILLAGE SYSTEMS ON EARLY SEEDLING GROWTH, FODDER YIELD AND ECONOMICS OF MAIZE

Fazal Munsif<sup>1</sup>, Muhammad Arif<sup>1</sup>, Nasrullah Khan<sup>3</sup>, Zahid Hussain<sup>2</sup>, Muhammad Waqas<sup>1</sup> and Kawsar Ali<sup>1</sup>

<sup>1</sup> Department of Agronomy<sup>2</sup>, Department of Weed Science, KPK Agricultural University Peshawar, Pakistan, <sup>3</sup> Department of Botany University of Karachi

Corresponding email: [nasrullahdushkhetifa@yahoo.com](mailto:nasrullahdushkhetifa@yahoo.com)

ABSTRACT

Reduced tillage has become an integral component of sustainable agriculture reducing input costs and soil loss, conserve energy, reduce soil erosion and labor costs, and elimination of extensive land preparation prior to planting. Therefore, in order to investigate the effect of tillage systems on early seedling growth, fodder yield and economic analysis, the experiment was conducted at Agricultural Research Farm of NWFP Agricultural University, Peshawar during spring 2007. The experiment was laid out in randomized complete block design having four replications. The tillage systems consisted of no-till, reduced tillage and deep tillage. The net plot size of 30 m by 20 m was planted at the seed rate of 60 kg ha<sup>-1</sup> as broadcast. Nitrogen and phosphorus were applied at the rate of 120 and 90 kg ha<sup>-1</sup>. Deep tillage resulted in greater emergence m<sup>-2</sup> (134). Reduced tillage produced taller plants (211 cm), more leaves per plant (9.3) and higher fresh and dry fodder yield of maize (130.5 & 48 t ha<sup>-1</sup>). Similarly, reduced tillage resulted in higher gross income (Rs. 65250) and net income (Rs. 63250). Higher value cost ratio (31.6) was recorded for reduced tillage followed by no-tillage (24.8), whereas deep tillage fetched lowest value cost ratio (11). It was concluded that reduced tillage resulted in higher fodder yield, net income and value cost ratio.

Key-words: Tillage, maize, seedling growth, fodder yield.

INTRODUCTION

Maize (*Zea mays* L.) is a dominant crop in the farming system in Khyber Pukhtoonkhwa and Pakistan because it is a staple food crop for most of the rural population as well as fodder for animals. Maize is cultivated both in spring and summer season as a dual purpose crop. Staggered planting from February to September helps cope with the fodder scarcity problems faced in May-June and October-November (Harris *et al.*, 2007). Its nutritious fodder is relished by all kinds of livestock, especially milch animals and exceeds all other summer fodder crops in average yield, dry matter and digestibility (Lakho *et al.*, 2004). The green fodder of maize is rich in vitamin-A and contains 1.56% protein, 0.30% fat, and 5.27% fiber (Chaudhry, 1982). It is a cash crop for growers, as around cities it is widely grown for sale as green fodder.

Tillage is considered the most effective farm activity for developing a desired soil structure. However it has become a controversial practice over the last few decades. For example Patil and Sheelavantar, (2006) and Papini *et al.* (2007) attained better plant growth and performance with deep ploughing. Halvorson *et al.* (2000) and Sainju *et al.* (2006) reported the same results with conventional ploughing while Dolan *et al.*, (2006); Gangwar *et al.*, (2006) and Nakamoto *et al.*, (2006) with no-tillage system. However, other authors have found little or no difference between the various tillage practices (Fischer *et al.*, 2002; Iqbal *et al.*, 2005; Wang and Dalal 2006). Reduced tillage can be efficient in saving more water for crop production (Habtegebrial *et al.*, 2007). It improves productivity and sustainability of arable land in temperate region (Nakamoto *et al.*, 2006). Improvement in soil organic C (Wright and Hons, 2005; Dolan *et al.*, 2006). N enrichment (Habtegebrial *et al.*, 2007) and slow release of nutrient upon gradual organic matter decomposition are associated with reduced tillage. However, both deep and reduced tillage have been shown to improve soil porosity and aeration (Hao *et al.*, 2001; Zorita, 2000), preserve greater soil moisture and nutrients for plant and microbes (Lopez-Bellido *et al.*, 2001; Patil and Sheelavantar, 2006) and hence ultimately had increased crop yield (Zorita, 2000).

This study was carried out to asses the financial feasibility of using various tillage systems and its effect on early seedling growth and fodder yield of maize.

MATERIALS AND METHODS

Experimental site

The effects of different tillage systems were assessed on early seedling growth, fodder yield and economic analysis in field experiment on sweet corn. The experiments were carried out at Agricultural Research Farm of KPK

ATTESTED  
*[Signature]*



Sarhad J. Agric. Vol.27, No.1, 2011

## PERFORMANCE OF MILLET VARIETIES UNDER DIFFERENT IRRIGATION LEVELS

SAIFULLAH\*, AMANULLAH JAN\*, FAZAL MUNSIF\*, MUHAMMAD ARIF\*,  
HAMAYOON KHAN\*, KAWSAR ALI\*, MUHAMMAD WAQAS\* and ABID ALI\*\*

\* Department of Agronomy, Khyber Pakhtunkhwa Agricultural University, Peshawar – Pakistan.  
\*\* Pakistan Tobacco Board, Mardan – Pakistan.  
E-mail: munsiffazal@yahoo.com

### ABSTRACT

Field experiment on performance of millet varieties (PARC MS-2, PARC MS-3, Compasit<sup>1</sup> and Local) under different irrigation levels (no irrigation, single irrigation at 15, 30, 45, double irrigation at 15 and 30, at 15 and 45 and at 30 and 45 days after sowing (DAS) was carried out at New Developmental Research Farm of NWFP Agricultural University, Peshawar, Pakistan during kharif 2006. The experiment was laid out in Randomized Complete Block Design with split plot arrangements replicated four times in a plot size of 5 m x 3 m. Irrigation levels were allotted to main plots while varieties to subplots. Significant differences were recorded for plant height, green fodder and dry fodder yield among the varieties, while emergence m<sup>-2</sup>, days to emergence and tiller plant<sup>-1</sup> were found non significant. Variety PARC-MS-2 had maximum plant height (238 cm), green fodder yield (74.31 t ha<sup>-1</sup>) and dry fodder yield (18.17 t ha<sup>-1</sup>) when compared with the rest of the varieties. Local cultivar with plant height (191 cm), green fodder (59.08 t ha<sup>-1</sup>) and dry fodder yield (15.27 t ha<sup>-1</sup>) was the lowest. Irrigation had significant effect on all the parameters except emergence m<sup>-2</sup> and days to emergence. Tillers plant<sup>-1</sup>, plant height, green fodder and dry fodder yields were significantly higher for double irrigation than single irrigation or no irrigation. Double irrigation given at either day had statistically similar tiller plant<sup>-1</sup>, plant height but its green fodder yield was maximum at irrigation given at 15 and 30 DAS and 15 and 45 DAS, while dry fodder yield was significantly greater at double irrigation given at 15 & 45 DAS. From these result it can be concluded that PARC MS-2 was highly fodder yielding variety, while double irrigation once at 15 DAS and other at 30 or 45 DAS for higher millet fodder yield seems feasible.

**Key Word:** Millet, irrigation, cultivars, plant height, green and dry fodder yield.

**Citation:** Saifullah, A. Jan, F. Munsif, M. Arif, H. Khan, K. Ali, M. Waqas and A. Ali. 2011. Performance of millet varieties under different irrigation levels. Sarhad J. Agric. 27(1): 1-7

### INTRODUCTION

Millet (*Pennisetum typhoidum* L.) is a summer annual forage crop consumed as a food as well as a fodder for livestock in the world and most important kharif fodder in Pakistan. It is high quality forage crop in the developed countries like USA and Australia, and considered as a new experimental forage crop in South America and Korea (Khairwal *et al.* 2007). It is being considered one of the most important fodder grown during summer season in Pakistan (Imran *et al.* 2007). It was grown on 593.3 thousand hectares with an annual production of 273.7 thousand tones, giving average green fodder yield of 508 kg per hectare. (Anonymous, 2003-04). In Pakistan at least 50% of the irrigated and 25% of the rainfed millet area is harvested exclusively for fodder before the grains are formed. The lower yield of millet in Pakistan is mainly due to lack of improved varieties and growing crop on marginal land and as rainfed crop because it is considered as hardy plant. Using improved varieties can increase three-fold green fodder and thus could feed double the number of animals per unit area compare to traditional fodder crops (Haqqani *et al.* 2003).

According to Andrews and Kumar (1992), millet has many advantages for cultivation due to its resistance to drought and heat and requires only a short growing season. Millet has great potential of developing to an excellent feed and forage crop cultivation (Andrew and Kumar, 1992) provided that attention is given to introduction of improved varieties, appropriate production technology including proper irrigation to increase yield per unit area. The potential advantage of millet is that it can be sown as late as June and hence may produce a satisfactory fodder yield during the time when fodder is in short supply particularly in period from mid-to-late summer (Chaudhry, 1994).

In order to meet the growing demand of green fodder for live-stock, it is essential to introduce high fodder yielding varieties of millet, as significant variation in yield of various varieties have been reported in literature. Cultivars vary considerably in their growth habits and yield (Chot *et al.*, 1988; Yrejewda, 1990; Akmal *et al.*, 1992; Naeem *et al.*, 1993). Byregowda (1990) observed a fresh fodder yield of 16 to 22 t ha<sup>-1</sup> comparing 13 millet genotypes. Naeem *et al.*, (2002) reported as high as 73 -82 tons ha<sup>-1</sup> fresh fodder yield in trail by evaluating nine millet varieties. This variation in yield is mainly because of plant height, number of

ATTESTED

40

## SEED PRIMING IN ZN SOLUTIONS ENHANCES EMERGENCE AND YIELD OF CHICKPEA

Muhammad Arif<sup>1</sup>, Muhammad Waqas<sup>1</sup>, Fazal Munsif<sup>2</sup>, Abid Ali<sup>3</sup>, Zahid Hussain<sup>4</sup>, Nasrullah Khan<sup>5</sup> and Abdul Samad<sup>2</sup>

<sup>1</sup>Department of Agronomy, Khyber Pakhtoon Khwan, Pakistan

<sup>2</sup>Agricultural Research Institute Tarnab, Peshawar, Khyber Pakhtoon Khwan, Pakistan

<sup>3</sup>Pakistan Tobacco Board Mardan, Khyber Pakhtoon Khwan, Pakistan

<sup>4</sup>Department of Weed Science, KPK Agricultural University, Peshawar, Khyber Pakhtoon Khwan, Pakistan

### ABSTRACT

In order to study the effect of zinc priming on chickpea, an experiment was conducted at Agricultural Research Farm of NWFP Agricultural University Peshawar during Rabi 2002-2003. The experiment was laid out in Randomized Complete Block design with four replications. The seeds of chickpea variety 'Karak-1' were primed in water as well as 0.05% and 0.075% Zn solutions. Dry seeds (non primed) were used as control treatment. A plot size of 15 m by 4 m with row to row distance of 30 cm was used. Analysis of the data indicated that seed priming with Zn significantly improved seeds emergence, grain yield and biological yield of chickpea. Seeds primed in 0.05% Zn solution resulted in highest emergence, grain and biological yields of chickpea. In conclusion it can be recommended that seed priming in Zn solutions does improve the seed emergence, grain yield and biological yield of chickpea crop.

**Keywords:** Chickpea, Zinc, Boron, seed priming, emergence, grain and biological yield.

### INTRODUCTION

Chickpea (*Cicer arietinum* L.), commonly known as gram, is the fifth most important legume crop in the world, after soybean, groundnut, dry bean and pea. It is the major pulse crop with respect to consumption and cultivated area in Pakistan. The annual production varied from 767.1 to 397 thousand tons due to fluctuation in its productivity during 1997-98 to 2000-01, respectively (Anonymous, 2001). Seed quality (viability and vigor) has got profound influence on the stand establishment and crop yield. Healthy plant with well developed root system can more effectively mobilize limiting nutrients from the soil and can better withstand adverse conditions (e.g. dry spells). Vigorous early seedling growth has been shown to be associated with higher yield (Harris *et al.*, 2000). The vigor of seeds can be improved by techniques generally known as seed priming, which enhances the speed and uniformity of germination (Heydecker *et al.*, 1975). Seed priming comprises the soaking of seed in water and drying back to the storage moisture until use. On-farm seed priming is a simple, low-cost, and risk-less technology that has been successful in improving emergence, seedling vigor and yield in a range of crops, including legumes (Harris *et al.*, 1999; 2001; Park *et al.*, 1999; Mussa *et al.*, 2001).

Crop production is also affected by soils deficient in P and Zn (Ryan, 1997). Especially during the early growth stages, lack of P and Zn retards seedling growth, rendering the young plantlets sensitive to the frequently encountered dry spells (Jones and Wahbi, 1992). Rapid establishment of healthy seedlings and an adequate supply of P and Zn are prerogative to reduce the risk of crop failure (Brown *et al.*, 1987). Nutrient priming has been proposed as a novel technique that combines the positive effects of seed priming with an improved nutrient supply (Al-Mударis and Jutzi, 1999). In nutrient priming, seeds are pretreated (primed) in solutions containing the deficient nutrients, instead of being soaked simply in water. This alternative approach involves soaking seeds in dilute solutions before sowing.

It is now well established that 'on-farm' seed priming with water alone is effective in substantially increasing yields of chickpea (Harris *et al.*, 1999; Mussa *et al.*, 2001) and wheat (Harris *et al.*, 2001) in South Asia. There are several advantages of using seed priming; micronutrients adequately supplied to the seeds, uneven application of zinc to the soil is avoided as each seed is exposed to the nutrient, uptake is guaranteed, and the amounts required are likely to be less in magnitude than that for soil application. Conversely, the risk of toxicity may be increased by priming. The instant research experiment was therefore conducted with the aim to evaluate the effect of seeds primed in Zn solutions on seeds emergence and yield of chickpea crop.

ATTESTED



**EFFECT OF VARIOUS TILLAGE METHODS AND NITROGEN MANAGEMENT ON WEEDS AND MAIZE PERFORMANCE**

**Kawsar Ali<sup>1</sup>, Shad Khan Khalil, Zahid Hussain<sup>2</sup>, Fazal Munsif<sup>1</sup>, Iftikhar ud din<sup>3</sup>, Muhammad Waqas<sup>1</sup> and Wagma<sup>4</sup>**

**ABSTRACT**

Weeds are the major source of yield loss in maize crop. To investigate the effect of different tillage practices and nitrogen (N) management techniques, an experiment was conducted at Agricultural Research Farm, Khyber Pakhtunkhwa Agricultural University Peshawar, Pakistan during summer 2010. The experiment was laid out in randomized complete block design with split plot arrangement having three replications. The experiment consisted of 10 fertilizer treatments i.e. control, sole nitrogen, farm yard manure (FYM), poultry manure (PM), 75% N (mineral) + 25% FYM, 50% mineral N + 50% FYM, 25 % mineral N + 75% FYM, 75% N + 25% PM, 50% mineral N + 50% PM, and 25% mineral N + 75% PM; and three tillage practices viz. reduced tillage (RT), conventional tillage (CT) and deep tillage (DT). Maize variety "Azam" was sown with a plot size of 4.5m x 5m for each experimental unit. Tillage practices were kept in main plots while N treatments were allotted to the sub plots. Tillage practices and N management significantly affected weed density, fresh and dry weed biomass and yield components of maize. Weed density and fresh and dry weed biomass were significantly higher for RT followed by CT and DT. Application of half mineral N and half PM produced the highest thousand grain weight, grain yield and biological yield of maize. In contrary, half mineral N + half PM resulted in lowest weed density, fresh weed biomass and dry weed biomass. Tillage also influenced yield and yield components of maize as highest thousand grain weight, grain yield and biological yield was produced by CT. It was concluded that management of organic and inorganic N application and CT has a beneficial effect on weed control and maize yield.

**Key words:** Maize, nitrogen, tillage, weeds, yield, *Zea mays* L.

**INTRODUCTION**

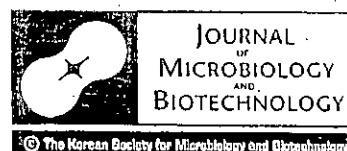
Maize (*Zea mays* L.) is a multipurpose crop that provides food for human and feed for animals especially poultry and livestock. It is a rich source of raw material for the industries where it is being extensively used for the preparation of dextrose, syrup and flakes

<sup>1</sup>Department of Agronomy, <sup>2</sup>Department of Weed Science, <sup>3</sup>Department of Statistics, Mathematics and Computer Science, <sup>4</sup>Department of Horticulture, Khyber Pakhtunkhwa Agricultural University Peshawar.  
Corresponding author: [krshah98@yahoo.com](mailto:krshah98@yahoo.com)

ATTESTED  
*[Signature]*

42

J. Microbiol. Biotechnol. (2011), 21(9), 893-902  
doi: 10.4014/jmb.1103.03012  
First published online 28 July 2011



## Salinity Stress Resistance Offered by Endophytic Fungal Interaction Between *Penicillium minioluteum* LHL09 and *Glycine max.* L

Khan, Abdul Latif<sup>1,2</sup>, Muhammad Hamayun<sup>3</sup>, Nadeem Ahmad<sup>4</sup>, Javid Hussain<sup>2,5</sup>, Sang-Mo Kang<sup>1</sup>, Yoon-Ha Kim<sup>1</sup>, Muhammad Adnan<sup>2</sup>, Dong-Sheng Tang<sup>6</sup>, Muhammad Waqas<sup>1</sup>, Ramalingam Radhakrishnan<sup>1</sup>, Young-Hyun Hwang<sup>1</sup>, and In-Jung Lee<sup>1\*</sup>

<sup>1</sup>School of Applied Biosciences, College of Agriculture and Life Sciences, Kyungpook National University, Daegu 701-702, Korea

<sup>2</sup>Kohat University of Science and Technology, Kohat, Pakistan

<sup>3</sup>Department of Botany, Abdul Wali Khan University, Mardan, Pakistan

<sup>4</sup>Department of Botany, Islamia College University, Peshawar, Pakistan

<sup>5</sup>Department of Biological Sciences and Chemistry, College of Arts and Sciences, University of Nizwa, Nizwa, Oman

<sup>6</sup>Key Laboratory of Agri-biodiversity and Pest Management, Yunnan Agricultural University, Kunming 650201, Yunnan, China

Received: March 7, 2011 / Revised: May 25, 2011 / Accepted: June 2, 2011

Endophytic fungi are little known for their role in gibberellins (GAs) synthesis and abiotic stress resistance in crop plants. We isolated 10 endophytes from the roots of field-grown soybean and screened their culture filtrates (CF) on the GAs biosynthesis mutant rice line - *Wairo-C*. CF bioassay showed that endophyte GMH-1B significantly promoted the growth of *Wairo-C* compared with controls. GMH-1B was identified as *Penicillium minioluteum* LHL09 on the basis of ITS regions rDNA sequence homology and phylogenetic analyses. GC/MS-SIM analysis of CF of *P. minioluteum* revealed the presence of bioactive GA<sub>1</sub> and GA<sub>2</sub>. In endophyte-soybean plant interaction, *P. minioluteum* association significantly promoted growth characteristics (shoot length, shoot fresh and dry biomasses, chlorophyll content, and leaf area) and nitrogen assimilation, with and without sodium chloride (NaCl)-induced salinity (70 and 140 mM) stress, as compared with control. Field-emission scanning electron microscopy showed active colonization of endophyte with host plants before and after stress treatments. In response to salinity stress, low endogenous abscisic acid and high salicylic acid accumulation in endophyte-associated plants elucidated the stress mitigation by *P. minioluteum*. The endophytic fungal symbiosis of *P. minioluteum* also increased the daidzein and genistein contents in the soybean as compared with control plants, under salt stress. Thus, *P. minioluteum* ameliorated the adverse effects of abiotic salinity stress and rescued soybean plant growth by influencing biosynthesis of the plant's hormones and flavonoids.

**Keywords:** *Penicillium minioluteum*, soybean plant growth, phytohormones, salt stress, daidzein, genistein

Endophytic fungi, either ascomycetes or basidiomycetes, live inside roots or tissues of the host plants without causing any disease symptoms or injury [4, 16, 33, 42]. These poorly known fungi represent a trove of unexplored biodiversity and are a frequently overlooked component of forest [5] and crop ecologies. In mutualistic mode, endophytic fungi increase plant fitness by dissuading herbivory and pathogenic attacks while also facilitating plant growth through nutrients uptake, water use efficiency, and curtailing of environmental stresses [6, 9, 38, 42, 52]. The endophytic fungi, in return, obtain access to the host plant's nutrients and dissemination to the next generation (e.g., members of Clavicipitaceous and Dikarya) [4, 24]. Endophytic fungi produce a wide array of biochemically important metabolites. Among these, endophytic fungi have been reported for synthesis of various plant hormones such as gibberellins (GAs) reported by Khan *et al.* [26, 27], Khan *et al.* [28], and Hamayun *et al.* [18, 19]. Currently, 136 GAs have been identified, and more than two dozen fungi, pathogenic and nonpathogenic, associated with plants and/or soil, have been reported as GAs producers [1, 7].

Salinity stress prevails throughout our agriculture lands affecting crops and their productivity. With the human population expanding, food demands have been at a sturdy rate and therefore, minimizing such stresses would be an urgent issue to resolve. Salt stress induces ionic and osmotic imbalance inside plant cells. Accumulation of attenued osmolytes transduces signals to aggregate phytohormones

\*Corresponding author

Phone: +82-53-950-5708; Fax: +82-53-958-6880;

E-mail: ijlee@knu.ac.kr

ATTESTED  
*[Signature]*

43

## *Exophiala* sp. LHL08 reprograms *Cucumis sativus* to higher growth under abiotic stresses

Abdul L. Khan<sup>a,b,†</sup>, Muhammad Hamayun<sup>c,†</sup>, Nadeem Ahmad<sup>d</sup>, Muhammad Waqas<sup>a</sup>, Sang-Mo Kang<sup>a</sup>, Yoon-Ha Kim<sup>a</sup> and In-Jung Lee<sup>a,\*</sup>

<sup>a</sup>School of Applied Biosciences, Kyungpook National University, Daegu, Republic of Korea  
<sup>b</sup>Department of Plant Sciences, Kohat University of Science & Technology, Kohat, Pakistan  
<sup>c</sup>Department of Botany, Abdul Wali Khan University, Mardan, Pakistan  
<sup>d</sup>Department of Botany, Islamia College University, Peshawar, Pakistan

Correspondence  
\*Corresponding author.  
e-mail: ijlee@knu.ac.kr

Received 25 April 2011;  
revised 23 June 2011

doi:10.1111/j.1399-3054.2011.01508.x

Endophytic fungi are potential sources of secondary metabolites; however, they are little known for phytohormones secretion and amelioration of plant growth under abiotic stresses. We isolated a novel endophyte from the roots of *Cucumis sativus* and identified it as a strain of *Exophiala* sp. by sequencing internal transcribed spacer/large subunit rDNA and phylogenetic analysis. Prior to identification, culture filtrate (CF) of *Exophiala* sp. has shown significant growth promotion of Waito-C (a gibberellins (GAs)-deficient mutant cultivar) and Dongjin-byeo (normal GAs biosynthesis cultivar) rice seedlings. CF analysis of *Exophiala* sp. showed the presence of physiologically active GAs (GA<sub>1</sub>, GA<sub>3</sub>, GA<sub>4</sub> and GA<sub>7</sub>) and inactive GAs (GA<sub>5</sub>, GA<sub>6</sub>, GA<sub>9</sub>, GA<sub>12</sub> and GA<sub>20</sub>). *Exophiala* sp. had higher GAs in its CF than wild-type strain of *Gibberella fujikuroi* except GA<sub>3</sub>. Influence of *Exophiala* sp. was assessed on cucumber plant's growth and endogenous abscisic acid (ABA), salicylic acid (SA) and bioactive GAs under salinity and drought stresses. *Exophiala* sp.-treated plants have shown significantly higher growth and rescued the host plants from stress promulgated water deficit, osmotic and cellular damage. The altered levels of stress-responsive ABA showed low level of stress confined in endophyte-applied plants than control. Elevated levels of SA and bioactive GAs (GA<sub>3</sub> and GA<sub>4</sub>) in endophyte-associated plants suggest stress-modulating response toward salinity and drought. In conclusion, symbiotic relations between *Exophiala* and cucumber have reprogrammed the host plant growth under abiotic stresses, thus indicating a possible threshold role of endophytic fungi in stress alleviation. This study could be extended for improving agricultural productivity under extreme environmental conditions.

**Abbreviations** – ABA, abscisic acid; BLASTN, basic local alignment search tool for nucleotide; CF, culture filtrate; DDW, double distilled water; DMRT, Duncan's multiple range test; EL, electrolytic leakage; GAs, gibberellins; GC/MS SIM, gas chromatography/mass spectrophotometry with selected ion monitoring; HPLC, high performance liquid chromatography; ITS, internal transcribed spacer; LSU, large subunit; MP, maximum parsimony; PDA, potato dextrose agar; PEG, polyethylene glycol; RWC, relative water content; SA, salicylic acid; SDW, shoot dry weight; SFW, shoot fresh weight.

<sup>†</sup>These authors contributed equally to the work.

ATTESTED

44

Article

## Endophytic Fungi Produce Gibberellins and Indoleacetic Acid and Promotes Host-Plant Growth during Stress

Muhammad Waqas <sup>1,2,†</sup>, Abdul Latif Khan <sup>1,3,†</sup>, Muhammad Kamran <sup>1</sup>, Muhammad Hamayun <sup>4</sup>, Sang-Mo Kang <sup>1</sup>, Yoon-Ha Kim <sup>1</sup> and In-Jung Lee <sup>1,\*</sup>

<sup>1</sup> School of Applied Biosciences, College of Agriculture and Life Sciences, Kyungpook National University, Daegu 702-701, Korea; E-Mails: agronomist89@gmail.com (M.W.); latifepm78@yahoo.co.uk (A.L.K.); m.kamran60@gmail.com (M.K.); kmoya@daum.net (S.-M.K.); kimyoonha@gmail.com (Y.-H.K.)

<sup>2</sup> Department of Agriculture Extension, Government of Khyber Pakhtunkhwa, Bunir 19290, Pakistan

<sup>3</sup> Department of Botany, Kohat University of Science and Technology, Kohat 26000, Pakistan

<sup>4</sup> Department of Botany, Abdul Wali Khan University, Mardan 23300, Pakistan; E-Mail: hamayun@awkum.edu.pk

† These authors contributed equally to this work.

\* Author to whom correspondence should be addressed; E-Mail: ijlee@knu.ac.kr; Tel.: +82-539-505-708; Fax: +82-539-586-880.

Received: 1 August 2012; in revised form: 17 August 2012 / Accepted: 3 September 2012 /

Published: 7 September 2012

**Abstract:** We isolated and examined two endophytic fungi for their potential to secrete phytohormones viz. gibberellins (GAs) and indoleacetic acid (IAA) and mitigate abiotic stresses like salinity and drought. The endophytic fungi *Phoma glomerata* LWL2 and *Penicillium* sp. LWL3 significantly promoted the shoot and allied growth attributes of GAs-deficient dwarf mutant *Waiteo-C* and *Dongjin-beyo* rice. Analysis of the pure cultures of these endophytic fungi showed biologically active GAs (GA<sub>1</sub>, GA<sub>3</sub>, GA<sub>4</sub> and GA<sub>7</sub>) in various quantities. The cultures of *P. glomerata* and *Penicillium* sp. also contained IAA. The culture application and endophytic-association with host-cucumber plants significantly increased the plant biomass and related growth parameters under sodium chloride and polyethylene glycol induced salinity and drought stress as compared to control plants. The endophytic symbiosis resulted in significantly higher assimilation of essential nutrients like potassium, calcium and magnesium as compared to control plants during salinity stress. Endophytic-association reduced the sodium toxicity and promoted the host-benefit ratio in

ATTESTED



45

Full Length Research Paper

## Assessment of endophytic fungi cultural filtrate on soybean seed germination

Muhammad Waqas<sup>1,2</sup>, Abdul Latif Khan<sup>1,3</sup>, Muhammad Hamayun<sup>1,4</sup>, Muhammad Kamran<sup>1,3</sup>, Sang-Mo Kang<sup>1</sup>, Yoon-Ha Kim<sup>1</sup> and In-Jung Lee<sup>1\*</sup>

<sup>1</sup>School of Applied Biosciences, Kyungpook National University, Daegu, Republic of Korea.

<sup>2</sup>Department of Agriculture Extension, Bunir, Pakistan.

<sup>3</sup>Department of Botany, Kohat University of Science and Technology, Kohat, Pakistan.

<sup>4</sup>Department of Botany, Abdul Wali Khan University, Mardan, Pakistan.

Accepted 22 June, 2012

Soybean seeds have high amount of isoflavones but its germination is often confronted with a variety of environmental problems resulting in low germination rate and growth. To overcome this in eco-friendly manner, we investigated the influence of cultural filtrate (CF) of gibberellins-producing endophytic fungi on soybean seed germination. Three endophytic fungi namely: *Chrysosporium pseudomerdarium*, *Aspergillus fumigatus* and *Paecilomyces* sp. were previously isolated from the roots of soybean plants. The culture filtrate application of the three endophyte resulted in significantly higher rate of soybean seed germination, germination percentage, relative seed germination percentage, peak value, germination value, shoot and root length, germination index and vigour index. Among the endophytes, *A. fumigatus* significantly increased the rate of germination, shoot and root length and vigour index. Same trend was noted in germination percentage and relative seed germination percentage for all the endophytic fungi. However, *C. pseudomerdarium* was the only one that enhanced germination index. The enhanced soybean seed germination by endophytes can be used for seed priming and hence improved crop plant growth under extreme environmental conditions.

**Key words:** *Chrysosporium pseudomerdarium*, *Aspergillus fumigatus*, *Paecilomyces* sp., soybean, seed germination.

### INTRODUCTION

Soybean (*Glycine max* L. Merr.) is considered as an important source of vegetable protein and oil (Moussa, 2004). High yield in annual crops depends on rapid and uniform field emergence of their seeds (Parera and Cantliffe, 1994; Subedi and Ma, 2005; Yari et al., 2010). Germination of seed start with the uptake of water by the inactive dry seed and cease with the protrusion of embryonic axis and sign of radical formation (Bewley, 1997). In agricultural ecosystem, early seed germination

set trend for seedling performance and ultimately proper plant establishment (Weitbrecht et al., 2011). To obtain better seeds germination and afterward improved plant growth, studies have elucidated the seed priming with plant growth regulators as reported by Wen et al. (2009) and Weihong (2004). There are some studies suggesting the use of plant hormones such as Gibberellins for seed priming (Wallace and Elizabeth, 2000; Debeaujon and Koornneef, 2000; Grappin et al., 2000). Among phytohormones, gibberellic acid (GA) are mostly responsible for cell division and elongation, activation of embryo, weakening of endosperm layer and mobilization of endosperm food reserves are its prominent action. In most species during seed development, GA biosynthesis results in build up and storage of bioactive GAs or precursors of inactive GAs (Groot and Karssen, 1987; Toyomasu et al., 1998; Kamiya and Garcia-

\*Corresponding author. E-mail: [ijlee@knu.ac.kr](mailto:ijlee@knu.ac.kr). Tel: + 82-53-950-5708. Fax: + 82-53-958-6880.

Abbreviation: CF, Cultural filtrate.

ATTESTED  
4

ORIGINAL PAPER

## Mutualistic association of *Paecilomyces formosus* LHL10 offers thermotolerance to *Cucumis sativus*

Abdul Latif Khan · Muhammad Hamayun · Ramalingam Radhakrishnan · Muhammad Waqas · Sang-Mo Kang · Yoon-Ha Kim · Jae-Ho Shin · Yeon-Sik Choo · Jong-Guk Kim · In-Jung Lee

Received: 25 May 2011 / Accepted: 17 August 2011  
© Springer Science+Business Media B.V. 2011

**Abstract** We investigated in this study the influence of an endophytic fungus, *Paecilomyces formosus* LHL10, on the thermotolerance of cucumber (*Cucumis sativus*) upon exposure to high (38°C) and low (8°C) temperature stresses. The results showed that endophyte-inoculated plants had significantly higher plant growth attributes under high-temperature stress. However, they were either low or insignificant in

non-inoculated control and inoculated plants with 8°C treatments. Lower stress-promulgated water deficit and cellular membrane damage were observed in endophyte-treated plants after 38°C treatment than in control plants under 8°C stress. Total polyphenol, reduced glutathione, and lipid peroxidation activities were reduced in endophyte-associated plants after exposure to 38°C as compared with control and 8°C-treated plants. The concentration of saturated fatty acids (palmitic-C16:0; stearic-C18:0) was lower in endophyte-treated plants with or without low-temperature stress, but after 8°C treatment increased compared with controls. Unsaturated fatty acids (oleic-C18:1; linoleic-C18:2; linolenic-C18:3 acids) were similar at normal conditions; however, at 38°C, C18:2 and C18:3 were decreased, and C18:1 was increased in endophyte-treated plants compared with controls, while the inverse relationship was found at 8°C. Low levels of abscisic acid in *P. formosus*-associated plants after 38°C treatments revealed stress tolerance compared with control and 8°C-treated plants. In contrast, salicylic acid was pronounced in endophyte-treated plants after low-temperature stress as compared to other treatments. The results provide evidence that the response to *P. formosus* association was beneficial at normal growth temperature and had varying effects in response to temperature stress.

A. L. Khan · R. Radhakrishnan · M. Waqas · S.-M. Kang · Y.-H. Kim · J.-H. Shin · I.-J. Lee (✉)  
School of Applied Biosciences, Kyungpook National University, Daegu 702-701, Republic of Korea  
e-mail: ijlee@knu.ac.kr

A. L. Khan  
Kohat University of Science and Technology, Kohat, Pakistan

M. Hamayun  
Department of Botany, Abdul Wali Khan University, Mardan, Pakistan

Y.-S. Choo  
Department of Biology, Kyungpook National University, Daegu, Republic of Korea

J.-G. Kim  
Department of Life Sciences and Biotechnology, Kyungpook National University, Daegu, Republic of Korea

**Keywords** *Paecilomyces formosus* LHL10 · Thermotolerance · Cucumber plant growth · Abscisic acid and salicylic acid · Fatty acids and antioxidants

ATTESTED  
[Signature]



### SILICON TREATMENT TO RICE (*ORYZA SATIVA* L. cv 'GOPUMBYEO') PLANTS DURING DIFFERENT GROWTH PERIODS AND ITS EFFECTS ON GROWTH AND GRAIN YIELD

YOON-HA KIM<sup>1</sup>, ABDUL LATIF KHAN<sup>1,2</sup>, ZABTA KHAN SHINWARI<sup>1</sup>, DUCK-HWAN KIM<sup>1</sup>, MUHAMMAD WAQAS<sup>1</sup>, MUHAMMAD KAMRAN<sup>1</sup> AND IN-JUNG LEE<sup>1\*</sup>

<sup>1</sup>School of Applied Biosciences, Kyungpook National University, Daegu, 702-701, Korea

<sup>2</sup>Department of Botany, Kohat University of Science and Technology, Kohat Pakistan

<sup>3</sup>Department of Biotechnology, Qaid-e-Azam University, Islamabad, Pakistan

\*Corresponding author: e-mail: ijlee@knu.ac.kr

#### Abstract

Silicon (Si) has been considered a beneficial element for plant growth. We have assessed the effects of Si application on rice (*Oryza sativa* L.) growth and its grain yield at field level. For this, we performed two experiments. In experiment 1, we applied Si of three different concentrations (liquid Si-10, 25 and 36%) to the seedbed of rice before transplantation into paddy field. The results of this experiment showed that Si application to rice seedbeds did not affect the rice plant height and shoot fresh weight but its application significantly increased the pushing resistance of rice plants from 12.2~16.7% as compared with water applied control plants. The lodging index of Si treated rice plants significantly decreased (13.7% on LS-25) as compared with control. Similarly, Si treated plants had significantly higher yield. Upon Si treatment (LS-36), the grain yield per 10 acre and panicles per plant were 15.1% and 6.3% higher than the water treated control plants respectively. The best concentration (LS-36%) revealed in the first experiment was foliar applied at 10 days before heading stage, initial tillering stage and panicle initiation stage to the rice leaves and we observed that shoot biomass was not significantly different between control and Si treated plants. However, significantly higher pushing resistance (10.5%~13.8%) and plant height (12.2%~16.7%) were observed while lower lodging index (7.6~7.8%) was recorded for Si treated plants as compared to control plants. Similarly, Si application increased the number of panicles per plant as well as the grain yield per 10 acre as compared to control. In conclusion, the Si application can significantly regulate plant growth and yield if applied at proper time with feasible concentration.

#### Introduction

In Asia, rice is one of the three most important food crops. Especially, in the vast monsoonal areas of tropical Asia, rice gives the highest food-staple cereal yield from a fixed land area of arable land (Datta, 1986; Köster *et al.*, 2009). Rice (*Oryza sativa* L.) is classified a typical monocotyledon plant and also separated as semi-aquatic plant because of its growing at both the seedling and adult stages (Kende *et al.*, 1998). Rice is known to accumulate high amount of silicon approximately 10% of its dry weight (Epstein, 1994, 1999). Silicon (Si) is second abundant element in soil and is an essential element for animals and has been implicated in optimal bone and connective tissue growth and development in the human body (Mitani *et al.*, 2008). In higher plants, Si is consumed from the rhizosphere through root in the form of Si(OH)<sub>4</sub> and SiO<sub>2</sub> while its uptake and accumulation varies greatly among different plant species (Kim *et al.*, 2011; Parveen & Hussain, 2008; Takahashi *et al.*, 1990). Through recent studies, uptake and transport of Si in plants have revealed by two ways i.e., (i) from apoplast into symplast and (ii) loading of Si into xylem (Hattori *et al.*, 2008). Rice has both types of transporter, and their high activity allows rice to take up considerable amounts of silicon from soil solution (Hattori *et al.*, 2008; Mitani & Ma, 2005). After Si uptake in plants, Si has various physiological actions to plants in its regulation of biotic and abiotic stresses.

Numerous studies have elucidated its beneficial effects on plant growth and development under biotic (diseased and pests) and abiotic stresses (salinity, metal toxicity, drought, radiation damage, nutrient imbalances,

high temperature, freezing, etc.) conditions (Kim *et al.*, 2011). Si has many effect which Si improve cell wall thickness below the cuticle and it also improves leaf angle, making leaves more erect, thus reducing self-shading, especially under high nitrogen rate (Mauad *et al.*, 2003). Si treatment has acted as effective improvements of leaf water status by decreasing excess transpiration via modification of stomatal action and reduction in cuticular transpiration during abiotic stress conditions (Agarie *et al.*, 1999; Hattori *et al.*, 2008; Matoh *et al.*, 1991). The beneficial effect of Si application other than rice plants has also been reported. Studies have revealed that Si is a beneficial element to higher plants, particularly for grasses and various cultivated crops like rice, wheat, tomato, cucumber (Ahmed *et al.*, 2011; Chen *et al.*, 2011; Epstein, 1994, 1999; Hamayun *et al.*, 2010; Hattori *et al.*, 2005; Kim *et al.*, 2011; Liang *et al.*, 1996, 1999, 2002; Parveen & Ashraf, 2010). In last decade or so, various studies have revealed the mitigating role of Si under various biotic (plant diseases and pests) and abiotic stresses (heavy metals, drought and salinity) to crop plants (Ahmed *et al.*, 2011; Chen *et al.*, 2011; Epstein, 1994, 1999; Hamayun *et al.*, 2010; Hattori *et al.*, 2005; Liang *et al.*, 1996, 1999, 2002; Parveen & Ashraf, 2010). In mitigation of abiotic stress, according to many research reports, Si has beneficial effects to be applied during cultivation of rice (Ando *et al.*, 2002; Savant *et al.*, 1997; Yoshida *et al.*, 1962). However, few reports also suggest that applying Si-containing fertilizers to paddy fields can suppress both leaf and panicle blast in irrigated rice (Dainoff *et al.*, 1991; Seebold *et al.*, 2000). Further studies are needed at field levels to elucidate the effects on the economically important agronomics. The effects of

ATTESTED  
*[Signature]*

48

## *Exophiala* sp.LHL08 association gives heat stress tolerance by avoiding oxidative damage to cucumber plants

Abdul Latif Khan · Muhammad Hamayun ·  
Muhammad Waqas · Sang-Mo Kang · Yoon-Ha Kim ·  
Duk-Hwan Kim · In-Jung Lee

Received: 18 August 2011 / Revised: 4 December 2011 / Accepted: 8 December 2011 / Published online: 21 December 2011  
© Springer-Verlag 2011

**Abstract** *Exophiala* sp. LHL08, a gibberellin-producing strain, was investigated to assess its effects on cucumber plant growth and heat (40°C) stress tolerance. The results reveal that *Exophiala* sp. associated plants had significantly higher plant growth attributes (shoot length, plant biomass, chlorophyll contents, and leaf area) than control under heat stress. Endophytic association helped the plants to obtain adequate water to reduce the leaf electrolytic leakage under stress. High-temperature-induced oxidative stress was less pronounced in *Exophiala* sp. associated plants as shown by enhanced levels of total polyphenol and reduced activities of glutathione, superoxide anion, and lipid peroxidation. To tolerate heat stress and rescue plant growth, the endophyte association significantly increased catalase and peroxidase activities of the host plants as compared to control plants. Contents of palmitic, stearic, oleic, and  $\alpha$ -linolenic were significantly decreased in the *Exophiala* sp.-inoculated plants than control plants under heat stress. Contents of flavonoids like genistein and daidzein were produced in

higher quantities, while glycitein content was almost same in endophyte-associated plants under heat stress than control plants. Contrarily, stress-responsive endogenous abscisic acid and jasmonic acid were significantly activated in non-inoculated control treatments as compared to endophyte-inoculated plants under heat stress. The findings of the study reveal that association of *Exophiala* sp. with cucumber host plants can modulate heat stress by influencing physiological and biochemical contents of plants under heat stress.

**Keywords** *Exophiala* sp.LHL08 · Heat stress · Phytohormones · Isoflavonoids · Fatty acids · Oxidative stress

### Introduction

Crop yield is often limited by extreme environmental conditions. Both high and low temperature can bring changes ranging from ionic/osmotic levels to phytohormonal and secondary metabolites (Wahid et al. 2007). Heat stress can (1) damage mitochondria and chloroplasts, (2) reduce water use efficiency, (3) decrease photosynthesis, and (4) decrease nutrients uptake while disrupting cellular structures (Wahid et al. 2007). High temperature can induce the synthesis of reactive oxygen species (ROS) such as superoxide radical, hydrogen peroxide, hydroxyl radical, etc. Reactive oxygen species can be a signaling molecule, but their uncontrolled production can negatively affect the cellular apparatus (Im et al. 2009; Zinn et al. 2010). To avoid oxidative damage, plants adapt de novo synthesis of compatible solutes acting as ROS scavenger. These scavengers include reduced glutathione (GSH), malondialdehyde (MDA), total polyphenols, catalase (CAT), peroxidase (POD), polyphenol oxidase (PPO), etc. These scavengers stabilize subcellular structures and buffers cellular redox potential under stress conditions (Wahid et al.

**Electronic supplementary material** The online version of this article (doi:10.1007/s00374-011-0649-y) contains supplementary material, which is available to authorized users.

A. L. Khan · M. Waqas · S.-M. Kang · Y.-H. Kim · D.-H. Kim ·  
I.-J. Lee (✉)  
School of Applied Biosciences, Kyungpook National University,  
Daegu 702-701, Republic of Korea  
e-mail: ijlee@knu.ac.kr

A. L. Khan  
Department of Plant Sciences,  
Kohat University of Science & Technology,  
Kohat, Pakistan

M. Hamayun  
Department of Botany, Abdul Wali Khan University Mardan,  
Mardan, Pakistan

ATTESTED  
*[Signature]*

### ROLE OF ENDOPHYTE *CHAETOMIUM GLOBOSUM* LK4 IN GROWTH OF *CAPSICUM ANNUUM* BY PRODUCTION OF GIBBERELLINS AND INDOLE ACETIC ACID

ABDUL LATIF KHAN<sup>1,2</sup>, ZABTA KHAN SHINWARI<sup>3</sup>, YOON-HA KIM<sup>1</sup>, MUHAMMAD WAQAS<sup>1</sup>, MUHAMMAD HAMAYUN<sup>4</sup>, MUHAMMAD KAMRAN<sup>1</sup> AND IN-JUNG LEE<sup>1\*</sup>

<sup>1</sup>School of Applied Biosciences, Kyungpook National University, Daegu, Republic of Korea

<sup>2</sup>Department of Botany, Kohat University of Science & Technology, Kohat Pakistan

<sup>3</sup>Department of Biotechnology, Quaid-e-Azam University, Islamabad Pakistan

<sup>4</sup>Department of Botany, Abdul Wali Khan University, Mardan, Pakistan

\*Corresponding author's e-mail: ijlee@knu.ac.kr; Tel: + 82-53-950-5708; Fax: + 82-53-958-6880

#### Abstract

Endophytic fungi represent a trove of unexplored natural source of plant hormones like gibberellins (GAs) and indoleacetic acid (IAA). In present study, we isolated eight endophytes from the roots of drought stressed pepper (*Capsicum annuum* L.) plants. To assess phytohormones secreting potential, culture filtrates (CF) of endophytes were screened on GAs biosynthesis mutant *Wairo-C* rice. Endophyte CAC-1G significantly promoted the shoot growth, chlorophyll content and biomass of *Wairo-C* rice seedlings as compared with CF of *Fusarium fujikuroi* and distilled water. CAC-1G was identified as strain of *Chaetomium globosum* LK4 by sequencing internal transcribed spacer regions and phylogenetic analysis of similar sequences. The CF analysis of *C. globosum* showed the presence of GAs (GA<sub>1</sub> 0.67±0.13 ng/ml; GA<sub>4</sub> 21.8±1.2 ng/ml; GA<sub>9</sub> 0.51±0.11 ng/ml; GA<sub>12</sub> 13.4±0.41 ng/ml; GA<sub>20</sub> 1.11±0.2 ng/ml) and IAA (16.71±1.42 µg/ml). The CF of *C. globosum* had higher GA<sub>4</sub>, GA<sub>12</sub> and GA<sub>20</sub> than the CF of *F. fujikuroi*. The CF containing propagules of *C. globosum* was applied to the host-pepper plants. The results revealed significantly higher shoot growth, chlorophyll content, plant biomass and leaf area as compared to fungal-free medium and water applied plants. The present results of *C. globosum* can be reciprocated for improved plant growth and yield at field levels.

#### Introduction

Endophytic fungi live asymptotically within plant tissues have been found in almost all plant species (Saikkonen *et al.*, 1998; Schulz & Boyle, 2005). These poorly known fungi represent a trove of unexplored biodiversity, and a frequently overlooked component of forest (Reinhardt, 2007; Arnold, 2008) and crop ecology (Khan *et al.*, 2011a). The endophyte-host interaction is mutualistic or neutral and may differ among hosts and on the basis of environmental conditions (Saikkonen *et al.*, 1998; Faeth & Fagan, 2002). Endophytic fungi draw three basic benefits from the host plants: nourishment, physical protection and adversities reproduction e.g. members of Clavicipitaceus and Dikarya (Hyde & Soyong, 2008). In return, the host plant is benefited by the endophyte through production of metabolites [(e.g. alkaloids, antibiotics, or toxins, growth regulators (Schulz & Boyle 2005, Khan *et al.*, 2011a)], nutrient composition inside tissues, plant hormonal balance, chemical composition of root exudates, physical modification of soil, disease resistance and protection against external calamities (Waller *et al.*, 2005; Rahman & Saiga, 2005; Oses *et al.*, 2008).

These endophytes have been found as a novel source of various kinds of bioactive secondary metabolites (Schulz *et al.*, 2002). However, there are few reports available about the endophytes secreting phytohormones like gibberellins (GAs), auxin etc. Previously, some endophytic fungal strains were reported to produce a variety of physiologically active and inactive GAs. This includes: *Fusarium fujikuroi*, *Sphaeloma manihoticola* (Bomke *et al.*, 2008) *Phaeosphaeria* sp. L487 (Kavvaide, 2006), *Phaeosphaeria* sp., *Neurospora crassa* (Rademacher 1994), *Sesamum indicum* (Choi *et al.*, 2005), *Cladosporium* sp. MH-6

(Hamayun *et al.*, 2010), *Aspergillus fumigatus* (Khan *et al.*, 2011a) *Penicillium funiculosum* (Khan *et al.*, 2011b), *Exophiala* sp. LHL08 (Khan *et al.*, 2011c), and *Curvularia protuberata* etc. These phytohormones producing endophytes have been also reported to play essential role in crop plant growth and metabolism. However, there is little information available on endophytes isolated from extreme environmental conditions.

Chilli pepper (*Capsicum annuum* L.) is an important vegetable as well as spice crop, used worldwide for domestic and commercial purposes. They are rich source of antioxidants, vitamin C, pro-vitamin A, E, and B (Bosland & Votava, 1999). Pepper is regarded as a sensitive to salinity and drought (Kanber *et al.*, 1992). With expanding human population, food demands have been at sturdy rate and therefore, maintaining plant growth is crucial for crop yield. Symbiosis of such endophytic fungi offers advantages to host plants in transport and assimilation biochemicals necessary for plant growth and counteract biotic and abiotic stresses (Schulz & Boyle, 2005; Waller *et al.*, 2005; Reinhardt, 2007; Khan *et al.*, 2011abc; Davitt *et al.*, 2011). Previously, three different endophytic fungal strains (*Aspergillus favus*, *Coniothyrium* sp., and *Nigrospora* sp.) were isolated from pepper plant which improved plant growth and protected plants against pathogenic attack. However, we failed to find any report of phytohormones producing endophytic fungi from the isolated from pepper plants. GA-producing fungal endophytes might have potential to increase crop yields due to increasing concern about the excessive use of fertilizers in agricultural and the subsequent negative effect on the environment. In present work, we aimed to isolate phytohormones producing bioactive endophytic fungal strain from the roots of drought stressed pepper plants. We screened the

ATTESTED  
*[Signature]*

50

Molecules 2012, 17, 7980-7988; doi:10.3390/molecules17077980

**molecules**

ISSN 1420-3049

www.mdpi.com/journal/molecules

Communication

## Rhizonin A from *Burkholderia* sp. KCTC11096 and Its Growth Promoting Role in Lettuce Seed Germination

Sang-Mo Kang <sup>1,†</sup>, Abdul Latif Khan <sup>1,2,†</sup>, Javid Hussain <sup>3,4</sup>, Liaqat Ali <sup>3</sup>, Muhammad Kamran <sup>1</sup>, Muhammad Waqas <sup>1</sup> and In-Jung Lee <sup>1,\*</sup>

<sup>1</sup> School of Applied Biosciences, Kyungpook National University, Daegu 702-701, Korea; E-Mails: kmoya@hanmail.net (S.-M.K.); latifepm78@yahoo.co.uk (A.L.K.); m.kamran60@gmail.com (M.K.); agronomist89@gmail.com (M.W.)

<sup>2</sup> Department of Botany, Kohat University of Science and Technology, Kohat 26000, Pakistan

<sup>3</sup> Department of Biological Sciences and Chemistry, College of Arts and Sciences, University of Nizwa, Nizwa 33, Oman; E-Mails: javidhej@unizwa.edu.com (J.H.); malikhejric@gmail.com (L.A.)

<sup>4</sup> Department of Chemistry, Kohat University of Science and Technology, Kohat 26000, Pakistan

<sup>†</sup> These authors contributed equally to this work.

\* Author to whom correspondence should be addressed; E-Mail: ijlee@knu.ac.kr; Tel.: +82-53-950-5708; Fax: +82-53-958-6880.

Received: 19 May 2012; in revised form: 19 June 2012 / Accepted: 28 June 2012 / Published: 3 July 2012

**Abstract:** We isolated and identified a gibberellin-producing *Burkholderia* sp. KCTC 11096 from agricultural field soils. The culture filtrate of plant growth promoting rhizobacteria (PGPR) significantly increased the germination and growth of lettuce and Chinese cabbage seeds. The ethyl acetate extract of the PGPR culture showed significantly higher rate of lettuce seed germination and growth as compared to the distilled water treated control. The ethyl acetate fraction of the *Burkholderia* sp. was subjected to bioassay-guided isolation and we obtained for the first time from a *Burkholderia* sp. the plant growth promoting compound rhizonin A (**1**), which was characterized through NMR and MS techniques. Application of various concentrations of **1** significantly promoted the lettuce seed germination as compared to control.

**Keyword:** *Burkholderia* sp. KCTC11096; rhizonin A; growth promotion; lettuce seed

ATTESTED  
*[Signature]*

(51)

Molecules 2013, 18, 15519-15530; doi:10.3390/molecules181215519

**molecules**

ISSN 1420-3049

www.mdpi.com/journal/molecules

Communication

## Seed Germination-Influencing Bioactive Secondary Metabolites Secreted by the Endophyte *Cladosporium cladosporioides* LWL5

Muhammad Waqas <sup>1,2,†</sup>, Abdul Latif Khan <sup>1,3,†</sup>, Liaqat Ali <sup>3</sup>, Sang-Mo Kang <sup>1</sup>, Yoon-Ha Kim <sup>1,4</sup> and In-Jung Lee <sup>1,\*</sup>

<sup>1</sup> School of Applied Biosciences, College of Agriculture and Life Sciences, Kyungpook National University, Daegu 702-701, Korea; E-Mails: agronomist89@gmail.com (M.W.); latifepm78@yahoo.co.uk (A.L.K.); kmoya@daum.net (S.-M.K.); kimyoonha7979@gmail.com (Y.-H.K.)

<sup>2</sup> Department of Agriculture Extension, Government of Khyber Pakhtunkhwa, Bunir 19290, Pakistan

<sup>3</sup> Department of Biological Sciences and Chemistry, College of Arts & Sciences, University of Nizwa, Nizwa 33, Oman; E-Mail: malikhejric@unizwa.edu.om (L.A.)

<sup>4</sup> International Agricultural Training Center, Kyungpook National University, Daegu 702-701, Korea

† These authors contributed equally to the work.

\* Author to whom correspondence should be addressed; E-Mail: ijlee@knu.ac.kr; Tel.: +82-539-505-708; Fax: +82-539-586-880.

Received: 8 October 2013; in revised form: 7 December 2013 / Accepted: 9 December 2013 / Published: 13 December 2013

**Abstract:** The present study was aimed to isolate bioactive metabolites produced by a fungal endophyte from *Helianthus annuus*, *Capsicum annuum*, and *Cucumis sativus* and to assess their role in seed germination. Culture filtrate of the endophyte HA-3B from *H. annuus* was significantly inhibitory towards the germination and growth of lettuce seeds. HA-3B was identified as *Cladosporium cladosporioides* LWL5 through molecular techniques. Different concentrations (100, 500 and 1000 ppm) of the ethyl acetate extract obtained from the culture inhibited the lettuce seed germination. The extract was subjected to column chromatography and a bioassay-guided isolation method, which yielded compounds **1**, **2** and an oily fraction. The oily fraction, subjected to fractionation and spectroscopic techniques, resulted in the identification of 31 different constituents. Compounds **1** and **2** were identified and characterized through MS and NMR spectroscopic techniques as benzoic acid. The bioassay results showed that this compound significantly inhibited the growth and germination of lettuce seeds. In conclusion, assessing the role of

ATTESTED  
*[Signature]*

## Fungal endophyte *Penicillium janthinellum* LK5 improves growth of ABA-deficient tomato under salinity

Abdul Latif Khan · Muhammad Waqas · Abdur Rahim Khan · Javid Hussain · Sang-Mo Kang · Syed Abdullah Gilani · Muhammad Hamayun · Jae-Ho Shin · Muhammad Kamran · Ahmed Al-Harrasi · Byung-Wook Yun · Muhammad Adnan · In-Jung Lee

Received: 20 November 2012 / Accepted: 13 May 2013  
© Springer Science+Business Media Dordrecht 2013

**Abstract** An endophytic fungus was isolated from the roots of tomato (*Solanum lycopersicum* Mill) and identified as *Penicillium janthinellum* LK5. The culture filtrate (CF) of *P. janthinellum* significantly increased the shoot length of gibberellins (GAs) deficient mutant *waito-c* and normal Dongjin-beyo rice seedlings as compared to control. The CF of *P. janthinellum* contained GAs (GA<sub>3</sub>, GA<sub>4</sub>, GA<sub>7</sub> and GA<sub>12</sub>). To assess endophyte-growth promoting and stress-tolerance potential, the CF along with the propagules of endophyte was applied to tomato-host and abscisic acid (ABA)-deficient mutant *Sitiens* plants under sodium chloride (NaCl) induced salinity stress. *Sitiens* plants had retarded growth under normal and salinity stress however its growth was much improved during *P. janthinellum*-association. The endophyte inoculation reduced the membrane injury by decreasing lipid peroxidation as compared

to non-inoculated control under salinity. Endophyte-associated *Sitiens* plants have significantly higher catalase, peroxidase and glutathione activities as compared to control. Endophyte-infected host and *Sitiens* plants had low level of sodium ion toxicity and high calcium contents in its root as compared to control. *P. janthinellum* LK5 helped the *Sitiens* plants to synthesis significantly higher ABA and reduced the level of jasmonic acid to modulate stress responses. The results suggest that endophytes-association can resist salinity stress by producing gibberellins and activating defensive mechanisms of host and *Sitiens* plants to achieve improved growth.

**Keywords** *Solanum lycopersicum* · *Sitiens* · Endophytism · Abscisic acid · Salinity

**Electronic supplementary material** The online version of this article (doi:10.1007/s11274-013-1378-1) contains supplementary material, which is available to authorized users.

A. L. Khan · J. Hussain · S. A. Gilani · A. Al-Harrasi  
Department of Biological Sciences and Chemistry,  
University of Nizwa, Nizwa, Oman

A. L. Khan · J. Hussain · M. Adnan  
Department of Botany, Kohat University of Science and  
Technology, Kohat, Pakistan

M. Waqas · A. R. Khan · S.-M. Kang · J.-H. Shin · M. Kamran ·  
B.-W. Yun · I.-J. Lee (✉)  
School of Applied Biosciences, Kyungpook National University,  
Daegu 702-701, Republic of Korea  
e-mail: ijlee@knu.ac.kr

M. Hamayun  
Department of Botany, Abdul Wali Khan University,  
Mardan, Pakistan

Published online: 11 July 2013

### Introduction

Salinity has devastating effects on crop plant's growth and yields. An estimated 20 % of world arable and approximately half of the irrigated agricultural lands are affected by salinity (Sairam and Tyagi 2004). Salinity causes production of various reactive oxygen species (ROS) like hydrogen peroxide and hydroxyl radicals etc. which can negatively affect the cellular functions. To counteract ROS production, plant adopts a defensive strategy by recruiting various antioxidants and related enzymes to minimize cellular toxicity (Mittler et al. 2004). Reduced glutathione (GSH), tocopherols, lipid peroxidation like melonodialdehyde (MDA), peroxidase (POD), catalase (CAT) and polyphenol oxidase (PPO) etc. can play an essential role in eradicating radicals from cell and convert them into stable byproducts (Mittler et al. 2004). Furthermore, the plants respond to stress by signal transductions of plant hormones

ATTESTED  
[Signature]

S3

RESEARCH ARTICLE Open Access

# Co-synergism of endophyte *Penicillium resedanum* LK6 with salicylic acid helped *Capsicum annuum* in biomass recovery and osmotic stress mitigation

Abdul Latif Khan<sup>1,2,3</sup>, Muhammad Waqas<sup>2</sup>, Muhammad Hamayun<sup>1</sup>, Ahmed Al-Harrasi<sup>1</sup>, Ahmed Al-Rawahi<sup>1</sup> and In-Jung Lee<sup>2\*</sup>

### Abstract

**Background:** Water-deficiency adversely affects crop growth by generating reactive oxygen species (ROS) at cellular level. To mitigate such stressful events, it was aimed to investigate the co-synergism of exogenous salicylic acid (SA) and symbiosis of endophytic fungus with *Capsicum annuum* L. (pepper).

**Results:** The findings of the study showed that exogenous SA ( $10^{-6}$  M) application to endophyte (*Penicillium resedanum* LK6) infected plants not only increased the shoot length and chlorophyll content but also improved the biomass recovery of pepper plants under polyethylene glycol (15%) induced osmotic stress (2, 4 and 8 days). Endophyte-infected plants had low cellular injury and high photosynthesis rate. SA also enhanced the colonization rate of endophyte in the host-plant roots. Endophyte and SA, in combination, reduced the production of ROS by increasing the total polyphenol, reduce glutathione, catalase, peroxidase and polyphenol oxidase as compared to control plants. Osmotic stress pronounced the lipid peroxidation and superoxide anions formation in control plants as compared to endophyte and SA-treated plants. The endogenous SA contents were significantly higher in pepper plants treated with endophyte and SA under osmotic stress as compared to control.

**Conclusion:** Endophytic fungal symbiosis and exogenous SA application can help the plants to relieve the adverse effects of osmotic stress by decreasing losses in biomass as compared to non-inoculated plants. These findings suggest that SA application positively impact microbial colonization while in combination, it reprograms the plant growth under various intervals of drought stress. Such symbiotic strategy can be useful for expanding agriculture production in drought prone lands.

**Keywords:** *Penicillium resedanum* LK6, Osmotic stress, Salicylic acid, Antioxidants, Biomass recovery, *Capsicum annuum* L

### Background

Water-deficient or drought stress conditions can drastically hinder the crop growth and yield. Exposure to extreme conditions brings changes inside plant tissues at ionic/osmotic, phytohormonal and secondary metabolites levels [1]. Continuous waves of drought cause an imbalance in the osmotic potential of the plant tissues, thus, inducing the synthesis of reactive oxygen species (ROS) [2]. To maintain the cellular redox potential and buffer the

negative effects of ROS, plant produce antioxidants like reduced glutathione (GSH), total polyphenols, catalase (CAT), peroxidase (POD) and polyphenol oxidase (PPO) etc [3]. Plants tend to accumulate higher antioxidants to avoid cellular damage. Additionally, the plant hormonal apparatus is activated to transduce stress signals during altered osmotic potential. Endogenous salicylic acid (SA) is known to develop defence-related responses during biotic stress [4,5] while exogenous application of SA has mostly showed abiotic stress tolerance for example, heat stress in mustard [6], chilling in maize [7], salinity in wheat [8] and drought in wheat and sunflower [9,10]. Exogenous

\* Correspondence: ijlee@knu.ac.kr

<sup>2</sup>School of Applied Biosciences, Kyungpook National University, Daegu, Republic of Korea

Full list of author information is available at the end of the article



ATTESTED  
*[Signature]*

54

RESEARCH ARTICLE

Analysis of Phytohormones and Phosphate Solubilization in *Photorhabdus* spp.

Ihsan Ullah, Abdur Rahim Khan, Gun-Seok Park, Jong-Hui Lim, Muhammad Waqas, In-Jung Lee, and Jae-Ho Shin

Received: 14 December 2012 / Revised: 2 February 2013 / Accepted: 4 February 2013 / Published Online: 28 February 2013  
© KoSFoST and Springer 2013

**Abstract** Bacteria of the genus *Photorhabdus* are nematodes (*Heterorhabditidae*) symbiont and highly entomopathogenic in nature. Present study was concerned to investigate the effects of *Photorhabdus temperata* M1021 and *Photorhabdus luminescens* TT01 on plant morphology and physiology ('Dongjin-beyo' and 'waito-c'). A significant ( $p < 0.05$ ) increase in the plant growth attributes including total plant length, root length, biomass, and chlorophyll contents were observed after treatment with culture extracts. Moreover indole-3-acetic acid (IAA) production by respective bacteria was confirmed by GC-MS analysis. IAA production through tryptophan independent pathway was confirmed in *P. temperata* M1021 and *P. luminescens* TT01. Phosphate solubilization capability was also investigated by growing them on pikovskaya (PVK) agar as well as on PVK liquid medium with pH 7 at  $28 \pm 2^\circ\text{C}$  for 14 days. IAA production and phosphate solubilization capability of these bacteria could be the potential factors for plant growth promotion.

**Keywords:** *Photorhabdus*, insecticidal bacteria, *Photorhabdus temperata* M1021, *Photorhabdus luminescens* TT01, GC-MS

**Introduction**

Members of the genus *Photorhabdus* are nematode-symbiotic, Gram-negative, and bioluminescent bacteria

(*Enterobacteriaceae*), symbiotically associated with nematodes (*Heterorhabditidae*) (1). The *Photorhabdus* genus consists of 3 bacterial species: *P. luminescens*, *P. temperata*, and *P. asymbiotica* (2). The first 2 species are nematode-symbiotic, while the third is a potential human pathogen (1). *Photorhabdus* genome has islands for the insecticidal toxin complexes and it produces numerous toxins (2-5). Beside the proteinous toxins, pathogenicity of the bacteria is thought to be due to the production of chemical compounds, such as pyrrothins known as xenorhabdins as well as the indole derivatives (3). There are bunch of indole derivatives including plant growth hormones, auxin(s), which are mainly synthesized either by plants themselves or by microbes (bacteria and fungi) (4,5). Auxins are known to regulate specific physiological responses, such as division, elongation, and differentiation of cells (6). Indole-3-acetic acid (IAA) is one of the most important members of auxin group which plays important role in microbe-microbe and microbe-plant signaling. Several recent reports indicate that IAA can be a signaling molecule in bacteria and therefore can have a direct effect on bacterial physiology (7). It has been found that bacteria synthesize IAA through several pathways, but mainly there are 2 pathways, tryptophan dependent and tryptophan independent for the production of IAA. The tryptophan independent pathway for the biosynthesis of IAA is branched from indole-3-glycerolphosphate or indole (8,9). Nutrients (macro and micro nutrients) play critical role in the plant growth and developments (10). Phosphorus is one of the major essential macronutrients for biological growth and development. Phosphorus is required to all living being in the form of phosphate ion (10). Plants are capable to directly absorb the phosphate ions from the rhizosphere (11). Because of the negative charge of phosphate ions, they are quickly absorbed after weathering

Ihsan Ullah, Abdur Rahim Khan, Gun-Seok Park, Jong-Hui Lim, Muhammad Waqas, In-Jung Lee, Jae-Ho Shin (✉)  
School of Applied Sciences, Kyungpook National University, Daegu 702-701, Korea  
E-mail: jhshin@knu.ac.kr

ATTACHED



## Fungal endophyte *Penicillium janthinellum* LK5 can reduce cadmium toxicity in *Solanum lycopersicum* (*Sitiens* and *Rhe*)

Abdul Latif Khan · Muhammad Waqas · Javid Hussain · Ahmed Al-Harrasi · In-Jung Lee

Received: 19 January 2013 / Revised: 22 June 2013 / Accepted: 1 July 2013  
© Springer-Verlag Berlin Heidelberg 2013

**Abstract** We investigated the role of gibberellins-producing endophyte *Penicillium janthinellum* LK5 associated with *Solanum lycopersicum* (host), abscisic acid (ABA)-deficient tomato mutant *Sitiens* and its wild-type Rheinlands Ruhm (*Rhe*) plants under cadmium (Cd) stress. A 100- $\mu$ M Cd application to host, *Sitiens* and *Rhe* reduced the shoot growth, chlorophyll content and stomatal conductance. However, these parameters were significantly ( $P < 0.0011$ ) higher (1.0- to 2.6-folds) in host, *Sitiens* and *Rhe* under endophytic association than in non-endophyte infected plants (control) under Cd stress. Furthermore, endophytic association minimized the Cd-induced membrane injury and oxidative stress to host, *Sitiens* and *Rhe* plants by reducing electrolytes and lipid peroxidation while increasing the content of reduced glutathione and catalase activities as compared to non-endophyte-infected plants. Stress-responsive ABA content significantly increased (~2-folds) in *Sitiens* and *Rhe* under endophyte association, while in host plants it was decreased under Cd stress. Salicylic acid content was ~1.7-fold higher in host, *Sitiens* and *Rhe* plants under Cd stress and endophyte association than in the control. Besides gibberellins production, the endophyte has the potential to solubilize phosphates (12.73  $\pm$  0.24 mg/l) since higher P was observed in the roots of *Sitiens*, *Rhe* and host plants. Similarly, nutrients like sulfur and calcium were more efficiently assimilated in roots of endophyte-associated plants than control under Cd stress. Conversely, Cd accumulation was significantly decreased ( $P < 0.001$ ) in the roots of endophyte-inoculated host, *Sitiens* and *Rhe* than control. In

conclusion, endophyte symbiosis can counteract heavy metal stress which can exert negative effects on plant growth.

**Keywords** *Solanum lycopersicum* · *Sitiens* · Endophytism · ABA · SA · Cadmium stress

### Introduction

Interactions among metals, microbes, and plants have attracted much attention because of the biotechnological potential of microorganisms to remove metals directly from polluted media and the possible role of microorganisms in promoting plant growth in metal contaminated soils (Ike et al. 2007; Rajkumar et al. 2009; Weyens et al. 2009; Deng et al. 2011). Among microbes, endophytic fungi are important due to their symptomless and mutualistic role with plants (Khan et al. 2011a). The presence of suitable genomic traits is also important for endophytes to establish their interactions with plants (Barret et al. 2011). These endophytes, besides producing plant growth regulators (like gibberellins and auxins), can help the host plants to counteract extreme environmental conditions (Schulz and Boyle 2005; Redman et al. 2011; Khan et al. 2012). The development of an endophytic association in roots changes the key aspects of plant physiology, including the nutrient composition of tissues, plant hormonal balance, chemical exudation from roots and potential to remove soil contaminants by enhancing phytoremediation (Xiao et al. 2010).

Crop plants exposed to Cd pollution exhibit retarded growth and yield. Cadmium damages the photosynthetic apparatus (John et al. 2009), lowers the chlorophyll content (Dong et al. 2006), inhibits the stomatal opening (Yadav 2010), and increases the production of reactive oxygen species (ROS; Schützendübel and Polle 2002). To counteract ROS, the plants activate antioxidants like catalase activity and reduced glutathione while the lipid peroxidation shows the degree of oxidative stress perceived during membrane

A. L. Khan · J. Hussain · A. Al-Harrasi  
Department of Biological Sciences and Chemistry, University of Nizwa, Nizwa 616, Oman

M. Waqas · I.-J. Lee (✉)  
School of Applied Biosciences, Kyungpook National University, Daegu 702-701, South Korea  
e-mail: ijlee@knu.ac.kr

ATTESTED  
*[Signature]*

**EFFECT OF CUTTING ON PRODUCTIVITY AND ASSOCIATED WEEDS OF CANOLA**

Kawsar Ali<sup>1</sup>, Muhammad Arif<sup>1\*</sup>, Zahid Khan<sup>2</sup>, Muhammad Tariq<sup>1</sup>, Muhammad Waqas<sup>3</sup>, Bakhtiar Gul<sup>4</sup>, Shahida Bibi<sup>4</sup>, Zia-ud-Din<sup>5</sup>, Murad Ali<sup>6</sup>, Bushra Shafi<sup>7</sup> and Muhammad Adnan<sup>8</sup>

**ABSTRACT**

Dual-purpose canola means cutting or grazing the crop and then letting it to re-grow and produce grain with no or little yield penalty similar to dual-purpose cereals. A field experiment was conducted to study the effect of cutting on seed and biomass yields, weed density, fresh and dry biomass of canola at New Developmental Farm of the University of Agriculture, Peshawar during winter 2009-10. The experiment consisted of cutting treatments i.e. cut and no cut was in Randomized Complete Block Design with three replications. Cutting treatments significantly suppressed weed density, weed fresh and dry biomass and seed and biomass yield of canola. Cutting of canola for fodder 70 days after sowing considerably trimmed down weed density and their fresh and dry biomass. Similarly, it also radically decreased seed and biomass yield of canola. It is concluded that canola can be used for both fodder and seed production but at the cost of about 27% yield penalty though weeds are suppressed.

**Key words:** Canola, cutting, seed yield, biomass yield, weed.

**Citation:** Ali, K., M. Arif, Z. Khan, M. Tariq, M. Waqas, B. Gul, S. Bibi, Zia-ud-Din, M. Ali, B. Shafi and M. Adnan. 2013. Effect of cutting on productivity and associated weeds of canola. Pak. J. Weed Sci. Res. 19(4): 393-401.

**INTRODUCTION**

Like other developing countries, Pakistan is also facing severe scarcity of edible oil and 70% of the supplies are met through import which results in huge loss of foreign exchange (Aslam *et al.*, 1996). Canola (*Brassica napus* L.) is a specific type of rape seed associated with high quality oil and meal. It contains 40-45% oil and 36-40%

<sup>1</sup>Dept. of Agronomy, <sup>4</sup>Dept. of Weed Science, <sup>5</sup>Dept. of Human Nutrition, <sup>6</sup>Dept. of Water Management, <sup>7</sup>Dept. of Rural Sociology,

<sup>8</sup>Dept. of SES, the University of Agriculture, Peshawar, Pakistan

<sup>2</sup>Department of Pharmacy, Federal Urdu University, Karachi, Pakistan

<sup>3</sup>Department of Agriculture Extension, Buner, Pakistan

\*Corresponding author's email: [marifkhan75@aup.edu.pk](mailto:marifkhan75@aup.edu.pk)

ATTESTED  
*[Signature]*

**EFFECT OF NITROGEN APPLICATION TIMINGS ON THE SEED YIELD OF BRASSICA CULTIVARS AND ASSOCIATED WEEDS**

Muhammad Tariq<sup>1</sup>, Zahid Khan<sup>2</sup>, Muhammad Arif<sup>1\*</sup>, Kawsar Ali<sup>1</sup>, Muhammad Waqas<sup>3</sup>, Khalid Naveed<sup>4</sup>, Murad Ali<sup>5</sup>, Murad Ali Khan<sup>6</sup>, Bushra Shafi<sup>7</sup> and Muhammad Adnan<sup>8</sup>

**ABSTRACT**

To study the effect of nitrogen application timings on Brassica cultivars, an experiment was conducted at New Developmental Farm, University of Agriculture, Peshawar, during winter 2011-12. The experiment consisted of three Brassica cultivars (Abasin-95, Dure-e-NIFA and NIFA Raya), three nitrogen application timings (all at sowing, half each at sowing and flowering, one third each at sowing, flowering and pod formation). Different cultivars showed significantly varied response for weed fresh and dry weight and seed yield. Abasin-95 cultivar showed the strong weeds growth suppression and resulted in lower weeds fresh and dry weight of the weeds and also produced higher seed yield. Nitrogen applied in two or three splits doses resulted in higher Brassica seed yield. Cutting declined weeds fresh and dry weight and seed yield of Brassica.

**Keywords:** Dual purpose Brassica, nitrogen timing, cultivars, weeds and yield.

**Citation:** Tariq, M., Z. Khan, M. Arif, K. Ali, M. Waqas, K. Naveed, M. Ali, M.A. Khan, B. Shafi and M. Adnan. 2013. Effect of nitrogen application timings on the seed yield of Brassica cultivars and associated weeds. Pak. J. Weed Sci. Res. 19(4): 493-502.

**INTRODUCTION**

Rapeseed (*Brassica napus* L.) being traditional oilseed crops of Pakistan are grown over large area in all the four provinces of the country under both irrigated and rainfed conditions (Khan *et al.*,

<sup>1</sup>Dept. of Agronomy, <sup>5</sup>Dept. of Water Management, <sup>6</sup>Dept. of Animal Health, <sup>7</sup>Dept. of Rural Sociology, <sup>8</sup>Dept. of SES; the University of Agriculture, Peshawar, Pakistan.

<sup>2</sup>Department of Pharmacy, Federal Urdu University, Karachi, Pakistan

<sup>3</sup>Department of Agriculture Extension, Bunir, Pakistan

<sup>4</sup>Department of Agricultural Sciences, University of Haripur, Pakistan

\*Corresponding author email: [marifkhan75@yahoo.com](mailto:marifkhan75@yahoo.com)

ATTESTED

8

Alexander von Humboldt



Auriga Group



USDA



Climate Change  
Adaptation  
Program

85