Live imaging of developmental processes in a living meristem of *Davidia involucrata* (Nyssaceae)

Markus Jerominek1*, Kester Bull-Hereñū2,3, Melanie Arndt1 and Regine Claßen-Bockhoff1

1 Institut für Spezielle Botanik, Johannes Gutenberg-Universität, Mainz, Germany
2 Escuela de Pedagogía en Biología y Ciencias, Universidad Central de Chile, Santiago de Chile, Chile
3 Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago de Chile, Chile

**Edited by:**
Elena M. Kramer, Harvard University, USA

**Reviewed by:**
Stewart Gillmor, CINVESTAV-IPN, Mexico
Jacques Dumais, Universidad Adolfo Ibáñez, Chile

*Correspondence:* Markus Jerominek, Institut für Spezielle Botanik, Johannes Gutenberg-Universität, Anselm-Franz-von-Bentzel-Weg 9 a, Mainz, 55099, Germany
e-mail: mail@spinningspecies.com

Morphogenesis in plants is usually reconstructed by scanning electron microscopy and histology of meristematic structures. These techniques are destructive and require many samples to obtain a consecutive series of states. Unfortunately, using this methodology the absolute timing of growth and complete relative initiation of organs remain obscure. To overcome this limitation, an *in vivo* observational method based on Epi-Illumination Light Microscopy (ELM) was developed and tested with a male inflorescence meristem (floral unit) of the handkerchief tree *Davidia involucrata* Baill. (Nyssaceae). We asked whether the most basal flowers of this floral unit arise in a basipetal sequence or, alternatively, are delayed in their development. The growing meristem was observed for 30 days, the longest live observation of a meristem achieved to date. The sequence of primordium initiation indicates a later initiation of the most basal flowers and not earlier or simultaneously as SEM images could suggest. *D. involucrata* exemplarily shows that live-ELM gives new insights into developmental processes of plants. In addition to morphogenetic questions such as the transition from vegetative to reproductive meristems or the absolute timing of ontogenetic processes, this method may also help to quantify cellular growth processes in the context of molecular physiology and developmental genetics studies.

**Keywords:** live imaging, *in vivo*, morphogenesis, floral unit meristem (FU meristem), epi-illumination light microscopy (ELM), *Davidia involucrata*, Nyssaceae

**INTRODUCTION**

One of the most significant technical advances of the last decades in plant sciences is the *in vivo* observation of developmental processes. *In vivo* techniques have the great advantage that they are non-destructive and allow imaging of phenomena as they occur within the plant body (Grandjean et al., 2004; Sijacic and Liu, 2010; Hiroi et al., 2013). Several innovations in light microscopy and fluorescence labeling technologies have offered amazing insights into developmental processes in meristematic tissues (Campilho et al., 2006; Reddy, 2008).

While these approaches primarily address gene expression or hormone flux issues (Grandjean et al., 2004; Heisler et al., 2005; Vernoux et al., 2011), traditional imaging techniques such as histology, scanning electron microscopy (SEM), epifluorescence light microscopy (ELM) and computer tomography (CT, Staedler et al., 2013) have succeeded in providing clear information regarding morphogenesis at the tissue level. Unfortunately, these techniques are normally destructive and necessarily imply the observation of many individuals in different developmental stages to reconstruct ontogenetic sequences. Thus, this approach demands some interpretation, since the same developing structure is not being observed among different samples.

Particularly, this can become a complex issue when reconstructing the development of numerically variable structures, e.g., condensed inflorescences known as “floral units” (Claßen-Bockhoff and Bull-Hereñu, 2013). In floral units (FU), flower primordia usually fractionate in either a centripetal (e.g., umbels in Apiaceae, (Bull-Hereñu and Claßen-Bockhoff, 2010)) or centrifugal sequence (e.g., cyathia in *Euphorbia*, (Prenner and Rudall, 2007)) (Figure 1A). If primordia appear almost simultaneously the sequence is interpreted from the arrangement and size of flower primordia, assuming that the smaller ones have been initiated later (Figure 1A). A direct size-age correlation is used because of the *a priori* assumption that all flower primordia share similar growth rates. However, simultaneous initiation of primordia but a slower growth rate in the basal primordia would create a false impression of centrifugal initiation (Figure 1B).

This is a case where traditional imaging techniques find their interpretational limit when trying to elucidate which ontogenetic process is actually occurring.

The floral unit of *Davidia involucrata* Baill. (Nyssaceae, Figure 1F) illustrates this conflict. During development, a number of flower primordia arise almost simultaneously on the FU meristem (Figure 1C); those in the equatorial zone are larger than the most basal ones (Figures 1D,E). As stated above it remains unclear whether the basal flower primordia in *D. involucrata*...