Rapid Induction of Water Stress in Wheat

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[Abstract] Traditional water stress evaluation studies in wheat are time consuming and can take up to several months to finish. A rapid phenotypic screening for water stress is important for accommodating time-bound water stress studies such as transient gene silencing studies in wheat. This method explains a procedure to induce water stress in young wheat plants within three weeks.

Materials and Reagents

1. Wheat seeds
2. Permanent marker pen
3. Green house handling gloves

Equipment

1. Plastic pots and potting mixture
2. Volumetric beaker (500 ml)
3. Petri dishes (11 cm diameter)
4. Growth chamber
5. Light incubators/Green house facility
6. Weighing scale

Procedure

1. We have to calculate the field capacity (FC) of the experimental set up to maintain proper water stress. FC is the amount of water in the soil remaining after water is removed by gravity following water saturation. For the purpose of estimating the FC for the experimental set up.
   a. Plastic pots holding 500 g of potting mixture are fully saturated with water, drained by gravity for 3 h and weighed.
   b. These pots are allowed to fully dry over a period of 12 days. These pots are weighed

again and the difference in weight constitutes the water held by soil in pot after gravitational drainage. This constitutes the approximation of FC for these pots. An average reading from 10 pots is assumed as the field capacity for this experimental setup (in our case FC was 245 g).

2. Once we establish the FC for our system, achieving 50% FC is the goal for inducing water stress
    a. 50% FC corresponds to half of the amount of water (measured in weight) for the calculated FC. This FC is achieved in our pots by maintaining the weight of the pots at a level equal to weight of dried down pots (with soil) plus half of the measured FC (measured as weight of water) calculated for these pots. Control plants were kept at 100% FC. In this experiment 100% FC can be achieved by adding the water equal the calculated FC to the dried down pots. All the pots are periodically (twice a day) weighed to maintain the water level (50% FC and 100% FC).
3. Preparation of wheat plants
    a. Sterilization and germination of wheat seeds: The wheat seeds are placed in petri dishes, covered with disinfecting (5% (w/v) sodium hypochlorite) for 15 min, stirred, drained, and washed four times with sterile deionized water. These seeds are placed on moist filter paper in petri dishes. Store these petri dishes in a dark place (preferably in a growth chamber) with stable room temperature (~25 °C).
    b. These seeds that germinated (~48 h) are then transplanted (3 seeds in a pot) into pots holding 500 g of potting mixture in a temperature-controlled growth room at 22–25 °C and relative humidity of 60% with a 12 h photoperiod with light intensity ranging from 300 to 400 μE/m^2/s. Prophylactic measures (disease free seeds, clean water for treatment, pest and pathogen free environment *etc*) are taken to maintain the plants disease and pest free.
    c. The ideal age to start this experiment is found to be 3-5 leaf stage in wheat (~15 days from germination initiation). The sample size (number of plants) is determined by the experimental design. This experiment used 24 plants in each treatment.
4. For this experiment, two subsets of plants (well watered set and water stressed set) are maintained.
    a. One set of plants (well watered set) is maintained at 100% field capacity (FC).
    b. The second set of plants is water stressed plants. Water stress is imposed on this set of plants by withholding water until 50% FC weight is achieved. Soil moisture regimes are monitored gravimetrically by weighing the pots every day. It was found that withholding water continuously for ~3 days could achieve a water level of 50% FC in our experimental setup.
5. No other enclosure for pots are necessary as evapotranspiration from the pots under

study was found to be statistically similar in all pots as conditions in the two treatments were identical (plant growth stage, pot size and growing conditions), except the treatment (water stress).

6. Withholding water up to 50% FC is found to induce the water stress phenotype in the experimental plants placed in 500 g of potting mix within the time frame of three weeks.
7. The 100% field capacity (FC) plants would give the control phenotype for well watered plants and 50% field capacity (FC) plants would show the water stressed phenotype (Figure 1).

Figure 1. The phenotype of well watered (100% field capacity) and water stressed (50% field capacity) plants after 7 days of stress induction. Plastic covers are used to prevent evaporation from high light during photography.

8. Stunted growth and chlorosis are observed in water stressed plants in comparison with well watered plants. To confirm and measure the water status in the plant, leaf relative water content (RWC) is estimated according to the method of Ekanayake *et al*. (1993).

Acknowledgments

This protocol is adapted from Ekanayake *et al*. (1993) and Manmathan *et al*. (2013).

References

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