

Accelerated leaf senescence and abscission are associated with drought in nature as a means to decrease canopy size. In perennial plants, this strategy contributes to the survival and the completion of the plant life-cycle under drought. In contrast, this strategy reduces the yields of annual crops, with concomitant economical losses. We hypothesized that it is possible to enhance the tolerance of plants to water deficit by delaying the drought-induced leaf senescence and abscission during the stress episode. Our working hypothesis is that the suppression of stress-induced senescence could enable plants to develop a vigorous acclimation response that would result in enhanced drought tolerance with less yield losses. The general objective of this proposal is to identify genes with significant roles in conferring drought tolerance in rice, and the generation of drought-tolerant and water-use-efficient rice plants in different varieties and backgrounds. The proposal is divided in two phases: **(I)** in the first phase, our efforts are focused on the development of different varieties of rice plants expressing $P_{SARK}::IPT$, and testing both in the greenhouse and in the field the ability of the $P_{SARK}::IPT$ plants to sustain drought and display increased water use efficiency; and **(II)** in the second phase, the $P_{SARK}::IPT$ plants will be used to identify and characterize genes that play determinant roles in plant drought tolerance.

The specific aims of this proposal are: (1) To test the efficacy of stress-induced cytokinin synthesis in conferring drought tolerance in upland and lowland rice varieties expressing $P_{SARK}::IPT$; These tests will include physiological; biochemical and molecular biological characterizations (growth, photosynthesis, nutrient assimilation, yields, etc.) under both greenhouse and field conditions; (2) To identify and characterize genes playing significant roles in the cytokinin-dependent acquired drought tolerance and factors affecting their activity; (3) To use forward-, reverse-genetics and TILLING to assess and confirm the role of the identified genes in drought tolerance.

Experimental approach:

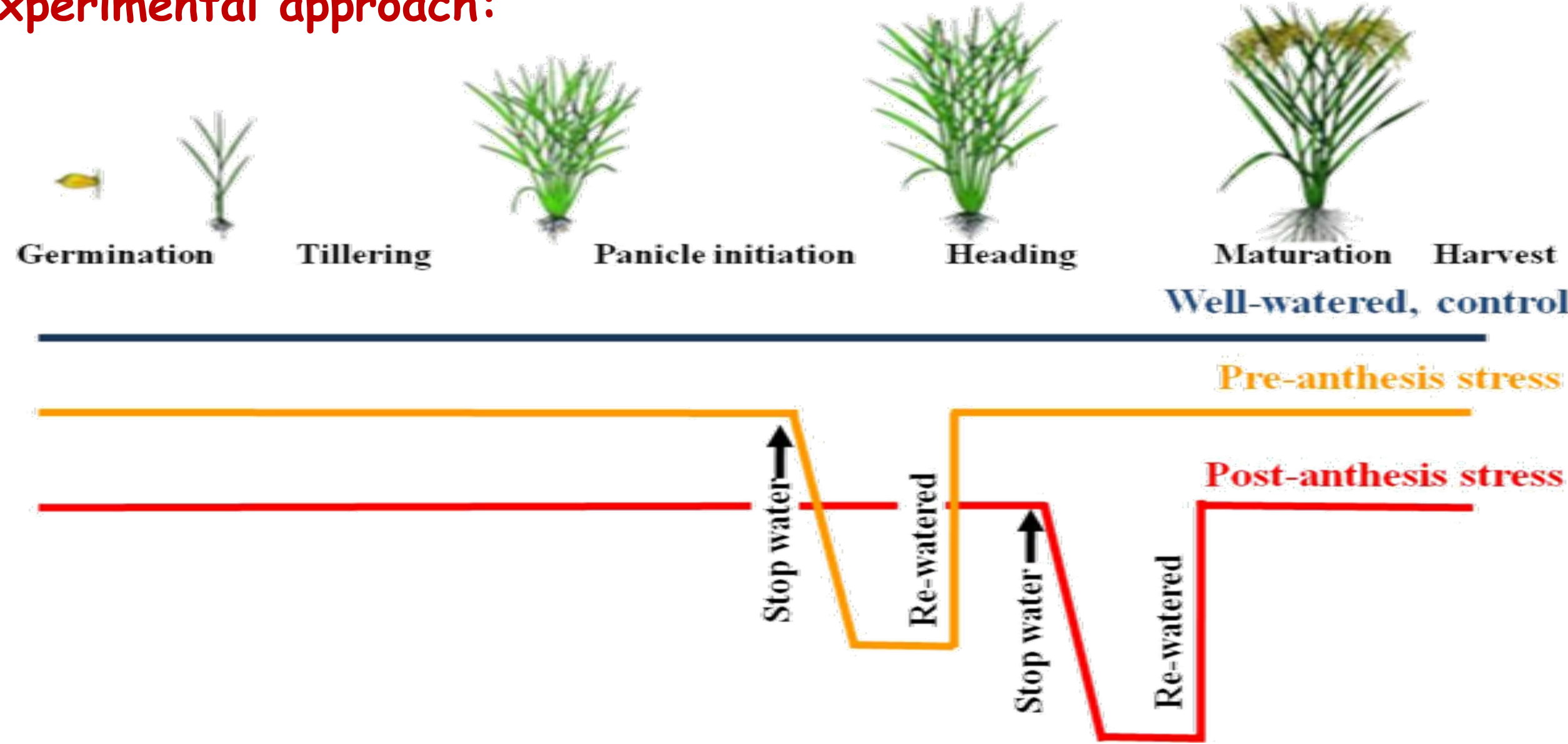


Figure 1. Water stress was applied at two developmental stages: (i) *pre-anthesis* (end of panicle initiation, booting stage), and (ii) *post-anthesis* (two weeks after panicle initiation), by slowly drying water-logged pots until visual stress symptoms (~12 days) appeared. Pots were then re-watered and plant productivity, morpho-physiology, and phenology parameters were collected from plants after maturation.

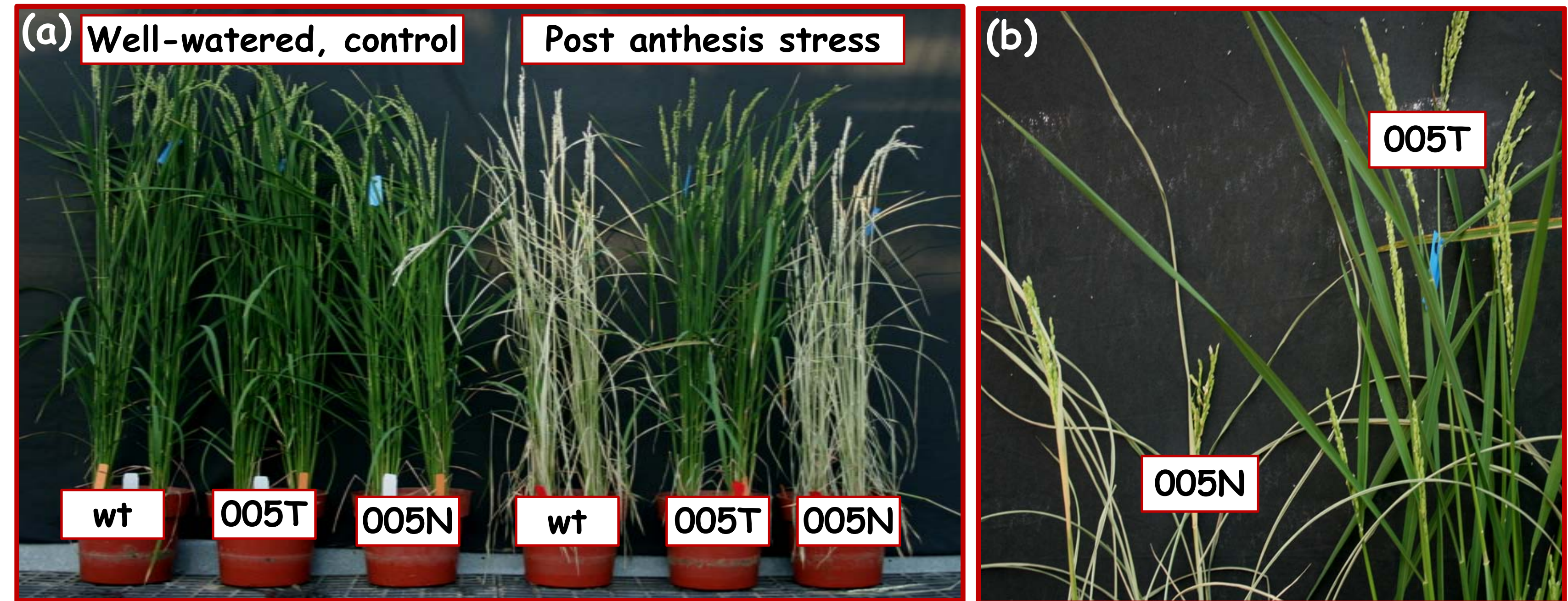


Figure 2. (A) Wild-type, null (005N) line and transgenic (005T) lines expressing $P_{SARK}::IPT$. Plants were grown under well-watered conditions (left) and were subjected to 8 days of water stress two weeks after anthesis followed by 3 days re-watering (right). (B) Effects of drought on panicles.

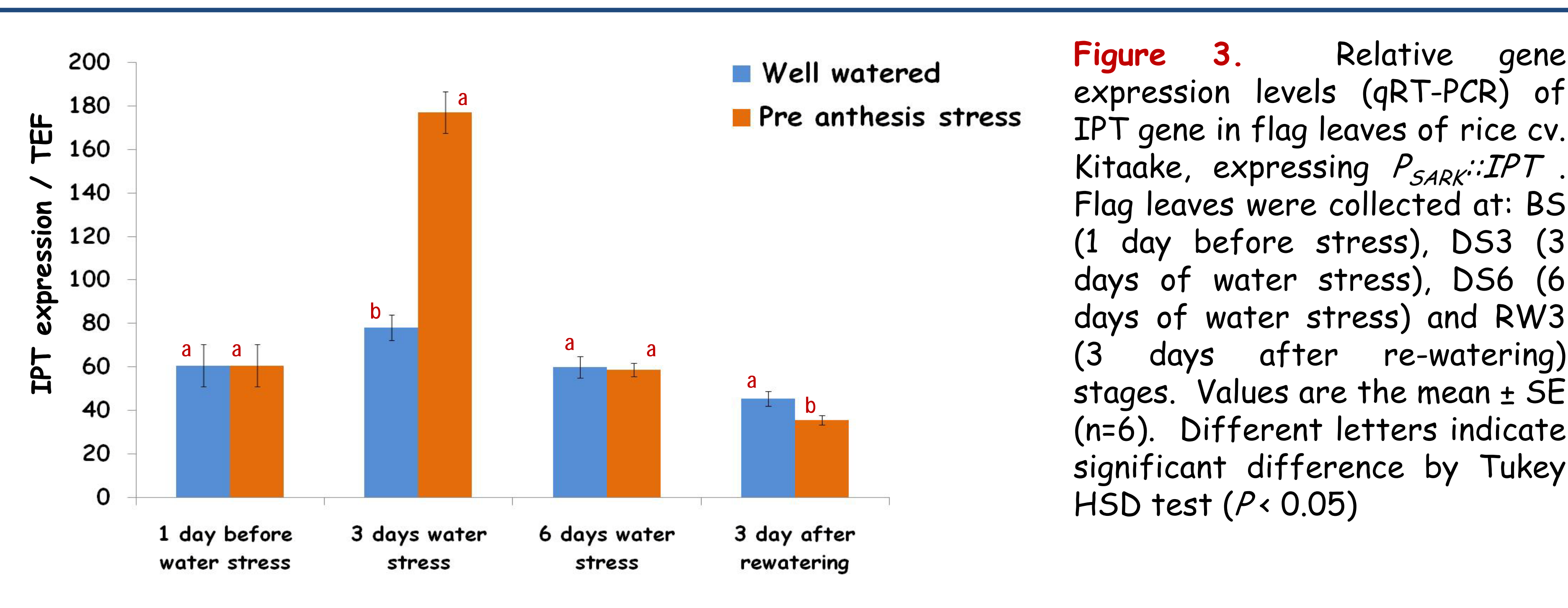


Figure 3. Relative gene expression levels (qRT-PCR) of IPT gene in flag leaves of rice cv. Kitaake, expressing $P_{SARK}::IPT$. Flag leaves were collected at: BS (1 day before stress), DS3 (3 days of water stress), DS6 (6 days of water stress) and RW3 (3 days after re-watering) stages. Values are the mean \pm SE (n=6). Different letters indicate significant difference by Tukey HSD test ($P < 0.05$).

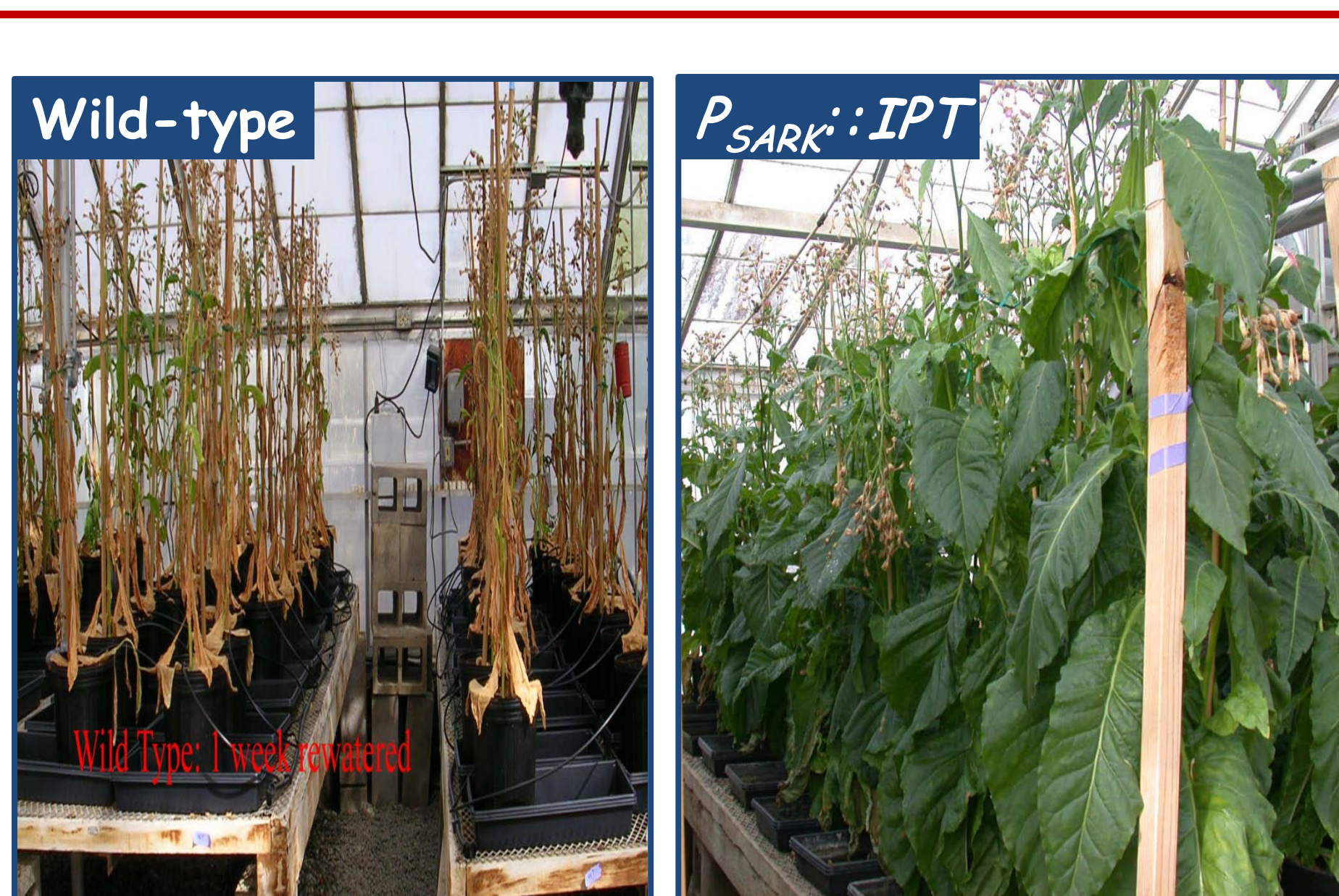


Figure 4. Wild-type and transgenic plant expressing $P_{SARK}::IPT$ after 15 days of drought followed by 7 days of re-watering.

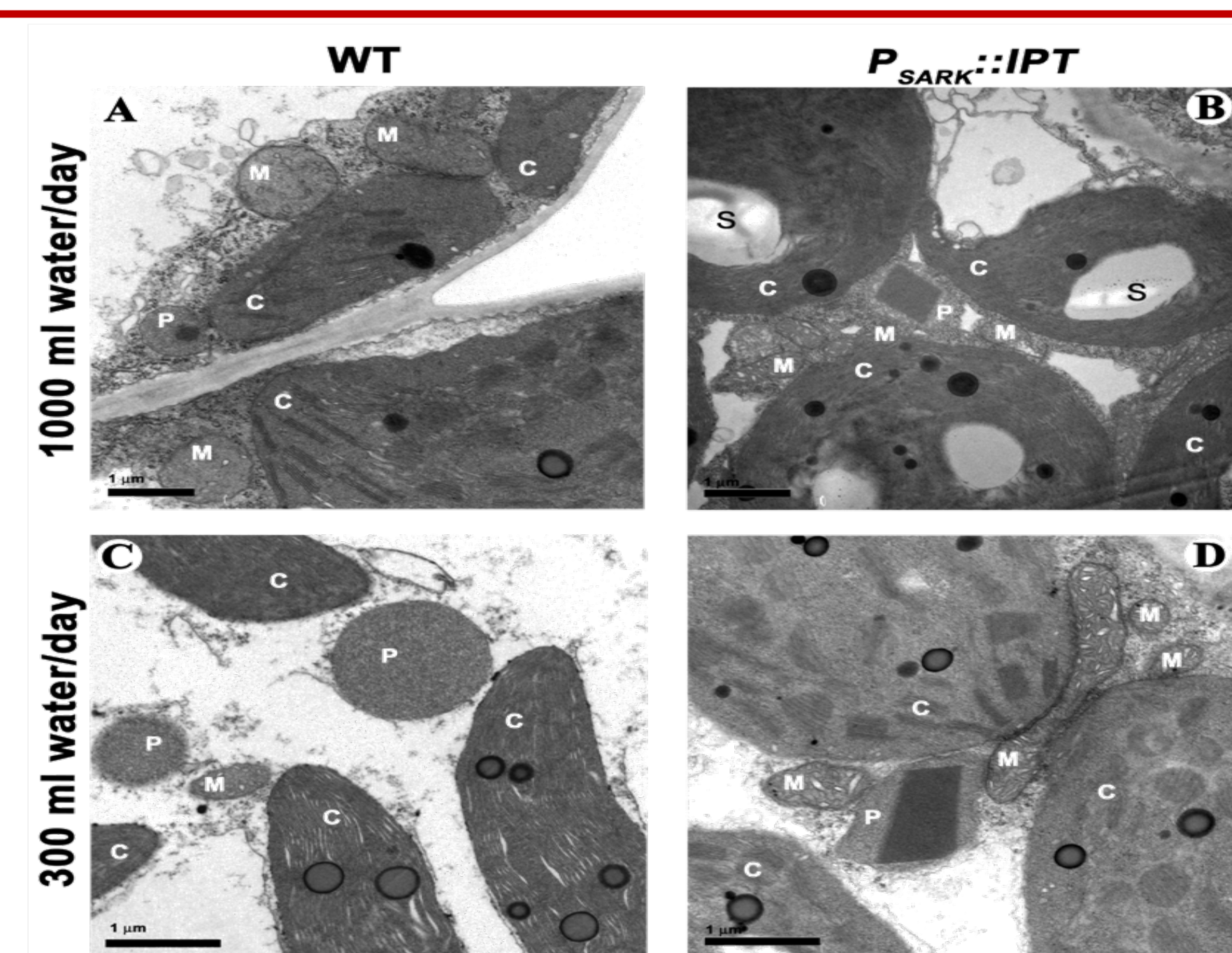


Figure 5. Electron micrographs of chloroplasts and surrounding organelles from wt (A,C) and transgenic $P_{SARK}::IPT$ plants (B,D) grown under optimal or restricted watering. C: chloroplast, M: mitochondria, P: peroxisome, S: Starch. Microphotographs were taken from leaf 8 of tobacco plants treated for 50 d. The crystalloids visible inside peroxisomes in transgenic plants (B,C) correspond to catalase. Scale bars = 1 μ m.

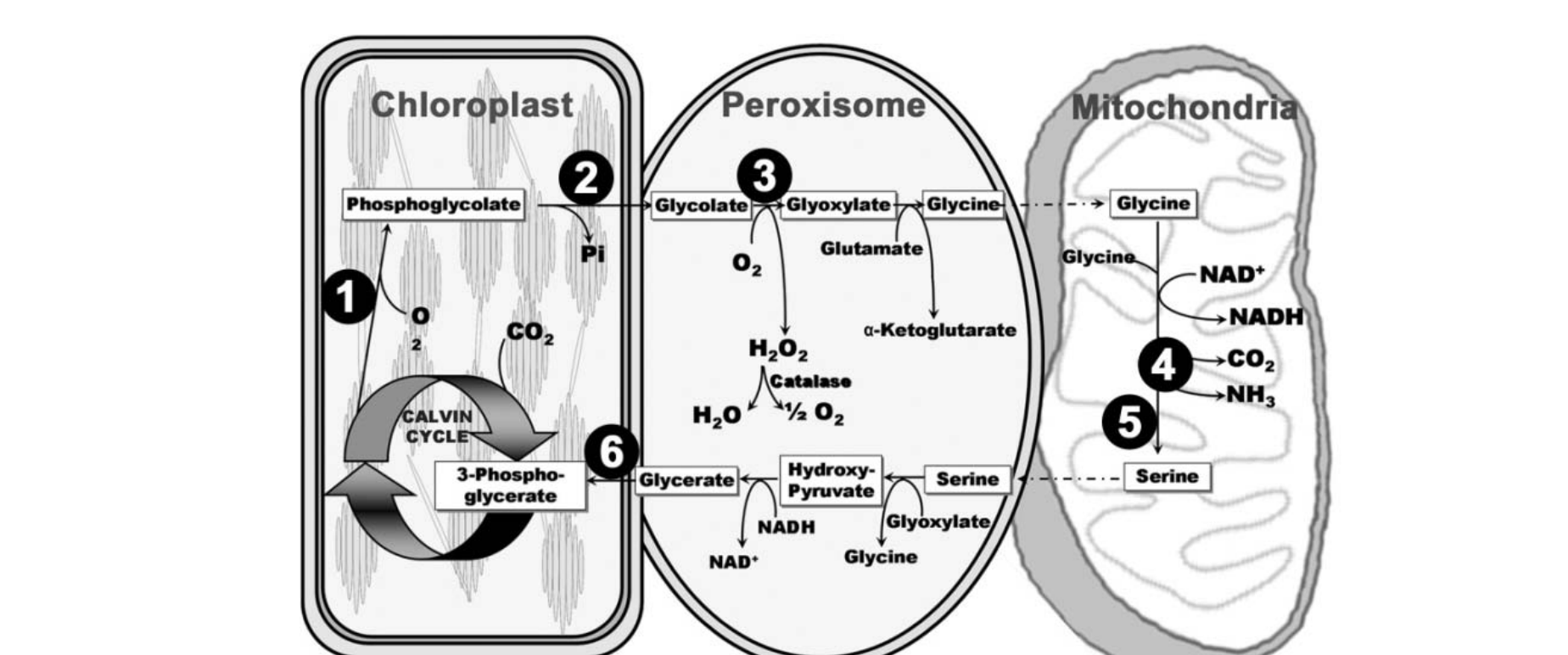


Figure 6. Scheme of the photorespiratory pathway of C_3 plants. Relative expression of selected transcripts related to photorespiration. (1) Rubisco small subunit, (2) phosphoglycolate phosphatase, (3) glycolate oxidase, (4) glycine decarboxylase, (5) serine hydroxymethyltransferase, (6) glycerate kinase. Values are the mean \pm SE (n=6). The analysis was performed using the 8th fully expanded leaf of individual plants.

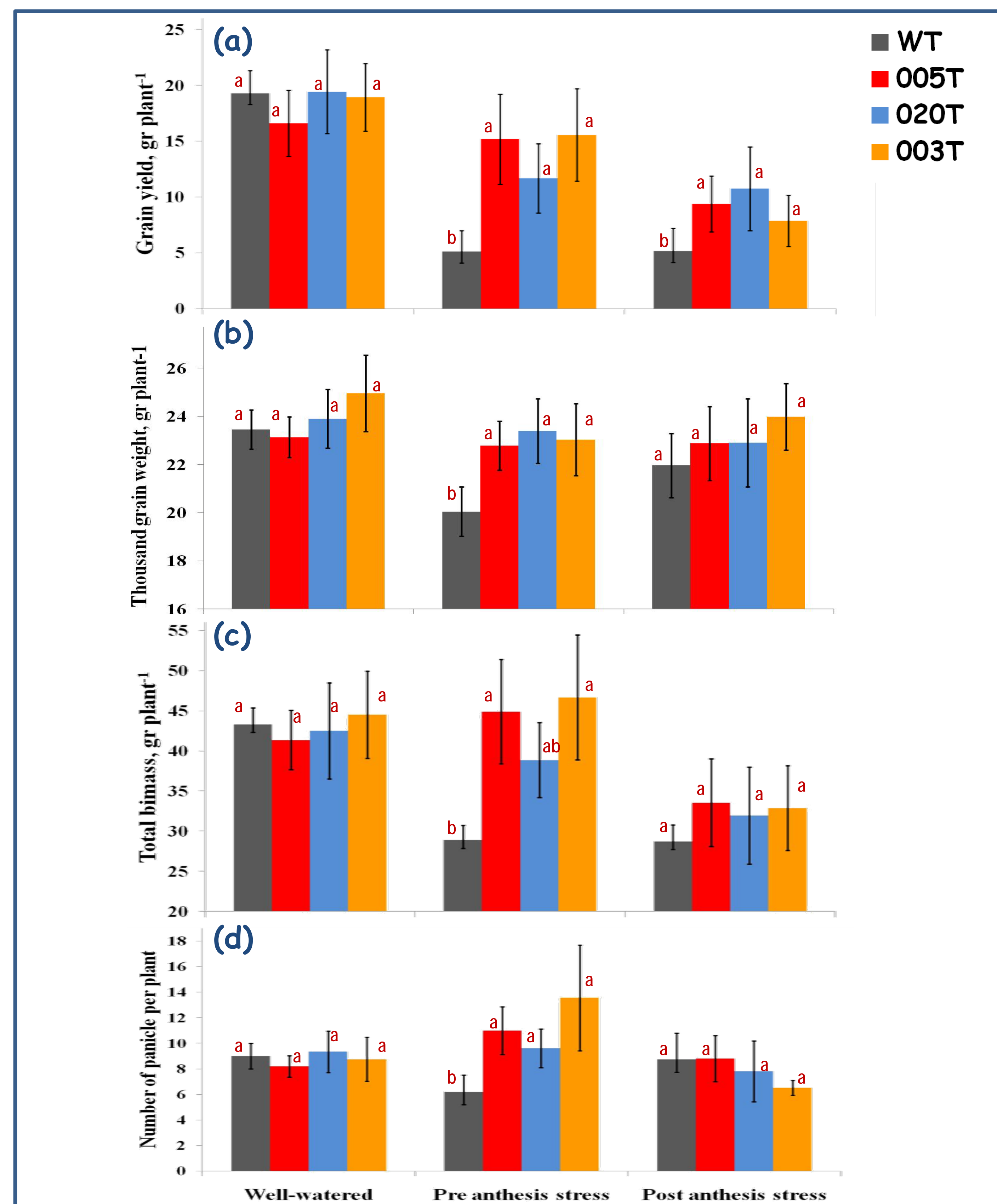
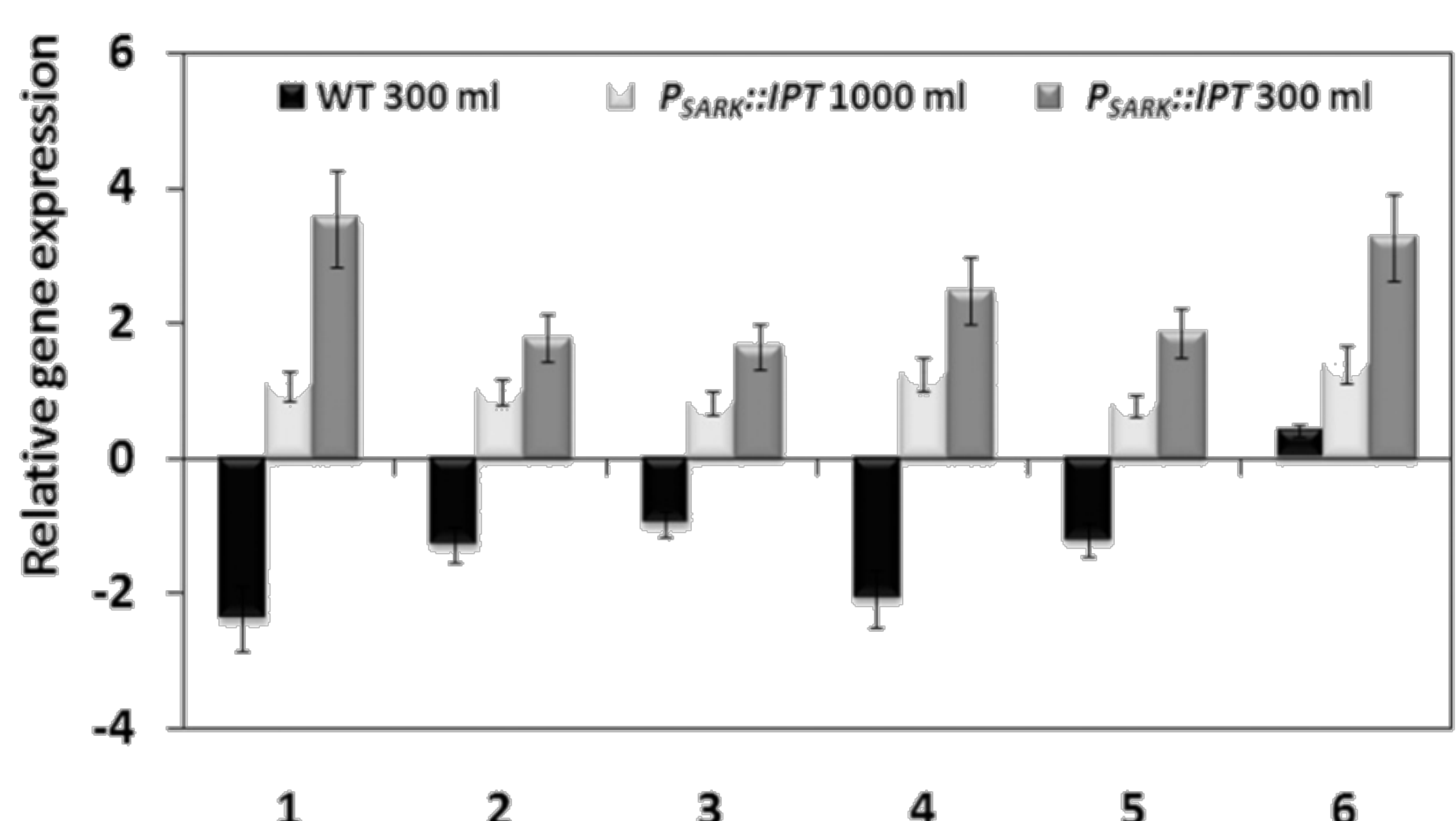


Figure 7. Phenotypal characterization of 3 transgenic lines expressing $P_{SARK}::IPT$ and wild type exposed to water stress at pre-anthesis and post-anthesis stage. (a) Grain yield per plant, (b) 1,000-grain weight, and (c) total biomass and (d) number of panicles. Values are mean \pm SD (n=6). Different letters indicate significant difference by Tukey LSD test ($P < 0.05$).

Conclusions

- Transgenic rice plant expressing $P_{SARK}::IPT$ showed delayed senescence which resulted in improved yield under water stress at the critical developmental stages.
- Under the pre-anthesis treatment, the transgenic plants produced up to 80% of grain yield (GY) and 98% of 1000-grain weight (TGW) as compared to the wt grown under well-watered conditions, whereas the wt plants showed a dramatic reduction in GY (26%) or TGW (85%).
- Our results clearly demonstrate that the delay of stress-induced senescence can be engineered into monocot crops with significant yield improvements.