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A review on sugarcane smut caused by *Sporisoriums citamineum* and its eco-friendly management

SHAILBALA SHARMA

Department of Plant Pathology, College of Agriculture, G.B. Pant University of Agriculture and Technology, Pantnagar-263 145 (U.S. Nagar, Uttarakhand)

ABSTRACT: Sugarcane is an important high value crop which gives high economic returns. It provides products like white crystal, khandsari, jaggery, pressmud, ethanoletc and shows industrial potential. Sugarcane is attacked by many biotic and abiotic factors which is responsible for lowering down the cane yield. Among the biotic factors, diseases caused by fungi are the major cause of concern. Among the various fungal diseases, sugarcane smut caused by pathogen *Sporisoriums citamineum* (Syn: *Ustilago scitamineum*) has become highly significant in all over the world. Infected whip produces diploid teliospore and transmit the fungus into the healthy plants. Generally, the whip is the combination of host and pathogen tissue. When plant becomes two to four month-old, whip (covered with transparent layer) emerges. Correct diagnosis of pathogen at correct time is essential specially to identify the pathogen, its pathogenesis, epidemiological studies and finally management aspect. Due to change in science and technology, various molecular diagnostic techniques have been developed and became reliable to study pathogen variability. Sugarcane smut considered as the most dreaded disease, if not manage properly, may affect the cane yield as well as juice quality. Considering the importance of disease, sett treatment with hot water is suggested for the disease management. Some field sanitation practices i.e., removal of infected clumps/plants from field, using disease free sugarcane setts etc. which may lower down inoculum. Disease resistance in plant also plays an important role. Combination of Chemotherapy, biological control, quarantine, biotechnological approaches etc with other practices can be an effective way against sugarcane smut.

Key words: Cane yield, chemotherapy, *Sporisoriums citamineum,* sugarcane, smut, pathogen, symptoms, thermotherapy, whip

Sugarcane is known as one of the important cash crops from agro-industrial point of view in India. Involvement of many biotic stresses includes pests and diseases that reduces sugarcane yield. Being a vegetatively propagated crop, sugarcane is attacked by many diseases and pathogens can easily be transmitted into healthy plant right from planting to harvesting (Anonymous, 2005). Sugarcane is perennial monoculture crop and harvested after 10 to 12 months. So it allows many systemic pathogens to proliferate and spread from one ratoon to the next season. In one study, Rott *et al*. (2000) reported that there have been approximately 240 diseases of sugarcane reported from all over the world and may causes severe cane yield reduction. In India, cane yield losses due to diseases are approximately 19-31 % (Jayashree *et al*., 2010).

After 1930, sugarcane smut became widespread in all the sugarcane growing areas and created severe problem in our country (Viswanathan *et al*., 2009).It causes considerable losses in juice quality and cane yield (Wada *et al*., 2016). Vicente *et al*. (2021) reported that this disease also causes increase in number of sick sprouts as well as size of the inoculum. Ramesh Sundar *et al*. (2012) also noticed that smut disease is mainly responsible to affect cane yield as well as qualitative attributes. In one report, it is mentioned that smut disease causes 30 - 40 % yield losses in plant crop and 70 % in ratoon crop. According to Mehra and Sahu (2015) smut caused upto 3 to 7 % reduction in sucrose content. Xiupend *et al*. (2019) also gave the same conclusion and said that that significant reduction in sucrose content is due to smut disease.

THE PATHOGEN

Smut fungus was first described as *Ustilago sacchari* by Rabenhorst in the year1870 but in India, it came into limelight by Sydow and Butler in the year 1906. In the year 1924, Sydow thoroughly studied and confirmed that smut fungus is also present in India. A smut fungus present in Java and Phillippines was different from *Ustilago sacchari* in terms of size of spores and so smut fungus was named as *Ustilagos*

citaminea. Later position of smut fungus was rearranged and it was renamed as *Sporisoriums citamineum* (Piepenbring *et al*., 2002). Generally, smut fungus belongs to Kingdom: Fungi, Phylum: Basidiomycota, Class: Ustilaginomycetes, Order: Ustilaginales, Family: Ustilaginaceae, Genus: *Sporisorium*(syn: *Ustilago*) and **s**pecies: *scitamineum* (Ramesh Sundar, *et al*., 2012).

HOW TO DETECT THE SMUT PATHOGEN

Earlier for detection of smut pathogen, microscopy combined with specific stains was commonly used practice. For detection of hyphae of smut fungus especially in nodal buds of sugarcane crop, a staining technique was developed by Sinha and Singh (1982) by using trypan blue dye. If some clones of smut pathogen escaped from infection, could easily be detected by using this dye. By using antiserum, a new ELISA technique was developed by Padmanabhan and Mohanraj (1994)with the aim to detect the smut infection. In this technique, a product taken from a *Sporisorium scitamineum* mating type allei (bE gene) is amplified by primer and main feature of this technique is that it is highly specific to *Sporosorium scitamineum.* To know the presence of smut hyphae, stained or cleared meristematic tissue of sugarcane is microscopically examined and Echaves-Badel (1991) reported that this is used to identify smut infected plant just before sorus formation.

Acevedo and Pinon (1996) developed an indirect immune-fluorescence technique which is mainly used to diagnose the presence of *Sporisorium scitamineum* infection in sugarcane. At present time, for fast, accurate detection and quantification of plant pathogens PCR based techniques are frequently used. Singh *et al*. (2004) reported that PCR technique gives the best results and significantly better than others for smut detection. Jorf and Izadi (2007) concluded that microscopic study along with PCR assay could be used efficiently to detect the presence of smut pathogen.

VARIABILITY

Variability in pathogen is responsible to evolve the new races of pathogen. In this context, Schenck (2003) reported that new races of smut pathogen emerged in Hawai. To study the variability in smut pathogen, combination of different molecular diagnostic tools could be used. It could be an appropriate and reliable approach. Braithwaite *et al*. (2004) revealed that AFLP (Amplified Fragment Length Polymorphism) could be useful to examine genetic variation between 38 isolates of test pathogen. Even simple sequence repeats (SSR or microsatellites) show higher sensitivity and may generate polymorphism to show the presence of other clusters.

Intra-species diversity within isolates of test pathogen taken from South Africa, reunion island, Hawaii and Guadeloupe was studied by using RAPDs, *b*E mating type gene detection, rDNA sequence analysis and spore morphological studies (Singh *et al*., 2004). By using microsatellites, genetic diversity and population structure of smut fungus could be investigated (Raboin *et al*., 2007).Different studies were conducted to know the presence of different physiological races of smut pathogen and finally revealed about the possible presence of smut race in Kenya country (Nzioki *et al*., 2010). Different new techniques were studied to assess genetic diversity. Gang-Hong *et al*. (2017) revealed that ISSR molecular marker technique is an efficient as well as economical. For better understanding of smut pathogen, study on genetic diversity is essential. It clearly provides a base for development of resistant varieties.

SYMPTOMATOLOGY

One of the most characteristic features of smut infected plant is emergence of whip like structure filled with grey to black powdery mass (Comstock, 2000). The whip may be few inches to few feet long and mainly develops from the terminal bud or from lateral shoots on infected stalks. The developing whip having powdery mass which is covered with transparent layer and it takes six to seven months to mature. When wind blows, transparent layer of the whip ruptures and release huge quantity of smut spores which are already present inside the whip. These smutted spores can easily be transmitted from

one infected plant to another healthy plant even by a gentle wind. Due to profuse tillering, the smut infected plants may have produced cylindrical or thin cane, spindly or more erect shoots with small narrow leaves which finally results poor cane formation. Two blooms period of the smut disease mainly in the month of May-June and October-November occur in Sub-tropical India.

Fig: Smut whip formation from the apical region of the stalk.

Fig: Smut infection in sugarcane plant

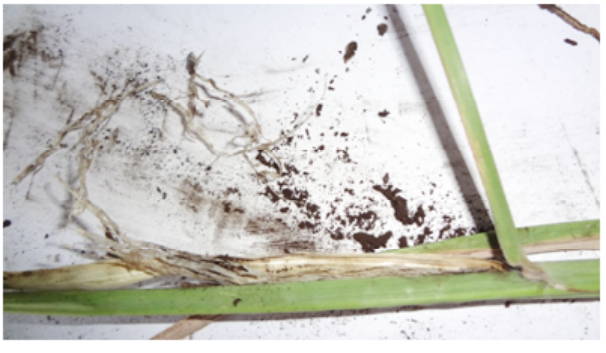

Fig: Release of black powdery mass from smut infected portion

EPIDEMIOLOGY

Smut fungus is present in the sugarcane setts in the dormant stage. So, primary infection occurs from nodal portion of the sugarcane. Sometimes contact of fungal spores with sugarcane setts after planting may also cause the infection. After the rupturing of transparent layer of whip, wind borne teliospores may reach nodal buds of standing cane may cause secondary infection. Alexander and Ramakrishanan (1978) reported that pathogen may remain viable for more than 10 years in dry conditions. Waller (1969) revealed that dispersal of spores is restricted during wet weather, disease may increase rapidly during hot weather with more irrigations. 30-35^0 C temperature and moderate rains favour the disease (Durairaj *et al*., 1972).

Teliospores produced in smut whip, may easily disseminate the disease. These teliospore require sufficient amount of water for germination (Waller, 1969).They produce promycelium and even undergo for meiosis process and finally produces four haploid sporidia. Pathogen produces two different mating types of sporidia due to its bipolar nature. These two different types of sporidia come together and form dikaryon. This dikaryon produces hyphae structure which penetrates and enters into the bud scales. It finally infects the meristematic tissue and induces formation of flowering structures in which it colonises and produce teliospores (Croft and Braithwaite, 2006). Now flowering structures is completely changed into a whip like sorus that come out between the leaf sheaths. The thin transparent layer made up of host epidermis covers the smutted powder. Finally spread of teliospore takes place through wind and spore reaches to healthy plants. By this way, disease cycle continues. The teliospores are generally 6.5 to 8 µm in size and reddish brown, round, sub-ovoid, smooth to moderately echinulate in shape. Spore production may remain continue for three to four months from a single sorus.It releases 108-109 spores per day.

It is also reported that there may be two possible cycles of infection in which primary infection takes place through dormant teliospores which are present in soil while secondary infection by wind or through unsanitary farming practices. These infection cycles result in the development of characteristic smut whips like structure. Waller (1969) reported that time

interval from infection to whip production is almost 6 months under field conditions. The pathogen perpetuates and spread by spores, planting material as well as by ratooning.

ENVIRONMENTAL FACTORS

Temperature about 20^0 to 31^0 C is considered as good for promycelium development. 31^0C temperature is suitable for disease development. The same temperature is an important for the production of infectious hyphae and sporidia (Bock, 1964).Sreeramulu (1973) reported that dispersal of spores is maximum during the day time. At 24^0 to 27^0 C temperature and 50 to 60 % relative humidity, maximum dispersal of spores take place. Windborne spores spread from one plant to other plant and cause infection in to the buds.

Bhuiyan *et al*. (2009) also reported that the optimum temperature around 30^0 C is required for spore germination. The isolates received from Australian and Thailand had different characteristic feature. Both strains were different so their temperature requirement will be also different for spore germination (Braithwaite *et al.,* 2004 and Raboin *et al.*, 2007). Mehra and Sahu, (2015) reported that 22.3^0 C is optimum temperature for disease development.

DISTRIBUTION

Natal, reported the first appearance of sugarcane smut in South Africa in the year 1877 (McMartin, 1945). Now this disease has been established in all sugarcane growing parts of the world. In our country, this disease is distributed in many parts of sugarcane growing areas of Andra Pradesh, Bihar, Delhi, Gujarat, Punjab, Karnataka, Madhya Pradesh, Maharashtra, Tamil Nadu, Uttar Pradesh, West Bengal (CABI/EPPO, 2008; EPPO, 2014). The smut disease of sugarcane is widely distributed all over the world. In countries like India, Egypt, Ethiopia, Kenya, Madagascar, Sri lanka, Thailand, Vietnam, Somalia, Afganisthan, Cambodia, Myanmar, Pakistan, Mozambique, Cuba, Queensland and South Africa sugarcane smut disease is distributed in wide spread pattern (Butler and Bisby, 1960; Ali, 1959; Lopez *et al*., 1979; IPPC 2015a; McMartin, 1945; CABI/EPPO, 2008 and EPPO, 2014).

HOST RANGE

Marchelo-d' Ragga and Ahmed, (2015) reported about the host range of pathogen and they clearly indicated that a few genotypes within the cultivated sugarcane species attacked by pathogen show the disease symptoms. Vanky (2000) also reported that different *Saccharum* spp, *Imperata* spp and *Erianthus* spp etc shows the host range of smut pathogen. Some scientists reported that the damage by pathogen depends on the susceptible and resistance nature of the plant species.In our country, smut is always reported in *Saccharum barberi* while *Saccharum spontaneum* is known as collateral host. It also has reservoir of inoculum (Braithwaite *et al.,* 2004).

ECONOMIC IMPORTANCE OF DISEASE

Smut pathogen is mainly present in sugarcane sett so considered as the sett-borne pathogen (Piepenbring *et al.*, 2002) which finally lowers down the cane productivity and causes the considerable loss in crop (Rott *et al.*, 2000). In main sugarcane crop, smut causes 30 to 40% yield reduction while in ratoon crop yield losses may reach up to 70% which also affect the juice. Rao *et al.* (1985) reported that single smut disease may cause 68 % to 80 % loss in cane yield while 32% reduction in juice quality in the main crop but intensity of these losses may increase in ratoons crop. Briceno *et al*. (2005) also reported that yield losses may reach upto 39 to 56% in planted crop while 52-73% reduction in cane yield is noticed in the ratoon crop. The reason behind the reduction in cane yield is reduction in number of millable canes and size of cane girth due to smut disease. De Armas *et al*. (2007) also reported that smut disease also affects the sucrose content and deteriorates the juice quality.

DISEASE MANAGEMENT

All the vegetatively propagated crop are more prone towards the disease infection. Likewise, sugarcane

is vulnerable to systemic infection by smut pathogen right from planting of crop and become a serious issue to sugarcane growers. For disease management, one should not depend on one management practice. Incorporation of various disease management methods like thermotherapy, cultural practices, host resistance, chemotherapy, biological control practices, quarantine regulations, different biotechnological approaches are always known as the best options and plays important role in disease management. Prevention of any disease is always better than cure of disease. So, at early as well as perfect stage of disease development, smut should be managed. Abera *et al*. (2009) and Firehun *et al*. (2009) gave the different package of practices such as continued monitoring, field sanitations which include rouging of smut affected stools, hot water treatment (at temperature 50^0 C for 2 hours' time period) of seed setts, chemical treatment of sugarcane setts, use of resistant sugarcane cultivars and avoidance of ratooning of affected fields etc.

Thermotherapy for disease management

Thermotherapy is one of the most reliable practice especially for management of sugarcane sett. Abera (2005) reported that disease can easily be managed through sett treatment at temperature 50^0 C for 2 hours. One can also maintain the temperature at 52^0 C for half an hour for sett treatment. Results of thermotherapy are highly satisfactory and proved that hot water treatment is effective against sugarcane smut. Some scientists worked in this direction and suggested that hot water treatment along with sett treatment with fungicide at the same time may avoid this problem. In hot water treatment, temperature and time factor plays an important role so optimum temperature and proper time must be maintained to manage the disease. This will help in destruction of pathogen from infected setts of sugarcane. Abera *et al*. (2009) suggested for raising nursery crop, host water treatment should be avoided up to some extent.

Cultural control

Proper cultural practices should be adopted to manage disease right from planting of crop. The various practices include use of disease-free and healthy seeds, complete destruction of diseased stools/plants, avoid the ratoon crop, fallowing, crop rotation etc reduce inoculum and lower down yield losses in the field. Field sanitation practices avoid disease spread as well as perpetuation of the pathogen (Kalaimani and Natarajan, 1990). Abera *et al*. (2009) reported that complete destruction of smutted infected stools or infected shoots at 10-15 days interval right from two months old crop up to harvesting period is consider as good practice.

Host resistance

Scortecci *et al*. (2012) and Ramesh Sundar *et al*. (2012) reported that use of disease resistant cultivars is one of the most efficient and effective way to manage the smut disease. Use of disease resistant cultivars is the best option but never forget to use the disease-free seeds. Genetic bases of resistant and susceptible cultivars have been used to manage the smut disease. There was smut outbreak in Kununurra which clearly gave the picture to encourage and develop the breeding programme. In Indonesian resistance screening trials, commercial varieties of sugarcane were screened through testing against the smut susceptible varieties (Croft *et al.,* 2000).Comstock, (2000) also showed the importance of resistant varieties for management of smut disease.

Due to development of new virulent strains of pathogen, most of disease resistant cultivars beak down. So, it is essential to know the resistance source in crop to flourish the newly released disease resistant varieties for commercial cultivation. Fontaniella *et al*. (2002) revealed that metabolites like glycoprotein stops the germination of teliospore. Legaz *et al*. (2005) reported that some other defence metabolites like β 1, 3 glucanase, chitinase, glycoproteins also prevent the teliospore germination of smut fungus. Santiago *et al*. (2010) reported that caffeic acid also affect the growth and physiology of both host and pathogen. Ramesh Sundar *et al*. (2012 b) focused towards the external and internal resistance in sugarcane crop against smut disease.

Ramesh Sundar *et al*. (2015) concluded that for successful management of smut, one should have correct information of resistant source. In this context, TaqMan quantitative real time polymerase chain reaction analysis can be the best option and it provides the most efficient and reliable resistant identification procedure against sugarcane smut (Su *et al*. 2016).

Chemotherapy for disease management

Sugarcane sett treatment with fungicides triademifon (0.1%) or propiconazole (0.1%) for two-hourtime interval can be suggested for an effective management of sugarcane smut (Bharathi, 2009-2010).Fungicides triademifon and propiconazole not only reduced the smut incidence but also increased the cane yield (Sundravadana *et al.,* 2011). Meena and Ramyabharathi (2012) also concluded that sugarcane sett treatment as well as foliar spray with fungicide triadimefon @ 0.1 % at 30, 45 and60 daysinterval just after planting gave the highest cane yield and reduced the smut infection. Fungicide triademifon @ 0.1% and propiconazole @ 0.1% clearly showed its effectivity against smut disease. The fungicides reduce the disease significantly (Shailbala *et al*., 2013, 2014).

Singh *et al*. (2014) reported that fungicide propiconazole (tilt)@ 0.2 % and emisan@ 0.25 % gave the best results to reduce the smut incidence. Sett treatment for 5 min dip with triazole fungicides, propiconazole andtriadimefon effectively manage the smut disease in seedcane of sugarcane (Bhuiyan *et al.,* 2012). Bhuiyan *et al*. (2015) also reported that fungicide flutrifol along with fertilizer reduced smut infection in sugarcane. Kishore *et al*. (2020) revealed that sugarcane sett treatment with combi fungicides like azoxystrobin + tebuconazole @0.1% has significantly lowered down the smut incidence as compared to other treatments.

Biological control for disease management

Fungus like *Fusarium moniliforme* var *subglutinans*, *Aspergillus niger*, *A. flavus* and *Penicillium*sppinhibits the teliospore germination of smut pathogen(Vaishnav *et al*., 1992).In one study, bio-agent *Trichoderma* spp. also showed the same activity and inhibited the spore germination in Cuba (Martinez *et al*., 1998). Not only the fungus but three species of beetle also attacked and fed on membrane nearby *Sporisoriums citamineum* whips and inhibited the spore germination (Sabalpara and Vaishnav, 1997).

Bio-agents like *Trichoderma* spp, *Aspergillus* spp, *Penicillium* spp showed the antagonistic potential against smut pathogen (Lal *et al*., 2000). *Trichoderma harzianum* and *Trichoderma viride* completely inhibited the mycelial growthof pathogen *in vitro* (Singh *et al*., 2014).

Quarantine regulations

Always avoid the entry of planting materials from risk prone areas. It must be strictly followed as routine practices which will avoid the entry of the most dreaded pest and pathogens in a new area. Jaroenthai *et al*. (2007) reported that out of total germplasm collected in Thailand, approximately 20 % of germplasm collections showed reduction of yield, CCS and brixvalue due to entry of sugarcane seeds from other country without following proper guidelines. Even in our country, no quarantine guidelines are followed and seed is taken from one state to other state without restriction. Que *et al*. (2012) advised that sugarcane seed must be tested against smut disease with permissible limit and then distributed to other areas. In all the sugarcane growing countries of the world, strict quarantine regulations must govern for the importation of sugarcane seed which will avoid the entry of smut pathogen from one place to another.

Biotechnological Approaches

Different types of molecular techniques were used to know the interaction between sugarcane crop and test pathogen at molecular level. These techniques include cDNA-AFLP(Que *et al*., 2011a), DDRT-PCR (Que *et al*., 2009a), cDNA microarray (Que *et al*., 2009b), TaqMan real-time PCR (Su *et al*., 2013a), Solexa se-quencing (Wu *et al*., 2013), RNA-Seq (Que *et al*., 2014a), genome sequencing (Que

et al., 2014b) and two-dimensional gel electrophoresis (2-DE) with matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-TOF/ MS) (Que *et al*., 2011b). By using cDNA-SRAP along with agarose gel electrophoresis technique, smut responsive gene can easily be identified which may play important role in resistance mechanism (Huang *et al*., 2015).

Ramesh Sundar *et al*. (2015) reported that some molecular techniques help to collect information on differentially expressed transcripts of sugarcane against test pathogen. These techniques include cDNA-AFLP, differential display techniques etc. There are clear-cut difference in the level of PR proteins like poly phenol oxidases, phenylalanine ammonia lyase, peroxidase, esterase, chitinase and β 1, 3 glucanase in sugarcane genotype/clones in susceptible and resistant cultivars against smut fungus (Esh *et al*., 2014). Su *et al*., (2014 b) studied and reported about the structural properties of chitinase gene obtained from RNA sequence analysis of interaction between host (sugarcane) and test pathogen.

CONCLUSION

Smut is one of the most dreaded diseases of sugarcane. Black or grey coloured whip like structure is the most distinguishing feature of this disease. Pathogen is mainly present in the setts so infected sugarcane seed setts are known as the primary source of inoculum while wind borne teliospores take responsibility of secondary spread. Smut disease causes reduction in cane yield and deterioration in juice quality. So integration of all the important management practices against smut of sugarcane is the best option. Hot water treatment and moist hot air treatment of sugarcane setts definitely help in destruction of smut pathogen. Involvement of recommended cultural methods mainly use of disease-free seeds, field sanitation etc helps to lower down the inoculum level. Development and use of smut resistant varieties are known as the best way to manage the disease. Sugarcane sett treatment with fungicides and bio-control agents also play important role in disease management. Sugarcane setts are used for planting purpose so it is essential to follow the quarantine regulation through which entry of infected seed material from one place to another place can be avoided. To get the precise and early detection of pathogen, different molecular tools are very much effective. Now a days these tools are considered as a pre-requisite for smut management. Therefore, combination of the best possible management practices is required to minimize the losses caused by sugarcane smut.

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