

AUXIN AND CYTOKININ PROFILES IN BARLEY TWEAKY SPIKE (*tw*) MUTANTS REVEAL AN AUXIN-SPECIFIC HORMONE DISTURBANCE

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Barley pleiotropic developmental tweeky spike (*tw*) mutants exhibit abnormal flower/inflorescence structure that has been associated with disturbances in auxin (Aux) physiology. Since spike development is regulated not by individual phytohormones but by their interactions in different spike segments, changes in Aux concentration may influence the distribution of other hormones along the spike axis and, conversely, Aux gradients may arise from imbalances in other phytohormone pathways. Barley inflorescence architecture is a key determinant of grain number and quality, and developmental mutants therefore provide an effective route to identify regulatory pathways controlling spike morphogenesis. The *tw* mutants comprise a recessive allelic series with pleiotropic spikes and floret defects, consistently associated with disrupted Aux regulation. In this study, we aimed to investigate the concentrations and distribution of phytohormones: indole-3-acetic acid (IAA) and cytokinins (CKs) among *tw* and wild-type backgrounds.

IAA and CKs concentrations were compared between *tw* mutants and their corresponding wild-type backgrounds in upper and lower spike segments. Across the *tw* alleles examined, mutants consistently had lower IAA levels than their corresponding wild types. In the 'Auksiniai 3' (A3) wild type, IAA was clearly higher in the upper spike segment than in the lower segment. In *tw*₉ and *tw*₁₀ (A3 background), this gradient almost disappeared because the upper and lower segments contained very similar IAA levels. Therefore, the IAA distribution along the spike axis was much more even. In contrast, CKs profiles were largely stable at the segment level: cis-Zeatin was the main detectable CKs, while trans-Zeatin was inconsistent and isopentenyladenine/dihydrozeatin were mostly marginal, with no genotype-consistent directional change separating *tw* mutants from wild types.

Overall, the *tw* mutants show consistent Aux deficiency and disrupted IAA distribution, while CKs profiles remain broadly similar to wild types, indicating that the *tw* developmental syndrome is mainly associated with altered Aux homeostasis rather than major changes in CKs content.

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