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Research Article

Field screening and identification of stable resistance sources in wheat germplasm against loose smut disease caused by *Ustilago segetum* var. *tritici*

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Abstract

Loose smut (LS) caused by *Ustilago segetum* var. *tritici* is an important disease of wheat (*Triticum* spp.) and accounts for 1-2% annual yield losses in India. The disease can be managed easily with the use of seed treatment with systematic fungicides, however, the application of these fungicides remained limited amongst farmers mainly due to their high price, limited knowledge about seed protectants as well as lack of any visible external symptoms on infected seeds. Additionally, seed dressing with protectants is hazardous to the environment and human health and thus not acceptable in organic wheat cultivation. The most affordable and environment friendly technique to protect wheat crop is to develop varieties that are resistant to loose smut disease. Therefore, research efforts have been made to screen one hundred and ninety-seven wheat genotypes obtained from gene bank (Germplasm resource unit, ICAR-Indian Institute of Wheat and Barley Research) under field conditions for LS disease using artificial inoculation technique. The results of the field trials for two consecutive years (2014-15 and 2015-2016) indicated that more than 13.74% genotypes (31) were found immune and 3.05 % (6) genotypes were highly resistant (HR). Only seventy seven genotypes (39.09%) showed resistance to loose smut and infection ranged from 0-5% under artificially inoculated conditions. There was forty-nine moderately susceptible genotype obtained from the experiment. Twenty-three genotypes (11.68%) were identified as susceptible (S). The results revealed that there are some promising LS resistance lines and could therefore be introduced to wheat breeders for further breeding as well as multi-locational trials for developing disease-resistant cultivars.

Keywords: Artificial inoculations, field screening, ‘Go-go’ method, loose smut, resistance breeding, wheat varieties

1. Introduction

Loose smut (LS) caused by *Ustilago segetum* var. *tritici* is one of the most important internally seed borne disease of wheat (*Triticum aestivum* L.). The disease is favored by moist and cool climate during anthesis (Wunderle *et al.*, 2012; Kashyap *et al.*, 2019). This fungus transforms the spike floral tissues to fungal teliospores, causing yield losses corresponding to the percent smutted spikes (Singh, 2018). The primary inoculum source of this fungal pathogen resides in the embryo of the wheat seeds (Kassa *et al.*, 2015) and documented to minimize 5–20 per cent profit even at an infection level of 1-2 % (Kashyap *et al.*, 2019). In North Western parts of India, Joshi and colleagues (1980) recorded LS incidence up to 10%. Globally, 5–10% LS incidence has also been reported from Russia, New Zealand, and USA (Menzies *et al.*, 2009; Kaur *et al.*, 2014). Nielsen and Thomas (1996) documented

15–30% annual yield losses as a consequence of *U. segetum* var. *tritici* infection in wheat.

The infection process and disease cycle of *U. segetum* var. *tritici* on wheat has been elaborately described by several workers (Wilcoxon and Saari, 1996; Ram and Singh, 2004; Abrahim 2019). Dikaryotic spores of *U. segetum* var. *tritici* land on the wheat floret, germinate and penetrate the ovary through feathery stigma during anthesis (Shinohara, 1976). Kumar *et al.* (2018) mentioned that the mycelia of *U. segetum* var. *tritici* live within the embryo of infected seeds and move systemically through the growing point of the tillers without displaying any visible symptoms. The symptoms become visible only after that emergence of spikes from the boot and recognized by black or brown powdery spore masses or sori forming on the inflorescences (Gautam *et al.*, 2020). It has been observed that the races that infect bread wheat are also reported pathogenic to other hosts such as *Aegilops* sp., *Secale cereale* and *Triticosecale* (a triticale cultivar) (Menzies *et al.*, 2016). Three races (T1, T10 and T11) of *Ustilago tritici* were identified from the Punjab State of India (Rewal and Jhooty, 1985). Published literature indicated that*U. segetum* var. *tritici* infection in wheat can be effectively managed by following seed treatment with systemic fungicides like Carboxin, Tebuconazole, Carbendazim, and Thifluzamide (Goel *et al.*, 2001; Sharma et al., 2001; Kashyap *et al.*, 2018; Kashyap *et al.*, 2022). Unfortunately, these chemical recommendations have not been properly adopted by farmers due to their high cost as well as lack of any visible symptoms on infected seeds (Singh *et al.*, 2017). Therefore, an effective and sustainable means to combat LS disease is to develop disease-resistant cultivars (Thambugala *et al.*, 2020). Further, it is important to mention here that India is bestowed with diverse agro-climatic conditions which make it rich in wheat germplasm adapted to various niches. The germplasm resource unit of ICAR–IIWBR, Karnal comprised of local landraces, trait specific genetic stocks including introgressions from wild relatives, exotic collections, released varieties, and improved germplasm. Keeping in view of aforementioned information the major objective of the present study is aimed for searching new resistance sources which confer resistance to LS disease. Therefore, efforts have been made to evaluate and identify the potential resistance source against loose smut disease under artificially inoculated field conditions.

2. Materials and Methods

2.1 Experimental site and plant materials

The experimental material consisted of 197 indigenous wheat germplasm accessions obtained from Germplasm Resource Unit (GRU), ICAR-Indian Institute of Wheat and Barley Research (IIWBR), Karnal, India. The lines were deposited in the GRU unit from different wheat programs of the country. For a comprehensive screening, these lines were evaluated for resistance against loose smut during 2014–2015 and 2015–2016 crop seasons at Experiment farm, ICAR-IIWBR, Karnal, India.

2.2 Experimental layout, inoculations and field screening procedure

The experiment was laid out in randomized block design with three replications. Each line was planted in a single row plot of one-meter length keeping 25 cm distance between rows. Artificial inoculations were done using smutted heads from the susceptible genotype (cv. Sonalika) which was inoculated in the previous year with LS teliospores at ICAR-IIWBR, Karnal. Five ears of each entry were inoculated at growth stage '59' of Zadoks' scale (Zadoks*et al.*, 1974) with LS teliospores using modified 'Go-go' method (Joshi *et al.*, 1988) during both the *rabi* cropping seasons. The crop was raised as per recommended agronomic practices and seeds were sown without treatment. Briefly, the florets of the ear to be inoculated were clipped open with scissors at the early anthesis stage. The ear was then covered with a parchment paper bag. Inoculations were performed by opening the bag by cutting from top and dusting spores from smutted ear-heads from the infected genotype (cv. Sonalika). After inoculation the cut end of the bag was stapled and on maturity inoculated seeds were harvested.

2.3 Data recording and disease rating scale

The seeds obtained during previous year were planted during the next season and record on smutted and healthy ears was maintained on tiller basis after ear emergence. Any plant with one or more smutted ears (completely or partially smutted) was recorded as infected. The per cent loose-smut infection (LSI) was calculated by counting the diseased and healthy ears on tiller basis. The loose smut incidence (LSI) in each line was determined as follows:

LSI (%) = Number of smutted plants / Total number of plants x 100

Categorization of resistance response was determined on the basis of disease rating scale (0-6) mentioned in Table 1. The cut-off for resistance/susceptibility was based on Nielsen (1987), who categorized wheat lines with 0–10 % LSI as resistant and wheat lines with >10 % LSI as susceptible.

Table 1: Disease rating scale employed for determining the loose smutresistance response in wheat genotype under field condition

Scale	LSI (%)	Reaction
0	0	Immune (I)
1	0.01-<1.0	Highly resistant (HR)
2	1.0-5.0	Resistant (R)
3	5.1-10.0	Moderately resistant (MR)
4	10.1-20.0	Moderately susceptible (MS)
5	21.0-50.0	Susceptible (S)
6	>50	Highly susceptible (HS)

LSI =Loose smut incidence based on percent smutted heads in total counted plants

3. Results and Discussion

Loose smut (LS) caused by *Ustilago segetum* var. *tritici* is one of the important diseases of wheat worldwide (Kashyap *et al*., 2019; Kumar *et al*., 2022). Practically, there is no LS resistant wheat cultivar under cultivation. The development of resistant wheat cultivars ensures the stability of production, and also ensures quality, cost effectiveness and offers sanitary and epidemiological safety in the wheat field. Therefore, identification and characterization of genetically diverse sources possessing LS disease resistance and their deployment in breeding programmes is essential to achieve durable disease management. The current study is aimed at the identification of effective and potentially new sources of disease resistance against LS disease in 197 indigenous wheat accessions conserved in the germplasm resource unit (GRU) of ICAR–IIWBR, Karnal.

Field data of the disease reactions revealed a range of response levels of the tested wheat genotypes to LS disease during both the seasons (2014-15 and 2015-16). The perusal of the field data demonstrated that the inoculation with *U. segetum* var. *tritici* teliospores confirmed 13.74% genotypes as immune (0.0% LSI, N= 31), while 11.68 % genotypes as susceptible (>21 % LSI, N= 23) (Fig. 1 and Table 2). Based on this cut-off limit, there were 125 lines resistant and 72 susceptible to *U. segetum* var. *tritici*. Further, it has been noticed that 48 genotypes (24.37% genotypes) showed moderately resistant (MR) and 49 (24.87 %) reflected moderately susceptible (MS) reaction. Such studies have been done by different workers for identifying sources of resistance. For instance, Mishra *et al.* (1990) reported that out of 92 cultivars that were inoculated artificially through needle method against loose smut of wheat caused by *U. segetum* var. *tritici*, 15 cultivars were found resistant and 3 were found moderately resistant to loose smut of wheat. Sherif *et al.* (1991) evaluated 96 wheat entries along with 10 Egyptian wheat varieties to loose smut and reported 14 entries as highly resistant. Further, they also concluded that the Egyptian wheat cultivars e.g. Giza 155, Giza160 and Giza 162 showed resistant reaction (0-5%), while Sakha 61 and Sakha 92 displayed susceptible reaction against LS disease. Later on, Singh et al. (2002) evaluated a total of 931 advanced lines of wheat against LS disease under artificially inoculated conditions and found 92 LS resistance lines with 0–5% infection score. It is worth to mention that a high number of LS resistant lines (77 lines; 39.09% lines) were identified in present study in comparison to earlier reports (Singh *et al.*, 2002; Sammour *et al.*, 2015), which clearly validate the fact that the set of analyzed germplasm lines has sufficient level of resistanceagainst LS diseases. The results of present study corroborate with the findings ofKumar *et al.* (2019) who screened 247 wheat lines and reported 93 accessions (37.65% genotypes) displaying resistance against LS disease. Gothwal and Pathak (1983) also screened wheat genotypes or varieties using artificial inoculation with mixture of *U. tritici* isolates. However, they did not get any success in getting immune genotypes. Interestingly, in present study, we obtained 31 genotypes (IC177785, IC564138, IC443626, IC542107, IC539112, IC415868, IC530072, IC539286, IC177716, IC228213, IC341362, IC47034, IC529011, IC529090, IC529499, IC529354, IC542127, IC529931, IC542040, IC529908, IC530120, IC443628, IC47021, IC529408, IC539569, IC398307, IC17770, IC564092, IC542104, IC529712, and IC560465) completely resistant and free from any disease incidence. Overall, resistant accessions identified in the current study have enriched the existing gene pool for LS resistance in wheat and will serve as a potential source for resistance in future.

Table 2: Categorization of indigenous germplasm lines against loose smut resistance based on two year (2014-15 and 2015-16) evaluation

Reaction type	Genotypes /Lines	Genotype(s) in category (%)	Mean LSI range (%)
Immune	IC177785, IC564138, IC443626, IC542107, IC539112, IC415868, IC530072, IC539286, IC177716, IC228213, IC341362, IC47034, IC529011, IC529090, IC529499, IC529354, IC542127, IC529931, IC542040, IC529908, IC530120, IC443628, IC47021, IC529408, IC539569, IC398307, IC17770, IC564092, IC542104, IC529712, IC560465	15.74	0.00
HR	IC539106, IC529733, IC415864, IC539171, IC524018, IC529854	3.05	0.01-0.95
R	IC329538, IC309885, IC128153, IC398298, IC530025, IC529355, IC547575, IC559913, IC177711, IC240800, IC529692, IC543400, IC530005, IC560464, IC543333, IC549356, IC445514, IC529357, IC529894, IC529360, IC549524, IC443771, IC483032, IC317314, IC582733, IC296431, IC539296, IC539109, IC529388, IC177776, IC281570, IC542078, IC393113, IC529484, IC574482, IC585661, IC443650, IC529805, IC543411, IC529296	20.30	1.06-4.90
MR	IC443760, IC445517, IC138587, IC445316, IC593162, IC529311, IC549359, IC539301, IC582716, IC539167, IC529331, IC539371, IC543344, IC177721, IC549928, IC524292, IC528930, IC547558, IC539568, IC542005, IC593574, IC547593, IC558801, IC443639, IC529292, IC138479, IC543331, IC560466, IC564113A, IC524302, IC252652, IC547563, IC177722, IC527929, IC240796, IC539353, IC310117, IC529429, IC549397, IC530109, IC128191, IC443674, IC539534, IC128252, IC524303, IC529509, IC401933, IC529452	24.37	5.03-9.96
MS	IC427183, IC549526, IC362213, IC443691, IC445490, IC539290, IC393883, IC539563, IC443736, IC443729, IC144920, IC529386, IC529886, IC553917, IC393878, IC529234, IC567640, IC309868, IC116275, IC566544, IC522507, IC539373, IC551389, IC530119, IC406519, IC541989, IC533416, IC564106, IC443640, IC426648, IC561181, IC582713, IC547619, IC539469, IC585666, IC574387, IC543345, IC547578, IC410038, IC529291, IC539473, IC128181, IC559930, IC529086, IC547683, IC542077, IC328473, IC177714, IC290187	24.87	10.05-19.46
S	IC539346, IC426651, IC75242, IC406724, IC405232, IC369874, IC564141, IC393883, IC549399, IC393885, IC524298, IC443625, IC309847, IC543349, IC546937, IC438390, IC443683, IC393119, IC529318, IC540910, IC402050, IC145620, IC564126A	11.68	20.39-41.18
HS	-	-	

LSI: Loose smut incidence; I: Immune; HR: Highly resistant; R: Resistant; MR: Moderately resistant; MS: Moderately susceptible; S: Susceptible; HS: Highly susceptible. Mean Infection score range (%) include the LSI % data of both the years.

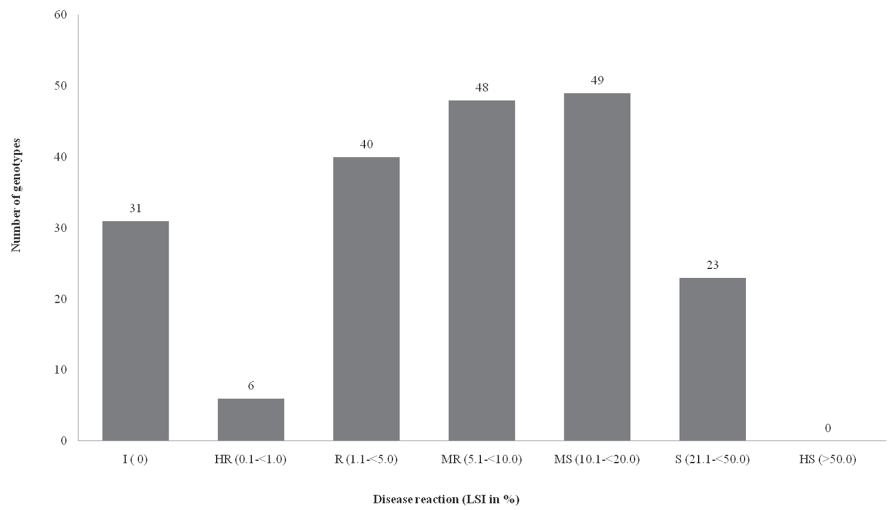

Fig 1: Histogram of loose smut disease response based on pooled average response of two year (2014–2015 and 2015–2016) data among 197 wheat accession inoculated with *Ustilago segetum* var. *tritici* teliospores during under field conditions. I: Immune; HR: Highly resistant; R: Resistant; MR: Moderately resistant; MS: Moderately susceptible; S: Susceptible; HS: Highly susceptible; LSI= Loose smut incidence in per cent

4. Conclusion

The results of present study lead to identification of wheat accessions having some degree of immune, resistant, and moderately resistant reaction to LS disease. Phytopathological evaluation on an artificial infection, 37genotypes (IC177785, IC564138, IC443626, IC542107, IC539112, IC415868, IC530072, IC539286, IC177716, IC228213, IC341362, IC47034, IC529011, IC529090, IC529499, IC529354, IC542127, IC529931, IC542040, IC529908, IC530120, IC443628, IC47021, IC529408, IC539569, IC398307, IC17770, IC564092, IC542104, IC529712, IC560465, IC539106, IC529733, IC415864, IC539171, IC524018 and IC529854) showed HR type of reaction that can be exploited in breeding programs as donors over time and space to develop disease resistant cultivars against loose smut disease in wheat.

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Contribution to this manuscript

All authors contributed to the article and approved the submitted version.

Ethical Compliance Statement

NA

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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