

Optimizing Salinity Screening Protocol in Alfalfa & Digitizing Root Phenotyping Using Deep Neural Network

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Soil salinity is a growing concern for profitable alfalfa (*Medicago sativa* L.) production in the Southwest United States, where recurrent drought and frequent irrigation accelerate salt accumulation in arable land. Salinity stress severely impairs germination and early seedling development of alfalfa. An efficient, accurate, and non-destructive screening protocol is critical to select salt-tolerant genotypes that can be utilized for developing salinity tolerant alfalfa populations. To optimize a germination-based salinity screening protocol, we evaluated three USDA alfalfa check cultivars (AZ-GERM SALT-II (highly resistant), Mesa-Sirsa (resistant), and Rambler (susceptible) under five NaCl concentrations (0, 1.25, 1.50, 1.75, and 2.00% w/v, corresponding to 0.0, 213.9, 256.7, 299.4, and 342.2 mM NaCl, respectively) in a randomized complete block design with ten replicates. Germination was assessed at 7 and 10 days after trial setup, with seedlings exhibiting radicle lengths ≥ 1 cm classified as germinated. Analysis of variance revealed highly significant effects of salinity, genotype, and their interaction ($P < 0.001$). Across all genotypes, germination decreased progressively with increasing salt concentration. In AZ-GERM SALT-II, it declined from about 95% under control conditions to near-complete inhibition (0–5%) at 2.00% NaCl. AZ-GERM SALT-II consistently showed better tolerance, maintaining approximately 79% and 49% germination at 1.25% and 1.50% NaCl, respectively. Mesa-Sirsa showed intermediate tolerance, whereas Rambler was highly sensitive even at lower salinity levels. Severe reductions in germination were observed at 1.75% NaCl, with near-complete germination failure at 2.00% across all genotypes. Despite these findings, fungal contamination remained a significant challenge, while germinated root counting and measurement remained manual, subjective, time-intensive, and error-prone. To minimize contamination, autoclaving and filter sterilization of salt solutions will be implemented, and multiple seed sterilization treatments (1% (v/v) NaOCl, 2% (v/v) H_2O_2 , 70% (v/v) ethanol; 1% (v/v) NaOCl mixed with 2% (v/v) H_2O_2 and 70% (v/v) ethanol mixed with 1% (v/v) NaOCl) with varying exposure durations are being evaluated under three salinity levels (0, 1.25, and 1.50%). Additionally, as an attempt to digitize germinated root measurement to hasten salinity screening, we propose integrating Deep Learning (DL) in Computer Vision (CV) models like, You Only Look Once (YOLO)-based architectures for automated germination detection and the Segment Anything Model 2 (SAM2) for precise root trait quantification. This integrated approach offers a scalable, objective solution to accelerate screening efficiency and enhance genetic gain in alfalfa salinity tolerance breeding programs

Keywords: Alfalfa (*Medicago sativa* L.), salinity screening, Deep Learning (DL), YOLO, SAM2, Computer Vision (CV)