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Eco-friendly Management of False Smut (*Ustilaginoidea virens*) of Rice

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False smut of rice caused by *Ustilaginoidea virens*, perfect sexual stage *Villosiclava virens. Ustilaginoidea virens* belongs Division – Ascomycota, Class – Sordariomycetes, Order – Hypocreales, Family – Incertaesedis. The yield losses due to RFS 1 -75% is measured. The symptoms are visible only after flowering when the fungus transforms into globose structures or yellowed carbonaceous masses of individual grains of panicle. These masses are dusty representing more than twice in diameter of normal grain. At early stage, development of fungus areshow yellow and then changed in dark green or almost black color, and explode releasing the spores of the fungal causal agent. The pathogen survives both in seed and also in collateral host. The fungus by the means of sclerotia and chlamydospores overwinters in soil. Ascospores produce by sclerotiaare primary source of infection to rice plants, whereas air-borne chlamydospores may cause secondary infection. The seed should be treated through biological control agents like *Trichoderma viride*, *Trichoderma virens*, *Trichoderma harzianum* and *Trichoderma reesei* against *Ustilaginoidea virens* find more effective to control diseases. Growing of resistant hybrids variety like VNR-211, GK-5025, HRI-140, IRH-74, PRSH-9018, KPH-467, RH-10428, 27P64 and KRH-4. By spraying of Carbenadazim 50% WP (0.1 %) at the booting stage. Treatment with Copper Oxy Chloride (Blitox) 50 WP (0.3%) and Propiconazole (tilt) 25 EC (0.1%) were found as the most effective to control false smut.

Introduction

False smut or green smut is a common disease of rice caused by *Ustilaginoidea virens* in rice growing regions of India. Epidemics of false smut disease of rice were reported in Tamil Nadu in India and later in many countries of world (Singh and Pophaly, 2010). False smut of rice caused by a fungal pathogen, *Ustilaginoidea virens* (Cooke) Takahashi, is a common grain disease of rice around the world. The disease was first reported from

Tinnevelli in Tamil Nadu, India by Cooke in 1878 (Ou, 1972).

In recent years, it has emerged as the most devastating grain disease in the majority of rice growing areas of the world. In India, the disease has been observed in severe form since 2001 in major rice growing states, viz., Haryana, Punjab, Uttar Pradesh, Uttaranchal, Tamil Nadu, Karnataka, Andhra Pradesh, Bihar, Jharkhand, Gujarat, Maharashtra, Jammu & Kashmir and Puducherry (Dodan and Singh, 1996 and Mandhare *et al.,* 2008). Antimitotic cyclic peptides, ustiloxin from its chlamydospores are produced by pathogen on infected grains, poisonous to both humans and animals (Nakamura *et al.,* 1994; Koiso *et al.,* 1994). Ascospores produce by sclerotiaare primary source of infection to rice plants, whereas air-borne chlamydospores may cause secondary infection (Ashizawa *et al.,* 2010). The pathogen survives as dormant structures such as sporeballs, chlamydospores, sclerotia etc. in soil, stubbles of the crop and also in collateral hosts (Singh and Dube, 1976; Yashoda and Anahosur (2000). The collateral hosts aid in the off-season active survival of the pathogen. The reported collateral hosts of *U. virens* include *Oryza officinalis, Digitaria marginata, Panicum trypheron, Echinochloa crusgalli*, *Imperata cylindrical* etc. (Rao and Reddy, 1955; Shetty and Shetty, 1985; Shetty and Shetty, 1987; Atia, 2004). There have been many reports of severe outbreaks of RFS since 2001 in many rice-growing provinces of China, such as Liaoning, Hubei, Sichuan, and Anhui; the yield loss ranged from 20 to 50% indifferent areas and varied with rice varieties. In 2005, the occurrence area of RFS disease was approximately 330,000 ha and a third of the panicles in Sichuan were affected (Lu *et al*., 2009).

RFS having causing significant yield losses globally has become an important disease of rice. The disease reduces yield, affects grain quality and imposes health hazards significantly in all rice producing areas. (Tanaka *et al.,* 2008), Infection by the pathogen transforms individual grains of infected panicles into initially orange, becoming yellowish green or greenish black at maturity (Ou, 1972; Lee and Gunnell, 1992).

Economic importance

Upadhyay and Singh (2013) reported that yield loss due to RFS disease from many rice growing areas ranges from 1 to 75%. (Pannu*et al.,* 2010) also reported losses up to 44 per cent in Punjab. In Uttar Pradesh, yield losses up to 44 percent were observed by (Singh and Dube, 1978). In some rice growing districts of Bihar, 15-50 percent losses occurs due to false smut of rice when comes as medium to severe form (Laha*et al.,* 2013). In Bangladesh 10-15% of annual production loss was due to false smut disease (Latif*et al.,* 2007). The disease causes both quantitative and qualitative losses. The losses in grain yield occur due to chaffiness, reduction in test weight and sterility of the spikelets neighbouring smut balls. The yield losses have been estimated to vary between 0.2 - 49% in different states of the country. (URL-1).

Host Range

The alternate host through which pathogen survivesare, barnyard grass (*Echinochloa crusgalli), Imperata cylindrical*, and common rice weed *Digitaria marginata*. (URL-1) During infection, *U. virens* produces mycotoxins such as Ustilotoxins A and B which contaminate rice seeds and straw, man and livestock are also harmed by these mycotoxins(Shan *et al.,* 2013). The fungus attacks some of the weed species that commonly occur in rice fields and may also serve as sources of inoculums (Atia, 2004). The pathogen survives as dormant structures such as sporeballs, chlamydospores, sclerotia etc. in soil, stubbles of the crop and also in collateral hosts (Singh and Dube, 1976; Yashoda and Anahosur, 2000).

Taxonomic Position

U. virens, the causal agent of false smut, belongs to the division Ascomycota, subdivision Pezizomycotina, and class Sordariomycetes (Nguyen *et al.,* 2012). *Ustilaginoidea virens* (Cooke) Takah., (1896) Synonyms *Tilletiaoryzae* Pat., (1887) *Sphacelotheca virens* Omori{?}, (1896) *Ustilagovirens* Cooke, (1878) *Ustilaginoidea oryzae* (Pat.) Bref., (1895) (URL-4).

Morphology of Pathogen

In order to study the detailed cultural characteristics of the pathogen, 5 mm mycelial discs were collected from ten days old culture of *U. virens* and inoculated at the center of PSA plates under aseptic cultural condition in three replicates (27 ± 2°C temp). (Manu *et al.,* 2017).The fungus produces ustilotoxin, a phytotoxin and a mycotoxin thus contaminating rice products (Koiso *et al.,* 1992 and Li *et al.,* 1995). Smut balls are initially yellow in colour and are covered by a membrane, later the membrane bursts and the colour changes to yellowish green and finally greenish black (Gulzar *et al.,* 2012). Chlamydospores formed in the masses of spores are spherical to elliptical, warty, of olive color, and 3 to 5 × 4 to 6 µm. Colonies on PDA developed in approximately 14–15 days.(Quintana *et al.,* 2012). 10- 15 days after rice anthesis the balls begin to appear. Balls consist of white hyphae, at the beginning, which later forms thick yellow, loose outer layer of chlamydospores in summer and early autumn, and an olive to black, hard outer layer in late autumn. With higher and lower temperature differences between day and night- especially in later autumn- sclerotia often form on the colony surfaces (Zhang *et al.,* 2013).

Symptom

Rice false smut disease is also known as green smut and considered as Lakshmi disease, because it was always found associated with bumper harvest. The false smut pathogen, *Ustilaginoidea virens*, infects rice at the time of panicle development and affects the young ovary of the individual spikelet transforming it into large, yellow to velvety green balls (smut balls) and the symptoms produced are visible from milky stage onwards. Initially, the smut balls are small in size and remain confined between glumes. They gradually enlarge and enclose the floral parts. The individual grain get converted into yellowish smut ball then changes to yellowish orange to green, olive green and greenish black on maturity. Powdery dark green spores are released when smut balls burst open (Biswas, 2001; Atia, 2004).

If the infection occurs before fertilization most of the glumes remain sterile without any visible sign of infection. Typical large, velvety, green smut balls develop when infection occurs after fertilization. The fructifications replacing the grains represent the conidial, pseudosclerotial and sclerotial stages of the pathogen. The pseudosclerotia (green smut balls) consist of mycelial tissue and spore masses, remnants of anthers and portions of palea and lemna. In general only few grains are affected in a panicle but the number may rise up to 100 in case of severe disease incidence (Ladhalakshmi *et al.,* 2012).The symptoms are visible only after flowering when the fungus transforms the individual grains of the panicle into globose structures or yellowed carbonaceous masses. These masses are dusty representing more than twice the diameter of normal grain and at early development are yellow and then acquire dark green or almost black color, and explode releasing the spores of the fungal causal agent (Quintana *et al.,* 2012). The disease has been reported on the male inflorescence of *Zea* *mays* and on wild species of Oryza (Mulder and Holliday, 1971).

Diseases cycle

Fungus *Ustilaginoidea virens* insight the disease. *U. virens* produces both sexual (sclerotia) and asexual (chlamydospores) stages in its life cycle. Sclerotia are the major source of primary inoculum. In nature, over wintered sclerotia germinate and produce ascospores and coincides with the anthesis of early sown rice crop. Ascospores feed on the floral parts and initiate infection. Air-borne chlamydospores play an important role in the secondary infection, which is a major part of disease cycle (URL-1). Various disease incidents and severity significantly varies between seasons and locations. Seed germination in smut-infected samples was 72.4% which is 21.45 per cent lower than the non - smutted samples (92.20%) (Gulzar and Sanghera *et al.*, 2012).

During the surveys common graminaceous grasses and sedges in the rice fields like *Echinochloa colona*, *Echinochloa crussgalli*, *Digitaria longifolia*, *Leersiahex andra*, *Fimbristylis milliaceae*, *Cyperus rotundus*, weedy rice etc. were observed for the presence of false smut disease. Observations were made on any other modes of active survival as well. (Rashmi *et al*., 2016).

The fungus overwinters as sclerotia in the soil. They germinate and produce spores that infect the grain. The disease first appears as a large gray to brownish green fruiting structure covered by a thin membrane that replaces one or more grains of the mature panicle (URL-3). Ascospores produce by sclerotia are primary source of infection to rice plants, whereas air-borne chlamydospores may cause secondary infection (Ashizawa *et al.*, 2010). The RFS pathogen grows and contaminates the infected plant tissues, including the stamen and filament. Thus, this is regarded as the plant stamen-filament disease (Tang *et al*., 2013).

Epidemiology

False smut (*Ustilaginoidea virens*) was observed during Oct II and III week and that coincided with average temperature 27.42^{o} C, relative humidity of 83 per cent and rainfall of 12.1 mm (Vakiti *et al.*, 2017). High relative humidity (>90%), temperature between 25 and 30°C and rainy days at the time of flowering are the favourable environmental factors for the disease infection, as mentioned by (Atia2004). Fungi can reproduce by selfing or outcrossing. In heterothallic ascomycetes such as *Tuber melanosporum* Vittad (Rubini *et al*., 2011). The maximum disease severity and incidence were occurred at temperature range of 32-24ºC, relative humidity (88-74%), rainfall (6.67-6.66 mm) and sunshine hrs (6.20-6.29 hrs) both in case of variety Sabourardhajal and 25th June sown crop whereas, minimum disease incidence and disease severity occurred when temperature and relative humidity was same but their sunshine hrs and rainfall was lower than both Sabourardhajal and 25th June sown crop in case of Sahbhagi variety and 15th July sowing (Priya *et al*., 2017).

Integrated diseases management

Prediction or forecasting Proper management strategies with non- chemical means are needed to be framed to control the disease. More emphasis should be given for achieving the resistance varieties and management of false smut needs. This review summarizes the present status of the disease and progress in the field of its integrated management by resistant varieties, exploration of resistance genes, chemical and non-chemical means of control including the use of bio-control agents (Sanjeet *et al*., 2018). Management of the disease has been achieved through cultural, biological and chemical control. (Pannu*et al.*, 2010; Mohiddin *et al.,* 2012) reported the effectiveness of various fungicide against false smut. According to farmers, inadequate management of these diseases resulted in poor production in crops (Nelson *et al*., 2001). The strategic management of the disease may be directed to the specific areas of the fields where there is a history of the disease through manipulating genotypes and transplanting time (Sarker *et al*., 2017).

Resistant cultivars

Research on rice false smut resistance screening and molecular mechanism of false smut resistance is not sufficient (Zhang *et al.*, 2014). Phenotyping of rice cultivars for false smut is based on scoring system as per the standard evaluation system (SES) scale of IRRI (2002).(Singh and Singh 2005) evaluated and screened 27 rice genotypes resistant to false smut from 98-rice germplasm (Yan *et al.,* 2014). Screened 186 rice hybrids to false smut resistance was done who identified few hybrids with low disease incidence. Screening of 125 rice genotypes by artificial inoculation of false smut (Kaur *et al.,* 2015) identified nine hybrids namely Hybrids VNR-211, GK-5025, HRI-140, IRH-74, PRSH-9018, KPH-467, RH-10428, 27P64 and KRH-4 which shown complete resistance to rice false smut.

Cultural control

Weeding and insecticides application were done at appropriate time for best management practices (Priya *et al*., 2017). Early transplanted rice had higher disease incidence when compared to late planting (Chhottaray, 1991; Dodan and Ram Singh, 1995).

Conservation tillage, continuous rice cropping and moderate nitrogen fertility rates reduced false smut disease in susceptible cultivars (Brooks *et al.,* 2009). Use of sclerotic free seeds for sowing and cleaning of bunds may help the farmers to reduce the initial occurrence of the disease.

In respect of cultivation practices, furrow irrigated rice cultivation system recorded less disease severity compared to flooded fields (Sanjeet *et al*., 2018). The mechanism behind is the reduction on the survival period of chlamydospores in soil and occurrence of physiological changes in the host plant in response to shift of rice cultivation from anaerobic to aerobic growing conditions (Brooks *et al.*, 2010). The management of the rice seedling and adult plants were the same as the conventional method except for an extra application of 75 kg/hm2 urea at the beginning of rice booting stage (Zhang *et al.*, 2014). Cultural practices like bunds and fields cleaning reduce the incidence as the disease has been reported on some of the weeds.

Biological control

Biological control should done by *Trichoderma viride*, *Trichoderma virens*, *Trichoderma harzianum* and *Trichoderma reesei* against *Ustilaginoidea viren* (Kannahi *et al.*, 2016). Raji *et al.*, (2016) studied plant extracts under *in vitro* against rice false smut pathogen which was considerably inhibited by bulb extract of garlic (*Allium sativum*), rhizome extract of turmeric (*Curcuma longa*), leaf extracts of lantana (*Lantana camara*) and bael (*Aeglemarmelos*), whereas plant oils of lemon grass (*Cymbopogon flexuous*) cinnamon (*Cinnamomum zeylanicum*), and palmarosa (*Cymbopogon martinii*) have completely inhibited the growth of *U. virens*. *Bacillus subtilis* (Liu *et al.*, 2007) was found effective against the fungus.

Andargie *et al.*, (2017) reported first time *Antennariella placitae* effective against rice false smut (*Ustilaginoidea virens*) both *in vitro* and *in vivo* condition.

Chemical control

(Liang *et al.*, 2014) found the control efficiency of 91.92 % by spraying 2.5 % Wenquning, a solution of validamycin suspension of *Bacillus subtilis*@ 4.5 Litre/ha at 6 days before heading. Bagga and Kaur (2006) evaluated and reported significant reduction in false smut incidence by spraying with Carbenadazim 50% WP (0.1 %) and fujione 40 EC (0.1, 0.2 and 0.3%) at the booting stage. Treatment with Propiconazole (tilt) 25 EC (0.1%) and Copper Oxy Chloride (Blitox) 50 WP (0.3%) were found as the most effective. Application of prochloraz + carbendazim followed by chlorothalonil was effective in controlling the false smut of rice (Mohiddin*et al.*, 2012) Spraying of chlorothalonil 75 WP (Kavach) @ 2 ml/l or Propiconazole 25 EC (Tilt or Result) during flowering reduce the disease incidence (URL-1). The fungicides evaluated exhibited 100% inhibition on the growth of *U. virens* eight days after inoculation *in vitro* in Nordox (copper fungicide), Mancozan super (Mancozeb 640 g /kg + Metalaxyl 80 g/kg), Suncozeb (80% WP Mancozeb) and Sidalco defender (435 g/l copper oxycholoride), however, after 23 days only Nordox inhibited mycelium growth (Daniel *et al*., 2017). (Researchers) reported that two sprays of 50% propiconazole EC at 300 g a.i. ha^{-1} and of 10% difenoconazole GR at 225 g a.i. ha^{-1}, exhibited the best control of rice false smut. Treated seed with trifloxystrobin (Trilex 2000), and propiconazole plus trifloxystrobin (Stratego) were also effective in reducing false smut with foliar application at the heading stage (X Zhou *et al.*, 2012).

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