The Pharma Innovation Journal 2022; 11(5): 669-672

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(5): 669-672

www.thepharmajournal.com
Received: 07-02-2022
Accepted: 16-03-2022

Priya Bhargava
Department of Plant Pathology, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh, India

SK Rana
Department of Plant Pathology, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh, India

Sachin Upamanyu
Department of Plant Pathology, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh, India

Corresponding Author:
Priya Bhargava
Department of Plant Pathology, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh, India

Impact of different inoculation techniques and levels of inoculum load of *Urocystis agropyri* (Preuss) A. A. Fisch. Waldh. On wheat

Priya Bhargava, SK Rana and Sachin Upamanyu

Abstract
In the present study, among different inoculation technique at four levels of *Urocystis agropyri* inoculum load, the maximum flag smut incidence (58.18%) was recorded in inoculation of germinating seed with dry inoculum @ 10 g inoculum/ kg seed followed by its 7.5 g inoculum/ kg seed (55.65%) and inoculation of seed with germinating inoculum at 10 and 7.5 g inoculum/ kg seed with 53.76 and 51.11 per cent, respectively and the least incidence (5.30%) in inoculation of germinating seed with germinating inoculum @ 2.5 g inoculum/ kg seed. However, within effect of inoculum load on seed health parameters, the highest inoculum load of 20 g inoculum/ kg seed showed maximum reduction on seed germination percentage (13.70%), seed vigour index (52.96%), seedling growth rate (54.41%) and seedling dry weight (6.73%) except speed of seedling germination i.e. maximum at 17.5 g inoculum/ kg seed. Yet, the least reduction in each case was noted at 5 g inoculum/ kg seed.

Keywords: *Urocystis agropyri*, flag smut, inoculation technique, inoculum load, seed health parameters

1. Introduction
Wheat is a major food crop that provides energy to 40 per cent of the world's population (Giraldo *et al.* 2019) [6] and is also known as the "King of cereals" due to its large area acquisition, productivity and spectacular position in the worldwide trade of food grains (Bhushan *et al.* 2013) [4]. Wheat area under cultivation in Himachal Pradesh is 0.32 million hectares, with production and productivity of 0.57 million tonnes and 1.77 metric ton/ha, respectively (Anonymous, 2019) [2]. Diseases are one of the key constraints in wheat production (Pal, 1951) [21], incurring losses of up to 20% globally each year (Serfling, 2017) [23]. Rust, loose smut, flag smut, Karnal bunt, common bunt, hill bunt, head scab, powdery mildew, leaf blight, and Septoria diseases are the most prevalent and major fungal diseases of wheat that cause significant loss in India. Smuts are the second most damaging wheat diseases, after rusts, in terms of yield loss.
Flag smut caused by *Urocystis agropyri* (Preuss) A.A. Fisch. Waldh. is a widespread disease of many wheat-growing regions of India. Its presence has been recorded in several states, including Punjab, Haryana, Himachal Pradesh, Madhya Pradesh, Uttar Pradesh, Delhi, Bihar, and Rajasthan (Goel *et al.* 1977) [7]. Severe incidence (up to 50%) of flag smut on wheat has been observed in low and mid-hill areas of Himachal Pradesh from many years (Basandrai *et al.* 1993) [3]. *U. agropyri* is seed and soil-borne in nature and remain viable for a year in the soil (Joshi *et al.* 1970) [14]. The presence of pathogen propagules in the soil and on seed surfaces highly influences progression or development of the disease (Madden, 1980; Goel and Jhooty, 1989; Khamari *et al.* 2019) [17, 9, 15]. Keeping this in view, the current study was conducted to assess the impact of inoculation techniques and levels of inoculum load of *U. agropyri* on wheat crop.

2. Material and Methods
The collected diseased plant samples were rubbed individually against iron wire mesh and sieved thoroughly by a fine muslin cloth to separate the inoculum/ teliospores in the laboratory after five months of storage. The extracted inoculum was then used for conducting the experiment.

2.1 Evaluation of inoculation techniques
In order to find out the most effective method of inoculation for *U. agropyri*, four different

inoculation methods were evaluated such as i) inoculating wheat grains directly with dry inoculum, ii) inoculating wheat seeds with germinating inoculum, iii) inoculating germinating grains with dry inoculum and iv) inoculating germinating grains with germinating inoculum at four different level of inoculum load *viz*., 2.5, 5.0, 7.5 and 10.0 g inoculum/ kg seed. The spore density of each level of inoculum load were counted using haemocytometer. The germination of inoculum was induced by presoaking them by dusting over the surface of sterile distilled water containing petriplates and incubated in dark at 19±1°C for seven days. After that, with the help of a clean glass rod, the presoaked teliospores from the Petri plates were transferred to glass cavity block containing 1 ml distilled water and small bits or tissues of the lower stem portion (1cm) of a fresh seedling (germination stimulant) of wheat variety PBW 343 (30 mg/ ml). Cover slips were placed over the cavity blocks and were incubated at 19±1°C (Goel and Jhooty, 1984) [8]. The infected seed lots were then sown (15 seeds/pot) in sterilized soil containing pots of 15 cm diameter. Four replications of each treatment were maintained in a green house at ambient temperature to monitor the progress of disease regularly. The pots were watered as per the requirement. The data was taken in terms of disease incidence (%) by counting infected and total number of tillers in a pot.

$$\text{Disease incidence (\%)} = \frac{\text{Number of smutted tillers}}{\text{Total number of observed tillers}} \times 100$$

2.2 Effect of inoculum load on seed health parameters

To Know the effect of inoculum load on seed health parameters, surface sterilized wheat seeds were inoculated with different levels of inoculum (5.0, 7.5, 10.0, 12.5, 15.0, 17.5, and 20.0 g inoculum/ kg seed) using the dry inoculum inoculation method. As a check, a healthy seed without inoculum was used. The rolled paper towel technique was used to examine the germination of infected and uninoculated (control) seedlings (ISTA, 1993) [12].

A total of 100 inoculated seeds were placed on moistened double-layered germination sheets supported by a butter paper sheet, with 10 seeds per row leaving adequate space between rows and sheet edges and were carefully rolled to avoid mingling of the seeds. Each treatment was replicated four times. To obtain optimum seed germination, the rolled sheets were placed in an incubator at 20±1°C for 10 days. After 10 days of incubation, all the morphologically normal seedlings were counted to calculate the per cent germination using the given formula (ISTA, 1985) [11].

Germination % = (Number of germinated seeds/ Number of total seeds) × 100

To calculate the speed of germination (emergence) of seedlings using formula given by Maguire (1962) [18], the initial count of normal seedlings was recorded on the 5th day of incubation and subsequently every day until the final count taken on the 10th day of incubation.

Speed of germination (emergence) of seedlings =
(Number of normal seedlings/ days of first count) +…+ (Number of normal seedlings/ days of last count)}

The seedling growth rate was calculated by measuring total length (shoot + root length) of five randomly selected seedlings of each treatment in mm on 5th and 10th day of incubation (Gupta, 1993) [10].

Seedling growth rate = (L1 - L2)/ T

Where, L1 = Total average length taken during first measurement, L2 = Total average length taken during second measurement and T = Number of days between first and second measurement

After the 10th day of seed incubation, ten seedlings from each treatment were taken, dried in a hot air oven at 100°C for 24 hours, and weighed using a digital electronic balance to determine the dry weight. Seed vigour index of each treatment was also obtained using the formula given by Abdul-Baki and Anderson (1973) [1].

Vigour index = Germination% × Seedling length (Shoot + Root length)

3. Result and Discussion

3.1 Evaluation of inoculation techniques

The data on evaluation of different inoculation techniques are presented in Table 1 and revealed that all the inoculation methods showed infection in the plant. Although, inoculation of germinating seed with dry inoculum provided the maximum disease incidence (48.11%) followed by inoculation of seed with germinating inoculum (43.57%) and inoculation of seed with dry inoculum with 43.57 and 15.20 per cent incidence. While, the minimum disease incidence of 7.85 per cent was given by inoculation of germinating seed with germinating inoculum. However, similar trend of the disease was recorded in all the techniques at four inoculum loads. The highest incidence of disease was obtained at 10 g inoculum/ kg seed and subsequently followed by its lower loads of inoculum i.e. 7.5, 5.0 and 2.5 g inoculum/ kg seed, respectively.

Among different inoculation techniques at four levels of inoculum load, inoculation of germinating seed with dry inoculum @ 10 g inoculum/ kg seed gave the maximum incidence of disease (58.18%) followed by its 7.5 g inoculum/ kg seed (55.65%), inoculation of seed with germinating inoculum at 10 and 7.5 g inoculum/ kg seed with 53.76 and 51.11 per cent, respectively. Inoculation of germinating seed with dry inoculum @ 5 g inoculum/ kg seed and inoculation of seed with germinating inoculum @ 5 g inoculum/ kg seed were the next best treatment after them 42.10 and 37.78 per cent disease incidence, respectively. While, inoculation of the germinating seed with germinating inoculum @ 2.5 g inoculum/ kg seed gave the least disease incidence of 5.30 per cent. Similarly, Noble (1924) [19] inoculated wheat seedlings with inoculum rather than seed and recorded maximum range (7.70 - 93.80%) of disease incidence in inoculation of seedlings with germinating spores followed by inoculation of seedlings with germination commencing spores (0.00-88.90%) and minimum (0.00-66.70%) in inoculation of wheat seedlings with presoaked teliospores/ inoculum in distilled water for 3 days at 20 °C temperature. Miller and Millikan

(1934) [20] also found that inoculation of seeds with either germinating spores or dry spores as the most effective technique to incite the disease among eight tested inoculation techniques with 34.5 and 20.0 per cent infection of the disease. Rewal *et al.* (1986) [22] reported seed inoculation and seed cum soil inoculation with dry spores yielded higher disease incidence as compared to soil inoculation with chopped flag smut infected plants alone. However, Shekhawat (2008) [24] recorded maximum incidence of disease (70.0%) in seed cum soil inoculation with pathogen inoculum @ 35 g / kg seed and 1g/ kg of soil in a pot.

Table 1: Evaluation of inoculation techniques for *Urocystis agropyri*

Technique	Disease incidence (%) with inoculum load (g inoculum/ kg seed)				
	2.5 (3×10^5)*	**5.0 (6×10^5)**	**7.5 (9×10^5)**	**10.0 (12×10^5)**	**Mean**
Inoculation of seed with dry inoculum	7.63 (16.00)**	11.43 (19.69)	17.36 (24.62)	24.37 (29.56)	15.20 (22.47)
Inoculation of seed with germinating inoculum	31.62 (34.21)	37.78 (37.91)	51.11 (45.64)	53.76 (47.16)	43.57 (41.23)
Inoculation of germinating seed with dry inoculum	36.51 (37.16)	42.10 (40.44)	55.65 (48.24)	58.18 (49.71)	48.11 (43.89)
Inoculation of germinating seed with germinating inoculum	05.30 (13.30)	06.75 (14.96)	07.56 (15.75)	11.79 (19.82)	07.85 (13.95)
CD (P=0.05)	Technique = 1.64 Inoculum load = 1.64 Technique × Inoculum load = 3.29				

* Figures within parentheses are spore density (spore/ g inoculum) of the pathogen
**Figures within parentheses are angular transformed values

3.2 Effect of inoculum load on seed health parameters

The data presented in Table 2 depicts the effect of inoculum load on different seed health parameters. The highest reduction in seed germination (13.70%), vigour index (52.96%) and dry weight of seedling (6.73%) was achieved with the maximum load of inoculum i.e. 20 g inoculum/ kg seed followed by 17.5 (13.44, 49.44 and 6.42%), 15.0 (13.18, 44.49 and 6.12%), 12.5 (6.98, 36.17 and 5.50%), 10.0 (4.65, 32.77 and 5.20%) and 7.5 (3.62, 21.29 and 4.89%) inoculum/ kg seed, respectively. While, the minimum reduction in germination of seed (4.59%), vigour index (52.96%) and dry weight of seedling (6.73%) were provided by 5.0 g inoculum/ kg seed.

In term of seedling growth rate, inoculum load of 20.0 g inoculum/ kg seed gave the highest reduction (54.41%) followed by 17.5 (45.20%), 10.0 (29.84%), 15.0 (24.72%), 12.5 (20.63%) and 7.5 (11.41%) g inoculum/ kg seed and the least reduction (5.36%) was achieved with 5.0 g inoculum/ kg seed. However, the inoculum load of 17.5 g inoculum/ kg seed yielded the highest reduction (15.01%) in speed of germination of seedling (seedling emergence) followed by 20.0 (14.59%), 15.0 (13.83%), 12.5 (8.84%), 10.0 (5.39%), 7.5 (5.75%) g inoculum/ kg seed and the least reduction (2.50%) with 5 g inoculum/ kg seed.

In a similar study, Jain (2000) [13] reported that all levels of inoculum load i.e. 2, 3, 4 and 5 g teliospores/ kg seed of *Sorosporium paspali thunbergii* (head smut pathogen), except 1 g teliospores/ kg seed, substantially decreased the germination of seed of kodo millet. However, Dharmveer and Panwar (2006) [5] did not noticed any adverse effect of tested inoculum load i.e. 10 and 20 g inoculum/ kg seed on per cent germination of inoculated seed in resistant varieties, although found a substantial reduction in per cent seed germination below the ISTA standard (85%) in susceptible varieties contrary to uninoculated seeds of each variety (control). Similar observation of their effect on speed of seedling germination/ emergence was also recorded by them i.e. no adverse effect in case of resistant varieties but a significant reduction in susceptible varieties than control. On the other hand, decrease in per cent seed germination with increasing inoculum load of *Macrophomina phaseoline* was obtained in sesame (Khanzada *et al.* 2012) [16] and okra (Khamari *et al.* 2019) [15].

Table 2: Effect of inoculum load on different seed health parameters

Inoculum load (g inoculum/ kg seed)	Germination of seed (%)	Reduction in germination of seed (%)	Vigour index of seed	Reduction in Vigour index of seed (%)	Dry weight of seedling (g)	Reduction in dry weight of seedling (%)	Seedling growth rate (mm/day)	Reduction in seedling growth rate (%)	Speed of germination of seedling (seedling emergence) (number/days)	Reduction in speed of germination of seedling (seedling emergence) (%)
5.0	96.75	1.29	1803.85	10.92	104.00	4.59	4.39	5.36	18.64	2.50
7.5	94.50	3.62	1703.15	21.29	103.67	4.89	4.11	11.41	18.02	5.75
10.0	93.50	4.65	1692.05	32.77	103.33	5.20	3.26	29.84	18.09	5.39
12.5	91.25	6.98	1590.95	36.17	103.00	5.50	3.69	20.63	17.43	8.84
15.0	85.25	13.18	1405.23	44.49	102.33	6.12	3.50	24.72	16.48	13.83
17.5	85.00	13.44	1381.08	49.44	102.00	6.42	2.54	45.20	16.25	15.01
20.0	84.75	13.70	1384.18	52.96	101.67	6.73	2.12	54.41	16.33	14.59
Control	98.00	-	1873.99	-	109.00	-	4.64	-	19.12	-
CD (P=0.05)	1.29	-	-	-	NS	-	0.11	-	0.43	-

4. Conclusions

Thus from the present study, it is evident that inoculating germinating seed with dry inoculum was the most efficient technique for inciting the disease. It also showed that an increase in the levels of inoculum load increased the incidence of disease as well as the seed health parameters such as per cent germination and vigour index of seed as well as speed of germination (emergence), growth rate and dry weight of seedling.

5. References

1. Abdul-Baki AA, Anderson JD. Vigour determination in soybean by multiple criteria. Crop Science. 1973;3:630-633.
2. Anonymous. Agricultural statistics at a glance 2019. Directorate of Economics and Statistics. Government of India, New Delhi, 2019, 1-108.
3. Basandrai AK, Sood AK, Sud AK. Prevalance of flag smut (*Urocystis agropyri*) of wheat in Himachal Pradesh. Plant Disease Research. 1993;8:85.
4. Bhushan B, Bharti S, Ojha A, Pandey M, Gourav SS, Tyagi BS, *et al*. Genetic variability, correlation coefficient and path analysis of some quantitative traits in bread wheat. Journal of Wheat Research. 2013;5:24-29.
5. Dharmveer, Panwar MS. Impact of Flag Smut Inoculum on Wheat Seedling Vigour. Seed Research -New Delhi. 2006;34:229-231.
6. Giraldo P, Benavente E, Manzano-Agugliaro F, Gimenez E. Worldwide Research Trends on Wheat and Barley: A Bibliometric Comparative Analysis. Agronomy. 2019;9:352.
7. Goel LB, Singh DV, Srivastava KD, Joshi LM, Nagrajan S. Smut and Bunts of wheat in India. Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi, 1977, 1-38.
8. Goel RK, Jhooty JS. Germination of teliospores of *Urocystis agropyri* causing flag smut of wheat. Indian Phytopathology. 1984;37:706-08.
9. Goel RK, Jhooty JS. Some factors affecting the incidence of flag smut of wheat. Indian Journal of Agricultural Research. 1989;23:200-204.
10. Gupta PC. Seed vigour testing. In: Handbook of seed testing (PK Agrawal, ed), Department of Agriculture and Cooperation, Ministry of Agriculture, GOI, New Delhi, 1993, 242-249.
11. International Seed Testing Association. International rules for seed testing. Seed Science and Technology. 1985;13:299-513.
12. International Seed Testing Association. International Rules for Seed Testing. Seed Science and Technology. 1993;21:1-288.
13. Jain AK. Effect of inoculum load on incidence of head smut in kodo millet. Journal of Mycology and Plant Pathology. 2000;30:121-122.
14. Joshi LM, Renfro BL, Saari EE, Wilcoxson RD, Raychaudhuri SP. Rust and Smut Diseases of Wheat in India. Plant Disease Reporter. 1970;54:391-394.
15. Khamari B, Satapathy SN, Patra C. Effect of inoculum load and duration of exposure to *Macrophomina phaseolina* on disease incidence of sesame. International Journal of Chemical Studies. 2019;7:1728-1730.
16. Khanzada AM, Lodhi AM, Shah N, Maitlo SA. Effect of different inoculation methods and inoculum levels of *Macrophomina phaseolina* on okra. E Sci. Journal of Plant Pathology. 2012;01:01-05.
17. Madden LV. Quantification of disease progression. Protection Ecology. 1980;2:159-176.
18. Maguire JD. Speed of germination-aid selection and evaluation for seedling emergence and vigor. Crop Science. 1962;2:176-177.
19. Miller WB, Millikan CR. Investigations on flag smut of wheat caused by *Urocystis tritici* koern. Part I Journal of Department of Agriculture, Victoria. 1934;32:365-380.
20. Noble RJ. Studies on the parasitism of *Urocystis tritici* Koern. the organism causing flag smut of wheat. Journal of Agricultural Research. 1924;27:451-490.
21. Pal BP. Wheat breeding investigations at Indian Agricultural Research Institute. The Journal of Indian Botanical Society. 1951;30:1-4.
22. Rewal HS, Goel RK, Jhooty JS. Seed and soil inoculation in relation to the incidence of flag smut in wheat. Indian Phytopathology. 1986;39:599-600.
23. Serfling A, Kopahnke D, Habekuss A, Novakazi F, Ordon F. Wheat diseases: an overview. In: Achieving sustainable cultivation of wheat. Breeding, quality traits, pests and diseases. (P Langridge ed), Burleigh Dodds Science Publishing, Cambridgeshire, 2017, 319-326.
24. Shekhawat PS. Investigations on flag smut (*Urocystis agropyri* (Preuss) Schrot) of wheat (*Triticum aestivum* L.). Ph D Thesis, Department of Plant Pathology, Rajasthan Agricultural University, Bikaner, India, 2008, 1-108.