

Fine-Tuning Drought Tolerance: Optimizing Soil Moisture Levels for Ascorbic Acid Analysis in Chickpea

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Abstract:

Chickpea (*Cicer arietinum* L.), a vital legume crop, faces significant challenges from drought stress, impacting its yield and quality. Recent research has unveiled a fascinating interplay between soil moisture and ascorbic acid (AsA), a potent antioxidant, in chickpea plants. This intricate relationship has opened new avenues for breeding drought-resilient varieties. AsA, commonly known as vitamin C, plays a crucial role in various plant physiological processes, including antioxidant defense, cell wall synthesis, and hormone synthesis. Under drought stress, plants experience oxidative stress due to increased production of reactive oxygen species (ROS). AsA acts as a vital antioxidant, scavenging these harmful ROS and mitigating oxidative damage to cellular components. A recently developed protocol pinpoints the optimal soil moisture level to assess genetic variation in endogenous AsA content, shedding light on its role in plant drought tolerance.

Protocol Development and Implementation:

This excerpt describes a well-defined experimental protocol for studying the effects of drought stress on chickpea. Here's a breakdown:

- **Well-Watered:** Both well-watered and water-stressed plants were initially maintained at 60% field capacity (FC). This ensures that all plants have access to adequate water during the initial growth phase and establishes a baseline for comparison.
- **Stress Induction:** Drought stress was induced by withholding irrigation. This gradually reduced soil moisture in the stressed pots, simulating a realistic drought scenario.
- **Monitoring and Control:** Soil moisture was continuously monitored in both sets of pots. The stress was allowed to progress until soil moisture stabilized at 20% FC after 12 days. This provides a consistent level of stress across all stressed plants.

- **Genotypic Responses:** This controlled environment allows researchers to precisely observe how different chickpea genotypes respond to the imposed drought stress. They can then analyze various parameters, including AsA content, growth, yield, and other physiological traits, to identify drought-tolerant varieties.

This protocol is crucial because:

- **Reproducibility:** It ensures consistent and reproducible experimental conditions, allowing for reliable comparisons between different genotypes.
- **Control:** By maintaining a constant initial moisture level and gradually reducing it, researchers can accurately control the severity and duration of drought stress.
- **Precision:** Continuous monitoring of soil moisture provides precise data on the water status of the plants, enabling a more accurate assessment of their drought responses.

Key Findings: AsA Accumulation Trends:

1. **Initial Response:** As soil moisture declined from 60% FC to 30±1% FC, both drought tolerant as well as drought-sensitive chickpea genotypes showed a similar increase in endogenous AsA levels. However, the genotypic differences became apparent beyond this critical point.
2. **Peaking AsA Levels:** At 30±1% FC, both genotypes reached their highest AsA accumulation. Drought tolerant genotype recorded significantly higher AsA than drought-sensitive genotype. This stage was determined as the optimal moisture level for assessing genetic variation in AsA.
3. **Decline at Severe Stress:** Under extreme stress conditions (20% FC), both genotypes exhibited reduced AsA levels. Drought tolerant genotype still outperformed drought-sensitive genotype. Notably, neither genotype could recover fully after rehydration, highlighting the critical nature of drought stress beyond this threshold.
4. **Validation:** Repeated experiments confirmed that leaf AsA accumulation in chickpea seedlings consistently peaks at 30±1% FC. This precision underscores the reliability of the protocol in identifying drought-related genotypic responses.

Implications for Chickpea Breeding:

The study provides a robust, reproducible method for integrating soil moisture and AsA analysis into drought-tolerance screening programs. The findings confirm that drought-



tolerant genotypes maintain higher AsA levels even under severe stress, emphasizing AsA's role as a biochemical marker for drought resilience.

Conclusion:

The optimized protocol for soil moisture levels not only refines the analysis of endogenous AsA but also lays the groundwork for targeted breeding strategies. By leveraging this knowledge, researchers can better identify and develop chickpea genotypes capable of thriving in water-limited environments. This advancement holds great promise for enhancing food security in drought-prone regions.

