E X R N A - A G

Bacterial Extracellular RNA-Mediated Reprogramming of Quinoa (*Chenopodium quinoa*) Seed Germination

Target Analysis · Mechanistic Models · Validation Strategy

31 Gene Targets Analyzed

REPORT PREPARED BY

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CONFIDENTIAL

ExRNA Research Report: Quinoa

Chenopodium quinoa — Bacterial Extracellular sRNA Target Analysis [CONFIDENTIAL]

> **CONFIDENTIAL** — Generated 2026-02-18 **Family**: Amaranthaceae | **Assembly**: ASM168347v1 **Treatment**: M-9 bacterial EPS solution **Analysis Status**: targets_identified

Executive Summary [CONFIDENTIAL]

This report presents the analysis of **31 predicted exRNA targets** in Chenopodium quinoa (quinoa). These transcripts were identified as potential targets of bacterial extracellular small RNAs (exRNAs) that may improve seed germination and seedling vigor when seeds are treated with M-9 bacterial EPS solution.

Target Distribution

PRIORITY	COUNT
High	4
Medium	4
Low	23
Total	**31**

Pathway Distribution

PATHWAY	TARGETS
Unknown	23
Defense Immunity	3

PATHWAY	TARGETS
Metabolic Priming	1
Transport Ion Homeostasis	1
Signaling	1
Photosynthesis	1
Hormone Signaling	1

High-Priority Targets [CONFIDENTIAL]

GENE ID	ANNOTATION	PATHWAY
AUR62010943	CqHAK5 - High-Affinity K+ transporter 5 (HAK/KUP/KT family)	transport_ion_homeostasis
AUR62044372	CqCNGC14 - Cyclic Nucleotide-Gated Channel 14 (Ca2+ signalin	signaling
AUR62015391	CqGUN4 - Tetrapyrrole-binding protein, chloroplastic (Mg-che	photosynthesis
AUR62003502	CqBRL2/VH1-like - BRI1-Like Receptor Kinase 2 (LRR-RLK; vasc	hormone_signaling

Pathway Analysis Summary [CONFIDENTIAL]

Pathway Analysis Index

TL;DR: Gene targets grouped by 14 biological pathways. Key pathways include hormone signaling, defense/immunity, epigenetics, ROS/redox, transport, and metabolic priming. Last Updated: 2026-02-18

Pathways [CONFIDENTIAL]

PATHWAY	FILE	TARGETS	SUMMARY
Cell Wall Remodeling	cell_wall_remodeling.md	3	Of course. As a plant systems biologist, I will analyze the coordinated downregu
Defense Immunity	defense_immunity.md	5	Of course. As a plant systems biologist, I will analyze this "Defense Immunity"
Dna Repair Replication	dna_repair_replication.md	6	Of course. As a plant systems biologist, I will analyze this pathway by integrat
Epigenetic Regulation	epigenetic_regulation.md	6	Of course. As a plant systems biologist, I will analyze this set of epigenetical
Hormone Signaling	hormone_signaling.md	3	Of course. As a plant systems biologist, I will analyze the coordinated function
Metabolic	metabolic_priming.md	15	Of course. As a plant systems biologist, I will analyze this set of downregulate
Organelle Biogenesis	organelle_biogenesis.md	6	Of course. As a plant systems biologist, I will analyze this "Organelle Biogenes
Protein Turnover	protein_turnover.md	11	Of course. As a plant systems biologist, I will analyze the coordinated downregu
Rna Processing	rna_processing.md	10	Of course. As a plant systems biologist, here is a pathway-level analysis of the
Ros Redox	ros_redox.md	3	Of course. As a plant systems biologist, here is a detailed pathway-level analys
Signaling	signaling.md	11	Of course. As a plant systems biologist, here is a pathway-level analysis of the
Transport Ion Homeostasis	transport_ion_homeostasis.md	18	Of course. As a plant systems biologist, I will analyze the "Transport Ion Homeo

PATHWAY	FILE	TARGETS	SUMMARY
Transposon Related	transposon_related.md	5	Of course. As a plant systems biologist, here is a detailed pathway-level analys
Unknown	unknown_function.md	7	Of course. As a plant systems biologist, I will analyze this gene set. The key c

Cross-Pathway Analysis [CONFIDENTIAL]

See cross_pathway_interactions.md

Theme Analysis Summary [CONFIDENTIAL]

Themes Index

> TL;DR: 6 cross-cutting biological themes extracted from target analysis. Last Updated: 2026-02-18

Themes [CONFIDENTIAL]

THEME	FILE	SUMMARY
Defense Downshift	defense_downshift.md	Theme analysis for defense downshift
Epigenetic Remodeling	epigenetic_remodeling.md	Theme analysis for epigenetic remodeling
ROS Optimization	ros_optimization.md	Theme analysis for ros optimization
Hormone Nodes	hormone_nodes.md	Theme analysis for hormone nodes
Transport / Ion Homeostasis	transport_ion_homeostasis.md	Theme analysis for transport / ion homeostasis
Metabolic Priming	metabolic_priming.md	Theme analysis for metabolic priming

Theme Interactions [CONFIDENTIAL]

See individual theme files for cross-theme analysis.

Synthesis: Ranked Targets [CONFIDENTIAL]

Ranked Target Analysis

Definitive Ranked Analysis: Bacterial exRNA Targets in Spinach Seed Germination

Executive Summary [CONFIDENTIAL]

This analysis encompasses 107 predicted gene targets across 14 functional pathways, all putatively downregulated by bacterial extracellular small RNAs (exRNAs) to improve *Spinacia oleracea* seed germination and early seedling vigor. The target landscape reveals a coherent, multi-layered systems-level reprogramming strategy rather than a collection of independent effects. The dominant biological theme is the **suppression of the dormancy-defense-growth tradeoff**: the bacterial exRNAs appear to collectively dismantle the seed's default "locked-down" state—characterized by epigenetic silencing, ABA-dominant hormone signaling, immune readiness, and metabolic stasis—and redirect resources toward radicle emergence and early growth.

The highest-confidence targets cluster around three mechanistic nodes with the strongest support from Arabidopsis functional genetics: (1) **hormone signaling** (ethylene receptor, LOX/JA pathway, cytokinin two-component relay), where downregulation directly shifts the ABA/GA/ethylene balance toward germination-permissive states; (2) **epigenetic regulation** (DNA methyltransferase, HIRA, SUVR5, PHD reader, GIS2), where downregulation dismantles transcriptional repression of germination-promoting loci; and (3) **ion/osmotic homeostasis** (CNGC, CCC cotransporters), where modulation of turgor and calcium signaling directly enables cell expansion. These three nodes are mechanistically interconnected and mutually reinforcing, suggesting that even partial downregulation of each would produce additive or synergistic germination benefits.

Critical caveats must be acknowledged throughout: (i) all target assignments are based on annotation similarity to Arabidopsis homologs, with no spinach-specific functional validation reported; (ii) the exRNA mechanism itself—cross-kingdom sRNA uptake, RISC loading in plant cells, and target silencing—remains incompletely validated in any plant system [SPECULATIVE for most targets]; (iii) the experimental treatment involves bacterial EPS (exopolysaccharides), which are known osmopriming and elicitor agents, representing a major confounder for any phenotypic attribution; and (iv) several annotated targets (reverse transcriptases, cry8Ba, DUF proteins) likely reflect annotation artifacts or transposable element fragments rather than bona fide germination regulators.

Ranking Methodology [CONFIDENTIAL]

Targets were ranked using a multi-criteria scoring framework. Each criterion was weighted by its contribution to mechanistic plausibility and phenotypic relevance:

CRITERION	WEIGHT	RATIONALE
Mechanistic directness — How directly does downregulation of this gene class affect germination rate/vigor based on established plant biology?	30%	Proximal effectors outrank distal modulators
Evidence quality in model systems — Strength of functional data for the Arabidopsis/plant homolog	25%	Arabidopsis T-DNA knockout/ overexpression phenotypes; biochemical data
Pathway priority assignment — Pathway-level "high/medium/low" priority from the provided analyses	20%	Reflects pathway-level emergent behavior weighting
Cross-pathway connectivity — Does this target sit at a hub connecting multiple pathways?	15%	Hub genes have amplified phenotypic impact
Annotation confidence — Is the spinach gene annotation reliable, or is it based on weak homology?	10%	Penalizes DUF proteins, unknown proteins, and likely TE fragments

Targets assigned "low" pathway priority AND with weak annotation AND no clear germination mechanism were placed in Tier 3 or flagged as low-confidence. All confidence ratings reflect the *prior probability* that downregulation of this specific gene in spinach seeds produces a measurable germination phenotype, not certainty of the exRNA mechanism itself.

Tier 1: Critical Targets (Expected Large Phenotypic Effect)

[CONFIDENTIAL]

These targets have strong mechanistic rationale, high-quality homolog evidence, and direct connections to core germination regulatory nodes.

1. SOV3g000150.1 — Ethylene Receptor

- **Mechanism**: Ethylene receptors (ETR1/ERS family) are **negative regulators** of ethylene signaling: receptor presence suppresses ethylene responses. Downregulation of the receptor therefore **activates** ethylene signaling constitutively. In seeds, ethylene promotes germination by antagonizing ABA signaling, reducing ABA

sensitivity, and promoting endosperm weakening. [KNOWN for receptor family; INFERRED for this specific germination context]

- **Evidence strength**: Strong
- **Key references**: *Arabidopsis* ETR1 (AT1G66340) loss-of-function mutants (*etr1-1*) show constitutive ethylene responses; ethylene promotes germination by suppressing ABI5 accumulation (Linkies et al. 2009, *Plant Cell*; Beaudoin et al. 2000, *Plant Cell*). ETR1 receptor downregulation mimics ethylene treatment. Tomato *Nr* (Never-ripe) ethylene receptor mutants confirm receptor-as-repressor model across species.
- **Confidence**: High
- **Confounders**: EPS from bacteria can itself trigger ethylene-like responses via MAMP signaling; the phenotypic contribution of exRNA vs. EPS cannot be separated without exRNA-only controls. [KNOWN confounder]

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2. SOV3g035520.1 — Lipoxygenase (LOX)

- **Mechanism**: LOX enzymes catalyze the first committed step in jasmonic acid (JA) biosynthesis (13-LOX branch) and also generate lipid peroxides that can reinforce dormancy. JA acts synergistically with ABA to inhibit germination. Downregulation of LOX reduces JA production, thereby relieving JA/ABA-mediated suppression of germination. Additionally, reduced lipid peroxidation preserves membrane integrity during imbibition. [KNOWN for LOX-JA-ABA axis; INFERRED for this specific LOX isoform's predominant role]
- **Evidence strength**: Strong
- **Key references**: *Arabidopsis* LOX2 (AT3G45140) and LOX3 (AT1G17420) contribute to JA biosynthesis; *lox3 lox4* double mutants show enhanced germination under stress (Caldelari et al. 2011). JA inhibition of germination via JAZ-MYC2-ABI5 module is well-established (Linkies & Leubner-Metzger 2012, *Plant J*).
- **Confidence**: High
- **Confounders**: LOX isoforms have distinct subcellular localizations and substrate preferences; without isoform-specific data for SOV3g035520.1, the JA-biosynthesis assignment is inferred from annotation. Some LOX isoforms are primarily involved in defense, not JA synthesis.

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3. SOV4g032870.1 — Histidine-containing Phosphotransfer Protein 1 (AHP-like)

- **Mechanism**: AHPs are central relay components of the plant two-component cytokinin signaling system (AHK receptor → AHP → ARR transcription factors). Cytokinin signaling during germination is complex, but specific AHP isoforms (particularly AHP1/2) have been shown to **negatively regulate** ABA responses by modulating type-A ARR expression, which feeds back to suppress ABA signaling. Downregulation of a specific AHP could shift the cytokinin relay toward a configuration that reduces ABA sensitivity and promotes germination. [INFERRED; the directionality depends critically on which AHP isoform and which ARR targets are involved]
- **Evidence strength**: Moderate

- **Key references**: *Arabidopsis* AHP1 (AT3G21510); Arabidopsis *ahp* mutants show altered ABA sensitivity during germination (Kushwah & Laxmi 2014, *Plant Physiol*). Type-A ARRs are negative regulators of cytokinin signaling and can modulate ABA responses.
- **Confidence**: Medium (directionality of effect is isoform-dependent and not fully resolved)
- **Confounders**: AHP proteins are phosphorylation relays with multiple downstream targets; downregulation could have pleiotropic effects on cytokinin homeostasis beyond ABA crosstalk.

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4. SOV1g033340.1 — DNA (Cytosine-5)-Methyltransferase

- **Mechanism**: [... truncated]

Synthesis: Causal Models [CONFIDENTIAL]

Causal Models

Alternative Causal Models: Bacterial exRNA-Mediated Germination Enhancement in *Spinacia oleracea*

Model 1: The Epigenetic Master Switch Model [CONFIDENTIAL]

Core hypothesis: Bacterial exRNAs primarily target the epigenetic silencing machinery that maintains seed dormancy, causing a cascading de-repression of germination-promoting loci; all other observed effects (hormonal shifts, defense suppression, metabolic activation) are downstream consequences of this chromatin remodeling.

Causal chain:

1. Bacterial exRNAs (likely 21–24 nt sRNAs) are delivered to seed cells during imbibition, potentially packaged in outer membrane vesicles (OMVs) or stabilized by EPS matrix association; they are loaded into the plant AGO1/AGO4-containing RISC complex in the cytoplasm and/or nucleus. [SPECULATIVE — cross-kingdom RISC loading demonstrated in limited systems, e.g., *Botrytis–Arabidopsis* (Weiberg et al. 2013, *Science*), but not validated for beneficial bacteria–seed interactions]
2. **Primary targets — the epigenetic gatekeepers — are downregulated:**
3. **SOV1g033340.1 (DNA cytosine-5-methyltransferase)** is silenced → maintenance methylation at CG and CHG contexts fails during the first rounds of DNA replication upon imbibition → passive demethylation of promoters of GA-responsive genes (e.g., *EXPANSIN*, *α-amylase* homologs), ABA catabolism genes (*CYP707A* family), and cell-cycle re-entry genes. [KNOWN that MET1/CMT3 loss causes global hypomethylation in *Arabidopsis*; INFERRED for these specific germination loci in spinach]
4. **SOV4g015450.1 (SUVR5-like H3K9 methyltransferase)** is silenced → loss of repressive H3K9me2 marks at heterochromatic and euchromatic loci → chromatin decompaction at dormancy-associated gene clusters. [KNOWN that SUVR5 contributes to H3K9me2 in *Arabidopsis* (Caro et al. 2012, *PLoS Genetics*); INFERRED for spinach dormancy loci]
5. **SOV6g036290.1 (HIRA histone chaperone)** is silenced → reduced deposition of histone variant H3.3 at specific loci; in the dormancy context, HIRA may maintain repressive nucleosome occupancy at germination-promoting genes, and its loss destabilizes these nucleosomes. [KNOWN that HIRA deposits H3.3 in *Arabidopsis*; SPECULATIVE that this specifically represses germination loci]

6. **SOV4g030590.1 (PHD-domain protein)** and **SOV4g038060.1 (GIS2 zinc finger)** are silenced → loss of "reader" proteins that recruit Polycomb Repressive Complex 2 (PRC2) and other repressive complexes to H3K4me0/H3K27me3-marked chromatin → further chromatin opening. [INFERRED from PHD-PRC2 interactions in *Arabidopsis*; SPECULATIVE for GIS2 in this context]

7. **Cascading transcriptional de-repression:**

8. Chromatin opening at hormone biosynthesis/signaling loci leads to increased GA biosynthesis (*GA3ox*, *GA20ox*), increased ABA catabolism (*CYP707A*), and increased ethylene biosynthesis (*ACS*, *ACO*) — effectively recapitulating the hormonal shift from ABA-dominant to GA/ethylene-dominant without directly targeting hormone pathway genes. [INFERRED — this is the known transcriptional program activated during *Arabidopsis* germination upon epigenetic de-repression]

9. Chromatin opening at defense regulon promoters paradoxically makes them accessible, but the simultaneous direct targeting of **EDR2** (SOV3g043450.1, SOV6g048760.1) and **MOS1** (SOV5g005530.1) by exRNAs ensures that immune signaling remains dampened even as chromatin opens — this represents a "selective unlocking" where growth genes are activated but defense genes are kept suppressed by a secondary layer of post-transcriptional silencing. [SPECULATIVE]

10. The hormone shift (↑GA, ↓ABA) then drives all downstream execution: cell wall loosening (via GA-induced α-amylase, expansins, XTHs), ROS window establishment, reserve mobilization, and water uptake.

11. **Net phenotypic outcome**: Accelerated and more uniform radicle emergence (improved T50 and germination synchrony) due to earlier and more complete activation of the germination transcriptional program. Seedling vigor is enhanced because the epigenetic "reset" also de-represses post-germination growth genes (photomorphogenesis, root elongation programs) ahead of schedule.

Supporting evidence: - DNA methylation dynamics are causally linked to dormancy cycling in *Arabidopsis*: seeds of *met1* and *cmt3* mutants show reduced dormancy [KNOWN] (Xiao et al. 2006; Zheng et al. 2012) - SUVR5 loss-of-function in *Arabidopsis* causes ectopic gene expression at normally silenced loci [KNOWN] (Caro et al. 2012) - The GA/ABA ratio is the master determinant of germination timing, and both pathways are epigenetically regulated [KNOWN] (Graeber et al. 2012, *Plant Cell*) - Cross-kingdom sRNA-mediated gene silencing has been demonstrated for fungal pathogens targeting plant immunity genes [KNOWN] (Weiberg et al. 2013) - 5 of 6 epigenetic pathway genes are ranked "high priority" in the target analysis, the highest density of any pathway

Weaknesses: - This model requires that epigenetic changes occur *fast enough* during imbibition (hours) to influence germination, yet passive demethylation requires at least one round of DNA replication; early germination in many species occurs without cell division (cell expansion only), so the timing is problematic [KNOWN constraint] - Does not directly explain why specific transport/ion homeostasis genes (CNGC, CCC cotransporters) are targeted — these would need to be coincidental targets or explained as secondary effects - Assumes that the bacterial exRNAs can access the nucleus and/or that cytoplasmic RISC can silence nuclear-localized transcripts of chromatin modifiers — mechanistically uncertain [SPECULATIVE] - Cannot distinguish from EPS-mediated osmopriming, which also accelerates imbibition and could independently activate demethylation pathways via earlier replication onset

Testable predictions: 1. **Bisulfite sequencing**: Seeds treated with bacterial exRNA [... truncated]

Synthesis: Confounder Analysis [CONFIDENTIAL]

Confounder Analysis

Critical Analysis of Confounders in the exRNA Germination Improvement System

Preamble [CONFIDENTIAL]

This analysis treats the claimed mechanism — bacterial extracellular small RNAs entering spinach seeds and silencing 109 plant target transcripts via antisense complementarity — as the hypothesis under scrutiny. The goal is to rigorously enumerate alternative explanations for the observed germination and vigor phenotypes, estimate their plausibility, and design controls to discriminate among them.

1. EPS Osmopriming Effect [CONFIDENTIAL]

Mechanism

[KNOWN] Exopolysaccharides (EPS) are high-molecular-weight hygroscopic polymers (typically 10^5–10^6 Da) that dramatically alter the water potential (ψ) of solutions. When seeds are imbibed in EPS solutions, the osmotic environment is fundamentally different from water alone. This is functionally equivalent to **osmopriming**, one of the oldest and most robust seed invigoration techniques in agriculture.

Specific physical chemistry: - EPS solutions create a controlled matric/osmotic potential (typically −0.5 to −1.5 MPa depending on concentration) that allows seeds to initiate Phase I and early Phase II imbibition without completing germination [KNOWN — reviewed in Bewley et al., 2013, *Seeds: Physiology of Development, Germination and Dormancy*, 3rd ed.] - During controlled hydration, seeds activate DNA repair enzymes (e.g., *OGG1*, *LIGASE IV*), antioxidant systems (SOD, CAT, APX), and synthesize mRNAs for germination-associated proteins without radicle protrusion [KNOWN] - Upon transfer to water, osmoprimed seeds germinate faster and more synchronously because they have already completed pre-germinative metabolic preparation [KNOWN]

Critically relevant: Many of the 109 identified "targets" fall in pathways that are **expected** to change during any priming treatment: - ROS/redox pathway genes — universally modulated during priming [KNOWN] - Hormone signaling (ABA catabolism, GA biosynthesis) — the canonical priming response involves ABA/GA ratio shifts [KNOWN] - Metabolic priming genes — by definition activated during osmopriming [KNOWN]

Expected Magnitude vs. Observed Effect

[KNOWN] Osmopriming with PEG, mannitol, or even NaCl solutions routinely produces: - 15–40% improvement in germination rate under stress conditions - 20–50% improvement in germination speed (T50 reduction) - Significant improvement in seedling vigor indices

[INFERRED] If the M-9 EPS solution has osmotic properties in the priming-effective range (−0.5 to −1.5 MPa), the **entire observed germination phenotype** could potentially be explained by osmopriming alone, without invoking any RNA-mediated mechanism.

Controls Needed

CONTROL	PURPOSE	PRIORITY
EPS solution matched for osmotic potential but **RNase A/III treated**	Eliminates RNA while preserving osmopriming	**CRITICAL**
PEG 8000 solution at identical ψ to M-9 EPS	Non-biological osmoticum control	**CRITICAL**
Heat-denatured EPS solution (autoclaved, 121°C, 20 min)	Denatures RNA and proteins but preserves polysaccharide backbone osmotic effects	HIGH
Measure ψ of M-9 EPS solution with osmometer	Quantify the osmotic contribution	**CRITICAL**

Evidence Level: [KNOWN] for the mechanism; [INFERRED] that it likely contributes substantially to the observed phenotype

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2. Polysaccharide Elicitor Effects [CONFIDENTIAL]

Known Defense/Growth Priming by Bacterial Polysaccharides

[KNOWN] Bacterial EPS and lipopolysaccharides (LPS) are well-characterized **microbe-associated molecular patterns (MAMPs)** that trigger plant innate immune responses:

- **EPS perception:** Plants recognize bacterial polysaccharides through receptor-like kinases (RLKs) including members of the LysM-RLK family (e.g., *CERK1/LYK5* in Arabidopsis) and lectin-type RLKs [KNOWN — Zipfel, 2014, *Curr. Opin. Plant Biol.*]
- **β-glucan recognition:** If the EPS contains β-1,3- or β-1,6-glucan motif [... truncated]

Synthesis: Validation Plan [CONFIDENTIAL]

Validation Plan

Comprehensive 4-Tier Validation Plan: Bacterial exRNA-Mediated Germination Enhancement in *Spinacia oleracea*

Preamble: Validation Philosophy [CONFIDENTIAL]

This plan is designed around **falsificationist logic**: each tier is structured to eliminate confounders before attributing phenotypes to the proposed exRNA mechanism. The ranked targets, causal models, and confounder analysis collectively define a **prior probability landscape** in which the EPS osmopriming effect [KNOWN, HIGH magnitude] and polysaccharide elicitor effects [KNOWN, MEDIUM magnitude] must be rigorously excluded before any RNA-mediated interpretation is defensible. The plan proceeds from phenotypic attribution (Tier 1) through molecular target validation (Tier 2) to mechanistic dissection (Tier 3) and translational application (Tier 4). No Tier 2 or higher experiments should be interpreted without Tier 1 confounder controls completed.

Epistemic labeling is maintained throughout: [KNOWN], [INFERRED], [SPECULATIVE] tag each prediction.

Tier 1: Essential Controls — Confounder Elimination and Phenotypic Attribution [CONFIDENTIAL]

These experiments must be completed and interpreted before proceeding. Their purpose is to determine what fraction of the germination phenotype, if any, requires intact RNA.

Experiment 1.1: RNA Integrity Ablation Control

Experiment: RNase-treated EPS solution germination assay

Hypothesis tested: Does the germination improvement require intact RNA in the bacterial exudate, or is it fully explained by the osmotic/polysaccharide properties of the EPS matrix?

Method: 1. Prepare M-9 bacterial culture supernatant/exudate at the standard treatment concentration used in the original experiment 2. Split into four treatment arms: - **T1 (Full treatment)**: Untreated M-9 exudate (positive control, replicates original experiment) - **T2 (RNase A + III)**: Exudate treated with RNase A (0.1 mg/mL, 37°C, 1 h) followed by RNase III (0.05 mg/mL, 37°C, 30 min) to degrade both ssRNA and dsRNA; confirm RNA degradation by Bioanalyzer or TapeStation on a parallel aliquot - **T3 (Proteinase K)**: Exudate treated with Proteinase K (0.1 mg/mL, 55°C, 1 h, then 95°C 10 min inactivation) to eliminate protein-based effectors while preserving RNA and EPS - **T4 (Water control)**: Sterile distilled water 3. Measure osmotic potential (ψ_s) of T1 and T2 using a vapor pressure osmometer (Wescor VAPRO or equivalent) to confirm RNase treatment does not alter osmolarity 4. Imbibe 50 spinach seeds per replicate (n = 6 biological replicates per treatment) on moistened filter paper in sealed Petri dishes at 15°C (standard spinach germination temperature) 5. Score germination (radicle ≥ 2 mm) at 24 h intervals for 10 days 6. Calculate: germination percentage (GP), mean germination time (MGT), T50, germination uniformity index (GUI), and seedling vigor index (SVI = germination % × mean radicle length at day 7) 7. Perform ANOVA with post-hoc Tukey HSD; significance threshold $\alpha = 0.05$

Expected result if exRNA mechanism is real: T1 > T2 ≈ T4 for all germination metrics; T3 ≈ T1 (protein ablation does not eliminate effect). The difference T1 − T2 quantifies the RNA-dependent component. [INFERRED — this is the minimum requirement to proceed]

Expected result if EPS osmopriming is the primary confounder: T1 ≈ T2 >> T4; RNase treatment does not reduce germination improvement because the osmotic environment, not the RNA, drives the phenotype. [KNOWN mechanism; INFERRED as likely outcome given EPS osmotic properties]

Expected result if polysaccharide elicitor effect is primary: T1 ≈ T2 ≈ T3 >> T4; neither RNA nor protein removal eliminates the effect, implicating the polysaccharide backbone. [INFERRED]

Critical confounders within this experiment: - RNase A/III may not penetrate OMVs if exRNAs are vesicle-packaged [KNOWN limitation — address in Experiment 1.3] - RNase treatment may alter EPS viscosity/osmolarity — [... truncated]

Methodology [CONFIDENTIAL]

1. **Target Identification**: Bacterial exRNA sequences aligned against Chenopodium quinoa transcriptome
2. **Gene Analysis (Stage 1)**: Individual gene function analysis via Gemini 2.5 Flash
3. **Pathway Mapping (Stage 2)**: Pathway-level grouping and interaction analysis via Gemini 2.5 Pro
4. **Literature Dive (Stage 3)**: Homolog research and deep literature review
5. **Theme Extraction (Stage 4)**: Cross-cutting biological theme identification
6. **Synthesis (Stage 5)**: Claude-powered ranking, causal modeling, and validation design

Generated by ExRNA Autonomous Research Platform Gemini (bulk research) + Claude (synthesis & critical review)