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A greenhouse screening technique to assess rust resistance in sorghum*

(*Keywords:* Puccinia purpurea, sorghum, screening technique, resistance)

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Abstract. Studies were conducted to determine the influence of plant growth stage, inoculum density, temperature, and relative humidity (RH) on development of rust (*Puccinia pupurea*) in sorghum (*Sorghum bicolor*). Rust development was maximum (>80% severity), when plants of a susceptible sorghum genotype (IS 18420) were inoculated at the four- to five-leaf stage with an inoculum concentration of 4×10^6 urediniospores per ml and incubated at 20–25 C under high RH (>90%) for 24 h. Disease severity (percentage leaf area covered with rust pustules) scores were taken 2 weeks after inoculation. Using this technique, 29 sorghum genotypes were screened for rust resistance in a greenhouse. This technique proved effective in discerning resistant and susceptible genotypes, and IS 3979, ICSH 110, ICSH 86647 and ICSH 871035 were identified resistant (< 20% rust severity) compared with a susceptible control IS 18420 (90% rust severity). This technique is simple and rapid, and can be used effectively and economically to screen, on a large scale, germplasm lines and breeding populations in the greenhouse.

1. Introduction

Rust of sorghum (*Sorghum bicolor* (L.) Moench.), caused by *Puccinia purpurea* Cooke, is an important leaf disease in areas where cool and humid weather prevails during crop maturity (Tarr, 1962). Under natural rust infection, grain yield losses up to 65% were estimated (Bandyopadhyay, 1986). Hepperly (1990) recorded a 29–50% loss in grain yield, and a 28–41% loss in 100-grain weight, due to rest infection.

Screening for rust resistance in sorghum has generally been conducted under natural infection conditions (Avadhani and Gowda, 1979; Laxmanan *et al.*, 1986; Patil *et al.*, 1988) with inconsistent results due to variable disease pressures across locations and years. Due to limited available information on host–pathogen–environment interaction an effective screening technique has not yet been developed. This paper reports the effects of plant growth stage, inoculum density, temperature, and relative humidity on rust infection leading to the development of a greenhouse screening technique for rust resistance in sorghum.

2. Materials and methods

2.1. *Inoculum production and inoculation*

Rust-infected sorghum leaves of IS 18420 were collected from the ICRISAT research farm, Patancheru, washed with tap water to remove old spores, blotted-dry, and incubated at 25°C in a humid chamber (>90% RH) made of plastic trays lined with pre-wetted blotter sheets. A fresh crop of urediniospores was collected, 48 h after incubation, into glass vials using a cyclone spore collector attached to an air suction pump (3·45 KPa).

Plants of a rust-susceptible sorghum inbred IS 18420 were raised in 18-cm diameter plastic pots filled with a mixture of vertisol (black loamy soil), sand and farm-yard manure (2 : 2 : 1). Four plants were maintained in each pot. Plants were fertilized with a basal dose of diammonium phosphate (3 g per pot), and later with Hoagland nutrient solution (Hoagland and Arnon, 1950) at weekly intervals. Plants were protected from shoot-fly and stem-borer attack by spraying with endosulfan as and when required.

Inoculum was prepared by suspending urediniospores in sterilized distilled water to which Tween 20 (polyoxyethylene sorbitan monolaurate) was added (two drops per 100 ml) as a wetting agent. Plants were spray-inoculated at the four- to five-leaf stage with the suspension (4×10^6 urediniospores per ml) and incubated at 20 C by covering individual pots with a pre-wetted polyethylene bag (>90% RH) for 24 h. After removing the polyethylene bag, the plants were then shifted to a greenhouse at 25 ± 2 C.

2.2. *Disease evaluation*

Rust severity was recorded visually on all leaves as percentage leaf area covered by the rust pustules, 14 days after inoculation. Mean rust severity over all plants in a pot was computed.

2.3. *Plant growth stage at inoculation*

The rust-susceptible inbred IS 18420 was sown in pots, as described before, at weekly intervals for 11 weeks. Plants were spray-inoculated with the urediniospore suspension (4×10^6 spores per ml) at 11 different growth stages, from two-leaf to flowering (Vanderlip and Reeves, 1972). The experiment was conducted in a randomized complete block design with three replications with four plants per pot per replication. Rust severity was scored 14 days after inoculation as described earlier.

2.4. *Inoculum concentrations*

Ten spore concentrations (41·7, 17·1, 11·7, 5·7, 1·9, 1·2, 0·9, 0·4, 0·2 and 0·1 $\times 10^5$ urediniospores per ml) were obtained by progressively diluting the stock urediniospore suspension

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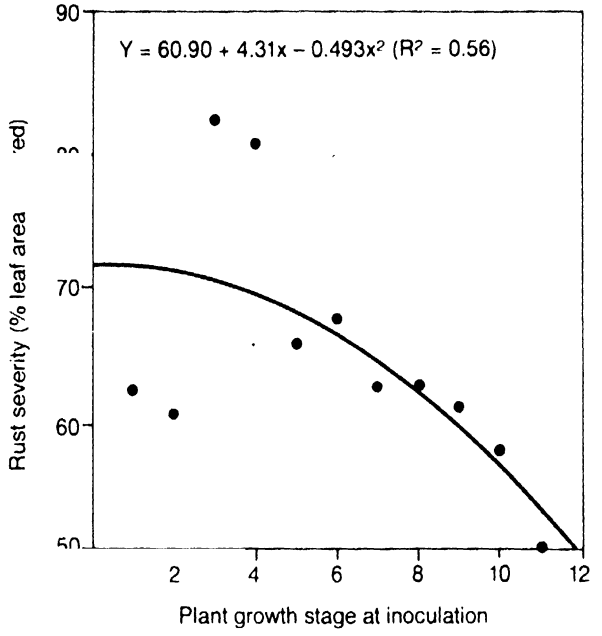

Figure 1. Relationship between rust severity (Y) and plant growth stages (X) in susceptible sorghum genotype IS 18420.

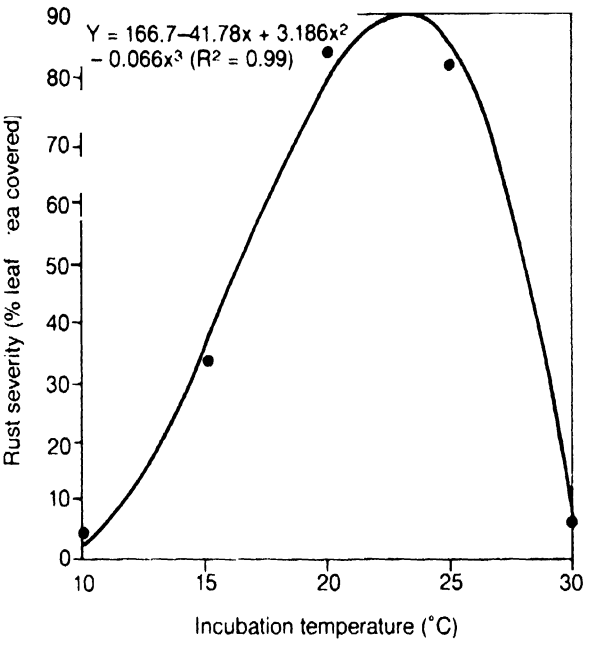

Figure 3. Relationship between rust severity (Y) and incubation temperature (X) in susceptible sorghum genotype IS 18420.

with sterile distilled water. Spore concentrations were adjusted using a haemocytometer. Plants were spray-inoculated with each inoculum concentration, and incubated. The experiment was conducted as a randomized complete block design with three replicates.

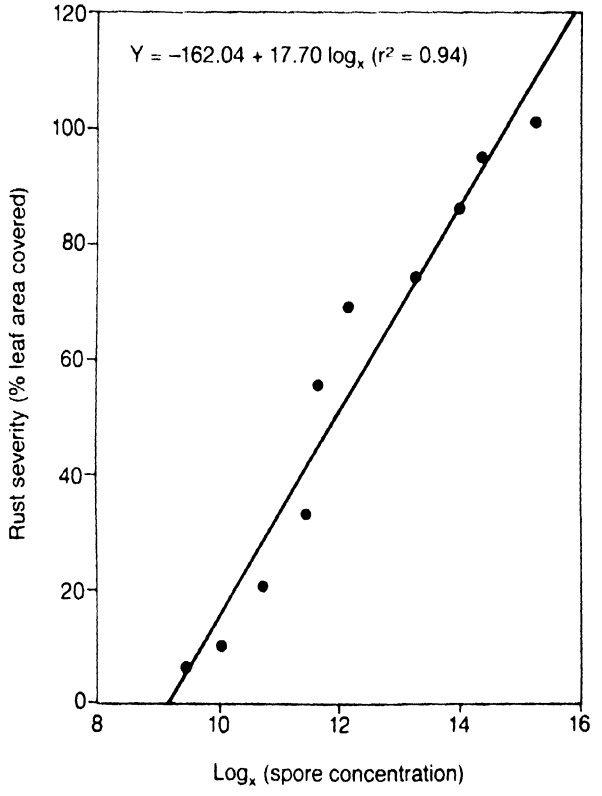

Figure 2. Relationship between rust severity (Y) and spore concentration (X) in susceptible sorghum genotype IS 18420.

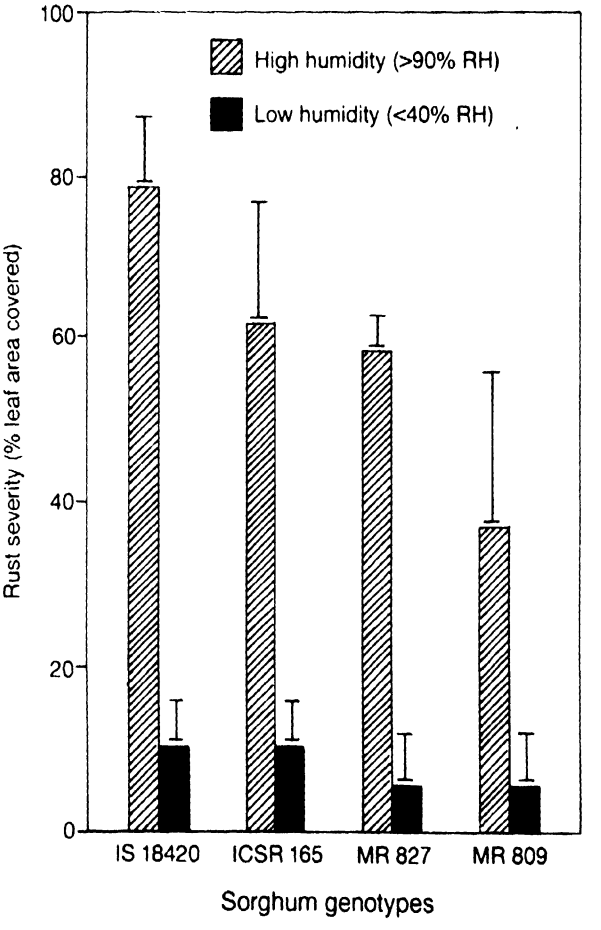

Figure 4. Levels of rust severity in four sorghum genotypes (IS 18420, ICSR 165, MR 827, and MR 809) at two post-inoculation humidity levels. Standard error of means are represented by vertical lines.

2.5. *Incubation temperatures*

Pot-grown plants of IS 18420 at the four- or five-leaf stage were acclimatized at different temperatures (5, 10, 15, 20, 25, 30, 35, and 40 C) for 24 h in Percival incubators (Percival Mfg. Co., Boone, Iowa, USA, Model No. I-35LL). They were then spray-inoculated with the urediniospore suspension and returned to the respective temperatures. The plants were covered with pre-wetted polyethylene bags for 24 h and then shifted to the greenhouse. Rust severity was scored as described earlier. The experiment was conducted in a randomized complete block desigh with four replications.

2.6. *Effect of post-inoculation relative humidity*

Pot-grown plants of four genotypes with different levels of rust susceptibility (IS 18420, ICSR 165, MR 827, and MR 809) were spray-inoculated with the urediniospores suspension and were either covered with pre-wetted polyethylene bags (>90% RH) or left uncovered (<40% RH) at 25 C in the Percival incubator, for 24 h. A small thermohygrometer was kept inside the incubator to measure the RH. The plants were later transferred to the greenhouse, and rust severity was scored. The experiment was conducted as a randomized complete block design with two RH regimes, four genotypes and three replicates.

2.7. *Greenhouse screening technique*

Based on the results from the above studies, a screening technique was developed which involved spray inoculation of pot-grown plants at the four- or five-leaf stage with a spore suspension (4×10^6 urediniospores per ml), incubation of inoculated plants at 20 C and >90% RH for 24 h, and scoring for rust severity 14 days later. Using this technique 29 sorghum genotypes comprising six ICRISAT Center bred varieties, 15 hybrids, three restorer lines, three germplasm accessions and a local cultivar, were screened for rust resistance. Rust susceptible genotype IS 18420 was used as an indicator check.

3. Results and discussion

3.1. *Effect of plant growth stage*

Plants were susceptible to rust at all the 11 plant-growth stages. However, significantly higher rust severity (>80%) was observed when plants were inoculated at the four- and five-leaf stages, which usually occur between 20 and 30 days after emergence, than when inoculated at other growth stages. A quadratic equation showed a good fit between rust severity and plant growth stage at inoculation (Figure 1). The quadratic coefficient indicates reduction in the rust severity, when inoculated at the later stages of plant growth. However, under field conditions, Soumini (1949)

Table 1. Rust severity (per cent leaf area covered with rust pustules) in 29 sorghum genotypes screened in the greenhouse at ICRISAT Center (IC), 1990

Genotype[a]	Pedigree	Origin	Rust severity (%)
IS 3979	SH DD Shallu	USA	7.5
ICSH 110	296 A × ICSR 33	ICRISAT	13.7
ICSH 86647	ICSA 70 × ICSR 161	ICRISAT	15.0
ICSH 871035	ICSA 102 × ICSR 165	ICRISAT	17.5
ICSV 88013	(PM 11344 × SPV 351)-27-1-1	ICRISAT	20.0
ICSH 638	ICSA 37 × ICSR 134	ICRISAT	53.7
ICSH 88065	ICSA 67 × ICSR 154	ICRISAT	55.0
MR 875	[(Bulk Y × 165) × CSV 4]-18-1	ICRISAT	55.0
ICSH 89123	ICSA 56 × ICSR 89028	ICRISAT	55.0
ICSH 871015	ICSA 90 × ICSR 165	ICRISAT	56.2
ICSV 88032	(ICSV 197 × ICSV 1)-27-1-1	ICRISAT	57.5
ICSH 228	(CSA 9 × ICSR 153	ICRISAT	60.0
ICSV 760	(148 × 555)-29-3-2-2	ICRISAT	61.2
ICSH 89120	ICSA 31 × ICSR 89022	ICRISAT	62.5
MR 809	[ET 2039 × (SC 108-3 × 148)]-29-3-1	ICRISAT	65.0
IS 73	DD Feterita	Mexico	66.2
ICSH 89122	ICSA 56 × ICSR 89027	ICRISAT	67.5
ICSH 871031	ICSA 101 × ICSR 165	ICRISAT	70.0
IS 3413	Sorgho Niska	Zaire	70.0
ICSV 655	(20/75)-1-1-5-1	ICRISAT	73.7
ICSR 165	SPV 422	ICRISAT	73.7
ICSH 871033	ICSA 102 × ICSR 172	ICRISAT	77.5
ICSV 430	(PS 21116 × SC 108-3)-2-2-4-1	ICRISAT	78.7
ICSV 745	(ICSV 197 × A 6250)-4-1-1-1	ICRISAT	83.7
ICSH 871001	ICSA 84 × ICSR 172	ICRISAT	86.2
Local sorghum (from farmer's field)		India	86.2
ICSH 88646	ICSA 70 × ICSR 162	ICRISAT	90.0
IS 18420	Khundi jowar	India	90.0
ICSH 87891	ICSA 73 × ICSR 174	ICRISAT	96.2
SE±			12.42

[a]ICSV = ICRISAT sorghum variety; ICSH = ICRISAT sorghum hybrid; ICSR = ICRISAT sorghum restorer line; MR = mould resistant line; IS = International sorghum accession.

reported high rust severity in 2–3-month-old sorghum plants when younger plants were completely free from the disease. Vidhyashekaran *et al.* (1971) made a similar observation on field-grown sorghum, and reported that the older plants (> 55 days) developed more rust than the younger ones. They suggested that the presence of high levels of hydrocyanic acid in the leaves of young sorghum plants imparted resistance to rust. In our study, however, plants as young as 20–30 days developed high rust severity under artificial inoculation. Differing levels of hydrocyanic acid, and other unknown factors related to rust resistance, might result in variable disease effects in different genotypes at different growth stages in field and greenhouse conditions. Headrick and Pataky (1985) reported that most of the sweet-corn hybrids evaluated were more susceptible to *Puccinia sorghi* at the seedings stage than at older growth stages.

3.2. *Effect of inoculum concentration*

The relationship between log(spore concentration) and rust severity was estimated assuming linearity and is shown in Figure 2. An inoculum concentration of about 4×10^6 urediniospores per ml produced maximum rust (100% severity) under favourable conditions of temperature and humidity. Rust, being a polycyclic disease, can develop very rapidly from a few initial infection foci and become quite severe within a few days after favourable environmental conditions. Inoculum threshold may not be a limiting factor for rapid rust development.

3.3. *Effect of temperature*

Rust development was maximum (81–84% severity) between 20 and 25 C, and was minimum (< 5% severity) at 10 and 30 C (Figure 3). There was no rust development at 5, 35, and 40 C and therefore, these temperatures were excluded from the regression analysis. The relationship between incubation temperature and rust severity was best represented by a third-order polynomial ($R^2 = 0.99$). Rust severity increased linearly with increase in temperature up to 23–24 C, and thereafter the severity decreased rapidly with further increase in temperature (Figure 3). In maize, under natural conditions, rust (*P. polysora*) development was maximum between 16 and 32 C (Ordonia-Sinohin and Exconde, 1981; Hollier and King, 1985). Similar results were obtained for other species of *Puccinia* (Mahindrapala, 1978), and for *P. sorghi*, causing rust in maize (Headrick and Pataky, 1986).

3.4. *Effect of relative humidity*

High RH (> 90%) was more favourable for rust development than low RH (< 40%) in all the four sorghum genotypes (Figure 4). Highly significant ($P =< 0.001$) variance for rust severity was observed among genotypes, humidity levels, and the genotype × humidity level interaction. However, larger variation was observed between humidities than the other two (genotypes and genotypes × humidity levels) interactions. Headrick and Pataky (1986) observed that 12 h of high RH (>90%), obtained by intermittent misting for 30 min each hour, favoured infection and development of *P. sorghi* on maize. Similar results were reported for *P. polysora* (Hollier and King, 1985).

Our results indicate that post-inoculation environment, particularly temperature and RH, play major roles in infection and development of rust in sorghum. A post-inoculation temperature of about 20 C and high RH (>90%) with an adequate level of initial inoculum could cause severe rust at all growth stages of sorghum. Plants that get infected in the early growth stages (two- and three-leaf stages) may die due to severe rust infection.

3.5. *Greenhouse screening*

Of 29 sorghum genotypes screened in the greenhouse, IS 3979 showed high rust resistance (7·5% severity) compared with 90% severity in the susceptible control IS 18420 and in ICSH 88646, 96% severity in ICSH 87891 (Table 1). A few other genotypes, ICSV 88013, ICSH 110, ICSH 88647, and ICSH 871035, showed < 20% rust severity. Several known susceptible genotypes (IS 18420 and local sorghum) showed high rust severity, confirming the effectiveness of the screening technique. The ANOVA further indicates highly significant ($P =< 0.001$) differences among the genotypes. It is recommended that the screening technique reported here can be used very effectively and precisely to screen, on a large scale, germplasm lines and segregating breeding materials for rust resistance in sorghum.

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