J. Soils and Crops 27 (1) 34-38, June 2017 ISSN 0971-2836

EPIDEMIOLOGY AND CULTURAL CHARACTERISTICS OF *Fusarium graminearum* CAUSING HEAD BLIGHT OF WHEAT

G. Raja Sekar[1] , M. Suriachandraselvan[2] and Sneha R. Patil[3]

ABSTRACT

Wheat (*Triticum aestivum* L.) is the most important cereal crop for the majority of world's population. It has been associated nearly with 17 causal organisms of which *Fusarium graminearum* is the principal pathogen responsible for head blight in many countries including India. The disease epidemiology, cultural and physiological characteristics of head blight of wheat was studied in 2015-2016 at Deptt. of Plant Pathology, AC&RI, Killikulum, T.N.A.U. The *Fusarium* head blight disease affected wheat plant samples were collected from IARI, Regional Research Station, Wellington, Coonoor, Nilgiri district, Tamil Nadu. The causal agent of *Fusarium* head blight (FHB) disease was isolated from the diseased wheat plant (ear head) using PDA medium and sub cultured by the single hyphal tip method. Cultural characteristics of *Fusarium graminearum* were studied in ten different solid and liquid media and also at nine different pH level and five different temperature levels of the medium. Three replications were maintained for each treatment. The radial growth of the mycelium and mycelial dry weight were assessed for all the cultural characteristics. The results revealed that the maximum mycelial growth of *F. graminearum* was found in complete medium exhibiting 90.00 mm radial mycelial growth. Potato dextrose broth was found to be the best by recording significantly the maximum mycelial dry weight of 1.60 g. Among the different pH levels tested, *F. graminearum* was found to grow well at pH 7.0 with mycelial growth of 90.00 mm. Significantly the maximum mycelial growth of 90.00 mm was observed at temperature 25°C.

(Key words: Wheat, *F. graminearum,* isolation, cultural characters)

INTRODUCTION

Wheat is infected nearly by fifty pathogens in our country (Weise, 1987). Among the fungal diseases, stripe rust /yellow rust (*Puccinia striiformis tritici*), karnal bunt (*Tilletia indica -Neovossia indica*), black point (*Alternaria alternata*), loose smut (*Ustilago nuda tritici*), powdery mildew (*Erysiphe graminis tritici*), head scab (*Fusarium graminearum*) are the most important diseases affecting wheat crop.

Head blight or Head scab is a destructive disease in the humid and sub humid wheat growing areas of the world. It has been associated nearly with 17 causal organisms, of which *F. graminearum* (Schwabe) is the principal pathogen responsible for head blight in many countries including India (Saharan *et al.,* 2002). Head blight or scab of wheat caused severe epidemics in many areas worldwide (Dubin *et al*., 1997). The disease has been especially destructive in the United States since 1991 and particularly caused severe epidemic during 1993 that accounted for a crop loss of up to $1 billion (McMullen *et al.,* 1997). Totally losses due to this disease in the USA during 1990s approached $ 2.6 billion (Windels, 2000). In India, wheat grain yield losses of 15.1-29.0 per cent have been reported from Arunachal Pradesh due to head blight incidence on different wheat varieties (Chaudhary *et al.,* 1991) and also in Punjab maximum yield loss of 21.6 per cent in wheat variety PBW 222 (Kaur *et al*., 2000).

F. graminearum does not produce microconidia and produces macroconidia only which is the characteristics features of this pathogen (Leslie and Summerell, 2006). It can survive as mycelium, ascospores, macroconidia and chlamydospores (Sutton, 1982). *F. graminearum* infects wheat spike tissues primarily through anthers that protrude from the glumes, then spreads to the epidermis of glumes and to the ovaries (Xu and Hideki, 1989). Anthesis is the period of greater susceptibility, because the high levels of choline and betaine produced in the extruded anthers stimulate the growth of *F. graminearum* and promote the infection of wheat spikes by the pathogen (Strange and Smith, 1978).

The earlier studies on head blight caused by *Fusarium graminearum* involved only reports of field observations on disease occurrence and its symptoms. Few workers have cultured a pathogen in laboratory and observed the colony morphology. However, there has been no systematic study involving disease epidemiology, effects of culture media, effects of abiotic factors like pH, temperature on growth and development of pathogen especially in case of wheat. Considering the wide host range and damage potential of *Fusarium graminearum,* the problem deserves immediate and effective measures of

1 and 3. P.G. Students, Deptt. of Plant Pathology, AC&RI, Killikulum, T.N.A.U.
2. Professor and Head, Deptt. of Plant Pathology, AC&RI, Killikulum, T.N.A.U

control so as to minimize the yield losses. The present study aimed to investigate the disease epidemiology and the effect of culture media and abiotic factors on the growth of *Fusarium graminearum*.

MATERIALS AND METHODS

Sample collection

The *Fusarium* head blight disease affected wheat plant samples were collected from IARI, Regional Research Station, Wellington, Coonoor, Nilgiri district, Tamil Nadu. The samples were collected in clean polythene bags and the samples were brought to the laboratory at Agricultural college and Research Institute, Killikulam, Vallanad for various studies during the year 2015-16.

Isolation, preparation and maintenance of pure culture of pathogen

The freshly infected wheat ear heads showing typical symptoms of *Fusarium* head blight disease collected from the field were used for isolation of the pathogen. The *Fusarium* infected ear heads were made into small pieces of three mm size cut along the edges of the lesions using a sterilized scalpel. The ear head pieces were surface sterilized with 0.1 per cent mercuric chloride solution for 30 seconds. These bits were then washed 3-5 times separately in repeated changes of sterile distilled water. The sterilized potato dextrose agar medium amended with 100 ppm of streptomycin sulphate (to avoid bacterial contamination) was prepared. The sterilized cooled (warm) medium (20 ml) was poured in to sterile petri plates (90mm) and allowed to solidify. The surface sterilized plant tissue bits were placed individually at equidistance at the rate of 3 bits plate^{-1}. All these works were carried out under aseptic conditions. The plates were incubated at room temperature (25 ± 2° C) for 5 days and observed for fungal growth. Identification of cultures was done by microscopic examination and by perithecial production. The growing fungal colony of each plant piece was sub cultured and purified by single hyphal tip method (Tutte, 1969). The pure culture of the pathogen was maintained on PDA slants for further use in this study. The stock cultures were maintained in PDA slants for long term storage under refrigerated condition at 4^0 C.

Effect of culture media on growth of *F. graminearum*

The growth characteristics of *F. graminearum* was tested in ten different solid and liquied media *viz.,* Potato dextrose agar (Ainsworth, 1961), Oatmeal agar medium (Booth, 1971), Czapek's Dox agar medium (Dox, 1910), V8 agar medium, Nutrient agar medium (Allen, 1953), Nash Snyder agar medium (Cho *et al.,* 2001), Yeast extract agar medium, Carrot agar medium (Klittich *et al.,* 1988), Asthana and Hawkers medium (Khilare and Rafi Ahmed, 2012) and Complete medium (Correll *et al.*, 1987). The prepared, sterilized and cooled (warm) media were poured @ 20 ml into sterilized petri plates (90 mm) and allowed to solidify. The pathogen, *F. graminearum* was inoculated at the centre of the plate by placing a nine mm actively growing PDA culture disc cut from a 5 day old culture by means of a sterilized cork borer. The plates were incubated at 25 ± 2^{0}C. Three replications were maintained for each treatment. The radial growth of the mycelium was measured when the mycelium covered the entire petri plate in any one of the treatments. The visual observation on pigment production was made 10 days after inoculation.

The culture broths for the above 10 media were prepared separately without adding agar agar. One hundred ml of each broth was distributed uniformly into 250 ml Erlenmeyer conical flasks separately and sterilized by autoclaving at 1.4 kg/cm---^{2} pressure for 20 minutes and cooled. Each flask was then inoculated with a nine mm actively growing complete medium culture disc cut from a 5day old culture of *F. graminearum* by means of a sterilized cork borer. The flasks were incubated at 25 ± 2^{0}C for 10 days for mycelial dry weight assay and three replications were maintained for each treatment. The mycelial mat was filtered through a pre weighed Whatman No.1 filter paper in each case and dried in hot air oven at 60° C until a constant weight was obtained. The mycelial dry weight was obtained separately by subtracting the weight of the filter paper alone from the total weight.

Effect of abiotic factors on growth of *F. graminearum*

The effect of pH of the medium on growth of *F. graminearum* was studied using complete medium. Complete medium was prepared and the pH of the medium was adjusted to different levels *viz.*, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5 using 0.1 N HCL or NaOH and 100ml medium was distributed uniformly into 250 ml conical flask and sterilized in an autoclave at 1.4 kg/cm^2 pressure for 20 minutes. Twenty ml of sterilized, melted and cooled (warm) medium from each pH level was poured into sterilized petri plates (90 mm) separately and the plates were rotated both clockwise and anticlockwise direction and allowed to solidify. A nine mm actively growing culture disc of *F. graminearum* cut from 5 day old culture using a sterilized cork borer was placed at the centre of the each petri plate under aseptic conditions and incubated at 25 ± 2 °C. Three replications were maintained for each pH level. The radial growth of the mycelium was measured when the mycelium covered the entire petri plates in any one of the treatment.

The effect of temperature on mycelial growth of *F. graminearum* was studied on complete medium. Complete medium was prepared and 100 ml medium was distributed uniformly into 250 ml conical flask and sterilized in an autoclave at 1.4 kg/cm^2 pressure for 20 minutes. Twenty ml of sterilized and cooled (warm) medium was poured into the sterilized petri plates (90 mm) separately and the plates were rotated both clockwise and anticlockwise direction and allowed to solidify. A nine mm actively growing culture disc of *F. graminearum* cut from 5 day old culture using a sterilized cork borer was placed at the centre of the each petri plate under aseptic conditions and incubated for 5 days at different temperature *viz.,* 15°C, 20°C, 25°C, 30°C and 35°C . Three replications were maintained for each

temperature. The radial growth of the mycelium was measured when the mycelium covered the entire petri plates in any one of the treatment.

The data were statistically analysed using the SPSS (Statistical Package for the Social Sciences) version 16.0. Prior to statistical analysis of variance (ANOVA) the percentage values were arcsine transformed. Data were subjected to analysis of variance (ANOVA) at significant levels ($P< 0.05$) and means were compared by Duncan's Multiple Range Test (DMRT). Wherever, the 'F' test were found significant, critical difference (C.D.) were worked out at 5 per cent level of probability for comparison of treatment means. The treatment effects were presented by making table of means with appropriate standard error (S.E.) and C.D. value.

RESULTS AND DISCUSSION

Epidemiology

The causal agent of wheat head blight disease *Fusarium* sp. was isolated from the diseased wheat plant (ear head), using PDA medium and sub cultured by the single hyphal tip method. The morphology of the mycelium initially appeared white colour. Two days later, mycelia turned pink and entire agar medium became typically reddish pink colour. Analysis of asexual spore showed that the pathogen produced only macroconidia and no microconidium was produced. This is the typical characteristics feature of *F. graminearum*. The isolated *Fusarium* sp. was identified based on morphological and cultural characters as *F. graminearum.* Similar to this result Wegulo *et al*. (2008) also showed that *F. graminearum* in agar cultures appeared white mycelial growth with grey, pink, brown and red pigments in agar. It produced asexual spores known as macroconidia in primarily fruiting structures known as sporodochia.

Effect of solid and liquid culture media on growth of *F. graminearum*

The growth of *F. graminearum* was tested in ten different solid media to find out the best medium for the maximum mycelial growth and the results are given in table 1 and fig.1 and 2. Mycelial growth was observed in all the tested solid media and showed significant variation. The maximum mycelial growth of *F. graminearum* was found in complete medium (89.00 mm) which was significantly superior over all other media. This was followed by nutrient agar medium (83.33 mm). Growth of the pathogen in oat meal agar (67.33) and PDA (64.33 mm) was at par with each other. The minimum growth was recorded in Czapek Dox agar (48.00 mm).

The experiment was also conducted to identify which liquid medium would support the maximum mycelial growth of *F. graminearum.* The tested 10 different liquid media in this study (Table 1 and Fig.3) showed significant variation in mycelial growth. Among them, Potato dextrose broth was found to be the best by recording significantly the maximum mycelial dry weight of 1.60 g. The next best broth which supported the mycelial dry weight was complete broth with mycelial dry weight of 1.18 g. Growth in Nash Snyder broth (0.12g), nutrient broth (0.13g), Czapek Dox broth (0.23g) and Oat meal broth (0.30g) was at par with each other. Asthana and Hawker's broth recorded the minimum mycelial dry weight of 0.07g.

The results obtained in the present study corroborates with the results obtained by earlier workers. Leslie and Summerell (2006) reported *Fusarium* species to grew well vegetatively on complete medium, but sporulation characters varied. But in contradict to the present results of Thompson *et al.* (2013) reported that the fungus *F. graminearum* developed and sporulated well on potato dextrose agar. Selvi and Sivakumar (2013) reported that *F. graminearum* grew faster in potato dextrose agar medium. Pradeep *et al.* (2013) reported that *Fusarium moniliforme* grew well in PDA and PD broth.

Among the different solid media studied, carrot agar and PDA medium supported the production of deep pink discolouration of the media to the maximum extent (Table 1). This result also indicated that the effect of different culture media on the mycotoxin production by *F. graminearum.* Among the media tested, carrot medium and PDA medium showed higher level of mycotoxin production than any other media tested. Thus, in this study, the discolouration of solid media indicating the higher level of mycotoxin production in carrot medium and PDA. Similar result has been reported by Pradeep *et al.* (2013) in *F. moniliforme* which showed that maximum mycelial growth and pigment production was best on PDA. Mycelium were whitish colour in RBA, NB, CZA medium and colour mycelial growth appear in PDA (Reddish brown), OMA (Dark pinkish), MA (violet), YMA (yellow) medium. Boonyapranai *et al.* (2008) also reported that in most studies of pigment biosynthesis, cultivation of fungi involved rich organic media of complex or indefinite composition. PDA is one of the most commonly used culture media, because of its simple formulation and its ability to support mycelial growth and pigment production for wide range of fungi.

Premalatha *et al*. (2012) reported PDA and PDB to be the best culture media for mycelial growth and pigment production. Most fungi thrive on PDA, but this can be too rich in nutrients, thus encouraging the mycelial growth with ultimate loss of sporulation (Annonymous, 1998). Conventionally, starch based media, such as PDA or malt extract agar are good substrates for the species of *Fusarium* and *Dematiaceous hypromycetes* to grow rapidly and produce abundant aerial mycelia.

Effect of abiotic factors on the growth of *F. graminearum*

Growth of *F. graminearum* was observed in nine different pH levels maintained in complete medium (Table 2 and Fig 4). Among the different pH levels tested, *F. graminearum* was found to grow well at pH 7.0 with mycelial growth of 90.00 mm which is significantly superior over all other levels of pH. This was followed by pH 7.5 which

Table 1. Growth and Mycotoxin synthesis of *F. graminearum* on different culture media

Sr.No	Culture media	Mycelial growth (mm) in solid media	Pigment production in solid media	Mycotoxin synthesis in solid media	Mycelial growth (g) in liquid media
1	Complete medium	90.00[a]	white colony growth and light pinkish discolouration	+	1.18[b]
2	Nutrient agar medium	83.33[b]	Sparsely white colony growth and no discolouration	-	0.13[de]
3	Nash Snyder agar medium	77.33[c]	White colony growth and no discolouration	-	0.12[de]
4	Carrot agar medium	73.33[c]	White dense colony growth and dark pinkish discolouration	+++	0.61[c]
5	Yeast extract agar medium,	74.67[c]	White colony growth and no discolouration (medium colour yellow)	-	0.41[cd]
6	Oatmeal agar medium	67.33[d]	White dense colony growth and light pink discolouration	+	0.30[de]
7	Potato dextrose agar medium	64.33[de]	Pinkish white dense colony growth and dark pinkish discolouration	+++	1.60[a]
8	V8 agar medium	59.33[e]	White colony growth and purple violet colour discolouration	++	0.34[cde]
9	Asthana & Hawkers medium	52.67[f]	White colony growth and light pinkish discolouration	+	0.07[e]
10	Czapek's Dox agar medium	48.00[f]	White colony growth and light pinkish discolouration	+	0.23[de]
	SE±	**1.95**			**0.087**
	CD (P=0.05)	**5.85**			**0.260**

The treatment means were compared using Duncan's Multiple Range Test (DMRT)
In a column, means followed by a common letter (s) are not significantly different (P=0.05)

- \- No toxin production
- \+ Less toxin production
- ++ Moderately toxin production
- +++ Highly toxin production

Table 2. Effect of different pH level and temperature on growth of *F. graminearum*

pH levels	Mycelial growth (mm)	Temperature	Mycelial growth (mm)
4.5	65.33[d]	15°C	38.33[d]
5.0	72.33[cd]	20°C	73.33[b]
5.5	72.67[cd]	25°C	90.00[a]
6.0	79.67[bc]	30°C	45.33[c]
6.5	82.00[b]	35°C	0.00[e]
7.0	90.00[a]	**SE±**	**1.18**
7.5	86.67[ab]	**CD (P=0.05)**	**3.50**
8.0	80.67[bc]		
8.5	79.33[bc]		
SE±	**2.49**		
CD (P=0.05)	**7.41**		

The treatment means were compared using Duncan's Multiple Range Test (DMRT)
In a column, means followed by a common letter (s) are not significantly different (P=0.05)

showed mycelial growth of 86.67 mm and 6.5 pH which showed mycelial growth of 82.00 mm which were at par with each other. Minimum mycelial growth was recorded at pH 4.5 (65.33 mm). The data in the present study revealed that *F. graminearum* was able to grow in a wide range of pH from 4.5 to 8.0 with a maximum growth at pH 7.0. This finding corroborates with the report of Khilare and Ahmed (2012) and Selvi and Sivakumar (2013), who also reported that the maximum growth of *F. graminearum* was recorded at pH 8.0.

F. graminearum grown on complete medium was maintained at five different temperatures to identify the suitable temperature for *F. graminearum.* The pathogen was placed at 15°C, 20°C, 25°C, 30°C and 35°C and the effect are presented in table 2 and Fig 4. Maximum significant mycelial growth of 90.00 mm was observed at temperature of 25°C followed by 20°C with mycelial growth of 73.33 mm. No mycelial growth was recorded at 35°C. In the present study, *F. graminearum* was found to grow well at temperature 25°C by recording the maximum growth of 90.00 mm and no growth was recorded in 35°C. Similar findings were recorded by Hudec and Muchova (2010), who reported that the growth of *Fusarium avenaceum, F. culmorum, F. graminearum*, and *F. poae* was the fastest at 25°C. The optimal temperature for *F. culmorum, F. graminearum* and *F. avenaceum* was between 20°C and 25°C, while *M. nivale* grew better at temperatures lower than 20°C (Brennan *et al*., 2003). Selvi and Sivakumar (2013) also reported that the maximum growth of *F. graminearum* occurred at 25°C.

Thus, it is summarized from this study that the maximum mycelial growth of *F. graminearum* was found in complete medium at pH 7.0 and temperature 25^{0}C by recording significantly maximum mycelial radial growth of 90.00 mm and in potato dextrose broth by recording significantly the maximum mycelial dry weight of 1.60 g.

REFERENCES

Annonymous, 1988. UKNCC, Growth and Media Manuals. Strain databases (www.ukncc.co.uk/).

Allen, M.B. 1953. The thermophilic aerobic spore-forming bacteria, Bacteriol. Rev., **17:** 125-173.

Ainsworth, G.C. 1961. Dictionary of Fungi. Common Wealth Mycological Institute, Kew, Swrrey, England, pp. 547.

Brennan J.M., B. Fagan and A. Van Maanen. 2003. Studies on *in vitro* growth and pathogenicity of European *Fusarium* fungi. European J. Pl Pathology, **109**: 577–587.

Boonyapranai, K.R., P. Tung, S. Lhieochaiphant and S. Phutrakul, 2008. Optimization of submerged culture for the production of naphthoquinones pigment by *Fusarium verticillioides.* Chiang Mai J. Sci. **35**: 457-466.

Booth, C. 1971. The Genus *Fusarium.* Commonwealth Agricultural Institute, Kew, Swrrey, UK**: 237**.

Chaudhary, R.G., S. Edison and Vishwadhar, 1991. Epidemiology and basic facts of severity, yield loss and grain quality deterioration due to ear blight of wheat in Arunachal Pradesh. Indian J. Phytopathol. **43**: 571-574.

Cho, J.H., J.C. Rupe, M.S. Cummings, E.E. Jr. Gbur, 2001. Isolation and identification of *Fusarium Solani* f.sp.glycines from soil on modified Nash and Snyder's medium. Plant disease **85**:256-260.

Correll, J.C., C.J.R. Klittich and J.F. Leslie, 1987. Nitrate non-utilizing mutants of *Fusarium oxysporum* and their use in vegetatively compatibility test. Phytopathology, **77**: 1640-1646.

Dox, A.W. 1910. U. S. Dept. Agr. Bur. Anim. Ind-Bull. pp.120.

Dubin, H.J., L. Gilchrist, J. Reeves and A. McNab. 1997. *Fusarium* head scab*:* global status and future prospects. Mexico, DF, CIMMYT, Mexico, DF pp. 130.

Hudec, K. and D. Muchova, 2010. Influence of temperature and species origin on *Fusarium* spp. And *Michrodochium nivale* pathogenicity to wheat seedlings. Plant Protect. Sci, **46**: 59-65.

Kaur, S., S.S. Chahal and N. Singh, 2000. Assessment of losses caused by head blight in wheat. J. Mycol. Plant Pathol. **30**: 204-206.

Khilare, V.C. and R. Ahmed. 2012. Effect of different media, pH and temperature on the growth of *fusarium oxysporum f.sp. ciceri* causing chickpea wilt. I.J.A.B.R., **2**: 99-102.

Klittich, C. J.R. and J.F.Leslie, 1988. Nitrate reduction mutants of *Fusarium monilijorme (Gibberella jujikuroi).* Genetics, **118:** 417-423.

Leslie, J.F. and B.A. Summerell. 2006. The Fusarium laboratory manual. Blackwell Professional; Ames, IA, USA.

McMullen, M., R. Jones and D. Gallenberg, 1997. Scab of wheat and barley: a re-emerging disease of devastating impact; Plant Dis., **81:** 1340-1348.

Pradeep S.F., M.S. Begam, M. Palaniswamy and B.V. Pradeep. 2013. Influence of Culture Media on Growth and Pigment Production by *Fusarium moniliforme* KUMBF1201 Isolated from Paddy Field Soil. World Applied Sci. J. **22**: 70-77.

Premalatha, B., F. Stanly Pradeep, B.V. Pradeep and M. Palaniswamy, 2012. Production and characterization of naphthoquinone pigment from *Fusarium moniliforme* MTCC6985. World J. Pharmaceutical Res. **1**: 1126-1142.

Saharan, M.S., J. Kumar, R. Tiwari, S. Nagarajan, S. Sharma and Priyamvada, 2002. Phenotypic, Pathogenic and molecular variation among *Fusarium* species/isolates associated with head scab of wheat in India; In proc. 2nd Int'l Group Meeting on Wheat Technologies for Warmer Areas, pp.: 51-52.

Selvi K. V. and T. Sivakumar, 2013. Isolation, identification and Characterization of Fusarium species from mangrove habitat of Pichavaram, Tamil Nadu, India. Int.J.Curr.Microbiol.App.Sci. **2**: 33-49.

Strange, R.N. and H. Smith, 1978. Specificity of choline and betaine as stimulants of *Fusarium graminearum.* Trans. Br. Mycol. Soc.**70**:187-192.

Sutton, J.C. 1982. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum.* Can. J. Plant Patho. **4**: 195-209.

Thompson, R.S., T.A.S. Aveling, and R. Blanco Prieto, 2013. A new semi-selective medium for *Fusarium graminearum, F. proliferatum, F. subglutinans* and *F. verticillioides* in maize seed. South African J. Bot. **84**: 94–101.

Tutte, J. 1969. Plant Pathological methods. Fungi and bacteria. Burgess publishing company, U.S.A. pp.229.

Wegulo, S.N., A.T. Jackson, P.S. Baenziger, M.P. Carlson and J.H. Nopsa, 2008. *Fusarium* head blight of wheat. University of Nebraska-Lincoln Extension.

Weise, M.V. 1987. *Compendium* of Wheat Diseases St. Paul. American phytopatho. Soc. pp.112.

Windels, C.E. 2000. Economic and Social impacts of *Fusarium* head blight: Changing farms and rural communities in Northern Great Plains: Phytopathology, **90**: 17-21.

Xu, Y.G. and N. Hideki, 1989. The infection process of the wheat scab pathogen. J. Nanjing Agric. Univ. **12**: 33-38.

Rec. on 10.10.2016 & Acc. on 10.11.2016