



MECHANICAL STRESS BEHIND FLORAL BUD CLOSURE AND PROTECTION

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Abstract

Among the different floral organs in plants, sepals are considered protective in nature, as they form the outermost layer and safeguard the reproductive structures. Sepals exhibit differential growth patterns influenced by environmental cues. Recently, Trinh et al. demonstrated that microtubule rearrangement in the transverse direction, in response to mechanical stress, leads to growth arrest at the sepal tip, resulting in the formation of a sepal hook (a reversible process) that encloses the floral bud. Therefore, this study reveals a novel mechanism of stress-mediated growth arrest involved in sepal hook formation, which prevents the opening of premature flowers and also protects flowers against other possible environmental stress (e.g., osmotic stress).

Keywords: Bud Protection, Bud Closure, Growth Arrest, Mechanical Stress, Microtubular Rearrangement, Sepal Tip

Introduction

The flower is the distinguishing and reproductive structure of angiosperms. The floral axis of a flower consists of petals, sepals, stamens, and pistils. Among these, sepals and petals are accessory structures primarily involved in protection, while stamens and pistils are the true reproductive organs. The transition from the vegetative to the reproductive stage in plants is marked by the onset of flowering and is considered a key developmental milestone (Lippmann et al. 2019). Flowering is a prerequisite for the formation of fruits and seeds. Beyond contributing structure, colour, and diversity to the environment, flowers also attract pollinators, thereby facilitating seed production and protection and ensuring the transmission of genetic material to subsequent generations. Various external factors influence floral development, with the most significant being the duration of light and darkness (van Doorn and Kamdee 2014). The phenomenon of flowering in plants depends on photoperiodism. Some plants, such as sugar beet, spinach, potato, and lettuce, require long days (long light exposure), while others like rice, onion, and soybean flower under short-day conditions.

In the process of understanding floral development, researchers have observed that after the vegetative growth phase, the shoot apical meristem or axillary shoot apical meristem can transition into either a group of flowers (inflorescence) or a single flower through the action of floral meristem identity genes. Flower development is a multistep process that leads to the formation of floral organs such as sepals, petals, stamens, and carpels (van Doorn and Kamdee 2014; Bowman and Moyroud 2024).

Research on floral development has revealed that certain physical forces influence the fate of developing floral organs. With the advancement of molecular biology, it has become evident that various genes, including APETALA1/2/3, PISTILLATA, SEPALLATA1/2/3, and AGAMOUS, play critical roles in the development of floral organs (Liu et al. 2010; Gong et al. 2017). It may seem counterintuitive, but mechanical forces do play a significant role in plant growth and development. Earlier studies have shown

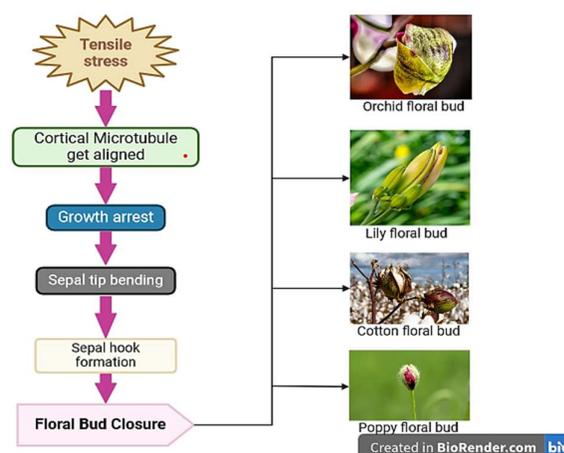
that flower development involves mechanical forces such as compression, tearing, torsion, and stretching (Hamant et al. 2008). Numerous examples support the involvement of mechanical forces in floral development. In *Lemboglossum bictoniense*, for instance, the labellum is positioned abaxially at the bottom of the flower due to torsion, which causes a 180° twist in the flower (Kauth et al. 2008). Additionally, artificial leaf primordia can be generated from meristematic tissue through bulging induced by mechanical forces (Trinh et al. 2024). In *Gossypium*, mechanical forces cause trichomes to interlock petals at overlapping regions, forming a protective mesh-like covering (Tan et al., 2016). Similarly, in *Arabidopsis*, a sepal hook is formed as a result of tensile stress at the sepal tip, enclosing the floral bud (Zhao et al. 2020). Upon further investigation, Trinh et al. (2024) reported that the cell wall at the tip of *Arabidopsis* sepals becomes stiffer, which contributes to inward bending and the formation of the sepal hook.

Involvement of tip cells in sepal curvature

The sepal is the first floral organ to develop, collectively forming the calyx of the flower (Tucker 2003). Its primary function is to protect the floral bud and support the developing petals. To investigate the properties of the sepal tip region during hook formation, Trinh et al. (2024) qualitatively examined the effects in a transgenic line, focusing on the marginal and inner portions of the sepal. They observed that the marginal cells and those in the inner regions appeared normal and were unaffected by the mutant line, except for one enlarged cell, which is likely the tip cell. Morphological observations revealed a reduction in sepal length, while the width remained unchanged. This reduction did not interfere with hook formation (Weber et al. 2015; Trinh et al. 2024). Furthermore, the authors demonstrated that the growth pattern of cells at the sepal tip determines cell fate during hook formation (Trinh et al. 2024).

Microtubules: a vital component in sepal hook formation at tip via their transverse orientation

Microtubules provide mechanical support and play a crucial role in cell division, cell expansion, and morphogenesis (Yan et al. 2023). In *Arabidopsis*, differential growth during sepal development generates transverse strain in the cortical microtubules, causing them to align at the tip and subsequently arrest growth. Trinh et al. (2024) investigated microtubules in the cortical region of the *Arabidopsis* sepal (Fujita et al. 2013) and reported that microtubules are essential for the formation of curvature or hook-like structures in the sepals. It has been observed that mutants with defects in the protein responsible for breaking down microtubules into small filaments-known as Katanin (Baral et al. 2021), lack the hook-like structure at the sepal tips. This results in the flattening of the sepal tip (Xu et al. 2024) and leads to early floral opening due to reduced growth at the tip region. To further investigate the role of cortical microtubules in sepal hook formation, Trinh and colleagues (2024) developed a transgenic line by expressing PHS1ΔP (a kinase associated with cortical microtubule depolymerization) under the WOX1 promoter. They found that the mutant lines exhibited a higher growth rate at the tip but failed to form sepal hooks. These observations indicate that the rearrangement of microtubules in the transverse direction at the sepal tip is crucial for hook formation, which in turn facilitates floral bud closure to protect the reproductive organs from environmental stresses (**Figure 1**).



Conclusion and future directions

Overall, the authors revealed that the growth pattern of tip cells and the transverse alignment of microtubules in the tip region are responsible for cell growth at the tip and the formation of the sepal hook in plants, ultimately leading to floral bud closure. The coordination between these two factors suggests that sepal hook formation is a self-regulated process, influenced primarily by changes in the growth pattern of tip cells.

To what extent external mechanical forces can help determine the fate of a growing cell remains an area that requires detailed investigation. Beyond this, questions remain: are specific signalling molecules or receptors involved in directing cortical microtubule alignment, or is this orientation governed by a plant organ's sensing mechanism? Do the neighbouring cells of sepal's support or oppose sepal alignment? Solving these questions may help in understanding the underlying molecular mechanisms of sepal hook formation, which could be important for protecting developing flowers from potential injuries.

List of abbreviations:

APETALA1/2/3, a floral homeotic gene encoding a MADS domain protein homologous to SRF transcription factors; PISTILLATA, a floral homeotic gene encoding a MADS domain transcription factor; SEPALLATA1/2/3, encodes a MADS box transcription factor involved flower and ovule development; AGAMOUS, a floral homeotic gene encoding a MADS domain transcription factor; PHS1 Δ P, a protein kinase associated with cortical microtubule depolymerization; WOX1, encodes a WUSCHEL-related homeobox gene family member with 65 amino acids in its homeodomain.

Declarations

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References

1. Baral, A., Aryal, B., Jonsson, K., Morris, E., Demes, E., Takatani, S., Verger, S., Xu, T., Bennett, M., Hamant, O., & Bhalerao, R. P. (2021). External mechanical cues reveal a katanin-independent mechanism behind auxin-mediated tissue bending in plants. *Developmental Cell*, 56(1), 67–80.
2. Bowman, J. L., & Moyroud, E. (2024). Reflections on the ABC model of flower development. *The Plant Cell*, 36(5), 1334–1357.
3. Fujita, S., Pytela, J., Hotta, T., Kato, T., Hamada, T., Akamatsu, R., Ishida, Y., Kutsuna, N., Hasezawa, S., Nomura, Y., & Nakagami, H. (2013). An atypical tubulin kinase mediates stress-induced microtubule depolymerization in *Arabidopsis*. *Current Biology*, 23(20), 1969–1978.
4. Gong, P., Ao, X., Liu, G., Cheng, F., & He, C. (2017). Duplication and whorl-specific down-regulation of the obligate AP3–PI heterodimer genes explain the origin of *Paeonia lactiflora* plants with spontaneous corolla mutation. *Plant and Cell Physiology*, 58(3), 411–425.
5. Hamant, O., Heisler, M. G., Jonsson, H., Krupinski, P., Uyttewaal, M., Bokov, P., Corson, F., Sahlin, P., Boudaoud, A., Meyerowitz, E. M., & Couder, Y. (2008). Developmental patterning by mechanical signals in *Arabidopsis*. *Science*, 322(5908), 1650–1655.

6. Kauth, P. J., Kane, M. E., & Vendrame, W. A. (2008). Asymbiotic germination response to photoperiod and nutritional media in six populations of *Calopogon tuberosus* var. *tuberosus* (Orchidaceae): Evidence for ecotypic differentiation. *Annals of Botany*, 102(5), 783–793.
7. Lippmann, R., Babben, S., Menger, A., Delker, C., & Quint, M. (2019). Development of wild and cultivated plants under global warming conditions. *Current Biology*, 29(24), 1326–1338.
8. Liu, C., Zhang, J., Zhang, N., Shan, H., Su, K., Zhang, J., Meng, Z., Kong, H., & Chen, Z. (2010). Interactions among proteins of floral MADS-box genes in basal eudicots: Implications for evolution of the regulatory network for flower development. *Molecular Biology and Evolution*, 27(7), 1598–1611.
9. Tan, J., Walford, S. A., Dennis, E. S., & Llewellyn, D. (2016). Trichomes control flower bud shape by linking together young petals. *Nature Plants*, 2(7), 1–5.
10. Trinh, D. C., Melogno, I., Martin, M., Trehin, C., Smith, R. S., & Hamant, O. (2024). *Arabidopsis* floral buds are locked through stress-induced sepal tip curving. *Nature Plants*, 10, 1258–1266.
11. Tucker, S. C. (2003). Floral development in legumes. *Plant Physiology*, 131(3), 911–926.
12. van Doorn, W. G., & Kamdee, C. (2014). Flower opening and closure: An update. *Journal of Experimental Botany*, 65(20), 5749–5757.
13. Weber, A., Braybrook, S., Huflejt, M., Mosca, G., Routier-Kierzkowska, A. L., & Smith, R. S. (2015). Measuring the mechanical properties of plant cells by combining micro-indentation with osmotic treatments. *Journal of Experimental Botany*, 66(11), 3229–3241.
14. Xu, S., He, X., Trinh, D. C., Zhang, X., Wu, X., Qiu, D., Zhou, M., Xiang, D., Roeder, A. H., Hamant, O., & Hong, L. (2024). A 3-component module maintains sepal flatness in *Arabidopsis*. *Current Biology*, 34(17), 4007–4020.
15. Yan, Y., Sun, Z., Yan, P., Wang, T., & Zhang, Y. (2023). Mechanical regulation of cortical microtubules in plant cells. *New Phytologist*, 239(5), 1609–1621.
16. Zhao, F., Du, F., Oliveri, H., Zhou, L., Ali, O., Chen, W., Feng, S., Wang, Q., Lü, S., Long, M., & Schneider, R. (2020). Microtubule-mediated wall anisotropy contributes to leaf blade flattening. *Current Biology*, 30(20), 3972–3985.

Figure Legend:

A schematic diagram of sepal tip bending and hook formation in plants induced due to mechanical stress at the time of growth and development, ultimately resulting into floral bud closure.