Supplementary 4

Protocol for the determination of osmotic potential in mesocotyl and primary root

Mesocotyl:

1. One cm of mesocotyl tissue is sectioned from each maize seedling taking the coleoptile node as a reference placing them in separately labeled Eppendorf tubes.
2. The plant material is immediately frozen with liquid nitrogen for 1 min. At the end of the time, the samples were kept for 10 min at room temperature (approximately at 25° C) and then they are centrifuged for 20 min at 14000 rpm (Eppendorf 5415C Microcentrifuge©)
3. Repeat step two five times.

Primary root:

1. Primary roots of each seedling were cut from its base, depositing three of them per Eppendorf tube.
2. Continue with the procedure described in the numerals two and three of the previous protocol for mesocotyl.
3. The osmolality of the samples was determined using a vapor pressure osmometer (Wescor VAPRO model 5600, Wescor, Inc., Logan UT USA) following the manufacturer's instructions. The measurements are obtained in mmol/Kg, therefore, for its conversion to bars, it was considered that 1OsM/Kg = - 25 bars. Values were validated using two control levels, one of 1000 mmol/Kg and the other of 290 mmol/Kg [32].