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EVALUATION OF WHEAT INOCULATION TECHNIQUES WITH NEOVOSSIA INDICA AND SCREENING OF WHEAT GERMPLASM AGAINST THE PATHOGEN

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Of the five Neovossia indica inoculation methods, boot inoculation with hypodermic syringe proved to be the best, followed by drop inoculation method of clipped florets, dip inoculation of unclipped florets and spray incoulation of heads, in that order, Screening of 80 wheat germplasm lines revealed 3 lines to be immune, 8 moderately resistant, 10 moderately susceptible, 21 susceptible and 38 highly susceptible.

INTRODUCTION

Artificial inoculation of crop germplasm with the inocula of their pathogens is a usual procedure for screening out sources of resistance against these pathogens. However, the successful screening depends upon the successful in fections which, in turn, depend upon the inoculation technique used. Thus various techniques for the inoculation of wheat germplasm with Neovossia indica (Mitra) Mundkar, the cause of Karnal bunt disease, were evaluated to know the most reliable and dependable inoculation method. Later by the use of this method, wheat germplasm was inoculated and screening for the sources of resistance against the pathogen was carried out.

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MATERIALS AND METHODS

Evaluation of inoculation methods

Ten seeds of a wheat cultivar Pak-81, highly susceptible to Karnal bunt disease, were sown in each of the five pots (size 12" x 18") and were kept in the greenhouse where the temperature ranged between 18-24°C and relative humidity was above 70 percent. After about one month, weak tillers were clipped away leaving behind 15 vigorously growing tillers per pot. When the plants reached boot and anthesis stage, 10 tillers/pot/inoculation method were inoculated with the sporidial suspension of Neovossia indica. The sporidial culture of N. indica was prepared by following the method of Torres et al., (1982). The sporidial suspension was prepared as follows.

Five sporidial culture slants, each in 250 ml flask, were taken; culture layers alongwith some PDA were removed and hand mashed thoroughly in 750 ml distilled water taken in a 1000 ml beaker. The sporidial suspension thus obtained was filtered through muslin cloth and the suspension was diluted further to get about 10,000 sporidia/ml of H_2O. This sporidial suspension was then used for each of the following inoculation methods.

i) Spray inoculation

When the plants reached the anthesis stage, florets of 10 wheat heads, exposed from the boot were spray inoculated with the help of an automizer.

ii) Syringe injection

At boot stage 2-3 ml of the sporidial suspension of Neovossia indica was injected into the boot of each of the ten tillers with hypodermic syringe.

iii) Drop inoculation of clipped florets

At anthesis stage individual florets of tenheads were clipped with the help of scissors to expose the stigmas and then each floret was inoculated with a drop of sporidial suspension.

iv) Dip inoculation of unclipped florets

Unclipped florets of ten heads at anthesis stage were dipped in the sporidial suspension of Neovossia indica.

v) Dip inoculation of clipped florets

Florets of ten heads were clipped to expose the stigmas and then these heads with clipped florets were dipped in the sporidial suspension.

The inoculated heads for each of the inoculation methods were covered with crossing bags. At maturity the heads were harvested and data on total number of diseased and healthy kernals were recorded for each inoculation method. The data on disease incidence in each of the five methods of inoculation were subjected to statistical analysis, using the Z-test, in order to visualize differences between the results of the methods of inoculation.

Screening of wheat germplasm for the sources of resistance agains N. indica

Eighty germplasm lines/commercial or local varieties/ advanced lines, received from Wheat Research Institute, Ayub Agricultural Research Institute, Faisalabad, were sown in single row sub plots, each 3 meter long. There was a 30 cm row spacing and 15 cm plant to plant distance. The test entries were boot inoculated by injecting 2-3 ml of sporidial suspension of Neovossia indica with a hypodermic syringe starting from the last week of February to mid March as and when a particular cultivar approached to its boot leaf stage. Boot inoculation was carried following the procedure of Singh and Krishna (1982) and Aujla et al., (1983). Five to ten heads of each test line were boot inoculated. The inoculated plants of each cultivar were tagged and labelled. To facilitate infection, field was irrigated to lower the temperature and to increase the relative himidity. Fortunately there were also frequent rains during the days of inoculation which further enhanced the chances of floral infection. Inoculated heads were harvested at maturity between 20 to 30th of April, 1988. Heads of each line were hand threshed and total number

of grains as well as the bunted grains of the inoculated heads were counted and disease incidence (percent seed infection) was thus calculated. The level of resistance/susceptibility of the test cultivars was assessed using the following modified disease rating scale of Moseman (1969; personal communications).

0 = No infection (immune)

1 = 0 - 0.2 percent infection (resistant)

2 = 0.21 - 1.5 percent infection (moderately resistant)

3 = 1.6 - 10 percent infection (moderately susceptible)

4 = 10 - 25 percent infection (susceptible)

5 = Above 25 percent infection (Highly susceptible)

RESULTS AND DISCUSSION

Evaluation of inoculation methods

Data on percent bunted grain achieved by various bunt inoculation methods are given in Table 1. Of the five Karnal bunt inoculation methods, boot inoculation with hypodermic syringe proved to be the best method as this method gave 84.16 percent grain infection by Karnal bunt. The second best method was drop inoculation method of clipped florets which gave 58.66 percent bunted wheat grains. The least effective methods were the dip inoculation method of clipped florets, dip inoculation method of unclipped florets and spray inoculation method of ear, in that order. Each of these method provided 36.10, 17.74 and 14.53 percent grain infection respectively. However, there was no statistical difference between the effectiveness of dip inoculation of unclipped florets and the spray inoculation of whole ear. The effectiveness and reliability of boot inoculation over some other methods of inoculation have also been reported (Chona et al., 1969; Aujla et al., 1983; Warham, 1984).

Screening of wheat germplasm by boot inoculation method for the sources of resistance against N. indica

Of the eighty cultivars evaluated, three cultivars including T.C.L. 83740, V-86257 and V-86354 remained totally free from

Table 1. *Percent bunted grain achieved by various methods of bunt inoculation*

Methods of inoculation	Bunted grains	Healthy grains	Percent bunted grains
Spray inoculation method	74	435	14.53 d*
Syringe injection or boot injection method	441	83	84.16 a
Drop inoculation of clipped florets	281	198	58.66 b
Dip inoculation of unclipped florets	96	445	17.74 d
Dip inoculation of clipped florets	178	315	36.10 c

Any two means having same letter do not differ at 5% level of significance (Z-test)

every kind of Karnal bunt infection and thus they were classed to be immune to Karnal bunt infection (Table 2). However, none of the cultivars was found to fall in the resistant class exhibiting 0.01 to 0.2 percent grain infection. Eight test lines i.e. V-85003, V-85028, V-85255, V-85409, V-86231, V-86326, V-86369 and V-86371 exhibited moderately resistant reaction (0.21 - 1.5 percent infection). The cultivars which expressed moderately susceptible reaction (1.6 - 10 percent infection) were V-83035, V-83134, V-83152-1, V-83156-3, V-84021, V-85060, V-85165, V-85195, V-85283 and V-86357. The cultivars which gave susceptible reaction (10 - 25 percent infection) were Dirk, M. Pak-65, Sa-75, B. silver, Lyp-73, Pb-75, C-591, C-518,, C-271, C-217, C-273,

Table 2. *Summary statement of wheat cultivars exhibiting various levels of resistance/susceptibility based on percent grain infection*

Levels of resistance/susceptibility (% grain infection					
Zero % grain infection (Immune)	0.01 - 0.20 percent infection (Resistant)	0.21 - 1.5 percent infection (Moderately resistant)	1.6 - 10 % grain infection (Moderately susceptible)	10 - 25 % grain infection (Suscept-ible)	Above 25 % grain (Highly suscept-ible)
TCL83740	Nil	V.85003	V.83134	Dirk	Arz
V.86257		V.85028	V.83156-3	M.Pak-65	Pavan
V.86354		V.85409	V.83035	SA-75	LU.26
		V.85255	V.84021	B.Silver	LU.26 S
		V.86369	V.85195	Lyp.73	LU.26 S-1
		V.86231	V.85165	Pb.85	WL-711
		V.86326	V.85060	C-591	Indus-79
		V.86371	V.83152-1	C-518	Pak-81
			V.85283	C-271	Pb. 81
			V.86357	C-217	BWP-79
				C-273	K.Noor-83
				Yacuura	Morrocco
					Potwar
				Sutileg 86	Barani-70
				Sandal	Chenab-70
				V.85096	Chenab-79
				V.84658	SA-42
				V.86115	Pb.76
				V.86061	C.228
				V.85292	FSBD 85
				V.86184	Wandanak
				V.87240	Pari-75
					Chakwal 86
					Barani-83
					V.83171
					V.84140
					V.85078
					V.85205
					V.85072-1
					V.84133
					V.85054
					V.86215
					V.86299
					V.85405
					V.86303
					V.86124
					V.86240
					V.87239

Yaccura, Sutleg 86, Sandal, V-84658, V-85096, V-85292, V-86061, V-86115, V-86184 and V-87240. The cultivars which exhibited very high degree of infection (25 to 100 percent) were regarded as highly susceptible; these were Arz, Pavan, LU-26, LU-26-S-1, WL-711, Indus-79, Pak-81, BWP-79, K. Noor-83, Morracco, Pothowar, Barani-70, Chanab-70, Chanab-79, SA-42, Pb-76, C-228, FSD-85, Wadanak, Pari-73, Chakwal-86, Barani-83, V-83171, V-84133, V-84414, V-85054, V-85072-1, V-85078, V-85205, V-85405, V-86124, V-86215, V-86240, V-86299, V-86303 and V-87239. The immunity (if it realy does exist) and the resistance of the germplasm could further be exploited for their incorporation into commercial wheat cultivars. Aujla et al., (1980) screened 286 genotypes with the boot inoculation technique and found 10 lines with only 1-5 percent grain infection. Earlier Gautam et al., (1977 a) tested 96 lines with the boot inoculation technique and reported 32 lines with zero infection, 10 with less than 1 percent infection, 38 with 1-5 percent and remaining 16 lines with over 5 percent infection. Gautam et al., (1977 b) screened 350 common wheat lines under field conditions and found 160 lines with no infection. Krishna and Singh (1983 d) screened 21 wheat cultivars against Karnal bunt by artificial inoculation and none was found free from the disease. Recently, Aujla et al., (1985) screened wheat germplasm under artificial epiphytotic conditions against Karnal bunt and reported 26 lines as disease free and 58 lines having infection range between 0-5 percent.

REFERENCES

Aujla, S.S., A.S. Grewal, K.S. Gill and I. Sharma. 1980. Effect of Karnal bunt on chappati making properties of wheat grains. Crop Improve. 7: 147-149.

Aujla, S.S., A.S. Grewal and I. Sharma. 1983. Yield and vigor components of wheat as affected by Karnal bunt pathogen (N. indica). Jour. Res. Pb. Agri. Univ. Ludhiana, India 20(3): 231-235.

Aujla, S.S., I. Sharma, K.S. Gill and A.S. Grewal. 1985. Variable resistance in wheat germplasm to Neovossia indica. 3rd National Seminar on Genetics and Wheat Improvement held at IARI. Regional Station Flowerdale Shimla from May 8-10, PP.7. (Abstr:).

Chona, B.L., R.L. Munjal and K.L. Adlakha. 1961. A method for screening wheat plants for resistance to Neovossia indica. Indian Phytopath. 14(1): 99-101.

Guatam, P.L., S. Pal, S.K. Malik and D.P. Saini. 1977a. Note on reaction of promising wheat strains of Karnal bunt. Pantnagar Jour. Res. 2(2): 228-229.

Guatam, P.L., T.B. Singh, S.K. Malik and S. Pal. 1977b. Screening of superior genetic stocks for karnal bunt resistance under field conditions. Genetics and Wheat Improvement (Ed. A.K. Gupta) New Delhi, India PP. 97-100.

Krishna, A. and R.A. Singh. 1983 d. Method of artificial inoculation and reaction of wheat cultivars to Karnal bunt. Indian Jour. Mycol. and Plant Pathol. 13(1) : 124-125.

Singh, R.A. and A. Krishna. 1982. Susceptible stage of inoculation and effect of Karnal bunt on viability of wheat seed. Indian Phytopath. 35: 54-56.

Torres, E., G. Bekel and J.M. Prescot. 1982. Karnal bunt. CIMMYT Report on Wheat Improvement. PP. 68-70.

Warham, E.J. 1984. A comparison of inoculation methods for Karnal bunt (Neovossia indica). Phytopathology 74(7): 856-857. (Abstr.).