Chapter 2

Strigolactone-mediated Stimulation of Secondary Xylem Proliferation in Stems

Javier Agusti

Abstract

Secondary xylem (wood) accounts for a large proportion of the terrestrial biomass. Understanding how secondary xylem develops and proliferates is a challenge to enhance our capacities for biomass production. Recent reports revealed that the plant hormone strigolactone is key for the development of secondary xylem. Here, I describe a protocol for strigolactone-mediated stimulation of secondary xylem proliferation in stems. The protocol has been tested in Arabidopsis and Eucalyptus and can be adjusted to other species.

Key words Strigolactone, Cambium, Secondary xylem, Lanolin, Stem, Arabidopsis, Eucalyptus

1 Introduction

The application of hormonal treatments has been instrumental over decades to enhance biomass production in agriculture. Understanding the precise effect of hormones on specific developmental processes and/or at specific developmental stages allows for establishing new treatments aimed at maximizing biomass yields.

Secondary xylem (wood) is the most abundant biological tissue in the planet. Auxin has long been known to be pivotal in secondary xylem proliferation by initiating the activity of the vascular cambium (*see* **Note 1**). It has been recently discovered that auxin-mediated cambium activity initiation requires strigolactone (SL) signaling and that, indeed, strigolactone alone is sufficient to initiate such cambial activity and to, consequently, stimulate secondary xylem proliferation [1].

Evidences indicate that lack of SL leads to enhanced branching [2] while SL accumulation leads to secondary xylem proliferation [1]. Given that branching and secondary xylem development are two processes resulting in large biomass accumulation, it has been suggested that plants regulate the type of biomass that they generate by regulating the amount of SL that they produce [3]. Therefore, altering the accumulation patterns of strigolactone in specific parts

Miguel de Lucas and J. Peter Etchells (eds.), *Xylem: Methods and Protocols*, Methods in Molecular Biology, vol. 1544,
DOI 10.1007/978-1-4939-6722-3_2,

of the plant could result in the alteration of biomass accumulation patterns. For example, strigolactone treatments in tree stems could lead to large accumulation of biomass in the form of wood. As a proof of principle of such hypothesis, stems of Arabidopsis plants treated with SL displayed enhanced secondary xylem accumulation [1]. This effect is conserved in trees and, therefore, SL treatments have been suggested as a method to improve our capacities for biomass production in the form of secondary xylem/wood [1]. An exemplar method to induce secondary xylem formation in Arabidopsis and Eucalyptus shoots through SL treatments is described in the next sections. The method can be adapted to other species.

2 Materials

2.1 Reagents

1. GR24: a strigolactone synthetic analog for which this protocol is optimized. When in powder, GR24 must be kept at room temperature protected from light. GR24 stock solutions must be prepared in acetone (see procedure below) and stored at −20 °C until used to minimize evaporation (*see* **Note 2**).
2. Acetone: used to (1) dissolve GR24 and (2) generate a liquid solution for stem treatments.
3. Tween 20: wet agent for liquid applications of GR24 (*see* **Note 3**).
4. Pen: to mark the treatment zone on the stem.
5. Paintbrush: to perform GR24 liquid applications.
6. Lanolin: a solid carrier for GR24 treatment (*see* **Note 4**).
7. Spatula (or similar tool): to deposit the GR24 on stems when using lanolin as a carrier.
8. Weighing boats: to (1) weight GR24 on a precision balance and (2) use as a support for mixing lanolin with GR24.
9. Soft tissue: to remove lanolin from the stems.
10. Razor blade: to collect treated stems for further analyses.
11. Paraformaldehyde (PFA): to generate a fixative for collected samples (for further microscopy analyses).
12. Microtome: to section samples for analyses.
13. Slides: to mount sectioned samples.
14. Toluidine blue: to stain sectioned samples.
15. Coverslips: to cover stained samples on slides.
16. Microscope: to observe/analyze samples.

2.2 Solutions

2.2.1 Preparing GR24 Stock Solution

1. Using a precision balance, weight sufficient GR24 to generate a stock solution with a final concentration between 5 and 10 mM.
2. Rapidly, solve the GR24 in the required volume of acetone to reach the desired concentration (at room temperature) and store the solution immediately at −20 °C.

2.2.2 Preparing GR24 Liquid Solution

1. Dilute GR24 from stock solution to the desired concentration (typically between 1–10 μM; *see* **Note 5**) in a 0.5% acetone and 0.1% Tween 20 solution.
2. A 0.5% acetone and 0.1% Tween 20 solution should be used as mock control.

2.3 Preparing GR24 Treatments Using Lanolin as Solid Carrier

1. *GR24 in lanolin*:
   (a) Heat lanolin (usually contained in a glass bottle) in a microwave until melted to liquid state.
   (b) Pipette 1 ml and transfer to a weighing boat.
   (c) Let lanolin cool down.
   (d) Before lanolin completely solidifies, add the appropriate volume of GR24 from the stock solution to reach the desired concentration (typically between 1–10 μM).
   (e) Mix well and proceed immediately to treat plants.
2. *Mock*:
   (a) Heat lanolin (as described in **step 1(a)**).
   (b) Pipette 1 ml and transfer to a weighing boat.
   (c) Let lanolin cool down.
   (d) Before lanolin completely solidifies, add the appropriate amount of acetone (exactly the same volume as the GR24 stock solution volume used in **step 1(d)**).

3 Method

All procedures should be carried out at room temperature unless otherwise stated.

3.1 Plant Material and Growth Conditions

All plants should grow in long day conditions (16 h light, 8 h dark) at 21 °C and with standard light. For both *Arabidopsis* and *Eucalyptus*, plants were treated when they reached a stature of 5–10 cm (*see* **Note 6**).

3.2 Strigolactone Treatments

Perform treatments on stems as described below. Treatments should be repeated every second day during 21 days (*see* **Note 7**), always in (exactly) the same position within the stem (Fig. 1). In

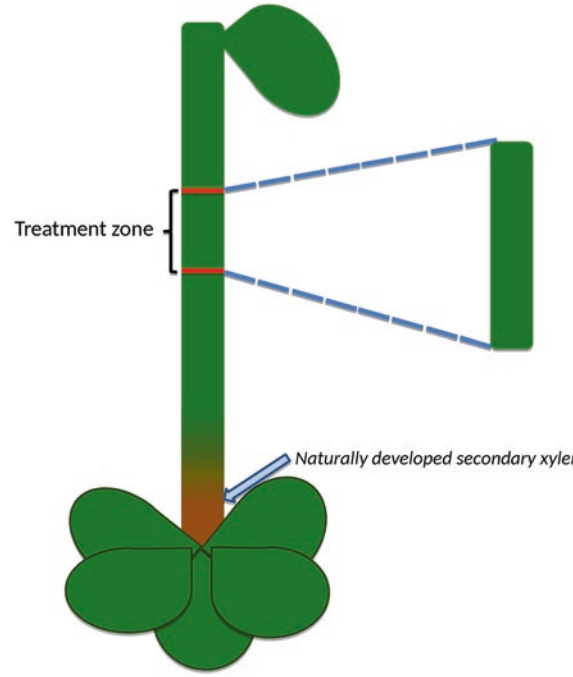

Fig. 1 Strigolactone treatment on Arabidopsis stem. *Red stripes* mark the treatment zone within the stem. The zone where secondary xylem develops in a natural manner is marked in *brown*. In Arabidopsis stems such zone comprises typically ~1 cm from the base of the stem in adult plants. Treated zones must be collected using a razor blade for further analyses

order to ensure that the treated zone in the stem is always exactly the same, it is recommendable to previously mark such zone using a regular marker. The treatment zone must be located in a position where secondary xylem does not develop in a natural manner (Fig. 1). GR24 can be applied in a liquid solution or using lanolin as a carrier. Both types of treatments work equally well in *Arabidopsis* and *Eucalyptus*. However, if working with other species it is recommendable to determine whether it is more convenient to apply GR24 in a liquid solution or with lanolin (depending on the nature of the species and on its stem morphology).

Treatments with GR24 in Liquid Solution

1. Determine the treatment position within the stem and mark it with the help of a pen (as described in Fig. 1).
2. Generate new, fresh liquid treatment solutions (0.5% acetone and 0.1% Tween 20 in water) before each treatment.
3. Separate an aliquot for mock control and an aliquot for GR24 treatment. Add the required amount of GR24 (from the stock solution) to the latter to reach the desired concentration (*see* **Note 5**).

4. Mix well and proceed immediately to treat the predetermined treatment zone of the stem (see **step 1** of this subheading) by literally "painting" it with the liquid solution, with the help of a paintbrush.
5. Perform mock control treatments in the same way (using the mock control solution). To avoid GR24 contaminations in the mock treatments, always use a different paintbrush to the one used for GR24 treatments.
6. Repeat GR24 and mock control treatments every second day, always in the exact same position of the stem, for 21 days.

Treatments with GR24 in Lanolin

1. Determine the treatment zone within the stem, as described in Fig. 1.
2. Apply the GR24 mixed in lanolin (generated as described in Subheading 2.2) on the treatment zone using a spatula.
3. Perform mock treatments exactly in the same manner.
4. Repeat GR24 and mock control treatments every second day, always in the exact same position of the stem, for 21 days. Before repeating each treatment, lanolin from the previous treatment must be gently removed using soft tissue.

3.3 Tissue Harvesting and Histological Analyses

Before harvesting, prepare a fresh aqueous solution of 4% paraformaldehyde (PFA) to be used as fixative (*see* **Note 8**).

1. Collect the treated area of the GR24 and mock treated plants with a razor blade (Fig. 1) and transfer the samples immediately to the fixative.
2. Fix samples in 4% PFA by (1) vacuum infiltration (15 min) and (2) overnight incubation at 4 °C.
3. Store samples at 4 °C until used (still within the fixative solution).
4. Process samples for wax embedding as described [4, 5].
5. Section samples using a microtome, mount them on water on slides, and leave at 42 °C overnight.
6. For best observation, staining with toluidine blue (0.05%) is recommended, following previously described protocols [4, 5].
7. Cover slides with coverslips and mounting medium and observe slides in a microscope using bright field and white light. Compare the anatomy of the mock and GR24 treated samples.

4 Notes

1. The vascular cambium is the meristem that gives rise to the secondary vascular tissues (i.e., secondary xylem and secondary phloem).
2. Acetone evaporates very quickly at room temperature; this is the reason why stock solutions should remain at −20 °C (acetone remains liquid at this temperature). However, even at −20 °C acetone experiences some evaporation. This is a problem because it can result in changes in the actual concentration of the stock solution. To solve this problem, it is recommended to check regularly the volume of the stock solution and to keep a strict track of the amount of stock solution used. Replace acetone if needed to keep the right concentration.
3. For this protocol we used Tween 20 as wet agent. However, other wet agents can be used without altering the effect.
4. Lanolin is an inert form of fat that has been extensively used as solid carrier for local application of specific chemicals and/or hormones in specific organs or spatial locations in plants. Lanolin alone has no effect on plants.
5. A GR24 1–10 μM concentrations is the best concentration range for xylem proliferation in Arabidopsis and Eucalyptus. However, the optimal concentration to be used might be different in other species.
6. GR24 treatment tends to work better in young plants. In Arabidopsis and Eucalyptus, the treatment yielded best results when applied on plants that were 5–10 cm tall.
7. Shorter treatments did not induce cambium activity initiation or xylem proliferation. It has been suggested that GR24 may be difficult to be taken up by epidermal cells in the stem. Therefore, treatments need to be prolonged in time.
8. Other fixatives (i.e., 70% EtOH) can be used.

References

1. Agusti J, Herold S, Schwarz M, Sanchez P, Ljung K, Dun EA, Brewer PB, Beveridge CA, Sieberer T, Sehr EM, Greb T (2011) Strigolactone signaling is required for auxin-dependent stimulation of secondary growth in plants. Proc Natl Acad Sci U S A 108(50):20242–20247
2. Gomez-Roldan V, Fermas S, Brewer PB, Puech-Pages V, Dun EA, Pillot JP, Letisse F, Matusova R, Danoun S, Portais J-C, Bowmeester H, Becard G, Beveridge CA, Rameau C, Rochange SF (2008) Strigolactone inhibition of shoot branching. Nature 455:189–195
3. Agusti J, Greb T (2013) Going with the wind—adaptive dynamics of secondary meristems in plants. Mech Dev 130(1):34–44
4. Agusti J, Lichtenberger R, Schwarz M, Nehlin L, Greb T (2011) Characterization of transcriptome remodelling during cambium formation identifies *MOL1* and *RUL1* as opposing regulators of secondary growth. PLoS Genet 7(2):e1001312. doi:10.1371/journal.pgen.1001312
5. Sehr EM, Agusti J, Lehner R, Farmer EE, Schwarz M, Greb T (2010) Analysis of secondary growth in the Arabidopsis shoot reveals a positive role of jasmonate signaling in cambium formation. Plant J 63:811–822

http://www.springer.com/978-1-4939-6720-9

Xylem
Methods and Protocols
Lucas, M. de; Etchells, P. (Eds.)
2017, X, 262 p. 50 illus., 37 illus. in color., Hardcover
ISBN: 978-1-4939-6720-9
A product of Humana Press