

Identification and localisation of glycosyl moieties of surface glycoproteins of teliospore wall of Karnal bunt (*Tilletia indica*) of wheat

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(Received 10 February 2012; final version received 9 March 2012)

This article aims to characterise and localise the glycosyl moieties of teliospore wall of *Tilletia indica* a quarantined fungal pathogens by biochemical and immunological approaches. Chemical enzyme modifier studies, followed by determination of structural configuration using phase contrast and SEM after periodate treatment, showed antigenic entities are glycoprotein in nature. Further characterisation using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) glycoprotein staining and western blotting using anti-teliospore antibodies showed two common proteins of molecular weight 28 and 40 kDa, which is also suggestive of glycoprotein nature of antigenic entities of teliospore wall. To study the binding patterns and localisation of glycosyl moieties on the teliospore walls, fluorescein isothiocyanate (FITC) labelled lectins [Wheat Germ Agglutinin (WGA) and Concanavalin A (Con A)] and anti-teliospore antibodies were used. The patterns of WGA and anti-teliospore antibodies binding with teliospore wall are almost similar and hence it is quite reasonable to suggest that immunodominant glycosyl entities of teliospore wall are acetylglucosamine in nature.

Keywords: Karnal bunt; *Tilletia indica*; glycoprotein; lectin; Con A; WGA

Introduction

Karnal bunt (KB) is the major disease of wheat (*Triticum aestivum* L., *T. durum* and triticale) caused by the sporadic fungus, *Tilletia indica* (Syn *Neovossia indica*), and named after the city of Karnal, India, where this fungal disease was first reported on a wheat crop in 1931 (Mitra, 1931). The disease has been reported in India, Pakistan, Iraq, Afghanistan, Nepal (Joshi, Singh, Srivastava, & Wilcoxon, 1983; Singh, 1994; Warham, 1986), Mexico (Warham, 1986), from geographically isolated areas in the USA (Ykema, Floyd, Palm, & Peterson, 1996) and in South Africa (Crous et al., 2001). Within India it occurs frequently in the north-western plains of Himachal Pradesh, Punjab, Uttar Pradesh and also prevalent in Tarai regions of Uttarakhand. Although the pathogen has never been found in climates that prevail in Europe despite many opportunities to spread internationally (Jones, 2007), it is deemed to be

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a significant threat to European wheat crops also (Sansford et al., 2006). Its presence is noted by kernels containing masses of small black spores (22–49 µm diameter) that emit a fishy odour, making the flour and other wheat products from KB-infected wheat less palatable (Warham, 1986). Wheat containing 3% bunted kernels is considered unfit for human consumption.

The disease has gained significant importance not only because of qualitative and quantitative losses (0.2–0.5%) but also for reluctance on the part of many countries to accept wheat from Indian subcontinent. Worldwide distribution of *T. indica* is limited (Garrett & Bowden, 2002) but numerous wheat importing countries have quarantines against the import of wheat with KB, hence the presence of KB in a region seriously affects the international grain trade (Garrett & Bowden, 2002). Rush et al. (2005) lists 71 countries other than the USA with specific regulations on the importation of wheat from countries with *T. indica*, either through national regulations, or through being a member of a regional plant protection organisation. A quarantine pest has been defined as ‘a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled’ (Anon., 2006).

The teliospore of KB is an infectious entity and can be detected in the grains by simple microscopic techniques, but the teliospores of this fungus closely resemble teliospores of fungi causing bunts in other crops like rye and rice. These pathogens have similar teliospore texture but they differ in size. Similarity in teliospore configuration makes it difficult to differentiate KB teliospores from the teliospores of other bunt fungi. In order to determine the correct identity of contaminating fungi, it is essential to develop specific diagnostic probes, which could be used by the seed certification labs and plant quarantine departments.

Significant progress has been made in our lab to develop the rapid formats for immunodiagnosis of KB teliospores by using high titre polyclonal antibody probes raised against intact teliospores in New Zealand white rabbits (Gupta, Kumar, Lakhchaura, & Garg, 2001; Kumar, Singh, & Garg, 1998). The developed immunodiagnostic formats viz. Seed Immunoblot Binding Assay (SIBA), Indirect Immunofluorescent staining tests, Dyed Latex Bead Agglutination Assay and Immuno-dipstick Assay can be successfully employed for the sensitive detection of seed borne inoculum of the fungus present either in bunted seeds or as loose teliospores on the seed surface (Gupta, Kumar, Singh, & Garg, 2000; Kesari & Kumar, 2003; Kesari, Mishra, Garg, & Kumar, 2005; Kumar et al., 1998). However, the major obstacle is the specific detection of KB teliospores due to cross-reactivity of anti-teliospore antibodies with other bunt and related fungal pathogens especially with teliospores of *Tilletia barclayana*, a non-quarantined rice fungal pathogen, *Tilletia foetida* and also with spores of rust fungus *Puccinia recondita* indicates the common antigens or similarity in antigen configuration of these pathogens (Kesari and Kumar, 2003).

High-quality immunological reagents (specific immunoprobe against infectious entities) are required for the development of specific immunodiagnostic procedure which can be used for the management of a disease. Therefore, it is mandatory to characterise the antigenic configuration of teliospore walls of *T. indica* (Gupta, Narwal, Umapathy, Mall, & Kumar, 2009). Our present study is based on earlier findings and focuses on characterisation of potential antigens of the teliospore wall of *T. indica* by biochemical and lectin binding studies using scanning electron and fluorescent microscope, in the anticipation that characterisation of such antigens,

could be useful in the development of specific diagnostic immunoprobes for designing, optimisation and refinement of immunodiagnostic kits for single step identification and differential diagnosis of *T. indica*.

Materials and methods

Collection of teliospores of Tilletia indica

The teliospores of *Tilletia indica* were extracted from KB infected seed of wheat with the help of brush, needle, forceps and surgical blades. The teliospores were collected in a paper sheet and the debris was separated from the teliospores/spores by using a sieve.

Preparation of polyclonal antibodies

Albino, New Zealand white rabbits procured from IVRI, Bareilly, were used for production of anti-teliospore antibodies. The rabbits were immunised, by administering five injections through subcutaneous, intramuscular and foot pad routes. The immunogen was prepared using intact teliospores in phosphate buffered saline (PBS, pH 7.4) and emulsified with an equal volume of either Freund's complete adjuvant (FCA) or Freund's incomplete adjuvant (FIA). The first injection was given with 20 mg of teliospore suspension emulsified in FCA followed by three injections at biweekly intervals of 10 mg of spores in FIA. After three booster doses, the rabbits were bled and antiserum was collected from one hyper-immunised rabbit and straw coloured clear supernatant was transferred into capped cryo-vials and stored at -20°C . The antibody reactivity with teliospores of KB was checked using indirect immunofluorescent assay.

Development of indirect IFA

Indirect immunofluorescent assay was developed with little modification of method developed by Gupta et al. (2009). 0.5 mg teliospores of *T. indica* were washed three times with PBS and suspended in 1.5 ml centrifuge tube. The centrifuge tubes were filled with PBS containing 5% skimmed milk for 2 hours at room temperature to prevent adventitious binding and were washed again with PBS with centrifugation at 1000 rpm for 5 min at 4°C . The polyclonal antibodies were diluted in PBS. The primary antibody (100 μl) was added and incubated for 1 hour 30 min at room temperature. Washing was again done thrice with PBS. Then 100 μl of fluorescein isothiocyanate (FITC) labelled secondary antibody (1:40 dilutions) was incubated for 1 hour at room temperature. The tubes were washed three times with PBS with centrifugation at 1000 rpm for 5 min at 4°C . Finally, the pellets were dissolved in 100 μl of PBS and suspended in fluorescent plate. The entire process after using FITC labelled antibody was performed in the dark. Fluorescent activity was assayed on subjecting the plate to Fluorescent plate reader FL $\times$ 800 (BIOTEK, Winooski, USA). The fluorescence signal was read after excitation at 480 nm filter and emission at 520 nm filter.

Biochemical characterisation of antigen(s) using immunofluorescent assay

The preliminary characterisation of antigen(s) was done using chemical modifiers and enzyme treatment studies (Gupta et al., 2001) using immunofluorescent assay.

The teliospores (0.5 mg) of *T. indica* were washed three times with PBS and suspended in PBS at a concentration of 1 mg/ml in centrifuge tubes. The tubes were kept in a boiling water bath for 15 min. The other tube was taken as control and kept at room temperature. After removing the tube from the water bath, it was cooled at room temperature and processed for IFA. To know the proteinaceous nature of antigen(s), intact teliospores were pelleted in 5 ml tubes and treated with trypsin in appropriate buffer (0.25% Trypsin in 40 mM Tris-HCl, pH 8.1, containing 50 mM CaCl_2) at a concentration of 1.0 mg/ml. Incubation was carried out for 60 min at 37°C. For control, the pellets were suspended in 1 ml of buffer omitted with the enzyme and incubated under the same conditions. After washing the pellet for three times with PBS the teliospores were processed for IFA.

Periodate treatment showed the carbohydrate nature of antigen. Intact teliospores were pelleted in 5 ml centrifuge tubes and treated with periodate (50 mM sodium periodate in 50 mM sodium acetate, pH 4.5) at room temperature for 2 hours. As a control the pellet was suspended in 1 ml of buffer omitted with sodium periodate and incubated under the same conditions. After washing the pellet for three times with PBS, the teliospores were processed for IFA.

Methanol treatment described the lipid nature of antigen. The teliospores were incubated with methanol and absolute ethanol for 60 min at 0–4°C and after washing three times with PBS, the pellet was processed for IFA.

To know the glycoprotein nature of antigen, sequential treatment of periodate and trypsin was done. Intact teliospores were pelleted in 5 ml glass tubes and treated with periodate (50 mM sodium periodate in 50 mM sodium acetate, pH 4.5) for 2 hours. The teliospores were washed four times with PBS and subsequently treated with trypsin (0.25% in 40 mM Tris-HCl, pH 8.1 containing 50 mM CaCl_2) for 1 hour at 37°C. For control, the pellets were suspended in PBS. After washing the pellets for three times in PBS, the teliospores were processed for IFA.

Surface configuration studies using phase contrast and scanning electron microscopy

The change in surface configuration of teliospore wall was determined after modification using periodate (50 mM periodate dissolved in 50 mM sodium acetate, pH 4.5) treatment. After washing steps slides were prepared and observed under phase contrast microscope (Nikon Eclipse 80i, Tokyo, Japan) followed by scanning electron microscopy. For SEM, prepared slide of teliospores was mounted on stubs with conductive carbon tape and coated with a 20 nm layer of gold in ion sputterer (JEOL, Tokyo, Japan). The teliospores without treatment and modified teliospores were examined with a JSM-6610LV scanning electron microscope (JEOL) operated at 2.5 kV. All photographs were taken in the secondary electron mode with the beam incident to the surface of the teliospores.

Isolation of spore wall proteins

The teliospores/spores (100 mg) were crushed with a pestle and mortar with liquid N_2 and 2 ml solubilising buffer (0.5% SDS). Approximately 100% breakages of teliospores were seen under Nikon microscope. Spore wall fragments were pelleted from the extract by centrifugation at 8450 g for 10 min. The resulting supernatant

was transferred to a clean tube and stored at -20°C . The protein was estimated by using the method of Bradford (1976).

SDS-PAGE analysis of spore wall's proteins

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was done for analysing proteins of teliospore walls. After electrophoresis, the gel was fixed and stained with Coomassie Brilliant Blue for 4–6 hours. The gel was destained thoroughly by soaking it in the methanol/acetic acid solution, changing the destaining solution three or four times.

Western blotting

For Western blot analysis, 20 μg of teliosporic protein along with pre-stained protein molecular weight marker was resolved and the proteins were transferred to a nitrocellulose (NC) membrane (0.45 μm) using semi-dried system a transfer apparatus (SCIE-PLAS, Cambridge, UK) at 2 mA/square cm for 90 min. The NC membrane was incubated in 5% skimmed milk in PBS solution at 37°C for two hours for blocking. The blocked membrane was probed with rabbit anti-teliospores antibody, diluted 1:250 in PBS for 2 hours at 37°C with constant shaking. Washing was performed four times with PBS allowing five minutes per wash, followed by incubation in goat anti-rabbit ALP conjugate for 45 min at 37°C with constant shaking. Washing was performed at least four times with 10 min at each wash, after which BCIP/NBT substrate was added for colour development. Thereafter, the reaction was stopped by washing the membrane in double distilled water and the results were observed.

Glycoprotein Staining

Teliosporic proteins were subjected for glycoprotein staining by using Thermo Scientific Pierce glycoprotein staining kit, which detects glycoprotein sugar moieties after electro transfer of electrophoresised proteins onto NC membranes. When treated with oxidising reagent (periodic acid), glycols present in glycoproteins were oxidised to aldehydes. After completing the procedure, the glycols were stained, yielding magenta bands with a light pink background.

Lectin and antibody binding studies using fluorescent microscopy

The teliospores were washed with PBS and stained FITC labelled lectins [Concanavilin A (Con A) dissolved in (0.01 M Tris-HCl, pH 7.2) containing 1 mM CaCl_2 , 1 mM MnCl_2 and Wheat Germ Agglutinin (WGA) dissolved in (PBS pH 7.4)] having specificities to bind with mannose and N-acetylglucosamine, respectively. Besides, teliospores were also stained with anti-teliospore antibody followed by FITC labelled secondary antibody. After washing, the teliospores were subjected for fixation on a glass slide covering with coverslip. The slides were prepared in the dark and subjected to fluorescent microscopy.

Results

Development of indirect IFA using anti-teliospore polyclonal antibodies

Indirect immunofluorescent assay was developed to check the reactivity and specificity of polyclonal antibodies generated against teliospores. For determination of optimum dilution for anti-teliospore antibody, 0.5 mg teliospores of antigen of *T. indica* were washed three times with PBS and suspended in centrifuge tubes with different sets of primary antibody dilution (1:100, 1:250, 1:500, 1:1000, 1: 2000) in triplicate. The Relative Fluorescence Unit (RFU) showed a linear decrease on increasing the dilutions of polyclonal anti-teliospore antibodies. A mid point from the antibody dilution curve was selected and 1:250 dilution was taken as optimum dilution of anti-teliospore antibody for further study (Figure 1).

Characterisation of nature of teliospore wall antigens of Tilletia indica using indirect IFA

To study the nature of antigens recognised by the rabbit anti-teliospores antiserum, the effect of various chemical and enzyme modified on the reactivity of antibodies using indirect IFA was investigated. The immunoreactivity of polyclonal antiserum with teliospores was not affected by methanol treatments showed minor loss of 6% reactivity. The enzyme (trypsin) and heat treatments reduced the immunoreactivity of teliospores of *T. indica* by 27% and 23%, respectively, while, periodate treatment of teliospores of *T. indica* caused 50% loss of immunoreactivity. The loss of immunoreactivity of teliospores of *T. indica* was further increased upto 58% after sequential treatment of periodate and trypsin (Figure 2, Table 1). The anti-teliospore antibody may recognise a glycoprotein via either protein moiety or the carbohydrate prosthetic group and may be due to the fact that the antigenic epitopes consists of both protein and carbohydrate entities. These results suggest a glycoprotein nature of antigen recognised by polyclonal antiserum due to multiple polysaccharide and protein epitopes or due to bridging epitopes made by both glycosyl and peptidal moieties.

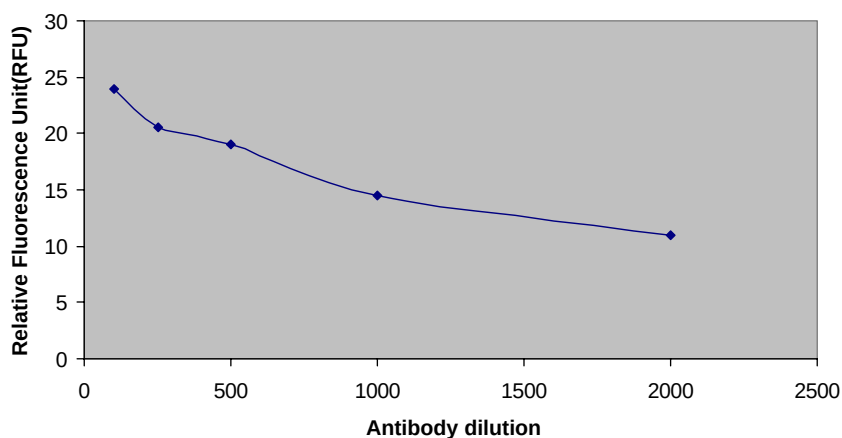


Figure 1. Determination of optimum antibody dilution of anti-teliospore antibodies for development of indirect immunofluorescent assay.

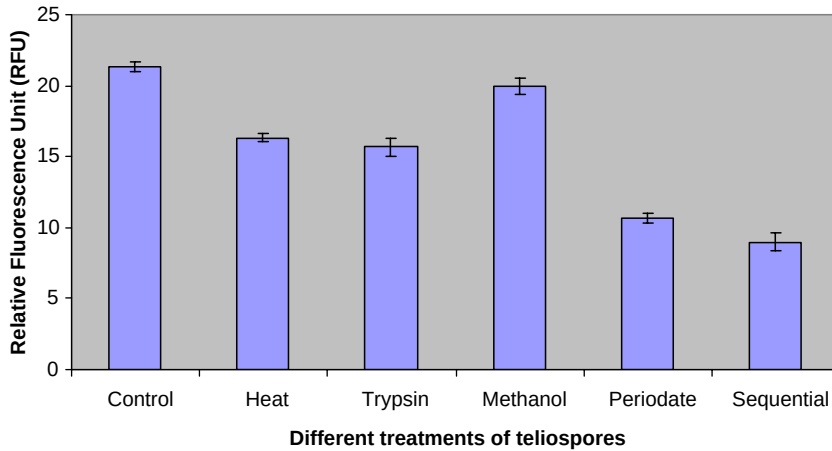


Figure 2. Preliminary characterisation of nature of teliosporic proteins of teliospore wall of *T. indica* after subjecting with various treatments (chemical and enzyme modifier studies) and determination of immunoreactivity by indirect immuno-fluorescent assay.

Table 1. Effect of various treatments on immunoreactivity of teliospores of *Tilletia indica* using anti-teliospores antibodies of *T. indica*.

Name of treatments	Relative Fluorescence Unit	
	(RFU) using <i>T. indica</i> antiserum	Loss of reactivity (%)
Control	21.33 ± 0.33	0
Heat	16.33 ± 0.33	23.4
Trypsin	15.66 ± 0.66	26.6
Methanol	10.67 ± 0.33	6.2
Periodate	20.00 ± 0.58	50.0
Sequential (periodate + trypsin)	9.00 ± 0.58	57.8

Phase contrast and scanning electron microscopy

The structural configuration of teliospore wall of *T. indica* was changed drastically after treatment with periodate when compared to the control teliospore treated with only PBS buffer as shown under scanning electron microscope (Figure 3). Besides the periodate treatment sequential treatment (periodate treatment followed by trypsin dissolved at concentration of 0.25% in 40 mM Tris-HCl buffer containing 50 mM CaCl₂) was also done but no difference was observed at microscope level (data not shown). The spore wall is mainly composed of proteins, lipids and polysaccharides, thus sodium periodate is used to oxidise simple sugars and polysaccharides present on the surface of teliospore wall of *T. indica*. It was observed that periodate treatment disturb the exoporum of the teliospore wall and lost their smooth grooves like structure and resulted into reticulated structure (Figure 3b). In phase contrast microscopy also, the difference on the surface of teliospore wall was observed as shown in Figure 4. It gave indirect evidence that due to oxidation, glycosyl moieties might be reduced and disturbed which resulted into loss in immunoreactivity after periodate treatment as shown in Table 1.

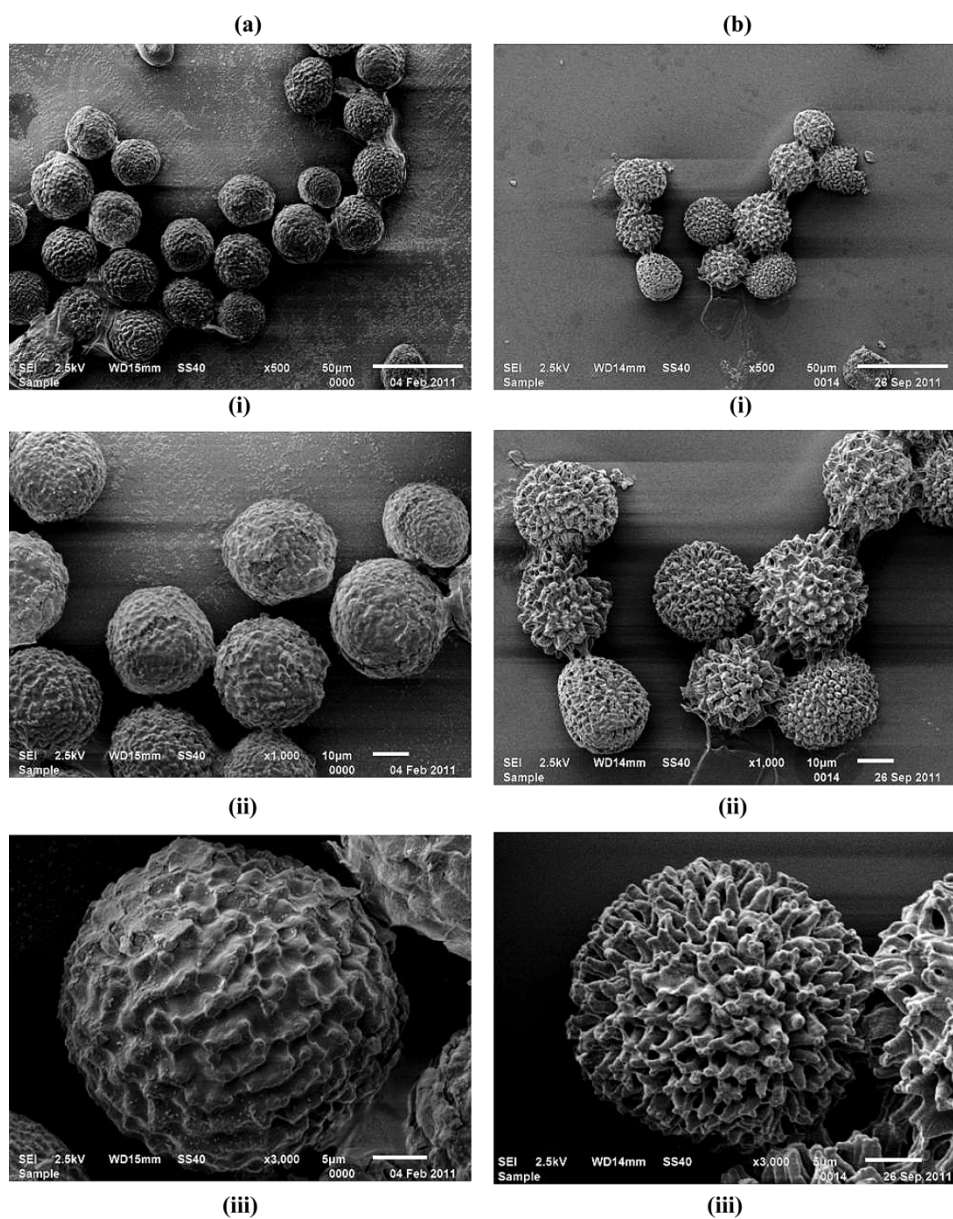
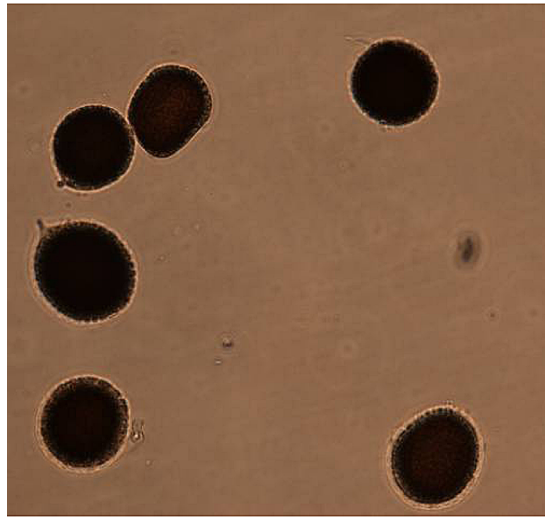


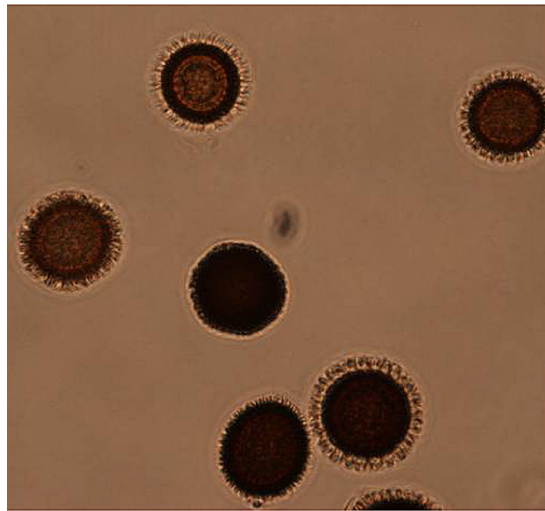
Figure 3. Effect of periodate treatment on the teliospore morphology of *T. indica* as observed under scanning electron microscopy (a) control at (1) 500 $\times$, (2) 1000 $\times$ and (3) 3000 $\times$ (b) after periodate treatment at (i) 500 $\times$, (ii) 1000 $\times$ and (iii) 3000 $\times$.

Western blotting

To determine the immunoreactivity of teliosporic proteins, western blotting was done using polyclonal antibodies raised against teliospore of *Tilletia indica*. Teliosporic protein of *T. indica* was separated by SDS-PAGE, transferred to NC



(a)



(b)

Figure 4. Effect of periodate treatment on the teliospore morphology of *T. indica* as observed under phase contrast microscope at $40\times$ magnification (a) control (b) after periodate treatment.

membranes and treated with anti-teliospore antibody. Several proteins with different molecular weights were detected in SDS-PAGE of teliosporic proteins however, after incubation of these proteins with anti-teliospore antibodies only five bands of approximately molecular weight 28, 40, 66, 68 and 83 kDa were detected (Figure 5a).

Glycoprotein staining

The 12% of SDS-PAGE was performed to the extracted teliosporic protein capable of resolving polypeptides and proteins ranging from 10 to 100 kD (Figure 5b). SDS-PAGE separated teliosporic proteins were transferred onto the membrane and stained by using the 'glycoprotein staining kit' which detects glycoprotein sugar moieties in NC membranes (Figure 5c). Glycoprotein staining showed three light magenta bands of approximately molecular weight 28, 40 and 48 kDa which confirmed the presence of glycoproteins in the configuration of teliospore wall of *Tilletia indica*.

Lectin binding studies

Immunolocalisation of glycosyl moieties on the teliospore wall of *Tilletia indica* was done using FITC labelled lectins namely WGA and Con A. Binding of the FITC labelled lectins to teliospore wall of *T. indica* seen under fluorescence microscope is shown in Figure 6. The interaction of FITC labelled WGA (specific to an acetylglucosamine) binding indicated the presence of chitin a polymer of acetylglucosamine. FITC labelled Con A bound to surface of teliospore wall of *Tilletia indica*, indicating the presence of mannosyl and glucosyl residues. The weak and ring shaped

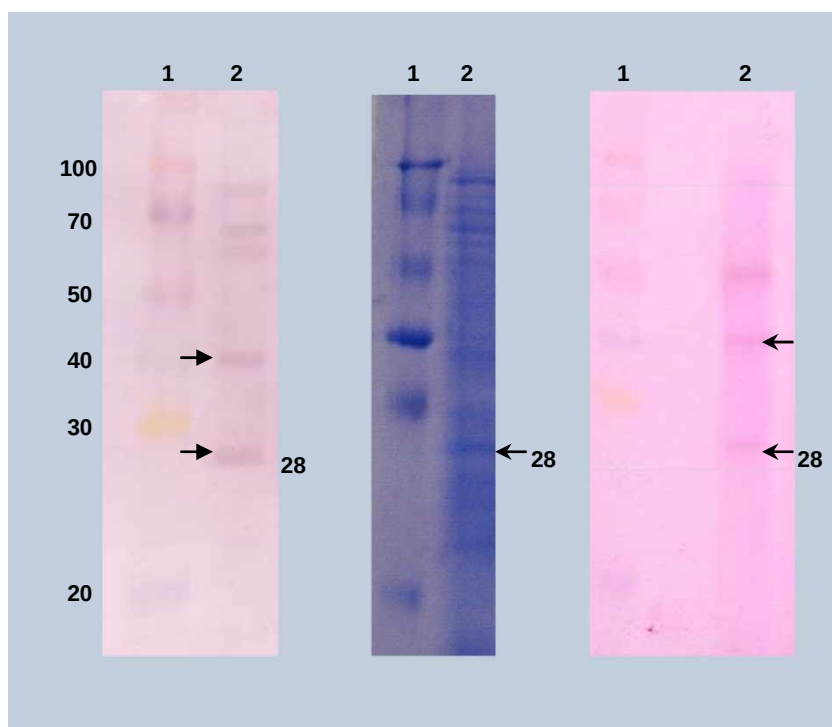


Figure 5. Characterisation of teliosporic proteins of teliospore wall of *T. indica* (a) western blot of teliosporic protein (Lane 1: Marker, Lane 2: teliosporic protein), (b) SDS-PAGE of teliosporic protein of *T. indica* (Lane 1: Marker, Lane 2: Protein) and (c) glycoprotein staining of teliosporic protein (Lane 1: marker, lane 2: teliosporic protein).

fluorescence with light patchy around the teliospores was observed in the binding of FITC labelled Con A with teliospore wall (Figure 6d). However, binding of WGA showed uniform prominent bright, green patchy (Figure 6c). These observations indicate that lectin is distributed all along the teliospore wall of *T. indica*. The patchy fluorescence indicated the presence of glycosyl residues on the grooves of the surface of teliosporewall of *T. indica*. In the binding with WGA, intense uniform fluorescent

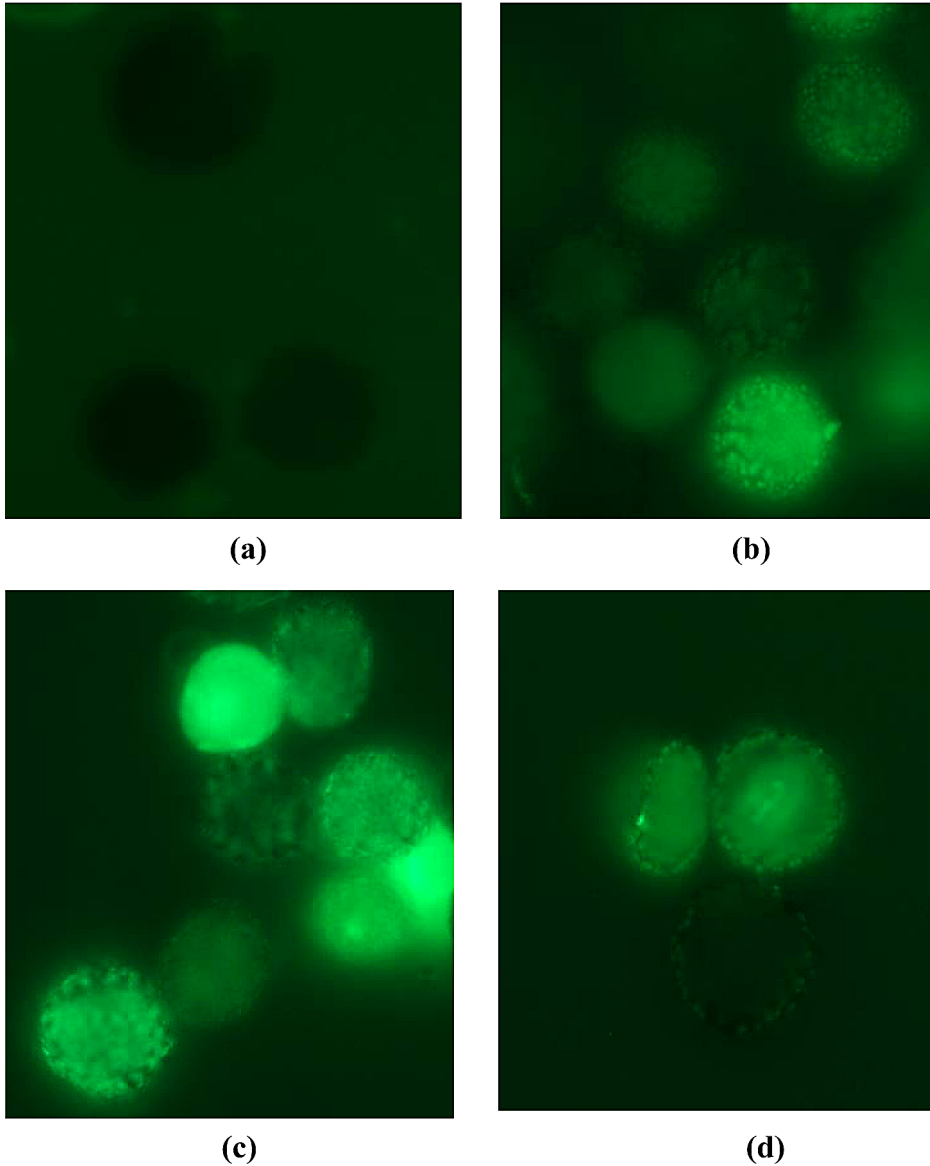


Figure 6. Immuno-localisation of glycosyl moieties on teliosporewall of *T. indica* by UV-fluorescent microscopy (a) control, teliospore without lectins, (b) Teliospores bound with anti-teliospore antibodies followed by FITC labelled secondary antibody and (c) Teliospores bound with FITC labelled WGA (d) Teliospores bound with FITC labelled Con A.

label was seen over the grooves surface of the teliosporewall revealing chitin on the surface at very high level. The binding study of anti-teliospore antibodies with teliospore surface also indicated the dark patchy fluorescence (Figure 6b). The binding pattern of WGA with teliospore wall showed similar binding pattern of anti-teliospore antibody with teliospore walls which suggested that immunodominant antigenic entities of teliospore wall are acetylglucosamine in nature.

Discussions

Early detection, correct identification and differential diagnosis of fungal pathogens are of primary importance in determining the most effective course of treatment to prevent the spread of fungi causing plant disease and post harvest storage rots. The correct diagnosis on the basis of symptoms alone is difficult for many diseases. Identification and enumeration of spores of fungi is also important especially for disease forecasting and development of control strategies. It is well established that differentiation of fungal pathogen is dependent on the qualitative and quantitative differences of specific markers present on the surface of the pathogens. The numbers of these markers are very small compared to common structural components of two fungal pathogens. It, therefore, becomes extremely difficult to pinpoint these markers in fungal spores/teliospores by conventional methods. Due to high specificity of antigen-antibody reaction, immunological approaches have great promise to study the structural components of teliospores and discriminate species. In the present investigation, an attempt was further made to characterise the antigens present on the teliospore wall of *T. indica* using indirect immuno fluorescent assay and further localise it with the help of scanning electron and fluorescent microscopy. An anti-teliosporic antibody was generated against intact teliospores in New Zealand white rabbits. These polyclonal antibodies were also employed to study the binding pattern using fluorescent immuno assay and which can also be employed for detection of KB.

To characterise the nature of antigen(s) of teliospore walls of *T. indica*, chemical modifiers and enzyme cleavage analysis were done using indirect IFA. The methanol treatment did not affect the reactivity of antigen of teliospore walls to anti-teliospore antibodies of *T. indica* (6%). However, comparatively increase in the loss of reactivity were observed in treatment with trypsin and heat (<27%). The periodate treatment also caused the loss of reactivity of anti-teliospore antibodies upto (50%) showing the carbohydrate nature of immunodominant antigenic determinant in the teliospore walls of *T. indica*. However, the highest loss of reactivity was observed in the sequential treatment of periodate and trypsin in the preliminary characterisation of teliosporewall of *T. indica* suggested glycoproteinaceous nature of antigen. Thus, it indicates that antibodies may recognise a glycoprotein via either the protein moiety or glycosyl moieties present in carbohydrate prosthetic groups. Gupta et al. (2009) also observed the presence of glycoprotein in the phenol extracted teliospore wall proteins of *T. indica* using indirect ELISA. Furthermore, in order to determine the structural configuration of teliospore wall of *T. indica* SEM studies were conducted which revealed the exposure of reticulated surface after periodate treatment. It also indicated the presence of antigenic epitopes on outer surface of teliospore wall which might be lost after treatments.

To confirm the existence and further characterisation of antigenic entities present on the teliospore surface, SDS-PAGE and glycoprotein staining were performed

which showed three magenta colour bands of size 28, 40 and 48 kDa. The same teliospore wall proteins were subjected for SDS-PAGE and western blotting which showed five immunoreactive bands of approximate molecular weights 28, 40, 66, 68 and 83 kDa. The most interesting observation was that two of these bands of molecular weight 28 and 40 kDa were found in teliospore wall protein using both glycoprotein staining as well as in western blotting. The similarity of two teliosporic proteins both by the SDS-PAGE western blot and glycoprotein staining studies suggested that antigenic entities are glycoproteinaceous in nature. Luster, Bonde, Peterson, Hack, and Schaad (1998) also showed that phenol extracted of teliospore wall proteins was in medium molecular weight range (37–43 kDa) and immunoreactive in nature. Further lectin binding studies were conducted to localise the glycosyl moieties on the surface of teliospore wall using immunofluorescence technique. The weak immunofluorescence was observed on the teliospore bound with Con A indicated the less presence of mannosyl or glycosyl residue. The binding pattern of WGA with teliospore wall showed similar binding pattern of anti-teliospore antibody with teliospore walls of *T. indica* indicated presence of chitin a polymer of an acetylglucosamine and suggested that immunodominant antigenic entities of teliospore wall are acetylglucosamine in nature. Chitin appears as the unique microsporidial polysaccharide and is associated with the thick inner layer of the spore wall, named the endospore (Bigliardi et al., 1996; Vavra & Larson, 1999). On combining the result of immunofluorescence, SDS glycoprotein staining and western blotting which support the SEM observations and suggested that teliospore surface configuration is enriched with acetylglucosamine, may be responsible for inducing an immune response against such antigenic entities present on the teliospore wall of *T. indica*.

Characterisation of such teliosporic proteins which are commonly identified by SDS-PAGE glycoprotein staining and western blotting, by subjecting them to peptide finger printing analysis by MALDI-TOF will enable to identify the nature of proteins and solve the complexity of these proteins. The teliosporic proteins of 28 and 40 kDa may be potential diagnostic antigens if subjected to peptide finger printing analysis will help in the identification of immunodominant epitopes for raising panel of specific monoclonal antibodies. After determining the specificity of sets of such monoclonal antibodies a diagnostic kit can be developed for detection of teliospores in infested wheat lots. Such kit has now mandatory requirements for seed certification and plant quarantine regulations.

Acknowledgements

The financial support provided by Department of Biotechnology, Government of India, in the form of research project is gratefully acknowledged. The authors are grateful to the Dean, College of Basic Sciences and Humanities, and the Director, Experiment Station, for providing all necessary help and facilities for carrying out this research and also thankful to Dr Prabha Pant, Assistant Professor, Department of Social Sciences, for the editing of this manuscript.

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