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Quantification and immunolocalization of auxin in *Prunus dulcis* (Mill.) D. A. Webb micrografts [†]

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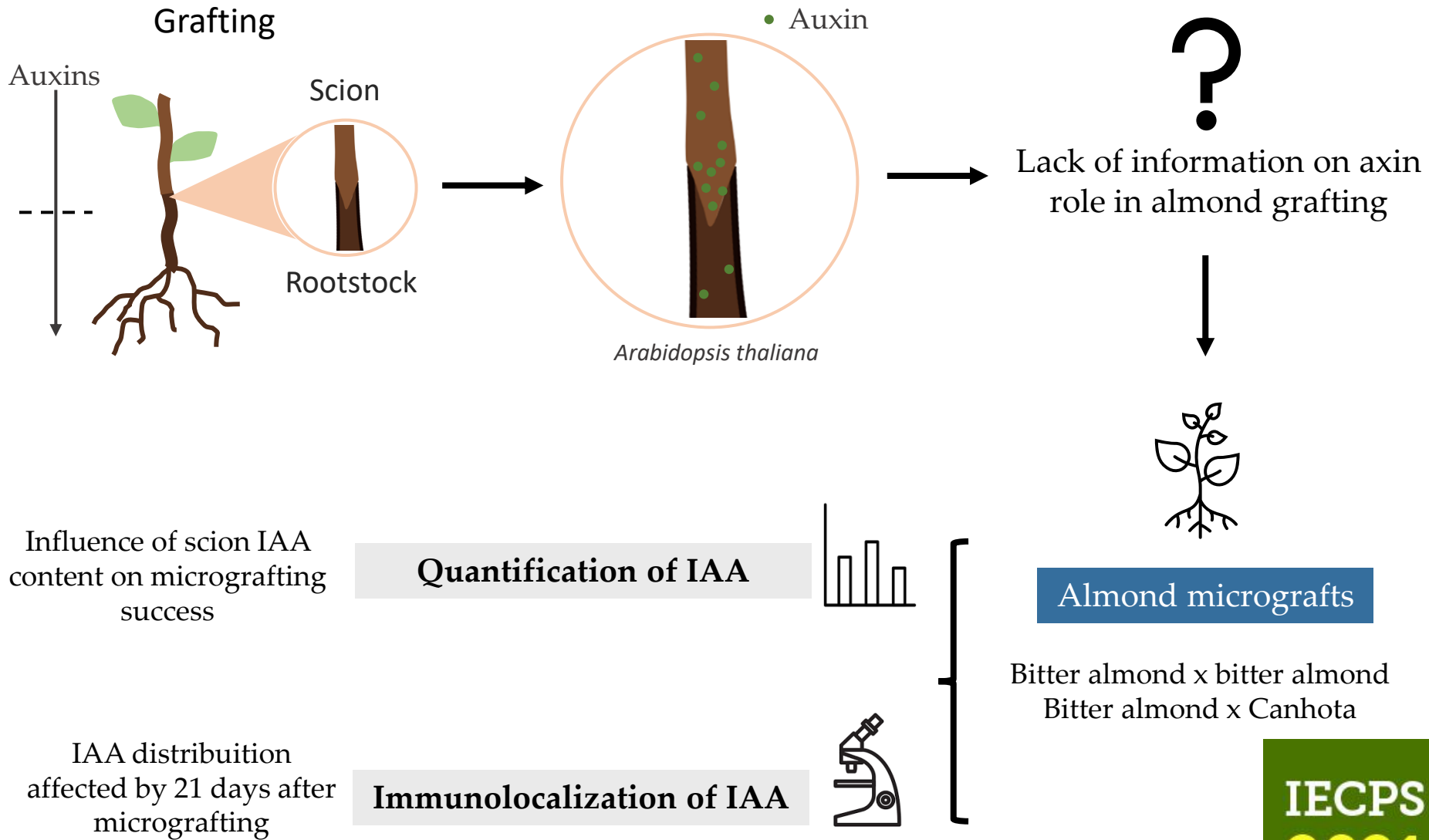
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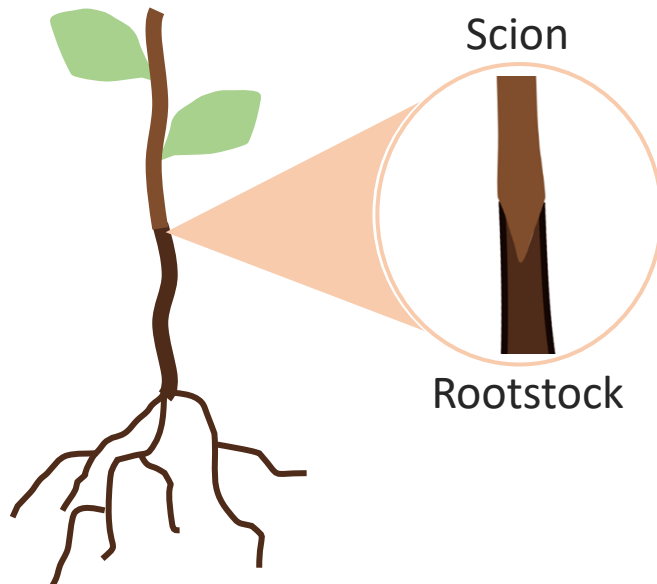
Abstract: In recent years almond production has increased due to the strong tendency of consumers towards plant-based products. The grafting propagation method is the most widely used for this *Prunus* species to increase production and fruit quality. During grafting, the plant vasculature is severely damaged thus affecting auxin transport. However, once the scion and the rootstock join, a complex process involving cell division and differentiation of vascular tissues establishes communication between the two parts. Nevertheless, wound healing and auxin-induced regulatory mechanisms involved in scion-rootstock interactions remain largely unknown. Thus, this work aimed to quantify and immunolocalize IAA (indole-3-acetic acid) in almond trees micrografts, before and 21 days after micrografting, an *in vitro* technique that allows a rapid graft union formation using *in vitro* established scions and rootstocks.

To achieve this goal, scions and rootstocks were *in vitro* established and micropropagated. Micrografts were successfully achieved from bitter almond homografts and bitter almond rootstocks x Canhota (Portuguese traditional variety). IAA quantification performed through Ehrlich reaction, showed a scion content of 1.292 ± 0.448 μg IAA/ mg FW (Canhota) and 5.505 ± 1.179 μg IAA/ mg FW (bitter almond) before micrografting, and the potential influence of these levels on micrograft success. Through IAA immunolocalization a possible accumulation at the graft union in the scion part was observed. The results obtained here are a step forward to the understanding of how scion and rootstock communicate in different almond tree micrograft combinations and how their communication is associated with graft success.

Keywords: Almond, Auxins, Immunohistochemistry, Indole-3-acetic acid, Micrografts

Introduction

Grafting is one of the main *Prunus dulcis* (Mill.) D. A. Webb propagation methods



Graft union formation

Ruptured cells collapse

Tissue adhesion

Cell dedifferentiation and division -
Callus formation

Regeneration of vascular tissues

[3]

Auxins are reported to be involved in the development of successful graft unions.

[5]



Auxin accumulation in the grafted zone is followed by cell differentiation and vascular reconnection between scion and rootstock.

[6]

Introduction

Objective

Evaluate auxin role in grafting of *P. dulcis*



Quantification and immunolocalization of endogenous indole-3-acetic acid (IAA)



Almond micrografts

Bitter almond x bitter almond
Bitter almond x Canhota

Results

Micrografting

Scion



Stock

MS + 3% w/v sucrose

21 days after micrografting



Scale bar, 1 cm

Bitter almond x bitter almond
60% success



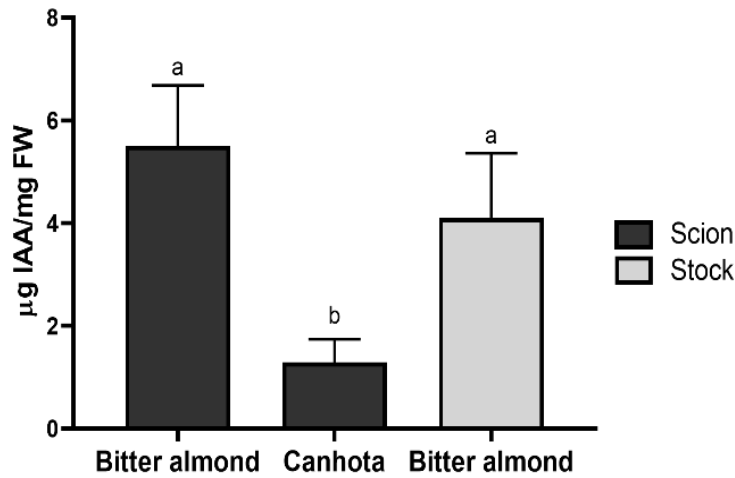
Scale bar, 1 cm

Bitter almond x Canhota
90% success

Results and discussion

IAA quantification

Cut but ungrafted scions and rootstock

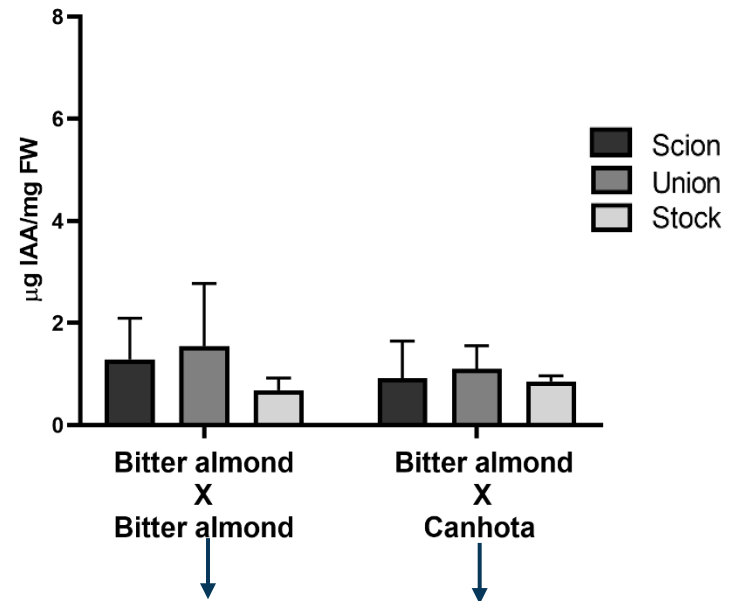


Scions used for micrografting with the same rootstock

Diferent micrografting success rates

Potential influence of initial scion IAA content on micrografting success

21 days after establishment



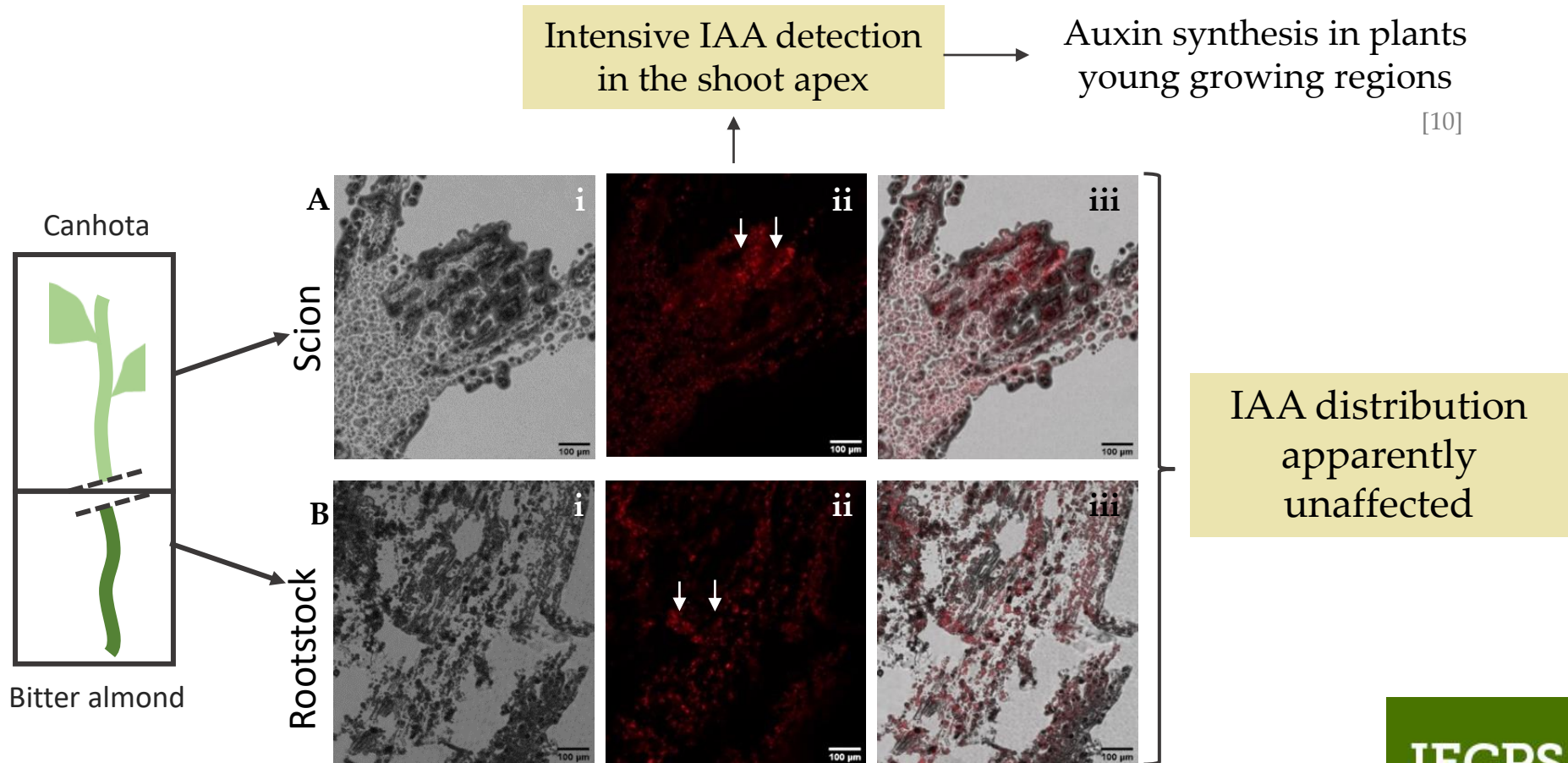
Tendency for IAA accumulation at the graft union

Role of auxins in graft compatibility and vascular regeneration

[6,7]

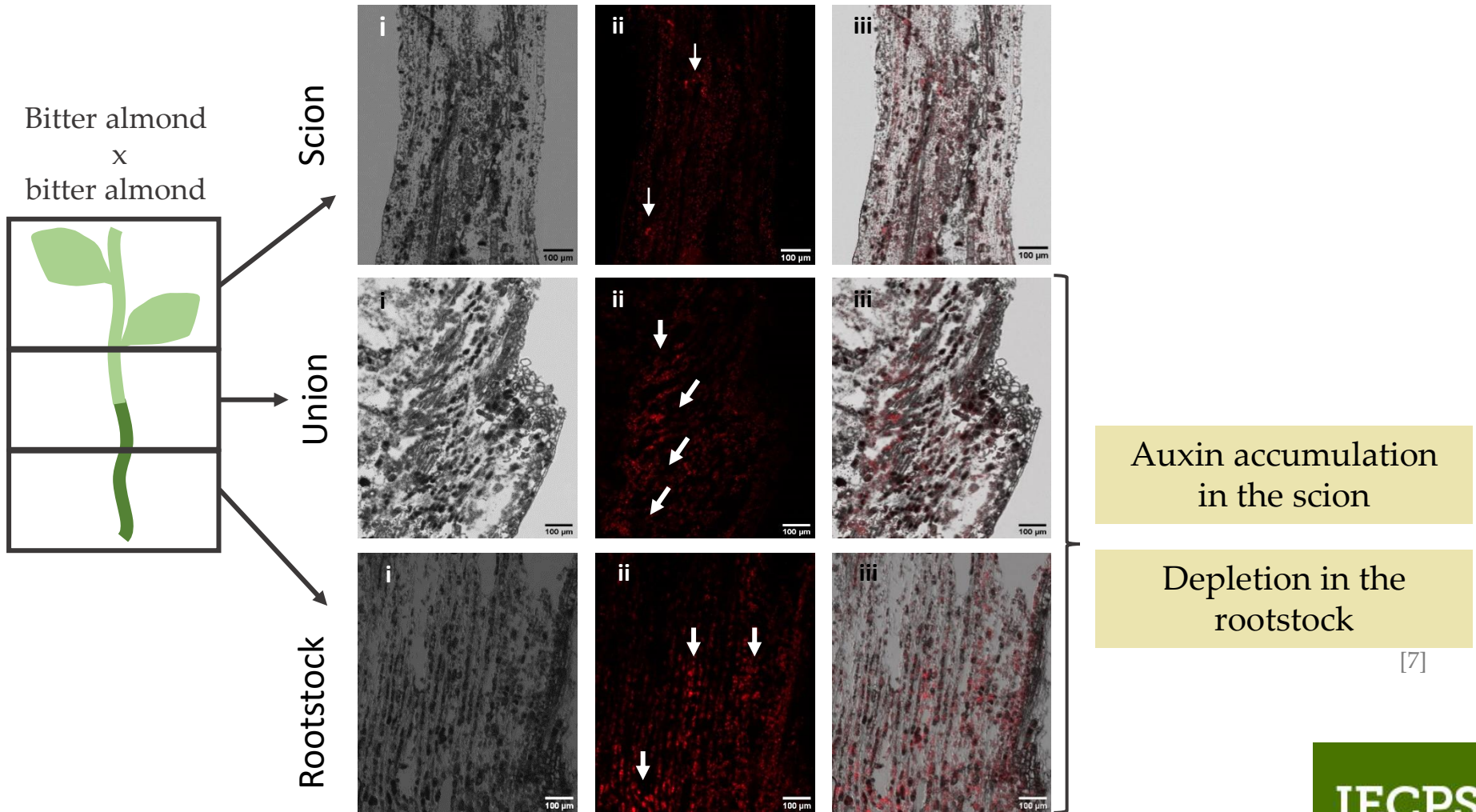
Results and discussion

IAA immunolocalization



Results and discussion

IAA immunolocalization



IAA distribution apparently affected by the initial cut

Conclusions

Possible influence of scion IAA initial levels on micrografting success

Tendency for IAA accumulation at the graft union 21 days after micrografting

IAA distribution dynamics possibly affect by 21 days after micrografting

Base for future studies on the molecular communication between scion and rootstock in almond grafts



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