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REF1: A breakthrough peptide signal unlocking plant regeneration and transformation

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ABSTRACT

This commentary highlights the groundbreaking discovery of REGENERATION FACTOR1 (REF1), a peptide that acts as a pivotal local wound signal promoting plant regeneration and transformation. REF1, perceived by the receptor kinase PORK1, activates the transcription factor SlWIND1 to drive callus formation and shoot regeneration. Unlike systemin, REF1 independently regulates local defense and regenerative pathways. The REF1-PORK1-SlWIND1 signaling module enhances regeneration efficiency in recalcitrant crops such as tomato and soybean, offering a promising strategy for agricultural biotechnology and crop improvement through enhanced transformation and regeneration.

Keywords: Plant regeneration | Plant transformation | PORK1 | REF1 | SlWIND1 | Tissue culture

Living organisms are frequently subjected to diverse environmental stressors that can cause significant physical damage, often resulting in partial or complete organ loss. Unlike animals, plants experience injuries more regularly and have evolved an extraordinary ability to regenerate—repairing damaged tissues and even regrowing entire organs or whole organisms [1-3]. Plants exhibit a variety of regenerative mechanisms in response to injury. In many cases, they can reconstruct shoot or root apical meristems following localized damage [3,4]. Even more remarkably, plants can regenerate entirely new organs or even complete organisms from small tissue fragments or even single cells through de novo organogenesis or somatic embryogenesis [5]. A key feature of de novo organogenesis is the reactivation of cell division at wound sites [4], often leading to the formation of a proliferative cell mass known as a callus. This structure serves as a critical foundation for subsequent regenerative processes [6]. Understanding the intrinsic regenerative capacity of plants provides essential insights for biotechnological applications, particularly in in vitro propagation and the controlled production of numerous plant species.

In a recent study, Yang et al [7] identify REGENERATION FACTOR1 (REF1), a peptide derived from the tomato *SlPep* precursor, as a critical local wound signal that promotes plant regeneration (Figure 1). Unlike the well-known peptide systemin, which primarily regulates systemic defense responses, REF1 operates independently of systemin to mediate both local defense and regenerative processes. The researchers demonstrate that REF1 is perceived by the receptor PORK1, activating the transcription factor SlWIND1, a master regulator of cellular reprogramming. This signaling cascade enhances callus formation and shoot regeneration, offering a novel method to improve the transformation efficiency of recalcitrant crops.

The research article presents compelling findings on the role of the peptide SlPep, derived from the tomato *PROPEP* (*PRP*) gene, as a systemin-independent local wound signal dubbed REGENERATION FACTOR 1 (REF1). The study demonstrates that while systemin primarily drives systemic defense responses, REF1 regulates local defense and regeneration processes. For instance, mutants defective in systemin biogenesis (*prs*) or signaling (*spr1-1*) retained intermediate local defense responses, as measured by wound-induced expression of the proteinase inhibitor gene *PI-II* in damaged leaves, but lacked systemic responses in undamaged leaves. In contrast, the *spr9* mutant, identified through map-based cloning as carrying a C146 deletion in *PRP* leading to a premature stop codon, showed compromised local defense responses alongside an intermediate systemic response. Introducing wild-type *PRP* into *spr9* restored both local and systemic defense capabilities, confirming *PRP's* role.

Beyond defense, REF1 emerged as a critical regulator of plant regeneration. The *spr9* and CRISPR-generated *prp* mutants exhibited severely impaired callus formation and shoot regeneration when cultured on callus-inducing medium (CIM) and shoot-inducing medium (SIM), showing minimal callus growth and quantified projection areas significantly lower than wild-type (WT) controls. Conversely, plants overexpressing *PRP* (*PRP-OE*) displayed significantly enhanced regeneration, with increased callus projection areas and a higher number of regenerated shoots per hypocotyl explant compared to WT. Exogenous application of REF1 not only rescued the regeneration defects in *prp* and *spr9* mutants but also boosted callus formation in WT plants in a dose-dependent manner, with effects observed at concentrations as low as 1 nM.

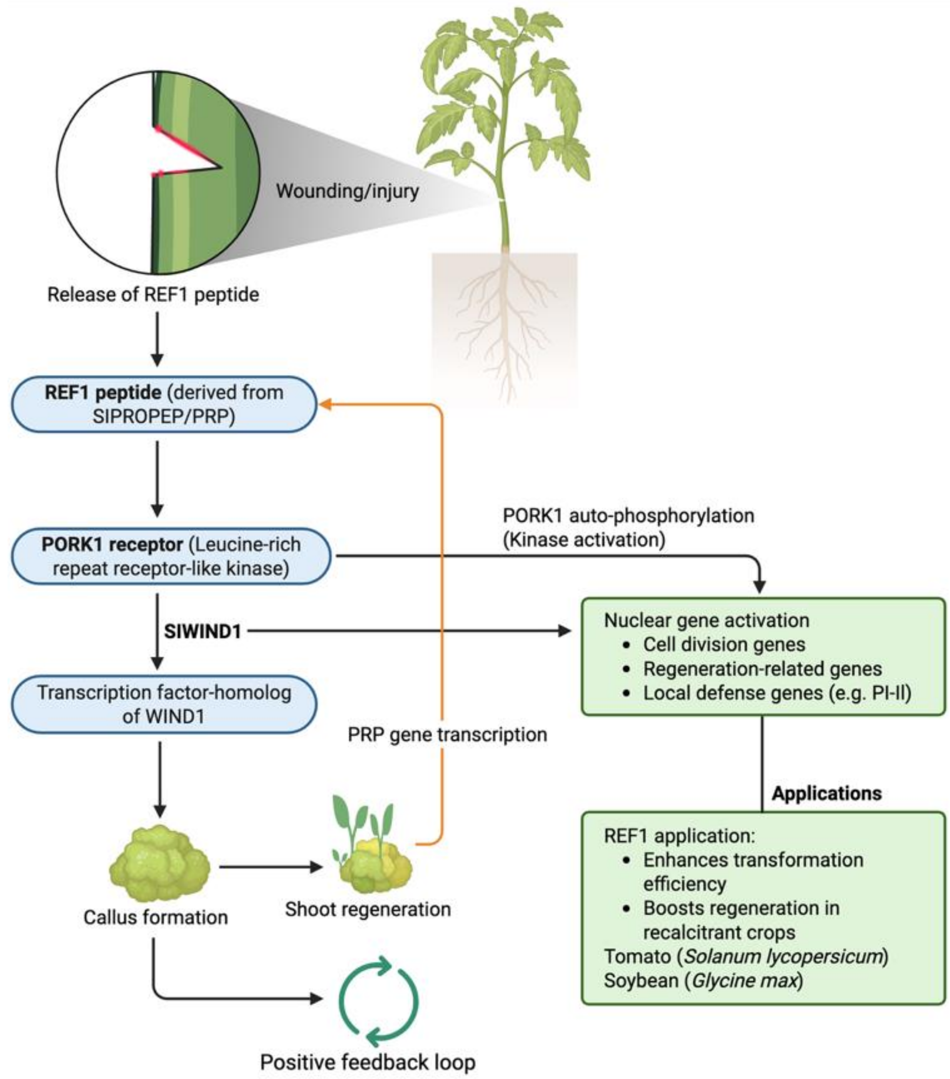

Figure 1: REF1 signaling pathway regulates local defense and regeneration in plants. Mechanical injury releases the REF1 peptide, which activates the PORK1 receptor (a leucine-rich repeat receptor-like kinase). This triggers nuclear gene activation for cell division, regeneration, and local defense. A WIND1-like transcription factor (SlWIND1) is also upregulated, promoting PRP gene transcription, callus formation, and shoot regeneration. The process creates a positive feedback loop that sustains and amplifies regeneration. REF1 application improves transformation efficiency and regeneration in crops like tomato and soybean.

The study further identified PORK1, a leucine-rich repeat receptor-like kinase (LRR-RLK), as the receptor for REF1. CRISPR-generated *pork1* mutants were fully responsive to systemin but insensitive to REF1 in both defense gene expression and root growth inhibition assays, indicating PORK1's specificity to REF1 signaling. These mutants also lost regeneration capacity, failing to form callus or regenerate shoots, while *PORK1-OE* plants showed enhanced callus and shoot formation compared to WT. Biochemical assays reinforced this interaction: the PORK1 ectodomain specifically pulled down biotinylated REF1 (but not systemin), and REF1 triggered autophosphorylation of PORK1's kinase domain in tobacco leaves, an effect absent in kinase-dead mutants (*PORK1^N365E*). Dose-response studies in Arabidopsis protoplasts expressing PORK1 revealed an EC_{50} of ~0.028 nM for REF1-induced expression of the *FRK1* reporter gene, underscoring PORK1's high affinity for REF1. Mechanistically, REF1-PORK1 signaling promotes regeneration by activating *SlWIND1*, the tomato homolog of the master transcription factor *WIND1*. Wound-induced *SlWIND1* expression, tracked via a *pSlWIND1::GUS* reporter, was enhanced by REF1 treatment in WT hypocotyls but compromised in *prp* and *pork1* mutants, as confirmed by RT-qPCR. Null *slwind1* mutants failed to regenerate, producing negligible callus or shoots, while *SlWIND1-OE* plants exhibited superior regeneration capacity. A feedback loop was also uncovered: *SlWIND1* transcriptionally activates PRP, amplifying REF1 production, as evidenced by increased *PRP* expression in *SlWIND1-OE* lines.

Finally, the study demonstrated REF1's practical application by boosting regeneration and transformation efficiency in recalcitrant crops. In *Solanum habrochaites* (accession LA1777), REF1 treatment increased callus formation and the number of transformed shoots, with statistical significance ($p<0.01$). Similarly, in soybean (*Glycine max* variety Dongnong-50), the homologous GmREF1 enhanced regeneration and transformation rates, validated by the detection of a transgenic-specific PCR product. Sequence alignment showed conserved residues across tomato, soybean, maize, and wheat REF1 peptides, suggesting broad applicability. Collectively, these results establish REF1 as a pivotal local wound signal that, through PORK1 and *SlWIND1*, drives plant regeneration, offering a simple, effective strategy to improve transformation in challenging crop species. This discovery not only advances our understanding of plant wound responses but also provides a transformative tool for agricultural biotechnology.

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AUTHOR CONTRIBUTIONS

Conceptualization: Q.S. and T.Y | Visualization: T.K. | Writing – original draft: Q.S. | Writing – review and editing: T.Y. The authors confirm their contributions to the paper as follows.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The author has nothing to report.

LINKED ARTICLE

This article is a Commentary on Wang *et al.* (2024) https://doi.org/10.1016/j.cell.2024.04.040

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