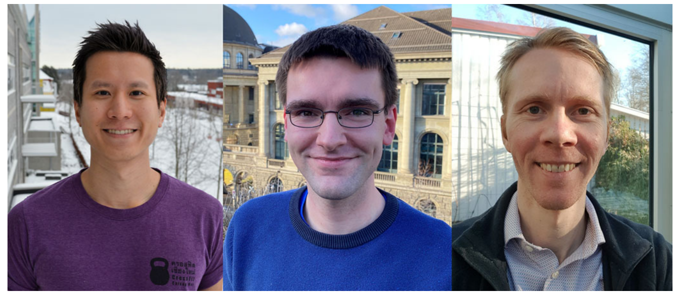
© 2022. Published by The Company of Biologists Ltd | Development (2022) 149, dev200650. doi:10.1242/dev.200650

INTERVIEW

The people behind the papers – Phanu Serivichyaswat, Kai Bartusch and Charles Melnyk

Although botanists and horticulturalists often use warm nurseries to increase graft success, little was known about the role of temperature in promoting wound healing and tissue regeneration. Now, a new paper in Development describes the molecular mechanism behind the temperature-dependent enhancement of grafting. We caught up with first authors Phanu Serivichyaswat and Kai Bartusch and corresponding author Charles Melnyk, Assistant Professor at Swedish University of Agricultural Sciences (SLU) in Uppsala, Sweden, to find out more about their research.



L-R: Phanu Serivichyaswat, Kai Bartusch and Charles Melnyk

Charles, can you give us your scientific biography and the questions your lab is trying to answer?

CM: Ever since I was young, I've had a strong interest in plants and joined the local field naturalist society before studying biochemistry at the University of Ottawa and plant sciences at the University of Cambridge. In Cambridge, I used grafting to look at the long-distance movement of RNAs and realised that, although grafting was being widely used in science and horticulture, we had little idea of how plants actually grafted. I took up a Junior Research Fellowship at Clare College in Cambridge to address this question. Later, I got a group leader position in Sweden, where my group now focuses on better understanding grafting in various plant species including *Arabidopsis*, conifers, monocots and nightshade family members. More recently, I've become interested in parasitic plants and trying to figure out how these plants can connect their vasculature to distantly related hosts.

Phanu and Kai, how did you come to work on this project and what drives your research today?

PS: When I was studying how changes in ambient temperatures affect flowering time with Ji Hoon Ahn at Korea University, I became inspired to study how temperature controls development of life forms, especially plants. When I met up with Charles for my PhD interview, I knew I wanted to further extend my interest in temperature sensing, so I developed a research proposal involving temperature and tissue regeneration. I thought it was a perfect marriage between the two exciting biological questions. Charles was supportive of the idea from the beginning and has provided me with the tools to work with grafting. Kai joined us during his internship, and the three of us started working together on this project. As for what drives my research, I am excited to develop a deeper understanding of the natural world, particularly through plant developmental biology.

KB: In parallel to my agricultural studies at Halle University, I was working as a student assistant in Marcel Quint's lab where I was trying to implement different grafting techniques to study temperature signalling pathways in plants. While optimising and developing new grafting procedures, I became more and more interested in the process of grafting itself, and which factors influence graft formation. When Charles visited Halle (Saale) in 2018, we started to discuss different aspects of grafting, and this led to my research internship in his group, where I started working on how temperature affects graft formation together with Phanu. When I was back in Halle, I continued this fascinating project in Marcel's lab as part of my Master's thesis. Eventually, this project triggered my passion for the plant vasculature, so I decided to pursue my PhD in the interdisciplinary field of plant vascular development and function at ETH Zürich.

What was known about the role of temperature in grafting success before your work?

CM: The external environment, including temperature, humidity and physical support, plays a really important role in wound healing and grafting success. People have been grafting *Arabidopsis* for years, and it's been known for some time that elevated temperatures make a difference, but no one really knew why or how this mechanism operated. People have observed the effect of temperature on graft success in quite a few species, so we knew it was important and worth trying to figure out using *Arabidopsis*.

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Can you give us the key results of the paper in a paragraph?

PS, KB & CM: The main finding of this work provides an explanation for how temperature can influence plant tissue regeneration. Using *Arabidopsis* as a model, our investigation revealed that warm temperatures increase PIF4 levels and activate YUC-mediated auxin production in the leaves. Using different grafting techniques and genotype combinations for the leaves, stem and rootstocks, we were able to see that, although auxin is generally required for a successful grafting, an extra boost of the

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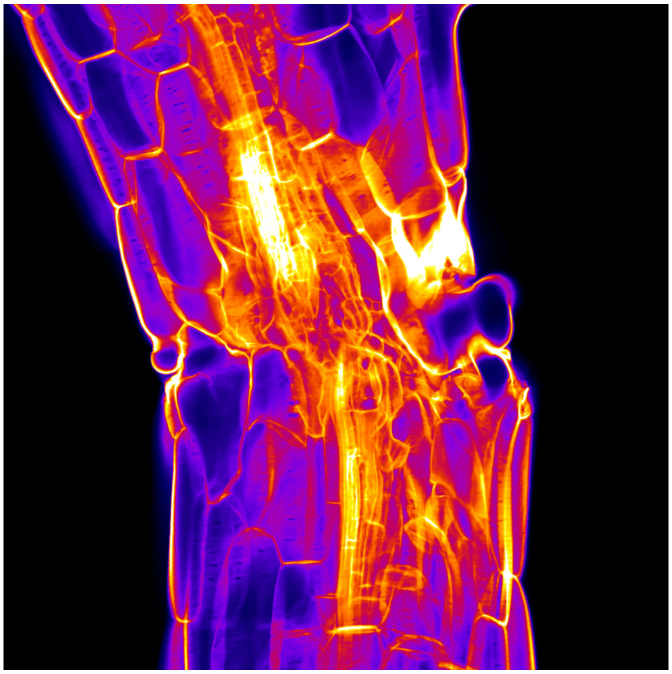

Optical cross section of an *Arabidopsis* hypocotyl graft junction showing regenerating vasculatures between cut tissues.

hormone levels in the leaves followed by transport to the graft junction was responsible for the induction effect at warm temperatures. We also found that parasitic plant infection, a regeneration process similar to grafting, was also enhanced by warm temperatures in a similar manner. Our findings suggest a conserved thermo-responsive auxin-dependent signalling pathway in grafting and plant parasitism.

When doing the research, did you have any particular result or eureka moment that has stuck with you?

PS: It was when I took confocal images of the graft junction of the auxin responsive reporter *pDR5::GFP* plants and detected much brighter fluorescent signals from the plants grafted at warm temperatures (see Fig. 3B in the article). It was far from the most technically challenging experiment of the project, but it was the first visual evidence confirming our hypothesis for the involvement of auxin in the thermo-induced graft regeneration. I knew then that we were on the right track.

KB: At the very beginning of the project, we had various hypotheses as to which pathways or factors might control temperature-enhanced graft formation. I remember one eureka moment when I was grafting different temperature signalling mutants in consecutive rounds in Charles' lab. At some point, I grafted the *pifQ* mutant and observed its grafting behaviour under the stereomicroscope. Immediately, I got the feeling 'that's the one' as I saw no difference between the temperature treatments. Later, it turned out that this was the first mutant we found showing no temperature-enhanced graft formation but exhibiting normal grafting dynamics at later time points. With this result, we knew that PIF4 is a central regulator of temperature-enhanced grafting.

And what about the flipside: any moments of frustration or despair?

PS: There was an evening at the lab when I mismanaged a grafting experiment, which meant I missed the last bus home. It was too cold and too far to walk (Swedish winter), and I did not have any food. I eventually had a hot chocolate from the coffee machine for my dinner and went to sleep in one of the nap rooms. Being stuck in the lab at night without food was quite a literal experience of despair.

KB: There was a phase of the project when I was testing whether auxin is involved in the thermo-induced graft formation using pharmacological treatments. Multiple attempts failed and I was not far from giving up this line of research. Fortunately, this frustration turned to success when I finally found a suitable way of how, where and when to align NPA plasters (which inhibit auxin transport) on petioles to reveal the role of leaf-derived auxin in grafting (see Fig. 3A in the article).

Is auxin required for graft regeneration at lower temperatures, and if so, what is the source of the auxin?

PS, KB & CM: Yes, we think that auxin is most definitely required for successful grafting. One challenge of working with auxin is that it is impossible to completely remove, or the plant will die. In our report, we could nicely show that inhibiting auxin transport or auxin production from the embryonic leaves blocked the temperature effects, but we know that auxin can also be produced in developing leaves and is also likely produced locally at the site of grafting. We haven't yet succeeded in disconnecting the role of auxin production in grafting at ambient temperatures from its role in keeping the plant alive, but we do know that blocking auxin response locally at the graft junction can prevent successful healing at both high and low temperatures.

Do you have any favourite candidates for the temperature enhancement of callus formation and tissue adhesion? Do you think that these processes will also involve long-range signalling?

PS, KB & CM: We were surprised to see that callus formation and tissue adhesion were not affected by the long-distance auxin signal. We think auxin is likely to be very important for callus and tissue attachment but that, at elevated temperatures, auxin is probably coming from additional sources. Perhaps auxin is locally derived or coming from the developing leaves. Another idea is that cell wall damage during cutting activates callus formation and tissue adhesion. In either case, temperature enhancement of callus and adhesion is probably a much more local response.

What's next for you after this paper?

PS: The results presented in this paper not only describe the thermo-responsive mechanism but have also given us a clue on the biology of grafting. However, much is still left to learn about the developmental process of graft regeneration. With my current project, I am trying to figure out the cellular and molecular processes behind graft development.

KB: While working on vascular regeneration for this paper and diving deep into the relevant literature, I realised that the underlying fundamental process of how plant cells differentiate into functional vasculature is not well understood. The major goals for my current PhD project are to identify new factors involved in phloem differentiation and to reveal their importance in phloem functionality and plant growth.

Where will this story take your lab next?

CM: The effects we saw with temperature on grafting and parasitic plant infections were really striking. I'd love to see whether we can

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use elevated temperature to improve grafting in other species including monocots and conifers. I'm also really interested to know why the effect of temperature in the leaves was so specific to vascular healing and why it didn't speed up healing more generally.

Finally, let's move outside the lab – what do you like to do in your spare time?

CM: Gardening is a big stress relief for me and, depending on the season, I go running, road biking or cross-country skiing.

PS: Besides constantly competing for my cat's attention, I enjoy attending techno music events.

KB: Ranging from hiking in the Swiss Alps to learning new languages, I enjoy lots of activities with friends and family.

Reference

Serivichyaswat, P. T., Bartusch, N., Leso, M., Musseau, C., Iwase, A., Chen, Y., Sugimoto, K., Quint, M. and Melnyk, C. W. (2022). High temperature perception in leaves promotes vascular regeneration and graft formation in distant tissues. *Development* **149**, dev200079. doi:10.1242/dev.200079