Developmental Biology 419 (2016) 114–120

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

Live confocal imaging of Arabidopsis flower buds

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ARTICLE INFO

Article history:
Received 1 February 2016
Received in revised form
11 March 2016
Accepted 14 March 2016
Available online 15 March 2016

Keywords:
Flower
Flower development
Flower meristem
Plant development
Confocal microscopy
Live confocal imaging
Sepals
Floral organs

ABSTRACT

Recent advances in confocal microscopy, coupled with the development of numerous fluorescent reporters, provide us with a powerful tool to study the development of plants. Live confocal imaging has been used extensively to further our understanding of the mechanisms underlying the formation of roots, shoots and leaves. However, it has not been widely applied to flowers, partly because of specific challenges associated with the imaging of flower buds. Here, we describe how to prepare and grow shoot apices of Arabidopsis *in vitro*, to perform both single-point and time-lapse imaging of live, developing flower buds with either an upright or an inverted confocal microscope.

1. Introduction

Formation of the underground and aerial tissues of flowering plants is primarily post-embryonic. New tissues and organs are continuously produced by meristems, which are groups of undifferentiated cells with a subpopulation of stem cells, situated at the tip of the roots and the shoots. All of the structures that grow below ground in the plant derive from the root apical meristem (RAM), while all of the structures that grow above ground derive from the shoot apical meristem (SAM). During vegetative growth, the SAM generates leaves on it flanks, while during the reproductive phase, it produces flower meristems (FMs), which develop into flowers. The FM, like the SAM, consists of a group of undifferentiated cells, with stem cells at its center. However, unlike the SAM, which produces lateral organs one at a time, the FM generates 16 floral organs with four different identities (four sepals, four petals, six stamens and two fused carpels), in a partially synchronous manner, before stem cells cease to be maintained (Smyth et al., 1990). Thus, the FM is a much more crowded space than the SAM, in which multiple developmental programs take place simultaneously, only separated by narrow boundaries.

Many key developmental regulators are expressed in only a subset of cells at a particular time in development. For decades, the spatial and temporal expression patterns of developmental genes were determined by techniques such as in situ hybridization and analysis of transgenic plants that contain fusions with promoters driving the expression of non-fluorescent reporters. While these techniques have allowed for considerable progress in our understanding of the mechanisms controlling the development of plants, they generally lack cellular resolution, and do not allow for the easy characterization of the expression of several genes in the same biological samples. These techniques can also only be applied to dead and fixed samples, which limit our capacity to fully grasp the dynamic processes of development. The recent advances in laser scanning confocal microscopy of live samples, combined with the development of multiple fluorescent reporters, provide us with a formidable tool to overcome these restrictions, as they give us the possibility to monitor the expression of multiple genes, in live tissues, with a fine cellular resolution. Live confocal imaging has been extensively used to further our understanding of the mechanisms underlying root and shoot growth, but with the exception of a few studies (*e.g.* Chandler et al., 2011; Roeder et al., 2010; Urbanus et al., 2009), it has not been widely applied to the study of flower development.

While the RAM is easily accessible, confocal imaging of the SAM or the FMs during reproductive growth requires the prior removal of siliques and older flowers to uncover the SAM and the youngest flower buds. Therefore, imaging the SAM and the FMs involves a similar procedure. However, imaging developing flower buds presents specific challenges, notably the presence of the sepals,

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http://dx.doi.org/10.1016/j.ydbio.2016.03.018
0012-1606/

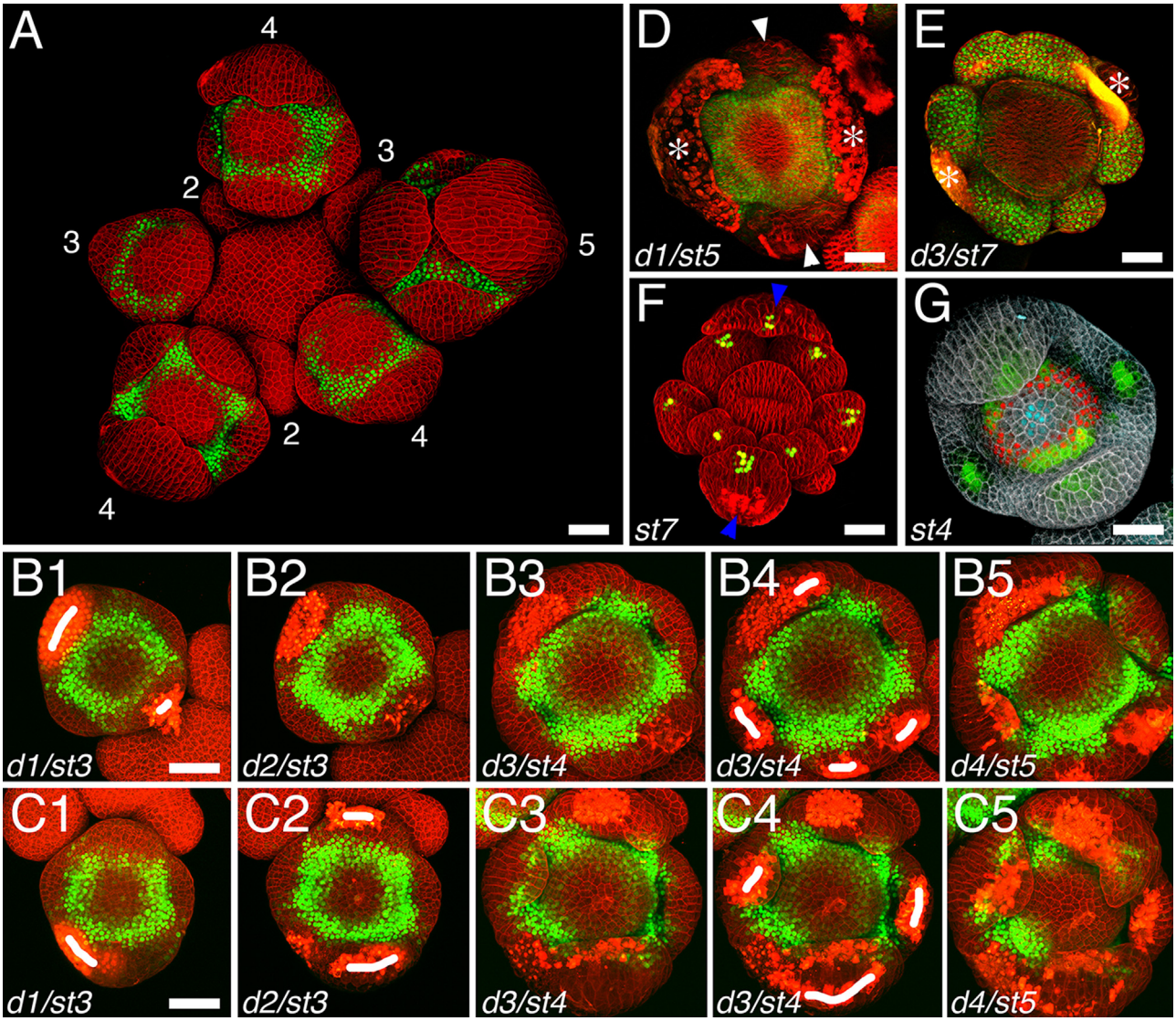

Fig. 1. Maximum intensity projections of confocal z-stacks of live flower buds. (A–E) flowers expressing a *Venus* reporter for the *APETALA3* gene (green); cell walls were stained with propidium iodide (red). (A) Inflorescence; numbers indicate floral stages; sepals in stage 4 and 5 flowers filter out the fluorescence of the *Venus* reporter, which normally forms a ring. Note that some flower buds appear tilted compared to the inflorescence. (B1–B5) 4-day time-lapse of an individual flower bud from stage 3 to stage 5; laser ablations (marked as white traits) performed on day 1 and day 3 were sufficient to prevent the sepals from covering the center of the flower bud at stage 5. (C1–C5) 4-day time-lapse of an individual flower bud from stage 3 to stage 5; laser ablations performed on day 1, 2 and 3 were insufficient to prevent the sepals from covering the center of the flower bud. (D–E) Individual flower bud after manual removal of the abaxial and adaxial sepals (D), and of all sepals (E); white arrowheads indicate remaining sepals; white asterisks indicate scars resulting from the removal of the sepals. (F) stage 7 *ap1-1* flower expressing a *DR5-3xVenusN7* reporter (green; (Vernoux et al., 2011)); plasma membranes were stained with FM4-64 (red); blue arrowheads indicate the leaf-like structures that replace sepals and do not cover the flower bud. (G) Stage 4 flower buds expressing fluorescent a *GFP* reporter for *DORNROSCHEN-LIKE* (green; (Chandler et al., 2011)), a *Venus* reporter for *SUPERMAN* (red) and a *dsRed* reporter for *CLAVATA3* (cyan; (Zhou et al., 2015)); cells walls were stained with propidium iodide (grey). d: day; st: stage. Bars = 25 μm.

which quickly grow to cover the FM and filter out the fluorescence from underlying tissues (Fig. 1A). Here, we explain how to prepare and grow shoot apices *in vitro* for both one-time and time-lapse confocal imaging of live flower buds, using either an upright or an inverted microscope.

2. Material

Tweezers (*e.g.* Dumont #5). Before use, sharpen the tweezers using a sharpening stone (*e.g.* Arkansas Sharpening Stone, Translucent, Grobet USA) and a drop of oil. Making the tweezers blade-like rather than pointed allows for better leverage on the flower buds to be removed.

Pin vise with straight stainless steel needles for dissecting sepals.

P10 and P1000 pipettes with appropriate tips.

Tissue paper (*e.g.* Kimwipes, Kimtech).

MS plates (1 × Murashige and Skoog basal salt mixture without vitamins, 0.8% agar, pH 5.8 with potassium hydroxide solution) for seed germination.

Dissecting dishes. Round dishes approximately 6 cm wide and 2 cm deep (*e.g.* plastic box, round, RD2, Electron Microscopy Science), filled with approximately 0.5 cm of 1% agarose.

Imaging dishes. For imaging with an upright confocal microscope, use a plastic box with a transparent lid (*e.g.* rectangular hinged boxes, 2-7/8" long, 2" wide, 1-1/4" deep, Durphy Packaging Co.), filled with 0.5 cm of imaging medium. For imaging with an inverted confocal microscope, use a small Petri dish (*e.g.* easy grip Petri dish, polystyrene, 3.5 cm wide, 1 cm deep, Falcon), filled exactly to the brim with imaging medium.

Imaging medium. For one-time imaging, use 1% agarose. For