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Kernel Smut of Rice: An Overview

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ABSTRACT Kernel smut (*Tilletia barclayana*) of rice also referred as paddy bunt (*Neovossia horrida*) is worldwide in distribution. In India, large number of seed samples get rejected as the tolerance levels for the disease are prescribed at 0.1 and 0.5 per cent for foundation and certified seed, respectively. In USA, the disease has been occurring persistently for the last 50 years in Arkansas and adjoining rice growing areas causing significant quantitative as well as qualitative losses. There is voluminous literature available on the distribution, economic importance, etiology, epidemiology, disease cycle and management of this disease which is reviewed in this article.

Key words: Kernel smut, paddy bunt, *Tilletia barclayana*, *Neovossia horrida*

Kernel smut [*Tilletia barclayana* (Bref) Sacc and Syd.] of rice (*Oryza sativa* L.) henceforth abbreviated as KS in this article also referred as paddy bunt [(*Neovossia horrida* (Tak.) Padwick and Khan] is a designated seed borne disease under the Indian Minimum Seed Certification Standards and the tolerance levels have been prescribed at 0.1 and 0.5 per cent, respectively, for Foundation and Certified Seed lots [1]. The disease causes quantitative as well as qualitative losses and is world wide in distribution. Extensive work has been carried out in India and abroad on different aspects. Completely smutted grains are lost during harvesting operations and add a lot of inoculum in the soil. Partially smutted grains, besides carrying the inoculum to the soil, also release bunt spores imparting grey colour to the milled rice and, adversely affect the market value of such rice. Rice samples containing more than 3 per cent bunted kernels are graded as *Smutty* [2] and penalized at mill.

GEOGRAPHICAL DISTRIBUTION

The disease was first reported by Takahashi [3] from Japan in 1896 and Anderson [4] from the U.S.A. in 1899. The disease has been observed from all the major rice growing countries *viz*., Atlantic Islands, Bangladesh, Brazil, China, Columbia, Cuba, Fiji, Formosa, Greece, Guyana, Indonesia, Italy, Java, Korea, Malaysia, Mexico, Myanmar, Nepal, North-East Argentina, North-East Brazil, Pakistan, Panama, Peru, Philippines, Surinam, Taiwan, Tajikistan, Trinidad and Sierra Leone, Venezuela, USSR, Uzbekistan, Vietnam and West Africa [5-18]. It was intercepted during routine phytosanitary inspection in Poland [19]. In USA, the disease has been reported to occur throughout rice growing regions, it is more important in Arkansas, Mississippi and Missouri states [20, 21].

In India, the disease was reported for the first-time by Butler [22] in 1913. Since then, it has spread to all the rice growing States including Assam [23], Punjab [23-24], Orissa [25-26], Rajasthan [27], Uttar Pradesh [23,28-30], Karnataka [31], Andhra Pradesh [32-33], Tamil Nadu [33], Bihar, Madhya Pradesh and Himachal Pradesh [34-35], Haryana [36], Maharashtra [37] and West Bengal [38-39].

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ECONOMIC LOSSES

In South Carolina, Fulton [40] reported 3-4 per cent grain infection causing serious damage to rice flour due to dark colour. A loss of 2-5 per cent was reported from Mandalay (Myanmar) by Su [41]. The disease has been occurring regularly, in Arkansas and causing significant loses [42-51]. An estimated loss of 15 million dollars was reported to have been suffered by the Texas rice producers by this disease only [52]. In Pakistan, severe panicle infection (87%) was reported from Sind and Punjab provinces [53].

In India, a heavy attack of bunt was recorded in a number of States. The incidence of the disease ranged from 8-63 per cent in Assam [23], 22-43 per cent in Tamil Nadu and 16-35 per cent in Andhra Pradesh [33]. Reddy and Reddy [33] observed that the disease infection percentage was higher (38-60 per cent) during *kharif* than in *rabi* (2-22 per cent). The National Seeds Corporation of India rejected a large number of seed lots in Orissa region as these were contaminated up to 35 per cent with the smut [54]. Large-scale rejection of quality seed in Punjab has also been reported for want of meeting the minimum prescribed tolerance standards during the last 15 years [55-58]. The disease has been making a regular appearance in Punjab and no variety or location was free from this disease [59]. Bunt infected seeds are responsible for poor germination, vigour and crop stand [60].

Of late, KS has become a major problem in rice hybrids [61-64] in India and China. Incidence of KS has been recorded up to 15-20 per cent on some of the CMS lines in Punjab which has proved a major bottleneck in seed production and release of rice hybrids. In China also, this disease is reported to cause 20-50 per cent losses in parental lines of hybrid rice [65-69].

DISEASE SYMPTOMS

The symptoms of the disease appear at the time of crop maturity. Only a few grains in panicle are infected. Normally, only a part of the grain is affected, but many times, the entire grain is replaced with a black powdery mass of bunt spores. Such grains show minute black pustules or streaks bursting through the glumes. In severe infections, a short beak or spur-like outgrowth is produced by the rupturing glumes resulting in scattering of black spores on to the other seeds and foliage (Fig. 1) and it is the easiest way to detect the disease in the field. Most often, in the field, infected grains exhibit no external symptoms, but if examined carefully, the infected seeds can be detected by their characteristic dull colour. However, for authenticated detection of infection, NaOH seed soak method is used [70-71].

PATHOGEN

Taxonomic position/historical account/present status

There have been different views regarding the nomenclature of the pathogen. Originally, the causal organism of the smut was named as *Tilletia horrida* by Takahashi [3] and he described the fungus as: "Spore masses pulverulent, black, produced within the ovaries and remain covered by the glumes. Spore shape described as globose, irregular rounded or sometimes broad elliptical, the round ones 18.5-23.0 µm in diameter and the elongated 22.5-26.0 x 18.0-22.0 µm in size. Epispores deep olive brown, opaque, thickly covered with conspicuous spines. The spines hyaline or slightly coloured, pointed at the apex, irregularly polygonal at the base more or less curved, 2.5-4.0 µm in height and 1.5-2.0 µm apart at their free ends. Sporidia filiform or needle-shaped, curved in various ways, 10-12 in number and 35-53 µm in length."

Later, a number of workers [22, 60, 71-75] conducted morphological and spore germination studies and indicated that there were several aspects in which this fungus was regarded as different from a typical *Tilletia*. They observed that the teliospores on germination produced a large number of sporidia in a single whorl on the promycelium and the primary sporidia did not fuse. The spores often had a short hyaline apiculus and a broad, hyaline, warty outer coat of a gelatinous nature.

The pathogen infected only a few grains in a panicle and often only a part of the grain. These observations lead Padwick and Khan [76] assign the fungus to the genus *Neovossia* and named it *Neovossia horrida* (Tak.) Padwick and Khan. Tullis

and Johnson [77] conducted further studies on spore germination and inoculated the pathogen to several grass hosts (*Pennisetum* spp.), on which they found that it produced spores essentially identical to those of *Neovossia barclayana* Brefeld. Thus, they agreed with Padwick and Khan that the fungus belonged to *Neovossia* and named it *Neovossia barclayana* which has priority. Fischer [78], however, retained the name *Tilletia horrida* Tak. However, these studies leave room for doubt regarding the validity of the results because they had inoculated the sporidia of the fungus on to young seedlings of *Pennisetum*, growing in Petri-plates, thinking that the pathogen was systemic in nature, whereas the pathogen causes local infection. Fischer and Halton [79] did not recognise the pathogen as a species of *Neovossia*. However, Duran and Fischer [80] agreed that the fungus on rice was the same as that found on grasses by Tullis and Johnson [77], but regarded the mature spores to be more of the genus *Tilletia* than *Neovossia*. They, therefore, disposed it as *Tillletia barclayana* (Bref.) Sacc and Syd., which is now generally used. Later on, Singh and Pavgi and Singh *et al.* [81-82] studied the cytology of teliospore germination and development of fungus and were of the opinion that it belonged to *Neovossia*. They reported that the fungus was homothallic. Recent studies based on phylogenetic analysis have revealed that *Neovossia* is not distinct from *Tilletia* and hence the species being described as or transferred to *Neovossia* have been included in *Tilletia* [83-84]. However, to date, both the names are being used by different workers. The fungus was reported to be homothallic based on presence of two nuclei in detached sporidia [30], nevertheless, later studies suggest the heterothallic nature of the fungus based on inoculation with single and paired monosporidia [85-86].

Etiology, spore germination and cytology

Chlamydospores (teliospores) of the fungus were described by Anderson [4] as spherical, 22-26 µm and covered with a hyaline envelope having 2 µm or more thickness. However, Tullis and Johnson [77] reported the spore diameter to be 23-35 µm, which upon germination produced 20-60 hyaline, filliform, non-septate primary sporidia (1.5-2.0 x 38.0 x 53.0 µm). Cell wall of teliospore was found to have four distinct layers [87]. Agarwal *et al.* [88] observed that the black spore mass is intermixed with some sterile cells, few to many, usually globose hyaline to yellowish, wall 2-4 µm thick, 10-30 µm diameter. Spores globose to sub-globose, initially light brown turning dark brown at maturity enclosed in a hyaline sheath with or without a short apiculus, mostly 17-25 µm in diameter. The authors have recorded the spore diameter in the range of 18-25 µm.

Teliospores of the fungus are thick-walled that germinate under specific conditions. The smut spores remain dormant for 4-5 months after the time of harvest [31, 73, 89]. The teliospores have also been reported to become dormant under cold conditions [90]. Whitney [91] reported that dormancy depended largely upon drying of teliospores after maturity. The authors of this article have also made similar observations while conducting spore germination studies. Previous unsuccessful attempts of spore germination by various workers may be due to insufficient ageing of spores [4, 22, 42, 60, 92-93].

Teng [71] found that the chlamydospores germinate near the surface of water drops in a Petri dish near a window, indicating the need for light and a good supply of oxygen. Several studies have indicated the stimulation of spore germination by treating with different chemicals like hydrogen peroxide [94]; HCI, NaOH, Mannitol [58, 95]; pre-soaking in water and giberellic acid at 25-200 ppm concentration [67-96]; heating of teliospores for 30 min at 60°C and 70°C [89, 97] and exposing the chlamydospores to sunlight, UV light for different periods, which included exposure to direct sunlight for 2 hrs [98], Flourescent light for 4-5 hrs [67, 89, 97]. Abundant germination was obtained after five days of light illumination for 10-12 h/day, or after 6-7 days when first 3-4 days were in the dark and the last three days under illumination for 10-12 hours [67, 98]. Whitney [91] recorded spore germination even under approximately 3 mm of water in a Petri dish, where the promycelium extrudes on to the water surface to produce whorl of primary sporidia. Similar aspects were also studied by the authors of this article. However, authors found constantly for four crop seasons (1993-1996) that teliospores could germinate within

3 days in Punjab when floated on a film of water in the Petri-dish at vulnerable stage of the crop (Table 1).

Table 1. Teliospore germination of *Tellitia barclayana* (on water film in Petri-dish) in the Punjab fields during the crop season

Date of placing Teliospore	Germination period (days)	Germination range (%)	Temperature range (°C)
July 1	10-12	4.5-9.5	24.5-38.8
July 15	7-12	12.0-22.0	23.0-37.5
August 1	5-6	19.0-26.0	23.0-37.5
August 15	3	18.5-22.5	24.0-36.0
September 1	3	19.0-26.0	21.5-33.0
September 15	4-5	21.5-23.5	20.0-33.0
September 30	9-11	8.5-11.5	18.0-32.0

The optimum temperature for teliospore germination was observed 25-30°C [67, 89]. The fungus grew well on PDA, oatmeal, rice extract, soil extract agar, 2 per cent water agar and in cavities made in water agar [99-101]. The production of both filliform and allantoid sporidia was maximum on PDA and the host extract media, minimum on Czapeck's media, while Richard's and glucose nitrate media did not favour the production of any type of sporidial production [100].

The mature spore contains a single diploid nucleus, that enlarges and undergoes successive divisions in the spore itself to produce 32 to 76 sporidia in a whorl at the tip of the promycelium. They are filliform or needle-shaped with flat base having pointed tip, curved in various ways and measure 38-53 µm in length and 1.3-1.5 µm in width. These do not fuse in pairs or form H-shaped structures as recorded in *Neovossia indica*, the causal agent of Karnal bunt of wheat. Fully mature uninucleate sporidia detach en masse from the tip of the promycelium that become undulate in shape and elongate a little more [81]. The single nucleus undergoes a mitotic division making the sporidium binucleate. Sometimes a septum is formed. On these sporidia, sterigma is developed which bear hyaline, curved, secondary sporidia measuring 7.5-13.0 x 1.2-1.8 µm in size. The two nuclei from the primary sporidium pass into the secondary sporidium. These nuclei undergo a conjugate division to form nuclei for subsequent secondary sporidia. On germination, the secondary sporidia produce infection hyphae, which is dikaryotic [34].

Variability in pathogen

Kumar *et al.* [102] recorded cultural and physiological variations in isolates of *T. barclayana* collected from various locations of Punjab. Pannu *et al.* [103] categorized the Punjab isolates in to four distinct groups, based on morphological, cultural and pathogenic variations. Intra population variations have also been observed in Punjab isolates using random polymorphic DNA analysis [104]. Genetic variations, based, both on RAPD and RFLP analysis, have also been observed by Pimental *et al.* [105], which indicated that *T. barclayana* population was comprised of two distinct taxa with one cluster corresponding to rice infecting isolate and other to isolates infecting species of *Panicum* and *Paspalum*.

Host range

Naturally occurring hosts as well as the hosts described by successful artificial inoculations [106] and synonomy with the species obtained from them [107] have been included in the host range which comprises, *Pennisetum alopecuroides*, *Pennisetum glaucum*, *Pennisetum selosum* and *Pennisetum orientale* var. *trifoliorurn* [78], wild rice, *Oryza barthii* [108]. *Oryza minuta* [109], *Brachiaria*, *Digitaria*, *Eriochloa*, *Panicum* and *Pennisetum* [80].

However, description of *Digitaria* Haller, Leersia Sw., *Panicum* L. spp. as a host has raised certain controversies, which can be interpretted in the light of original misidentification of the pathogen as *T. corona* which at the time was reported to occur on the species in question [84]. A high degree of host specificity has been reported in *T. indica* and *T. barclayana* isolates infecting wheat and rice, respectively [110].

Detection technique

The infected seed with ruptured glumes can easily be detected due to presence of black mass of adhered smut spores (Fig. 1). However, such seeds are very few and majority of seeds with partial infection do not rupture and cannot be detected visually. A 'Sodium Hydroxide Seed Soak Method' has been recommended for the effective detection

of bunted seeds [69, 70]. The seeds are soaked in 0.2 per cent solution of NaOH at 20-25°C. After 24 hr, the solution is decanted and the seeds are spread on white blotting sheets. Infected seeds look shiny jet black-green (Fig. 1) and are separated with the help of a forceps. For quarantine purposes, the seed wash and centrifuge extraction has been useful [111]. Pneumatic Separation of infected grains has been reported by Duhan *et al.* [112] in which infected kernels are separated by blast of air at varying speeds. The immuno-assay based techniques for teliospore wall proteins [113], immuno dip-stick test [114] as well as PCR based Restriction Fragment Length Polymorphism (RFLP) and Internal Transcribed Spacer Sequence (ITS) method [115] are being explored for the detection of *Tilletia* spp. On the basis of immuno reactive bands, *T. barclayana* was found to be distantly related to other morphologically similar taxa viz. *T. indica* and *T. walkeri*, both of which were observed to be closely related. Various techniques involving biochemical characterization of surface components [116], light and scanning electron microscopy [117] and image analysis of bleached teliospores [118] have been evaluated for differentiation of *T. barclayana* from other related *Tilletia* spp. A simple method for direct extraction of DNA from un-germinated teliospore by manually cracking it under stereomicroscope prior to adding to PCR mixture has been reported by McDonald *et al.* [119], which avoids difficulty and time delay in having to germinate teliospores prior to extracting DNA from a mycelial mat.

DISEASE CYCLE AND SPREAD

Chlamydospores (Teliospores) of the fungus are the primary source of infection, which survive in the soil or are carried through infected seed [11,34]. Sharma *et al.* [63] reported that at soil surface, the chlamydospores remained viable for two years and trace germination was recorded even after 3 years. However, at 5-15 cm soil depth the spores remained viable for more than one year and in traces up to second year. Nevertheless, under laboratory conditions, when the spores were stored in glass bottles, they remained viable for four years and in traces up to the fifth year. Liang *et al.* [67] reported that chlamydospores lost viability after three years of indoor storage.

The chlamydospores on germination produce primary sporidia, which further give rise to secondary sporidia, either directly or from the mycelium produced. The secondary sporidia are the primary infection propagules and responsible for the floret infection. Secondary sporidia are sickle-shaped and discharged forcibly for easy dissemination and spread of the disease [23, 28, 84, 100, 120-122]. Pan *et al.* [66] and Shu [64] have emphasised the significance of epiphytic budding of secondary sporidia in disease development. They reported that secondary sporidia produced directly by teliospores are not the main source of inoculum, but the epiphytic budding occurred over a long period on the plant and sporidia produced at that time were the only effective disease causing propagules. Epiphytic budding of secondary sporidia occured on the surfaces of rice and other weed hosts from April to October [123].

Nature of infection

Anderson [4] and Butler [22] recorded the fungal mycelium within the stem and believed that the disease was systemic. They thought that like the other species of *Tilletia*, the fungus entered the host tissue while still below ground. Reyes [60] concluded that only smutted seed produce smutted plants. Later, Choudhury [23], using a vacuum method, inoculated 97 rice panicles and found that 72 of them became infected. He, therefore, concluded that the fungus caused local infection of florets by sporidia and did not cause systemic infection. These findings were later confirmed and supported by various workers [51, 53, 77, 120, 124]. Singh and Pavgi [125] precisely studied the process of infection and reported that the sporidia first lodge on the stigma, then penetrate the style reaching the chalazal end of the ovary: the hyphae remain between the aleuron layer and seed coat, digesting the endosperm and making room for the developing sorus. The embryo is not invaded. After germination, if the sporidia do not find suitable host within 72 hours, they die [126]. However, recent studies conducted by Goates [127] have shown the contrasting results. He reported that the secondary sporidia on Petri dish lids held up to 46-49 days at 20-25 cm above the soil surface in the canopy, readily regenerated even after diurnal exposure to temperature above 38°C and relative

humidity below 10 per cent indicating tendency of sporidia to withstand extensive dry periods in field conditions and then to regenerate and infect plants under humid and rainy conditions. These studies are of great significance from epidemiological point of view.

Meteorological factors affecting disease development

A temperature range of 25-30°C and relative humidity of 85 per cent or more with intermittent showers at the time of ear emergence have been reported to be favourable for infection [28, 30, 51, 85]. Kaurav and Mathur [26], in India, observed that one week after the flowering, rain for one or two days, temperature between 24-33°C, relative humidity 88-90 per cent and sunshine for 9-10 hours favoured the disease development. Cartwright *et al.* [46] observed that the fields at different locations in the Arkansas state, having light rains at the time of flowering had more severe kernel bunt infection. Anita *et al.* [128] reported a positive correlation between the duration of panicle wetness and KS incidence. The authors while analyzing the historical data (unpublished) on disease incidence and weather elements at Ludhiana of Punjab, (India) for 14 years (1991-2004) found that the maximum temperature and sun shine hours were positively correlated with the disease development whereas the rainfall and the number of rainy days were negatively correlated for the same period. The correlation of 33rd Standard Meteorological Week (the stage of crop vulnerability to pathogen) indicated that the range of temperature from 25.1-33.5°C, relative humidity from 69-87% and sunshine hours of 7.5 were conducive to disease development.

Artificial creation of kernel smut

For effective screening of breeding material as well as fungicides, artificial creation of the disease is very important. Different methods for artificial creation of the disease have been employed and tested by number of workers. Choudhary [23], using a vacuum method, inoculated 97 rice panicles with sporidia and found that 72 of them became infected. Templeton [51,120] confirmed that the sporidia infected the open flowers. Akhtar and Sarwar [129] followed a partial vacuum method used by Hassan [53] for inoculations and observed up to 84 per cent infection. Singh and Rai [130], under glass house conditions, obtained 12.33 per cent grain infection with syringe inoculations and 6.33 per cent with spray inoculations, respectively, at boot and anthesis stage with an inoculum load of 2500 sporidia/ml. Harada *et al.* [131] obtained 27.8-60.0 per cent grain infection with syringe inoculations at boot stage under greenhouse conditions with inoculum load of 2×10^5 sporidia/ml.

Fan and Xu [68] used 80,000 sporidia/ml for inoculations of hybrid rice. However, in the U.S.A., the emphasis has been laid on natural mist inoculation techniques for screening of germplasm. The cultivars were inoculated at late booting to early heading and misted during the nights [44, 46, 48] for the evaluation of germplasm against KS of rice.

Sharma *et al.* [132] conducted detailed studies on culturing of the pathogen and artificial creation of KS in Punjab (Plate 2) using different combinations of spore types, inoculum load, time of inoculation and stage of inoculation. Inoculum load (secondary sporidia/ml) ranging from 4×10^5 to 8×10^5 was ideal for syringe as well as spray inoculations under irrigated rice farming system. Boot inoculations in the evening (at late boot or early heading stage) and spray (at 50 per cent anthesis stage) were the best that produced 44.8 and 11 per cent grain infection, respectively.

DISEASE MANAGEMENT

There are many reports suggesting host resistance and other means of disease management.

Host resistance

To have long-term control of the disease, identification of resistant sources is a must. Extensive work on the screening of germplasm and identification of sources of resistance has been conducted in USA, especially at Arkansas Agricultural Experiment Station where the disease has been occurring persistently for the last 50 years. Besides, a number of genotypes have been screened from time to time in other countries like India, Philippines, Italy, China and Pakistan.

Reyes [60], while screening a large number of

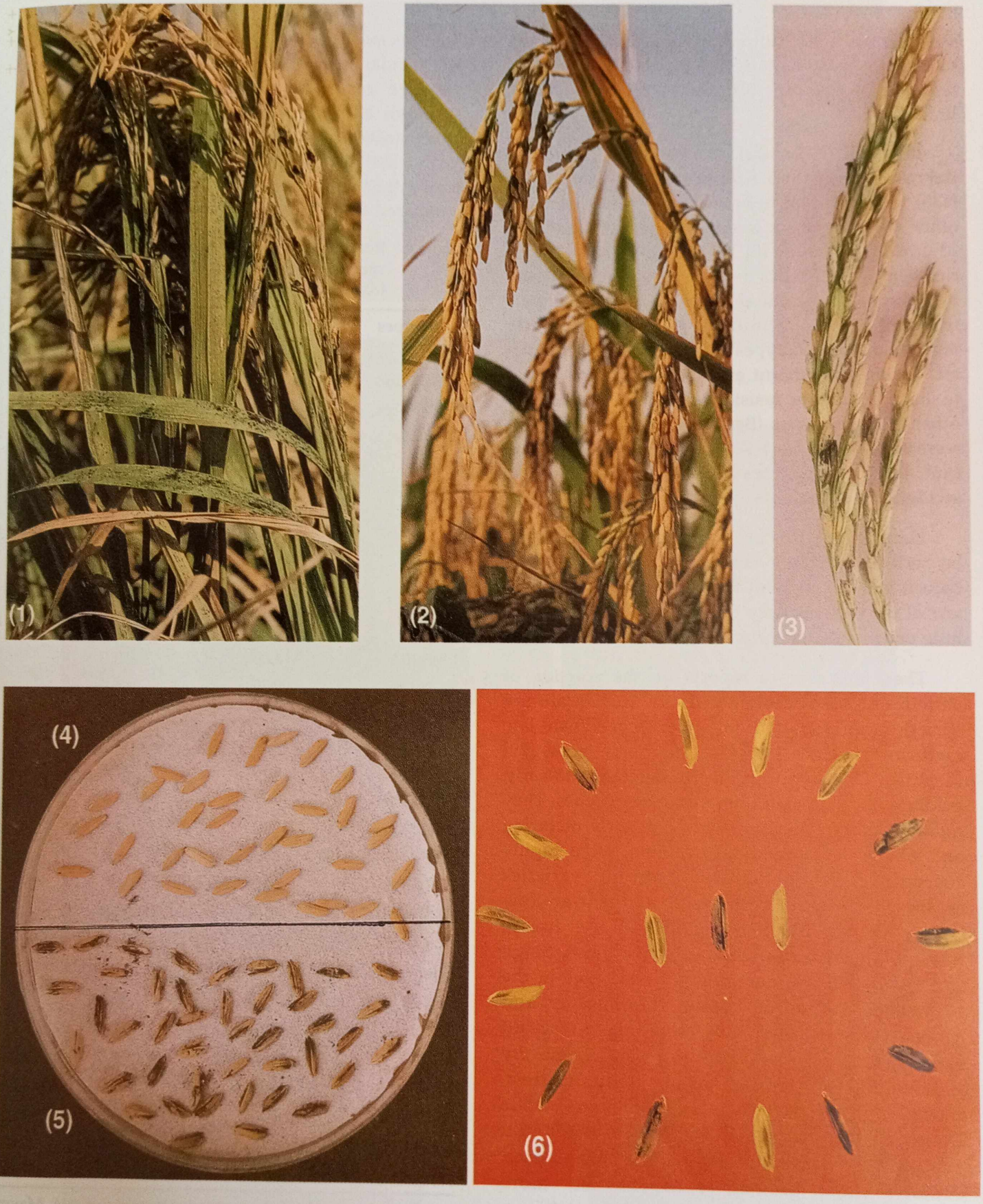

Fig. 1. (1) Smut spores scattered on foliage, (2) Severely infected seeds, (3) Infected seeds showing characteristic dull colour, (4) Healthy seeds, (5) Fully smutted seeds, (6) Seeds showing partial infection after soaking in sodium hydroxide

varieties in the Philippines, showed Elon-elon variety to be apparently immune to KS. In Italy, less than 1 per cent infection was shown in 47 lines [133].

In U.S.A. (Arkansas), rice varieties, Tora [134], Arkrose, Zenith and Saturn [135-137] and Wells [138] were reported to be resistant, whereas, many other cultivars were found less resistant [49, 50, 139]. Rutger *et al.* [140] have found that all the 9 rice genotypes (indica 1 - indica 9) being released by Arkansas Agricultural Experimental Station were resistant to KS. Similarly, out of 5 recently registered rice genotypes in Arkansas, 3 genotypes *viz.* spring rice; ahrent and wells rice were found to have moderate resistance [141-143] while the other two genotypes (Banks rice and Francis rice) were susceptible [144-145]. Templeton [51] found that late heading resulted in higher disease incidence in Arkansas.

Hassan [53] in Pakistan found 7 varieties to be resistant (0.2 per cent grain infection), while Baloch and Bhatti [146] failed to find resistance in any of the varieties they tested. Bisessar [147] found little or no smut in early sown varieties in Guyana.

There are diverse reports on the reaction of varieties to bunt infection in India. Early maturing varieties are reported to suffer more than late maturing varieties [28]. Chauhan and Verma [29] identified 22 resistant genotypes belonging to early, medium and late maturing group .of varieties. They reported that in Punjab, 76 per cent early maturing and 62 per cent medium varieties were highly susceptible, while the late maturing varieties escape the infection. However, Kameshwar Row [25], in Orissa, found that severity of the disease was higher on medium duration varieties. Singh and Pavgi [30] did not find any resistance in the 41 varieties tested in Assam. Muthusamy and Ahmed [148] reported IR36 as the most resistant variety among the 19 cultivars tested. Trimurthy and Singh [149] found Pankaj, Jagriti, Garima, Patel85 and Safri 17 as resistant cultivars, while Srinivasan [150] identified AD5426, Adl6674 and AD 14758 as resistant varieties against the disease. Usually, short grained cultivars had more resistance as compared to long grained cultivars [151]. The susceptibility of long grained cultivars was correlated partially with long duration of anthesis and wide angle of floret opening [91, 152]. KS incidence and severity was found to have positive correlation with anthesis period and negative correlation was observed with pollen concentration [153], indicating that rice genotypes with shorter anthesis period and higher concentration of pollen are less prone to KS as compared to genotypes having other combinations.

Table 2. Reaction of different genotypes of rice to kernel smut and duration of floret opening in Punjab (Average of data for 1992, 93)

Genotypes	Ks (%)	Duration (minutes) of floret opening
CMS Lines		
PMS 1A	7.95	97.0
PMS 2A	9.60	159.5
PMS 3A	13.50	95.0
PMS 4A	8.45	100.5
PMS 5A	15.60	102.5
PMS 6A	8.45	153.5
PMS 7A	4.05	171.0
PMS 8A	9.55	92.0
PMS 9A	2.45	98.5
PMS 10A	6.05	153.0
IR 58025A	0.55	141.0
Restorer Lines		
PAU 1106-21-3R	2.07	38.5
PAU 1106-21-4R	0.57	32.0
PAU 1106-5-4-1R	0.59	41.0
PAU 1126-47-2-2R	2.44	49.5
PAU 11 26-47-2- IR	0.25	49.0
IR 3192R	0.88	31.5
IR 31802 R	1.25	36.5
IR 82841 R	0.63	39.0
Conventional Varieties		
PR 103	1.23	54.5
PR 106	0.61	44.0
PR 108	1.82	50.5
PR 109	1.06	51.0
PR 110	0.97	56.5
IR 8	1.21	50.5
Jaya	1.58	47.5

Adopted from the article entitled, "Kernel Smut-A Major Constraint in Hybrid Seed Production of Rice and its Remedial Measures", *Seed Research* 27(1): 82-90 [63].

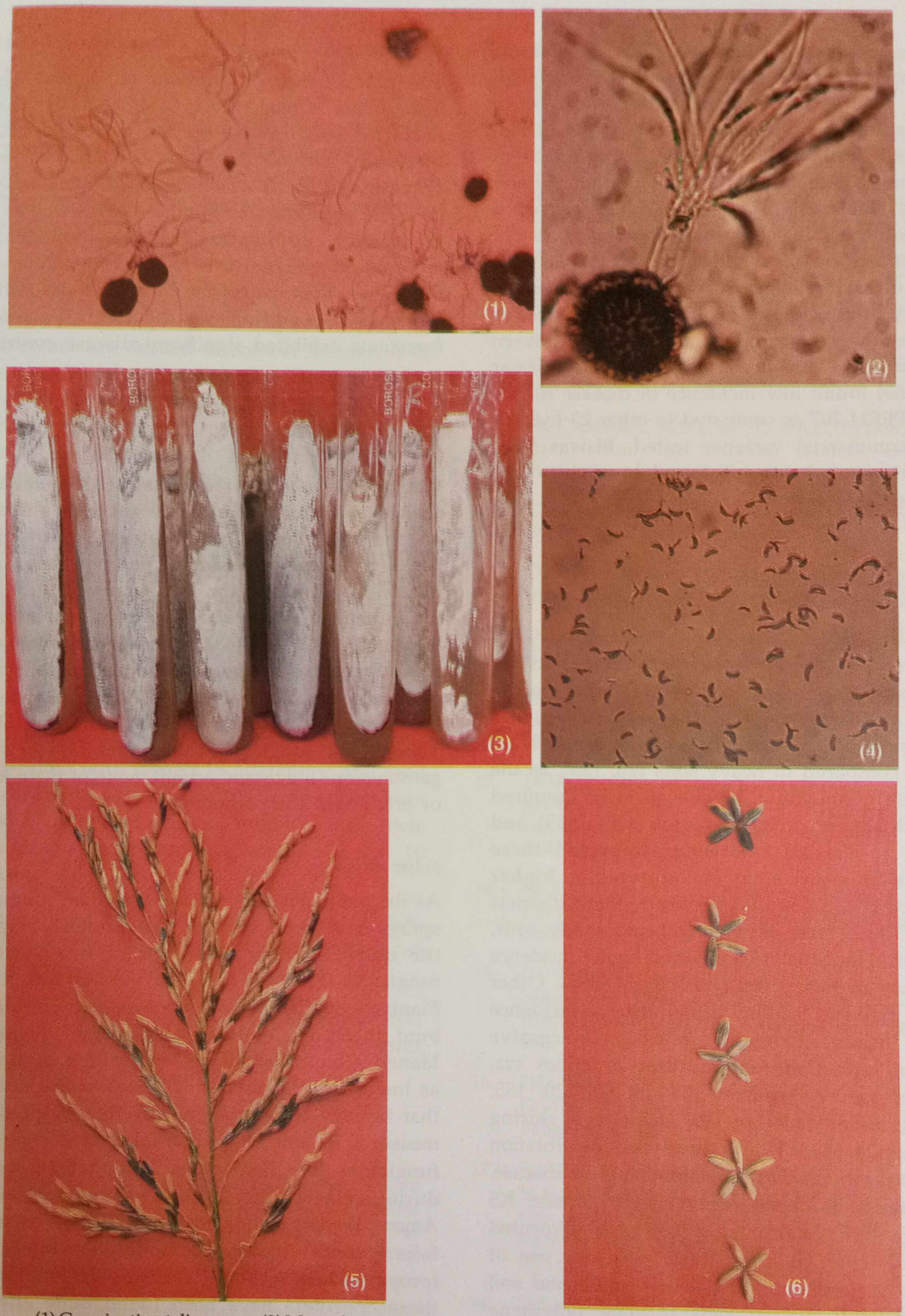

Fig. 2. (1) Germinating teliospores, (2) Magnified view of germinated teliospore, (3) Mycelial cultures, (4) Secondary sporidia, (5) Artificially inoculated panicle (NaOH soaked), (6) Grades of seed infection - Adapted from Seed Research, 29(2): 210-214 (132)

The cytoplasmic male sterile lines and hybrids were found to be more susceptible to KS with high disease severity and incidence as compared to inbred cultivars [154]. Sharma *et al.* [63] and Pan *et al.* [66] attributed the higher susceptibility of CMS lines to exceptionally longer duration of floret opening (Table 2). In Punjab, KS has proved a major bottleneck in seed production and release of rice hybrids [62-64], nevertheless, CMS line 58025A, in-spite of having longer duration of floret opening, had very low incidence of KS which is indicative of some other inherent factors too, which govern the resistance and need to be exploited. Kumar *et al.* [155] found low incidence of disease in rice hybrid PERH 207 as compared to other 23 hybrids and 4 commercial varieties tested. Biswas [156] tested 41 rice hybrids and found 16 of them free from the disease.

BIOLOGICAL CONTROL

Cultural practices

The disease can be effectively managed by alteration of various cultural practices that lead to disease development. The disease incidence has been reported to be common in light, sandy loam soils as compared to heavy soils [28, 126], as the teliospore germination is believed to be favoured in such soils. Sharma and Gill [55] and Sharma *et al.* [59] indirectly supported these findings as they have encountered a higher incidence of the disease in South-western districts of the Punjab state having light and sandy soils. However, Templeton [157] reported higher incidence of disease in heavy soils than light soils. Other cultural practices leading to high disease incidence include heavy nitrogen application, excessive irrigation etc. Different cultural practices *viz.* judicious use of fertilizers [24, 28, 63, 120, 135, 158-160], maintenance of low water level during flowering [24, 91, 161] and three-year crop rotation [126] are recommended for minimizing the disease. Late sowing has been observed to increase KS incidence [162]. Sharma *et al* [63] have advocated seed production at safer areas, judicious use of nitrogenous fertilizers, flag leaf clipping and soil solarisation of nursery beds through polythene mulching for the management of KS in hybrid rice.

Bio-Control

The performance of different bio-control agents against the pathogen was evaluated by Sharma *et al.* [163-164]. The culture filtrates of *Trichoderma harzianum, Trichoderma viride, Gliocladium virens and Gliocladiwn deliquescens* exhibited a fungistatic effect on spore germination. Simultaneous as well as pre-inoculation application of the homogenized cultures of antagonists showed better control of the disease than the culture filtrates under artificial inoculation conditions in the field. *Trichoderma harzianum* exhibited significant disease control.

CHEMICAL CONTROL

Seed treatment

Walker [92] recommended that seed material be soaked in water and all the light grains floating on the top should be discarded, followed by soaking of heavier seed in 2 per cent solution of potassium sulphide for two hours. Reyes [165] recommended hot water treatment. Later, many workers [51, 63, 166-168] tried seed treatment with many fungicides but found them ineffective because of non-systemic nature of the disease and occurrence of infection only at flowering stage. Though, the fungicides cause inhibition of spore germination (fungistatic action), none of them killed or eradicated the pathogen.

Foliar sprays

As the pathogen causes floret infection, fungicidal sprays have been tried at boot/anthesis stage of the crop. Templeton [137] tried two systemic fungicides (Plantvax and Vitavax) and found that Plantvax applied at late boot stage reduced the bunt infection. Whitney [166] found Benomyl, Maneb, Chlorothalonil or Triphenyltin hydroxide as ineffective. Ospischev [10] was of the opinion that besides crop rotation, effective prophylactic measures in time should be taken with non-toxic fungicides like Benlate or Thiophanate methyl during anthesis after careful weather forecasting. Among the large number of fungicides screened as foliar sprays, triazole compounds namely, Tilt (propiconazole), Folicur (tebuconazole) and Control (hexaconazole) have been found quite effective [55, 63, 169-170]. These fungicides provided

considerable control of the disease, when applied at the rate of 0.1 per cent twice at 5 per cent flower initiation stage and 10 days thereafter. Folicur, Tilt and Score 10 (difenoconazole) have been reported to inhibit sporidial production under *in-vitro* conditions [171]. However, application of gibbrellic acid has also been observed to reduce the incidence of KS [172].

FUTURE NEEDS

- Long-term strategies pertaining to breeding for resistance are needed to combat disease. It is, thus, essential to continue the screening of the disease germplasm against the prevalent pathotypes for obtaining more stable resistance. Besides, biochemical and morphological components of resistance need to be studied extensively.
- Modern biotechnical methods may also serve as important tools for transferring resistant gene fragments from highly resistant sources.
- Threshold level of teliospores on the seed and in the soil to cause infection should be determined.
- Monitoring and Surveillance of the disease is important with respect to quarantine and seed certification measures. This aspect has been taken up by first and last author of this article under National Seed Project for the last 10 years.
- Molecular diagnostic methods are required to be developed to detect the pathogen introduced as seed contaminant
- As a short-term strategy, the search should continue for exploring the new and safer chemicals for foliar sprays.

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