REVISED SALT TOLERANCE OF GERMINATING ALFALFA SEEDS STANDARD TEST

Test accepted: June 2024
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PROCEDURES

Plant..... Scarified seeds not previously treated with fungicides or inoculated with bacteria
Container..... 100-mm Petri dishes containing a single piece of Whatman no. 2 filter paper
Media..... Seeds are germinated in two concentrations of NaCl: 0.00 and 1.25% (wt/v) in deionized or distilled water; these salt treatments correspond to 0.0 and 213.9 mM NaCl solutions, respectively. Fifty scarified seeds are placed in a Petri dish and 4.5 mL of an appropriate salt solution is added. (*See helpful information for alternatives*)
No. of Reps..... 4 replications in a randomized complete block design with a minimum of 200 seeds total
Germination..... Apply Parafilm to seal each Petri dish to prevent evaporation. Place the dishes in a dark growth chamber or germination cabinet maintained at 25°C. (*See helpful information for alternatives*)
Counts..... Germinated and hard seeds are counted after 7 days. Count at 10 days If new germination is still occurring at 0% NaCl.

DATA ANALYSIS

Germination in each dish is computed by subtracting the number of hard seeds from 50 to obtain a corrected total. Divide the number of germinated seeds by the corrected total to obtain the fraction germinated. For each replicate, the corrected total germination of all susceptible/tolerant checks in each control treatment (i.e., 0% NaCl) must exceed 70%, otherwise, the replicate must be re-run. Adjusted germination is computed by dividing the fraction germinated in each dish at 1.25% NaCl by the germination of the same accession in the same replicate as the control (0.0 mM NaCl) treatment and multiplying by 100 to convert to percent. Adjusted germination percent (AGP) data from the same trial are evaluated by analysis of variance, or t-test procedures, and results must demonstrate a significant difference at $P<0.05$ between Rambler (susceptible check) and Mesa-Sirsa (tolerant check), and if included, between Rambler and the high tolerance check (AZ-GERM SALT-II). Before tolerance can be claimed for a new cultivar, its AGP must be significantly greater than the susceptible check and statistically equivalent to, or greater than, the tolerant check. Use Tukey's HSD or Fisher's LSD post-hoc tests for separating means. The AGP of all checks must also reside within their acceptable range of reaction. The statistic to be used as the standard descriptor is the AGP at 1.25% NaCl.

CHECK CULTIVARS

	Approximate Expected Adjusted Germination (%)	Acceptable Range of Reaction (%)
High Tolerance	---------- at 1.25% NaCl ----------	
AZ-GERM SALT-II	100	95-100
Tolerant		
Mesa-Sirsa	75	55-95
Susceptible		
Rambler	7	0-15

HELPFUL INFORMATION

If desired, the number of seeds per Petri dish can be reduced to facilitate counting as long as at least 200 seeds are evaluated. Testers may elect to use an additional treatment of 1.5% NaCl in addition to 0% and 1.25%. Petri dishes could be kept in a sealed dark box in a room with a temperature held at 25°C or place the Petri dishes in a ziptop bag in darkness at 25°C. Care should be taken that air movement within the chamber is not restricted excessively or condensation may occur inside the dishes. If bacteria/fungus contamination is a problem, seeds of each entry can be surface sterilized by placing them into a mesh bag in a 1% bleach solution for 5 minutes, followed by three rinses in water at 2 minutes each. The 1% bleach solution is prepared using 1 mL household bleach at 5.25 to 7.5% sodium hypochlorite into 99 mL water. Prior to placing these seeds in a Petri dish, the seed/bag should be blotted on clean paper towels to remove excess water. Nondormant germplasm is more tolerant than dormant germplasm. If notable variation in seed size exists, select "healthy and plump" seeds for conducting the test.

Alfalfa seed germination under different salt concentrations is considered an adaptation trait to soil salinity, where salts present in the solution generate osmotic, ionic, and oxidative stresses affecting seed germination and seedling growth. A standardized test to characterize salt tolerance of germinating alfalfa seeds (Rumbaugh, 1991) measured seed germination of new cultivars and check varieties in eight solutions of varying NaCl concentration (range: 0-2%) to determine the salt concentration required to inhibit germination of 50% of the viable seeds, i.e., IC_{50} values. This method requires costly equipment (vapor pressure osmometer) to verify osmotic values of the multiple solutions. The revised test uses lower-cost methodology where the vapor pressure osmometer verification step and IC_{50} value estimation process are eliminated. The revised protocol is easier to conduct, lower in cost, able to discriminate between susceptible and resistant checks, and establishes statistical criteria that must be met to declare that a germplasm possesses seed germination salt tolerance. It is also uses checks with consistent responses.

Comments: The authors encourage including additional elite salt sensitive checks as they are identified. As seeds become available for potential new salt sensitive and tolerant/high-tolerance checks, they and the established Rambler and Mesa-Sirsa checks should be provided to all individuals requesting seed for this standard test. Recipients of the established and pending checks will be required to provide data for their germination percentage at 0% and 1.25% NaCl, and the adjusted germination percentage, to the NAAIC. Evaluation of the potential new checks by multiple labs will be used to collectively establish expected adjusted germination percentage, and acceptable ranges of reaction, for their inclusion in a future amended protocol. It is recommended that 2 grams of seed of each check be provided for each germplasm request given the small number of seeds required for each test.

REFERENCES

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