PSM Microbiology
2017 | Volume 2 | Issue 3 | Pages 63-73
ISSN: 2518-3834 (Online)

www.psmpublishers.org

Research Article **Open Access**

Rice Chitinase Gene Expression in Genetically Engineered Potato Confers Resistance against *Fusarium solani* and *Rhizictonia solani*

Madiha Zaynab[1*], Sonia Kanwal[7], Iqbal Hussain[5], Muhammad Qasim[2], Ali Noman[3], Umer Iqbal[5], Ghulam Muhammad Ali[5], Khalida Bahadar[5], Atka Jamil[5], Kalsoom Sughra[8], Nazia Rehman[5], Mahmooda Buriro[8], Safdar Abbas[10], Mohsin Ali[6], Anwaar Hayder Alvi[9], Muhammad Anwar[4], Muhammad Ifnan Khan[3], Muhammad Tayyab[3]

[1]College of Life Science, [2]College of Plant Protection, [3]College of Crop Science, [4]College of Horticulture, Fujian Agriculture and Forestry University, Fuzhou 350002, PR China.
[5]National Agriculture Research Centre, Islamabad 44000, Pakistan.
[6]Department of Plant Pathology, [7]Institute of Horticulture Sciences, University of Agriculture, Faisalabad 38040, Pakistan.
[8]Department of Agronomy, Sindh Agricultural University, Tando jam, Sindh Pakistan.
[9]Institute of Molecular Biology & Biotechnology, Bahauddin Zakariya University, Multan, Pakistan.
[10]Department of Biochemistry, Quid-e-Azam University, Islamabad, Pakistan.

Received: 03.Aug.2017; Accepted: 04.Oct.2017; Published Online: 02.Nov.2017
***Corresponding author: Madiha Zaynab; Email: madiha.zaynab14@gmail.com**

Abstract
The present study was planned to evaluate the transgenic potato plants containing chitinase (RCG-3) gene transformed through *Agro bacterium* for resistance against *Rhizoctonia Solani* and *Fusarium solani. In vitro*, plants were cultured for multiplication and then pathogenicity of transgenic potato plants harboring chitinase gene were carried out with fungal pathogens *(R. solani* and *F. solani)*. The fungal pathogens were isolated from infected tubers and purified by single spore technique. For multiplication of pathogens wheat straw method was used for *R.solani* and sorghum seed method was used for *F.solani.* The inoculation of fungal infection (*R. solani*) and (*F. solani*) transgenic and non-transgenic plants were performed in laboratory and controlled greenhouse conditions (Containment). Results showed that transgenic potato plants containing chitinase (RCG-3) gene transformed through *Agrobacterium* showed resistance against *R. Solani* and *F. solani* compared to non-transgenic plants. It was concluded that rice chitinase gene expression in potato confers enhanced resistance against two major fungal diseases of potato in Pakistan.
Keywords: Dry rot, Fusarium wilt, Tissue culture, Transgenic, Vector.

Cite this article: Zaynab, M., Kanwal, S., Hussain, I., Qasim, M., Noman, A., Iqbal, U., Ali, G.M., Bahadar, K., Jamil, A., Sughra, K., Rehman, N., Buriro, M., Abbas, S., Ali, M., Alvi, A.H., Anwar, M., Khan, M.I., Tayyab, M., 2017. Rice Chitinase Gene Expression in Genetically Engineered Potato Confers Resistance against *Fusarium solani* and *Rhizictonia solani*. PSM Microbiol., 2(3): 63-73.

INTRODUCTION

The potato (*Solanum tuberosum* L.) is the fourth largest crop ranked after rice, wheat and maize (Okello *et al.,* 2016). Potato farming system in Pakistan is not easy because culture, socioeconomic, climate and agronomic conditions are not same (Zanoni, 1991). In Pakistan, potato yield is much low as compared to other developing countries (Butt *et al.,* 2008). There are many factors responsible for the less potato production, diseases being one of the major contributors. There are many other causes of low yield, one of which is its susceptibility to attack by pathogens such as fungi, viruses, bacteria, insects and mycoplasma (Ahmad *et al.,* 2007; Islam *et al.,* 2016).

In Pakistan, the commonly occurring fungal diseases in potato are early blight, late blight (Khan *et al.,* 1985), black scurf (Rauf *et al.,* 2007), wilts(Shafique and Shafique, 2012). Fungi are far more complex organisms than viruses or bacteria and have developed numerous strategies to survive in nature, which include saprotrophy, necrotrophy, hemibiotrophy and biotrophy (Atkinson *et al.,* 2003). It has been reported that more than 25% developing potatoes are killed by *Fusarium*, and up to 60% potato tubers were infected in storage (Wharton *et al.,* 2007). *Rhizoctonia solani* species is another important pathogen which causes black scurf on potato tubers, which produces both hyphae and sclerotia. For so many years, workers have thoroughly sorted out the genetic basis of disease resistance, biochemical pathways, breeding and cloning of resistant genes. Potato has complex genetic makeup, so traditional methods of breeding for resistance induction are complicated in it. Genetic engineering provides a tool for

adding disease resistance to existing cultivars (Punja, 2001).

Fungal pathogen produced cell wall degrading enzyme during plant infection such as Polygalacturonase and various toxins such as mycotoxin and oxalic acid. Inactivation of enzyme and toxin may be a target for transgenic resistance against fungal pathogens. Thus, a potato genetic transformation was experimented via *Agrobacterium tumefaciens*, with the help of a binary vector pBI333-EN4-RCG3 (Hussain, 2007).

Transformation of the chitinase gene in different plant species showed resistance against different fungal pathogens (Nishizawa and Hibi, 1991). Chitinases hydrolyze the N-acetyl glucosamine polymer chitin, and they occur in diverse plant tissues. The chitinase expression in combination with one or several different antifungal proteins should have a greater effect on reducing disease development, given the complexities of fungal-plant cell interactions and resistance responses in plants (Punja and Zhang., 1993). The present study was carried out for *in vitro* multiplication of transgenic potato containing RCG-3 gene transformed through *Agrobacterium* and evaluation of these transgenic potato plants against *R. solani* and *F. solani.*

MATERIALS AND METHODS

Transgenic potato plants

Confirmed transgenic *in vitro* potato plants of varieties Hermes and Desiree containing *chitinase* RCG-3 gene and hygromycin resistant gene were obtained from tissue culture laboratory, NIGAB, National Agricultural Research Center, Islamabad. These potato plants were transformed using *Agrobacterium* mediated transformation. The transgenic plants were selected on hygromycin. All the PCR + ve plants for the presence of chitinase and hygromycin gene were used for this study.

Explants

Nodes were excised at both ends of 0.1-0.5cm in size under aseptic conditions (Hussain, 2007) from each variety and cultured for multiplication.

In vitro multiplication

In vitro transgenic plants of the potato varieties Hermes and Desiree were cultured for multiplication through tissue culture before the pathogenicity test. These transgenic plants were used for pathoginecity test against the fungal pathogens *F. solani* and *R. solani.* Nodal fragments were cultured on Murashige-Skoogsolid containing 1mg/l GA3, 30g/l sucrose and 2.2 gm/lgel grow (Phytotech). Murashige-Skoog (1962) media with micro, macro elements and vitamins was used. Murashige-Skoogmedium, comprising macronutrients and micronutrients were supplemented with growth regulators. The pH of media was adjusted to 5.8 to 5.9. The media was boiled after additions of gel grow (2.2gm/l) and dispensed into glass jars/test tubes. The nodal explants after fifteen days were transferred on fresh media. Growth and multiplication were examined after every five weeks. Five nodes were cultured 250 ml per flask containing 30 ml media. Rate of multiplication and growth rate of shoots was monitored regularly until shoot reached to the size of 8-10 cm.

Culture incubation

Cultures were incubated at 25+2 ºC under 16 hours photoperiod in growth room with light intensity of 2000 lux.

Transplantation and Acclimatization of plantlets under *ex vivo* conditions

Complete rooted transgenic and non-transgenic plants were removed from the tissue culture vessels and washed gently in water to remove all excessive medium and sucrose traces to discourage infection. The plantlets were blotted lightly on filter papers and then were planted into pots containing (peat moss and soil in 1:1) in containment facility of NARC where relative humidity was maintained 80-85% by covering with polythene bags, light intensity was about 30% of natural light and temperature range from 20-25ºC. The plantlets were regularly irrigated with water after every two to three days of interval.

Pathogenicity

The Pathogenicity of transgenic potato plants harboring chitinase gene were screened for resistance against fungal pathogens *(R. solani* and *F. solani)* in Fungal Pathology Laboratory at Crop Diseases Research Institute (CDRI), NARC. Potato Dextrose Agar (PDA) media containing streptomycin was used for fungal growth.

Isolation of *Rhizoctonia solani* and *Fusarium solani*

Potato infected tubers showing typical symptoms of disease were selected for isolation of *R. solani* and *F. solani.* Infected tubers of potato were obtained from National Potato Program (NPP) and local markets. Tubers were surface sterilized with 1% Clorox for 1 min and then rinsed with distilled water for washing and then dried by keeping on sterile filter papers. After drying the upper layers of tuber having black scurf and bearing sclerotia were carefully removed with a sterile knife and cultured under aseptic condition. Sclerotia were transferred on potato dextrose agar (PDA) medium (Attaullah *et al.,* 2012). Plates were sealed with parafilm to avoid contamination. The petri plates were kept for incubation at 25˚C for 7 days until colonies of *Rhizoctonia solani* were appeared.

Identification and Purification of *Rhizoctonia solani* and *Fusarium solani*

The pathogen colonies were identified under, stereoscope (Watanabe, 2002). Single spore culture technique was used to purify the culture for further multiplication. The single spore from pure colony was

placed on agar medium and then incubated at 25^{o}C for 7 days. The fungal isolates were introduced in the healthy plants and were isolated from the plants that showed symptoms of disease and were observed under microscope for confirmation. *R. solani* and *F. solani* were re-isolated from infected plants and further multiplied after purification from single colony on PDA media by following the Koch's Postulates (Koch, 1880).

Multiplication of *Rhizoctonia solani* culture

Multiplication of *R. solani* was carried on wheat straw for pathogenicity. Wheat straw was washed and kept open overnight for drying. Flasks were filled with wheat straw and closed the mouth tightly with cotton plug. Wheat straw was autoclaved at 121^{0}C for 15 minutes at 15psi. Each flask containing wheat straw was inoculated with two plugs of 5-mm diameter of the 1 week old culture. The flasks were immediately closed and incubated at 28^{0}C for 20 days and regularly monitored the growth of the fungi. *R. solani* after the multiplication on wheat straw, the straw was used as fungal inoculum for the potato plants infection.

Multiplication of *Fusarium solani* culture

Sorghum grains were washed and removed all water after soaking overnight. Flasks were filled with sorghum seeds and left out for drying after closing with a cotton plug. The flasks were immediately closed after inoculation with *F. solani* and incubated at 28^{0}C for 20 days and regularly monitored for infection.

Pathogenicity of *Rhizoctonia solani* and *Fusarium solani*

Out of the 24 plants of the Desiree variety, 12 were given inoculum of *F. solani* and the remaining 12 pants were given the inoculum of *R. solani*. Similarly the 24 plants of the Desiree variety, 12 were given inoculum of *F. solani* and the remaining 12 pants were given the inoculum of *R. solani.*

Pot culture assay in Laboratory

The transgenic and non-transgenic potato plants were removed from flask carefully and washed under tap water to remove the debris. The inoculation of fungal infection (*R. solani* and *F. solani*) to transgenic and non-transgenic plants was performed in laboratory. Then 5% w/w of the inoculum was introduced in the sterilized soil. The potato plants were then grown in infested soil. The plants were regularly monitored for plant growth and disease symptoms. Plants were kept for a month and data were collected.

Pot culture assay in containment

The transgenic and non-transgenic potato plants were removed from flasks carefully and washed under tap water to remove the debris. The inoculation of fungal infection (*R. solani* and *F. solani)*) to transgenic and non-transgenic plants was performed in greenhouse condition (containment). 5% w/w of the inoculum was introduced in the sterilized soil. The potato plants were grown in infested soil. They were shifted in a greenhouse with relative humidity 80-85%, natural light intensity were about 30% and temperature ranges from 20-25^{o}C. Each pot contains one plant having five replicates. The plants were regularly monitored for plant growth and disease symptoms. Plants were kept for a month and data was collected.

Disease incidence

Disease incidence was calculated with the following formula:

$$\text{Disease incidence} = \frac{\text{Number of infected plant}}{\text{Total number of potato plants}} \times 100$$

Disease severity was calculated according to the disease rating scale (Tsror and Peretz-Alon, 2005).

Transgenic and non-transgenic plants were evaluated regularly to compare tolerance level to *R. solani* in laboratory and greenhouse condition.

Statistical analysis

All field experiments were carried out in randomized completely block design (RCBD) and lab experiments were carried out in completely randomized design (CRD). Each experiment was replicated thrice. One-Way analysis of variance (ANOVA) was applied to the mean values to observe significance of treatments by using statistical program IBM-SPSS, at a significance level of 0.05 (Field, 2013). Similarly, the significance of transgenic plants for other characteristics (height, number of nodes and number of leaves) was analysed by applying t-test, using IBM-SPSS. Disease severity was calculated via measuring mean values and applying Vakalounakis, Chaerani and Pandey's formula.

RESULTS

Background of transgenic potato plants

The *in vitro* potato plants of Hermes and Desiree varieties were acquired from tissue culture lab, NIGAB, National Agricultural Research Center, Islamabad. The potato plants were already transformed *Agrobacterium* strain LB4404 which contained binary vector having plasmid pB1333-EN4-RCG3 (Figure 1) with RCG-3 and Hygromycin gene for selection which was acquired from Dr. Yoko Nshizawa National Institute of Agro biological Resources (NIAR), Tskuba, and Ibaraki, Japan. The size of the vector was 1.1-kb DNA fragment. It was encoded by Cht-3 from a genomic rice clone (RCG3) and hygromycin resistance gene driven by enhanced cauliflower mosaic virus (CaMV) 35S promoter, possessing (EN4) tandemly repeated enhancer region (-290 to -90) of its own.

The transgenic potato plants harboring the chitinase gene showing PCR +ve product for chitinase and

hygromycin primers (Figure 2 and 3) were used in this study.

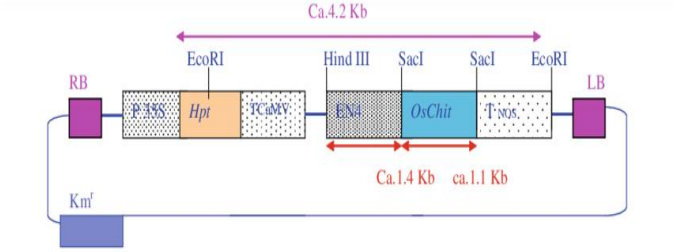

Fig. 1. Plasmid pB1333-EN4-RCG3 used in the study

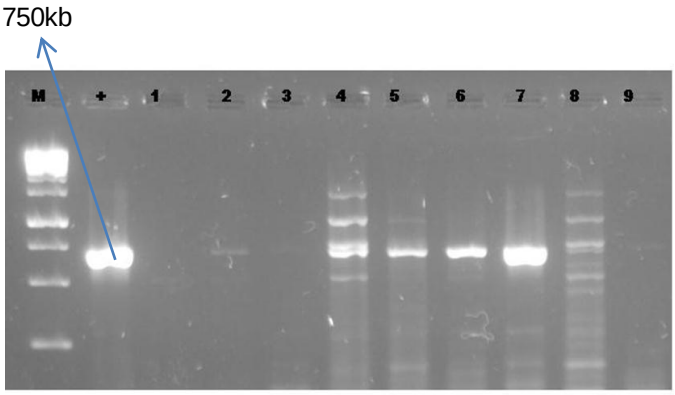

Fig. 2. PCR amplification of Rice Chitinase gene of Desiree variety. Lane M: One kb Marker (Fermentas Lithuania; Lane +: Positive control; Lane 1 to 8: Transgenic plants that survive on selection media; Lane 9 : Control non-transformed plant DNA

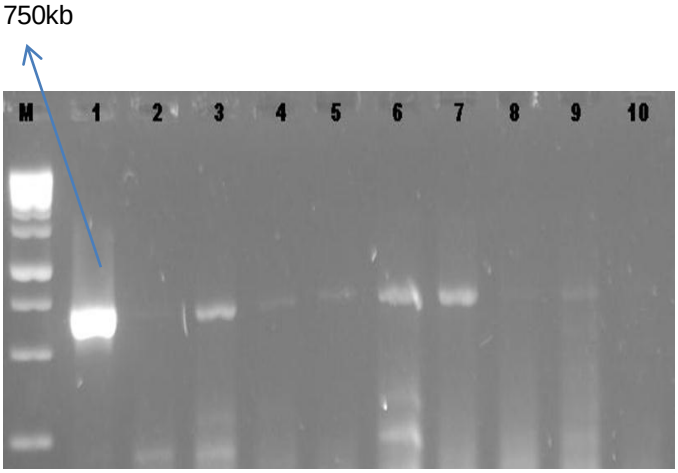

Fig. 3. PCR amplification of Rice Chitinase gene of Hermes variety. Lane M: One kb Marker (Fermentas Lithuania; Lane +: Positive control; Lane 1 to 8: Transgenic plants that survive on selection media; Lane 9 : Control non-transformed plant DNA

***In Vitro* Multiplication**

Transgenic *in vitro* potato plants harboring (chitinase RCG-3 gene) were multiplied by nodal fragmentation method on MS solid media (Figure 4). The cultures were incubated for three weeks and data recorded was growth of multiple shoots, no of nodes, plant height and no of leaves. The plants showed initial response by emergence of new leaves after third day of culturing in all media composition tested. After four weeks the plant growth showed variable response on media. Shoot multiplication was achieved after four weeks from the both varieties (Figure 5).

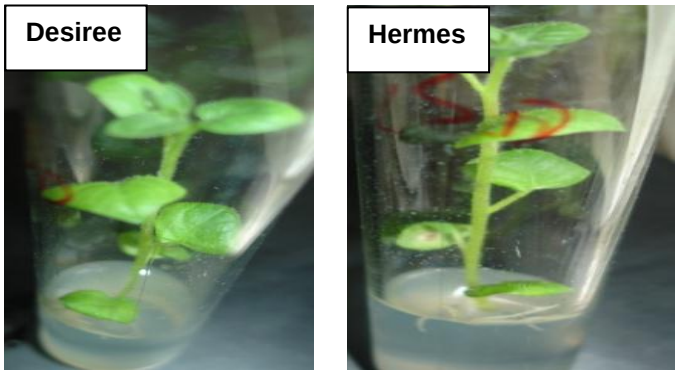

Fig. 4. Transgenic potato plants before multiplication

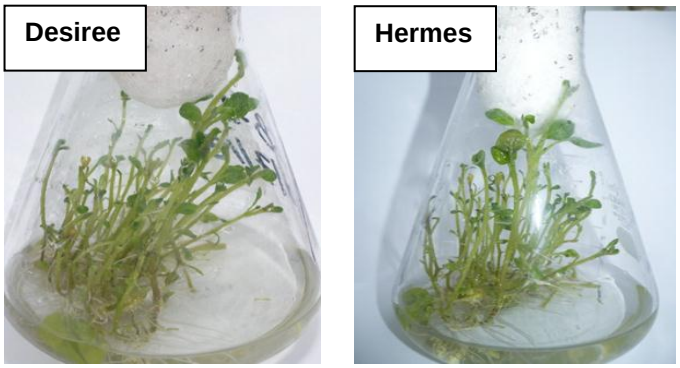

Fig. 5. *In vitro* multiplication of transgenic potato

There was a mean value difference between two varieties in height, number of leaves and number of nodes after using same concentration of GA_3 in same condition. Variety Desiree produced 10.111cm tall plants while variety Hermes produced 7.333 cm tall as in table 1. Number of nodes in Desiree variety was observed 8.4444 and Number of nodes is Hermes variety was observed 6.0000. Variety Desiree produced 10.667 leaves per plant while mean value of number of leaves in variety Hermes was 8.222 as in Table 1. The Desiree variety showed better height, number of nodes and number of leaves than Hermes ($p< 0.05$).

Table 1. Mean values of plant height, number of leaves and number of nodes between Desiree and Hermes prior infection

Variety	Plant Height (cm)	Number of leaves	Number of nodes
Desiree	10.111	10.667	8.4444
Hermes	7.333	8.222	6.0000
p= 0.05 α	0.0017	0.0001	0.0021

Pathogenicity

Isolation of *Rhizoctonia solani and Fusarium solani*

The fungal pathogens cultured on PDA media showed growth after third day of incubation. The colonies of *Rhizoctonia solani* and *Fusarium solani* were fully grown after keeping the Petri plates for incubation at 25˚C for 7 days as shown in Figures 6, and 7.

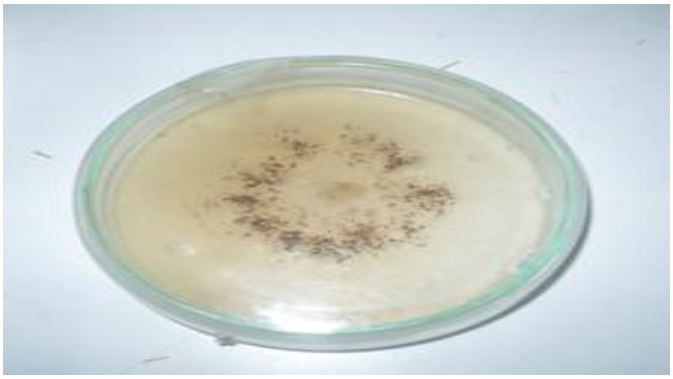

Fig. 6.Colonies of *R. solani*

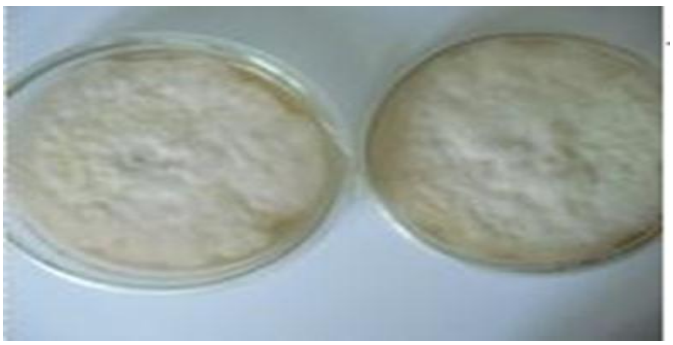

Fig. 8. Colonies of *F. solani*

Purification of *Rhizoctonia solani and Fusarium solani*

Pure colonies of *F. solani* and *R. solani* were obtained after 7 days under incubation at 25^{o}C which were picked using stereoscope by single spore technique. The colonies were observed under microscope which confirmed the specific isolates of *R. solani* and *F. solani* (Figure 8).

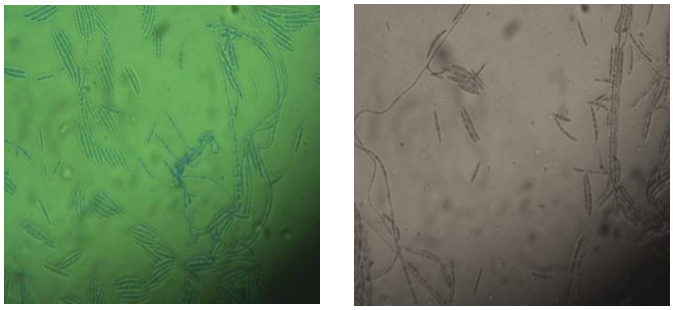

Fig. 8. Microscopic image of pathogens (left: *Rhizoctonia solani and* right: *Fusarium solani*)

Multiplication of pathogen culture

After the maximum growth of fungal pathogen on PDA media which was achieved after seven days of culturing and further multiplication of *R. solani* was carried out on wheat straw and *F. solani* on sorghum seed (Figures 9 and 10). Proper fungal inoculums were applied for infection to the potato plants after15- 20 days under incubation at 25˚C.

Fig. 9. Multiplication of *R.solani* on Wheat Straw

Fig. 10. Multiplication of *F. solani* on Sorghum seeds

Highest disease incidence based on lesions was observed in non-transgenic plants of Desiree variety (87.48%) while lowest in transgenic plants of Hermes variety (12.45%) in green house conditions with lowest disease severity (Table 2).

Table 2. Resistance rating is based on calculated disease incidence and severity

S.No.	Plants	Variety	Disease Incidence	Severity	Disease Reaction
1	Transgenic in Greenhouse	Desiree	20.8%	2	Resistance
		Hermes	12.45%	1	High resistance
2	Non-Transgenic in Greenhouse	Desiree	87.48%	5	High Susceptible
		Hermes	66.66%	5	High Susceptible
3	Transgenic in Lab	Desiree	29.15%	2	Resistance
		Hermes	20.83%	2	Resistance
4	Non-Transgenic in Lab	Desiree	79.16%	5	High susceptible
		Hermes	62.49%	5	High Susceptible

1= healthy plants, 2= several black and brown lesions, 3= up to 15% plants is covered with lesions, 4= up to 60% plants is covered with lesions, 5= > 60 % plants is covered with lesions (Tsror and Peretz-Alon, 2005).

Transgenic plants infected with *R. solani* showed highest level of disease incidence (33.3%) in Desiree variety grown in laboratory with several black and brown lesions while lowest disease incidence was shown by healthy plants of Hermes variety (16.6%) in greenhouse conditions. While transgenic plants infected with *F. solani* showed highest level of disease incidence (25%) in Desiree variety grown in laboratory with several black and brown lesions while lowest disease incidence was shown by healthy plants of Hermes variety (8.3%) in greenhouse conditions (Table 3).

Non-transgenic plants infected with *R. solani* showed highest level of disease incidence (91.66%) in Desiree variety grown in laboratory with more than 60 % lesions while lowest disease incidence was shown by plants of Hermes variety (66.66%) with more than 60% lesions grown in laboratory conditions. While non-transgenic plants infected with *F. solani* showed highest level of disease incidence (91.66%) in Desiree variety grown in greenhouse with more than 60% lesions while lowest disease incidence was shown by plants of both Hermes and Desiree varieties (58.33%) with more than 60% lesions in greenhouse and laboratory conditions respectively (Table 3). Disease incidence and severity was observed high in non-transformed plants in green house and lab conditions while these were less in transformed plants in green house and lab conditions.

After 30 days of infection it was observed that all the non –transgenic plants showed wilting. The symptoms of disease started from the leaves and all the plants died after 30 days. The transgenic potato plants harboring chitinase RCG-3 gene survived after 30 days of the infection. New leaves come from the tip of the shoot. Disease incidence of transformed plants were low while disease incidence in non-transformed plants were high similarly disease severity in transformed plants were low as compared to the non-transformed plants as showed in Figures 11 and 12.

Table 3. Resistance rating is based on calculated disease incidence and severity of potato plants infected by *Fusarium solani* and *Rhizictonia solani*

Growth conditions	Varieties	*Rhizoctonia solani*		*Fusarium solani*	
		Incidence%	Severity	Incidence%	Severity
Transgenic in Green House	Desiree	25	2	16.6	2
	Hermes	16.6	1	8.3	1
Non transgenic in Green House	Desiree	83.3	5	91.66	5
	Hermes	75	5	58.33	5
Transgenic in lab	Desiree	33.3	2	25	2
	Hermes	25	2	16.66	2
Non transgenic in Lab	Desiree	91.66	5	66.66	5
	Hermes	66.66	5	58.33	5

1= healthy plants, 2= several black and brown lesions, 3= up to 15% plants is covered with lesions, 4= up to 60% plants is covered with lesions, 5= > 60 % plants is covered with lesions (Tsror and Peretz-Alon, 2005).

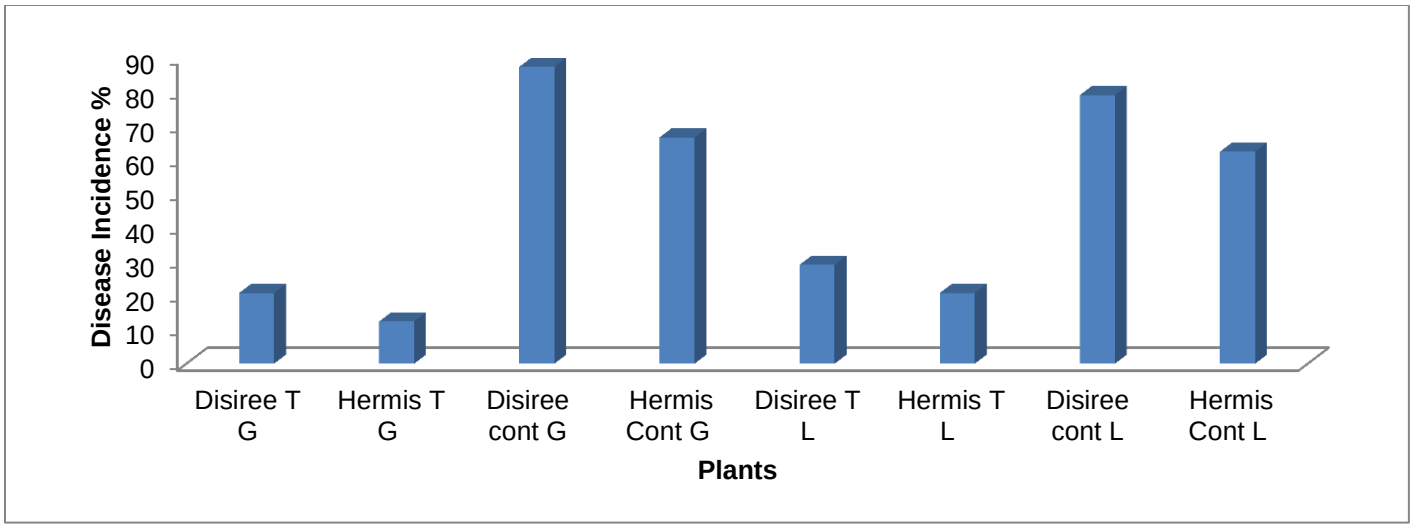

Fig. 11. Disease incidence (%) rating of transgenic and non-transgenic plants infected by *F. solani* and *R. solani* in green house and lab
T G = Transgenic in Green House, Cont G = Control plants in Green House, T L=Transgenic in Lab, Cont L= Control plants in Lab

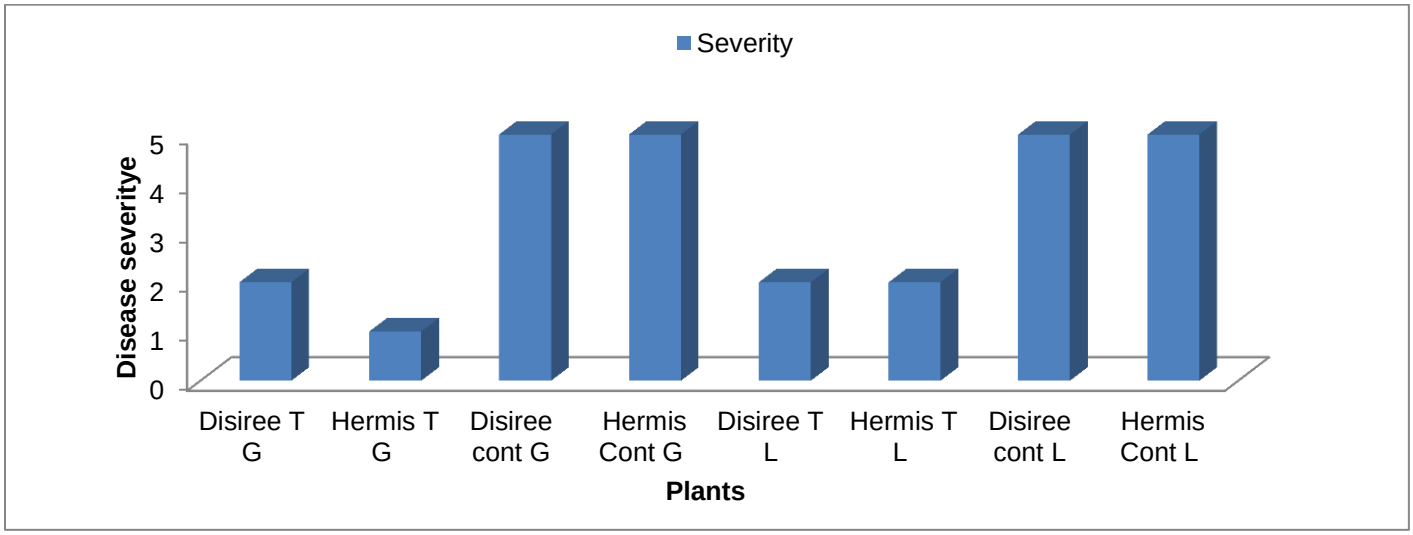

Fig. 12. Severity of transgenic and non-transgenic potato plants infected by *F. solani* and *R. solani* in green house and Lab
T G = Transgenic in Green House, Cont G = Control plants in Green House, T L=Transgenic in Lab, Cont L= Control plants in Lab

Mean height comparison of transgenic and non-transgenic plants grown in greenhouse conditions showed that there was no similarity between them. Transformed plants had highest value of mean plant height (11.479cm) and number of leaves (7.9375) indicating that height and number of leaves of transformed plants were highly significant (p=0.0000 at 0.05 α) than those of non-transformed plants (Table 4).

Table 4. Mean comparison of transgenic and non-transgenic plants (height and number of leaves) in green house (containment).

Variety	Mean height (cm)	Mean no. of leaves
Transgenic	11.479	7.9375
Non-transgenic	5.417	4.6042

Mean height comparison of transgenic and non-transgenic plants grown in laboratory conditions showed that there was no similarity between them. Transformed plants had highest value of mean plant height (10.208cm) and number of leaves (7.27) indicating that height and number of leaves of transformed plants were highly significant (p=0.0000 at 0.05 α) than those of non-transformed plants (Table 5).

Table 5. Mean comparison of transgenic and non-transgenic plants height and number of leaves in lab experiment

Variety	Mean height	Mean no. of leaves
Transgenic	10.208	7.27
Non transgenic	4.542	4.35

In the light of results the transformed plants showed high resistance and non-transformed showed high susceptibility to disease. Disease incidence and disease severity was high in non-transformed plants and low in transformed plants. The alternate hypothesis was proved which suggested the possibility of no similarity in response to the pathogenic attack. The transgenic plants harboring (*chitinase* RCG-3 gene) showed resistance against *F.solani* and *R. solani* (Figures 13, 14 and 15). The pathogenicity of the isolates was evaluated 30 days after inoculation of the plants by presence or absence of symptoms confirms the species involved. The transgenic plants harboring (*chitinase* RCG-3 gene) showed result that favor the alternate hypothesis and reject null hypothesis.

a **b**

Fig. 13. (a) Transgenic potato plants with fungal pathogen *R. solani* (b) Non-transgenic potato plants with fungal pathogen *R. solani*

a **b**

Fig. 14. (a) transgenic potato plants with fungal pathogen *F. solani* (b) Non-transgenic potato plants with fungal pathogen *F. solani*

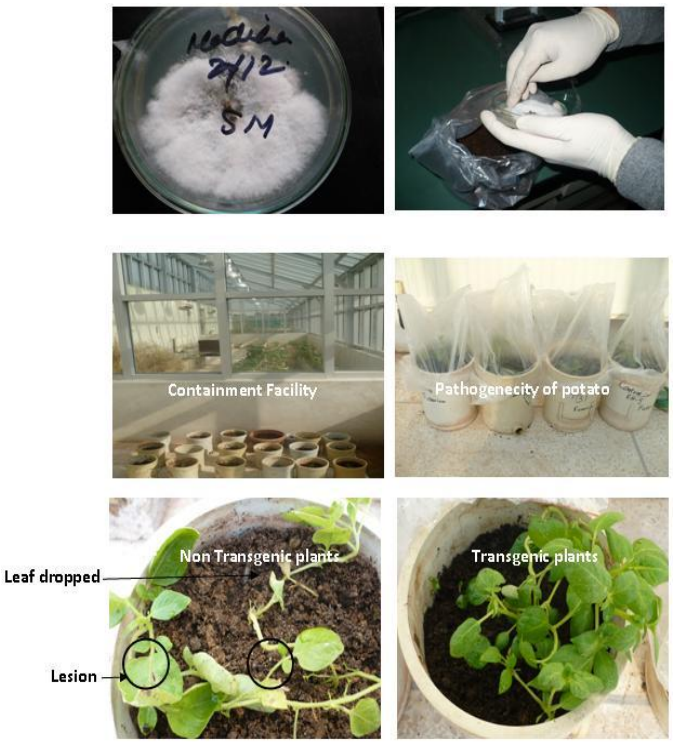

Fig: 15. Evaluation of transgenic potato against *Fusarium solani* and *Rhizictonia solani*

DISCUSSION

Potato (*Solanum tuberosum* L.) is an important food crop other than cereals. Its production has been threatened by several fungal, bacterial and viral diseases. Fungal pathogens cause 20% yield loss reported by (Baker *etal*. 1997; Walter *et al*., 2011). Numerous fungicides have been utilized to prevent fungal diseases but their use is not environmental friendly and tolerant strains of pathogens have been emerged. Therefore, new cultivars are required which show tolerance to these fungal pathogens.

Transgenic *in vitro* potato plants harboring (chitinase RCG-3 gene) and non-transgenic potato plants were tested for the fungal infection against fungal pathogens *(Rhizoctonia solani and Fusarium solani).* Differences were observed in number of node, number of leaves and height of plants. Desiree variety showed more number of nodes, number of leaves and height of plants. The difference might have been due to varietal genetic makeup of varieties. These results showed similarity with those of Gerson et al. (1997). The results in the present study showed differences for number of leaves and plant height in both of varieties (Desiree and Hermes) on MS media containing GA_3 concentration. The results agree with Rabbani (2001) in which that GA_3 and BAP (Benzyl amino purine) effects on *in vitro* multiplication of potato variety Desiree. In this study Desiree showed higher multiplication rate on solid media

than Hermes with same GA_3 concentration disagree with Sarkar and Mustafa (2002) reported that multiplication rate was higher using liquid medium containing Ca-pantothnic acid 2.0 mg/liter, biotin 1.0mg/L and GA_3 0.4 mg/L, 0.05 mg/l and 2 % sucrose.

Transgenic potato plants harboring (chitinase RCG-3 gene) survived up to 30 days after providing the infection with *F. solani*. Disease severity and disease incidence was low and new leaves came from the tip of the shoot of the transgenic potato plants. There were no wiltings observed in transgenic plants. High disease incidence and disease severity were observed in non-transgenic plants. Symptoms of dry rot disease were observed on non-transformed plants after 30 days of infection. Our results were supported by Romberg and Davis (2007) who reported that the fungal pathogen *F. solani* responsible to cause disease *Fusarium* dry rot in potato while *Fusarium* dry rot disease was not observed in transformed plants because of the antifungal activities of chitinase RCG-3 gene.

R. solani is main pathogen cause black scurf in potato plants. Non transgenic plants showed high disease incidence and disease severity and showed symptoms of disease after infection of *R. solani*. Our result of non-transgenic plants were supported by Hide et al. (2008) who reported *R. solani* is soil borne fungal pathogen responsible to cause black scurf in potato plants. Inoculum was used for the production of black scurf disease in potato plants and inoculum in non-transgenic plants was able to induce black scurf disease, our study was confirmed by Tsror and Peretz-Alon (2005) who reported that inoculum presence induce black scurf disease incidence and severity. Desiree variety of plants was highly susceptible against *R. solani,* our result supported by the Naz et al. (2008) who reported Disiree variety was susceptible against *R.solani.* Presence of chitinase showed resistance against *R. solani.* After the 30 days of infection of the *R. solani* to the transgenic plants, black scurf disease was low in transgenic plants due to the presence of chitinase in transgenic plants showed tolerance against *R. solani* agree with Pereira and Foster (2004) reported the transformed plants with chitinase have increased level of tolerance to *Rhizoctonia solani.*

Our results are similar to previous studies (Hussain, 2007; Ahmad *et al.,* 2012; Munir *et al.,* 2016), who produced transgenic potato harboring rice chitinase gene and those transgenic potato plants showed resistant against local Pakistani Fungal isolates. Resistance observed against different fungal diseases in transgenic tobacco (Lin *et al.,* 1995) rice (Karmakar *et al.,* 2016; Rajesh *et al.,* 2016) and potato (Ghosh *et al.,* 2016). Jabeen et al. (2015) reported that rice chitinase gene enhanced resistance against fungal pathogen *Fusarium oxysporum* and *Alternaria solani* in tomato. However it was also observed that transgenic banana plant was produced harboring rice chitinase gene, showing resistance against fungal pathogen (Maziah *et al.,* 2007; Kovacs *et al.,* 2013). Moreover, it was noted that transgenic peanut and tomato plants indicated the resistance to various pathogenic fungi, like late leaf spot, wilt and early blight (Prasad *et al.,* 2013; Jabeen *et al.,* 2015).

It was concluded that rice chitinase gene expression in potato confers enhanced resistance against two major fungal diseases of potato in Pakistan. Potato plants harboring chitinase gene showed high disease resistant and low disease susceptibility as compared to non-transformed potato plant in Pakistan.

CONCLUSION AND RECOMMENDATIONS

Non-transgenic potato plants cause disease against *Rhizictonia solani* and *Fusarium solani*. Transgenic potato plants showed resistance against *R. solani* and *F. solani*. Chitinase gene showed its function in transgenic plants. Chitinase degrade chitin which is a major constituent of fungal wall. *R. solani* and *F. solani* both have chitin containing wall. Transgenic plants containing Chitinase gene which degrade fungal cell wall and inhibit fungal growth. *R. solani* and *F. solani* could not produce disease due to presence of Chitinase RCG3gene. These transgenic potato plants should be tested for, their toxicity or lethal effect on other living organism by following bio safety rules.

ACKNOWLEDGEMENT

We are really thanks to tissue culture laboratory, NIGAB, National Agricultural Research Center, Islamabad, Pakistan, for providing support to this work.

CONFLICT OF INTEREST

There is no conflict of interests regarding the publication of this paper.

REFERENCES

Ahmad, M.Z., Hussain, I., Muhammad, A., Ali, S., Ali, G.M., Roomi, S., Zia, M.A., Ijaz, A., 2012. Factor affecting Agrobacterium-mediated transformation of rice chitinase gene in *Solanumtuberosum* L. Afric. J. Biotech. 11: 9716-9723.

Ahmad, Y.A., Costet, J., Daugrois, H., Nibouche, S., Letourmy, P., Girard, J.C., Rott, P., 2007. Variation in infection capacity and in virulence exists between genotypes of Sugarcane yellow leaf virus. Plant Dis., 91: 253-259.

Atkinson, H., Dale, J., Harding, R., Kiggundu, A.,.Kunert, K., Muchwezi, J., Sagi, L., Viljoen, A., 2003. Engineering resistance to pathogenic fungi.Genetic transformation strategies to address the major constraints to banana and plantain production in Africa. International Plant Genetic Resources Institute (IPGRI), 125: 1-38.

Attaullah, S., Husain, I., Ali, F., Khan, A., Khan, G., 2012. Comparison of different techniques for the isolation of

Rhizoctonia solani from infected potato tubers. Greener J. Agric. Sci., 2: 212-219.

Baker, B., Zambryski, P., Staskawicz, B., Dinesh-Kumar, S.P., 1997. Signaling in Plant Microbe Interactions. Sci., 276:726-733.

Butt, T.M., Sahi, S.T., Chaudhary, K., Muhammad, S., 2008. Role of mass media for enhancing potato production in District Okara of Pakistan. Ind. Res. J. Ext. Edu., 8: 16-18.

Field, A., 2013. Discovering statistics using IBM SPSS statistics, Sage.

Gerson, R.L.F., Andrade, L.B., Oliveira, M.F., Muller, N.T.G., Faria, T.C., 1997. *In Vitro* multiplication of potato (*Solanum tuberosum* L.) cv. Cristal under different MS Salt and sucrose concentrations. Hortic. Sci., 32(3): 471.

Ghosh, S., Molla, K.A., Karmakar, S., Datta, S.K., Datta, K., 2016. Enhanced resistance to late blight pathogen conferred by expression of rice oxalate oxidase 4 gene in transgenic potato. Plant Cell, Tissue Organ. Cul., 126: 429-437.

Hide, G.A., Hirstand, J.M., Stedman, O.J.,1973. Effects of black scurf (*Rhizoctonia solani*) on potatoes. Ann. Appl. Biol., 74(2): 139–148.

Hussain, I., 2007. Development of resistance against fungal pathogens in potato by genetic transformation. PMAS-Arid Agriculture University, Rawalpindi.

Islam, W., Awais, M., Noman, A.,Wu, Z.,2016. Success of Bio Products against Bacterial Leaf Blight Disease of Rice caused by *Xanthomonas oryzae* pv. oryzae. PSM Microbiol., 1: 50-55.

Jabeen, N., Chaudhary, Z., Gulfraz, M., Rashid, H.,Mirza,B., 2015. Expression of Rice Chitinase Gene in Genetically Engineered Tomato Confers Enhanced Resistance to Fusarium Wilt and Early Blight. Plant Pathol. J., 31: 252-258.

Karmakar, S., Moll, K.A., Chanda, P.K., Sarkar, S.N., Datta, S., Datta K., 2016. Green tissue-specific co-expression of chitinase and oxalateoxidase 4 genes in rice for enhanced resistance against sheath blight. Planta., 243: 115-130.

Khan, B., Haq, I., Iftikhar, S., Aslam, M., 1985. Occurrence of late blight of potato in Pakistan. Pak. J. Bot. 17(1): 163.

Koch, R., 1880. Investigations into the etiology of traumatic infective diseases.New Sydenham Society.

Kovács, G., Sági, L., Jacon, G., Arinaitwe, G., Busogoro, J.P., Thiry, E., Strosse, H., Swennen, R., Remy, S., 2013. Expression of a rice chitinase gene in transgenic banana ('Gros Michel', AAA genome group) confers resistance to black leaf streak disease. Trans. Res., 22: 117-130.

Lin, W., Anuratha, C., Datta, K., Potrykus, I., Muthukrishnan, S., Datta, S.K., 1995. Genetic engineering of rice for resistance to sheath blight. Nat. Biotech., 13: 686-691.

Maziah, M., Sreeramanan, S., Puad, A., Sariah, M., 2007. Production of transgenic banana cultivar, Rastali (AAB) via Agrobacterium-mediated transformation with rice chitinase gene. J. Plant Sci., 2: 504-517.

Munir, F., Hayashi, S., Batley, J., Naqvi, S.M.S.,Mahmood, T., 2016. Germin-like protein 2 gene promoter from rice is responsive to fungal pathogens in transgenic potato plants. Fun. Integr. Geno., 16(1): 19-27.

Murashige, T., Skoog, F.,1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Planta. 15: 473-497.

Naz, F., Rauf, C.A., Abbasi, N.A., Haque, I., Ahmad, I., 2008. Influence of inoculum levels of *Rhizoctonia solani* and susceptibility on new potato germplasm. Pak. J. Bot., 40(5): 2199-2209.

Nishizawa, Y., Hibi, T., 1991. Rice chitinase gene: cDNA cloning and stress-induced expression. Plant Sci., 76: 211-218.

Okello, J.J., Zhou, Y., Kwikiriza, N., Ogutu, S.O., Barker, I., Schulte-Geldermann, E., Atieno, E., Ahmed, J.T., 2016. Determinants of the Use of Certified Seed Potato among Smallholder Farmers: The Case of Potato Growers in Central and Eastern Kenya, Agricul. 6: 55.

Prasad, K., Bhatnagar-Mathur, P., Waliyar, F., Sharma, K.K., 2013. Overexpression of a chitinase gene in transgenic peanut confers enhanced resistance to major soil borne and foliar fungal pathogens. J. Plant Biochem. Biotech., 22: 222-233.

Punja, Z.K., 2001. Genetic engineering of plants to enhance resistance to fungal pathogens-a review of progress and future prospects, Can. J. Plant Pathol. 23: 216-235.

Punja, Z.K., Zhang, Y.Y., 1993. Plant chitinases and their roles in resistance to fungal diseases. J. Nemat., 25: 526-540.

Rabbani, A., Askari, B.,Abbasi, N.,Bhatti,M.,Quraishi, A., 2001. Effect of growth regulators on in vitro multiplication of potato. Int. J. Agric. Biol., 3:181-182.

Rajesh, T., Maruthasalam, S., Kalpana, K., Poovannan,K., Kumar, E., Kokiladevi, D., Sudhakar, R.,Samiyappan, P., 2016. Stability of sheath blight resistance in transgenic ASD16 rice lines expressing a rice chi11 gene encoding chitinase. Biologia Plantarum. 60: 749-756.

Rauf, C.A., Ashraf, M., Ahmad, I., 2007. Management of black scurf disease of potato. Pak. J . Bot., 39(4): 1353-1357.

Romberg, M.K., Davis, R.M., 2007. Host range and phylogeny of *Fusarium solani* from potato and tomato in California. Plant Dis., 91: 585-592.

Sarkar, R.H., Mustafa, B.M., 2002.Regeneration and *Agrobacterium* –mediated genetic transformation of two indigenous potato varieties of Bangladesh. Plant Tissue culture.12(1):69-77.

Shafique, S., Shafique, S., 2012. Biological Control Potential of *Parthenium hysterophorus* against *Fusarium solani*- A Cause of Fusarium Wilt in Potato. International Conference on Applied Life Sciences (ICALS2012): 315-320.

Tsror, L., Peretz-Alon, I., 2005. The influence of inoculum source of *Rhizoctonia solani* on development of black scurf on potato. J. Phytopathol., 153: 240-244.

Walter, R.S., Rosemary, L., Gary, D.F., Weingartner, D.P., 2011. Compendium of potato diseases (APS compendium of plant disease series). APS Press, The American Phytopathological Society, St. Paul.

Watanabi, T., 2002. Pictorial atlas of soil and seed fungi morphologies of cultured fungi and Key to Species.2nd edi. CRC press USA.

Wharton, P., Hammerschmidt, R., Kirk, W., 2007. Fusarium dry rot, Michigan State University Extension.

Zanoni, U., 1991. Potato atlas and compendium of Pakistan: Potato production by agro-ecological zones. Publisher: Pakistan Agricultural Research Council; 2nd edition, p. 258.