Understanding capitulum development:

Gerbera hybrida inflorescence meristem as an experimental system

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ABSTRACT

Inflorescences, the flower-bearing structures in plants, show enormous diversity in nature. Their architecture, in terms of number and arrangement (phyllotaxis) of flowers, play a central role in reproductive success and adaptation of plants, as well as yield in crops. We apply Compositae capitula, or flower heads, as our model system to study inflorescence development. The unique architecture of capitula, often composed of morphologically and functionally distinct types of flowers, is considered the key innovation for the evolutionary success of this largest family of flowering plants. Moreover, the arrangement of individual flowers in intersecting spirals in capitula represent an iconic example of the geometric regularity found in nature. Our aim is to explore the gene regulatory networks that control patterning of the Compositae inflorescence meristems and their subsequent development. In this brief review, we summarize the recent technological developments and tools that allow us to explore and follow meristem patterning early on – already before we can see any visual changes in them. These include visualization of capitulum development by X-ray micro-computed tomography (micro-CT), live-imaging of dissected meristems on tissue culture media, and application of laser ablation to disrupt meristem organization and to follow its re-patterning. We anticipate that these methods are applicable to distinct species to promote comparative studies and understanding of developmental diversity of capitula within Compositae.

Keywords: laser ablation, live-imaging, meristem, micro-CT

INTRODUCTION

Gerbera (*Gerbera* × *hybrida* Hort.) is a highly popular ornamental crop. Our group has explored this species since the late 1980's, first developing an *Agrobacterium*-mediated genetic transformation method in collaboration with a Finnish company motivated by the extreme hype in plant biotechnology at that time. At the beginning, we focused on understanding the regulation of anthocyanin pigmentation patterns. Only later we, as molecular biologists, fully realized the uniqueness of the system. With advanced imaging technologies combined with genomics and molecular tools, gerbera has emerged as a model for plant developmental studies expanding them beyond the conventional models (Figure 1).

Most recently, our focus has turned to meristems, the small growing points that give rise to different types of organs in plants such as leaves and flowers. Exploring meristem patterning has proven to be most informative to understand capitulum development. The gerbera inflorescence meristems (IM) initiate hundreds of florets in an iconic phyllotactic pattern forming regular left- and right-curving spirals (Zhang et al., 2021a). Intriguingly, the spiral numbers represent two consecutive numbers of the Fibonacci series. We have visualized the plant hormone auxin in transgenic gerbera using the *DR5rev*::3XVENUS-N7 reporter (Heisler et al., 2005), and shown how auxin defines the positions of future florets (see the fluorescent signals on IMs visualized by confocal and light-sheet microscopy in Figure 1) – this happens before we see the bulging florets by conventional

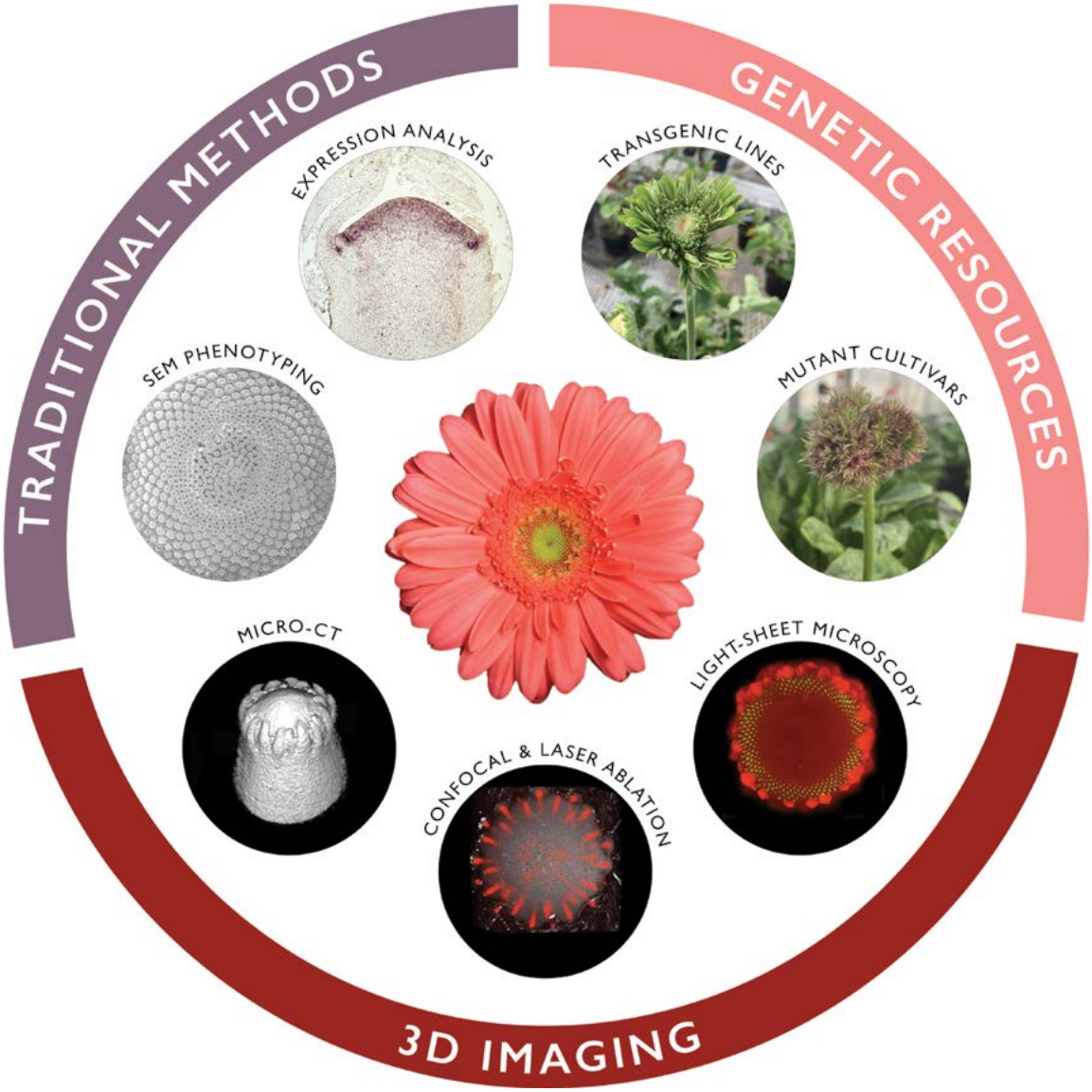

Figure 1. Available research methods and resources for studying *Gerbera* capitulum development. 3D imaging methods including micro-CT, confocal and light-sheet microscopy were recently optimized and integrated among the more conventional methods.

scanning electron microscopy (SEM). Combining the *in vivo* microscopic data with X-ray micro-computed tomography (micro-CT) based growth analysis, a 3D computational model for capitulum phyllotaxis was established in collaboration with Prof. Prusinkiewicz's team at the University of Calgary (Zhang et al., 2021a). Our recent molecular data shows that many genes regulating flower development have been recruited to regulate IM development in gerbera (Zhao et al., 2016; Zhang et al., 2017; Zhao et al., 2020). Altogether the data supports the botanical hypotheses suggesting that the capitulum may have evolved from a single, determinate meristem (Classen-Bockhoff & Bull-Hereñu, 2013). Based on these data, we propose that the giant gerbera IM provides a useful model to understand meristem patterning beyond the conventional model species.

Here, our aim is to introduce selected experimental approaches that facilitate our aims to understand capitulum development, and that potentially can be applied in other species to explore the developmental diversity in Compositae.

Gerbera: a model system

Gerbera, a well-known ornamental crop, has turned out to be a working model for plant developmental studies. The vegetatively propagated plants are easy to grow in greenhouses. They flower continuously and produce material for sampling. Genetic modification provides a key tool to discover functions of genes of interest.

Gerbera hybrida cultivar 'Terra Regina', Greenhouse at the Viikki Campus, University of Helsinki
Photo by Teng Zhang

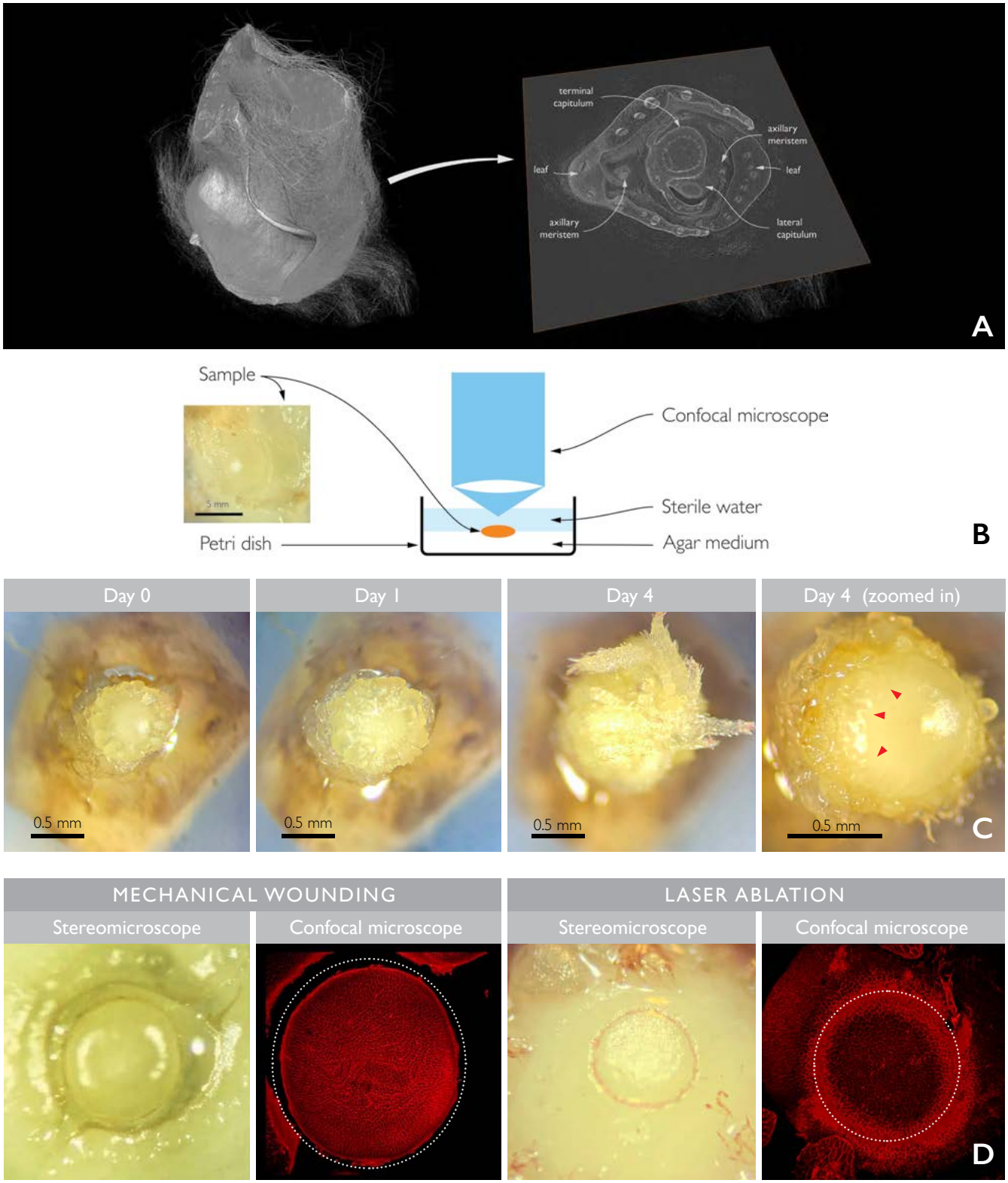

Figure 2. Micro-CT, live-imaging and wounding experiments conducted on developing capitula. **A.** Micro-CT segmentation helps to locate the developing gerbera IMs in the rosette. **B.** Sample setup for confocal microscopic imaging. The image on the left shows a sample sector that contains the shiny, dome-shaped meristem. **C.** Stereomicroscope images showing a growing *Gerbera* IM explant *in vitro*. The IM at 4-day timepoint is covered by involucral bracts and the zoom-in image shows initiating floret primordia (red arrows). **D.** Side-by-side stereomicroscope and confocal microscope images from the wounding experiments performed by mechanical wounding and by laser ablation. Cylindrical cuts are marked by the dashed line in the confocal microscope images.

TOWARDS 4D PHENOTYPING OF GERBERA CAPITULUM DEVELOPMENT

To understand the phyllotactic patterning of the gerbera capitulum, we had to first determine how to properly find and dissect the IM samples. At the earliest developmental stage, the diameter of the IM is about 300-500 µm. The IMs are hidden within the rosette leaves, below the soil, and they are covered by protective hairs (Figure 2A). We applied micro-CT scanning to follow the early capitulum growth. For micro-CT, sample fixation is done as for the regular SEM but most importantly, the structures can be targeted by X-rays without very accurate dissection of the surrounding tissues. These scans were very informative as we learned from the 3D reconstructed images to precisely locate the meristems (Figure 2A), and later to expose them under the stereomicroscope (Figure 2B) and to dissect them apart from the hairy leaf axils. Based on our experience, micro-CT scans are valuable for creating detailed 3D phenotypic data and could easily be applied to any species of interest.

Live-imaging is another key method that allows visualization of developmental events with the addition of the time component, i.e., 4D. This methodology has been widely applied to analyze the dynamic growth of the *Arabidopsis thaliana* (L.) Heynh. shoot apical meristem (Heisler & Ohno, 2014), as well as organs such as leaves (Kierzkowski et al., 2019) and stamens (Silveira et al., 2021). Live-imaging of capitula requires that they are separated from the leaf rosettes and grown on tissue culture media. For this purpose, we dissected the rosette sectors (3 × 3 mm in size) containing an intact IM (Figure 2B, C). Since the sectors were collected from the greenhouse, Plant Preservative Mixture treatment (PPM™, a kind suggestion from Dr. Siobhan Braybrook) was used to eliminate contamination during culturing. Using a modified gerbera multiplication medium, we were able to follow the growth of the IM explants *in vitro* up to two weeks (Zhang et al., 2021a). This allowed us to capture confocal microscopic stacks from the same IM explant in multiple time points, and thus the development could be rendered with cellular resolution. The live-imaging method allowed us to follow the propagation of the DR5 auxin signals from cell-to-cell during meristem growth. We discovered that the DR5 signal (representing the emerging bract initia) moves not only radially but also laterally through the cells between its two neighboring maxima in the expanding meristem. This movement was found to be the key for the emergence of Fibonacci numbers of auxin maxima during the early stages of meristem patterning (Zhang et al., 2021a).

REVISITING THE CLASSIC EXPERIMENTS ON CAPITULUM WITH NEW TECHNIQUES

We have always been fascinated by the pioneering experiments in sunflower in the 1980s to manipulate capitulum development. Among these, mechanical wounding of the capitulum resulted in *de novo* patterning from the wound margins, i.e., initiation of new bracts, ray and disc florets (Palmer & Marc, 1982; Hernandez & Palmer, 1988). After optimizing the live-imaging method for gerbera, we revisited these experiments. We conducted cylindrical cuts using a modified syringe tip and further adopted laser ablation to achieve more precise wounding of gerbera IMs (Figure 2D). By wounding of the IMs of the DR5 auxin reporter lines (Zhang et al., 2021b), our results faithfully recapitulated the wound responses previously observed in sunflower and showed conserved changes at the cellular level similar to those observed in the *Arabidopsis* IM (Caggiano et al., 2017). Besides wounding, exogenous application of hormones such as cytokinin (Hernandez, 1996) or physical compression (Hernandez & Green, 1993) can also alter capitulum development. Revisiting these experiments with modern techniques, combined with molecular level studies, could provide new insights into mechanisms of *de novo* patterning.

Despite its complexity, the large IM of gerbera provides an additional model to understand spatio-temporal patterning of meristems beyond the traditional models like Arabidopsis and tomato. The major challenge is to understand how changes in the functions of key developmental genes and their regulatory networks have led to the enormous morphological diversity of flowering plants. This requires comparative studies across multiple species – hopefully including new models of Compositae in the future.

Capitula diversity: gerbera and more

Compositae is the largest among the flowering plant families. Understanding evolution of the huge morphological diversity within this family waits for comparative genomic and molecular level studies. Such knowledge facilitates crop breeding and helps to sustain global biodiversity.

Gerbera hybrida cultivar 'Terra Regina', Greenhouse at the Viikki Campus, University of Helsinki
Photo by Paula Elomaa

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LITERATURE CITED

Caggiano, M.P., Yu, X., Bhatia, N., Larsson, A., Ram, H., Ohno, C.K., Sappl, P., Meyerowitz, E.M., Jönsson, H. & Heisler, M.G. 2017. Cell type boundaries organize plant development. *eLife* 6: 1-32.

Classen-Bockhoff, R. & Bull-Hereñu, K. 2013. Towards an ontogenic understanding of inflorescence diversity. *Ann. Bot.* 112: 1523–1542.

Heisler, M.G., Ohno, C., Das, P., Sieber, P., Reddy, G.V., Long, J.A. & Meyerowitz, E.M. 2005. Patterns of auxin transport and gene expression during primordium development revealed by live imaging of the *Arabidopsis* inflorescence meristem. *Curr. Biol.* 15: 1899–1911.

Heisler M.G. & Ohno C. 2014. Live-imaging of the *Arabidopsis* inflorescence meristem. Pp. 431–440 in: Riechmann J., Wellmer F. (eds) Flower Development. Methods in Molecular Biology (Methods and Protocols), vol 1110. Humana Press, New York, NY.

Hernandez, L.F. 1996. Morphogenesis in sunflower (*Helianthus annuus L.*) as affected by exogenous application of plant growth regulators. *AgriScientia* 13: 3–11.

Hernandez, L.F. & Palmer, J.H. 1988. Regeneration of the sunflower capitulum after cylindrical wounding of the receptacle. *Am. J. Bot.* 75:1253–1261.

Hernandez, L.F., & Green, P.B. 1993. Transductions for the expression of structural pattern: analysis in sunflower. *Plant Cell* 5:1725–1738.

Kierzkowski, D., Runions, A., Vuolo, F., Strauss, S., Lymbouridou, R., Routier-Kierzkowska, A-L., Wilson-Sánchez, D., Jenke, H., Galinha, C., Mosca, G., Zhang, Z., Canales, C., Dello Ioio, R., Huijser, P., Smith, R.S. & Tsiantis, M. 2019. A growth-based framework for leaf shape development and diversity, *Cell* 177: 1405–1418.

Palmer, J.H. & Marc, J. 1982. Wound-induced initiation of involucral bracts and florets in the developing sunflower inflorescence. *Plant Cell Physiol.* 23:1401–1409.

Silveira, S.R., Le Gloanec, C., Gómez-Felipe, A., Routier-Kierzkowska, A-L. & Kierzkowski, D. 2021. Live-imaging provides an atlas of cellular growth dynamics in the stamen. *Plant Phys.*: kiab363.

Zhang, T., Zhao, Y., Juntheikki, I., Mouhu, K., Broholm, S.K., Rijpkema, A.S,, Kins, L., Lan, T., Albert, V.A., Teeri, T.H. & Elomaa, P. 2017. Dissecting functions of *SEPALLATA* like MADS box genes in patterning of the pseudanthial inflorescence of *Gerbera hybrida*. *New Phytol.* 216: 939–954.

Zhang, T., Cieslak, M., Owens, A., Wang, F., Broholm, S.K., Teeri, T.H., Elomaa, P. & Prusinkiewicz, P. 2021a. Phyllotactic patterning of gerbera flower heads. *Proc. Nat. Acad. Sci. USA* 118: e2016304118.

Zhang, T., Wang, F. & Elomaa, P. 2021b. Repatterning of the inflorescence meristem in *Gerbera hybrida* after wounding. *J. Plant Res.* 134: 431–440.

Zhao, Y., Zhang, T., Broholm, S.K., Tähtiharju, S., Mouhu, K., Albert, V.A., Teeri, T.H. & Elomaa, P. 2016. Evolutionary co-option of floral meristem identity genes for patterning of the flower-like Asteraceae inflorescence. *Plant Phys.* 172: 284–296.

Zhao, Y., Broholm, S.K., Wang, F., Rijpkema, A.S., Lan, T., Albert, V.A., Teeri, T.H. & Elomaa, P. 2020. TCP and MADS-box transcription factor networks regulate heteromorphic flower type identity in *Gerbera hybrida*. *Plant Phys.* 184: 1455–1468.