Table 16 - Methods: Seedling reaction to Tan Spot. C. Kent Evans and Robert M. Hunger, Plant Pathology Department, Oklahoma State University, Stillwater.

The reactions of entries in the 1993 SRPN to tan spot caused by *Pyrenophora tritici-repentis* (PTR) were determined in two tests conducted in the greenhouse. Specific test conditions and procedures were as follows:

Isolates: Three single ascospore isolates of PTR were used to produce inoculum. These isolates were obtained from naturally infested wheat straw collected in 1991 from different wheat producing regions in Oklahoma. Each isolate produced abundant conidia in vitro, and caused the type lesions typically associated with tan spot on susceptible wheats.

Inoculum preparation: Inoculum was prepared by combining equal amounts of conidia from each of the three PTR isolates to a final concentration of 1000 conidia/ml + 1 drop of Tween 20/100 ml. Inoculum was prepared as described previously (Phytopathology 1991. 81:1238). The inoculum that results from this procedure was nearly free of other fungal propagules such as conidiophores and hyphae.

Inoculation of wheat: The nursery was tested twice in the greenhouse. Three genotypes were included as resistant ('Red Chief', 'Agrotricum') and susceptible ('TAM-105') checks. Ten seeds of each entry were planted as a clump in soil contained in wooden flats, which were planted in a randomized complete block design with four replications. When the first leaf was fully expanded, seedlings were inoculated with the conidial suspension using a DeVilbiss sprayer (model #5601D) until incipient runoff. Following inoculation, plants were allowed to dry for one hour and then placed in a mist chamber that provided near 100% relative humidity. After 24 hr in the mist chamber, plants were placed on greenhouse benches.

Determination of response to tan spot: The length of the largest lesion in the middle 50% of the first leaf was determined after eight days using a dial caliper with an accuracy range of ±0.05 mm. One measurement was made on each of four leaves from each clump of seedlings. Measurements were made from the border of the visible edge of the chlorotic or necrotic lesions longest dimension, which generally was oriented parallel with the leaf axis. Statistical analyses were conducted on the mean of the four measurements made per entry.