International Journal of Scientific Research in Biological Sciences **Research Paper**
Vol.6, Issue.3, pp.56-64, June (2019) **E-ISSN:** 2347-7520
DOI: https://doi.org/10.26438/ijsrbs/v6i3.5664

To Raise the Polyclonal Immuno-Probe(s) and Development of Enzyme Immuno-Assay for Detection of Cross Reactivity of *Alternaria brassicae* and their Different Isolates

Amardeep Singh[1*], Gyanika Shukla[2] and Shailendra Singh Gaurav[3]

[1,2,3] Dept. of Genetics and Plant Breeding, Chaudhary Charan Singh University, Meerut, India

Corresponding author: amardeep95singh@gmail.com, Mobile No. +91-8126849594

Available online at: www.isroset.org

Received: 06/May/2019, Accepted: 17/Jun/2019, Online: 30/Jun/2019

Abstract- *Alternaria brassicae* is a fungus commonly found in South-East Asia, is one of the most common plant pathogenic fungi in *Brassica* crops. Its toxic activities have been proved. Hence, a sensitive, rapid and inexpensive screening test to detect *Alternaria brassicae* in agricultural commodities is necessary to protect *Brassica* crops. In the present study, the proteins of *Alternaria brassicae* were isolated, prepared, characterized and used as an antigen to raise polyclonal antibodies (pAbs) against it. Rabbits were immunized by the antigen in combination with Freund's adjuvant. Immuno blotting analysis was performed to determine antibody specificity towards the antigen. Hybridoma supernatants were screened by enzyme-linked immuno- sorbent assay (ELISA), dot-blot and slot-blot tests which confirmed the presence of specific Antigen-Antibody interactions among pAbs and *Alternaria brassicae* antigens. It was observed that most of the pAbs cross-reacted with the fungi. It was concluded that development of highly specific monoclonal antibodies (mAb) against the specific strain of *Alternaria brassicae* can be done in near future. It will be used for the disease diagnostic and identification of infection level.

Keywords: *Alternaria brassicae,* Alternaria blight, Immuno blotting, ELISA, DOT BLOT, SLOT BLOT.

I. INTRODUCTION

The family *Brassicaceae* includes a number of vegetables of worldwide importance and most of these are native to Europe, Middle East or Asia [1]. It is extensively grown traditionally as a pure crop as well as intercrop (mixed crop) in marginal and sub-marginal soils in the eastern, northern and north western states of India. The crop is grown during Rabi season under irrigated as well as rain-fed conditions. Despite considerable increase in productivity, a wide gap exists between yield potential and yield realized at farmer's field, which is largely due to biotic and abiotic stresses. Among biotic stress, white rust caused by *Albugo candida,* Kuntze and Alternaria blight caused by *Alternaria brassicae* have been reported to be most wide spread and destructive fungal diseases of rapeseed-mustard throughout the world [2]. Aflatoxins which are readily found in plant pathogenic fungi are acutely toxic, immunosuppressive, mutagenic, teratogenic and carcinogenic compounds that are present [3]. *Brassica* (rapeseed-mustard) is the second most important edible oilseed crop in India after groundnut and accounts for nearly 30% of the total oilseeds produced in the country [4]**.** When compared to other edible oil crops, rapeseed/ mustard oil has the lowest amount of harmful saturated fatty acids. It also contains adequate amounts of the two essential fatty acids that are linoleic and linolenic, which are not present in many of the other edible oils [5]. The North Central Regional Plant Introduction Station has maintained about 2000 accessions of *Brassica* representing 20 species. *Brassica* oils are generally rich in long multi-unsaturated omega 3 fatty acids [6]. The world's production of rapeseed mustard has been increasing at a rapid rate in several countries largely in response to the continuing increase in demand for edible oils and its products [7]**.** Many diseases are known to occur on *Brassica* crops in India [8]. The production of Rapeseed-Mustard is widely affected by rapeseed-mustard diseases *viz* Alternaria blight [*Alternaria brassicae* (Berk.) Sacc.], white rust [*Albugo candida* (Pers.) Kuntze] and White rot [*Sclerotinia sclerotiorum* (Lib.) de Bary]. Alternaria blight is a common disease in rapeseed mustard not only in India but also in all the continent of the world with no proven source of resistance against the disease reported till date in any of the host [9]. Chlorotic toxin was identified as destruxin B in *Alternaria brassicae*, which activates a signal transduction pathway leading to perturbations in cell cycle and expression of cell death proteins like p53 and Caspases [10].

© 2019, IJSRBS All Rights Reserved

Interestingly, the *Alternaria brassicae* toxin mediated cell death pathway is antagonized by cytokinin, zeatin in cell culture of *Brassica* [11].

Early detection and correct identification of fungal pathogens is of primary importance in determining the most effective course of treatment to prevent the spread of fungi causing plant disease and post harvest storage losses. For preventing this problem polyclonal antibodies (pAbs) were raised against total lypholised antimycelial fungal protein in rabbit. Antigens are substances that are foreign to the body and induce an immune response. Bacteria, viruses and fungi are common source of environmental antigens encountered in daily life. Exposure to fungal mycelial antigens can elicit both humoral and cellular immune responses. During a humoral immune response, most antigens activate many different lymphocytes B, which then proliferate and differentiate into individual clones of antibody-secreting plasma cells; such an immune response can be referred to as polyclonal.

Alternaria brassicae is recognized as an important aeroallergen indoors and outdoors. Exposure to the fungus has been identified by Enzyme Linked Immuno Sorbent Assay (ELISA), dot blot, and slot blot etc. A report concluded that 95% to 99% of American homes contained detectable amounts of *Alternaria* antigens when analyzed with polyclonal antibodies (pAbs) based ELISA [12].

The present study was conducted in 'Plant Stress laboratory' of Department of Molecular Biology and Genetic Engineering (MBGE), Pantnagar (Uttrakhand, India), with the objective to develop the disease diagnostic methods and identification of infection level of *Alternaria brassicae* in *Brassica* crops.

II. MATERIALS AND METHODS

Collection of samples

Infection was identified as the infected leaves shows typical brown to black spots on surface of crops. *A. brassicae* infected leaves from different varieties of *B. juncea* (ASHIRWAD, DIVYA, JD6, KANTI, KRANTI, LOCAL MUSTERED, NDRE4, PRE2007, PRE2010 and VARUNA) were collected (3gm wet and 400mg dry sample) and preserved in Plant Stress lab of Department of MBGE, Pantnagar. Fungal mycelia were mechanically lysed by grinding in liquid N_2 with pestle and mortar. Extraction buffer is added to further lyse fungal cells and solubilise the fungal proteins. Enzyme systems for fungal/yeast cell lysis are usually a mixture of several different enzymes, including one or more β (1→3) glucanase (lytic and nonlytic), protease, β (1→6) glucanase, mannanase and chitinase, which act synergistically for lysing the cell wall. Enzymatic cell lysis of yeast begins with binding of the lytic proteases to the outer manno-protein layer of the wall. The protease opens up the protein structure, releasing wall proteins and mannans and exposing the glucan surface below. The glucanase then attacks the inner wall and solubilizes the glucan. *In vitro*, this enzyme cannot lyse fungi in absence of reducing agents, such as dithiothreitol or b-mercaptoethanol, because the breakage of disulphide bridges between mannose residues and wall proteins is necessary for appropriate exposition of the inner glucan layer [13]. Extraction buffer contains 50 mM Tris-base, 50 mM EDTA, 0.39% ascorbic acid, 0.3% β-mercaptoethanol, 2mM Phenyl Methane Sulfonyl Fluoride (PMSF), 4% Polyvinyl Pyrolidone (PVP).

Preparation of anti-mycelial antibodies against whole lyophilised fungal mycelium

Fifteen days old mycelia were harvested from liquid PDB culture media. It was lyophilised and stored at -20˚C for further use. Complete Freund's adjuvant (CFA) and Freund's incomplete adjuvant (FIA) adjuvants were used. Phosphate buffer saline (PBS) buffer (0.15 M, pH 7.4) was used to prepare the antigen emulsions for immunization. For the production of polyclonal antibodies, ten different fractions of *A. brassicae* were used to generate antisera. These fractions represent distinct approaches to produce polyclonal antisera against surface antigen.

Preparation of antiserum

The blood samples (2 ml) were first clotted at room temperature by resting them in glass tubes for 2hr and kept overnight at 4˚C. The clear sera were decanted into 1.5 mL tubes and were spun at 4000 rpm for 10 min at 4˚C to get rid of any remaining red blood cells. The straw colour clear supernatant was transferred into separately capped cryovials and stored at -20˚C.

Protein estimation

Total soluble protein was estimated in all the samples using Bradford's method [14]. In this method 12 glass test tubes were taken with concentration 5, 10, 20, 40, 60, 100 µl of Bovine Serum Albumin (BSA) in duplicate. Add extraction buffer/ double distilled water to make volume up to 300µl. Add 3 ml of dye in each tube. Incubate them for 15 min at room temperature.

© 2019, IJSRBS All Rights Reserved

Absorbance of all samples was taken at 595 nm in spectrophotometer. Standard graph between the BSA concentration and absorbance was drawn.

Slot Blot Analysis for Antigen-Antibody Interaction

Slot blot is an efficient technique for detection of proteins and it is very rapid and semi quantitative technique in nature. In this method nitrocellulose (NC) membrane was washed in sterile distilled water, dried and imprint made by dot blot apparatus. Aliquots of sample (250ng) containing required amount of antigen of *A. brassicae* were spotted on to the glossy/dull surface of nitrocellulose membrane and allowed to dry for 15 min. NC membrane was treated with 5.0 % blocking solution for 2 hrs and further washed thrice with PBS (0.15M, pH 7.5) and 0.05% skimmed milk for 5 min. Then NC membrane was incubated with primary antibody in dilution buffer (1:250) at room temperature for 2 hrs and then washed thrice with PBS (0.15M, pH 7.5) and 0.05% skimmed milk for 5 min. Further the NC membrane was incubated with alkaline phosphatase conjugated to secondary antibody for 1 hr. Antibody was diluted to 1:1000 in antibody dilution buffer. The membrane was blotted 3 times (5min. each) in the PBS+0.05% skimmed milk. Colour development substrate solution was added and incubated for 10 min. The reaction was stopped by adding distilled water and removing the substrate. Different isolate samples were dropped on nitrocellulose membrane and held till the sample dried.

Indirect ELISA for cross reactivity patterns of different isolates of *A. brassicae*.

Lyophilized mycelium were taken and dissolved in PBS at the rate of 100 mg/ml. This was homogenized in motorized hard homogenizer. Resultant solution was used as antigen. Polystyrene micro plates were coated with mycelia fragments. Antigen fractions were dissolved in coating buffer into different concentrations. About 100µl of respective antigen was delivered to a well. Antigen coated plates were incubated for 1 hr at room temperature followed with overnight incubation at 4˚C. The plates were washed thrice with PBS+Tween-20 (0.01%). The plates were filled with blocking solution containing PBS and 5% skimmed milk and incubated for 2 hr at room temperature. Washing was done to prevent adventitious binding. About 100µl of anti-mycelial primary antibodies (raised against respective antigens) were added and incubated for 2 hrs at room temperature. The antibody dilution was done with PBS + 0.025% skimmed milk (1:250). Again washing was done thrice with PBS + Tween-20 (0.01%) + 0.25% skimmed milk. Further, 100µl of 1:1000 diluted alkaline phosphate conjugated secondary antibodies were incubated for 2 hr at room temperature. The plates were washed thrice with PBS containing 0.25% skimmed milk + Tween-20 (0.01%). Alkaline phosphate activity was assayed with p-nitrophenyl phosphate sodium salt dissolved in substrate buffer (1 mg/ml). The plates were incubated for 30 min in dark and the reaction was stopped with 100µl of 1.5 M NaOH solution. This allowed soluble and secreted antigens to bind to the ELISA well surface and thus to be retained in the ELISA well until the completion of the assay. Optical density (OD) was determined spectrophotometrically at 405 nm after a substrate incubation time (SIT) of 30 minutes using an Ultra Microplate Reader, Model ELx800 (BIO-TEK Instruments, Inc.,Winooski, VT).

III. RESULTS AND DISCUSSION

Antibodies have been used in studies of plant pathogen interaction for many years. Using *Alternaria blight* as a model fungus we sought to demonstrate that polyclonal antibodies (pAbs) can be used to investigate immunological cross-reactivity patterns among fungi.

Development of anti-mycelial antibodies against total lyophilized mycelial antigen

Polyclonal Ab (IgG1) reacted only with spore preparations but not mycelia of *A. blight* or propagules of any other fungus. The polyclonal antisera developed to different fungal immunogen preparations displayed a range of specificity similar to results obtained by Wycoffk *et al* (1987) used a similar method to raise antibodies to mycopathogens but their antigenic material was not necessarily limited to surface molecules, since they included Merthiolate in the bathing solution [15]. Also, Hardhama *et al* (1987) in their experiment raised Antisera SBR1A and SBR1B, against nongerminated urediniospores that reacted positively with both *Phytophthora pachyrhizi* and *P. meibomiae* urediniospores in ELISA; while antiserum SBR2, produced against intact germinated urediniospores, reacted only with *P. pachyrhizi* urediniospores. Antisera SBR3 and SBR4, produced against crude extract of pulverized germinated urediniospores and cell walls of pulverized germinated urediniospores, respectively [16]. Very little is known about the sites and nature of species-specific antigens in fungi but our results are consistent with those of Notermans et al (1986) and Dewey et al (1989), who raised mAbs to extracellular glycoproteins of *P. megasperma* f. sp. *Glycinea* and zoospores of *P. cinnamomi,* respectively [17, 18]. Dewey *et al* (1990) developed a relatively specific antiserum that recognized only species of *Penicillium* [19]. However, it has now been shown that it is possible to raise mAbs of varying specificity against fungi and use these to develop immunoassays [20, 21].

Protein quantification of ten different isolates of *A. brassicae* using Bradford method

© 2019, IJSRBS All Rights Reserved

Total protein ware extracted from the lyophilised fungal mycelia of nine isolates of *A. brassica* by using 0.15 M PBS. The amount of protein was estimated by Bradford method of each isolates as shown in table 1. The concentration of protein obtained from different samples was shown in table 2. Figure 1 was used to determine the protein content in the unknown sample with the help of graph.

Table 1: Bradford method

BSA stock solution	Extraction buffer/double distilled water	Total Volume (μl)	Dye	O.D (595nm)	Conc.
5μl	295μl	300μl	3ml	0.038	1μg
10μl	290μl	300μl	3ml	0.072	2μg
20μl	280μl	300μl	3ml	0.095	4μg
40μl	260μl	300μl	3ml	0.160	8μg
60μl	240μl	300μl	3ml	0.219	12μg
100μl	200μl	300μl	3ml	0.287	20μg
Blank	300 μl	300μl	3ml	0.293	-----

Table 2: Determination of protein concentration of *A. brassicae* isolates

S. No	*A. brassicae* isolates	Absorbance OD at 595 nm	Protein concentration (mg/ml)
1	PRE-07	0.8213	2mg/ml
2	JD-6	0.8198	1.94mg/ml
3	KRANTI	0.7896	1.8mg/ml
4	LM	0.7765	1.74mg/ml
5	NDRE-4	0.6797	1.59mg/ml
6	AW	0.7832	1.78mg/ml
7	DIVYA	0.8190	1.93mg/ml
8	PRE-10	0.6587	1.49mg/ml
9	VARUNA	0.8076	1.90mg/ml
10	KANTI	0.5187	1.22mg/ml

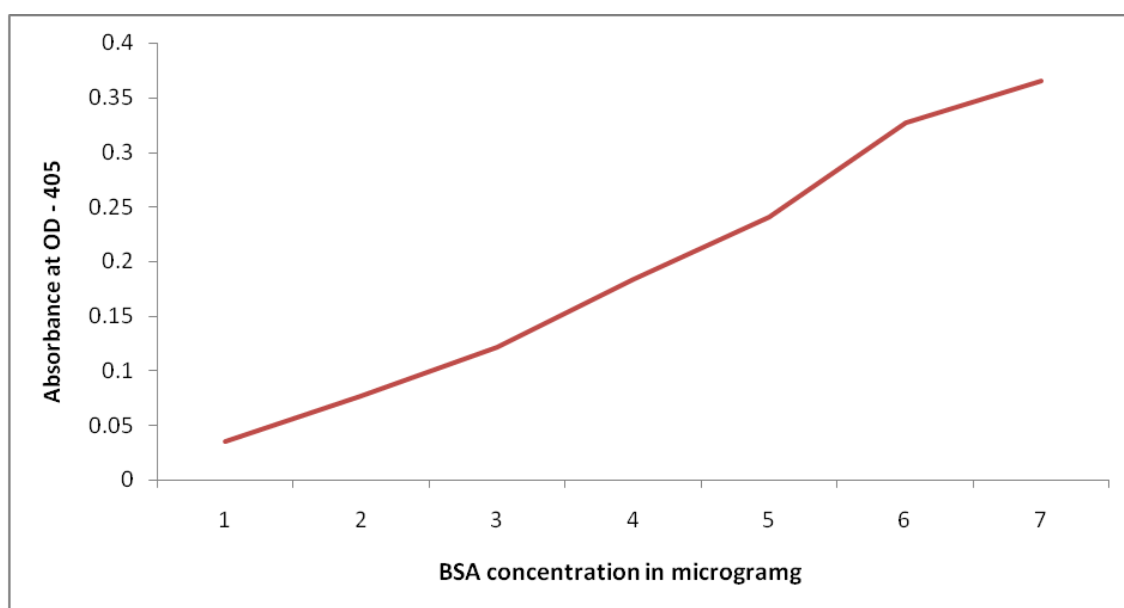


Figure 1: Preparation of standard curve.

© 2019, IJSRBS All Rights Reserved

Optimization of Appropriate Concentration of Mycelial Antigens

Indirect ELISA was performed for checking the cross reactivity in *A. brassicae* isolates causative agents of Alternaria blight disease of *Brassica*. The graph was plotted (O. D. at 405) against mycelial antigen in ng as shown in figure 2 and it was observed that the 250 ng of antigen concentration was found optimum for the development of immuno-assay using anti-mycelial antibodies by ELISA. Tabular representation of antigen kinetics of fungal antigen is shown in table 3.

Table 3: Tabular representation of antigen kinetics of fungal antigen.

Mycelial antigen in ng	Absorbance OD at 405 nm
50	0.638
100	0.659
250	0.7545
500	0.6080
1000	0.4515
2000	0.4595
4000	0.3835
8000	0.378

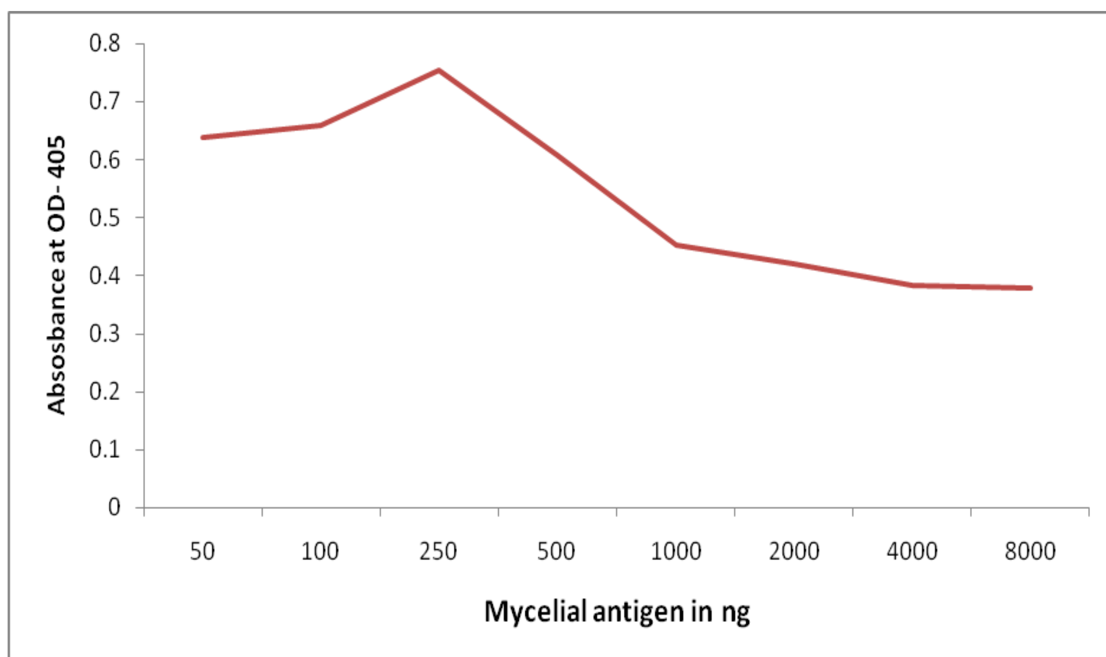


Figure 2: Antigen kinetics for determination of optimal mycelial antigen concentration of *A. brassicae* for development of immuno-assay using anti-mycelial antibodies.

Determination of Titre of Anti-Mycelial Antibody

In order to determine the cross reactivity of *A. brassicae* isolate/strains, mycelial-antigen antibody from all isolates of (1:50, 1:100, 1:250, 1: 500, 1:1000, 1:2500, 1:5000, 1:10000, and 1:20000) were subjected to indirect ELISA. Thus 1:5000 was determined as titre of anti-mycelial antibody raised against mycelial antigen. As per the results obtained through indirect ELISA based colorimetric test, it was concluded that 1:250 antibody dilution was appropriate for performing indirect ELISA as show in figure 3. Tabular representation of anti-mycelial antibodies dilution as indirect ELISA Absorbance values (405 nm) is represented in table 4.

Table 4: Tabular representation of anti-mycelial antibodies dilution as indirect ELISA Absorbance values (405 nm)

Anti-mycelial antibodies dilution	Absorbance at 405 nm
1:100	0.946
1:250	0.873
1:500	0.842
1:1000	0.663
1:2500	0.480

© 2019, IJSRBS All Rights Reserved

1:5000	0.169
1:10000	0.146
1:20000	0.021

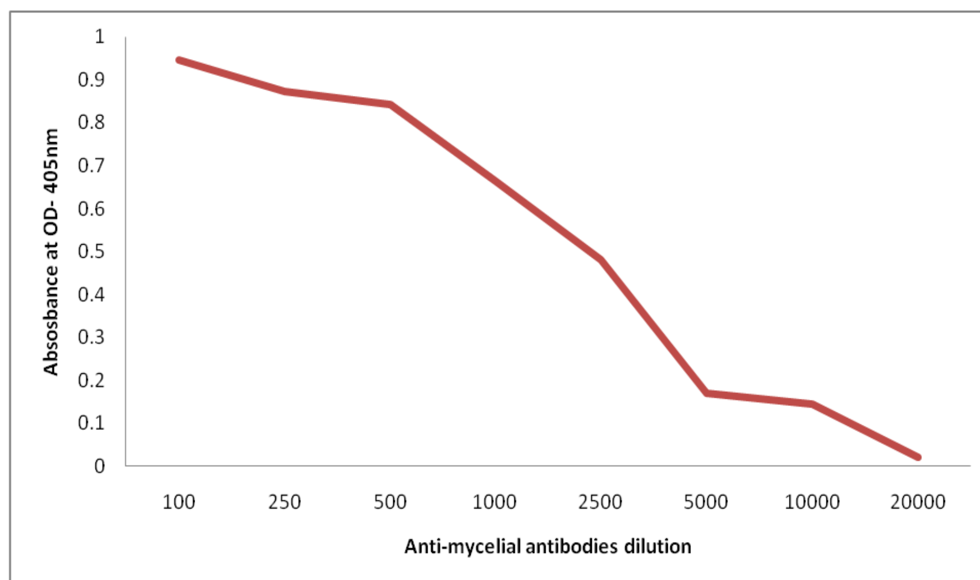
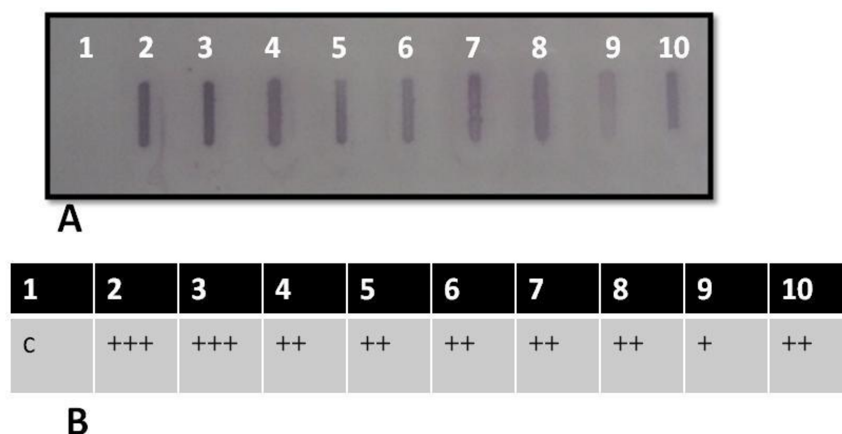


Figure 3: Determination of titre of anti-mycelial antibody for development of Indirect ELISA by Performing Antibody dilution curve analysis.

Detection of Antigen-Antibody Complexes Through Slot Blot

Cross-reactivity of *Alternaria brassicae* antiserum was tested against 250 ng antigens of 10 isolates (PRE-7, JD-6, KR, LM, KANTI ND-4, AW, DV, PRE-10, VR) of *Alternaria brassicae*. Total protein was extracted from lyophilized mycelia pathogen and cross reactivity patterns with anti-mycelial serum show positive response by immuno Slot Blot technique. Purple color was observed with all the tested *Alternaria brassicae* isolates. On the basis of color intensity, two isolates (PRE-07, JD-6) showed maximum antigen-antibody interaction; six isolates (KRANTI, LM, AW, DV, VR) showed intermediate interaction while PRE-10 showed minimum interaction as shown in figure 4.



1	2	3	4	5	6	7	8	9	10
C	+++	+++	++	++	++	++	++	+	++

Figure 4: Cross reactivity studies between different strains of *A. brassicae* by Slot Blot assay using anti-mycelial antibody.

Cross Reactivity Patterns of Different Isolates of *Alternaria Brassicae* through Indirect ELISA

A. brassicae exhibits high diversity, among as well as within the species. These variations similarly reflect in their pathogenic behaviours. Different *A. brassicae* show different virulence levels. These intra-species pathogen fungi showed cross-reactivity with anti-mycelial antibodies raised against whole mycelial antigen. PRE-2007 and VARUNA showed maximum reactivity

© 2019, IJSRBS All Rights Reserved

with anti-mycelial antibodies strongly cross reacting with mycelium while PRE-2010 showed minimum cross reactivity with antibodies as shown in figure 5. Tabular representation of cross reactivity of *A. brassicae* isolates is shown in table 5.

Table 5: Tabular representation of cross reactivity of *A. brassicae* isolates.

***Alternaria brassicae* isolates**	**Absorbance at 405 nm**
PRE-07	0.597
JD-6	0.567
KRANTI	0.521
LOCAL MUSTARD	0.476
MNRE-4	0.498
ASHIRWAD	0.489
DIVYA	0.505
PRE-10	0.441
VARUNA	0.571
KANTI	0.456

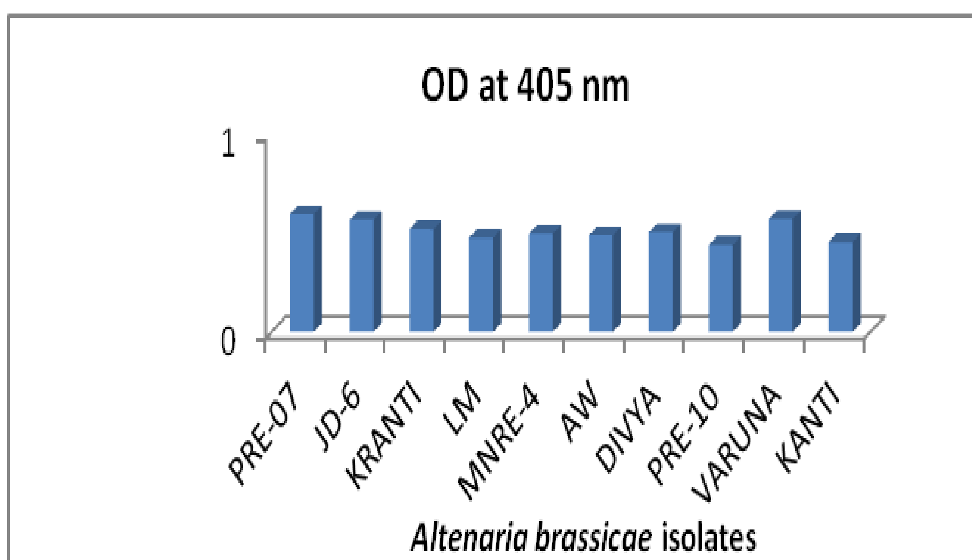


Figure 5: Cross reactivity studies between different strains of *A. brassicae* by indirect ELISA assay using anti-mycelial antibody and Dilution of primary antibody was 1:250, Dilution of secondary antibody was 1:1000, Antigen concentration was 250ng.

In an alternative approach, a prototype rotating-arm sampler was developed and designed for use with ELISA, to detect and enumerate airborne spores [22]. ELISA has been proven to be useful for monitoring the antigenic properties in the process of population shift during the determination of genetic diversity among the pathogens and subsequently for immune-pathotyping [23].

SDS-PAGE Analysis of Mycelial Proteins of Different Isolates of *Alternaria Brassicae*

The SDS-PAGE analysis of proteins showed distinct banding pattern for each isolates. A 12% SDS-PAGE gel was used which was capable of resolving polypeptides and proteins ranging from 10 kDa to 100 kDa. Only proteins in the range of 14-100 kDa were detected in the mycelial proteins of *A. brassicae* by coomassie brilliant blue (R) staining (Figure 6). Unique bands were detected in lane 4, lane 6 and lane 10 with molecular weights of 35 kDa, 66 kDa and 29 kDa respectively, which might be useful in making differential diagnosis with other isolates of *A. brassicae* and other fungal pathogens.

Many common bands were observed in PRE-07, Kanti, Divya, Varuna, JD6. These were categorised into low, medium and high molecular weight range that are 20-35 kDa, 38-50 kDa and 60-100kDa respectively. The mycelial protein of *A. brassicae* showed 4 bands in low molecular weight range, 6 in medium molecular weight range and 5 in high molecular weight range.

© 2019, IJSRBS All Rights Reserved

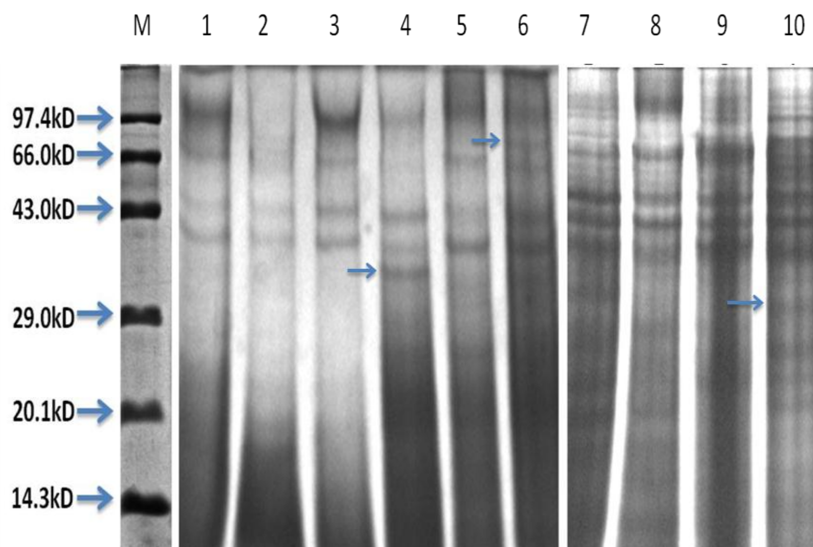


Figure 6: SDS-PAGE protein profiling of *Alternaria brassicae* antigens (Total buffer soluble protein), Loading, M- Medium range molecular weight marker, Lane 1-PRE-7, Lane 2-Kanti, Lane3-PRE-10, Lane4-ASH, Lane 5- Divya, Lane 6-Varuna, Lane 7-JD6, Lane 8-KRANTI, Lane9-LM, Lane10-NDRE4,(30µg/well).

IV. CONCLUSION

Serological (antibody based) techniques have been used for identification and taxonomic classification of plant pathogen as well as for diagnosing infection levels. Immunoassays have been very successfully used for a number of years for the detection of diseased plants. In this study, the potential development of polyclonal antibodies was done; it was highly reactive but non-specific for intra-species and inter-species identification pathogen of *A. Brassicae*. Antimycelial polyclonal antibodies can be used for the detection of phylogenetic relationship between strain and species of *A. brassicae.* With the results and outcomes of 2D-PAGE (two dimensional-polyacrylamide gel electrophoresis), GC (Gas Chromatography) and SDS-PAGE profiling, we conclude that highly specific monoclonal antibodies against the specific strain of *A. brassicae* may be developed in near future. It will be useful for the disease diagnostic and identification of infection level of pathogen.

ACKNOWLEDGEMENT

The authors of this paper are highly thankful to Dr. Gohar Taj (Associate Professor at Department of Molecular Biology and Genetic Engineering, College of Basic Sciences and Humanities, GBPUA&T, Pantnagar, UK, India) for kindly providing the place of research work and facilitating with all the necessities whenever required.

REFERENCES

[1] G. R. Dixon, “Origins and diversity of *Brassica* and its relatives”. Vegetable Brassicas and related crucifers, pp.1-33, 2006.
[2] Abhishek, R. Prasad, D. Kumar, V. Kumar, M. Kumar, D. Kumar, “Symptoms and Management of White Rust/ Blister of Indian Mustard (*Albugo candida*)”, International Journal of Current Microbiology and Applied Sciences Vol. 6, Issue 10, pp. 1094-1100, 2017.
[3] C. M. Abraham, T. M. Thomas, “Emerging fungal contaminants isolated and identified from raw fennel seeds”, International Journal of Scientific Research in Biological Sciences Vol. 5, Issue 3, pp. 32-35, 2018.
[4] P. D. Meena, R. P. Awasthi, C. Chattopadhayay, S. J. Kolte, A. Kumar, “Alternaria blight: a chronic disease in rapeseed-mustard”, Journal of Oilseed Brassica Vol. 1, Issue 1, pp. 1-11, 2010.
[5] S. C. Manchanda, S. J. Passi, “Selecting healthy edible oil in the Indian context”, Indian heart journal Vol. 68, Issue 4, pp. 447-449, 2016.
[6] R.O. Vles, “Nutritional characteristics and food uses of vegetable oils”, Oil Crops of the World, Their Breeding and Utilization pp.63-86, 1989.
[7] G. S. Saharan, “Management of rapeseed and mustard diseases”, Advances in oilseeds research Vol. 1, pp.152-188, 1992.
[8] P. D. Meena, C. Chattopadhyay, V. R. Kumar, R. L. Meena, U. S. Rana, “Spore behaviour in atmosphere and trends in variability of *Alternaria brassicae* population in India”, Journal of Mycology and Plant Pathology Vol. 35, p.511, 2005.
[9] A. Agarwal, G. K. Garg, S. Devi, D.P. Mishra, U. S. Singh, “Ultrastructural changes in *Brassica juncea* leaves upon treatment with *Alternaria brasicae* toxin”, Journal of Plant Biochemistry and Biotechnology Vol. 6, pp. 25-28, 1997.
[10] O. Salazar, C. Basso, P. Barba, C. Orellana, J.A. Asenjo, “Improvement of the lytic properties of a b-1, 3- glucanase by directed evolution”, Molecular Biotechnology Vol. 33, pp. 211–220, 2006.

© 2019, IJSRBS All Rights Reserved

[11] K. Marmath, P. Giri, S. Sharma, D. Pandey, A. Kumar, "Induction of MAPK-4 during zeatin-Alternaria brassicae challenge in host, non-host and tolerant transgenic brassica against alternaria blight and *In-Silico* prediction of its upstream kinases", Journal of Natural Science, Biology and Medicine Vol. 2 Issue 3, 2011.

[12] D. Schmechel, B. J. Green, F. M. Blachere, E. Janotka, D. H. Beezhold, "Analytical bias of cross-reactive polyclonal antibodies for environmental immunoassays of *Alternaria alternate*", Journal of Allergy and Clinical Immunology Vol. 121, Issue 3, pp.763-768, 2008.

[13] O. Salazar, J. A. Asenjo, "Enzymatic lysis of microbial cells", Biotechnology letters Vol. 29, Issue 7, pp.985-994, 2007.

[14] M. M. Bradford, "A rapid and sensitive method for the estimation of microgram quantities of protein utilizing the principle of protein- dye binding", Analytical Biochemistry, Vol. 72, pp. 248-254, 1976.

[15] L. Wycoffk, J. Jellison, A. R. Ayers, "Monoclonal antibodies to glycoprotein antigens of a fungal plant pathogen *Phytophthora megasperma* f. sp. Glycinea", Plant Physiology Vol. 85, pp. 508- 515, 1987.

[16] E. Suzaki, J. L. Perkin, "Monoclonal antibodies to isolate-, species-, and genus-specific components on the surface of zoospores and cysts of the fungus *Phytophthora cinnamomi*", Canadian Journal of Botany Vol. 64, pp.311-321, 1986.

[17] S. Notermans, G. Wieten, H. W. B. Engel, F. M. Rombouts, P. Hoogerhout, J. H. Van Boom, J.H., "Purification and properties of extracellular polysaccharide (EPS) antigens produced by different mould species", Journal of applied bacteriology, Vol. 62, Issue 2, pp.157-166, 1987.

[18] F. M. Dewey, C. J. Munday, C. M. Brasier, "Monoclonal antibodies to specific components of the Dutch elm disease pathogen *Ophiostoma ulmi*", Plant pathology Vol. 38, Issue 1, pp.9-20, 1989.

[19] F. M. Dewey, M. M. MacDonald, S. I. Phillips, R. A. Priestley, "Development of monoclonal-antibody-ELISA and -DIP-STICK immunoassays for *Penicillium islandicum* in rice grains", Journal of General Microbiology Vol. 136, pp. 753-760, 1990.

[20] D. Schmechel, H. A. McCartney, N. Magan, G. Marshall, "A novel approach for immunomonitoring airborne fungal pathogens", In British Crop Protection Council Symposium Proceedings, 1996.

[21] D. Schmechel, H. A. McCartney, N. Magan, "The production and characterization of monoclonal antibodies against *Alternaria brassicae* (Berk.) Sacc., the cause of dark leaf and pod spot in oilseed rape", Food and Agricultural Immunology Vol. 9, pp. 219-232, 1997.

[22] S. Li, R.R. Marquardt, D. Abramson, "Immunochemical detection of molds: a review", Journal of Food Protection Vol. 63, pp. 281-291, 2000.

[23] F. M. Dewey, M. Macdonaldm, S. I Phillips, "Development of monoclonal-antibody-ELISA, -DOT-BLOT and -DIP-STICK immunoassays for *Humicola lanuginosa* in rice", Journal of General Microbiology Vol. 135, pp. 361-374, 1989.

AUTHORS' PROFILE

Mr. Amardeep Singh is presently pursuing PhD from Department of Genetics and Plant Breeding, Chaudhary Charan Singh University, Meerut (CCSU), UP, India. He worked as 'Research Assistant' at the 'Department of Biotechnology', CCSU, in a CST (UP) funded project during the years 2015 to 2018. He also worked as 'Guest Faculty' in the 'Department of Biotechnology', CCSU. He has contributed more than 10 research and review articles in national and international journals. His active areas of research are biotechnology, microbiology, immunology, bio-nanotechnology, genetics, plant breeding etc. He has received six 'Young Scientist Awards' in various international seminars and conferences for his contribution in academics and research.

Ms. Gyanika Shukla is presently pursuing PhD from Department of Genetics and Plant Breeding, Chaudhary Charan Singh University, Meerut (UP, India). She is also working as 'Guest Faculty' in the 'Department of Biotechnology', CCSU. She has qualified ICAR–NET in the year 2018. She had worked as Assistant Professor in Department of Biotechnology under Faculty of Science, Swami Vivekanand Subharti University, Meerut (NAAC 'A' Accredited) during the years 2013 to 2017. She has contributed more than 20 research and review articles in national and international journals. Her active areas of research and study are biotechnology, microbiology, bio-nanotechnology, genetics, plant breeding etc. She has received 'Young Achiever award' in "International Conference on allied and applied biotechnology" (ICAABT), held on 1-2 April, 2017, for her contribution in academics and research.

Prof. (Dr.) Shailendra Singh Gaurav is presently working as Professor and Head of the Department of Genetics and Plant Breeding, Chaudhary Charan Singh University (CCSU), Meerut (UP, India). Additionally, he is Coordinator of 'Department of Biotechnology' and 'Department of Seed Science & Technology' at CCSU. He is also the Nodal Officer of 'Intellectual Property Cell' and 'All India Survey on Higher Education'. Recently, he has been nominated as EC member of University of Allahabad, UP. He was awarded PhD in the year 2004, from Indian Agricultural Research Institute (IARI), Pusa campus, New Delhi. He has qualified ICAR-NET thrice in the years 1998, 1999 and 2001. He has more than 20 years experience in research, academics and administration. He is receiver of many fellowships and awards. He is an active editorial team member of 6 international/national journals. He has supervised 12 Doctorate degrees and more than 20 M.Phil degrees. He has more than 80 publications in various international/national journals and conferences. His active areas of research are Seed Science & Technology, Plant Biotechnology, Seed Pathology, Bio-nanotechnology.

© 2019, IJSRBS All Rights Reserved